B.6.1.1 Toxicokinetic and metabolism studies in rats that were not previously evaluated in the EU 1st study (1995)
Reference:
IIA, 5.1.1/01
Report:
(1995) HR-001: Metabolism in the rat
Data owner: Arysta Lifescience SAS Report No.: SNY 332/951256
Date:1995-08-16, not published, ASB2012-11379
Guidelines:
Japanese MAFF, 59 NohSan, Notification No. 4200 (1985)
OECD 417
US-EPA FIFRA 85-1
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Materials and methods
Non-labelled test material:
HR-001
Identification:
N-(phosphonomethyl)glycine (CAS No. 1071-83-6)
Description:
Solid
Lot/Batch #:
061221
Purity:
98.9 %
Stability of test compound:
Expiry date 1996-12-20
Radiolabelled test material:
[14C] - HR001
Identification:
[14C] - N-(phosphonomethyl)glycine (CAS No. 1071-83-6)
Position of radiolabel:
N-(phosphono[14C]methyl)glycine
Lot/Batch #:
Not reported
Purity:
> 98 % (TLC, followed by radioscanning)
Specific activity:
327.7 µCi/mg, 56 mCi/mmol
Stability of test compound:
Not reported
Reference substances:

Identification:
Aminomethylphosphonic acid (AMPA) (CAS No. 1066- 51-9)
Description:
Solid
Lot/Batch #:
09203L2
Purity:
99 %
Stability of test compound:
Not reported
Radiolabelled reference substance:
Identification:
[14C] -Aminomethylphosphonic acid (14C-AMPA)
Position of radiolabel:
Amino[14C]methyl
Lot/Batch #:
No
Purity:
97.4 %
Specific activity:
2.0 GBq/mmol, 54 mCi/mmol
Stability of test compound:
Not reported
Vehicle and/or positive control:
Water, solubility was increased by addition of sodium hydrogen carbonate
Test animals:
Species:
Rat
Strain:
Sprague-Dawley
Source:
Age:
6-8 weeks (males), 7-9 weeks (females)
Sex:
Males and females
Weight at dosing:
approximately 200 g
Acclimation period:
At least 5 days
Diet/Food:
Standard Laboratory Diet LAD 1 (Special Diet Services, Witham, Essex, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
During acclimatisation:
Individual housing in suspended, wire bottom, stainless steel cages.

After dosing:
Excretion-balance experiments - individually in glass metabolism cages
Blood/plasma kinetics - in stainless steel battery cages Tissue distribution – in stainless steel battery cages
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 40 - 60 % Air changes: not reported 12-hour light/dark cycle
Study design and methods
In life dates: not reported
Animal assignment and treatment: Preliminary excretion studies
In two independent experiments two rats (1 male, 1 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 0-6, 6-24, and every 24 h for 7 days in receivers cooled with solid CO2. Faeces were collected every 24 h for 7 days. Expired air was passed through traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed every 24 h for 7 days after dosing. The interior of the cages were washed with water at sacrifice after 7 days. Samples were analysed immediately or were stored at -20
°C until taken for analysis.
Animal assignment and treatment: Excretion studies
In two independent experiments 10 rats (5 male, 5 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study. Blood was drawn by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following tissues/organs were taken or sampled for radioactivity measurement:
Adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.
Animal assignment and treatment: Plasma concentrations
In two independent experiments 18 rats (9 male, 9 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage. The animals were divided into three groups of six (3 per sex) and blood samples (0.5 mL) were taken from the tail vein into heparinised tubes at the following times from each group.
Group 1: prior to administration, 1, 4, 24 and 96 h
Group 2: 0.25, 2, 6, 48 and 120 h
Group 3: 0.5, 3, 12, 72 and 168 h
Each group was sacrificed upon completion of the specified sampling schedule.
Animal assignment and treatment: Quantitative tissue distribution
In two independent experiments 12 rats (6 male, 6 female) received single oral doses of either 10 or 600 mg/kg bw/day by gavage. The animals were divided into two groups of six (3 per sex) and sacrificed by cervical dislocation 6 and 18 h (low dose) or 3 and 9 h (high dose) after dosing, depending on the peak plasma concentrations and half the plasma concentration derived in the blood/plasma kinetics experiments. Data for 168 h (7 days) was provided by the excretion studies. Blood samples were taken by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following

tissues/organs were taken or sampled for radioactivity measurement: adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.

Measurement of radioactivity

Faeces were initially extracted by homogenisation with chloroform : 1N HCl (1:1, v/v) followed by further extracts with 1N HCl. After centrifugation radioactivity was measured in both extracts and residues. Samples of urine, plasma, solvent extracts contents of expired air traps, cage washings and other liquid samples were mixed with Special Scintillator MI-31 (Packard Instrument Co. Ltd, Reading, UK) for measurement of radioactivity by liquid scintillation counting (LSC). Samples of faecal residues, gastro-intestinal tract, liver, spleen and whole blood were combusted, absorbed, mixed with scintillation cocktail and analyzed thereafter. Carcasses were digested for 48 h at 55 °C in a solution of 2M NaOH in 30 % Methanol containing Triton X-405 (10 % v/v). Tissue samples suitable for solubilisation were incubated at around 50 °C for 18-24 h with solubiliser and mixed with scintillation cocktail and analyzed thereafter. Radioactivity with less than twice background counts was considered to be below the limit of accurate quantification when performing LSC.

Isolation of the major urinary and faecal metabolites

Samples of urine and faecal extracts from male and female rats were pooled and analysed directly by TLC or HPLC. Radiolabelled metabolite products formed in the rat were identified by co-chromatographic comparison using different systems with the reference compound aminomethyl phosphonic acid (AMPA) or 14C-AMPA.

Thin layer chromatography (TLC)

TLC was carried out on pre-layered Merck cellulose F plates (0.2 mm, BDH Chemicals Ltd., Poole, UK) using the following development systems:

System 1: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid (55 : 35: 5: 3.5 : 2, v/v/v/w/v)

System 2: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid (65 : 35: 2.5: 3.5 : 2, v/v/v/w/v)

System 5: Methanol : water : acetic acid (67 : 33 : 1, v/v/v)

Radioactivity was detected with a Berthold Linear Analyser controlled by a computer system (Berthold Instruments (UK)) and proportions of radioactive components were measured by integrating the areas under the peaks on the radio chromatogram following subtraction of background levels. Alternatively, components were detected and quantified using a Fuji BAS 2000 Bioimage Analyser. The produced images of radioactive TLC plates were processed to generate quantitative data.

High performance liquid chromatography (HPLC)

Two HPLC methods were used. HPLC system 1 (gradient elution method; column: Sperisorp SAX HPLC column (Hichrom, UK) and guard column, eluent A: water, eluent B: 0.75 M KH_2PO_4, pH 3.35) and HPLC system 2 (isocratic method; column: glyphosate analytical column (BioRad, USA), eluent: 0.005 M KH_2PO_4 + 4 % methanol v/v, pH 2.1) were both linked to an UV- and a radio-detector. A Compaq Prolinea computer with Labchrom software was used to collect and process data from the UV and radio detectors. Samples were co- injected with a mixture of the reference standards. Fractions were collected and radio assayed by LSC.

Results and discussion

Excretion and retention of radioactivity

A preliminary study on two rats per dose (male/female) indicated that more than 90 % of the administered radioactivity was excreted within 7 days by both the low and the high dose group after a single application of the test substance. Almost no radioactivity could be detected in expired air (about 0.15 %).

The main study with 10 rats per dose confirmed the initial observation (see Table B.6.1-1). During the 7 days observation period 23 % and 19 % (male/female) were excreted in the urine of the low dose group. Slightly higher percentages, 30 % and 29 % (male/female), of total administered radioactivity were detected in urine of the high dose group. The main portion of the radioactivity was detected at both dose levels within the first 48 h in males and females (21 % and 18 %, 10 mg/kg bw; 28 % and 27 %, 600 mg/kg bw). In both dose groups about 75 % of the administered radioactivity could be detected in the faeces of males and females within 7 days (75 % and 84 %, 10 mg/kg bw; 75 % and 74 %, 600 mg/kg bw). Again most of the radioactivity was detected within 48 h after dosing (72 % and 82 %, 10 mg/kg bw; 72 % and 69 %, 600 mg/kg bw). About 0.3 % of the radioactivity remained in the carcasses of the sacrificed animals after 7 days. Thus, in male and female rats almost all the administered radioactivity was excreted via in the urine and faeces within 7 days (97 % and 104 %, 10 mg/kg bw; 105 % and 104 %, 600 mg/kg bw).

Table B.6.1-1: Excretion balance (in mean % of applied dose) up to 168 h post dosing

Balance/Excretion	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
Urine 0-6	2.63	3.25	11.55	9.08
Urine 6-24	15.85	12.69	13.85	13.36
Urine 24-48	2.82	2.41	2.33	4.40
Urine 48-72	0.54	0.44	0.59	1.07
Urine 72-96	0.24			

0.19
0.30
0.40
Urine 96-120
0.15
0.13
0.21
0.24
Urine 120-144
0.09
0.07
0.17
0.17
Urine 144-168
0.07
0.05
0.13
0.18
Cage wash
0.12
0.14
1.13
0.60
Subtotal
urine + cage wash
22.51
19.37
30.26
29.50
Faeces 0-24
60.28
74.59
58.94
46.28
Faeces 24-48
11.72
7.56
13.41
22.87
Faeces 48-72
1.18
1.34
1.36
3.83
Faeces 72-96
0.29

0.36
0.35
0.47
Faeces 96-120
0.17
0.27
0.36
0.23
Faeces 120-144
0.35
0.08
0.08
0.12
Faeces 144-168
0.64
0.10
0.15
0.35
Subtotal faeces
74.63
84.30
74.65
74.15
Carcass
0.33
0.27
0.31
0.39
Total
97.47
103.94
105.22
104.04
Concentration of radioactivity in the plasma
After a single oral dose of 10 mg/kg bw 14C HR-001 to rats peak mean concentrations of radioactivity in plasma occurred at 6 and 2 h in males (0.22 µg equiv./mL) and females (0.28 µg equiv./mL), respectively. The absorption rate constants were 0.2963 h-1 in males and 0.4239 h-1 in females. Concentrations declined with an approximate half-life of 8.3 h in males and 7.8 h in females. The area under the concentration versus time curve (AUCt) was 3.2 and
3.7 µg equiv./mL *h in males and females, respectively (see Table B.6.1-2).
After a single oral dose of 600 mg/kg bw 14C HR-001 to rats peak mean concentrations of radioactivity in plasma occurred at 3 h in males (26 µg equiv./mL) and females (29 µg equiv./mL), respectively (see Table B.6.1-3). The absorption rate constants were with 0.2845 h-1 in males and 0.4477 h-1 in females comparable with the low dose group, thus absorption did not increase with dose. Concentrations declined with an approximate half-life of 5.9 h in males. The terminal half life could not be calculated for females of the high dose group due to rapid clearance

from plasma. The area under the concentration versus time curve (AUCt) was calculated at 400 and 355 µg equiv./mL*h in males and females, respectively. These values were around 120 fold higher than the AUCt obtained in the low dose group.

Table B.6.1-2: Kinetic parameters in plasma after single oral doses of 10 or 600 mg/kg bw

	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
Cmax (µg equiv./mL)	0.2219	0.2789	25.97	28.84
Tmax (h)	6.00	2.00	3.00	3.00
AUCt (µg equiv./mL*h)	3.20	3.70	399.90	355.30
AUC (µg equiv./mL*h)	3.80	4.20	419.00	*
Terminal rate constant (h-1)	0.0840	0.0887	0.1174	*
Terminal half life (h)	8.30	7.80	5.90	*
Absorption rate constant (h-1)	0.2963	0.4239	0.2845	0.4477

* could not be calculated

Measurements in whole blood in general lead to the same result. Distribution of radioactivity in tissue

Radioactivity concentrations in tissues were very low at all times (see Table B.6.1-3 and Table B.6.1-4). There was no indication of accumulation of radioactivity in any tissue. Only the gastrointestinal tract (GIT) the stomach, muscles and the kidneys, the organs of excretion contained higher concentrations of radioactivity than the plasma. High levels of radioactivity were detected in the content of stomach and GIT. At 7 days p.a. the radioactivity in most tissues had decreased to around the limit of detection. Highest remaining concentrations were detected in carcass (<0.4 %).

Table B.6.1-3: Radioactivity in tissues after single oral dose of 10 mg/kg bw (in mean % of applied dose, except bone and skin expressed as % of applied dose/g)

Tissue
Males
Females
6 h (n=3)
18 h (n=3)
168 h (n=5)
6 h (n=3)
18 h (n=3)
168 h (n=5)
Adrenal glands
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Bone
0.12
0.10
0.02
0.10
0.09
0.03
Bone marrow
0.01
0.01
<0.01
0.01
0.01
0.01
Brain
<0.01
<0.01
<0.01
<0.01
<0.01

<0.01
Carcass
2.00
2.69
0.33
1.69
3.03
0.27
Eyes
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Fat (abdominal)
0.06
0.04
0.01
0.04
0.03
0.01
Gastrointestinal tract
19.05
10.04
0.01
16.47
5.41
0.01
GIT contents
31.56
4.89
0.01
34.54
14.30
0.01
Heart
0.01
<0.01
<0.01
0.01
<0.01
<0.01
Kidneys
0.79
0.36

<0.01
0.67
0.26
<0.01
Liver
0.07
0.09
0.01
0.06
0.07
0.01
Lungs
0.01
0.01
<0.01
0.01
0.01
<0.01
Lymph nodes
0.09
0.05
<0.01
0.04
0.04
<0.01
Muscle (skeletal)
0.23
0.13
0.04
0.24
0.11
<0.03
Tissue
Males
Females
6 h (n=3)
18 h (n=3)
168 h (n=5)
6 h (n=3)
18 h (n=3)
168 h (n=5)
Ovaries
-
-
-
<0.01

<0.01
<0.01
Pancreas
0.02
<0.01
<0.01
0.01
<0.01
<0.01
Pituitary gland
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Plasma
0.12
0.03
<0.01
0.13
0.03
<0.01
Skin
0.01
0.01
<0.01
0.01
0.01
<0.01
Spleen
0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Stomach
3.47
0.60
0.60
2.56
0.62
<0.01
Stomach contents
25.16

5.05
0.01
22.90
6.96
0.01
Testes
0.01
0.01
<0.01
-
-
-
Thymus
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Thyroid
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Urinary bladder
0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Uterus
-
-
-
0.01
<0.01
<0.01
Whole blood
0.20
0.04
<0.03
0.15
0.05

<0.03
Table B.6.1-4: Radioactivity in tissues after single oral dose of 600 mg/kg bw (in mean % of applied dose, except bone and skin expressed as mean % of applied dose/g)
Tissue
Males
Females
3 h (n=3)
9 h (n=3)
168 h (n=5)
3 h (n=3)
9 h (n=3)
168 h (n=5)
Adrenal glands
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Bone
0.09
0.10
0.02
0.09
0.05
0.02
Bone marrow
0.01
0.01
<0.01
0.01
0.01
<0.01
Brain
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Carcass
1.87
1.70
0.31
2.85
2.41

0.39
Eyes
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Fat (abdominal)
0.09
0.05
<0.01
0.09
0.02
<0.01
Gastrointestinal tract
19.71
9.99
0.01
20.90
9.33
0.01
GIT contents
30.48
13.19
0.02
22.65
12.86
0.03
Heart
0.01
<0.01
<0.01
0.01
<0.01
<0.01
Kidneys
1.00
0.55
<0.01
0.82
0.21
<0.01
Liver
0.06
0.14

0.01
0.07
0.06
0.02
Lungs
0.02
0.01
<0.01
0.02
0.01
<0.01
Lymph nodes
0.07
0.02
<0.01
0.04
0.01
<0.01
Muscle (skeletal)
0.38
0.19
<0.05
0.48
0.18
<0.05
Ovaries
-
-
-
<0.01
<0.01
<0.01
Pancreas
0.01
0.01
<0.01
0.01
0.01
<0.01
Pituitary gland
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01

Plasma
0.26
0.07
<0.01
0.30
<0.01
<0.01
Skin
0.01
<0.01
<0.01
0.01
<0.01
<0.01
Spleen
0.01
0.01
<0.01
<0.01
<0.01
<0.01
Stomach
3.53
3.36
<0.01
4.33
3.14
<0.01
Stomach contents
28.73
32.70
0.02
34.20
45.01
0.02
Testes
0.01
0.01
<0.01
-
-
-
Thymus
<0.01
<0.01
<0.01

<0.01
<0.01
<0.01
Thyroid
<0.01
<0.01
<0.01
<0.01
<0.01
<0.01
Urinary bladder
0.01
0.01
<0.01
<0.01
<0.01
<0.01
Uterus
-
-
-
0.01
<0.01
<0.01
Whole blood
0.28
0.08
0.01
0.30
0.05
<0.01

Proportion of radioactive components in urine
Major urinary (18 - 27 %) and faecal (65 - 78 %) component was the parent compound. One further minor component was also observed in urine (0.1 – 0.3 %) and identified as aminome- thyl phosphonic acid by TLC and HPLC co-chromatography. In faeces two further minor components were detected (1-2 %, low dose; 0.3-0.6 %, high dose). One of them could be identified as aminomethyl phosphonic acid by TLC and HPLC co-chromatography.
Conclusion by the Notifiers
After oral administration of glyphosate (HR-001) at least about 25 % are absorbed. Absorption was similar in both sexes. About 75 % and 25 % of the parent compound are excreted via faeces and urine, respectively. There was no indication for accumulation of glyphosate.
RMS comments
The study is considered acceptable. As in some other studies of this section, it is not clear if the animals were fasted before sacrifice but this will not have affected the outcome of the study. The results confirmed previous knowledge, in particular with regard to rather poor oral absorption from

the gut (based on renal excretion), distribution of radioactivity to the various body compartments with certain affinity to the bones, virtually complete excretion and very minor metabolism. The identity of the second minor component in faeces (beside AMPA) should have been elucidated but it may be assumed to be a transformation product of bacterial activity in the gut. Unfortunately, no experimental group receiving multiple treatment was included in this study. Thus, final proof for the absence of an accumulating potential cannot be taken from the results of this study alone. However, such investigations have been performed by other researchers (1996 (TOX2000-1979); 1992 (TOX9300343); , 1988 (TOX9552356)) giving sufficient information on this endpoint.
2nd study , 1996)
Reference: IIA, 5.1.1/02
Report: . (1996) [14C]-Glyphosate:
Absorption, distribution, metabolism and excretion following oral administration to the rat
Data owner: Nufarm Report No.: 1413/2-1011
Date:1996-10-23, not published, ASB2012-11380 Guidelines: Japanese MAFF, 59 NohSan, Notification No. 4200 (1995) Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Non-labelled test material:
Identification:
Glyphosate
Description:
Not reported
Lot/Batch #:
08808TG and H95D161A
Purity:
96 % and 95.3 %, respectively
Stability of test compound:
Not reported
Radiolabelled test material:
Identification:
[14C] – glyphosate
Position of radiolabel:
N-(phosphono[14C]methyl)glycine
Description:
Not reported
Lot/Batch #:
24, lot 3 and 25, lot 4-7
Purity:
> 99 % (HPLC and TLC)
Specific activity:
310 µCi/mg, 53 mCi/mmol
Stability of test compound:
Stable over 24 h under the conditions of the study
Reference substance:
Identification:

Aminomethylphosphonic acid (AMPA) (CAS No. 1066- 51-9)
Description:
Not reported
Lot/Batch #:
50526010
Purity:
Not reported
Stability of test compound:
Not reported
Vehicle:
Deionised water
Test animals:
Species:
Rat
Strain:
Sprague-Dawley (Crl:CD BR)
Source:
Age:
6-10 weeks
Sex:
Males and females
Weight at dosing:
179 - 280 g (males) and 167 - 205 g (females)
Acclimation period:
Approximately 1 week
Diet/Food:
SQC Rat and Mouse Maintenance Diet No. 1, Expanded (Special Diet Services, Stepfield, Witham, Essex, UK), ad libitum
Diet was removed the evening before and returned 4 h after administration.
Water:
Tap water, ad libitum
Housing:
During acclimatisation:
Groups of 5 per cage, in wire floor polypropylene cages suspended over polypropylene dirt trays containing wood saw dust
After dosing:
Excretion-balance experiments - individually in glass metabolism cages
Blood/plasma kinetics - in wire floor cages Tissue distribution – in wire floor cages
Environmental conditions:
Temperature: 21 ± 2 °C (24 and 26 °C on two consecutive days) This deviation did not affect the study outcome Humidity: 40 - 70 %
Air changes: not reported 12-hour light/dark cycle
Study design and methods
In life dates: not reported
Animal assignment and treatment: Preliminary excretion study
Four fasted rats (2 males, 2 females) received single oral doses of 100 mg/kg bw/day by gavage and

were placed in glass metabolism cages immediately thereafter. Urine was collected at 0-12, 12-24, and every 24 h for 7 days in receivers cooled with solid CO2. Faeces were collected every 24 h for 7 days. Expired air was passed through duplicate traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed after 12, 24, 48 and 72 h after dosing. The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Animal assignment and treatment: Excretion studies

In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw/day by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study.

Animal assignment and treatment: Plasma concentrations

In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw/day by gavage. Blood samples (0.1 mL) were taken from the tail vein into heparinised tubes at the following times from each animal:

Prior to administration, and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h after administration. Blood was centrifuges to separate plasma and radioactivity was determined in plasma.

Animal assignment and treatment: Quantitative tissue distribution

In two independent experiments 24 fasted rats (12 male, 12 female) received single oral doses of either 1 or 100 mg/kg bw/day by gavage. The animals were divided into four groups of six (3 per sex) and sacrificed 4, 12, 24 and 72 (low dose) or 4, 6, 24 and 72 h (high dose) after dosing. Animals were exsanguinated under halothane anaesthesia. Following tissues/organs were taken or sampled for radioactivity measurements:

Adrenals, bone, brain, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, muscle (quadriceps), gonads, plasma, skin, spleen, and residual carcass

The contents of the gastrointestinal tract was analysed separately.

Animal assignment and treatment: Biliary excretion study

16 rats were cannulated (8 males, 8 females), of which 14 received single oral doses of 1 mg/kg bw/day by gavage. Following incision animals were placed in metabolism cages and allowed to recover for 24 h. Fully recovered animals were dosed after they were fasted overnight, and bile, urine and faeces were taken from the animals at the following times:

Bile: prior to administration, 0 - 1, 1 - 4, 4 - 6, 6 - 12, 12 - 24 and 24 - 48 h p.a. Urine, faeces: 0 - 24 and 24 - 48 h p.a. in vessels cooled with solid CO2.

The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Measurement of radioactivity

Pooled faecal samples were extracted with water prior to solid phase extraction. Urine samples were diluted with water prior to solid phase extraction. Solid phase extraction was performed using columns, conditioned with methanol and de-ionised water. The samples were loaded onto the cartridge washed with de-ionised water, methanol and again water. Radioactivity was eluted using formic acid (5 % v/v). The eluate was freeze dried and reconstituted in water prior to HPLC analysis and where appropriate LC - MS (samples of 100 mg/kg dose group).

A suitable volume of solubilising agent was added to tissue samples. After an appropriate incubation time, liquid scintillant was added and samples were subjected to liquid scintillation counting (LSC). Samples of faecal residues, cage debris homogenates, blood and bone were combusted, absorbed, mixed with scintillation cocktail and analysed by LSC thereafter. Combustion and trapping efficiencies were found to be in excess of 96 % and all reported data are, therefore, uncorrected. Radioassays

were performed in duplicats.
Isolation of the major urinary and faecal metabolites
Samples of urine and faecal extracts from male and female rats of the excretion studies were pooled and analysed directly by HPLC. Representative samples were then submitted for analysis by mass spectrometry. The samples were analysed for the presence of glyphosate and the potential metabolite aminomethyl phosphonic acid (AMPA). Following samples were pooled and analysed for each dose group and sex:
Excretion study: Urine 12 - 24 h, Faeces 24 - 48 h
Biliary excretion study: Urine 24 - 48 h, Faeces 24 - 48 h
High performance liquid chromatography (HPLC)
The gradient elution method was used for sample analysis (column: Sperisorp SAX 250 x 4.6 mm id, eluent A: water, eluent B: 0.75 M KH_2PO_4, pH 3.35). The system was linked to a radio-detector. Following HPLC analysis, representative samples were submitted for analysis by mass spectroscopy (samples of 100 mg/kg bw dose group).
Liquid chromatography - Mass Spectrometry (LC - MS)
A VG Quattro triple quadrupole mass spectrometer with electrospray LCMS interface connected to a Jasco ternary gradient HPLC system and a Lablogic A-Ram radio detector were used.
Mode: positive ion electrospray Scan range: m/z 50 – 250
Mobile phases: water or 1 M formic acid
Glyphosate was detected using Multiple Reaction Monitoring (MRM) of m/z 170 / 88. AMPA was detected using Selected Ion Recording (SIR) of m/z 112.
Results and discussion
Excretion and retention of radioactivity
In a preliminary study with a single dose of 100 mg/kg bw (two rats/sex) the mean total recovery of radioactivity within 7 days was 100.3 % (male) and 95.15 % (female). No relevant radioactivity could be detected in expired air or carcass.
The initial observation was confirmed in the main study with 10 rats per dose (see Table B.6.1-5). Mean total recovery of radioactivity in rats receiving a single dose of 1 mg/kg bw was 98.31 % in males and 98.81 % in females. Elimination of radioactivity was almost complete within the first 48 h after dosing. The major route of elimination after oral dosing was faeces with 72.62 % and 62.39 % recovered in males and females, respectively, with most of the radioactivity being excreted within the first 24 h after dosing, suggesting this proportion of the dose was not systemically absorbed. During the 7 days observation period 24.92 % (male) and 34.86% (female) of radioactivity were recovered in the urine, representing the systemically absorbed dose.
After administration of 100 mg/kg bw to rats mean total recovery of radioactivity was 96.31 % in males and 98.50 % in females. Elimination of radioactivity in the urine (including cage wash 53.27 % in males and 55.04 % in females) was quantitatively more significant compared to the low dose group. Faecal elimination accounted for 41.23 % in males and 42.37 % in females. Again most of the radioactivity was recovered within the first 48 h after dosing.
Table B.6.1-5: Excretion balance (in mean % of applied dose) up to 168 h post dosing
Balance/Excretion
1 mg/kg bw
100 mg/kg bw
Males
Females
Males

Females
Urine 0-12
9.52
15.47
31.30
34.93
Urine 12-24
6.14
7.59
4.68
4.46
Urine 24-48
2.10
3.03
2.40
2.32
Urine 48-72
0.35
0.56
0.46
0.71
Urine 72-96
0.15
0.20
0.27
0.33
Urine 96-120
0.09
0.14
0.14
0.15
Urine 120-144
0.06
0.10
0.10
0.10
Urine 144-168
0.04
0.06
0.08
0.07
Cage wash
6.48
7.71
13.85
11.96

Subtotal urine + cage wash
24.92
34.86
53.27
55.04
Faeces 0-24
63.93
49.69
30.46
32.28
Faeces 24-48
7.21
10.93
9.96
4.46
Faeces 48-72
0.65
1.46
0.55
1.10
Faeces 72-96
0.09
0.16
0.12
4.42
Faeces 96-120
0.03
0.06
0.06
0.06
Faeces 120-144
ND
0.07
0.06
0.04
Faeces 144-168
0.71
0.02
0.03
0.01
Subtotal faeces
72.62
62.39
41.23
42.37
Cage debris

0.03
0.58
0.98
0.10
Balance/Excretion
1 mg/kg bw
100 mg/kg bw
Males
Females
Males
Females
Carcass
0.75
0.98
0.84
0.98
Total
98.31
98.81
96.31
98.50
Biliary excretion of radioactivity
Biliary excretion was determined in biliary cannulated rats receiving 1 mg/ kg bw. Within 48 h 94.63 % and 95.99 % of radioactivity were recovered in males and females, respectively. Major route of elimination was faeces. Negligible amounts of radioactivity were detected in the bile (see Table B.6.1-6) providing strong evidence that low doses of systemic glyphosate are eliminated almost exclusively in the urine.
Table B.6.1-6: Excretion balance (in mean % of applied dose) at 48 h post dosing in biliary excretion study
1 mg/kg bw
Males
Females
Urine
27.45
24.21
Faeces
55.33
60.97
Bile
0.031
0.076
Cage wash
6.571
6.769
Cage debris
0.262

0.146
Carcass
4.989
3.817
Total
94.63
95.99
Concentration of radioactivity in the plasma
Following a single oral dose of 1 mg/kg bw of the test substance, low levels of radioactivity were detected in plasma (see Table B.6.1-7). Concentrations of radioactivity declined rapidly such that the levels of radioactivity were below the detection limit in most animals by 24 h. The mean terminal elimination half-lives were 10.86 h and 8.07 h with corresponding AUC of
0.319 and 0.340 µg equiv./mL*h in males and females respectively. As the elimination half- lives could not be calculated for several animals of the high dose group mean AUC0-24 (0.257 and 0.338 µg equiv./mL*h in males and females) were calculated to compare the results of both groups.
Following a single oral dose of 100 mg/kg bw of the test substance mean maximal plasma concentration of 8.91 (male) and 7.63 µg equiv./mL (female) were observed 2-4 h post-dose in males and 4 h post dose in females (see Table B.6.1-7). Mean AUC0-24 were 58.2 and 50.7
µg equiv./mL*h in males and females, respectively. Levels of radioactivity were below the detection limit in males by 48 and in females by 72 h.
Table B.6.1-7: Kinetic parameters in plasma after single oral dose of 1 or 100 mg/kg bw (n = 5)
1 mg/kg bw
100 mg/kg bw
Males
Females
Males
Females
Cmax (µg equiv./mL)
0.016
0.037
8.909
7.634
Tmax (h)
3.900
8.000
3.600
4.000
AUC0-24 (µg equiv./mL*h)
0.257
0.338
58.200
50.700
AUC (µg equiv./mL*h)
0.319
0.340
*

*
Terminal half life (h)
10.860
8.065
*
*
* could not be calculated
Distribution of radioactivity in tissue
After administration of 1 mg/kg bw radioactivity concentrations were detected in all tissues by 4 h post-dose (see Table B.6.1-8 and Table B.6.1-9). Apart from the gastrointestinal tract (and content) and carcass, the kidney was the only tissue with a notable content of radioactivity throughout the observation period. By 72 h, post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity. After administration of 100 mg/kg bw all tissues were exposed to radiolabelled material by 4 h post-dose (see Table B.6.1-10 and Table B.6.1-11). Again, only gastrointestinal tract, carcass and kidney contained significant amounts of radioactivity. By 72 h post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity.
Table B.6.1-8: Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean µg equiv./g)
Tissue
Males
Females
4 h
12 h
24 h
72 h
4 h
12 h
24 h
72 h
Adrenals
0.014
0.024
0.020
0.009
0.023
0.031
0.022
0.009
Blood
0.010
0.015
0.001
0.002
0.020

0.009
0.002
<0.001
Bone
0.062
0.105
0.201
0.123
0.091
0.140
0.134
0.112
Brain
<0.001
0.003
0.003
0.002
0.002
0.001
0.002
0.002
Carcass
0.021
0.028
0.049
0.016
0.035
0.076
0.045
0.024
Fat
0.022
0.005
0.003
0.002
0.013
0.010
0.006
0.002
GIT + contents
13.040
1.333
1.272
0.026
11.630
3.531

1.314
0.075
Heart
0.006
0.004
0.003
0.002
0.010
0.006
0.004
0.001
Kidney
0.463
0.380
0.307
0.020
0.424
0.387
0.129
0.012
Liver
0.012
0.013
0.022
0.012
0.016
0.018
0.015
0.012
Lung
0.009
0.009
0.013
0.006
0.019
0.013
0.009
0.006
Muscle
0.003
0.001
0.002
<0.001
0.006
0.003
0.002

0.001
Ovaries
-
-
-
-
0.031
0.018
0.021
0.007
Plasma
0.017
0.011
0.006
<0.001
0.027
0.015
0.004
<0.001
Skin
0.010
0.026
0.016
0.006
0.029
0.016
0.106
0.014
Spleen
0.004
0.009
0.010
0.005
0.010
0.009
0.010
0.005
Testes
0.004
0.002
0.001
0.001
-
-
-
-

Table B.6.1-9: Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean % of applied dose)

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
Adrenals	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Brain	<0.001	0.003	0.002	0.002	0.002	0.001	0.002	0.002
Carcass	1.236	1.668	3.048	1.045	1.887	4.115	2.542	1.405
GIT + contents	94.310	17.670	12.990	0.342	89.940			

41.740
13.760
0.910
Heart
0.002
0.001
0.001
0.001
0.005
0.003
0.002
0.001
Kidney
0.392
0.304
0.255
0.016
0.348
0.341
0.110
0.011
Liver
0.040
0.050
0.113
0.059
0.055
0.070
0.073
0.057
Lung
0.005
0.005
0.007
0.003
0.012
0.008
0.005
0.004
Ovaries
-
-
-
-
0.002
0.001

0.001
<0.001
Spleen
0.001
0.002
0.002
0.001
0.002
0.002
0.002
0.001
Testes
0.004
0.002
0.002
0.001
-
-
-
-

Table B.6.1-10: Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean µg equiv./g)

Tissue
Males
Females
4 h
6 h
24 h
72 h
4 h
6 h
24 h
72 h
Adrenals
2.936
5.610
1.856
0.338
8.161
7.244
1.522
0.504
Blood
4.545
4.900
0.016
ND

5.719
1.923
0.218
ND
Bone
24.660
31.360
18.600
11.140
35.450
24.420
17.010
10.420
Brain
0.344
0.699
0.269
0.221
0.619
0.630
0.293
0.215
Carcass
6.097
26.530
4.978
1.843
10.910
38.410
7.206
3.057
Fat
1.366
1.547
0.290
0.120
3.826
2.042
0.393
0.115
GIT+contents
1155.000
544.600
47.750
1.279
1057.000

401.800
59.580
4.320
Heart
2.063
3.424
0.363
0.140
3.704
2.282
0.314
0.092
Kidneys
105.500
127.700
17.440
1.433
132.200
55.770
10.800
1.191
Liver
2.942
4.970
1.831
1.165
5.105
5.564
1.552
0.981
Lung
3.495
4.206
1.069
0.423
6.476
4.623
0.999
0.443
Muscle
0.827
0.887
0.168
0.026
1.698
1.141

0.213
0.051
Ovaries
-
-
-
-
7.532
5.407
1.260
0.438
Plasma
6.479
5.406
0.359
ND
10.830
3.033
0.403
ND
Skin
2.884
3.520
1.293
0.313
6.106
22.480
1.543
0.435
Spleen
1.277
2.678
0.974
0.479
2.337
1.237
0.937
0.395
Testes
0.949
0.942
0.203
0.104
-
-
-

-
ND not detected
Table B.6.1-11: Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean % of applied dose)
Tissue
Males
Females
4 h
6 h
24 h
72 h
4 h
6 h
24 h
72 h
Adrenals
0.001
0.001
<0.001
<0.001
0.002
0.001
<0.001
<0.001
Brain
0.003
0.005
0.002
0.002
0.006
0.006
0.003
0.002
Carcass
4.620
8.549
2.402
1.014
8.288
5.879
2.752
1.254
GIT + contents
85.430
64.870
5.456

0.199
75.050
48.910
7.509
0.676
Heart
0.009
0.015
0.001
0.001
0.016
0.009
0.001
<0.001
Kidney
0.870
1.109
0.151
0.012
1.165
0.535
0.096
0.011
Liver
0.104
0.180
0.110
0.060
0.183
0.214
0.088
0.050
Lung
0.027
0.024
0.006
0.003
0.040
0.027
0.007
0.003
Ovaries
-
-
-
-

0.004
0.002
0.001
<0.001
Spleen
0.003
0.007
0.002
0.001
0.006
0.003
0.003
0.001
Testes
0.011
0.010
0.003
0.001
-
-
-
-
Metabolite profiling
After analysis of the pooled samples by HPLC a comparison of chromatograms indicated that the metabolism of the compound was not influenced by the sex or dose level. The peak with the majority of radioactivity could be allocated to [14C]-glyphosate standard. A peak with
<1 % of the total radioactivity was thought to correspond to AMPA. The presence of glyphosate could be confirmed by mass spectroscopy, whereas the presence of AMPA could not be verified due to technical problems.
Conclusion by the Notifiers
After oral administration of glyphosate absorption, distribution, metabolism and excretion were independent of dose level and sex. Absorption was limited and distribution was rapid and extensive. Metabolism was negligible. Elimination was essentially complete within 48 h, with the majority of radioactivity recovered in faeces, likely being the unabsorbed dose. The
remaining radioactivity was excreted with the urine. There was no indication for accumulation of glyphosate.
RMS comments
The study is considered acceptable. Again, poor absortion at least of the low dose, wide distribution and fast excretion of the compound as well as the virtual absence of metabolism were confirmed. Limited oral absorption rate was further supported by the very low amount of biliary excretion that was seldom investigated in ADME studies with glyphosate. In contrast, as compared to other studies, urinary elimination following the high dose was strikingly high. A much higher percentage of radioactivity than in other studies was found in cagewash and may be summed up with the material excreted in urine. The rather high organ/tissue residues in bile-cannulated rats at termination are due to the shorter duration of this experiment and are equal to what was measured in non-cannulated rats after 24 or 72 hours. It should be emphasised that the by far highest residues were

found in bone since this was not mentioned in the GTF dossier. Elimination of bone-bound residues was apparently slower than of the radioactivity that had been retained in other tissues. Unfortunately, no experimental group receiving multiple treatment was included in this study. Thus, final proof for the absence of an accumulating potential cannot be taken from the results of this study alone. However, such investigations have been performed by other researchers (1996 (TOX2000-1979); 1992 (TOX9300343);
, 1988 (TOX9552356)) giving sufficient information on this endpoint.
The following studies (1996 (TOX2000-1977, TOX2000-1978, TOX2000-1979, TOX2000-1980), 1996 (TOX2000-1981)) have been performed to adress certain aspects of toxicokinetics and metabolism of glyphosate. Taken together, they allow comprehensive evaluation.
3d study (1996)
Reference: IIA, 5.1.1/03
Report: (1996) Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat
Data owner: Syngenta Report No.: /4940
Date:1996-04-26, not published, TOX2000-1977
Guidelines: MAFF (Japan) Metabolism Study (1985), OECD 417 (1984), US- EPA FIFRA 85-1, EEC B.36 (1987)
Deviations: None
GLP: Yes
Acceptability: See RMS comment.
Materials and methods
Non-labelled test material:
Glyphosate acid
Identification:
N-phosphonomethyl glycine
Description:
White solid
Lot/Batch #:
Y04707/045
Purity:
99.2 %
Stability of test compound:
Stable throughout the experiment
Radiolabelled test material:
Identification:
[14C]–glyphosate acid
Position of radiolabel:
N-(phosphono[14C]methyl)glycine
Lot/Batch #:
Y04707/047
Purity:
> 98 %
Specific activity:
1.580 GBq/mMol
Stability of test compound:

Stable throughout the experiment
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rat
Strain:
Alpk:APfSD
Source:
Age:
Not reported
Sex:
Male/female
Weight at dosing:
195 - 235 g
Acclimation period:
At least 5 days
Diet/Food:
PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
During acclimatisation:
Groups of the same sex, in stock rat cages, 24 h prior to dosing transfer individually into metabolism cages After dosing:
Excretion-balance experiments - individually in metabolism cages
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 55 ± 15 % Air changes: 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: not reported
Animal assignment and treatment:(Excretion study and quantitative tissue distribution)
Ten rats (5 male, 5 female) received a single oral dose of 10 mg [14C]-glyphosate acid/kg bw/day (10 mL/kg, 0.6 MBq/kg of dosing solution) by gavage and were placed back in glass metabolism cages immediately thereafter. Urine was collected at 6, 12, 24, 36, 48 and 72 h after dosing in receivers cooled with solid CO2. Faeces were collected at 12, 24, 36, 48 and 72 h. The interior of the cages were washed with water after each collection time. At the end of the study cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20 °C until taken for analysis.
Animals were exsanguinated by cardiac puncture under halothane anaesthesia. Two blood samples were collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements: bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, heart, kidneys, liver, lungs, muscle (femoral), gonads, spleen, salivary glands, residual carcass.
Measurement of radioactivity
Samples of urine, cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of

anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus contents were homogenised. Liver, fat and residual carcass were than solubilised in Soluene- 350® whereas GIT plus contents were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation

Samples were oxidised in a Packard Tricarb sample oxidiser. The [14C]-carbon dioxide generated was absorbed into Carbo-sorb E® and mixed with Permafluor E+® scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)

Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3® and counted for [14C]-radioactivity to a 1% standard deviation of the count or for a maximum of 10 min, whichever occurred first. The results obtained were corrected for background activity and counting efficiency using [135Ba] as the external source.

Results and discussion

Excretion and retention of radioactivity

After a single oral dose to rats, excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (means of 77.8 % in males, and 80.7 % in females). In the urine, means of 11.5 % and 9.4 % of the radioactivity were eliminated in the first 24 h in male and female rats, respectively. Within the observation period of 72 h, means of 101.8 % (male) and 99.6 % (female) of the administered radioactivity were excreted (see Table B.6.1-12). There were no differences in the cumulative excretion patterns between the sexes.

Table B.6.1-12: Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	10 mg/kg bw (oral gavage)	
	Males	Females
Urine 0-6	3.7	3.5
Urine 6-12	4.5	3.3
Urine 12-24	3.3	2.6
Urine 24-36	0.8	0.7
Urine 36-48	0.4	0.4
Urine 48-72	0.3	0.2
Cage wash	0.3	

0.4
Subtotal urine + cage wash
13.3
11.1
Faeces 0-12
42.3
48.1
Faeces 12-24
35.5
32.6
Faeces 24-36
6.6
3.9
Faeces 36-48
2.8
2.9
Faeces 48-72
1.3
1.2
Subtotal faeces
88.5
88.7
Total
101.8
99.6
Distribution of radioactivity in tissues
The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 0.51 µg equiv./g (male) and 0.40 µg equiv./g (female), followed by the the intestinal tract plus contents with 0.15 µg equiv./g (males and females). Lower mean concentrations between 0.07 and 0.01 µg equiv./g were found in kidneys, liver, lungs, spleen, salivary glands and ovaries. Mean concentrations of 0.06 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of either sex. All other concentrations were either similar to or lower than the corresponding blood concentrations (see Table B.6.1-13). The mean total percentage of administered radioactivity present in all of the tissues examined and the residual carcass was 0.6 % for males and 0.5 % for females. The amounts in the intestinal tract plus contents were about 0.2 % for both sexes.
Table B.6.1-13: Radioactivity in tissues after a single oral dose of 10 mg/kg bw at 72 h
Tissue
Males
Females
% of dose
µg equiv./g
% of dose
µg equiv./g
Blood
N/A
0.011

N/A
0.009
Bone (femur)
N/A
0.511
N/A
0.395
Brain
0.001
0.011
0.001
0.009
Fat (abdominal)
N/A
0.007
N/A
<0.004
Heart
<0.001
0.012
<0.001
0.011
Kidneys
0.007
0.068
0.004
0.049
Liver
0.036
0.059
0.022
0.044
Lungs
0.002
0.031
0.001
0.026
Muscle (femoral)
N/A
0.007
N/A
0.006
Ovary
-
-
<0.001

0.024
Plasma
N/A
N/A
N/A
<0.004
Residual Carcass
0.542
0.062
0.458
0.056
Salivary glands
<0.001
0.017
<0.001
0.018
Spleen
0.001
0.026
0.001
0.024
Testes
0.001
0.007
-
-
Total
0.590
N/A
0.488
N/A
GIT plus contents
0.186
0.152
0.172
0.152
N/A not applicable
Conclusion by the Notifiers
After a single oral dose glyphosate acid was excreted rapidly and predominantly in faeces. Elimination was essentially complete within 72 h. There was no indication for accumulation of glyphosate acid.
RMS comments
The study is considered acceptable. Sufficient data was provided to describe toxicokinetics and tissue residues of glyphosate following administration of a low dose of 10 mg/kg bw. Plasma kinetics was not followed and metabolism was not investigated but enough information on these parameters is available from other sources.

Taking into account the negligible biliary excretion (, 1996 (ASB2012-11380); 1996 (TOX2000-1981)), oral absorption rate is estimated on the basis of renal elimination. As compared to other studies in which the same dose was applied (1995 (ASB2012-11379); , 1988 (TOX9552356)), urinary excretion in this study was remarkably low, accounted accounting for only 50 to 60% of the previously measured excretion rates in both sexes. The reason for this difference is not known but the lower figure established by (1996, TOX2000-1977) must not be ignored when correction of the AOEL for an internal dose is considered.
Affinity of the compound to bone tissue was confirmed once more.
4th study (1996)
Reference: IIA, 5.1.1/04
Report: (1996) Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat
Data owner: Syngenta Report No.:
Date:1996-06-19, not published, TOX2000-1978
Guidelines: MAFF (Japan) Metabolism Study (1985), OECD 417 (1984), US- EPA FIFRA 85-1, EEC B.36 (1987)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Non-labelled test material:
Glyphosate acid
Identification:
N-phosphonomethyl glycine
Description:
White solid
Lot/Batch #:
Y04707/048
Purity:
99.5 %
Stability of test compound:
Stable throughout the experiment
Radiolabelled test material:
Identification:
[14C]–glyphosate acid
Position of radiolabel:
N-(phosphono[14C]methyl)glycine
Lot/Batch #:
Y04707/047
Purity:
> 98 %
Specific activity:
1.580GBq/mMol
Stability of test compound:
Stable throughout the experiment
Vehicle and/or positive control:

Deionised water
Test animals:
Species:
Rat
Strain:
Alpk:APfSD
Source:
Age:
Not reported
Sex:
Male/female
Weight at dosing:
182 - 235 g
Acclimation period:
At least 4 days
Diet/Food:
PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
During acclimatisation:
Groups of 6 per cage and sex, in stock rat cages After dosing:
Excretion-balance experiments - individually in metabolism cages
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: not reported
Animal assignment and treatment (Excretion study and quantitative tissue distribution)
Ten non-fasted rats (5 male, 5 female) received single oral doses of 1000 mg/kg bw/day (10 mL/kg, 6MBq/kg) by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 6, 12, 24, 36, 48 and 72 h after dosing in receivers cooled with solid CO2. Faeces were collected at 12, 24, 36, 48 and 72 h. The interior of the cages were washed with water after each collection time. At the end of the study cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20 °C until taken for analysis.
Animals were exsanguinated by cardiac puncture under halothane anaesthesia. Two blood samples were collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements:
Bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, heart, kidneys, liver, lungs, muscle (femoral), gonads, spleen, salivary glands, residual carcass.
Measurement of radioactivity
Samples of urine cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus

contents were homogenised. Liver and fat were than solubilised in Soluene-350® whereas GIT and residual carcass were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation

Samples were oxidised in a Packard Tricarb sample oxidiser. The [14C]-carbon dioxide generated was absorbed into Carbo-sorb E® and mixed with Permafluor E+® scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)

Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3® and counted for a maximum of 10 min in Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficiency using [135Ba] as the external source.

Results and discussion

Excretion and retention of radioactivity

Excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (78.7 % in males, and 71.3 % in females). In the urine, means of 15.3 % and 16.0 % of the radioactivity were eliminated in the first 24 h in males and females, respectively. Within the observation period of 72 h, means of 106.4 % (male) and 102.3 % (female) of the administered radioactivity were excreted in total (see Table B.6.1-14).

Table B.6.1-14: Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	1000 mg/kg bw	
	Males	Females
Urine 0-6	7.9	9.7
Urine 6-12	5.0	3.9
Urine 12-24	2.5	2.4
Urine 24-36	0.7	0.8
Urine 36-48	0.4	0.5
Urine 48-72	0.3	0.3
Cage wash	0.1	0.2
Subtotal urine + cage wash	16.9	17.8
Faeces 0-12		

36.4
19.7
Faeces 12-24
42.2
51.6
Faeces 24-36
6.6
8.5
Faeces 36-48
2.9
3.5
Faeces 48-72
1.4
1.3
Subtotal faeces
89.5
84.6
Total
106.4
102.4
Distribution of radioactivity in tissues
The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 49.8 µg equiv./g (male) and 44.9 µg equiv./g (female), followed by the gastrointestinal tract (and contents) with 13.3 µg equiv./g (male and 16.3 µg equiv./g (female). Lower mean concentrations between 6.6 and 1.1 µg equiv./g were found in kidneys, liver, heart, lungs, spleen, brain, gonads and salivary glands of both sexes. Mean concentrations of 4.8 and 5.9 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of males and females respectively. All other concentrations were either similar to or lower than the corresponding blood concentrations (see Table B.6.1-15).
Table B.6.1-15: Radioactivity in tissues after single oral dose of 1000 mg/kg bw at 72 h
Males
Females
Tissue
% of dose
µg equiv./g
% of dose
µg equiv./g
Blood
N/A
0.894
N/A
0.803
Bone
N/A
49.792
N/A

44.925
Brain
0.001
1.233
0.001
1.164
Fat
N/A
0.536
N/A
0.496
GIT plus contents
0.2
13.276
0.219
16.329
Heart
0.001
1.111
0.001
1.254
Kidneys
0.007
6.511
0.005
6.046
Liver
0.039
5.480
0.029
5.226
Lungs
0.002
2.870
0.002
3.535
Muscle
N/A
0.816
N/A
0.825
Ovary
-
-
<0.001
2.940

Plasma
N/A
<0.392
N/A
<0.396
Residual carcass
0.466
4.772
0.537
5.858
Salivary glands
<0.001
1.811
<0.001
2.089
Spleen
0.001
2.441
0.001
3.106
Testes
0.001
0.905
-
-
N/A not applicable

Conclusion by the Notifiers
Oral doses of glyphosate acid were excreted rapidly and predominantly in the faeces. Elimination was essentially complete within 48 h. The remaining radioactivity was excreted with the urine. Negligible traces of radioactivity (<0.6 %) were still present in the tissues and residual carcass at 72 h, with bone representing the highest tissue residue. Thus, there was no indication for accumulation of glyphosate acid.
RMS comments
The study is considered acceptable. The conclusion is agreed with. Again, oral absorption rate (expressed as percentage of urinary excretion) was rather low in this study but there is no direct comparison with other studies from other laboratories possible since 1000 mg/kg bw was by far the highest dose that was administered in ADME studies. However, the urinary excretion rates were in line with data obtained by (1996, TOX2000-1981).
Plasma kinetics was not followed and metabolism was not investigated but enough information on these parameters is available from other sources.
The tissue distribution pattern observed by other researchers was confirmed.
5th study (1996)
Reference: IIA, 5.1.3/03
Report: (1996) Glyphosate acid: Biotransformation in the rat
Data owner: Syngenta Report No.: /P/5058

Date: 1996-06-28, not published, TOX2000-1981
Guidelines: OECD 417 (1984)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Non-labelled test material:
Glyphosate acid
Identification:
N-(phosphonomethyl) glycine
Description:
White solid
Lot/Batch #:
Y04707/048
Purity:
99.5 % w/w
Stability of test compound:
Not reported
Radiolabelled test material:
[14C]-phosphonomethyl-labelled glyphosate acid
Identification:
[14C]-phosphonomethyl-labelled glyphosate acid
Description:
Not reported
Lot/Batch #:
Y04707/047
Purity:
97.8 %
Specific activity :
1.580 GBq/mMol
Stability of test compound:
The test substance was shown to be stable in vehicle for longer than the period of use during this study.
Reference substance:
Identification:
Aminomethylphosphonic acid (AMPA)
Description:
Not reported
Lot/Batch #:
Not reported
Purity:
Not reported
Stability of test compound:
Not reported
Test animals:
Species:

Rat
Strain:
Alpk:APfSD
Source:
Age:
Not reported
Sex:
Males and females
Weight at dosing:
260- 305 g
Acclimation period:
At least 4 days in stock rat cages and 24 hours prior to surgery in metabolism cages
Diet/Food:
Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
Housed individually in glass metabolism cages
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: At least 12 change/hour 12-hour light/dark cycle
Study design and methods
In life dates: 1995-11-26 to 1996 May
Animal assignment and treatment
Two male and two female non-fasted rats were administered with a single oral dose of 1000 mg [14C]-glyphosate acid/kg by gavage after bile duct cannulation. 48 hours after dosing all animals were sacrificed.
Bile duct cannulation
The abdominal cavity was opened after anaesthesia and the bile duct exposed. A fine plastic cannula was inserted into the bile duct and externalised by passing through the abdominal wall and under the skin to an exit point at the back of the neck. The incisions in the abdominal and body walls were saturated and the exposed cannula was protected within a flexible metal sheath anchored to the skin at the back of the neck. Following surgery each animal was returned to its cage and allowed to recover overnight prior dosing.
Dosing Formulation Analysis
The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The radiochemical purity of the [14C]-labelled test substance was determined by the high performance liquid chromatography (HPLC) following formulation in the dosing.
Collection of excreta and bile
Urine only was collected 6 hours after dosing, and separately together with faeces 12, 24, 36 and 48 hours after dosing from all surviving animals.
Bile was collected at 2, 4, 6, 8, 12, 24, 36 and 48 hours after dosing.
Faecal samples were analysed by sample oxidation followed by liquid scintillation counting whereas samples of urine and bile were analysed without intermediate processing.
Quantification of metabolites

Urine and faecal samples obtained from previous excretion and tissue distribution studies (1996 TOX2000-1977, TOX2000-1978, TOX2000-1979) over 72 hours were used for the quantification of metabolites.

Urine samples were combined by taking a fixed percentage by weight to give separate male and female pools for each of the sample collections intervals. Subsamples of these pools were further combined to give pools representing the entire sample collection period. Each pool was analysed by TLC and HPLC. A representative urine sample was analysed by 1H-NMR. Faecal samples were combined in the same way as described above for urine samples. Subsamples of pooled faecal samples were mixed with distilled water and sonicated for several hours, the samples were filtered through filter paper and the solid material was re- extracted a second time with distilled water and a third time with 10% aqueous HCl. Extract volumes were measured and aliquots taken for scintillation counting to allow the calculation of extraction efficiencies.

Measurement of radioactivity Liquid scintillation counting

Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [133Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.

Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel (60F254) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15:
15 v/v/v/v).

Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser or a Bioscan System 200 imaging scanner. Glyphosate acid and AMPA standards were located by spraying the plates with a solution of 300 mg ninhydrin in 100 mL of butanol and 3 mL of glacial acetic acid.

High performance liquid chromatography (HPLC) Two different HPLC methods were employed:

HPLC method 1 was used for the analysis of dosing solutions. Prior to analysis samples were derivatised. A Hichrom S5NH column (250 x 4.6 mm) was eluted with acetonitrile buffered with 25 mMol aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of
1.5 mL/min.

HPLC method 2 was used for the analysis of urine and faecal extracts and for the quantification of glyphosate acid and AMPA. Prior to analysis samples were filtered. A Biorad's HLRC acid analysis column (250 x 4.6 mm) was eluted with 5 mM aqueous potassium dihydrogen phosphate with 4 % methanol at a flow rat of 0.5 mL/min.

Radioactivity was detected in both methods by liquid cell.

Proton Nuclear Magnetic Resonance Spectroscopy (1H-NMR)

Proton and phosphorus NMR spectra were acquired using a Brucker 400MHz instrument. Samples of glyphosate acid and AMPA were dissolved in D2O and analysed by both phosphorus and proton NMR. Control urine and urine from a bile duct cannulated rat administered an oral dose of glyphosate acid were analysed by phosphorus NMR. The urine sample from the rat that had been administered glyphosate was subsequently fortified with AMPA then glyphosate acid and reanalysed by phosphorus NMR.

Data evaluation

Dosing and excretion of radioactivity data were processed using the Debra computerised acquisition and processing system. Metabolites were quantified using the Flo_One integration software for

HPLC.

Results and discussion

Excretion of radioactivity

The results showed that 48 hours after dosing, excreted means of the administered dose in the urine and faeces amounted to 20.8 % and 39.1 % in males and 16.3 % and 30.5 % in females, respectively, whereas biliary excretion of radioactivity was negligible (see Table B.6.1-16).

The total excreted radioactivity after 48 hours accounted for 62.5 % and 52.0 % in males and females, respectively.

Table B.6.1-16: Excretion of radioactivity in urine, faeces and bile by male and female bile duct cannuled rats given a single oral dose of 1000 mg [14C]- glyphosate acid/kg bw (mean of two rats expressed as % of applied dose)

Males rats
Female rats
% of applied dose
% of applied dose
Time after dosing
(hours)
Urine
Faeces
Bile
Urine
Faeces
Bile
0-2
N/A
N/A
0.004
N/A
N/A
0.002
2-4
N/A
N/A
0.004
N/A
N/A
0.011
4-6
N/A
N/A
0.002
N/A
N/A
0.011
0-6
2.137

N/A
N/A
8.718
N/A
N/A
6-8
N/A
N/A
0.005
N/A
N/A
0.005
0-12
N/A
3.776
N/A
N/A
1.392
N/A
6-12
6.765
N/A
N/A
2.495
N/A
N/A
8-12
N/A
N/A
0.008
N/A
N/A
0.007
12-24
5.432
12.333
0.016
3.631
12.115
0.010
24-36
3.468
18.079
0.009
1.004
8.712

0.008
36-48
3.013
4.946
0.007
0.427
8.325
0.007
0-48
20.815
39.134
0.055
16.275
30.544
0.062
Cage wash
at 48 hours
2.534 (mean)
5.097 (mean)
Total
excreted
62.538
51.978
Values are expressed as percentages of administered dose and are then mean of two rats
Characterisation of radioactivity
The negligible levels of radioactivity in bile samples made chromatographic analysis unnecessary. Corresponding to this result, faecal extracts analysed by HPLC and NMR confirmed the radioactivity as glyphosate acid.
Analyses by chromatography and phosphorus NMR of urine pools from former studies (1996, TOX2000-1977, TOX2000-1978, TOX2000-1979) covering the 0-72 hour
period demonstrated a single peak identified as glyphosate acid. Earlier timepoints demonstrated a second peak identified as aminomethyl phosphonic acid (AMPA) that occurred in measurable quantities.
The percentages of dose accounted for glyphosate acid and AMPA following a low, high or repeated dose of glyphosate acid are given in Table B.6.1-17. For glyphosate acid and AMPA, the values range from 63.3-95.3 % and 0.07-0.66 %, respectively.
Table B.6.1-17: Percentage of administered radioactivity identified as glyphosate acid and AMPA
Low dose study 10 mg/kg bw
High dose study 1000 mg/kg bw
Repeated dose study 10 mg/kg bw
Male
Female
Male
Female
Male
Female

(% of applied radioactivity)
Urine
Glyphosate
acid
12.71
10.51
16.00
16.73
10.46
10.47
AMPA
0.19
0.11
0.63
0.66
0.07
0.08
Faeces
Glyphosate
acid
74.80
55.22
79.25
63.88
52.86
72.09
Total
Glyphosate
acid
87.52
65.73
95.25
80.61
63.33
82.57
AMPA
0.19
0.11
0.63
0.66
0.07
0.08
Conclusion by the Notifiers
Following an oral dose of glyphosate acid to rats approximately 10-20 % of the dose was absorbed. The unabsorbed glyphosate acid was excreted unchanged in faeces. The absorbed dose was excreted in urine as glyphosate and trace amounts of aminomethyl phosphonic acid (AMPA).

RMS comments
The study is considered acceptable. Data obtained in bile-canulated rats and on metabolism amend the information provided by (1996, TOX2000-1977, TOX2000-1978) and confirm low urinary excretion (although some underestimation is possible since 2-5 – 5 % radioactivity in cage was was not included), negligible biliary excretion also following administration of a high dose and virtually absent metabolism.
6th study (1996)
Reference: IIA, 5.1.3/01
Report: (1996) Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing
Data owner: Syngenta Report No.: /P/4944
Date: 1996-05-22, not published, TOX2000-1979
Guidelines: OECD 417 (1984)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Non-labelled test material:
Glyphosate acid
Identification:
N-phosphonomethyl glycine
Description:
White solid
Lot/Batch #:
Y04707/045
Purity:
99.2 % w/w
Stability of test compound:
Not reported
Radiolabelled test material:
[14C]-phosphonomethyl-labelled glyphosate acid
Identification:
[14C]-phosphonomethyl-labelled glyphosate acid
Description:
Not reported
Lot/Batch #:
Y04707/047
Purity:
> 98 %
Specific activity :
1.580 GBq/mMol
Stability of test compound:
The test substance was shown to be stable in the vehicle for longer than a period of use during the study.
Vehicle and/or positive control:
Deionised water

Test animals:
Species:
Rat
Strain:
Alpk:APfSD
Source:
Age:
Not reported
Sex:
Males and females
Weight at dosing:
225 - 328 g
Acclimation period:
At least 4 days prior to the study start and 24 hours prior to dosing with the radiolabelled preparation
Diet/Food:
Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Wiltham, Essex, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
After administration of the 14th unlabelled dose: Individually in stainless steel metabolism cages
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: At least 12 change/hour 12-hour light/dark cycle
Study design and methods
In life dates: 1995-10-16 to 1996-03-26
Animal assignment and treatment
Eight male and eight female non-fasted rats received a single oral dose of the unlabelled test substance (10 mg/kg) daily for 14 days by gavage. 24 hours after the 14th dose of unlabelled glyphosate acid, five male rats and five female rats were given a single oral dose (10 mg/kg) of [14C]-phosphonomethyl glyphosate acid. 72 hours after dosing, the rats were killed and the residual radioactivity was measured in blood, selected tissues and in the residual carcasses.
Dosing Formulation Analysis
The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [14C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) high performance liquid chromatography (HPLC).
Collection of excreta
Urine only was collected at 6 hours after dosing, while urine together with faeces were separately collected at 12, 24, 36, 48 and 72 hours after dosing.
Urine collections comprised rinsing of each cage at each time point together with a thorough washing at the end of the study. Faecal samples were analysed by sample oxidation followed by liquid scintillation counting whereas samples of urine and cage washings were analysed without intermediate processing.
Collection of blood and tissues

72 hours after dosing, the rats were sacrificed and a blood sample was retained and divided into two portions. A portion of each blood sample was centrifuged to obtain plasma, which was analysed for liquid scintillation counting. Whole blood was analysed by sample oxidation.
The following tissues together with representative samples of fat (abdominal), bone (femur), and muscle (femoral) were removed from each rat: brain, liver, testes or ovaries, lungs, heart, spleen, kidneys, salivary glands, intestinal tract plus contents and residual carcasses.
Measurement of radioactivity - Liquid scintillation counting
Radioactivity was measured by liquid scintillation counting by means of Packard Tricarb instruments. The results obtained from the counting were corrected for background activity and counting efficacy using [133Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.
Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.
Thin layer chromatography (TLC)
TLC was conducted using a normal phase silica-gel (60F254) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15:5 v/v/v/v). Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.
High performance liquid chromatography (HPLC)
To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mMol aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min. Radioactivity was detected using an on- line flow detector (liquid cell) and with UV absorption at 230 nm.
Data evaluation
Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample which was twice the liquid scintillation counter's background rate. For the purpose of calculating group mean results, individual values below the LOD are accepted as being equal to the limit of detection. Means which include one or more values which are below the LOD are reported as "<" the mean result and without a standard deviation. The limit of detection obtained for all tissues in this study was 0.004/µg equivalents glyphosate acid/g of tissue (µg equiv/g). This value is based upon a sample size of 200 mg of all determinations. Organs of less than this weight were analysed as a single sample and hence this figure represent a limiting value.
Results and discussion
Excretion of radioactivity
The results showed that the excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 hours after dosing (average of 80.6 % for males and 85.8 % for females).
Excretion of radioactivity in the urine during this period accounted for means of 9.2 % and 9.1 % of the administered dose in the male and female rats respectively. The total percentage of the administered radioactivity eliminated in excreta 72 hours after dosing were means of 97.5 % for males and 101.7 % for females (Table B.6.1-18)
Comparison of the cumulative excretion data showed that there were no marked differences in the rates of excretion of radioactivity in the urine or faeces for male and female rats.

Table B.6.1-18: Excretion of radioactivity in urine and faeces in male and female rats

Excretion of radioactivity [%]				
Males				
Time after dosing (hours)	Urine		Faeces	
	Meana	SD	Mean	SD
0-6	3.1	0.8	N/A	N/A
6-12	2.7	0.7	N/A	N/A
0-12	N/A	N/A	50.2	15.5
12-24	3.4	1.6	30.3	9.0
24-36	0.9	0.3	3.6	1.5
36-48	0.3	<0.1	1.3	0.7
48-72	0.2	<0.1	1.1	0.6
0-72				

10.6
3.0
86.6
5.2
Mean
SD
N/A
N/A
Cage wash at 72 h
0.2
<0.1
N/A
N/A
Total excreted
97.5
2.7
N/A
N/A
Excretion of radioactivity [%]
Females
Time after dosing (hours)
Urine
Faeces
Meanb
SD
Mean
SD
0-6
3.3
0.6
N/A
N/A
6-12
2.5
0.3
N/A
N/A
0-12
N/A
N/A
44.7
34.1
12-24
3.2
0.6
41.0

31.1
24-36
0.9
0.3
2.7
1.1
36-48
0.4
0.2
1.2
0.2
48-72
0.3
0.1
1.1
0.7
0-72
10.7
1.1
90.7
4.2
Mean
SD
N/A
N/A
Cage wash at 72 h
0.2
<0.1
N/A
N/A
Total excreted
101.7
4.0
N/A
N/A
N/A not applicable
a Mean of 4 animals
b Mean of 5 animals
Tissue distribution of radioactivity
The highest tissue concentration of radioactivity was found in bone with a mean concentration of 0.36 µg equiv/g for males and 0.35 µg equiv/g for females, followed by the intestinal tract plus contents, kidneys, liver, lungs, salivary glands, and ovaries.
Mean concentrations of 0.05 µg equiv/g were found in the residual carcass of either sex which also includes the remaining skeletal bone.
All other concentrations were either similar to or lower than the corresponding blood concentrations.

The mean total percentage of administered radioactivity present in all of the tissues examined and the residual carcass was 0.5 % for males and 0.4 % for females. The amounts present in the intestinal tract plus contents were 0.1 % for males and females (see Table B.6.1-19)

Table B.6.1-19: Tissue and carcass residues of radioactivity in male and female rats

Residue of radioactivity				
Males				
Tissue	% radioactivity of dose		µg equivalents/g	
	Mean a	SD	Mean	SD
Brain	0.001	<0.001	0.010	0.002
Testes	0.001	<0.001	0.007	0.001
Heart	<0.001	<0.001	0.011	0.002
Kidneys	0.005	0.002	0.061	0.015
Liver	0.031	0.009	0.055	0.014
Lungs	0.001	<0.001	0.026	0.004
Spleen	0.001	<0.001		

0.022
0.003
Salivary glands
<0.001
<0.001
0.019
0.004
Bone (femur)
N/A
N/A
0.358
0.177
Fat (abdominal)
N/A
N/A
0.008
0.001
Muscle (femoral)
N/A
N/A
0.008
0.001
Blood
N/A
N/A
0.014
0.006
Plasma
N/A
N/A
<0.004
Residual carcass
0.423
0.090
0.050
0.011
Total
0.463
0.101
N/A
N/A
Intestinal tract plus
contents
0.108
0.040
0.109

0.041
Residue of radioactivity
Females
Tissue
% radioactivity of dose
µg equivalents/g
Mean b
SD
Mean
SD
Brain
0.001
<0.001
0.010
0.002
Ovaries
<0.001
<0.001
0.026
0.006
Heart
<0.001
<0.001
0.012
0.004
Kidneys
0.004
0.001
0.049
0.011
Liver
0.021
0.005
0.045
0.010
Lungs
0.001
<0.001
0.029
0.006
Spleen
0.001
<0.001
0.025
0.006
Salivary glands

<0.001
<0.001
0.027
0.006
Bone (femur)
N/A
N/A
0.345
0.081
Fat (abdominal)
N/A
N/A
0.006
0.002
Muscle (femoral)
N/A
N/A
0.007
0.002
Blood
N/A
N/A
0.010
0.002
Plasma
N/A
N/A
<0.005
Residual carcass
0.382
0.067
0.046
0.008
Total
0.411
0.073
N/A
N/A
Intestinal tract plus contents
0.115
0.014
0.117
0.015
N/A not applicable
Residual carcass values include partial tissue percentages
a Mean of 4 animals

b Mean of 5 animals
Recovery of radioactivity
The total mean percentage recoveries, including excreta, tissues and residual carcass was 98.0 % for male rats and 102.2 % for females.
Conclusion by the Notifiers
Comparison of the results with those obtained at the same dose level but without pre-administration of unlabelled test substance (1996, TOX2000-1977) showed no significant differences on either the routes or rates of elimination after oral dosing. In both studies the test substance was excreted rapidly and predominantly in the faeces by rats of both sex and low amounts of radioactivity were detected in all the tissue examined.
RMS comments
The study is considered acceptable. No significant differences were observed when the results were compared to the data obtained following single administration. In particular, there was no evidence of accumulation when, e.g., the tissue residues were considered. Urinary excretion that may be taken as surrogate for oral absorption was even lower than in the single-dose experiments without pre-treatment, and so was radioactivity in cage wash.
7th study (1996)
Reference:
IIA, 5.1.3/02
Report:
1996) Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg)
Data owner: Syngenta Report No.: /P/4943
Date: 1996-06-10, not published, TOX2000-1980
Guidelines:
OECD 417 (1984)
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Materials and methods
Non-labelled test material:
Glyphosate acid
Identification:
N-phosphonomethyl glycine
Description:
White solid
Lot/Batch #:
Y04707/045
Purity:
99.2 % w/w
Stability of test compound:
Not reported
Radiolabelled test material:
[14C]-phosphonomethyl-labelled glyphosate acid

Identification:
[14C]-phosphonomethyl-labelled glyphosate acid
Description:
Not reported
Lot/Batch #:
Y04707/047
Purity:
> 96 %
Specific activity :
1.580 GBq/mMol
Stability of test compound:
The test substance was shown to be stable in the vehicle for longer than a period of use during the study
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rat
Strain:
Alpk:APfSD
Source:
Age:
Not reported
Sex:
Males and females
Weight at dosing:
215- 271 g
Acclimation period:
At least 5 days in stock rat cages and 24 hours prior to dosing in metabolism cages
Diet/Food:
Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
Housed individually in glass metabolism cages
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: At least 12 change/hour 12-hour light/dark cycle
Study design and methods
In life dates: 1995-10-25 to 1996-04-04
Animal assignment and treatment
Two male and two female non-fasted rats were administered with a single oral dose of 10 mg [14C]-glyphosate acid/kg by gavage. 24 and 48 hours after dosing, a heterosexual pair was killed and a qualitative whole body autoradiogram was performed. In addition, radioactivity was measured in urine, faeces and exhaled air.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [14C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) high performance liquid chromatography (HPLC).

Collection of excreta

Urine only was collected 6 hours after dosing, and separately together with faeces 12, 24, 36 and 48 hours after dosing from all surviving animals.

Urine collections comprised rinsing of each cage at each time point together with a thorough washing at the end of the study.

Faecal samples were analysed by sample oxidation followed by liquid scintillation counting whereas samples of urine and cage washings were analysed without intermediate processing.

Collection of exhaled air

The exhaled air from one heterosexual pair was passed through sodium hydroxide to trap any radioactivity expired as [14C]-carbon dioxide. Subsamples of the contents of each trap were removed for radiochemical analysis at 6, 12 and 24 hours after dosing and were taken, without further processing, for liquid scintillation counting.

Whole body autoradiography

Immediately after scheduled sacrifice, each carcass was frozen rapidly and embedded in blocks of 2 % (w/v) aqueous carboxymethylcellulose. Longitudinal sagittal sections, 30 µm thick, were taken, mounted on adhesive tape and freeze-dried for approximately 48 hours. Autoradiograms were prepared by contact with autoradiographic film and exposed for periods of 2, 4 or 6 weeks.

Measurement of radioactivity - Liquid scintillation counting

Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [133Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.

Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel (60F254) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15:5 v/v/v/v). Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.

High performance liquid chromatography (HPLC)

To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mMol aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min. Radioactivity was detected using an on- line flow detector (liquid cell) and with UV absorption at 230 nm.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample, which was twice the liquid scintillation counter's background rate. The LOD for each carbon dioxide trap in this study was 0.01 % of the administered dose.

Results and discussion

Excretion of radioactivity

The results showed that 24 hours after dosing, excreted means of the administered dose in the urine and faeces amounted to 22.3 % and 55.5 % in males and 11.9 % and 83.8 % in females, respectively, whereas less than 0.2 % was excreted as carbon dioxide.

48 hours after dosing, excreted means of the administered dose in the urine and faeces increased to 34.0 % and 60.5 % in males and 12.5 % and 91.2 % in females, respectively.

The results of the excreted radioactivity in urine, faeces and exhaled air expressed as percentages of the administered radioactivity, together with the results for cage washings, are listed in Table B.6.1-20.

Table B.6.1-20: Excretion of radioactivity in urine, faeces, cage wash and expired air by male and female rats

Time After Dosing (hours)	Males				Females			
	Rat 1	Rat 2	Rat 3	Rat 4				
	(% of applied dose)				(% of applied dose)			
Urine								
0-6	5.00	8.21	4.06	3.67				
6-12	5.30	8.67	4.32	4.12				
12-24	7.57	9.92	4.39	3.23				
24-36	N/A	4.30	N/A	0.93				
36-48	N/A	2.90	N/A					

0.54
Total
17.86
34.00
12.77
12.48
Faeces
0-12
30.62
0.21
54.27
32.63
12-24
28.69
51.48
26.03
54.76
24-36
N/A
1.65
N/A
2.71
36-48
N/A
7.12
N/A
1.13
Total
59.32
60.46
80.30
91.23
Exhaled 14CO2
0-6
0.07
N/A
0.08
N/A
6-12
0.02
N/A
0.04
N/A
12-24
0.02
N/A

0.03
N/A
Total
0.11
N/A
0.14
N/A
Terminal Cage wash
1.12
0.98
1.43
0.41
Total
78.40
95.44
94.64
104.12
N/A not applicable
Whole body autoradiography
The whole body autoradiograms showed no marked differences in the distribution of radioactivity between male and female rats. The greatest intensity of labelling was present in the bone for both sexes, followed by the intestinal tract and the kidneys 24 hours after dosing with lesser to negligible amounts being present after 48 hours.
Conclusion by the Notifiers
Orally dosed glyphosate acid was excreted rapidly and predominantly in the faeces. 48 hours after dosing the greatest intensity of radiolabelling was in the bone and intestinal tract plus contents.
RMS comments
The study is considered supplementary despite its good quality. However, the number of animals of one per sex and timepoint is too low for definitive conclusions. All the parameters examined (i.e., excretion and distribution) had been adressed in other studies before. This previous knowledge was confirmed. Oral absorption was generally low but showed a remarkable interindividual variability.
B.6.1.2 Re-evaluation of previously known studies (mentioned in the 1998 DAR, ASB2010-10302) by the RMS
For the first EU evaluation of glyphosate, two separate “pairs” of ADME studies have been submitted which consisted of an in-life part, i.e., a complete ADME study according to OECD 417, and a subsequent metabolism part in which the metabolite pattern in urine and faeces was investigated.
• Monsanto studies
In life part: (1988, TOX9552356)
Metabolism part: (1988, TOX9552357)
• ADAMA studies
In life part: (1995, TOX9650071)
Metabolism (and organ/tissue distribution) part: (1995, TOX9552251)
Furthermore, on behalf of Cheminova, (1992, TOX9300343) performed a full-range ADME study in rats.
Re-evaluation by the RMS revealed that, although partly carried out before GLP became compulsory, all these studies mostly comply to modern standards and provide reliable information on kinetics and

metabolism of glyphosate in the rat. In line with the original DAR, the studies by (1988, TOX9552356), by (1988,
TOX9552357) and by (1992, TOX9300343) are still regarded as fully acceptable by the RMS allowing comprehensive evaluation.
In contrast, quality rating of the studies by (1995, TOX9650071) and
(1995, TOX9552251) has been declined to “supplementary”. This is due to the fact that radioactive residues in the organs, tissues and carcass were only investigated in one group receiving a low intravenous dose and, thus, do not reflect real exposure conditions. Even for this group that was treated by a highly artifical route, radioactivity in bone was not
determined. Furthermore, search for metabolites was confined to urine samples taken from high dose animals and possible abundance of faecal metabolites was not considered.
Surprisingly, there was a different concentration given for the low oral dose by (0.2 mg/kg bw) and (0.3 mg/kg bw).
In addition, it must be emphasised that this study [as well as those by (1995, ASB2012-11379) and by (1996, ASB2012-11380)] did not include a group of rats receiving multiple administrations. Thus, a possible different kinetic behaviour following repeated exposure would not have been elucidated and information from other sources must be taken into account to cover this endpoint.
It could be argued that (1995, TOX9650071) had employed a rather low dose of only 0.2 mg/kg bw(/d) but the use of this concentration might even better reflect real exposure conditions than higher “low” doses would do. The advantage of that many ADME studies with glyphosate is in covering a wide dose range.
A preliminary study by (1992, TOX9552358) included determination of pharmacokinetic parameters in the blood and whole-body autoradiography at a low dose of 30 mg/kg bw and is, because of its limited focus, now considered supplementary, too.
Two more (and rather old) studies were considered supplementary during the previous evaluation yet:
A study by (1973, TOX9552355) is unique in that way that it was the only toxicokinetic experiment in which glyphosate was administered (for two weeks) to rats via the diet. Indeed, the dietary dose levels of 1 to 100 ppm appear rather low but these concentrations might reflect an actual residue concentration.
A second study by the same authors (, 1973, TOX9552353) is about toxicokinetics of glyphosate in male rabbits following single oral (gavage) administration of low doses in the range of 5.7 – 8.8 mg/kg bw. The results indicate similarities and differences between species such as a lower urinary clearance rate and higher tissue retention in rabbits as compared to the rat.
Although no batch number and no purity of the applied glyphosate was given and, therefore, in principle, assessment of these studies should have been turned into “unacceptable” now, the RMS suggests to keep them available as sources of useful additional information.
In contrast, there was another study also considered “supplementary” in the original DAR for which this rating actually cannot be maintained:
The metabolism study by (1990, TOX9551961) is considered not acceptable due to serious reporting deficiencies. Thus, no information on the test material (batch, purity) is given and the applied dose not mentioned. The application method is not clear since on one hand ”gastric lavage” (gavage?) is stated as the route but on the next page it is said that ”rats were fed with the radioactive material”. Description of animals and animal handling is very poor. No information on toxicokinetics was obtained but this was apparently beyond the scope of the study that was performed to find out wheter glyphosate was metabolised or not.

Likewise, a brief information (, 1973, TOX9552354) concerning kinetics and (absent) metabolism of aminomethylphosphonic acid (AMPA) is very poor and not acceptable from a today's regulatory view.

B.6.2.1 Acute oral toxicity

Glyphosate acid and its salts have been extensively tested for acute toxicity, skin and eye irritation, and skin sensitisation. An amount of 145 acute studies was submitted either for the previous EU evaluation or in the present GTF dossier.

For the previous EU evaluation, a large number of oral toxicity studies in rats and mice were submitted that had been conducted with either glyphosate acid or its salts. In the current GTF dossier, a variety of additional studies in rats with administration of glyphosate acid and two more studies in rats and mice with the IPA salt were provided.

Table B.6.2-1: Summary of acute oral toxicity studies with glyphosate acid

Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicl e	LD50 (mg/kg bw)	Main effects
Studies from the 2001 evaluation						
Annex B- 5.2.1.1.1, Glyphosate Monograph 1995 (Sanachem) TOX9650909	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	> 2000 (limit test)	Slightly congested lungs, spleno-megaly, Liver: centri- lobular congestion
Annex B- 5.2.1.1.1, Glyphosate Monograph 1994 (Herbex) TOX9500245	Rat, Sprague Dawley	1/sex/2000## 5/sex/2000	95	Arachi s oil		

> 2000
(limit test)
No findings
Reference (Data owner)
Species Strain
Number of animals / Dose levels
(mg/kg bw)
Purity (%)
Vehicl e
LD50
(mg/kg bw)
Main effects
Annex B-
Rat,
5/sex/0
97.2
water
> 5000
♂: heart weights
5.2.1.1.1,
Glyphosate
Wistar
5/sex/5000
(limit test)
Monograph
1994
(Alkaloida)
TOX9650142
Annex B-
Rat,
2/sex/250##
2/sex/500##
2/sex/1000##
2/sex/3000##
2/sex/5000## 5/sex/5000
95
CMC
> 5000
Piloerection,
5.2.1.1.1,
Sprague
(limit test)
subdued
Glyphosate
Dawley
behaviour,

Monograph
hunched
appearance
, 1995
(SIN)
TOX9500377
Annex B-
Rat,
1/sex/2000##
>97
water
> 2000
No findings
5.2.1.1.1,
Sprague
5/sex/2000
(limit test)
Glyphosate
Dawley
Monograph
, 1992
(Barclay)
TOX9551810
Annex B-
Rat,
5/sex/2500
96.8
Peanut
> 7500
7500 mg/kg bw:
5.2.1.1.1,
Wistar
5/sex/5000
oil
(estimated)
mortality (2/5
Glyphosate
5/sex/7500
♂, 2/5 ♀);
Monograph
lethargy, ataxia,
1991
dyspnoea,
(ADM)
weight loss
TOX9551088

Studies from the 2001 evaluation
Annex B- 5.2.1.1.1,
Glyphosate Monograph
1990 (AGC) TOX9500261
Rat, CD
5/sex/0 5/sex/3000 5/sex/5000 5/sex/8000
98.1
1% CMC
> 8000
≥ 5000 mg/kg bw: decreased activity, abnormal gait and/or limb position
Annex B- 5.2.1.1.1,
Glyphosate Monograph
Rat, Sprague Dawley
5/sex/5000
98.6
0.5 % CMC
> 5000
(limit test)
Piloerection, reduced activity, ataxia (♂ only)
1989
(CHE) TOX9552319
Studies not reviewed in the
IIA 5.2.1/01
Rat,
5/females/5000
96.4
water
> 5000
decreased
2009
Sprague
(limit test)
activity,
(HAG)
Dawley
diarrhoea,
ASB2012-
piloerection,
11381
polyuria,
salivation
Reference (Data owner)
Species Strain
Number of animals / Dose levels
(mg/kg bw)
Purity (%)

Vehicl e
LD50
(mg/kg bw)
Main effects
IIA 5.2.1/02
Rat,
5/sex/5000
95.68
0.5 %
> 5000
decreased
Sprague
CMC
(limit test)
spontaneous
, 1995
Dawley
motor activity
(ALS)
and salivation
ASB2012-
11382
IIA 5.2.1/03
Mice,
5/sex/5000
95.68
0.5 %
> 5000
decreased
ICR
CMC
(limit test)
spontaneous
, 1995
motor activity,
ASB2012-
sedation and
11383
crouching
position
IIA 5.2.1/04
Rat,
3 females/2000
96.66
water
> 2000

No findings
2009
Wistar
(step 1)
(EXC)
3 females/2000
ASB2012-
(step 2)
11384
IIA 5.2.1/05
Rat, CD
3 females/2000
98.8
0.8 %
> 2000
No findings
(step 1)
hydro-
(limit test)
2009 (HAG)
3 females/2000
xypro-
ASB2012-
(step 2)
pylme-
11385
thylcel-
lulose
Studies not reviewed in the 2001 evaluation
IIA 5.2.1/06
2010 (HAG) ASB2012-
11386
Rat, CD
3 females/2000
(step 1)
3 females/2000
(step 2)
96.4
0.8 % hydro- xypro- pylme-
thylcel- lulose
> 2000
(limit test)
No findings
IIA 5.2.1/07
2010 (HAG) ASB2012-
11387

Rat, CD
3 females/2000
(step 1)
3 females/2000
(step 2)
97.3
0.8 % hydro- xypro- pylme- thylcel-
lulose
> 2000
(limit test)
No findings
IIA 5.2.1/08
2005
(HAG)
ASB2012- 11388
Rat, Sprague- Dawley
3 females/5000
97.23
water
> 5000
(limit test)
Diarrhea, ano- genital & facial staining, reduced faecal
volume
IIA 5.2.1/09
2008 (HAG) ASB2012- 11389
Rat, Wistar
3 females/2000
(step 1)
3 females/2000
(step 2)
98.05
water
> 2000
(limit test)
No findings
IIA 5.2.1/10
2007 (NUF) ASB2012- 11390
Rat, HanRcc: WIST
2 x 3 ♀/2000
95.1
PEG 300
> 2000
(limit test)
Slightly ruffled fur
Studies not reviewed in
IIA 5.2.1/11

1988 (MON) Z35389
Rat, Sprague Dawley
5/sex/5000
97.76
water
> 5000
diarrhea, apparent urinary incontinence and hair loss on
the abdomen
Reference (Data owner)
Species Strain
Number of animals / Dose levels
(mg/kg bw)
Purity (%)
Vehicl e
LD50
(mg/kg bw)
Main effects
IIA 5.2.1/12
Rat,
5/sex/2500
99
water
> 5000
Mortalities: 1/10
Wistar
5/sex/3500
1/10, 3/10,7/10,
., 1979
5/sex/5000
10/10 at 2500,
(MON)
5/sex/7000
3500, 5000,
Z35541
5/sex/9900
7000 and 9900
mg/kg bw;
clinical signs:
ataxia,
convulsions,
muscle tremors,
red nasal
discharge, clear
oral discharge,
urinary staining
of the abdomen,

soft stool,
piloerection,
lethargy, and
fecal
staining of the
abdomen
IIA 5.2.1/13
Rat
5/sex/5000
95.6
water
> 5000
No findings
1996
(SYN)
TOX2000-
1982
IIA 5.2.1/14
Rat
3 ♀/5000
96.1
Water
> 5000
Ruffled fur,
2007
hunched posture
(SYN)
ASB2012-
11391
IIA 5.2.1/15
Rat
3 ♀/5000
96.3
0.5 %
>5000
No findings
Tavaszi, 2011
CMC
(
ASB2012-
11392
IIA 5.21/18
Rat
5 ♀/2000
85.8
DMS

> 2000
Hunched
2014
(fixed dose
posture
(Albaugh
method)
Europe Sàrl)
ASB2014-
9147
Studies from the 2001 evaluation
Annex B- 5.2.1.1.2,
Glyphosate Monograph
1991 (ADM) TOX9551089
Mice, Swiss albino
5/sex/2500 5/sex/5000 5/sex/7500
96.8
Peanut oil
> 7500
≥ 2500 mg/kg bw: mortality, lethargy, ataxia, dyspnoe, weight loss
Annex B-
5.2.1.1.2,
Glyphosate Monograph
Mice,
Charles River
5/sex/2000
techni
cal
0.5 %
CMC
> 2000
(limit test)
Piloerection,
hunched posture, hypoactivity
1994 (I.Pi. Ci) TOX9551624
Reference (Data owner)
Species Strain
Number of animals / Dose levels
(mg/kg bw)
Purity (%)
Vehicl e
LD50
(mg/kg bw)
Main effects
Annex B- 5.2.1.1.2,
Glyphosate Monograph

1991 (CHE)TOX95 52320
Mice, Bom:NM RI
5/sex/2000
98.6
water
> 2000
(limit test)
Piloerection, sedation
CMC = carboxymethylcellulose
Table B.6.2-2: Summary of acute oral toxicity studies with glyphosate salts
Reference
Species
Number of
Salt
Vehicl
LD50
Main effects
(Data owner)
Strain
animals / Dose
type
e
(mg/kg
levels
Purity
bw)
(mg/kg bw)
(%)
Studies from the 2001 evaluation
Annex B- 5.2.1.2.1,
Glyphosate Monograph
1995
(Sanachem) TOX9650910
Rat, Sprague Dawley
5/sex/2000
IPA 61.8
none
> 2000
(limit test)
severely congested lungs, splenomegaly, hepatomegaly with centrilo- bular conges- tion, subcap-
suloar renal
petechiae
Annex B- 5.2.1.2.1,
Glyphosate Monograph
1994 (MON / CHE)
TOX9552322

Rat, Sprague Dawley
5/sex/2000
IPA 62.2
none
> 5000
(limit test)
No findings
Annex B- 5.2.1.2.1,
Glyphosate Monograph
Rat, Wistar
5/sex/2000
IPA
62% in water
none
> 2000
(limit test)
No findings
1989 (I.Pi.Ci) TOX9551623
Studies from the 2001 evaluation
Annex B-
Rat,
5/sex/5000
IPA
none
> 5000
Pale colored
5.2.1.2.1,
Sprague-
65
(limit test)
kidneys and
Glyphosate
Dawley
hydronephrosis
Monograph
in few ♀
1981
(MON / CHE)
TOX9552321
Reference (Data owner)
Species Strain
Number of animals / Dose levels
(mg/kg bw)
Salt type
Purity (%)
Vehicl e

LD50
(mg/kg bw)
Main effects
Annex B- 5.2.1.2.2,
Glyphosate Monograph
1987 (SIN) TOX9500376
Mice, ICR
5/sex/0 (PEG) 5/sex/3125 5/sex/3625 5/sex/4125 5/sex/4625 5/sex/5125
IPA 41
PEG 200
3669
(confidenc e interval: 3489 –
3858)
Dose-related mortality in all IPA-treated groups; immobility, tremor, hyperemia of the ears
Study from the 2001 evaluation
Annex B- 5.2.1.2.2,
Glyphosate Monograph
1987 (SIN) TOX9500375
Mice, ICR
5/sex/0 (PEG) 5/sex/3125 5/sex/3625 5/sex/4125 5/sex/4625 5/sex/5125
IPA 64
PEG 200
4373
(confidenc e interval: 4144 –
4644)
Dose-related mortality in all IPA-treated groups; immobility, tremor, hyperemia of the ears
Studies not reviewed in the 2001 evaluation
IIA 5.2.1/16
1995 (MON) ASB2012- 11393
Mice CD-1
5/sex/5000
IPA 62.34
none
> 5000
Slight reduction in body weight gain in males at 5000 mg/kg/day versus controls
IIA 5.2.1/17
1999
(NUF) ASB2012-
11394
Rat, Sprague Dawley
5/sex/5000
IPA 62%
none
> 5000

(limit test)
♀: anogenital staining, soft faeces or diarrhoea
Study from the 2001 evaluation
Annex B- 5.2.1.2.1,
Glyphosate Monograph
1987 (MON / CHE) Z85869 & TOX9552323
Rat, Sprague- Dawley
1/sex/2000 5/sex/5000 5/sex/7500
NH4-
salt 90.8
none
4613
(confidenc e interval: 3511 –
5716)
5000 and
7500 mg/kg bw: mortality; ataxia, decreased activity, diarrhea, labored breathing, color changes of lungs (dark red), GI tract of dead rats with
dark/red fluid
Tier II summaries are presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
Reference: IIA, 5.2.1/01
Report: (2009) Glyphosate: Acute Oral Toxicity Study (UDP) In Rats.
Data owner: Helm AG Report No.: 12170-08
Date: 2009-03-11, not published ASB2012-11381
Guidelines: US EPA OPPTS 870.1100 Equivalent to OECD 425 (2008).
Deviations: Humidity was in the range of 33-89 % instead of 30-70 %. This deviation did not affect the study outcome
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate Tech Grade Mixed 5-Batch
Description:
White powder
Lot/Batch #:
080704-1 thru 5
Purity:
96.40 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Deionised water
Test animals:
Species:

Rat albino
Strain:
Sprague-Dawley
Source:
Age:
7 - 8 weeks
Sex:
Female
Weight at dosing:
160 - 187 g
Acclimation period:
5 days
Diet/Food:
Formulab #5008 (PMI Feeds Inc.), ad libitum except for approx. 16 h before dosing
Water:
Tap water, ad libitum
Housing:
Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 89 % Air changes: 10 - 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: 2008-11-11 to 2008-11-27 Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The dosing volume was 12.5 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanised by an overdose of CO2. All study animals were subjected to gross necropsy and all abnormalities were recorded.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs in one animal included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident at Day 8.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The oral LD50 of the test material (glyphosate) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and the evaluation is agreed.
Reference:
IIA, 5.2.1/02
Report:

(1995) HR-001: Acute Oral Toxicity Study In Rats.
Data owner: Arysta Life Sciences Report No.: IET 94-0134
Date: 1995-02-20, not published ASB2012-11382
Guidelines:
OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
Deviations:
None
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate technical, Code: HR-001
Description:
White crystal
Lot/Batch #:
940908-1
Purity:
95.68%
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
0.5% carboxymethyl-cellulose (CMC)
Test animals:
Species:
Rat
Strain:
Sprague-Dawley (Crj:CD), SPF
Source:
Age:
5 weeks
Sex:
Males and females
Weight at dosing:
♂ 168 - 179 g; ♀ 125 - 142 g
Acclimation period:
7 days
Diet/Food:
Pellet Diet MF (Oriental Yeast Co., Japan), ad libitum except for an overnight fast before dosing and about 3 h after dosing
Water:
Tap water, ad libitum
Housing:
Wire-mesh stainless steel cages in groups of 5 animals/sex/cage.

Environmental conditions:
Temperature: 23 ± 3°C Humidity: 55 ± 15%
Air changes: 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: 1995-01-24 to 1995-02-07
Animal assignment and treatment:
A group of five fasted rats per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anesthesia and subjected to gross necropsy.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Decreased spontaneous motor activity was observed in five males and three females as well as salivation in one male. These signs were observed at 1 and 3 hours after the administration.
Body weight: No body weight losses were recorded on Day 7 and 14 after administration when compared with the body weights on the day of administration.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The oral LD50 of the test material (HR-001) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, HR-001 is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and the evaluation is agreed.
Reference: IIA, 5.2.1/03
Report: (1995) HR-001: Acute Oral Toxicity Study In Mice.
Data owner: Arysta Life Sciences Report No.: IET 94-0133
Date: 1995-02-20, not published ASB2012-11383
Guidelines: OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate technical, Code: HR-001
Description:
White crystal
Lot/Batch #:
940908-1
Purity:
95.68 %
Stability of test compound:

No data given in the report.
Vehicle and/or positive control:
0.5 % carboxymethyl-cellulose (CMC)
Test animals:
Species:
Mice
Strain:
ICR (Crj:CD-1), SPF
Source:
Age:
6 weeks
Sex:
Males and females
Weight at dosing:
♂ 29.4 - 32.7 g; ♀ 22.8 - 25.8 g
Acclimation period:
7 days
Diet/Food:
Pellet Diet MF (Oriental Yeast Co., Japan), ad libitum
except for approx. 2 h before dosing, and 3 h after dosing
Water:
Tap water, ad libitum
Housing:
Aluminium cages with wire-mesh floors in groups of 5 animals/sex/cage.
Environmental conditions:
Temperature: 23 ± 3 °C
Humidity: 55 ± 15 % Air changes: 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: 1995-01-24 to 1995-02-07
Animal assignment and treatment:
A group of five fasted mice per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anesthesia and subjected to gross necropsy.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Decreased spontaneous motor activity was observed in one male and one female as well as sedation and crouching position in another male. These signs were observed at 1 and 3 hours after the administration.
Body weight: 7 days after administration, a slight body weight loss (0.5 g) was observed in one male when compared with the body weight on the day of administration. No body weight losses were recorded in any animal 14 days after the administration.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers
The oral LD50 of the test material (HR-001) in mice was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, HR-001 is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and the oral LD50 of > 5000 mg/kg bw in mice is agreed.
Reference: IIA, 5.2.1/04
Report: (2009) Glyphosate Technical: Acute Oral Toxicity Study in Rats.
Data owner: Excel Report No.: C22864
Date: 2009-04-02, not published ASB2012-11384
Guidelines: OECD 423 (2001)
Commission Regulation (EC) No 440/2008 (2008), method B.1 tris
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate Technical
Description:
Solid
Lot/Batch #:
GI-1045
Purity:
96.66 %
Stability of test compound:
(Stable under storage conditions.) Expiry date: July 2010.
Vehicle and/
or positive control:
Purified water
Test animals:
Species:
Rat
Strain:
HanRcc: WIST (SPF)
Source:
Age:
11 weeks
Sex:
Female
Weight at dosing:
181.0 – 198.7 g
Acclimation period:
7 days
Diet/Food:
Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 61/08 (Provimi Kliba

AG, 4303 Kaiseaugst / Switzerland) ad libitum (except for the overnight fasting period rior to intubation and approximately 3-4 hours post dose).
Water:
Tap water, ad libitum
Housing:
In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Shill AG, 4132 Muttenz / Switzerland).
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 % Air changes: 10 - 15/hour
12-hour light/dark cycle
Study design and methods
In life dates: 2009-02-13 to 2009-02-03 and 2009-02-05
Animal assignment and treatment:
Two groups of three fasted females each received the test material at a dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical signs of toxicity were made at least five times on the day of dosing (Day
1) and at twice daily thereafter during days 2-15. Individual body weights were recorded just prior to dosing and on Days 8 and 15.
On Day 15 after dosing, each animal was euthanised by CO2 asphyxiation. All study animals were subjected to gross necropsy and all abnormalities were recorded.
Results and discussion
Mortality: No deaths occurred during the study.
Clinical observations: No clinical signs were observed during the course of the study.
Body weight: The body weight of the animals was within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at necropsy.
Conclusion by the Notifiers
The oral LD50 of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate technical is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and the estimated oral LD50 > 2000 mg/kg bw is agreed.
Reference: IIA, 5.2.1/05
Report: (2009) Acute Oral Toxicity Study of Glyphosate TC in Rats.
Data owner: Helm AG Report No.: 23910
Date: 2009-06-16, not published ASB2012-11385
Guidelines: EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.
Deviations: A personnel change in the Head of the Quality Assurance Unit did not affect the validity of the study outcome.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate TC
Identification:

Glyphosate technical grade
Description:
White powder
Lot/Batch #:
20080801
Purity:
98.8 %
Stability of test compound:
2010-08-01
Vehicle and/or positive control:
0.8 % aqueous hydroxypropylmethylcellulose gel
Test animals:
Species:
Rat albino
Strain / Stock:
CD / Crl:CD(SD)
Source:
Age:
50 - 51 days
Sex:
Female
Weight at dosing:
167 - 186 g
Acclimation period:
5 days
Diet/Food:
ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), ad libitum except for approx. 16 h before dosing
Water:
Tap water, ad libitum
Housing:
Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:
Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 % 12-hour light/dark cycle
Study design and methods:
In life dates: 2009-02-04 to 2009-03-04
Animal assignment and treatment:
A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6, and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.
On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological

findings were noted at necropsy.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The oral LD50 of the test material (Glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and evaluation is agreed, the oral LD50 is greater than 2000 mg/kg bw.
Reference: IIA, 5.2.1/06
Report: (2010) Acute Oral Toxicity Study of Glyphosate TC in Rats.
Data owner: Helm AG Report No.: 24874
Date: 2010-01-06, not published ASB201211386
Guidelines: EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.
Deviations: There were no deviations from the study plan.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate TC
Identification:
Glyphosate technical grade
Description:
White powder
Lot/Batch #:
2009051501
Purity:
96.4 %
Stability of test compound:
2011-05-15
Vehicle and/
or positive control:
0.8 % aqueous hydroxypropylmethylcellulose
Test animals:
Species:
Rat albino
Strain / Stock:
CD / Crl:CD(SD)
Source:
Age:
Approx. 7 weeks

Sex:
Female
Weight at dosing:
171 - 192 g
Acclimation period:
5 days
Diet/Food:
ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), ad libitum except for approx. 16 h before dosing
Water:
Tap water, ad libitum
Housing:
Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:
Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 % 12-hour light/dark cycle
Study design and methods:
In life dates: 2010-10-15 to 2010-11-10
Animal assignment and treatment:
A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.
On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The oral LD50 of the test material (Glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and the oral LD50 >2000 mg/kg bw is agreed.
Reference: IIA, 5.2.1/07
Report: (2010)
Acute Oral Toxicity Study of Glyphosate TC in Rats.
Data owner: Helm AG Report No.: 24602
Date: 2010-02-19, not published ASB2012-11387
Guidelines: EC method B.1 tris (2004/73/EC), OECD 423 (ATC method) and OPPTS 870.1100.

Deviations: There were no deviations from the study plan.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate TC
Identification:
Glyphosate technical grade
Description:
White powder
Lot/Batch #:
20090506
Purity:
97.3 %
Stability of test compound:
May 2011
Vehicle and/or positive control:
0.8 % aqueous hydroxypropylmethylcellulose
Test animals:
Species:
Rat albino
Strain / Stock:
CD / Crl:CD(SD)
Source:
Age:
Approx. 7 - 8 weeks
Sex:
Female
Weight at dosing:
154 - 196 g
Acclimation period:
5 days
Diet/Food:
ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), ad libitum except for approx. 16 h before dosing
Water:
Tap water, ad libitum
Housing:
Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:
Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 % 12-hour light/dark cycle
Study design and methods:
In life dates: 2009-10-26 to 2009-11-24
Animal assignment and treatment:
A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by

oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6, and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.
On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The oral LD50 of the test material (Glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and based on the results the oral LD50 >2000 mg/kg bw/d is agreed.
Reference: IIA, 5.2.1/08
Report: (2005)
Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats.
Data owner: Helm AG Report No.: 15274
Date: 2005-04-04, not published ASB2012-11388
Guidelines: US EPA OPPTS 870.1100 (2002)
OECD 425 (2001).
Deviations: There were no deviations from the study plan.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate Acid Technical
Identification:
Glyphosate Acid Technical
Description:
White crystalline powder
Lot/Batch #:
040205
Purity:
97.23 %
Stability of test compound:
Test substance was expected to be stable for the duration of testing.
Vehicle and/or positive control:
Distilled water

Test animals:
Species:
Rat albino
Strain:
Sprague-Dawley derived
Source:
Age:
11 weeks
Sex:
Female
Weight at dosing:
222 - 235 g
Acclimation period:
21 or 23 days
Diet/Food:
Purina Rodent Chow #5012, ad libitum except for overnight fasting before dosing
Water:
Filtered tap water, ad libitum
Housing:
Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:
Temperature: 19-23 °C
12-hour light/dark cycle
Study design and methods:
In life dates: 2004-05-04 to 2004-05-20
Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The test substance was administered as a 50 % w/w suspension in distilled water. Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanised by an overdose of CO2. All study animals were subjected to gross necropsy and all abnormalities were recorded.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs noted for all animals included diarrhea, ano-genital and facial staining, and/or reduced fecal volume. All animals recovered by Day 4 and appeared active and healthy for the remainder of the 14-day observation period.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The oral LD50 of the test material (glyphosate acid technical) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised

criteria, Glyphosate Acid Technical is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and the evaluation is agreed.
Reference:
IIA, 5.2.1/09
Report:
2008) Acute Oral Toxicity Study in
Data owner: Helm AG
Report No.: RF-3996.305.475.07
Date: 2008-09-16, not published ASB2012-11389
Guidelines:
OECD guideline 423 (2001).
Deviations:
The experimental phase initiation and conclusion dates were
updated. This deviation did not affect the study outcome.
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Glyphosate Technical
Identification:
Glyphosate Technical
Description:
Solid
Lot/Batch #:
20070606
Purity:
98.05 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rat albino
Strain:
Wistar Hannover
Source:
Age:
8 - 9 weeks
Sex:
Female
Weight at dosing:
172 - 205 g

Acclimation period:
6 days
Diet/Food:
Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), ad libitum except for fasting overnight before dosing
Water:
Filtered drinking water, ad libitum
Housing:
Groups of three rats per cage were held in polypropylene rodents cages with wire mesh tops and bedding material.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 % Air changes: min. 10/hour 12-hour light/dark cycle
Study design and methods
In life dates: 2007-09-12 to 2008-06-11
Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 2000 mg/kg bw by oral gavage in a stepwise manner. Observations for mortality and clinical/behavioural signs of toxicity were made once within the first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were recorded just prior to dosing (Day 0) and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized by an overdose of CO2. All study animals were subjected to gross necropsy and all abnormalities were recorded.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs of toxicity were observed in females treated with 2000 mg/kg bw.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The acute oral LD50 cutoff-value of the test material (glyphosate technical) in female rats was estimated to be 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria Glyphosate Technical is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable. The limit dose of 2000 mg/kg bw (not 5000 mg/kg bw/d) did not cause signs of toxicity, the acute oral LD50 is >2000 mg/kg bw.
Reference: IIA, 5.2.1/10
Report: (2007)
GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study in rats
Data owner: Nufarm Report No.: BO2272
Date: 2007-03-01, unpublished ASB2012-11390
Guidelines: Japanese guideline Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute oral toxicity studies. Guideline 2"1-1 Notification 12 NohSan No. 8147, as partly revised in 16-Shouan- 9260, on 16 March 2005. English translation by ACIS on 17 Oct 2005.

Directive 2004173/EC, 8.1 tris "Acute Oral Toxicity-Acute Toxic Class Method", April 29, 2004.
OECD Guidelines for the Testing of Chemicals, Number 423 "Acute Oral Toxicity - Acute Toxic Class Method", adopted 17 December 2001.
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate Technical (NUP 05068)
Description:
White powder
Lot/Batch #:
200609062
Purity:
95.1 %
Stability of test compound:
Stable under storage conditions.
Vehicle and/or positive control:
Polyethylene glycol 300 (PEG 300)
Test animals:
Species:
Rat
Strain:
HanRcc:WIST (SPF)
Source:
Age:
11 weeks
Sex:
Female
Weight at dosing:
160 - 187 gg
Acclimation period:
5 days
Diet/Food:
Pelleted standard Provimi Kliba 3433 rat/mouse maintenance
diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/Switzerland) ad libitum.
Water:
Tap water, ad libitum
Housing:
In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Schill AG, CH-4132 MuttenzlSwitzerland).
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle

Study design and methods:
In life dates: 2006-12-12 to 2007-01-04
Animal assignment and treatment:
The animals received a single dose of the test item by oral gavage administration at 2000 mg/kg body weight after being fasted for approximately 18 to 19 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing. The dosing volume was 10 mL/kg body weight. OBSERVATIONS for Mortality and Viability: Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.
Body weights: On test days 1 (prior to administration), 8 and 15.
Clinical signs: Daily during the acclimatisation period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Once daily during days 2-15. All abnormalities were recorded.
NECROPSY: All animals were killed at the end of the observation period by Carbon dioxide asphyxiation.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs in one animal included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident at Day 8.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The median lethal dose of Glyphosate Technical (NUP 05068) after single oral administration to female rats, observed over a period of 14 days is: LD50 (female rat) > than 2000 mg/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate Technical (NUP 05068) is not to be classified for this end point.
Comment by RMS:
The study is considered acceptable and evaluation is agreed.
Reference: IIA, 5.2.1/11
Report: . (1988) Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats
Data owner: Monsanto Monsanto Report No.: FD-88-29 Date: 1988-06-08, not published Z35389
Guidelines: US EPA 81-1
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate
Description:
White powder
Lot/Batch #:
XLI-55
Purity:

97.76 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Distilled water
Test animals:
Species:
Rat
Strain:
Sprague-Dawley
Source:
Age:
Not specified
Sex:
Male and female
Weight at dosing:
♂ 300 - 332 g; ♀ 217 - 222 g
Acclimation period:
At least 5 days
Diet/Food:
NIH Open Formula 07 Rat and Mouse Diet, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), ad libitum (except when fasted overnight prior to dosing)
Water:
Tap water, libitum
Housing:
Wire mesh cages
Environmental conditions:
Temperature: 20 – 23.9 ºC
Humidity: 40 – 70 % Air changes: Not specified
Light cycle: 12 hour light/dark cycle
Study design and methods:
In life dates: 1988-04-05 to 1988-04-19
Animal assignment and treatment:
Groups of five male and five female rats received the test material at a dose level of 5000 mg/kg body weight by oral gavage as a 50 % w/v aqueous suspension. Observations for mortality and signs of toxicity were made three times on the day of dose administration and twice daily thereafter. Body weights were recorded prior dose administration on study day 1, and on days 8 and 15 (terminal sacrifice). A gross necropsy was performed on all animals at the time terminal sacrifice (day 15) and all abnormalities were recorded.
Results and discussion
Mortality: No mortalities occurred.
Clinical observations: Clinical signs included diarrhoea, apparent urinary incontinence, and hair loss on the abdomen.
Body weight: Body weight gain was noted for all animals.
Necropsy: No internal abnormalities were noted during gross necropsy examination of the animals.
Conclusion by the Notifiers

The oral LD50 of the test material (glyphosate) in rats was estimated to be 5000 mg/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate should not be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and evaluation is agreed.
Reference: IIA, 5.2.1/12
Report: (1979) Acute Oral
Toxicity Study In Rats.
Data owner: Monsanto
Monsanto Report No.: BND-77-428 Date: 1979-08-06, not published Z35541
Guidelines: None (pre-guideline)
Deviations: Not specified
GLP: no (pre-GLP)
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate Technical
Description:
Fine white powder
Lot/Batch #:
XHI-180
Purity:
99 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Distilled water
Test animals:
Species:
Rat
Strain:
Wistar
Source:
Age:
Not specified
Sex:
Male and female
Weight at dosing:
225 - 294 g
Acclimation period:
Not specified
Diet/Food:
ad libitum (except when fasted for approximately 18 hours prior to dosing)
Water:

ad libitum
Housing:
Individually
Environmental conditions:
Temperature: Not specified Humidity: Not specified Air changes: Not specified Light cycle: Not specified
Study design and methods:
In life dates: Not specified
Animal assignment and treatment:
Groups of five male and five female rats received the test material at a dose levels of 2.5, 3.5, 5.0, 7.0, and 9.9 g/kg body weight by oral gavage. The test material was administered by oral intubation as a 25 % w/v solution in distilled water. Observations for mortality and overt signs of effect were made at 0-2 and 4-6 hours following dosing and twice daily thereafter (early morning and late afternoon) for fourteen days. Body weights were recorded prior to fasting, on Day 7, and on Day 14 of the study. A gross necropsy was performed on all animals at the time of death or terminal sacrifice (Day 14). All abnormalities were recorded.
Results and discussion
Mortality: Mortalities in the study are as indicated below in Table B.6.2-3.
Table B.6.2-3: Summary of Mortality
Dose Level
(g/kg body weight)
Mortality/Total Number of Animals Dosed
2.5
1/10
3.5
1/10
5.0
3/10
7.0
8/10
9.9
10/10
Clinical observations: Clinical signs included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and fecal staining of the abdomen.
Body weight: For the 2.5, 3.5, 5.0, and 7.0 g/kg body weight dose levels, although some animals lost weight between 7 and 14 days, all surviving animals gained weight throughout the study.
Necropsy: A summary of the gross necropsy findings for the decedents and the animals necropsied at the conclusion of the 14-day observation period is presented in Table B.6.2-4.
Table B.6.2-4: Summary of Necropsy Findings
Dose Level
(g/kg body weight)
Animals Necropsied at 14 Days
Decedents
2.5
Discoloured lungs, liver, and/or kidneys

Urinary and faecal staining of the abdomen Discoloured lungs
Fluid filled stomach
Fluid filled and/or distended intestines
3.5
Discoloured lungs, liver, and/or kidneys Air filled intestines
Discoloured lungs
5.0
No observations
Oral and/or nasal discharge
Urinary and/or faecal staining of the abdomen
Discoloured lungs and/or liver
Fluid filled and/or discoloured stomach and/or intestines
7.0
Discoloured lungs, liver, and/or kidneys Air filled intestines
Oral discharge
Fluid filled intestines and/or stomach Discoloured liver, and/or kidneys Urinary and/or faecal staining of the
abdomen
9.9

Not applicable
Discoloured lungs, liver, and/or kidneys Fluid filled intestines and/or stomach Oral and/or nasal discharge
Urinary staining of the abdomen
Conclusion by the Notifiers
The oral LD50 of the test material (glyphosate) in rats was estimated to be 5.6 g/kg body weight with 95 % confidence limits of 4.9 to 6.3 g/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for acute oral toxicity.
Comment by RMS:
This old study was conducted prior to GLP and not according to any Test Guideline, subsequently some reporting deficiencies were apparent. Therefore, this study is considered
to be supplementary. Under the conditions of the present study the oral LD50 value of > 5000 mg/kg bw is agreed.
Reference: IIA, 5.2.1/13
Report: (1996) Glyphosate Acid: Acute Oral Toxicity Study In Rats.
Data owner: Syngenta Report No.: CTL/P/4660
Date: 1996-08-23, not published TOX2000-1982
Guidelines: OECD 425 (2001)
US EPA OPPTS 870.1100 (2002)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS.
Materials and methods
Test material:
Identification:

Glyphosate Acid
Description:
Technical, white solid
Lot/Batch #:
P24
Purity:
95.6 %
Stability of test compound:
The test substance was used within the expiry date.
Vehicle and/
or positive control:
Deionised water
Test animals:
Species:
Rat
Strain:
Alpk:APfSD (Wistar-derived)
Source:
Age:
Young adult
Sex:
Male and female
Weight at dosing:
233-260 g (males), 197-225 g (females)
Acclimation period:
At least 6 days
Diet/Food:
Diet (PCD), supplied by Special Diet Services Limited, Witham, Essex, UK ad libitum (except overnight immediately prior to dosing).
Water:
Mains water ad libitum
Housing:
5/cage, sexes separately in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study.
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 40 – 70 %
Air changes: Approximately 25-30/hour 12-hour light/dark cycle
Study design and methods:
In life dates: 1995-03-16 to 1995-03-30
Animal assignment and treatment:
In an acute oral toxicity study, a group of five male and five female, fasted, young adult Alpk:APfSD (Wistar-derived) rats were given a single oral dose of 5000 mg/kg bw of glyphosate acid by gavage. The test substance was diluted in deionised water. The volume of the dose was calculated for each animal according to its weight at the time of dosing and a standard volume of 10 mL/kg of the dosing preparation was administered.

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic toxicity once within 2 hours of dosing and again between 4 and 7 hours after dosing. Subsequent observations were made daily, up to day 15.
The animals were weighed on the day before dosing (day -1), immediately before dosing (day 1) and on days 3, 8, 8 and 15.
All animals were subjected to an examination post mortem. This involved an external observation and a careful examination of all thoracic and abdominal viscera.
Results and discussion
Mortality: There were no mortalities.
Clinical observations: There were no signs of systemic toxicity.
Body weight: All animals lost weight initially due to the pre-dose fast, but all had exceeded their initial weight by day 3, and apart from a transient weight loss in one female, continued to gain weight throughout the remainder of the study.
Necropsy: Red or mottled areas in the lung or red areas in the thymus were seen in three males and two females. These are common spontaneous findings in rats of this age and strain and are considered not to be treatment-related.
Conclusion by the Notifiers
The oral LD50 of the test material (glyphosate acid) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate acid is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and evaluation is agreed.
Reference:
IIA, 5.2.1/14
Report:
(2007) Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure). Data owner: Syngenta Report No.: B02755
Date: 2007-02-08, not published ASB2012-11391
Guidelines:
OECD 425 (2001)
US EPA OPPTS 870.1100 (2002)
Japanese MAFF 12 NohSan No. 8147
Deviations:
None
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate technical material
Description:
Technical, white powder
Lot/Batch #:

0507
Purity:
96.1 %
Stability of test compound:
Re-certification date August 2008. Stable under storage conditions (room temperature range 20 ± 5 °C, protected from light and humidity).
Vehicle and/or positive control:
Purified water (deionised water processed and treated by the PURELAB Option-R unit which links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation).
Test animals:
Species:
Rat
Strain:
HanRcc:WIST (SPF)
Source:
Age:
11 weeks
Sex:
Female
Weight at dosing:
183.0-188.9 g
Acclimation period:
5-7 days
Diet/Food:
Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) ad libitum (except for pre-dose fast).
Water:
Community tap water ad libitum
Housing:
Individually in Makrolon type-3 cages with standard softwood bedding
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle
Study design and methods:
In life dates: 2006-12-06 to 2006-12-26
Animal assignment and treatment:
In an acute oral toxicity study, a group of three, fasted, 11 week old, HanRcc:WIST (SPF), female rats was given a single oral dose of glyphosate technical material (96.1 % w/w glyphosate acid) at a concentration of 5000 mg/kg body weight by gavage. The test substance was diluted in vehicle (purified water) and dosed at a volume dosage of 10 mL/kg body weight.
Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. The first animal was treated at a dose level of 5000 mg/kg body weight. As no mortality or significant clinical signs were observed, two additional animals were sequentially dosed at 5000 mg/kg such that a total of 3 animals were tested. No mortalities were observed, therefore the study was terminated.
The animals were examined daily during the acclimatisation period and mortality, viability and

clinical signs were recorded. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2-15. Mortality/viability was recorded once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.
Body weights were recorded on day -1 (prior to removal of food), day 1 (prior to administration) and on days 8 and 15.
All animals were killed at the end of the observation period by carbon dioxide asphyxiation, necropsied and examined macroscopically.
Results and discussion
Mortality: There were no mortalities.
Clinical observations: Slight ruffled fur was noted in all animals from the 30-minute reading to the 5-hour reading and persisted in one animal until test day 3. Hunched posture was also noted in the animals from the 1- or 2-hour reading to the 5-hour reading.
Body weight: The body weight of the animals was within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy.
Conclusion by the Notifiers
The oral LD50 of the test material (glyphosate technical material) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical material is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and the estimated oral LD50 >5000 mg/kg bw is agreed.
Reference:
IIA, 5.2.1/15
Report:
2011) Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure)
Data owner: Syngenta Report No.: 10/218-001P
Date: 2011-04-15, not published ASB2012-11392
Guidelines:
OECD 425 (2008)
OPPTS 870.1100 (2002)
Deviations:
None
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
Technical, dry white powder
Lot/Batch #:
569753(BX20070911)

Purity:
96.3 %
Stability of test compound:
Stable under storage conditions (room temperature range
<30 °C), recertification date end August 2011
Vehicle and/or positive control:
0.5% Carboxymethylcellulose (CMC)
Test animals:
Species:
Rat
Strain:
RjHan:WI
Source:
Age:
10-11 weeks
Sex:
Female
Weight at dosing:
228-231 g
Acclimation period:
At least 21 days
Diet/Food:
ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany ad libitum (except for pre-dose fast)
Water:
Tap water ad libitum
Housing:
Individually in Type II. polypropylene/polycarbonate cages with Lignocel Bedding for Laboratory Animals
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 % Air changes: 15 - 20/hour 12-hour light/dark cycle
Study design and methods:
In life dates: 2011-01-20 to 2011-02-10
Animal assignment and treatment:
In an acute oral toxicity study, a group of three, fasted, 10-11 week old, RjHan:WI female rats was given a single oral dose of glyphosate technical (96.3 % w/w glyphosate technical) at a concentration of 5000 mg/kg body weight by gavage. The test substance was diluted in vehicle (0.5 % carboxymethylcellulose) and administered at a dosing volume of 10 mL/kg.
Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. Treatment of an animal at the next dose was only performed when no significant clinical signs were noted in the previous animal.
All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3, 4 and 6 hours after treatment on day 1 and once daily for 14 days thereafter.
Body weights were recorded on day -1 (prior to removal of food), day 0 (prior to administration) and

on days 7 and 14.
All animals were exsanguinated under pentobarbital anaesthesia at the end of the observation period, necropsied and examined macroscopically.
Results and discussion
Mortality: There were no mortalities.
Clinical observations: No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw.
Body weight: The body weight of the animals was within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy.
Conclusion by the Notifiers
The oral LD50 of the test material (glyphosate technical) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate technical is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and evaluation is agreed.
Reference: IIA, 5.2.1/18
Report:
Fixed Dose Method
Data owner: Albaugh Europe Sàrl
Report No.: 4140853
Date: 2014-00-01, not published ASB2014-9147
Guidelines: OECD 420 (2001)
Method B1 bis (EC) No.440/2008
Deviations: Homogeneity, concentration or stability or test item formulation not determined
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
Technical, white crystalline solid
Lot/Batch #:
04062014
Purity:
85.79 %
Vehicle and/or positive control:
Dimethyl sulphoxide
Test animals:
Species:
Rat
Strain:
RccHanTM:Wistar
Source:
Age:
8-12 weeks

Sex:
Female
Weight at dosing:
141 - 171 g
Acclimation period:
At least 5 days
Water:
Tap water ad libitum
Housing:
Animals were housed in groups of up to four
Environmental conditions:
Temperature: 19 to 25 °C Humidity: 30 - 70 %
Air changes: at least 15 /hour 12-hour light/dark cycle
Study design and methods:
In life dates: 2014-07-10 to 2014-07-30
Animal assignment and treatment:
Female Wistar (RccHanTM:Wist) strain rats were supplied by Harlan Laboratories UK Ltd., Oxon, UK. On receipt the animals were randomly allocated to cages. The females were nulliparous and non-pregnant. The body weight variation did not exceed ±20 % of the body weight of the initially dosed animal.
With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food (2014C Teklad Global Rodent diet supplied by Harlan Laboratories UK Ltd., Oxon, UK) was allowed throughout the study.
For the purpose of the study the test item was freshly prepared, as required, as a dispersion/suspension in dimethyl sulphoxide. Dimethyl sulphoxide was used because the test item did not dissolve/suspend in distilled water or arachis oil BP.
The test item was formulated within two hours of being applied to the test system. It is assumed that the formulation was stable for this duration.
No analysis was conducted to determine the homogeneity, concentration or stability of the test item formulation. This is an exception with regard to GLP and has been reflected in the GLP compliance statement.
Using available information on the toxicity of the test item, 2000 mg/kg bwwas chosen as the starting dose (one animal) and administered at a dosing volume of 10 mL/kg. In the absence of toxicity at a dose level of 2000 mg/kg bw, an additional group of four animals was treated. All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe.
Clinical observation were made ½, 1, 2, and 4 hours after dosing and then daily for fourteen days. Morbidity and mortality checks were made twice daily.
Individual body weights were recorded on day 0 (the day of dosing) and on days 7 and 14.
At the end of the observation period the animals were killed by cervical dislocation. All animals were subjected to gross necropsy. This consisted of an external examination and opening of the abdominal and thoracic cavities. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.
Results and discussion
Mortality: There were no deaths.
Clinical observations: No signs of sytemic toxicity were noted in the initial treated animal during the observation period. Hunched posture was noted during the day of dosing in four additional treated

animals.
Body weight: All animals showed expected gains in body weight over the observation period.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy. No tissues were retained.
Conclusion by the RMS
The study is considered to be acceptable. The oral LD50 of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate technical is not to be classified for acute oral toxicity.
Reference: IIA, 5.2.1/16
Report: 1995) Acute Toxicity Study of MON
0139 by Oral Administration in Mice.
Data owner: Monsanto
Monsanto Report No.: XX-95-205 Date: 1995-10-05, not published ASB2012-11393
Guidelines: JMAFF 59 NohSan No. 4200 (January 28, 1985)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
MON 0139
Description:
Light yellow viscous solution
Lot/Batch #:
LBRV-11092
Purity:
62.34% (isopropyl amine salt of glyphosate)
Stability of test compound:
Stable under room temperature, expiry July, 1996
Vehicle and/or positive control:
Water for injection
Test animals:
Species:
Mouse
Strain:
Crj:CD-1(ICR)
Source:
Age:
6 weeks
Sex:
Male and female
Weight at dosing:
♂ 31.1 – 34.5 g; ♀ 22.2 – 26.2 g
Acclimation period:
Approximately

Diet/Food:
CRF-1 pelleted diet, sterilised by radiation (Oriental Yeast Co., Ltd.), ad libitum except during fasting prior to dosing
Water:
Tap water; ad libitum
Housing:
Plastic cages with wood chip bedding in groups of 5 (groups of 5 or 6 during quarantine/acclimation)
Environmental conditions:
Temperature: 23 ± 3 ºC
Humidity: 50 ± 20 %
Air changes: 11 – 13 per hour
Light cycle: 12 hour illumination per day
Study design and methods:
In life dates: 1995-08-16 – 1995-09-06
Animal assignment and treatment:
Preliminary study: During the quarantine/acclimatization period, a preliminary study was conducted using 9 males and 9 females. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage, adjusting the dose volume according to each dose level. Three male and female animals were dosed with 1000, 2000 or 5000 mg/kg body weight. The animals were fed again after administration, and had free access to water throughout the experimental period.
Main Study:
In the preliminary study, no deaths were observed in either sex in any of the dose groups. Based on these results, the dose level of 5000 mg/kg body weight was selected for the main test. The animals were ranked by individual body weights and randomly assigned to groups so as to ensure the homogeneity of group means as far as possible. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage. The animals in the 5000 mg/kg body weight group and control group were given 0.041 mL/10 g body weight of test article and 'water for injection', respectively. Each group consisted of 5 animals per sex. Animals were fed again after administration, and had free access to water throughout the experimental period.
The animals were observed frequently for the first 6 hours after administration, and then once daily for 14 days for mortality, signs of toxicity and abnormalities. Body weights were recorded prior to fasting, immediately before dosing, and on days 1, 2, 3, 7, 10 and 14 after dosing. A gross necropsy was performed on all animals at the time of terminal sacrifice at the end of the 14-day observation period.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs of toxicity were observed.
Body weight: In males, a slight tendency toward retardation of body weight gain as compared with the control group was observed in the 5000 mg/kg body weight group from 7 days after administration (see Table B.6.2-5). In females, no compound-related changes were observed in the 5000 mg/kg body weight group.
Table B.6.2-5: Summary of male body weights
MON 0139 Dose (mg/kg body weight)
Days After Administration

0a
0
1
2
3
7
10
14
0
34.4
32.6
34.5
35.2
35.4
37.3
38.1
38.8
5000
34.8
32.8
34.2
34.1
34.4
35.1
35.9
37.3
a Prior to fasting
Necropsy: No abnormalities were observed.
Conclusion by the Notifiers
The oral LD50 of the test material (MON 0139) in mice was >5000 mg/kg body weight. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, MON 0139 is not to be classified for acute oral toxicity.
Comment by RMS:
The study is considered acceptable and the oral LD50 > 5000 mg/kg bw in mice is agreed.
Reference: IIA, 5.2.1/17
Report: (1999) NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test
Study No.: 7907
Date: 1999-09-16, unpublished ASB2012-11394
Guidelines: US EPA Health Effects Test Guidelines, OPPTS 870.1100 (1998)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
NUP5a99 62 %

Description:
clear viscous amber liquid
Lot/Batch #:
Drum Sample E
Purity:
62 %
Stability of test compound:
No data available
Vehicle and/
or positive control:
None
Test animals:
Species:
Rat
Strain:
Sprague-Dawley derived, albino
Source:
Age:
Not specified
Sex:
5 males and 5 females
Weight at dosing:
Young adult/males 227-254 grams and females 178-200 grams at
experimental startg
Acclimation period:
14 days
Diet/Food:
Purina Rodent Chow #5012
Water:
Tap water, ad libitum
Housing:
singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage
and was changed at least three times per week.
Environmental conditions:
Temperature: 22-24 °C Humidity: not specified Air changes: not specified
12-hour light/dark cycle
Study design and methods
In life dates: 1999-08-03 – 1999-08-17
Animal assignment and treatment:
Prior to dosing, a group of animals was fasted for approximately 17.25 hours by removing feed from their cages. After weighing and clinical examination, ten (five male and five female) healthy rats were selected for test. Individual doses were calculated based on the initial bodyweights, taking into account the specific gravity (determined by PSL) of the test substance. Each animal received 5000 mg/kg of the test substance via gavage. Feed was replaced approximately 3.5 hours after dosing. The day of administration was considered Day zero of the study. Animals were weighed prior to test

substance administration (initial) and again on Days 7 and 14 (termination). Clinical signs were recorded at 1,3 and 22 hours post- dosing and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and product safety labs central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma. All rats were euthanized via CO2 inhalation on Day 14. Gross necropsies were performed on all animals.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: Most females exhibited anogenital staining and two females exhibited soft feces or diarrhea, but recovered by Day 2.

Body weight: Body weight gain was unaffected by the administration of the test substance.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The single dose acute oral LD50 of NUP5a99 62 % glyphosate MUP is greater than 5000 mg/kg of bodyweight.

Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, NUP5a99 62 % glyphosate MOP is not to be classified for this endpoint.

Comment by RMS:

The study is considered acceptable and the estimated oral LD50 is greater than 5000 mg/kg bw.

Comment by GTF on the first draft of the RAR (July 2013):

The Rat LD50oral and Rat LD50dermal are listed as >2000 mg/kg bw whereas the data presented concludes LD50-values above 5000 mg/kg. This would also be consistent with the values presented in summary Table B.6.13-2 in Volume 3 B.6.

RMS comment (August 2013):

To conclude on the LD50 value >2000 mg/kg bw seems to be more appropriate, because not all different batches were tested up to 5000 mg/kg bw. In any case Glyphosate is considered not to be classified as acute oral or dermal toxic according to GHS categories.

B.6.2.2 Acute percutaneous toxicity

Similarly, for the previous EU evaluation, a multitude of dermal toxicity studies in rats and rabbits were provided using glyphosate acid and its salts. Additional studies in rats with glyphosate acid were submitted for the current re-evaluation.

Table B.6.2-6: Summary of acute dermal toxicity studies with glyphosate acid

Reference (Data owner)

Species Strain

Number of animals / Dose

Purity (%)

Vehicle

LD50

(mg/kg

Main effects

levels

bw)

(mg/kg bw)

Studies from the 2001 evaluation

Annex B- 5.2.2.1.1,
Glyphosate Monograph
1995
(Sanachem) TOX9650910
Rat, Sprague Dawley
5/sex/2000
97.6
Cotton seed oil
> 2000
(limit test)
splenomegaly, Liver: centri- lobular congestion
Annex B- 5.2.2.1.1,
Glyphosate Monograph
,
1994 (SIN) TOX9500378
Rat, Sprague Dawley
5/sex/2000
95
Suspen- ded (50%
w/w) in natrosol (1% w/w
in water)
> 2000
(limit test)
No findings
Annex B- 5.2.2.1.1,
Glyphosate Monograph
1994
(Herbex) TOX9500246
Rat, Sprague Dawley
5/sex/2000
95
none
> 2000
(limit test)
No findings
Studies from the 2001 evaluation
Annex B-
Rat,
2/sex/0
97.2
water
> 2000
No findings
5.2.2.1.1,
Wistar
5/sex/2000

(limit
Glyphosate
test)
Monograph
1994
(ALkaloida)
TOX9650143
Reference (Data owner)
Species Strain
Number of animals / Dose levels
(mg/kg bw)
Purity (%)
Vehicle
LD50
(mg/kg bw)
Main effects
Annex B- 5.2.2.1.1,
Glyphosate Monograph
1992
(Barclay) TOX9551813
Rat, Sprague- Dawley
5/sex/2000
> 97
none
> 2000
(limit test)
No findings
Annex B- 5.2.2.1.1,
Glyphosate Monograph
1991 (ADM TOX9551090
Rat, Wistar
5/sex/2500 5/sex/5000
96.8
Water (slurry)
> 5000
body weight loss
Annex B- 5.2.2.1.1,
Glyphosate Monograph
1990 (AGC) TOX9551793
Rat, CD
5/sex/0 5/sex/3000 5/sex/5000 5/sex/8000
98.1
0.9 % saline
> 8000
No findings
Studies not reviewed in the 2001 evaluation

IIA 5.2.2/01
1989 (CHE) TOX9300328
Rat, Sprague Dawley
5/sex/2000
98.6
Water for moiste- ning
> 2000
(limit test)
No mortalities, body weight loss in one female, scab formation at application site;
0.5 h-1d after dosing reduced
activity and piloerection
IIA 5.2.2/02
, 2009 (HAG) ASB2012- 11395
Rat,
Sprague Dawley
5/sex/5050
96.4
water
> 5050
body weight
loss in 1 male and 1 female
IIA 5.2.2/03
1995 (ALS) ASB2012- 11396
Rat, SD
5/sex/2000
95.68
water
> 2000
(limit test)
No findings
IIA 5.2.2/04
2009
(EXC) ASB 2012-
11397
Rat,
HanRcc: WIST
5/sex/2000
96.66
water
> 2000
No mortalities,
no signs of systemic toxicity; in 4 females slight local signs (erythema, scaling and
scabs) at the application sites
Reference (Data owner)
Species Strain
Number of animals / Dose levels

(mg/kg bw)
Purity (%)
Vehicle
LD50
(mg/kg bw)
Main effects
Studies not reviewed in the 2001 evaluation
IIA 5.2.2/05
Rat, CD
5/sex/2000
98.8
water

> 2000
No findings
2009 (HAG)
ASB2012- 11398
IIA 5.2.2/06
Rat, CD
5/sex/2000
96.4
water
> 2000
No findings
2010 (HAG) ASB2012-
11399
IIA 5.2.2/07
Rat, CD
5/sex/2000
97.3
water
> 2000
No findings
2010 (HAG) ASB2012- 11400
IIA 5.2.2/08
2005 (HAG) ASB2012-
11401
Rat,
Sprague Dawley
5/sex/5000
97.23
water
> 5000
No findings
IIA 5.2.2/09
2008 (HAG) ASB2012- 11402

Rat,
Wistar Hannove r
5/sex/2000
98.05
water
(for moisto- ning)
> 2000
No findings
IIA 5.2.2/10
2007 (NUF) ASB2012- 11403
Rat,
HanRcc: WIST
5/sex/2000
95.1
PEG 300
> 2000
(limit test)
No findings
IIA 5.2.2/11
1996 (SYN) TOX2000- 1983
Rat
5/sex/2000
95.6
Moiste-
ned with deionise d water
> 2000
Slight erythema
in 1♂, small scabs in 1 ♀
IIA 5.2.2/12
2007 (SYN) ASB2012- 11404
Rat
5/sex/5000
96.1
Moiste-
ned with purified water
> 5000
No findings
IIA 5.2.2/13
2011 (SYN) ASB2012- 11405
Rat
5/sex/5000
96.3
Moiste-
ned with purified water
> 5000
No findings

Reference (Data owner)
Species Strain
Number of animals / Dose levels
(mg/kg bw)
Purity (%)
Vehicle
LD50
(mg/kg bw)
Main effects
Studies from the 2001 evaluation
Annex B-
Rabbit,
5/sex/5000
97.8
Moiste-
> 5000
Mortality (1 ♀);
5.2.2.1.2,
NZW
ned with
anorexia,
Glyphosate
saline
diarrhea, soft
Monograph
stool
,
1988 (MON)
TOX9552325
Table B.6.2-7: Summary of acute dermal toxicity studies with glyphosate salts
Reference (Data owner)
Species Strain
Number of animals / Dose levels
(mg/kg bw)
Salt type Purity
(%)
Vehicle
LD50
(mg/kg bw)
Main effects
Studies from the 2001 evaluation
Annex B- 5.2.2.2.1,
Glyphosate Monograph
1995
(Sanachem) TOX9650910
Rat, Sprague Dawley

5/sex/2000
IPA 61.8
none
> 2000
(limit test)
severely congested lungs, splenomegaly, hepatomegaly with centrilobular congestion, subcapsuloar renal petechiae
Annex B- 5.2.2.2.1,
Glyphosate Monograph
1989 (I.Pi.Ci.) TOX9551625
Rat, Wistar
5/sex/2000
IPA 62%
none
> 2000
(limit test)
Erythema maculate (1♂), scales (1 ♀)
Annex B- 5.2.2.2.2,
Glyphosate Monograph
1987 (MON / CHE) TOX9552327
Rabbit NZW
5/sex/5000
Ammo nium salt 90.8
Moiste ned with saline
> 5000
(limit test)
Mortality (1♀); diarrhea, soft stool
Studies from the 2001 evaluation
Annex B- 5.2.2.2.2,
Glyphosate Monograph
1987 (MON / CHE) TOX9552328
Rabbit NZW
5/sex/5000
Sodiu m salt 80.3
Saline (1mL/g
)
> 5000
(limit test)
Soft stool
Annex B-
5.2.2.2.2,
Glyphosate Monograph
1981
(MON / CHE) TOX9552326
Rabbit
NZW

5/sex/5000
IPA
65
none
> 5000
(limit test)
No findings
Tier II summaries are presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation please refer to the monograph.
Reference:
IIA, 5.2.2/01
Report:
(1989) Acute dermal toxicity (limit) test in rats
Data owner: Cheminova A/S Report No.: 5884
Date: 1989-06-21, not published TOX9300328
Guidelines:
OECD, EEC, EPA1
Deviations:
None
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
White powder
Lot/Batch #:
206-Jak-25-1
Purity:
No data given in the report.
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Water
Test animals:
Species:
Rats
Strain:
Sprague-Dawley
Source:
Age:
8-10 weeks

Sex:
Males and females (nulliparous and non-pregnant)
Weight at dosing:
♂ 212 - 240 g; ♀ 188 - 234 g
Acclimation period:
6 days
Diet/Food:
Expanded Rat and Mouse Maintenance Diet, ad libitum
Water:
Tap water, ad libitum
Housing:
polypropylene cages with mesh floors in groups of 5 animals/cage.
1 Guideline numbers are not specified in the report, however the study is compliant with OECD 402, EEC B3 and EPA 81-2 with the exception of a slightly different test item application procedure.
Environmental conditions:
Temperature: 19 - 22 °C
Humidity: 49 %
12-hour light/dark cycle
Study design and methods
In life dates: 1989-06-06 to 1989-06-21
Animal assignment and treatment:
A group of 5 male and 5 female rats was prepared by clipping the backs free of hair, approximately 24 hours before application of the test material. Care was taken to avoid abrading the skin.
Glyphosate technical was administered dermally in a single application under occlusion at a dose level of 2000 mg/kg bw.
The test material was applied evenly onto gauze dressing, which was applied to the shaved back of each rat. The trunk of the rat was then encircled with a strip of non-irritating tape. After 24 hours the tape was removed and the skin was wiped with a water-dampened tissue to remove excess test material.
The rats were observed frequently on the day of dosing and once daily for 14 days following dosing. They were weighed immediately prior to dosing, 7 days after dosing and at sacrifice at the end of the 14-day observation period.
At the end of the observation period and sacrifice by carbon dioxide asphyxiation, each animal was subjected to a gross post mortem examination.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs noted 30 minutes to 1 day after dosing included piloerection and reduced activity. Scab formation was noted at test sites 2 to 14 days after dosing.
Body weight: Body weight gains with the exception of one animal, which lost weight, were acceptable.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The dermal LD50 of the test material, glyphosate technical, in Sprague-Dawley Rats was estimated to be greater than 2000 mg/kg bw.
Comment by RMS:

In the study report no specific Test Guideline was mentioned, however, the study was considered acceptable. Under the condition of this study, the estimated dermal LD50 > 2000 mg/kg bw is agreed.
Reference:
IIA, 5.2.2/02
Report:
(2009) Glyphosate – Acute Dermal Toxicity Study in Rats.
Data owner: Helm AG Report No.: 12171-08
Date: 2009-03-11 GLP:
not published
ASB2012-11395
Guidelines:
US EPA OPPTS 870.1200 (1998)
Deviations:
Humidity was in the range of 30-86% instead of 30-70%. This
deviation did not affect the study outcome.
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate Tech Grade Mixed 5-Batch
Description:
White powder
Lot/Batch #:
080704-1 thru 5
Purity:
96.71 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rat albino
Strain:
Sprague-Dawley
Source:
Age:
Approx. 8 weeks
Sex:
5 males and 5 females
Weight at dosing:
Males: 299 - 348 g

Females: 189 - 207 g
Acclimation period:
5 days
Diet/Food:
Formulab #5008 (PMI Feeds Inc.), ad libitum
Water:
Tap water, ad libitum
Housing:
Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 %
Air changes: 10 - 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: 2008-12-04 to 2008-12-18
Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 5050 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. Observations for evidence of dermal irritation were made at approximately 60 minutes after removal of wrappings, and on Days 4, 7, 11 and 14. On Day 14 after dosing, animals were euthanized by an overdose of CO2. All study animals were subjected to gross necropsy and all abnormalities were recorded.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 5050 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: Body weight gain was unaffected by the administration of the test substance, with the exception of 2 animals that lost or failed to gain weight during the study..
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The dermal LD50 of the test material (glyphosate) in rats was estimated to be greater than 5050 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate is not to be classified for acute dermal toxicity.
Comment by RMS:
The study is considered acceptable and the dermal LD50 >5000 mg/kg bw is agreed.
Reference:
IIA, 5.2.2/03
Report:
1995, HR-001: Acute dermal toxicity study in rats.
Data owner: Arysta LifeScience Report No.: 94-0154
Date: 1995-03-14, not published ASB2012-11396
Guidelines:
U.S. EPA FIFRA Guideline Subdivision F 81-2

Deviations:
None
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate technical. Code HR-001
Description:
White crystal
Lot/Batch #:
Batch n° 940908-1
Purity:
95.68 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rat
Strain:
Specific pathogen free SD rats (Crj:CD)
Source:
Age:
6 weeks
Sex:
Male and female
Weight at dosing:
248 – 268 g in males and 178 – 198 g in females
Acclimation period:
9 days
Diet/Food:
Certified diet pellet MF (Oriental yeast Co.)
Water:
Tap water, ad libitum
Housing:
Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:
Temperature: 24.3 ± 0.3 °C
Humidity: 53 - 55 %
Air changes: 12 times/hour 12-hour light/dark cycle
Study design and methods

In life dates: 1995-01-31 – 1995-02-23
Animal assignment and treatment:
The test material was prepared as a suspension in deionised water. The suspension was applied on the shave skin of 5 males and 5 females specific pathogen free SD rats (Crj:CD) at a dose level of 2000 mg/kg. Mortality and clinical signs were recorded 1, 3 and 6 hours after administration and at least once daily thereafter until the termination of the 14-day observation period. All animals were weighed on the day of administration and on days 7 and
14 after administration. The surviving animals were euthanized after completion of the observation period (day 14) and examined for gross abnormalities. All animals were subjected to necropsy.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were noted in any animals of 0 and 2000 mg/kg groups.
Body weight: All animals gained their body weights on days 7 and 14 after administration. Necropsy: There was no macroscopic abnormality in any surviving animals at final necropsy after completion of the observation period.
Conclusion by the Notifiers
The oral LD50 of the test material (glyphosate) in rats was estimated to be greater than 2000 mg/kg bw. However, the protocol of this study does not allow to confirm that glyphosate is not to be classified for acute oral toxicity based on the EU and the OECD Globally Harmonized System (GHS) classification criteria.
Comment by RMS:
The study is considered acceptable and the dermal LD50 >2000 mg/kg bw is agreed.
Reference:
IIA, 5.2.2/04
Report:
2009, Glyphosate Technical: Acute Dermal Toxicity Study in Rat.
Data owner:
Excel
Report No.: C22875
Date: 2009-04-02, not published
ASB2012-11397
Guidelines:
OECD 402 (1987)
Commission Regulation (EC) No 440/2008 (2008); method B.3
Deviations:
Weight of female animals was outside of the range specified in the
guideline (200-300 g).
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate Technical
Description:

Solid
Lot/Batch #:
GI-1045
Purity:
96.66 %
Stability of test compound:
No data given in the report. (Stable in purified water for 2 days.)
Expiry date: July 2010
Vehicle and/or positive control:
Purified water
Test animals:
Species:
Rat
Strain:
HanRcc: WIST (SPF)
Source:
Age:
Males: 9 weeks
Females: 11 weeks
Sex:
Males and female
Weight at dosing:
189.8 – 258.3 g
Acclimation period:
7 days
Diet/Food:
Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 61/08 (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland) ad libitum.
Water:
Tap water, ad libitum
Housing:
During acclimatisation in groups of five per sex in Makrolon type-4 cages with standard softwood bedding. Individually in Makrolon type-3 cages with standard softwood bedding (“Lignocel”, Schill AG, 4132 Muttenz / Switzerland) during treatment and observation.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle
Study design and methods:
In life dates: 2009-01-20 to 2009-02-03
Animal assignment and treatment:
Single dose of 2000 mg/kg bw/day of test substance (glyphosate technical) was applied dermally to an area of clipped skin (approx. 10 % of body surface area) of five male and five female young adult rats. The treatment area was covered with a semi-occlusive dressing. Application volume was 4 mL/kg bw. Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dapped off with disposable paper towels. The animals were evaluated for effects on the day of dosing (Day 1) at 30 minutes and at 1, 2, 3 and 5 hours after

application and once daily during days 2-15. Clinical observations, dermal findings, body weights and gross post mortem examinations were recorded.
Results and discussion
Mortality: No deaths occurred during the study.
Clinical observations: No systemic clinical signs were observed during the course of the study.
Body weight: A slight body weight loss (0.3-0.8 %) was observed in two females between acclimatisation and treatment start. The animals recovered until the end of the study. In spite of this body weight loss, the body weights of all animals were considered to be within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at necropsy.
Skin observations: No local dermal signs were observed in any of the treated male animals. After removal of the patch, a very slight erythema was noted in four females on test day 4 and persisted up to test days 6, 11 or 12. Scaling was observed in the same four females on test day 4 and persisted up to test days 10, 11 and 12. Scabs were recorded in two females on test day 9 that persisted to test day 11.
Conclusion by the Notifiers
The dermal LD50 of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical is not to be classified for acute dermal toxicity.
Comment by RMS:
According to the study report the body weight of female rats were in the range of 189.8 and 208.8 (mean 199.6) and therefore only slightly outside the suggested weight range. Under the present conditions the study is considered acceptable and the dermal LD50 > 2000 mg/kg bw is agreed.
Reference: IIA, 5.2.2/05
Report: 2009, Acute Dermal Toxicity Study of Glyphosate TC in CD Rats.
Hamburg, Germany Data owner: Helm AG Report No.: 23912
Date: 2009-06-16, not published ASB2012-11398
Guidelines: EC method B.3. (92/69/EEC), OECD 402 (1987) and US EPA OPPTS 870.1200 (1998)
Deviations: A personnel change in the Head of the Quality Assurance Unit did not affect the validity of the study outcome.
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Glyphosate TC
Identification:
Glyphosate technical grade
Description:
White powder
Lot/Batch #:
20080801
Purity:
98.8 %

Stability of test compound:
2010-08-01
Vehicle and/or positive control:
Aqua ad iniectabilia
Test animals:
Species:
Rat albino
Strain:
CD / Crl:CD(SD)
Source:
Age:
Males: 51 days
Females: 65 days
Sex:
5 males and 5 females
Weight at dosing:
Males: 224 - 234 g
Females: 200 - 217 g
Acclimation period:
5 days
Diet/Food:
ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), ad libitum except for approx. 16 h before administration
Water:
Tap water, ad libitum
Housing:
Individual housing in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:
Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 % Air changes: 12 - 18/hour
12-hour light/dark cycle
Study design and methods
In life dates: 2009-02-04 to 2009-03-04
Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). The administration volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.
Results and discussion
Mortality: There were no mortalities during the study.

Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The dermal LD50 of the test material (glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute dermal toxicity.
Comment by RMS:
The study is considered acceptable and the dermal LD50> 2000 mg/kg bw is agreed.
Reference: IIA, 5.2.2/06
Report: 2010, Acute Dermal Toxicity Study of Glyphosate TC in CD Rats.
Data owner: Helm AG Report No.: 24876
Date: 2010-01-06, not published ASB2012-11399
Guidelines: EC method B.3. (92/69/EEC), OECD 402 (1987) and US EPA OPPTS 870.1200 (1998)
Deviations: There were no deviations from the study plan.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate TC
Identification:
Glyphosate technical grade
Description:
White powder
Lot/Batch #:
2009051501
Purity:
96.4 %
Stability of test compound:
2011-05-15
Vehicle and/or positive control:
Aqua ad iniectabilia
Test animals:
Species:
Rat albino
Strain:
CD / Crl:CD(SD)
Source:
Age:
Males: approx. 7 weeks Females: approx. 9 weeks
Sex:
5 males and 5 females
Weight at dosing:
Males: 233 - 249 g

Females: 211 - 229 g
Acclimation period:
5 days
Diet/Food:
ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), ad libitum except for approx. 16 h before administration
Water:
Tap water, ad libitum
Housing:
Individual housing in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:
Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 % 12-hour light/dark cycle
Study design and methods
In life dates: 2009-10-15 to 2009-11-12
Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). The administration volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The dermal LD50 of the test material (glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, Glyphosate TC is not to be classified for acute dermal toxicity.
Comment by RMS:
The study is considered acceptable and the dermal LD50 >2000 mg/kg bw is agreed.
Reference:
IIA, 5.2.2/07
Report:
2010,
Acute Dermal Toxicity Study of Glyphosate TC in CD Rats.
Data owner: Helm AG Report No.: 24604
Date: 2010-02-19, not published ASB2012-11400

Guidelines:
EC method B.3. (92/69/EEC), OECD 402 (1987) and US EPA
OPPTS 870.1200 (1998)
Deviations:
There were no deviations from the study plan.
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Glyphosate TC
Identification:
Glyphosate technical grade
Description:
White powder
Lot/Batch #:
20090506
Purity:
97.3 %
Stability of test compound:
May 2011
Vehicle and/or positive control:
Aqua ad iniectabilia
Test animals:
Species:
Rat albino
Strain:
CD / Crl:CD(SD)
Source:
Age:
Males: approx. 7 weeks Females: approx. 9 weeks
Sex:
5 males and 5 females
Weight at dosing:
Males: 278 - 292 g
Females: 202 - 225 g
Acclimation period:
5 days
Diet/Food:
ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), ad libitum except for approx. 16 h before administration
Water:
Tap water, ad libitum
Housing:
Individual housing in MAKROLON cages (type III plus) with granulated textured wood as bedding

material.
Environmental conditions:
Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 % 12-hour light/dark cycle
Study design and methods
In life dates: 2009-10-26 to 2009-11-12
Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). The administration volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The dermal LD50 of the test material (glyphosate TC) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute dermal toxicity.
Comment by RMS:
The study is considered acceptable and the dermal LD50 >2000 mg/kg bw is agreed.
Reference: IIA, 5.2.2/08
Report: 2005 Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats – Limit Test.
Data owner: Helm AG Report No.: 15275
Date: 2005-04-04, not published ASB2012-11401
Guidelines: US EPA OPPTS 870.1200 (1998)
OECD 402 (1987).
Deviations: There were no deviations from the study plan.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate Acid Technical
Identification:
Glyphosate Acid Technical
Description:
White crystalline powder
Lot/Batch #:

040205
Purity:
97.23 %
Stability of test compound:
Test substance was expected to be stable for the duration of testing.
Vehicle and/or positive control:
Distilled water
Test animals:
Species:
Rat albino
Strain:
Sprague-Dawley derived
Source:
Age:
8 weeks
Sex:
5 males and 5 females
Weight at dosing:
Males: 231 - 264 g
Females: 193 - 200 g
Acclimation period:
8 days
Diet/Food:
Purina Rodent Chow #5012, ad libitum
Water:
Filtered tap water, ad libitum
Housing:
Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:
Temperature: 19-23 °C
12-hour light/dark cycle
Study design and methods
In life dates: 2004-05-05 to 2004-05-19
Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 5000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanized by an overdose of CO2. All study animals were subjected to gross necropsy and all abnormalities were recorded.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 5000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The dermal LD50 of the test material (glyphosate acid technical) in rats was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate acid technical is not to be classified for acute dermal toxicity.
Comment by RMS:
The study is considered acceptable and the dermal LD50 > 5000 mg/kg bw is agreed.
Reference: IIA, 5.2.2/09
Report: 2008, Acute Dermal Toxicity Study in Wistar Hannover Rats for Glyphosate Technical.
Data owner: Helm AG
Report No.: RF-3996.310.456.07
Date: 2008-07-04, not published ASB2012-11402
Guidelines: OECD guideline 402 (1987)
Deviations: 1. The experimental phase initiation and experimental phase conclusion dates were updated.
2. One female rat weighed less than 200 g on the day of test item application.
These deviations did not affect the study outcome.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate Technical
Identification:
Glyphosate Technical
Description:
Solid
Lot/Batch #:
20070606
Purity:
98.05 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rat albino
Strain:
Wistar Hannover
Source:
Age:
9 - 11 weeks
Sex:
5 males and 5 females

Weight at dosing:
Males: 266 - 298 g
Females: 199 - 213 g
Acclimation period:
7 days
Diet/Food:
Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), ad libitum
Water:
Filtered drinking water, ad libitum
Housing:
Individual housing in polypropylene rodents cages with wire mesh tops and bedding material.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 % Air changes: min. 10/hour 12-hour light/dark cycle
Study design and methods
In life dates: 2007-09-11 to 2008-06-11 Animal assignment and treatment:
One dose level group of 5 male and 5 female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once for 24 hours on the shaved intact dorsal skin of the rats (ca. 1/10 of body surface). Observations for mortality and clinical/behavioural signs of toxicity were made once within the first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were determined before the application of the test item (Day 0) and on days 7 and 14. On Day 14 after dosing, each animal was euthanized by an overdose of CO2. All study animals were subjected to gross necropsy and all abnormalities were recorded.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single dermal administration of 2000 mg/kg bw to 5 male and 5 female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.
Body weight: All animals gained the expected body weight, except for two females in the second week of the observation.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The dermal LD50 of the test material (glyphosate technical) in rats was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical is not to be classified for acute dermal toxicity.
Comment by RMS:
The study is considered acceptable and the dermal LD50 >2000 mg/kg bw is agreed.
Reference: IIA, 5.2.2/10
Report: 2007, GLYPHOSATE TECHNICAL (NUP05068):
Acute dermal toxicity study in rats
Data owner: Nufarm RCC Study No.: B02283
Date: 2007-03-01, unpublished ASB2012-11403
Guidelines: Japanese guideline Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute Dermal Toxicity Studies, Guideline 2-1-2. Notification 12 NohSan No. 8147, as partly revised in 16-Shouan- 9260, on 16 March 2005.

English translation by AGIS on 17 Oct 2005
Directive 92/69/EEC, B.3. "Acute Toxicity-Dermal", July 31, 1992. OECD Guidelines for Testing of Chemicals, Section 4, Number 402 "Acute Dermal Toxicity",
adopted February 24,1987.
Deviations:
None
GLP:

yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate Technical (NUP 05068)
Description:
Solid
Lot/Batch #:
200609062
Purity:
95.1 %
Stability of test compound:
Stable under storage conditions.
Vehicle and/or positive control:
Polyethylene glycol 300 (PEG 300)
Test animals:
Species:
Rat
Strain:
HanRcc:WIST (SPF)
Source:
Age:
Males: 8 weeks
Females: 11 weeks
Sex:
Male / Female
Weight at dosing:
194.8-254.3g
Acclimation period:
6 days
Diet/Food:
Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH- 4303 Kaiseraugst/ Switzerland) ad libitum
Water:
Tap water, ad libitum
Housing:

During acclimatization in groups of five per sex in Makrolon type-4 cages with standard softwood bedding. Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132
uttenz) during treatment and observation.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30-70 % Air changes: 10 - 15/hour 12-hour light/dark cycle
Study design and methods
In life dates: 12-DEC-2006 to 02-JAN-2007
Animal assignment and treatment:
One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface. Only those animals without injury or irritation on the skin were used in the test. On test day 1, the test item was applied at a dose of 2000 mg/kg body weight evenly on the intact skin with a syringe and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an
elastic adhesive bandage. Application volume/kg body weight: 6 mL Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the reaction sites were assessed. The fur of all animals was shaved on test days 4 and 9 just after the assessment of the reaction to facilitate the skin reading for the next day. Rationale: Dermal administration was used as this is one possible route of human exposure during manufacture, handling and use of the test item.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No systemic or local signs of toxicity were observed during the study period.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The median lethal dose of glyphosate technical (NUP 05068) after single dermal administration to rats of both sexes, observed over a period of 14 days is: Dermal LD50 (rat) > 2000 mg/kg body weight.
Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for this end point.
Comment by RMS:
The study is considered acceptable and the dermal LD50 >2000 mg/kg bw is agreed.
Reference: IIA, 5.2.2/11
Report: 1996, Glyphosate Acid: Acute Dermal Toxicity Study In The Rat.
Data owner: Syngenta Report No.: /P/4664
Date: 1996-08-23, not published TOX2000-1983
Guidelines: OECD guideline 402 (1987), OPPTS 870.1200 (1998): 92/69/EEC
B.3 (1992)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:

Glyphosate acid
Description:
Technical, white solid
Lot/Batch number:
P24
Purity:
95.6 % w/wi
CAS#:
Not reported
Stability of test compound:
The test substance was used within the expiry date
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rat
Strain:
Alpk:APfSD (Wistar-derived)
Age/weight at dosing:
Young adult / 250-274 g (males), 203-216 g (females)
Source:
Housing:
Individually in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the
study.
Acclimatisation period:
At least 6 days
Diet:
Diet (PCD), supplied by Special Diet Services Limited,
Witham, Essex, UK ad libitum.
Water:
Mains water ad libitum
Environmental conditions:
Temperature: 21±2 °C
Humidity: 40-70 %
Air changes: Approximately 25-30/hour Photoperiod: 12 hours dark / 12 hours light
Study design and methods
In-life dates: Start: 16 March 1995 End: 30 March 1995
Animal assignment and treatment: In an acute dermal toxicity study, a group of five male and five female, young adult Alpk:APfSD (Wistar-derived) rats were given a single dermal application of 2000 mg/kg bw of glyphosate acid.
Sixteen to thirty-two hours before application, the hair was removed by clipping from an area on the dorso-lumbar region of each rat (approximately 10 cm x 5 cm). The appropriate amount of test substance was moistened to a dry paste with 0.6-0.8 mL of deionised water. Approximately half the application site was covered by test substance (equivalent to 20.0-
21.9 mg/cm2 for males and 16.2-17.3 mg/cm2 for females). The application site was covered with a

4-ply gauze patch (approximately 7 cm x 7 cm) and kept in place for 24 hours using an occlusive dressing. The gauze patch was covered by a piece of plastic film (7 cm x 7 cm), held in place using adhesive bandage (25 cm x 7.5 cm) secured by two pieces of PVC tape.
At the end of the 24 hour contact period, the dressings were carefully removed and the skin cleansed of any residual test substance using clean warm water.
Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic toxicity once between one and four hours of dosing. Subsequent observations were made daily, up to day 15.
The animals were weighed immediately before dosing (day 1) and on days 3, 8, 8 and 15. All animals were subjected to an examination post mortem. This involved an external observation and a careful examination of all thoracic and abdominal viscera.
Statistics: The dermal LD50 was estimated (limit test, no mortalities).
Results and discussion
Mortality: There were no mortalities.
Clinical observations: There were no significant signs of systemic toxicity (only urinary incontinence due to bandaging). There were practically no signs of skin irritation. One male showed slight erythema on days 2 and 3 and one female had small scabs from day 3 to 8.
Body weight: Two males and three females lost weight initially, but all had exceeded their initial weight by day 5, and except for one female, continued to gain weight throughout the remainder of the study. One female lost weight slightly from day 5.
Necropsy: Red mottled lungs were seen in one female. This is a common spontaneous finding in rats of this age and strain and is considered not to be treatment-related.
Conclusion by the Notifiers:
The acute dermal LD50 of glyphosate acid is in excess of 2000 mg/kg to male and female rats (limit test, no mortality).
Comment by RMS:
The study is considered acceptable and the dermal LD50 > 2000 mg/kg bw is agreed.
Reference: IIA, 5.2.2/12
Report: 2007, Glyphosate technical material: Acute dermal toxicity study in rats.
Data owner: Syngenta Report No.: B02766
Date: 2007-02-08, not published ASB2012-11404
Guidelines: OECD 402 (1987), JMAFF 12 NohSan 8147 (2000), US EPA Health
Effects Test Guidelines, OPPTS 870.1200 (1998)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate technical material
Description:
Technical, white powder
Lot/Batch number:
0507
Purity:
96.1 % w/w glyphosate acid
CAS#:

Not reported
Stability of test compound:
Re-certification date August 2008. Stable under storage conditions (room temperature range 20±5°C, protected from
light and humidity).
Vehicle and/or positive control:
The test item was applied moistened with purified water before application.
Test animals:
Species:
Rat
Strain:
HanRcc:WIST (SPF)
Age/weight at dosing:
8 weeks (males), 11 weeks (females) / 247.0-222.7 g (males),-
191.3-204.2 g (females)
Source:
Housing:
Individually in Makrolon type-3 cages with standard softwood
bedding
Acclimatisation period:
7 days
Diet:
Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland)
ad libitum
Water:
Community tap water ad libitum
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30-70 %
Air changes: 10-15 air changes per hour Photoperiod: 12 hours light / 12 hours dark
Study design and methods
In-life dates: Start: 07 December 2006 End: 22 December 2006
Animal assignment and treatment: In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female HanRcc:WIST (SPF) rats were treated with glyphosate technical material (96.1 % w/w glyphosate technical) at 5000 mg/kg by dermal application. The test item was moistened with purified water before application. The application period was 24 hours.
One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface. Only those animals without injury or irritation on the skin were used in the test.
A single animal of each sex was treated first. As no deaths and neither severe local effects nor severe systemic symptoms were observed after the 24-hour exposure, the test was completed using the four remaining male and female animals for an exposure period of 24 hours.
On test day 1, the mass of the dose (5000 mg/kg) was calculated for each animal on the basis of its body weight. The appropriate amount of test item was weighed out on a suitable precision balance

into a plastic weighing boat and moistened to a dry paste with a minimum amount of purified water (0.5 to 0.6 mL) to allow good skin contact. The dry paste was applied evenly on the intact skin of the clipped area and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with tape. The area of skin covered by the test item was approximately 8 cm2 for the males and females.
Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the reaction sites were assessed.
The fur of all males and females was shaved, on test day 6 (female no. 2), on test days 5 and 9 (male no. 1), on test days 4 and 8 (males nos. 3 - 6 and females nos. 7 - 10) just after the assessment of the reaction to facilitate the skin reading for the next day.
The animals were checked daily for mortality/viability during the acclimatization period, at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.
Clinical observations were recorded daily during the acclimatisation period, at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on test day 1 and once daily during days 2-15. The animals were examined daily for local signs at the application site.
Body weights were recorded on test days 1 (prior to administration), 8 and 15.
All animals were killed at the end of the observation period by Carbon dioxide asphyxiation and discarded after macroscopic examinations were performed. No organs or tissues were retained.
Statistics: Not applicable (limit test, no mortalities).
Results and discussion
Mortality: There were no mortalities.
Clinical observations: No systemic signs or local signs of irritation were noted during the course of the study.
Body weight: The body weights of the animals were within the range commonly recorded for this strain and age.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy.
Conclusion by the Notifiers:
The acute dermal LD50 of glyphosate technical material after a single dermal administration to male and female HanRcc:WIST (SPF) rats, observed over a period of 14 days was greater than 5000 mg/kg body weight (limit test, no mortalities).
Comment by RMS:
The study is considered acceptable and the dermal LD50 is estimated greater than 5000 mg/kg bw
Reference: IIA, 5.2.2/13
Report: 2011, Glyphosate technical: Acute dermal toxicity study in rats; Final report amendment 1.
Data owner: Syngenta Report No.: 10/218-002P
Date: 2011-04-13, not published ASB2012-11405
Guidelines: OECD 402 (1987): OPPTS 870.1200 (1998): EC 440/2008 (2008)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test Material:
Glyphosate technical
Description:

Technical, dry white powder
Lot/Batch number:
569753 (BX20070911)
Purity:
96.3 % w/w Glyphosate technical
CAS#:
Not reported
Stability of test compound:
Stable under storage conditions (room temperature range
<30 °C), recertification date end August 2011
Vehicle and/or positive control:
None (tested as supplied)
Test animals:
Species:
Rat
Strain:
RjHan:(WI) Wistar
Age/weight at dosing:
Young adult / 220-259 g
Source:
Housing:
Individually in Type II. polypropylene/polycarbonate cages
with Lignocel Bedding for Laboratory Animals
Acclimatisation period:
6 days
Diet:
ssniff® SM R/M-Z+H "Autoclavable complete feed for rats
and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest
Germany ad libitum
Water:
Tap water ad libitum
Environmental conditions:
Temperature: 20.7-24.0 °C
Humidity: 39-65 %
Air changes: 15-20 air changes per hour Photoperiod: 12 hours light / 12 hours dark
Study design and methods
In-life dates: Start: 06 October 2010 End: 20 October 2010
Animal assignment and treatment: In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female RjHan:(WI) Wistar rats were treated with a single administration of glyphosate technical (96.3 % w/w glyphosate technical) at 5000 mg/kg by dermal application. The test item was applied as supplied. The application period was 24 hours, followed by a 14-day observation period.
The backs of the animals were shaved (approximately 10 % area of the total body surface) approximately 24 hours prior to the treatment. Only those animals without injury or irritation on the skin were used in the test. On test day 0, the test item was applied at a single dose of 5000 mg/kg body weight applied uniformly over the skin and remained on the skin throughout a 24- hour

exposure period. The test item was moistened with water to ensure good contact with the skin. Sterile gauze pads were placed on the skin of rats at the site of application. These gauze pads were kept in contact with the skin by a patch with adhesive hypoallergenic plaster. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24 hours. At the end of the exposure period, residual test item was removed, using body temperature water.
A clinical examination was performed on the day of treatment, at 1 and 5 hours after the application of the test item, and once each day for 14 days thereafter.
Observations included the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behaviour pattern. Particular attention was directed to the observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.
The body weight of all animals was recorded on Day 0 (beginning of the experiment) and on Days 7 and 14.
All animals were anaesthetised with Euthasol®40 % and exsanguinated. After examination of the external appearance, the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. Any gross macroscopic findings were recorded.
Statistics: Not applicable (limit test, no mortalities).
Results and discussion
Mortality: There were no mortalities.
Clinical observations: There were no clinical signs noted in any animals throughout the study.
No treatment related skin irritation was observed in any animal throughout the study.
Body weight: There were no effects on body weight and body weight gain during the observation period.
Necropsy: No macroscopic findings were recorded at the scheduled necropsy.
Conclusion by the Notifiers
The acute dermal LD50 of glyphosate technical after a single dermal administration to male and female RjHan:WI Wistar rats, observed over a period of 14 days was greater than 5000 mg/kg body weight (limit test, no mortalities).
Comment by RMS:
The study is considered acceptable and the dermal LD50 > 5000 mg/kg bw is agreed.

Comment by GTF on the first draft of the RAR (July 2013):
The Rat LD50oral and Rat LD50dermal are listed as >2000 mg/kg bw whereas the data presented concludes LD50-values above 5000 mg/kg. This would also be consistent with the values presented in summary Table B.6.13-2 in Volume 3 B6.
RMS comment (August 2013):
To conclude on the LD50 value >2000 mg/kg bw seems to be more appropriate, because not all different batches were tested up to 5000 mg/kg bw. In any case glyphosate is considered not to be classified as acute oral or dermal toxic according to GHS categories.
B.6.2.3 Acute inhalation toxicity
For the previous EU evaluation, a multitude of acute inhalation toxicity studies in rats were provided using glyphosate acid and its IPA salt. For the current re-evaluation, additonally eleven inhalation studies with glyphosate acid and two with glyphosate salts (one for potassium salt) were submitted.
Table B.6.2-8: Summary of acute inhalation toxicity studies with glyphosate acid
Reference (Data owner)
Species Strain

Number of animals / Dose level
(mg/L)
Purit y (%)
Vehicle
LC50
(mg/L air)
Main effects
Studies from the 2001 evaluation
Annex B- 5.2.3.1,
Glyphosate Monograph
1995
(Herbex) TOX9500247
Rat, Sprague Dawley
5/sex/5.35
95
Compres sed air;
4 h nose- only
> 5.35
Wet fur, hunched posture, piloerection, incidents of decreased respiratory rate, ptosis, brown stained fur (head)
Reference (Data owner)
Species Strain
Number of animals / Dose level
(mg/L)
Purit y (%)
Vehicle
LC50
(mg/L air)
Main effects
Annex B- 5.2.3.1,
Glyphosate Monograph
1994
(Alkaloida) TOX9650144
Rat,
5/sex/0
97.2
Watery
> 2.876
Trachea: lymphoid
Wistar
5/sex/1.138
aerosol;
cell infiltration,
5/sex/2.876
4 h
mucous

exposure
lung: congestion,
, route
haemorrhages,
not
oedema
stated
liver: mononuclear
cell infiltrations,
congestion
kidney: congestion,
nephrocalcinosis
Annex B-
Rat,
5/sex/4.98
98.6
Dust
> 4.98
No adverse
5.2.3.1,
Sprague
aerosol;
findings
Glyphosate
Dawley
4 h snout
Monograph
only
,
1989 (CHE)
TOX9552329
Studies not reviewed in the 2001 evaluation
IIA, 5.2.3/01
2010 (HAG) ASB2012-
11406
Rat, CD
5/sex/5.18
97.3
4 h nose only (MMAD
: 4.63
µm)
> 5.18
(limit test)
Slight tremor, slight dyspnoea
IIA 5.2.3/02
1995 (ALS) ASB2012- 11407

Rat, Fischer F344
5/sex/5.48
97.56
Dust,4 h whole body (MMAD
: 4.8 µm)
> 5.48
Wet and soiled fur (periocular and nasorostral)
IIA 5.2.3/03
2009 (EXC) ASB2012-
11408
Rat
5/sex/5.04
96.66
Dust, 4 h, nose- only, (MMAD
5.25 µm)
> 5.04
Increased respiratory rate, hunched posture, pilo-erection, wet
fur
IIA 5.2.3/04
2009 (HAG)
ASB2012- 11409
Rat, CD
5/sex/5.12 (dust)
98.8
4h (MMAD
: 6.62µm)
> 5.12
(limit test)
Slight dyspnoea and ataxia during exposure
IIA 5.2.3/05
2010 (HAG) ASB2012- 11410
Rat, CD
5/sex/5.02
96.4
4h (MMAD
: 4.2µm)
> 5.02
Slight dyspnoea, slight ataxia and slight tremor during exposure until 3 h after
exposure
Studies not reviewed in the 2001 evaluation
IIA 5.2.3/06
2009 (HAG) ASB2012- 11411
Rat, Sprague- Dawley
5/sex/2.24
96.4
4 h (MMAD

: 2.6 µm)
>2.24
(limit test)
No findings
IIA 5.2.3/07
2005 (HAG) ASB2012-
11412
Rat,
Sprague- Dawley
5/sex/2.04
97.23
4 h
(MMAD
: 2.5 µm)
> 2.04
(limit test)
No findings
Reference (Data owner)
Species Strain
Number of animals / Dose level
(mg/L)
Purit y (%)
Vehicle
LC50
(mg/L air)
Main effects
IIA 5.2.3/08
2008
(HAG)
ASB2012- 11413
Rat, Wistar Hannove r
5/sex/5.21
98.05
4 h (MMAD
: 18.2- 19.9µm)
> 5.21
(study not accepta- ble)
Wheeze and dyspnoea
IIA 5.2.3/09
2007 (NUF) ASB2012-
11414
Rat,
albino
5/sex/3.252
(highest techn. attain.)
95.1

4 h
(MMAD
: 2.95 –
3.05 µm)
> 3.252
Salivation in
males, breathing effects in both sexes, body weight
loss
IIA 5.2.3/10
1996 (SYN) TOX2000- 1984
Rat
5/sex/4.43
5/sex/2.47
95.6
4 h,
nose- only, (MMAD
: 2.91
and 3.41
µm)
> 4.43
Mortality: 2♂ &
2♀ at 4.43 mg/L. Irregular breathing, splayed gait, shaking & reduced righting reflex
IIA 5.2.11/
2011 (SYN) ASB2012- 11415
Rat
5/sex/5.04
96.9
4 h nose-
only (MMAD
: 3.65
µm)
> 5.04
Mortality: 1♂ on
day 4. Laboured and noisy respiration, respiratory rate increase, gasping respiration, sneezing, decreased activity and thin body appearance observed until
day 3.

Table B.6.2-9: Summary of acute inhalation toxicity studies with glyphosate salts

Reference (Data owner)
Species Strain
Number of animals / Dose level
(mg/L)
Purit y (%)
Vehicle
LC50
(mg/L air)
Main effects

Studies from the 2001 evaluation
Annex B- 5.2.3.2,
Glyphosate Monograph
1994 (MON/CHE) TOX9552331
Rat, Sprague Dawley
5/sex/4.24 (max. attainable conc.)
IPA 62
aerosol; 4 h nose- only
> 4.24
Lung: dark areas or multiple dark foci in 6 of 10 rats
Annex B-
5.2.3.2,
Glyphosate Monograph
Rat,
Wistar
5/sex/4.1
5/sex/4.42 5/sex/6.49
IPA
62%
in water
Nebu-
lised; 4 h nose-only
> 6.49
Nose bleeding,
ruffled fur
,
1989 (I. Pi.
Ci.) TOX9551626
Reference (Data owner)
Species Strain
Number of animals / Dose level
(mg/L)
Purit y (%)
Vehicle
LC50
(mg/L air)
Main effects
Annex B- 5.2.3.2,
Glyphosate Monograph
1988 (MON / CHE) TOX9552332
Rat, Sprague Dawley
5/sex/1.9 (max. attainable conc.)
Amm onium salt 85.5
Aerosol / vapor; 4 h whole body
> 1.9
Hyperactivity, perinasal encrustion

Annex B- 5.2.3.2,
Glyphosate Monograph
1987 (MON / CHE) TOX9552330
Rat, Sprague- Dawley
5/sex/1.3 (max. attainable conc.)
IPA 53.8
Aerosol / vapor; 4 h whole body
> 1.3
Mortality (1♀); yellow/brown nasal discharge, local and/or generalised hairloss, slight decreased body weight
Studies not reviewed in the 2001 evaluation
IIA 5.2.3/12
1999 (NUF) ASB2012- 11416
Rat, Sprague Dawley
Isopropylamine glyphosate 5/sex/2.08
IPA 62
air / 4 h nose-only (MMAD
2.6 µm)
> 2.08
During exposure: ocular and nasal discharge, hunched posture and hypoactivity. After exposure no findings
IIA 5.2.3/13
2004 (MON) ASB2012- 11417
Rat.
Sprague- Dawley
K-salt of
glyphosate 5/sex/2.21 5/sex/5.27
K-salt
57.8% (= 47.2%
glyph o- sate)
Aerosol /
4 h nose- only (MMADs:
2.9 µm,
3.8 µm)
> 5.27
2.21 mg/L:
congested brea- thing, dark material around eyes and nose, few faeces
5.27 mg/L:
congested brea- thing,
Tier II summaries are presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation please refer to the monograph.
Reference: IIA, 5.2.3/01
Report: 2010, Acute Inhalation Toxicity Study of Glyphosate TC In Rats
Data owner: Helm AG Report No.: 24603
Date: 2010-06-03, not published ASB2012-11406

Guidelines: EC method B.2, OECD 403, EPA Health Effects Test Guidelines, OPPTS 870.1300
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate TC
Description:
White powder
Lot/Batch #:
20090506
Purity:
97.3 %
Stability of test compound:
At room temperature in the dark stable until May 2011.
Vehicle and/or positive control:
none
Test animals:
Species:
Rat
Strain:
CD/Crl:CD (SD)
Source:
Age:
approx. 7 - 9 weeks
Sex:
Males and females
Weight at dosing:
♂ 234 - 270 g; ♀ 208 - 244 g
Acclimation period:
At least 5 days
Diet/Food:
ssniff RIM-H V1 534 (ssniff Spezialdiäten GmbH, Soest, Germany), ad libitum (except 16 h before exposure)
Water:
tap water, ad libitum
Housing:
In groups of 2-3 animals per cage in Makrolon type III plus cages with granulated textured wood bedding.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 55 ± 15 % Air changes: no data 12 hours light/dark cycle
Study design and methods
In life dates: 2010-02-02 to 2010-02-16
Test atmosphere generation:

A dust atmosphere was produced from the test material using a rotating brush dust generator and compressed air.
Exposure chamber conditions:
The cylindrical exposure chamber had a volume of approximately 40 L. The actual dust concentration was measured four times gravimetrically with an air sample filter (Minisart SM 17598; 0.45 pm) and pump (Vacuubrand, MZ 2C, Vacuubrand, Germany) controlled by a rotameter. Dust samples were taken once every hour during the exposure. For that purpose, a probe was placed close to the animals' noses in the inhalation chamber and air was sucked through the air sample filter at a constant flow of air of 5 L/min for 1 minute. The filters were weighed before and after sampling on an analytical balance (accuracy 0.1 mg).
Chamber airflow rates ranged from 800 to 900 L/h, providing ≥ 12 air changes per hour.
Particle size distribution:
A Malvern Spraytec Lasersystem (Malvern Instruments, Germany) was employed for the determination of the particle size distribution of the particle diameter (volume) in the exposure air. The particle size distribution of the test atmospheres was measured using a cascade impactor two times during the exposure period. The results were as follows:
Table B.6.2-10: Details of test atmosphere
Mean achieved actual concentration (HPLC)
Actual concentration (gravimetric method)
MMAD
GSD
Respirable amount particle size ≤ 4 µm
(mg/L air)
(mg/L air)
(µm)
(mg/L air)
(%)
5.18
5.05
4.633
3.02
1.08
20.8
MMAD = mean mass median aerodynamic diameter GSD = geometric standard deviation
The generated dust had a mass median aerodynamic diameter (MMAD) of 4.633 µm as determined with a cascade impactor. The Geometric Standard Deviation (GSD) of the MMAD was calculated as 3.02. No smaller MMAD and GSD could be obtained with the test item supplied.
Animal assignment and treatment:
A group of five fasted rats per sex received the test material at a dose level of 5.18 mg/L using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure. Observations for mortality and clinical/behavioural signs of toxicity were made at least once per day for 14 days. Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanised and all study animals were subjected to gross necropsy.
Results and discussion
Mortality: No deaths occurred.
Clinical observations: Clinical signs of toxicity included slight tremor and slight dyspnoea immediately

until 3 hours after end of exposure.
Body weight: All animals gained the expected body weight. Necropsy: No pathological findings were noted at necropsy.
Conclusion by the Notifiers
The acute inhalation LC50 for the test substance glyphosate TC was calculated to be greater than 5.18 mg/L. According to EU and OECD Globally Harmonised System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity.
Comment by RMS:
The MMAD and GSD are greater than recommended in OECD 403, however the explanation given by the authors is acceptable. Under the present conditions the study is considered acceptable and the acute inhalation LC50 >5 mg/L air/4 hours is agreed.
Reference:
IIA, 5.2.3/02
Report:
1995,
HR-001: Acute inhalation toxicity study in rats.
Data owner: Arysta LifeScience Report No.: 94-0155, not published
ASB2012-11407
Guidelines:
U.S. EPA FIFRA Guideline Subdivision F
Deviations:
None
GLP:
yes
Acceptability:
Please see comment RMS
Materials and methods
Test material:
Identification:
Glyphosate TC
Description:
Whitish crystals
Lot/Batch #:
T-941209
Purity:
97.56 %
Stability of test compound:
Not mentioned in the report.
Vehicle and/or positive control:
None
Test animals:
Species:
Rat
Strain:
F344/DuCrj
Source:

Age:
8 weeks
Sex:
Males and females
Weight at dosing:
♂ 176 - 187 g; ♀ 138 - 144 g
Acclimation period:
8 days
Diet/Food:
ssniff RIM-H V1 534 (ssniff Spezialdiäten GmbH, Soest, Germany), ad libitum (except 16 h before exposure)
Water:
tap water, ad libitum
Housing:
By group of 5 animals of the same sex in stainless steel wire cages during pre- and post-exposure periods.
Individually in stainless steel wire cages during exposure.
Environmental conditions:
Temperature: 22 ± 2 °C
Humidity: 55 ± 1 %
Air changes: 10/hour 12 hours light/dark cycle
Study design and methods
In life dates: 1995-03-28 to 1995-04-20
Test atmosphere generation:
The dust was generated by a turn-table type dust feeder with compressed air from air compressor. The compressed air was supplied to the dust feeder through an air filter. The air was introduced into the chamber as diluting air after filtering it through a HEPA filter.
Exposure chamber conditions:
The nominal atmospheric concentration of HR-001 was calculating by dividing the total amount of the test substance supplied to the duct feeder during the 4-hour exposure by the total air volume delivered during the exposure.
The actual atmospheric concentration was measured gravimetrically.
Particle size distribution:
The results for the air samples taken for the determination of particle size distribution are given in Table B.6.2-11.
Table B.6.2-11: Particule size distribution
Exposure group
Time of sampling
Analytical concentration
Particule size
mg/L
(min)
(mg/L)
MMAD2 (µm)
g3
5.48

60
6.54
5.0
1.6
120
4.80
180
5.11
4.6
1.8
Mean
S.D.4
5.48
0.93
4.8
0.3
1.7
0.1
MMAD : Mass median aerodynamic diameter
Mg: geometric standard deviation SD: Standard deviation
The results showed that particles size of HR-001 expressed as the mean value of NMAD during the exposure was 4.8 µm (Tg = 1.7). Thus more than 91 % of the test substance dust consisted of particles present consisted of inhalable particles
Animal assignment and treatment:
Groups of 5 male and 5 female specific pathogen free Fisher rats (F344/DuCrj) were exposed (whole-body) continuously for 4 hours to test substance mist containing concentrations of HR-001 at 5.48 mg/l. The flow rate was stable at approximately 100 l/min. Mortality and signs of reaction to treatment were recorded during a subsequent 14-day observation period. All animals were observed for clinical signs at 2 hours after the initiation of exposure, immediately and at 2 hours after the termination of exposure. In addition, animals were observed for lethality at 4 hours after the termination of exposure. All animals were weighed shortly before the exposure and on days 7 and 14. The surviving animals were euthanized on the following day (day 15). All animals were subjected to necropsy.
Results and discussion
Mortality: There were no deaths in either sex at the tested concentration 5.48 mg/L.
Clinical observations: No notable serious changes were observed as clinical signs. Wetted and soiled fur in the periocular and nasorostral regions were not considered to be particularly caused by HR-001 because the changes were slight in degree and are frequently observed in tha acute inhalation toxicity study.
Body weight: All animals gained weights, reflecting their good healthy conditions. Necropsy: No abnormalities were observed in any animal of either sex at necropsy.
Conclusion by the Notifiers
The acute inhalation LC50 for the test substance glyphosate HR-001 was calculated to be greater than 5.48 mg/L. According to EU and OECD Globally Harmonised System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity
2 Mass median aerodynamic diameter

3 geometric standard deviation
4 Standard deviation

Comment by RMS:
The study is considered acceptable and the acute inhalation LC50 > 5 mg/L air/4 hours is agreed.
Reference: IIA, 5.2.3/03
Report: 2009, Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat
Data owner: Excel Report No.: 2743/0001
Date: 2009-06-22, not published ASB2012-11408
Guidelines: OECD 403 (1981)
Commission Regulation (EC) No 440/2008 (2008), method B.2 (2008)
Deviations: Particle size diameter larger than required by the test guidelines.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate Tech
Description:
White powder
Lot/Batch #:
GI-1045
Purity:
96.66 %
Stability of test compound:
Expiration in July 2010.
Vehicle and/or positive control:
Not relevant
Test animals:
Species:
Rat
Strain:
HsdRccHan™ : WIST
Source:
Age:
Approx. 8 - 12 weeks
Sex:
Male and female
Weight at dosing:
178 - 350 g
Acclimation period:
5 days
Diet/Food:
With the exception of the exposure period, free access to food (Harlan 2014 Rodent Diet, Harlan UK Limited, Oxon, UK) was allowed throughout the study.
Water:

With the exception of the exposure period, free access to
drinking water was allowed throughout the study.
Housing:
Housed in groups of five by sex in solid-floor polupropylene cages with stainless steel lids, furnished with softwood flakes (Datesand Ltd., Cheshire, UK) and provided with environmental enrichment items: wooden chew blocks and cardboard “fun tunnels” (Datesand Ltd., Cheshire, UK).
Environmental conditions:
Temperature: 19 – 25 °C
Humidity: 30 – 70 %
Air changes: At least 15/hour 12-hour light/dark cycle
Study design and methods
In life dates: 2009-05-06 to 2009-05-12
Animal assignment and treatment:
Five male and female rats were exposed to one dose level of an aerosol atmosphere of glyphosate. The single 5 mg/L four hour exposure was “nose only” at a mean actual concentration of 5.04 ± 0.37 mg/L (nominal concentration was 27.3 mg/L).
Operational conditions (flow rate, oxygen levels, temperature, and humidity in the inhalation systems) were checked throughout the exposure period. All animals were observed for clinical signs at hourly intervals during exposure, immediately on removal from the restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for 14 days. Individual body weights were recorded prior to treatment on the day of exposure and on Days 7 and 14. At the end of the fourteen day observation period the animals were killed by intravenous overdose of sodium pentobarbitone. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.
The chamber flow rate was maintained at 45 L/min providing 90 air changes per hour.
Results and discussion
Test atmosphere
The particle size analysis of the atmosphere drawn from the animals’ breathing zone, was as follows:
Table B.6.2-12: Details of test atmosphere
Mean Achieved Atmoshpere Concentration (mg/L)
Mean Mass Median Aerodynamic Diameter
((m)
Inhalable Fraction (%
<4 <m)
Geometric Standard Deviation
5.04
5.25
41.1
3.35
It is noted that the achieved particle size is larger than required by the test guidelines.
During characterisation, changes were made to the generation system (addition of particle sizes separator) and grinding techniques in an attempt to increase the inhalable portion of the test material. However, this reduced the achieved concentration, and therefore, also reduced the actual concentration of particles <4 <m. It was, therefore, preferable to expose the animals to a higher concentration of test material, even though this also increased the mean mass median aerodynamic

diameter, as this resulted in the animals being exposed to the highest possible concentration of particles <4 <m.
Mortality: There were no mortalities during the study.
Clinical observations: Signs of hunched posture and piloerection are commonly seen in animals for short periods on removal from the chamber following 4-hour inhalation studies. Wet fur is commonly recorded both during and for a short period after exposure. These observations are considered to be associated with the restraint procedure and, in isolation, are not indicative of toxicity.
In addition to the observations considered to be due to the restraint procedure, increased respiratory rate was noted in all animals during exposure, on removal from the chamber and one hour post-exposure.
Body weight: Normal bodyweight development was noted during the course of the study. Necropsy: No macroscopic abnormalities were detected at necropsy.
Conclusion by the Notifiers
The inhalation LC50 (4 hours) of the test material (glyphosate tech) in rats was estimated to be greater than 5.04 mg/L. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate tech is not to be classified for acute inhalation toxicity.
Comment by RMS:
Under the present conditions the study is considered acceptable and the acute inhalation LC50 >5 mg/L air/4 hours is agreed.
Reference: IIA, 5.2.3/04
Report: 2009, Acute Inhalation Toxicity Study of Glyphosate TC in Rats.
Data owner: Helm AG Report No.: 23911
Date: 2009-11-09
not published ASB2012-11409
Guidelines: EC method B.2. (92/69/EEC), OECD 403 and OPPTS 870.1300.
Deviations: There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate TC
Identification:
Glyphosate technical grade
Description:
White powder
Lot/Batch #:
20080801
Purity:
98.8 %
Stability of test compound:
2010-08-01
Test animals:
Species:
Rat albino
Strain / Stock:

CD / Crl:CD(SD)
Source:
Age:
Males: 52 days
Females: 66 days
Sex:
5 male and 5 female
Weight at dosing:
Males: 240 - 267 g
Females: 209 - 216 g
Acclimation period:
5 days
Diet/Food:
ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), ad libitum except for approx. 16 h before dosing
Water:
Tap water, ad libitum
Housing:
Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:
Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: 2009-02-04 to 2009-07-30
Animal assignment and treatment:
The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was micronized before administration and the dust was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15 minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. A laser measured the size of the individual particles or individual aerosol drops. Animals were exposed four 4 hours to an actual concentration of 5.12 mg/L air (determined by HPLC).
After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.
Results and discussion
Dust concentration and particle size distribution
The actual dust concentration of 5.12 mg glyphosate TC/L air was measured at the animals' nose and was determined by HPLC.
Laser measurement revealed the following particle size distribution during the exposure:
Diameter

Actual concentration
5.12 mg/L air
d[10]
5.64 µm
d[50]
6.62 µm
d[90]
8.10 µm
[xx] = percentage of cumulative particle size distribution
No finer dust concentration of the test item could be generated.
Mortality: There were no mortalities during the study.
Clinical observations: A 4-hour exposure to glyphosate TC at the concentration of 5.12 mg/L revealed slight dyspnoea and ataxia in all 5 of 5 male and 5 of 5 female animals immediately until 60 minutes after the end of exposure.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The acute inhalation LC50 of the test material (glyphosate TC) in rats (males and females combined) was estimated to be greater than 5.12 mg/L air/4 hours. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute inhalation toxicity.
Comment by RMS:
Under the present conditions the study is considered acceptable and the acute inhalation LC50 >5 mg/L air/4 hours is agreed.
Reference: IIA, 5.2.3/05
Report: 2010 Acute Inhalation Toxicity Study of Glyphosate TC in Rats.
Data owner: Helm AG Report No.: 24875
Date: 2010-06-03, not published ASB2010-11410
Guidelines: EC method B.2. (92/69/EEC), OECD 403 and OPPTS 870.1300.
Deviations: There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate TC
Identification:
Glyphosate technical grade
Description:
White powder
Lot/Batch #:
20080801
Purity:
96.4%
Stability of test compound:

May 2011
Test animals:
Species:
Rat albino
Strain / Stock:
CD / Crl:CD(SD)
Source:
Age:
Males: approx. 7 weeks Females: approx. 9 weeks
Sex:
5 male and 5 female
Weight at dosing:
Males: 270 - 282 g
Females: 220 - 251 g
Acclimation period:
5 days
Diet/Food:
ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), ad libitum except for approx. 16 h before dosing
Water:
Tap water, ad libitum
Housing:
Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:
Temperature: 22 ± 3 °C
Rel. humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: 2009-10-15 to 2010-02-18
Animal assignment and treatment:
The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15 minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. Animals were exposed four 4 hours to an actual concentration of 5.02 mg/L air (determined by HPLC). A laser measured the size of the individual particles or individual aerosol drops. The particle size distribution for the estimation of the Mass Median Aerodynamic Diameter (MMAD) was carried out twice during the exposure period using a cascade impactor. The median particle size distribution of the test item was determined with a Malvern Sizer.
After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

Results and discussion
Dust concentration and particle size distribution Dust concentration and particle size distribution
The actual dust concentration of 5.02 mg glyphosate TC/L air was measured at the animals' nose and was determined by HPLC. The mean actual exposure concentration of glyphosate TC was as follows:
Table B.6.2-13: Details of test atmosphere
Actual concentration (HPLC)
[mg/L air]
Actual concentration (gravimetric method) [mg/L air]
MMAD
[µm]
Respirable amount particle size
≤4 µm
[mg/L air]
[%]
5.02
4.99
4.197 ± 2.64
1.03
20.5
No smaller MMAD could be obtained with the test item and no higher fraction of respirable particles could be obtained.
Laser measurement revealed the following particle size distribution during the exposure:
Diameter
Actual concentration
5.02 mg/L air
d[10]
12.51 µm
d[50]
37.15 µm
d[90]
86.42 µm
[xx] = percentage of cumulative particle size distribution
The particle size distribution of the delivered test item was d[50] = 14.5 µm. Mortality: There were no mortalities during the study.
Clinical observations: A 4-hour exposure to glyphosate TC at the concentration of 5.02 mg/L revealed slight ataxia, slight tremor and slight dyspnoea immediately until 3 hours after the end of exposure.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The acute inhalation LC50 of the test material (glyphosate TC) in rats (males and females combined) was estimated to be greater than 5.02 mg/L air/4 hours. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate TC is not to be classified for acute inhalation toxicity.
Comment by RMS:
Under the present conditions the study is considered acceptable and the acute inhalation LC50

>5 mg/L air/4 hours is agreed.
Reference:
IIA, 5.2.3/06
Report:
2009
Glyphosate – Acute Inhalation Toxicity Study in Rats. Stillmeadow, Inc., Sugar Land, TX, US
Data owner: Helm AG
Report No.: 12107-08
Date: 2009-03-09, not published ASB2012-11411
Guidelines:
US EPA OPPTS 870.1300.
Deviations:
Humidity was in the range of 33-89 % instead of 30-70 %. Female weight was outside the protocol range. These minor deviations did
not affect the study outcome.
GLP:
yes
Acceptability:
Please see comment by RMS.
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate Tech Grade Mixed 5-Batch
Description:
White powder
Lot/Batch #:
080704-1 thru 5
Purity:
96.71 %
Stability of test compound:
No data given in the report.
Test animals:
Species:
Rat albino
Strain / Stock:
Sprague-Dawley
Source:
Age:
Approx. 7-8 weeks
Sex:
5 male and 5 female
Weight at dosing:
Males: 262 - 289 g
Females: 172 - 191 g
Acclimation period:

5 days
Diet/Food:
Formulab #5008 (PMI Feeds Inc.), ad libitum except during the exposure period
Water:
Tap water, ad libitum except during the exposure period
Housing:
Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 % Air changes: 10 - 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: 2008-11-07 to 2008-11-21
Animal assignment and treatment:
The study was carried out using a 500 L nose-only stainless steel, dynamic flow inhalation chamber with 25 ports in 5 rows. Polycarbonate tubes were inserted into 10 designated individual ports. The test substance was ground for 10 hours and dried prior to exposure. The aerosol was generated from the undiluted test substance by a Venturi Aspirator and sprayed directly into the exposure chamber. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber. Animals were exposed to the aerosol for a period of 4
hours. The dust concentration in the inhalation chamber was determined gravimetrically twice per hour and nominally at the end of the exposure. Particle size, taken from the breathing zone of the animals, was determined twice during the exposure using a cascade impactor, and the mass median aerodynamic diameter (MMAD) and particle size distribution were calculated.
Observations for mortality and signs of pharmacological and/or toxicological effects were made frequently on the day of exposure and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to the inhalation exposure and on Days 7 and 14. On Day 14 after completion of exposure, all animals were euthanized by an intraperitoneal injection, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.
Results and discussion
Dust concentration and particle size distribution: The exposure concentration was determined to be 2.24 mg/L with an average MMAD of 2.6 µm.
Mortality: There were no mortalities during the study.
Clinical observations: The only prominent in-life observations were piloerection and activity decrease. Animals were asymptomatic by Day 4.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The acute inhalation LC50 of the test material (glyphosate) in rats (males and females combined) was estimated to be greater than 2.24 mg/L air/4 hours.
Comment by RMS:
The study is considered acceptable and the acute inhalation LC50 > 2.24 mg/L air/4 hours is agreed.
Reference: IIA, 5.2.3/07
Report: 2005,
Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats

– Limit Test.
Data owner: Helm AG Report No.: 15276
Date: 2005-04-04, not published ASB2012-11412
Guidelines: OPPTS 870.1300 (1998), OECD 403 and JMAFF 59 NohSan No. 4200 (1985).
Deviations: There were no deviations from the Study Plan.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate acid technical
Identification:
Glyphosate acid technical
Description:
White crystalline powder

Lot/Batch #:
040205
Purity:
97.23 %
Stability of test compound:
Test substance was expected to be stable for the duration of testing.
Test animals:
Species:
Rat albino
Strain / Stock:
Sprague-Dawley derived
Source:
Age:
9-10 weeks
Sex:
5 male and 5 female
Weight at dosing:
Males: 280 - 318 g
Females: 205 - 224 g
Acclimation period:
13 days
Diet/Food:
Purina Rodent Chow #5012, ad libitum
Water:
Filtered tap water, ad libitum
Housing:
Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:
Temperature: 19-23 °C

12-hour light/dark cycle
Study design and methods:
In life dates: 2003-05-17 to 2003-05-24
Animal assignment and treatment:
The study was carried out using a nose-only inhalation chamber with an internal volume of approximately 6.7 L and approximately 283 air changes per hour during the study. Animals were individually housed in polycarbonate holding tubes. The test item was micronized before administration and aerosolized using a dust generator which was directly connected to the inhalation chamber. Gravimetric samples were withdrawn at 6 intervals from the breathing zone of the animals to gravimetrically determine the dust concentration in the inhalation chamber. Particle size distribution of the test atmosphere was determined with an Andersen Cascade Impactor. Samples were withdrawn from the breathing zone of the animals at two intervals. Animals were exposed four 4 hours and 1 minute to an actual concentration of
2.04 mg/L air (determined gravimetrically).
Observations for mortality and clinical/behavioural signs of toxicity were made upon removal from the exposure chamber and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to test substance exposure and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanized by an overdose of CO2. All study animals were subjected to gross necropsy and all abnormalities were recorded.
Results and discussion
Dust concentration and particle size distribution: The gravimetric and nominal chamber concentrations were 2.04 and 8.99 mg/L, respectively. the mass median aerodynamic diameter was estimated to be 2.5 µm based on the particle size distribution as measured with an Andersen Cascade Impactor.
Mortality: There were no mortalities during the study.
Clinical observations: All animals appeared active and healthy upon removal from the exposure chamber and over the entire 14-day observation period.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers:
The acute inhalation LC50 of the test material (glyphosate acid technical) in rats (males and females combined) was estimated to be greater than 2.04 mg/L air/4 hours.
Comment by RMS:
The study is considered acceptable and the acute inhalation LC50 >2.04 mg/L air/4 hours is agreed.
Reference: IIA, 5.2.3/08
Report: 2008,
Acute Inhalation Toxicity Test of Glyphosate Technical in Rats
(Rattus norvegicus).
Data owner: Helm AG
Report No.: RF-3996.309.377.07
Date: 2008-09-11, not published ASB2012-11413
Guidelines: OECD guideline 403
Deviations: The experimental phase initiation and conclusion dates were updated. This deviation did not affect the study outcome.
GLP: yes

Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate Technical
Identification:
Glyphosate Technical
Description:
Solid
Lot/Batch #:
20070606
Purity:
98.05 %
Stability of test compound:
No data given in the report.
Test animals:
Species:
Rat albino (Rattus norvegicus)
Strain / Stock:
Wistar Hannover
Source:
Age:
Males: 9 weeks
Females: 11 weeks
Sex:
5 males and 5 females
Weight at dosing:
Males: 262 - 291 g
Females: 178 - 208
Acclimation period:
9 days
Diet/Food:
Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), ad libitum
Water:
Potable drinking water, ad libitum
Housing:
Polypropylene rodents cages with autoclaved wood shavings and stainless steel mesh lids containing five rats of each sex per cage.
Environmental conditions:
Temperature: 19-25 °C
Humidity: 30 - 70 %
Air changes: 10-15/hour 12-hour light/dark cycle
Study design and methods:
In life dates: 2008-06-06 to 2008-06-20
Animal assignment and treatment:
The study was carried out using an inhalation chamber with a nose-only exposure of the animals. The test item was aerosolised. Exposure started by locating the rats (5 male and 5 female animals) into

the exposure chamber. Animals were exposed to the aerosol at the maximum attainable concentration (5.211 mg/L) for a period of 4 hours. The actual concentration in the inhalation chamber was determined gravimetrically by taking eight equally time-spaced air samples from the breathing zone. Aerodynamic particle size distribution was determined two times using a Seven Stage Cascade Impactor.
After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made right after the exposure, and thereafter each working day. On Day 14 after completion of exposure, all animals were euthanised in a carbon dioxide chamber, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.
Results and discussion
Dust concentration and particle size distribution
The mean actual concentration was 5.211 mg/L. The actual concentration of the test item in each sample was within the ±15 % interval from the mean actual concentration, indicating that the test atmosphere was held stable over the 4-hour exposure period.
Analysis of the particle size distribution of samples from the breathing zone indicates that 4.72 to 5.15 % of the mass collected from the aerosol were within the respirable size range. The MMAD ranged from 18.555 to 19.901 µm and the geometric standard deviation (GSD) ranged from 2.869 to 2.914.
Mortality: There were no mortalities during the study.
Clinical observations: Clinical signs observed during the 14-day observation period included wheeze and dyspnoea. These acute respiratory signs started within the first day and reverted within the fourth day of the observation period.
Body weight: The mean body weight increased for both sexes, except for the males on the first post-exposure day. All animals exceeded their initial body weight by the conclusion of the experimental phase.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers
The acute inhalation LC50 of the test material (glyphosate Technical) in rats (males and females combined) was estimated to be greater than 5.211 mg/L air/4 hours. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate technical is not to be classified for acute inhalation toxicity.
Comment by RMS:
The study is considered not acceptable: The MMAD ranged from 18.555 to 19.901 µm, which is far above what is recommended in OECD 403 (MMAD ranging from 1 to 4 µm) and therefore, only 4.72 to 5.15 % of the particles were within the respirable size range. No explanation was given by the author why the standard could not be achieved.
Reference: IIA, 5.2.3/09
Report: 2007, Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats
Data owner: Nufarm Report No.: B02327
Date: 2007-04-02, unpublished ASB2012-11414
Guidelines: European Communities, Directive 92/69/EEC, Part B.2 "Acute Toxicity (Inhalation)", published
December 29, 1992.
- OECD Guidelines for Testing of Chemicals, Section 4, No. 403: "Acute Inhalation Toxicity",

adopted May 12, 1981.
- U.S. Environmental Protection Agency, Health Effects Test Guidelines OPPTS 870.1300, Acute Inhalation Toxicity, August 1998.
- Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute Inhalation Toxicity Studies Guideline 2-1-3. Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005. English translation by ACIS on 17 Oct 2005

Deviations: The following, minor deviations from the study plan were considered not to have compromised the quality, integrity or outcome of the study.
- The reference to the JMAFF inhalation test guideline was altered on request of the Sponsor. However, this did not affect the contents of the JMAFF inhalation test guideline.
- In the animal room, on brief occasions (for a total of less than 2 hours) the relative humidity was slightly higher than the upper limit of the target range given in the study plan.
- On the day of inhalation exposure (test day 1), the total aerosol generation period lasted 4 hours and 30 minutes, because a test aerosol was generated also for 30 minutes prior to the beginning of the exposure. This 30-minute pre-exposure aerosol generation period was used for fine-tuning of the settings of the aerosol generation and exposure system for the inhalation exposure. Consequently the nominal test atmosphere concentration was determined for the total of 4 hours and 30 minutes of aerosol generation (30 min pre- exposure aerosol generation without animals being present plus 4 h inhalation exposure of the animals).

GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate Technical (NUP 05068)
Description:
White powder
Lot/Batch #:
200609062
Purity:
95.1 %
Stability of test compound:
Stable under storage conditions.
Vehicle and/or positive control:
None
Test animals:
Species:
Rat
Strain:
HanRcc:WIST (SPF)
Source:
Age:

Males: 9 weeks
Females: 10 weeks
Sex:
Male / Female
Weight at dosing:
Males 241.6 – 257.4 g
Females 200.6 – 219.8g
Acclimation period:
5 days
Diet/Food:
Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH- 4303 Kaiseraugst/ Switzerland) ad libitum
Water:
Tap water, ad libitum
Housing:
During acclimatization in groups of five per sex in Makrolon type-4 cages with standard softwood bedding.
Environmental conditions:
Temperature: 19-20 °C
Humidity: 35-78 % Air changes: 10 - 15/hour 12-hour light/dark cycle
Study design and methods
In life dates: 14-DEC-2006 to 28-DEC-2006
Animal assignment and treatment:
A dust aerosol was generated from the milled and pre-dried test item using a rotating brush aerosol generator (CR 3020, CR Équipements SA, CH-1295 Tannay, Switzerland) connected to a micronising jet mill. No extra diluent air was added. The generated aerosol was discharged into the exposure chamber through a 63Ni charge neutraliser. The achieved mean aerosol concentration of 3.252 mg/L air administered for 4 hours was considered to represent the highest technically achievable concentration suitable for acute inhalation toxicity testing in rodents. An increase in aerosol concentration by an increased supply of test item to the rotating brush of the aerosol generator would have led to complete blockage of the rotating brush (which had happened in a pre-study technical trial not performed under GLP), and consequently to complete blockage of the aerosol generation and exposure system. Two generator cylinders containing test item were needed, in order to generate the highest technically achievable aerosol concentration over a 4-hour and 30-minute aerosol generation period.
The test atmosphere enters the top under slight positive pressure and is distributed to the entrance of each feed tube. It is then delivered through these tubes to the animal’s nose. The nhalation exposure system is located inside a ducted extraction cabinet. Test atmosphere samples for the gravimetric measurements of the test item concentration and particle size distribution, and for the measurement of temperature, relative humidity and oxygen concentration, were collected directly from the feed tube in the breathing zone of the animals, at an empty port of the exposure chamber delivering "fresh" test item to the animal's nose. This approach was chosen in order to obtain representative samples of what was delivered to the animals.
The particle size distribution was determined twice during the exposure using a Mercer 7 stage cascade impactor (Model 02-130, In-Tox Products Inc., Albuquerque, New Mexico, U.S.A.). Representative samples of the test atmosphere were drawn through the impactor with a flow rate of

1.0 L/min and the particles deposited according to their aerodynamic size onto stainless steel slips and the final filter stage (Type HVLP, Polyvinylidenedifluoride membrane, pore size 0.45 µm), on each stage of the impactor. To obtain the mass deposited on each stage of the impactor, the
steel slips and the final filter stage were carefully weighed before and after sampling using a Mettler MX5 analytical balance (Mettler AG, CH-8604 Volketswil, Switzerland). The total mass (µg) deposited in the impactor was then calculated by adding together the mass deposited on each of the stainless steel slips and the final filter stage. As the Effective Cut-off Diameters (ECD) represent the lower size limit of the particles collected on each stage, the cumulative percent less than the indicated size was tabulated as a function of the ECDB. This data was used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) using Microsoft Excel software. The target range for the MMAD was 1 to 4 µm, and was achieved.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: The following clinical signs were recorded during and/or after the inhalation exposure, whereby the whole range of the stated severity grades was not necessarily recorded in each affected animal: Salivation, moderate in degree, and deep respiration in two male animals (nos. 3 & 5), and breath sounds [rales], slight to marked in degree, in three male (nos. 1, 3 & 5) and two female animals (nos. 6 & 10).

The findings of salivation and deep respiration were seen at approximately 3 and 4 hours after exposure start, when the animals were restrained in the exposure tubes. Deep respiration was still evident one hour afterwards, at approximately one hour after the end of the exposure period. Breath sounds [rales] were only noticed at approximately one hour after the end of the exposure period and on the day afterwards (test day 2) after the animals had returned to their housing cages. By two days after the inhalation exposure (test day 3) all clinical signs had cleared, and all animals remained free from clinical signs until the scheduled necropsy day (test day 15).

Body weight: Losses in body weight were evident in three of five male animals (mean loss in the affected males –3.0 %) and three of five female animals (mean loss in the affected females –2.1 %), and retardation in body weight gain in one other male animal (+0.8 % weight gain) over the first three days following the inhalation exposure (test days 1 to 4). The effects on body weight were only transient and were followed by normal body weight gain in all animals.

Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

Conclusion by the Notifiers

The LC50 of glyphosate technical (NUP 05068) for acute 4-hour inhalation toxicity in male and female rats observed for a period of 15 days, was estimated to be greater than 3.252 mg/L air (gravimetrically determined mean aerosol concentration). This concentration was considered to represent the highest technically achievable aerosol concentration suitable for acute inhalation toxicity testing in rodents.

Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

Comment by RMS:

The study is considered acceptable and the acute inhalation LC50 >3.252 mg/L air/4 hours is agreed.

Reference: IIA, 5.2.3/10

Report: 1996, Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats

Data owner: Syngenta Report No.: /P/4882

Date: 1996-04-29, not published TOX2000-1984
Guidelines: OECD 403 (1981): OPPTS 870.1300 (1998): 92/69/EEC B.2 (1992)
+ amendment 93/21/EEC (1993)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Table B.6.2-14: Details of test atmosphere
Target concentration mg/L
Achieved particulate concentration
mg/L
MMAD* Mm
GSD+
2
2.47- 2 0.15
3.57, 3.03
1.94, 1.90
5
4.43 + 1.297
2.91, 3.41
1.74, 2.04
* Mass Median Aerodynamic Diameter (*m)
+ Geometric Standard Deviation
Two males and two females exposed to 4.43 mg glyphosate acid/L were found dead or were terminated in extremis during the observation period, the remaining animals in this group survived until scheduled termination. Clinical signs indicative of moderate toxicity were seen in this group. All surviving animals had regained their initial bodyweight by the end of the study.
Similar but less severe clinical signs were seen in animals exposed to 2.47 mg/L, all animals survived and showed complete recovery by the end of the study. All animals exposed to
2.47 mg/L survived to scheduled termination
It was concluded that the acute inhalation LC50 of glyphosate acid exceeded 2.47 mg/L for male rats and exceeded 4.43 mg/L for female rats.
Materials and methods
Test material:
Identification:
Glyphosate acid
Description:
Technical; white solid
Lot/Batch #:
P25
Purity:
95.6 % w/w
Stability of test compound:
Confirmed by Sponsor
Vehicle and/
or positive control:
None

Test animals:
Species:
Rat
Strain:
Alpk:APfSD
Source:
Age:
Young adult; 9 - 12 weeks old at delivery
Sex:
Males and females
Weight at dosing:
243-365 g (males); 210-247 g (females) at the start of exposure
Acclimation period:
At least five days
Diet/Food:
PCD diet (Special Diet Services Limited, Witham, Essex, UK) ad libitum except during exposure.
Water:
Mains water ad libitum except during exposure.
Housing:
5 per cage, sexes separately, except during exposure, in rat racks suitable for animals of the strain and weight range expected during the study.
Environmental conditions:
Temperature: 19-20 °C
Humidity: 40-70 %
Air changes: at least 15/hour Photoperiod: 12-hour light/dark cycle
Study design and methods
In-life dates: Start: 22 November 1995 End: 11 March 1996
Exposure conditions: Trial generations were carried out prior to the start of the study in order to determine the appropriate generation system and conditions, to determine the appropriate target concentration that could be achieved, or if not, what was the maximum stable attainable concentration, to obtain data on the aerodynamic particle size of the atmosphere generated, to determine an appropriate method of analysis of glyphosate acid. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of glyphosate acid.
Animal assignment and treatment: The study consisted of two main study groups of 5rats/sex/group exposed nose-only for a single four-hour period to glyphosate acid at target particulate concentrations of 5 mg/L and 2 mg/L. Prior to the start of the study the rats were examined to ensure that they were physically normal and exhibited normal activity. During exposure they were observed frequently and, at the end of the 4-hour exposure period, each rat was given a detailed clinical examination. They were also subjected to detailed clinical observations, daily during a 14-day observation period. The bodyweight of each rat was recorded on day -1, 1, 8 and prior to termination on day 15. All rats were killed on day 15 and subjected to a gross examination post mortem involving external observation and careful internal examination of all thoracic and abdominal viscera.
Generation of the test atmosphere / chamber description: Before exposure of the test animals, the atmosphere was shown to have been acceptably stable. The test atmosphere was generated using a modified Wright's dust-feed mechanism. Clean, dry air was passed through the dust feed at a nominal flow rate of 2.5 L/minute (at normal temperature and pressure) and carried the atmosphere

to the exposure chamber, having an internal volume of 27.6 litres. Since diluting air was not employed, the flow rate through the exposure chamber was the same as that employed in the generation of the test atmosphere. Air flows were monitored and recorded at approximately 30 minute intervals using variable area flow-meters and were altered as necessary to maintain target concentration. Animals were exposed nose-only to the atmosphere. They were restrained in polycarbonate tubes (Battelle, Switzerland), which were inserted into the Perspex exposure chamber. The chamber was covered with an aluminium cone and stood on an aluminium base.

Test atmosphere concentration: The particulate concentration of the test atmosphere, close to the animals' breathing zone, was measured gravimetrically at frequent intervals during the exposure period. This was done by drawing the test atmosphere, at a known flow rate, for a known time, through a 25 mm diameter, polyvinyl chloride (PVC) GLA 5000 filter housed in a Delrin open-faced filter holder. The filter was weighed before and after the sample was taken. The concentration was calculated as follows:

post wt (mg) - pre wt (mg)

Concentration (mg/L) =

time (minutes) x airflow (L/minute)

Pre wt = weight of filter prior to sampling Post wt = weight of filter after sampling

Particle size determination: The aerodynamic particle size distribution of the test atmosphere was measured twice during the exposure period, using a Marple Cascade Impactor, which aerodynamically separates airborne particles into pre-determined size ranges. Using a microcomputer, the data were transformed using a log/probit transform and a linear regression derived from the cumulative data. The linear regression line was then used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

Table B.6.2-15: Summary of acute study test atmosphere characteristics

Parameter	Target concentration 5 mg/L	Target concentration 2 mg/L
Measured particulate concentration	4.43 ± 1.30 mg/L	2.47 ± 0.15 mg/L
% total particulate	96.9 ± 4.2	98.5 ± 7.7
Analysed concentration of glyphosate acid(mg/L)	Mean 4.27 ± 1.15	Mean 2.43 ± 0.19
Particle size MMAD; GSD	2.91, 3.41µm; 1.74, 2.04	3.57, 3.03µm; 1.94, 1.90
Size range (µm)	% by weight in range	% by weight in range
Run 1 (1hr 35min into exposure)		
Run 2 (3hr 29min		

into exposure)
Run 1 (54min into
exposure)
Run 2 (2hr 59min
into exposure)
Analysed

Gravi-
metric
Analysed
Gravi-
metric
Analysed
Gravi-
metric
Analysed
Grav-
imetric
Particles > 9.8 µm (% w/w)
0.9
0.7
5.1
4.3
3.1
3.1
2.0
2.0
Particles 9.8-6.0 µm (% w/w)
21.1
20.6
26.3
23.8
20.9
23.0
16.7
16.4
Particles 6.0-3.5 µm (% w/w)
34.4
35.5
34.3
31.3
47.9
46.4
37.7
36.8
Particles 3.5-1.55 µm (%

w/w)
32.1
32.3
21.3
28.0
19.5
18.8
38.0
36.9
Particles 1.55-0.93 µm (%
w/w)
7.5
7.6
9.1
8.6
5.9
5.4
3.6
4.0
Particles 0.93-0.52 µm (%
w/w)
2.2
2.3
2.7
2.3
1.9
2.0
1.3
1.6
Particles P0.52 µm (% w/w)
1.5
1.0
1.3
1.8
0.8
1.3
0.8
2.4
Flow rate (whole system)
2.5 L/min
Temperature
14.7 – 21.7 °C
Humidity
25 - 65 %
- Percentages are calculated as follows:
Gravimetric: weight trapped at each size range x 100

Total weight trapped Statistics: The acute inhalation LC50 was estimated.
Results and discussion
Mortality: Two males and two females exposed to 4.43 mg/L were found dead or were terminated in extremis on days 5, 6 or 9 of the study, the remaining animals in this group survived until scheduled termination.
There were no mortalities at 2.47 mg/L.
Table B.6.2-16: Mortality / animals treated
Target exposure
Cumulative mortality (Number dead / total)
concentration mg/L
Day number
Males
Females
Combined
5
5
1/5
0/5
1/10
6
2/5
1/5
2/10
9
2/5
2/5
3/10
Day 14
2/5
2/5
4/10
2
Day 14
0/5
0/5
0/10
Clinical observations: Abnormalities generally associated with restraint (wet fur) were seen in all animals during exposure. Clinical changes seen were salivation, irregular breathing and auditory hypoaesthesia, these effects were considered to be related to treatment.
Immediately after exposure, abnormalities generally associated with restraint (hunched posture, piloerection and wet fur) were seen in both males and females. At an exposure concentration of 4.43 mg/L the clinical abnormalities seen in both sexes included breathing irregularities, reduced righting reflex, shaking, splayed gait andwere considered to be indicative of moderate toxicity.
At an exposure concentration of 2.47 mg/L the number of adverse clinical changes observed was reduced in both sexes. Those abnormalities observed were similar to those seen in animals exposed to 4.43 mg/L glyphosate acid

The clinical condition of most animals appeared to have improved by day 5 of the study, with the exception of 2 males and 2 females exposed to 4.43 mg/L. There was generally an improvement in clinical condition during the remainder of the study.
Bodyweight: Animals showed a treatment related reduction in bodyweight. At an exposure concentration of 4.43 mg/L all animals had exceeded their initial bodyweight by the end of the study. At an exposure concentration of 2.47 mg/L all animals had exceeded their initial weight by day 8 of the study.
Necropsy: In the animals exposed to 4.43 mg/L that died or were killed prior to termination, the two males found dead had dark lungs (probably a result of agonal congestion), the lungs of the females were normal.
At scheduled termination, the lungs of rats exposed to 4.43 mg/L were normal. One female exposed to 2.47 mg/L had red spots on the lungs and another female had dark lungs. These findings are considered to be incidental to treatment. Changes at necropsy in a variety of tissues in males exposed to 2.47 mg/L were of low incidence and were considered to be unrelated to treatment.
Conclusion by the Notifiers:
It was concluded that the acute inhalation LC50 of glyphosate acid exceeded 2.47 and exceeded 4.43 mg/L for male and female rats.
Comment by RMS:
The study is considered acceptable and the acute inhalation LC50 >4.43 mg/L air/4 hours is agreed.
Reference: IIA, 5.2.3/11
Report: 2011,
Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat
Data owner: Syngenta Report No.: 11/054-004P Date: 2011-06-06
GLP:
not published ASB2012-11415
Guidelines: OECD 403 (1981): OPPTS 870.1300 (1998): 440/2008 B.2 (2008)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
Technical; dry white powder
Lot/Batch #:
614034 (20100609\Milled)
Purity:
96.9 % w/w glyphosate technical
Stability of test compound:
Stable under storage conditions (room temperature range
<30 °C), recertification date end January 2014
Vehicle and/or positive control:
None
Test animals:
Species:

Rat
Strain:
RjHan:WI
Source:
Age:
8-10 weeks
Sex:
Males and females
Weight at dosing:
229-386 g
Acclimation period:
At least five days
Diet/Food:
ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany ad libitum (except during exposure)
Water:
Tap water ad libitum (except during exposure)
Housing:
In groups of 5 (or 2 in the case of sighting exposure), by sex, in solid-floor cages (Type III) with stainless steel mesh lids and softwood flake bedding.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30-70 %
Air changes: 15-20 air changes per hour Photoperiod: 12 hours light / 12 hours dark
Study design and methods
In-life dates: Start: 14 April 2011 End: 28 April 2011
Exposure conditions: Prior to animal exposures, test material atmospheres were generated within the exposure chamber. During these technical trials, air-flow settings and test material input rates were adjusted to achieve the required atmospheric characteristics. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of Glyphosate Technical.
Exposure system: The animals were exposed, nose-only, to an atmosphere of the test item using a TSE Rodent Exposure System (TSE Systems GmbH, Bad Homburg, Germany). This system comprises of 2, concentric anodised aluminium chambers and a computer control system incorporating pressure detectors and mass flow controllers.
Fresh aerosol from the generation system was constantly supplied to the inner plenum (distribution chamber) of the exposure system from where, under positive pressure, it was distributed to the individual exposure ports. The animals were held in polycarbonate restraint tubes located around the chamber which allowed only the animal's nares to enter the exposure port. After passing through the animal's breathing zone, used aerosol entered the outer cylinder from where it was exhausted through a suitable filter system. Atmosphere generation was therefore dynamic.
Airflows and relative pressures within the system were constantly monitored and controlled by the computer system thus ensuring a uniform distribution and constant flow of fresh aerosol to each exposure port (breathing zone). The flow of air through each port was at least
0.7 L/min. This flow rate was considered adequate to minimise re-breathing of the test atmosphere as it is about twice the respiratory minute volume of a rat.
Homogeneity of the test atmosphere within the test chamber and amongst the exposure ports was

not specifically determined during this study. However, chambers of this design have been fully validated and have shown to produce evenly distributed atmospheres in the animals' breathing zones.
Exposure procedure: Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber. Only the nose of each animal was exposed to the test atmosphere. Following an equilibration period of at least the theoretical chamber equilibration time (T99), a group of 10 rats (5 male and 5 female) was exposed to a target atmosphere concentration 5 mg/L for a period of at least 4 hours.
Generation of the test atmosphere / chamber description: The test item was aerosolised using a rotating brush powder disperser (Palas GmbH, Karlsruhe, Germany) located at the top of the exposure chamber. Compressed air was supplied by means of an oil-free compressor and passed through a suitable filter system prior to introduction to the dust generator.
Test atmosphere concentration: The test atmosphere was sampled at regular intervals during each exposure period. Samples were taken from an unoccupied exposure port (representing the animal's breathing zone) by pulling a suitable, known volume of test atmosphere through weighed GF10 glass fibre filters. The difference in the pre and post sampling weights, divided by the volume of atmosphere sampled, was equal to the actual achieved test atmosphere concentration.
The nominal concentration was calculated by dividing the mass of test material disseminated into the chamber by the total volume of air that through the chamber during the same period.
Particle size determination: The particle size of the test atmosphere was determined three times during the exposure period using a 7-stage impactor of Mercer style (which employs an inertial separation technique to isolate particles in the discrete aerodynamic size ranges). Samples were taken from an unoccupied exposure port (representing the animal's breathing zone).
The collection substrates and the backup filter were weighed before and after sampling and the weight of test item, collected at each stage, calculated by this difference.
The total amount collected for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 0.55, 0.96, 1.55, 2.11, 3.56, 6.66 and 10.55 µm was calculated.
From these data, using software supplied with the impactor (TSE Systems GmbH, Bad Homburg, Germany), the Mass Median Aerodynamic Diameter (MMAD), and Geometric Standard Deviation were calculated. In addition, the proportion (%) of aerosol less than 4µm (considered to be the inhalable portion) was determined.
Table B.6.2-17: Summary of main study test atmosphere characteristics
Parameter
Target concentration 5 mg/L
Mean achieved concentration (mg/L)
5.04 ± 0.17
Nominal (mg/L)
7.71
Particle size MMAD; GSD
3.65 µm; 2,24
Inhalable fraction (% < 4 µm)
54.4
% by weight in range #
Size range (µm)
Total mass/stage (mg)

Cumulative mass (%)
<0.55
0.35
2.05
0.55 – 0.96
0.30
3.81
0.96 – 1.55
0.91
9.13
1.55 – 2.11
1.90
20.26
2.11 – 3.56
5.43
52.05
3.56 – 6.66
4.69
79.51
6.66 – 10.55
2.06
91.57
>10.55
1.44
100.00
T99 (Minimum Acceptable Equilibration
Time)
1 minute
Chamber volume (inner plenum)
3.85 L
Air Flow In (Inner Plenum) (L/min)
20.0-20.6
Air Flow Out (Inner Plenum) (L/min)
19.4 – 38.4
Temperature
21.6 – 24.7 °C
Humidity
3.9 – 10.2 % (n = 3)
Oxygen Concentration (%)
19.6 – 20.3
Carbon Dioxide
0.1 – 0.8
Sighting studies: Two sighting exposures using 2 male and 2 female rats were performed before the main study due to insufficient information about the test item's inhalation toxicity. Animal assignment and treatment: Five male and 5 females were exposed to a target aerosol concentration of 5 mg/L glyphosate technical. The animals were exposed for 4 hours using a nose-only exposure

system, followed by a 14 day observation period. The day of exposure was designated Day 0.
Animals were checked hourly during exposure, 1 hour after exposure and twice daily (early and late in the working day) during the 14 days of the observation period for morbidity and/or mortality. All animals were observed for clinical signs at hourly intervals during exposure, as soon as practically possible following removal from restraint at the end of exposure, 1 hour
after exposure and subsequently once daily for 14 days. The body weight of each rat was recorded prior to treatment on the day of exposure (day 0) and on Days 1, 3, 7 and 14.
At the end of the 14 day observation period, the animals were sacrificed by exsanguination under anaesthesia and a gross macroscopic examination was performed, which included a detailed examination of the abdominal and thoracic cavities. Special attention was given to the respiratory tract for macroscopic signs of irritancy or local toxicity.
Statistics: The acute inhalation LC50 was calculated from the mortality data.
Results and discussion
Mortality: One male rat died following a 4 hour exposure to 5.04 mg/L glyphosate technical. Clinical observations: Wet fur and fur staining were commonly recorded on the day of and the day following exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be treatment related.
Significant clinical signs were recorded on day of exposure and the following day included laboured and noisy respiration, respiratory rate increased, gasping respiration, sneezing, decreased activity, thin body appearance (weak/wasted).
The majority of animals recovered from Day 3.
Body weight: Normal body weight gain was noted for all surviving animals from Day 1, with the exception of one male where a slight bodyweight loss was recorded during first week of the observation period.
Necropsy: There were no macroscopic abnormalities in animals surviving to scheduled termination. A specific cause of death was not determined for the single male that died in the main study.
Conclusion by the Notifiers:
Under the experimental conditions of this study, a single death occurred in a group of 10 rats exposed to a mean achieved atmosphere of 5.04 mg/L for 4 hours. The acute inhalation LC50 of glyphosate technical, in Wistar RjHan: (WI) strain rats is considered to be greater than
5.04 mg/L.
Comment by RMS:
The study is considered acceptable and the acute inhalation LC50 >5.04 mg/L air/4 hours is agreed.
Reference: IIA, 5.2.3/12
Report: 1999, NUP5a99 62% glyphosate MUP: Acute inhalation toxicity study in rats – Limit test
Data owner: Nufarm Study No.: 7909
Date: 1999-09-16, unpublished ASB2012-11416
Guidelines: Health Effects Test Guidelines, OPPTS 870.1300 (1998)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
NUP5a99 62 % glyphosate MUP
Description:

clear viscous amber liquid
Lot/Batch #:
Drum Sample E
Purity:
62 %
Stability of test compound:
No data available
Vehicle and/
or positive control:
None
Test animals:
Species:
Rat
Strain:
Sprague-Dawley derived, albino
Source:
Age:
Not specified
Sex:
5 males and 5 females
Weight at dosing:
males 224-256 grams and females 179-201 gramsg
Acclimation period:
10 days
Diet/Food:
Purina Rodent Chow #5012
Water:
Tap water, ad libitum
Housing:
singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage
and was changed at least three times per week.
Environmental conditions:
Temperature: 22-24 °C Humidity: not specified Air changes: not specified
12-hour light/dark cycle
Study design and methods
In life dates: August 6-20, 1999
Animal assignment and treatment:
Prior to initiation of the full inhalation study, pre-test trials were conducted to establish generation procedures for achieving as closely as possible the desired chamber concentration (2.0 mg/L) and desired particle size distribution (mass median aerodynamic diameter ≤4 /µm). The animals were placed in a rectangular whole body plexiglass chamber with a volume of 150 liters with prechamber operated under slight negative pressure, and were exposed to the test atmosphere for 4 hours and 15 minutes. The exposure period was extended beyond 4 hours to allow the chamber to reach equilibrium (T99). The times for 90 and 99 % equilibration of the chamber atmosphere were 7.5 and 15.1 minutes, respectively. The gravimetric and nominal chamber concentrations were 2.08 and

18.38 mg/L, respectively. The mass median aerodynamic diameter was estimated to be 2.6 microns based on the particle size distribution as measured with an Andersen Cascade Impactor. At the end of the exposure period, the generation was terminated and the chamber was operated for a further 15 minutes with clean air. At the end of this period the animals were removed from the chamber. Prior to being returned to their cages, excess test substance was removed from the fur of each animal.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: In-chamber animal observations included ocular and nasal discharge, hunched posture and hypoactivity. Apart from test substance noted on the fur, all animals recovered from the above symptoms upon removal from the exposure chamber and appeared active and healthy throughout the study.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Necropsy: The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.
Conclusion by the Notifiers:
The single exposure acute inhalation LC50 ofNUP5a99 62 % glyphosate MUP is >2.08 mg/L. Based on the EU and the OECD Globally Harmonised System (GHS) classification criteria, glyphosate is not to be classified for this end point.
Comment by RMS:
The study is considered acceptable and the acute inhalation LC50 > 2.08 mg/L air/4 hours is agreed.
Reference: IIA, 5.2.3/13
Report: 2004,
An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623
Data owner: Monsanto
Monsanto Report No.: SB-2003-116 Date: 2004-02-06, not published ASB2012-11417
Guidelines: EC method B.2, OECD 403, EPA Health Effects Test Guidelines, OPPTS 870.1300, JMAFF 12 Nohsan No. 8147
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
MON 78623
Description:
Clear colourless liquid (pipet), light amber liquid (bulk)
Lot/Batch #:
GLP-0306-14124-F
Purity:
47.2 % glyphosate (57.8 % potassium salt of glyphosate)
Stability of test compound:
Expiry June, 2004
Vehicle and/
or positive control:
None

Test animals:
Species:
Rat
Strain:
Sprague Dawley
Source:
Age:
8-9 weeks
Sex:
Males and females
Weight at dosing:
♂ 276 – 312 g; ♀ 182 – 210 g
Acclimation period:
At least 5 days
Diet/Food:
PMI Certified Rodent Chow #5002 (PMI Nutrition International), ad libitum (except during acclimatisation to the exposure tubes and during the exposure)
Water:
Tap water, ad libitum (except during acclimatisation to the exposure tubes and during the exposure)
Housing:
Individually housed in suspended stainless steel cages
Environmental conditions:
Temperature: 19 - 23 °C
Humidity: 31 - 65 %
Air changes: 10-15 per hour
Light cycle: 12 hours light/dark cycle
Study design and methods
In life dates: 2003-10-29 to 2003-12-29
Test atmosphere generation:
The test aerosol was generated with a Master Flex Pump and Pump Head (7523-30 and 77200-60) and a Pistol Spraying System. Conditioned high pressure external air was used in generating the test atmosphere. The aerosol was blown through a 5 L Elutriator, the Multi- Stage 10 L nose-only inhalation chamber and then vented from the chamber to an air treatment system which consisted of a prefilter, a HEPA filter, a charcoal bed and a water scrubbing tower.
Exposure chamber conditions:
Air flow readings were recorded at the initiation of the T99 equilibration period, at approximately 30-minute intervals during each aerosol exposure and at the conclusion of the de-equilibration period. The aerosol concentration was measured at the beginning of each aerosol exposure (after equilibration), at approximate 30-minute intervals during the aerosol exposure, and at the conclusion of each aerosol exposure (before de-equilibration). Samples of the test article aerosol were collected in the inhalation chamber by gravimetric technique. Both gravimetric and analytical aerosol concentrations were determined. A 5 L sample of the aerosol was drawn from the breathing zone of the animals in the chamber through a preweighed glass fiber filter. For the analytical concentration, the gravimetrically obtained samples were analysed by liquid chromatography for the non-volatile glyphosate component of the test article. These analyses were performed in order to determine the analytical (actual) concentrations of the aerosol in the chamber for each sampling period. Chamber

oxygen content was measured and recorded at approximate 30-minute intervals during each aerosol exposure using a GC-501 Oxygen Detector.

Particle size distribution:

The aerosol aerodynamic particle-size distribution was determined three times during each aerosol exposure using the ITP 7 Stage Cascade Impactor. Each stage of the impactor was fitted with a preweighed glass fiber filter. Five liters per minute of the chamber air were drawn through the impactor and the change in weight of each filter was then determined and recorded. The mean particle-size distribution was subsequently determined using an Excel
computer adaptation of the manual method. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and percentage of particles ≤ 4.0 µ were then determined. The results were as follows:

Table B.6.2-18: Details of test atmosphere

Mean Achieved Actual Concentration (analytical method) (mg/L)	MMAD (µm)	GSD	Respirable Amount Particle Size ≤ 4 µm (%)
2.21	2.9	2.18	67
5.27	3.8	2.20	53

MMAD = mean mass median aerodynamic diameter GSD = geometric standard deviation

Animal assignment and treatment:

The animals chosen for study use were randomly selected from healthy stock animals using a computerized random numbers table to avoid potential bias. On day 0, the animals chosen for the limit test were weighed, placed in a nose-only exposure tube and allowed to acclimate to the exposure tube for at least one hour. Animals that appeared to have been acclimated to the exposure tube (i.e., minimal struggling and no inversion) were considered to be acceptable. Animals that did not appear to acclimate to the exposure tube were not acceptable. All animals were removed from the exposure tubes and returned to their cages.

The acceptable animals were then placed in exposure tubes and the tubes inserted into the Multi-Stage 10 L nose-only inhalation chamber and the test article aerosolised at the following levels:

Table B.6.2-19: Dose Levels

Analytical Exposure Level (mg/L)	No. of Animals	
	Male	Female
2.21	5	

5
5.27
5
5
Each aerosol exposure consisted of a 3-minute T99 equilibration period, a 240-minute exposure period and a 3-minute de-equilibration period equal to the T99 equilibration period. After each aerosol exposure, animals were removed from the exposure tubes and residual test article was removed from the animal's exterior surfaces (where practical) by wiping the haircoat with a towel. The animals were then returned to ad libitum feed and water.
The limit test animals were observed for clinical abnormalities during each aerosol exposure (no positive clinical observations were noted during either exposure), two times on study day 0 (post-exposure) and daily thereafter (days 1-14). Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanized and all study animals were subjected to gross necropsy.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The most notable clinical abnormalities observed for the 2.21 mg/L dose level included transient incidences of congested breathing and dark material around the facial area.
The most notable clinical abnormalities observed for the 5.27 mg/L dose level included transient incidences of congested breathing and few faeces.
Body weight: Body weight gain was noted for all animals for the 2.21 mg/L dose level.
For the 5.27 mg/L dose level, slight body weight loss was noted for two females during the day 0 to 7 body weight interval and for one female during the day 7 to 14 body weight interval. Body weight gain was noted for all other animals and all animals exceeded their initial body weight at study termination.
Necropsy: No gross internal findings were observed at necropsy for the 2.21 mg/L and
5.27 mg/L dose levels on study day 14.
Conclusion by the Notifiers
The acute inhalation LC50 for the test substance MON 78623 was estimated to be greater than 5.27 mg/L. According to EU and OECD Globally Harmonised System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity.
Comment by RMS:
The study is considered acceptable and the acute inhalation LC50 > 5.25 mg/L air/4 hours is agreed.
B.6.2.4 Skin irritation
According to the previous EU evaluation, glyphosate acid and its salts were considered non- irritant to intact skin and only slightly irritant to abraded skin. For the current re-evaluation, 13 additional studies with glyphosate acid on rabbits were submitted.
Table B.6.2-20: Summary of skin irritation studies with glyphosate acid
Reference (Data owner)
Species Strain
Number and
/or sex of animals /
Purity [%]
Amount applied / Exposure conditions
Results

Studies from the 2001 evaluation
Annex B- 5.2.5.1,
Glyphosate Monograph
1994
(Alkaloida) TOX9650145
Rabbit NZW
4
97.2
0.5 g; intact + abraded skin
Very slight irritation
Annex B-
5.2.5.1,
Glyphosate Monograph
1991 (I. Pi. Ci.) TOX9551627
Rabbit
NZW
3 ♂
98
0.5 g moistened with
saline; intact skin
Very slight
irritation
Reference (Data owner)
Species Strain
Number and
/or sex of animals /
Purity [%]
Amount applied / Exposure conditions
Results
Annex B- 5.2.5.1,
Glyphosate Monograph
1991
(ADM) TOX9551092
Rabbit NZW
2 ♂, 1 ♀
96.8
0.5 g; intact skin
Non irritant
Annex B- 5.2.5.1,
Glyphosate Monograph
1990 (AGC)
TOX9551794
Rabbit NZW
3 ♂
98.1
0.5 g moistened with saline; intact + abraded skin

Non irritant
Studies from the 2001 evaluation
Annex B- 5.2.5.1,
Glyphosate Monograph
, 1989 (CHE) TOX9552333
Rabbit NZW
2 ♂, 4 ♀
98.6
0.5 g moistened with water; intact skin
Non irritant
Studies not reviewed in the 2001 evaluation
IIA 5.2.4/01
2007
(NUF)
ASB2012- 11418
Rabbit NZW
1 ♂, 2 ♀
95.1
0.5 g moistened with
0.5 mL water; intact skin
Non irritant
IIA 5.2.4/02
2009 (HAG) ASB2012- 11419
Rabbit
Himalaya n
3 ♂
96.4
0.5 g moistened with
water; intact skin
Non irritant
IIA 5.2.4/03
Hideo, 1995 (
ASB2012- 11420
Rabbit
NZW
6 ♀
97.56
0.5 g moistened with
0.5 mL water; intact skin
Non irritant
IIA 5.2.4/04
2009 (HAG) ASB2012-
11421
Rabbit
Himalaya n
3 ♂

98.8
0.5 g moistened with
water; intact skin
Non irritant
IIA 5.2.4/05
2010 (HAG)
ASB2012- 11422
Rabbit
Himalaya n
3 ♂
97.3
0.5 g moistened with
water; intact skin
Non irritant
IIA 5.2.4/06
2009 (HAG) ASB2012- 11423
Rabbit
NZW
1 ♂, 2 ♀
96.4
0.5 g moistened with
water; intact skin
Non irritant
IIA 5.2.4/07
2005 (HAG) ASB2012-
11424
Rabbit,
NZW
3 ♂
97.23
0.5 g moistened with
water; intact skin
Slightly irritating
Reference (Data owner)
Species Strain
Number and
/or sex of animals /
Purity [%]
Amount applied / Exposure conditions
Results
Studies not reviewed in the 2001 evaluation
IIA 5.2.4/08
Rabbit, NZW
3 ♀
98.05
0.5 g moistened with water; intact skin

Non irritant
2008 (HAG)
ASB2012- 11425
IIA 5.2.4/09
, 1988
(MON) Z35394
Rabbit NZW
3 ♂, 3 ♀
97.76
0.5 g moistened with saline; intact skin; semi-occluded
Non irritant
IIA 5.2.4/10
1979 (MON) Z35544
Rabbit
3 ♂, 3 ♀
98.5
0.5 g moistened with water; intact skin; occluded
Primary dermal irritation index 0.1 (study not
acceptable)
IIA 5.2.4/11
1996 (SYN) TOX2000-1985
Rabbit, NZW
6 ♀
95.6
0.5 g moistened with
0.5 mL water; intact skin
Non irritant
Studies not reviewed in the 2001 evaluation
IIA 5.2.4/12
2007 (SYN) ASB2012-
11426
Rabbit NZW
1 ♂, 2 ♀
96.1
0.5 g moistened with
0.5 mL water; intact skin
Non irritant
IIA 5.2.4/13
2011 (SYN) ASB2012-
11427
Rabbit
NZW
3 ♂
96.3
0.5 g moistened with
water; intact skin

Mild Irritant
(Primary dermal irritation index 0.11)
NZW = New Zealand White
Table 6.2-21: Summary of skin irritation studies with glyphosate salts
Reference (Data owner)
Species Strain
Number and
/or sex of animals /
Purity [%]
Amount applied /
Exposure conditions
Results
Studies from the 2001 evaluation
Annex B- 5.2.5.1,
Glyphosate Monograph Dreher, 1994
(MON / CHE) TOX9552335
Rabbit NZW
1 ♂, 5 ♀
IPA 62%
0.5 mL (pure)
Slight irritation
Annex B-
5.2.5.1,
Glyphosate Monograph Snell, 1994 (Herbex)
TOX9500248
Rabbit NZW
2 ♂, 1 ♀
IPA 360
g/L
0.5 mL; intact skin
Non irritant
Reference (Data owner)
Species Strain
Number and
/or sex of animals /
Purity [%]
Amount applied /
Exposure conditions
Results
Annex B- 5.2.5.1,
Glyphosate Monograph
1989 (I.Pi.Ci.) TOX9551628
Rabbit NZW
3 ♂, 3 ♀
IPA 62
0.5 mL (pure); ♂: intact skin; ♀:

Slight irritant
Annex B-
5.2.5.1,
Glyphosate Monograph
1987 (MON/CHE)
TOX9552336
Rabbit NZW
6
NH4-salt
90.8
0.5 g moistened with
saline
Non irritant
NZW = New Zealand White
Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation please refer to the Monograph.
Reference: IIA, 5.2.4/01
Report: 2007, Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)
Study No.: B02294 Data owner: Nufarm
Date: 2007-03-01, unpublished ASB2012-11418
Guidelines: OECD 404 (2002); Commission Directive 2004/73/EC B.4 (2004),
JMAFF guideline 2-1-4 (2005)
Deviations: Yes
The test patch used had a surface of 16 cm2 instead of 6 cm2.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate technical
Identification:
NUP 05068
Description:
Solid
Lot/Batch #:
200609062
Purity:
95.1%
Stability of test compound:
Stable under storage conditions (20 ± 5 °C); Expiry date: 2008-09-14
Vehicle and/
or positive control:
Purified water
Test animals:
Species:

Rabbit
Strain:
New Zealand White, SPF
Source:
Age:
13 weeks (male); 14 weeks (females)
Sex:
One male and two females
Weight at dosing:
2.662 kg (male), 2.637 kg and 2.97 kg (females)
Acclimation period:
At least five days
Diet/Food:
Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust), ad libitum
Water:
Tap water, ad libitum
Housing:
Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blooks and haysticks were provided for gnawing.
Environmental conditions:
Temperature: 17 - 23 °C
Humidity: 30 - 70 % Air changes: 10 - 15/hour 12 hours light/dark cycle
Study design and methods
In life dates: 2007-01-04 to 2007-01-15
Animal assignment and treatment:
The test was conducted using young adult New Zealand albino rabbits (1 male, 2 females). The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance was moistened with approximately
0.5 mL of purified water was applied to the intact skin of the clipped left flank of the rabbits on an approx. 16-cm2 gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed and the skin was cleaned with lukewarm tap water.
Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours after removal of the patch. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.
Results and discussion
Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study. Body weight: All rabbits showed the expected body weight gain.
Necropsy: No necropsy was performed.
Skin observations: No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.

Conclusion by the Notifiers:
Based on the EU classification criteria, glyphosate technical (NUP 05068) is not to be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (NUP 05068) is also not classified for skin irritation.
Comment by RMS:
The study is considered acceptable and the tested substance not irritant.
Reference: IIA, 5.2.4/02
Report: 2009, Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC In Rabbits
Data owner: Helm AG Report No.: 24877
Date: 2009-11-27, unpublished ASB2012-11419
Guidelines: OECD 404 (2002); Commission Directive 2004/73/EC B.4 (2004),
OPPTS 870.2500 (1998)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate TC
Description:
White powder
Lot/Batch #:
2009051501
Purity:
96.4 %
Stability of test compound:
At room temperature in the dark stable until May 15, 2011.
Vehicle and/or positive control:
Purified water
Test animals:
Species:
Rabbit
Strain:
Himalayan
Source:
Age:
Approx. 7.5 - 8.5 months
Sex:
Males
Weight at dosing:
2.6 - 3.2 kg
Acclimation period:
At least 20 days.
Diet/Food:
ssniff K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), ad libitum before and after the exposure period

Water:
Tap water, ad libitum before and after the exposure period
Housing:
Individual housing
Environmental conditions:
Temperature: 20 ± 3 °C
Humidity: 30 - 70 % Air changes: no data 12 hours light/dark cycle
Study design and methods
In life dates: 2009-10-15 to 2009-10-23 Animal assignment and treatment:
The test was conducted using three young male adult Himalayan albino rabbits. The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance was moistened with purified water and applied to the intact skin of the rabbits on an approx. 6-cm2 gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed. No residual test item had to be removed.
Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours after removal of the patch.
Results and discussion
Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study. Body weight: All rabbits showed the expected body weight gain.
Necropsy: No necropsy was performed.
Skin observations: No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.
Conclusion by the Notifiers
Based on the scores for erythema and oedema and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, glyphosate TC is not to be classified for skin irritation.
Comment by RMS:
The study is considered acceptable and the tested substance not irritant.
Reference:
IIA, 5.2.4/03
Report:
1995, HR-001: Primary Dermal irritation study in rabbits.
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Report No.: IET 95-0035
Data owner: Arysta LifeScience Date: 1995-06-28, unpublished ASB2012-11420
Guidelines:
U.S. EPA FIFRA Guideline Subdivision F MAFF 59 NohSan No. 4200 (1985)
Draize method
Deviations:
None
GLP:
yes

Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Glyphosate technical
Identification:
HR-001
Description:
White crystal
Lot/Batch #:
T-941209
Purity:
97.56 %
Stability of test compound:
Not mentioned in the report
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rabbit
Strain:
New Zealand White, SPF
Source:
Age:
12 weeks
Sex:
Six females
Weight at dosing:
2.512 (2408 – 2686) kg
Acclimation period:
18 days
Diet/Food:
Pellet Diet GC4 (Oriental Yeast Co., Ltd.)
Water:
Water filtrated and sterilized, ad libitum
Housing:
Individually in stainless steel cages with feed hoppers and drinking water bowls.
Environmental conditions:
Temperature: 23.9 - 24 °C
Humidity: 52.8 – 56.6 % Air changes: 15/hour
12 hours light/dark cycle
Study design and methods
In life dates: 1995-05-16 – 1995-05-19
Animal assignment and treatment:
Glyphosate (0.5 g) moistened with 0.5 mL of deionised water was then applied to the closely- clipped dorso-lumbar region of 6 New Zealand rabbits and covered by a semi-occlusive gauze patch for 4

hours. At the end of the exposure period, the patch was removed and the treatment site was washed with distilled water to remove any residual test substance. All animals were observed for primary dermal irritation 1, 24, 48 and 72 hours after removal of the patch. Degree of erythema and edema relative to treatment were recorded during a subsequent 72- hour observation period. Body weights were measured prior to application, and after the final observation.
Results and discussion
Mortality: No mortality occurred.
Clinical observations: Clinical signs were not observed.
Body weight: All rabbits showed the expected body weight gain. Necropsy: No necropsy was performed.
Skin observations: : No signs of erythema, eschar, edema and any other evidence of irritation were observed in either the test sunstance treated site or the negative control site at any time during the observation period.
The observation period was therefore completed after 72 hours.
Conclusion by the Notifiers
Based on the EU classification criteria, glyphosate technical (HR-001) is not to be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate technical (HR-001) is also not classified for skin irritation.
Comment by RMS:
The study is considered acceptable and the tested substance not irritant.
Reference: IIA, 5.2.4/04
Report: 2009, Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits
Data owner: Helm AG Report No.: LPT 23913
Date: 2009-04-30, unpublished ASB2012-11421
Guidelines: OECD 404 (2002)
US EPA OPPTS 870.2500
Deviations: Personnel change of the head of the Quality Assurance Unit: until January 31, 2009: Dipl. Biol. S. Steuer
as of February 1, 2009: Dr. med. vet. K. R. habil. Sultan. This minor deviation did not have any effect on the validity and integrity of the scientific results obtained in this study
GLP: yes

Acceptability: Please see comment by RMS.
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate TC
Description:
White powder
Lot/Batch #:
20080801
Purity:
98.8 %
Stability of test compound:
No data given in the report.

Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rabbit
Strain:
Himalayan
Source:
Age:
Approx. 4 - 5 months
Sex:
Male animals
Weight at dosing:
Animal no. l : 4.4 kg Animal no. 2: 4.0 kg
Animal no. 3: 3.8 kg
Acclimation period:
At least 20 adaptation days
Diet/Food:
Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available ad libitum before and after the exposure period.
Water:
Tap water, ad libitum
Housing:
The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH,
16352 Schönwalde, Germany).
Environmental conditions:
Temperature: 20 ± 3 °C
Humidity: 30 - 70 % 12-hour light/dark cycle
Study design and methods
In life dates: 2009-02-04 to 2009-02-13 Animal assignment and treatment:
Approximately 24 hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.
A dose of 500 mg of the test item was applied to the test site (area: approx. 6 cm^2).
The test item was applied to the test site and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control.
Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers.
At the end of the exposure time no residual test item had to be removed. As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours.
As neither a corrosive effect nor a severe irritant effect was observed after a four hour exposure, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.
Results and discussion

Mortality: There were no mortalities during the study.
Clinical observations: The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72 hours after patch removal. None of the three rabbits showed any significant test item-related lesions at these examination time points.
Conclusion by the Notifiers
Under the present test conditions, none of three rabbits exposed for 4 hours to 500 mg Glyphosate TC/patch (semi-occlusive conditions) showed any test item-related changes. There were no systemic intolerance reactions.
According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions, Glyphosate TC was non - irritating to skin, hence, no labelling is required.
Comment by RMS:
The study is considered acceptable and the tested substance not irritant.
Reference: IIA, 5.2.4/05
Report: 2010, Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits
Data owner: Helm AG Report No.: 24605
Date: 2010-01-06, unpublished ASB2012-11422
Guidelines: OECD 404 (2002)
US EPA OPPTS 870.2500
Deviations: No deviations from the Study Plan
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate TC
Description:
White powder
Lot/Batch #:
20090506
Purity:
97.3 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Rabbit
Strain:
Himalayan
Source:
Age:
Approx. 6 - 7 months

Sex:
Male animals
Weight at dosing:
Animal no. l : 2.9 kg Animal no. 2: 2.4 kg
Animal no. 3: 2.5 kg
Acclimation period:
At least 20 adaptation days
Diet/Food:
Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available ad libitum before and after the exposure period.
Water:
Tap water, ad libitum
Housing:
The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH,
16352 Schönwalde, Germany).
Environmental conditions:
Temperature: 20 ± 3 °C
Humidity: 30 - 70 % 12-hour light/dark cycle
Study design and methods:
In life dates: 2009-10-26 to 2009-11-06
Animal assignment and treatment:
Approximately 24 hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.
A dose of 500 mg of the test item was applied to the test site (area: approx. 6 cm^2).
The test item was applied to the test site and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control.
Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers.
At the end of the exposure time no residual test item had to be removed. As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours.
As neither a corrosive effect nor a severe irritant effect was observed after a four hour exposure, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72 hours after patch removal. None of the three rabbits showed any significant test item-related lesions at these examination time points.
Conclusion by the Notifiers
Under the present test conditions, none of three rabbits exposed for 4 hours to 500 mg Glyphosate TC/patch (semi-occlusive conditions) showed any test item-related changes. There were no systemic intolerance reactions.
According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the

approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under
the present test conditions, Glyphosate TC was non - irritating to skin, hence, no labelling is required.
Comment by RMS:
The study is considered acceptable and the tested substance not irritant.
Reference: IIA, 5.2.4/06
Report: 2009, Glyphosate – Acute Dermal Irritation Study in Rabbits
Report No..: 12173-08
Date: 2009-03-11, unpublished ASB2012-11423
Guidelines: US EPA OPPTS 870.2500 Equivalent to OECD 404 (2002).
Deviations: Humidity was in the range of 43-92% instead of 30-70%. This deviation did not affect the study outcome
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate Tech Grade Mixed 5-Batch
Description:
White powder
Lot/Batch #:
080704-1 thru 5
Purity:
96.4%
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Deionised water
Test animals:
Species:
Albino rabbit
Strain:
New Zealand White
Source:
Age:
Approx. 3 months
Sex:
1 male and 2 females (nulliparous and non-pregnant)
Weight at dosing:
Male: 2.000 kg; Females: 2.600 kg
Acclimation period:
5 days
Diet/Food:
PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily
Water:

Tap water, ad libitum
Housing:
Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:
Temperature: 20 ± 3 °C
Humidity: 30 - 70 % Air changes: 10 - 12/hour 12-hour light/dark cycle
Study design and methods:
In life dates: 2008-11-03 to 2008-11-14
Animal assignment and treatment:
Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 x 8 cm. Only those animals with exposure areas free of pre-existing skin irritation or defects were selected for testing. A single intact exposure site was selected as the test site while the contralateral intact site served as a control site.
On Day 0, 500 mg of test substance moistened with 0.2 mL of deionized water was applied to each test site and covered with a 4 ply, 2.5 x 2.5 cm surgical gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopedic stockinette) which was secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance. After four hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The test sites were observed for erythema and edema formation, and any other dermal defects or irritation at 1, 24, 48 and 72 hours after unwrap.
Body weight: Body weight gain was unaffected by the administration of the test substance.
Conclusion by the Notifiers
The primary irritation index of 0.0 out of a possible 8.0 was obtained from the 1, 24, 48 and 72 hour observations and was used to give Glyphosate a descriptive rating of non-irritating. Based on the 72-hour observations only, Glyphosate is assigned to Toxicity Category IV.
Comment by RMS:
The study is considered acceptable and the tested substance not irritant.
Reference: IIA, 5.2.4/07
Report: 2005, Glyphosate Acid Technical – Primary Skin Irritation Study in Rabbits
Report No.: PSL 15278
Date: 2005-04-04, unpublished ASB2012-11424
Guidelines: US EPA OPPTS 870.2500 Equivalent to OECD 404 (2002).
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate Acid Technical
Description:

White crystalline powder
Lot/Batch #:
040205
Purity:
97.23 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
Distilled water
Test animals:
Species:
Rabbit
Strain:
New Zealand albino
Source:
Age:
Young adult
Sex:
Male
Weight at dosing:
No data given in the report.
Acclimation period:
21 days
Diet/Food:
Pelleted Purina Rabbit Chow #5326
Water:
Tap water, ad libitum
Housing:
The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent Guide for the Care and Use of Laboratory Animals DHEW (NIH). Litter paper was placed beneath the cage and was changed at least three times per week
Environmental conditions:
Temperature: 18-22 °C
12-hour light/dark cycle
Study design and methods
In life dates: 2004-05-05 to 2004-05-08
Animal assignment and treatment:
On the day before application, a group of animals was prepared by clipping (Oster model #A5-small)
the dorsal area and the trunk. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy animals without preexisting skin irritation were selected for test. Prior to application, the test substance was moistened with distilled water to achieve a dry paste by preparing a 70 % w/w mixture. Five-tenths of a gram of the test substance (0.71 g of the test mixture) was placed on a l-inch x l-inch, 4-ply gauze pad and applied to one 6-cm^2 intact dose site on each animal. The pad and entire trunk of each

animal were then wrapped with semi-occlusive 3-inch Micropore tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.
After 4 hours of exposure to the test substance, the pads and collars were removed and the test sites were gently cleansed of any residual test substance. Individual dose sites were scored according to the Draize scoring system at approximately 1 , 24, 48, and 72 hours after patch removal.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: All animals appeared active and healthy. Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. Two of three sites were free from irritation throughout the study. One hour after patch removal, one animal exhibited very slight erythema. Dermal irritation cleared from this animal by 24 hours.
Conclusion by the Notifiers
Under the conditions of this study, Glyphosate Acid Technical is classified as slightly irritating to the skin.
Comment by RMS:
The study is considered acceptable. The test substance provoke very slight erythema in one animal one hour after patch removal, which was cleared within 24 hours. According to current EU and GHS criteria classification as 'irritating to skin' is not warranted.
Reference: IIA, 5.2.4/08
Report: 2008, Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical
Report No.: RF-3996.311.476.07
Date: 2008-09-23, unpublished ASB2012-11425
Guidelines: OECD 404 (2002).
Deviations: The experimental phase initiation and experimental phase conclusion dates were updated
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate Technical
Description:
Solid
Lot/Batch #:
20070606
Purity:
98.05 %
Stability of test compound:
No data given in the report.
Vehicle and/
or positive control:
Deionised water

Test animals:
Species:
Rabbit
Strain:
New Zealand White
Source:
Age:
17 weeks
Sex:
Female
Weight at dosing:
2.907 - 3.145 kg
Acclimation period:
5 to 6 days
Diet/Food:
Pelleted and autoclaved commercial diet for rabbits (Guabi, Mogiana Alimentos S.A. - Brazil)
Water:
Tap water, ad libitum
Housing:
The animals were housed individually in galvanized
steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.
Environmental conditions:
Temperature: 17 - 22 °C
Humidity: 30 - 70 % Air changes: 10 - 15/hour
12-hour light/dark cycle
Study design and methods
In life dates: 2008-05-20 to 2008-05-24
Animal assignment and treatment:
Each animal provisionally selected for the test was prepared by clipping the fur from the back approximately 24-hr prior to the application of the test item, using a small animal clipper (Oster model Golden A5, Electric Razor) with great care taken to avoid abrading the skin during the clipping procedure, so as not to alter its permeability. The clipped area was large enough to allow clear visualisation of the test site. After being clipped, visual examination of the skin confirmed the skin was intact and healthy. 0.5 g of the test item was applied over the skin of each animal. The test item was first placed onto a moistened gauze dressing, which was applied over a small section of the test area (approximately 6 cm^2) in such a manner that there was good contact and uniform distribution of the test item on the skin. After application, the gauze was held in the test site by an adhesive and non-irritating tape. Removal and ingestion of the test item was prevented by placing a suitable adhesive tape (semi-occlusive dressing) around the trunk and test area. Adjacent untreated shaved areas of the skin were used as the negative control. After the 4-hour exposure period, the gauze patches were removed, any residual test item washed using physiological saline and then the treated areas examined for signs of irritation at specified intervals. The test was performed initially using one single animal for evaluation of any irritant/corrosive effect of the test item to the skin. Because no severe dermal reaction was observed in the initial test, two additional animals were tested to confirm the response.

Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Animals' skin was examined for signs of erythema, eschars and edema formation at approximately 1, 24, 48 and 72 hours after removal of the patch. No signs of dermal irritation were observed at any of the time points in any of the animals tested.
Body weight: All animals presented gain in body weight during the observation period.
Conclusion by the Notifiers:
Under the test conditions, the test item Glyphosate technical when applied on the skin of the rabbits did not cause any dermal irritation. No treatment-related behavioral or clinical alterations were noted during the observation period.
Comment by RMS:
The study is considered acceptable and the test substance not irritant.
Reference: IIA, 5.2.4/09
Report: 1988b, Primary Dermal Irritation
Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits
Data owner: Monsanto Monsanto Report No.: FD-88-29 Date: 1988-06-08, Unpublished Z35394
Guidelines: US EPA 81-5
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate
Description:
White powder
Lot/Batch #:
XLI-55
Purity:
97.76 %
Stability of test compound:
Stored at room temperature
Vehicle and/or positive control:
Physiological saline
Test animals:
Species:
Rabbit
Strain:
New Zealand White
Source:
Age:
Young adult
Sex:
Three males and three females
Weight at dosing:
Between 2 and 3 kg

Acclimation period:
At least five days
Diet/Food:
NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Inc., Gardners, PA, US), ad libitum
Water:
Tap water, ad libitum
Housing:
Individually in stainless steel mesh cages
Environmental conditions:
Temperature: 20 – 23.9 °C
Humidity: 40 - 60 % Air changes: Not specified
Light cycle: 12 hours light/dark cycle
Study design and methods:
In life dates: 1998-04-11 to 1998-04-14
Animal assignment and treatment:
Six healthy animals weighing between two and three kilograms were selected randomly from the acclimated colony and assigned to the test group. Selection suitability was based on health, weight requirement and of dorsal skin for testing. The fur on the back of each rabbit was clipped with an electric clipper on the day prior to dose administration. The test article (0.5 g moistened with 0.5 mL physiological saline) was applied topically to each of two intact dorsal test sites per rabbit. Immediately after dosing, the test sites were semi–occluded with a one–inch square gauze patch held in place with tape. The animals were collared during the exposure period to prevent removal of the patches. The patches and collars were removed four hours after dose administration and the exposure sites gently wiped with gauze to remove as much non-absorbed test article as possible. Dermal irritation was evaluated at 0.5, 24, 48 and 72 hours after patch removal. Erythema and edema were scored separately according to the Draize method. The animals were observed twice daily for mortality at least five hours apart. Body weights were obtained on study day 1 prior to dose administration. At study termination, the animals were euthanised by intra- cardiac injection of sodium pentobarbital and discarded.
A mean primary irritation score was calculated at each scoring interval from individual scores obtained from the test animals. Six individual animal scores were calculated from dermal irritation readings taken at 0.5, 24, 48 ad 72 hours after patch removal. Individual animal scores were obtained at each scoring interval by adding the total erythema and eschar formation scores from both application sites to the total edema formation scores from both sites and dividing by two. The mean of the six individual animal scores represents the mean primary irritation score at each interval.
Results and discussion
Mortality: No mortality occurred. Clinical observations: Not reported. Body weight: Not reported.
Necropsy: No necropsy was performed.
Skin observations: No dermal irritation was noted following test substance application.
Conclusion by the Notifiers:
Based on the EU classification criteria, glyphosate is not to be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate is also not classified for skin irritation.
Comment by RMS:
The study is considered acceptable and the test substance not irritant.
Reference:

IIA, 5.2.4/10
Report:
1979, Primary
Dermal Irritation in Rabbits Data owner: Monsanto
Monsanto Report No.: BND-77-428 Date: 1979-08-06, Unpublished Z35544
Guidelines:
Not specified
Deviations:
Not specified
GLP:
no (pre-GLP)
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate Technical
Description:
Fine white powder
Lot/Batch #:
XLI-180
Purity:
99 %
Stability of test compound:
Not specified
Vehicle and/or positive control:
Distilled water
Test animals:
Species:
Rabbit
Strain:
New Zealand White
Source:
Age:
Not specified
Sex:
Three males and three females
Weight at dosing:
2.25 – 2.80 kg
Acclimation period:
Not specified
Diet/Food:
Not specified
Water:
Not specified
Housing:

Not specified
Environmental conditions:
Temperature: Not specified Humidity: Not specified Air changes: Not specified Light cycle: Not specified
Study design and methods:
In life dates: Not specified.
Animal assignment and treatment:
Six albino rabbits were closely clipped over the back and sides with an electric clipper. There were four test sites per rabbit, each site 1" x 1" in area. Two sites, one on each side of the spinal column were abraded, while the remaining two sites were left intact. The abrasions were sufficiently deep so as to penetrate the stratum corneum, but not so deep as to disturb the derma or produce bleeding. The test material was administered as a 25 % w/v solution in distilled water. In all cases
0.5 mL of the test substance was applied beneath a surgical gauze square, 1" x 1", eight single layers thick, placed directly on the test site and secured with tape. The animals were then wrapped with plastic sheeting secured with masking tape to help contain the test material. After 24 hours the sheeting and gauze patches were removed.
Observations for signs of dermal irritation or systemic toxicity were recorded at 24 and 72 hours after application. At each observation all treated sites were scored for erythema, edema and eschar formation
Results and discussion
Mortality: No mortality occurred. Clinical observations: Not reported. Body weight: Not reported.
Necropsy: No necropsy was performed.
Skin observations: In the intact skin, one animal had very slight erythema (score of 1) at 24 hours following test substance application. There was no other irritation noted for intact skin at 24 or 72 hours.
Conclusion by the Notifiers
Based on the EU classification criteria, glyphosate should not be classified for skin irritation. According to the OECD Globally Harmonized System (GHS) classification criteria glyphosate is also not classified for skin irritation.
Comment by RMS:
The study is considered not acceptable due to several deficiencies (pre-GLP, no guideline, observation period too short, clinical signs & body weight not reported).
Reference: IIA, 5.2.4/11
Report: 1996, Glyphosate Acid: Skin Irritation to the Rabbit Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK
Data owner: Syngenta Report No.: /P/4695
Date: 1996-08-23, not published TOX2000-1985
Guidelines: OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4
(2004)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test Material:
Glyphosate acid
Description:

Technical, white solid
Lot/Batch number:
P24
Purity:
95.6 % w/wi
CAS#:
Not reported
Stability of test compound:
The test substance was used within the expiry date
Vehicle and/or positive
control:
Deionised water (for moistening)
Test Animals:
Species
Rabbit
Strain
New Zealand White albino
Age/weight at dosing
Young adult / 3001-4386 g
Source
.
Housing
Individually in aluminium sheet cages in racks suitable for animals of this strain and the weight range expected during
the course of the study.
Acclimatisation period
At least 6 days
Diet
STANRAB SQC, (Special Diet Services Limited, Stepfield,
Witham, Essex, UK) ad libitum
Water
Mains water ad libitum
Environmental conditions
Temperature: 17+2 °C
Humidity: 40-70 %
Air changes: Approximately 25/hour Photoperiod: 12 hours light / 12 hours dark
Study design and methods
In-life dates: Start: 9 March 1995 End: 28 April 1995
Animal assignment and treatment: In a primary skin irritation study, Glyphosate acid (95.6 % w/w) was applied by semi-occlusive application of 500 mg to the intact skin of the left flank of each of six female, young adult New Zealand White rabbits.
Approximately one day before treatment, the left flank was clipped with an electric clipper, exposing an area of approximately 7 cm x 13 cm.
On the day of treatment, 500 mg of glyphosate acid (95.6 % w/w) (moistened with approximately 0.5 mL deionised water) was applied to the test site (approximately 2.5 cm x
2.5 cm) on the left flank of the rabbit. The treated area was covered with a piece of 8-ply surgical

gauze (approximate size 2.5cm x 2.5cm), which was secured by two strips of surgical tape (approximate size 1 cm x 8cm). This was covered by a piece of impermeable rubber sheeting (approximate size 35cm x 13cm) wrapped once around the trunk of the animal and secured with adhesive impermeable polyethylene tape (7.5cm wide).

The dressings were left in position for approximately four hours. The application site was gently cleansed free of any residual test substance using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

The Draize scale was used to assess the degree of erythema and oedema at the application sites approximately 30-60 minutes, 1, 2 and 3 days after removal of the dressings. Any other signs of skin irritation were also noted.

Results and discussion

There were no signs of ill-health and no signs of skin irritation in any animal during the study.

Table B.6.2-22: Individual and mean skin irritation scores of glyphosate acid according to the Draize scheme

Time
Erythema
Oedema
Animal number
1
2
3
4
5
9
1
2
3
4
5
9
after 1 hour
0
0
0
0
0
0
0
0
0
0
0
0
after 24 hours
0
0

0
0
0
0
0
0
0
0
0
0
after 48 hours
0
0
0
0
0
0
0
0
0
0
0
0
after 72 hours
0
0
0
0
0
0
0
0
0
0
0
0
0
mean score 24-72 h
0
0
0
0
0
0
0
0
0
0

0
0
0
Conclusion by the Notifiers:
Glyphosate acid is non-irritant following a single four-hour application to rabbit skin.
Comment by RMS:
The study is considered acceptable and the test substance is considered not irritant.
Reference: IIA, 5.2.4/12
Report: 2007, Glyphosate Technical Material: Primary Skin Irritation Study In Rabbits (4-Hour Semi-Occlusive Application)
Data owner: Syngenta Report No.: R61837/1010
Date: 2007-02-08, not published ASB2012-11426
Guidelines: OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4
(2004)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test Material:
Glyphosate Technical Material
Description:
Technical, white powder
Lot/Batch number:
0507
Purity:
96.1 % w/w glyphosate acid
CAS#:
Stability of test compound:
Stable under storage conditions of room temperature (range of
20 oC C 5 oC), protected from light and humidity.
Vehicle and/or positive
control:
The test substance was moistened with purified water before
application.
Test Animals:
Species
Rabbit
Strain
New Zealand White (SPF)
Age/weight at dosing
Male: 10-11 weeks / 2440 g; Females: 15-16 weeks / 2749
and 2815 g
Source
Housing
Individually in stainless steel cages equipped with feed
hoppers and drinking water bowls.

Acclimatisation period
5/6 days
Diet
Pelleted standard Provimi Kliba 3418 rabbit maintenance diet
ad libitum.
Water
Community tap water ad libitum
Environmental conditions
Temperature: 17-23 °C
Humidity: 30-70 %
Air changes: 10-15 per hour.
Photoperiod: 12 hours light, 12 hours dark.
Study design and methods
In-life dates: Start: 18 December 2006 End: 22 December 2006
Animal assignment and treatment: Three young adult (1 male and two female) New Zealand White rabbits were used in the study. As it was suspected that the test substance might produce irritancy, a single animal (one female) was treated first. As no corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals for an exposure period of 4 hours.
Four days before treatment, the left flank was clipped, exposing an area of approximately 100 cm2 (10 cm x 10 cm). The skin of the animals was examined one day before treatment, and regrown fur of all animals was clipped again. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.
On the day of treatment, 0.5 g of Glyphosate Technical Material was placed on a surgical gauze patch (2.5 cm x 2.5 cm). This gauze patch was applied to the intact skin of the clipped area. The patch was covered with a semi-occlusive dressing which was was wrapped around the abdomen and anchored with tape.
The duration of treatment was 4 hours after which the dressing was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible at that time.
Observations for viability, mortality and clinical signs were carried out daily from acclimatisation of the animals to the termination of the study. Bodyweights of individual animals were recorded at the start of the acclimatisation period, on the day of application and at termination of the observation period.
The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, approximately 1, 24, 48 and 72 hours, after removal of the dressing, gauze patch and test substance. To evaluate the irritation potential of the test substance, the mean values of erythema/eschar and oedema formation were calculated for each animal using the scores between 24 and 72 hours. The Primary Irritation Index (P.I.I.) was calculated by adding together the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of available figures.
Results and discussion
No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. The test substance did not elicit any skin reactions at the application site of any animal at any of the observation times (all scores 0). The individual mean score for erythema/eschar and oedema for each of the three animals was therefore 0. No staining produced by the test item of the

treated skin was observed and no alterations of the treated skin or corrosive effects were evident. The bodyweights of the rabbits were considered to be within the normal range of variability.
Conclusion by the Notifiers:
The application of Glyphosate Technical Material to the intact skin resulted in no signs of irritation. According to Draize classification criteria Glyphosate Technical Material is considered to be “not irritant” to rabbit skin (P.I.I. = 0.00).
Comment by RMS:
Under the present conditions the study is considered acceptable and the tested substance not irritant.
Reference: IIA, 5.2.4/13
Report: 2011, Glyphosate technical: Primary skin irritation study in rabbits
Data owner: Syngenta Report No.: 10/218-006N Date: 2011-04-13
not published ASB2012-11427
Guidelines: OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)
Deviations: None
GLP: yes

Acceptability: Please see comment by RMS
Materials and methods
Test Material:
Glyphosate technical
Description:
Technical, dry white powder
Lot/Batch number:
569753 (BX20070911)
Purity:
96.3 % w/w Glyphosate technical
CAS#:
Not reported
Stability of test compound:
Stable under storage conditions (room temperature range <30 °C), recertification date end August 2011
Vehicle and/or positive control:
None
Test Animals:
Species
Rabbit
Strain
New Zealand White
Age/weight at dosing
Approximately 12 weeks / 2995-3095 g
Source
Housing
Individually in metal cages

Acclimatisation period
5 days
Diet
Purina Base – Lap gr. diet (AgribrandsEurope Hungary PLC,
H-5300 Karcag, Madarasi út, Hungary) ad libitum
Water
Municipal tap water ad libitum
Environmental conditions
Temperature: 17-20 °C
Humidity: 30-70 %
Air changes: 15-20/hour
Photoperiod: 12 hours light/12 hours dark
Study design and methods
In-life dates: Start: 02 November 2010 End: 05 November 2010
Animal assignment and treatment: In a primary dermal irritation study, three male, young adult, New Zealand White rabbits were each given a dermal application of 0.5 g of undiluted Glyphosate Technical (96.3 % w/w Glyphosate technical).
Approximately 24 hours prior to the test the hair was clipped from the back and flanks of the animals with an electric clipper, exposing an area approximately 10 cm x 10cm. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.
On the day of treatment, 0.5g of glyphosate technical was placed on a surgical gauze pad (approximately 2.5 cm x 2.5 cm). This gauze pad was applied to the intact skin of the clipped area and was kept in contact with the skin by a patch with a surrounding adhesive hypoallergenic plaster. The entire trunk of the animals was then wrapped with plastic wrap held in place with an elastic stocking. The dressing was left in place for 4 hours after which it was removed and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible.
As it was suspected that the test item might produce irritancy, a single animal was treated first. As no corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals.
The animals were checked daily for signs of systemic toxicity and mortality. Body weights were recorded on the day of application and at termination of observations.
The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004, which was based on the Draize scoring system approximately 1, 24, 48 and 72 hours after the removal of the dressing, gauze patch and test item. The mean score was calculated across 3 scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately. An animal was positive when the mean score was 2 or greater. The test was positive for irritation when at least 2 animals were positive for the same endpoint (erythema/eschar or oedema).
The Cumulative Scores for the Skin Irritation Scores were calculated and represent the sum of all numerical scores for each animal at each time point. The resulting Mean Cumulative Skin Irritation Score was calculated for all animals at each time point.
The Primary Irritation Index (P.I.I.) was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of available figures.
Results and discussion

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. The bodyweights of all rabbits were considered to be within the normal range of variability. There was no staining of the skin, alterations or corrosive effects.
At the observations 1 and 24 hours after patch removal, very slight erythema (score 1) was observed in one animal. No signs of irritation were observed in the other treated animals throughout the study.
As no signs of irritation were observed 72 hours after patch removal, the study was terminated after the 72 hour observation.
Table B.6.2-23: Individual and mean skin irritation scores of glyphosate technical
Time
Erythema
Oedema
Animal number
606
622
620
606
622
620
after 1 hour
0
1
0
0
0
0
after 24 hours
0
1
0
0
0
0
after 48 hours
0
0
0
0
0
0
after 72 hours
0
0
0
0
0

0
mean score 24-72 h
0
0.3
0
0
0
0
Conclusion by the Notifiers:
According to the Draize classification criteria, glyphosate technical is considered to be a “mild-irritant” to rabbit skin (P.I.I. = 0.11).
Comment by RMS:
The study is considered acceptable. The test substance provoke slight erythema in one animal one hour after patch removal, which was cleared within 48 hours. According to current EU and GHS criteria classification as ‘irritating to skin’ is not warranted.
Comment by GTF on the first draft of the RAR (July 2013):
RMS stated: “Three of the new studies revealed mild or slight irritating effects. GTF: non-irritant to inact skin and only slightly irritant to abraded skin.
RMS comment (August 2013):
It is clearly stated that no classification and labelling is necessary and that in the majority of studies no evidence of skin irritation was obtained. Furthermore, studies conducted by
(2005, ASB2012-11424) and (2011, ASB2012-11427) showed slight or mild effects at intact skin.

B.6.2.5 Eye irritation
Eye irritation was examined in numerous studies in rabbits that were performed either with the acid or its different salts. In additon to the previous evaluation a huge number of further studies were provided for the current re-evaluation. To conclude on all study results, glyphosate acid was tested to be a strong eye irritant and classification and labelling (Xi/R41 or Cat. 1, H318) is needed.
Table B.6.2-24: Summary of eye irritation studies with glyphosate acid
Reference (Data owner)
Species Strain
Number and /or
sex of animals
Purit y [%]
Amount applied
Exposure conditions
Effects / Result
Studies from the 2001 evaluation
Annex B- 5.2.6.1,
Glyphosate Monograph
1994
(Herbex) TOX9500249
Rabbit NZW
1 ♀
95
0.1 mL

Due to strong ocular effects the test was stopped for humane reasons after 1 hour
irritant
Annex B- 5.2.6.1,
Glyphosate Monograph
Rabbit NZW
4 ♀
97.2
100 mg (pure)
Significant ocular lesions, especially chemosis irritant
1994
(Alkaloida) TOX9650146
Annex B- 5.2.6.1,
Glyphosate Monograph
1991 (ADM) TOX9551093
Rabbit NZW
2 ♂, 1 ♀
96.8
100 mg
Mortality (1♀: severe enteritis and opacity); significant ocular lesions that were not reversible within 3 weeks
moderately irritating
Annex B- 5.2.6.1,
Glyphosate Monograph
1991 (I. Pi.
Ci.) Z101610
Rabbit NZW
3 ♂
98
100 mg (pure)
Several ocular effects (score 1 & 2) that were reversible within 3 days
slightly irritant
Studies from the 2001 evaluation
Annex B- 5.2.6.1,
Glyphosate Monograph
1990 (AGC)
TOX9500264
Rabbit NZW
3 ♀
98.1
100 mg
Corneal opacity not reversible within 7 days (2♀), iris lesions not reversible within 6 days (1♀), conjunctivcal redness not reversible within 6 days (3 ♀) slightly irritant
Annex B-
5.2.6.1,
Glyphosate Monograph
J , 1989 (CHE) TOX9552338

Rabbit
NZW
1 ♂
98.6
100 mg (pure)
Due to strong ocular effects the
test was stopped for humane reasons after treatment of the first of six animals moderately to severely irritating
IIA 5.2.5
1996 (CHE) TOX1999-881
Rabbit
NZW
6 ♂, 3 ♀
98.2
65 mg
one group (3♂, 3 ♀) eyes were not washed; one group (3♂) eyes were washed 30
sec after treatment
Severely irritating (washed
eyes)
moderately irritating (non- washed eyes)
Reference (Data owner)
Species Strain
Number and /or
sex of animals
Purit y [%]
Amount applied
Exposure conditions
Effects / Result
Studies not reviewed in the 2001 evaluation
IIA 5.2.5/01
2007 (NUF) ASB2012- 11428
Rabbit NZW
1 ♂ 2 ♀
95.1
100 mg
Marked, early-onset and transient ocular changes, like very slight to slight corneal opacity, slight to marked conjunctival redness, conjunctival chemosis, reddening of the sclera and discharge. All eye effects were reversible within 10 days after instillation. No signs of corrosion or staining were observed in any eye.
irritating
IIA 5.2.5/02
2009 (HAG) ASB2012- 11429
Rabbit Himalaya n
3 ♂
96.4
100 mg (eyes were

rinsed 1 h post- application) (study considered supplementary)
slight and fully reversible ocular changes (≤ grade 1); all eye effects were reversible within 8 days after instillation. No signs of corrosion or staining were observed in any eye
Non irritant
IIA 5.2.5/03
1995 (ALS) ASB2012- 11430
Rabbit NZW
12 ♀
97.56
100 mg (pure) (one group without irrigation of eyes, two groups with irrigation of eyes at different time intervals after
application)
Severely irritating; animals in the irrigation groups showed reduced eye irritation and faster recovery
IIA 5.2.5/04
2009
(EXC) (Expert statement) ASB2012-
11431
Rabbit
Not applicable
96.66
Test solution: 1% in purified water
Not performed since pH of test item solution was < 2 (corrosive)
Studies not reviewed in the 2001 evaluation
IIA 5.2.5/05
2009 (HAG) ASB2012- 11432
Rabbit Himalaya n
3 ♂
98.8
100 mg (eyes were
rinsed 1 h post- application) (study considered
supplementary)
Non irritant
IIA 5.2.5/06
2010 (HAG) ASB2012- 11433
Rabbit
Himalaya n
3 ♂
97.3
100 mg
(eyes were rinsed 1 h post- application) (study considered
supplementary)
Non irritant
Reference (Data owner)
Species Strain
Number and /or
sex of animals

Purit y [%]
Amount applied
Exposure conditions
Effects / Result
IIA 5.2.5/07
2009 (HAG)
ASB2012- 11434
Rabbit NZW
2♂, 1 ♀
96.4
0.1 mL (93.2
mg)
Irritant
IIA 5.2.5/08
2005 (HAG) ASB2012-
11435
Rabbit
NZW
3 ♂
97.23
0.1 mL (60 mg)
Irritant
IIA 5.2.5/09
Rabbit
NZW
1 ♂ 2 ♀
98.05
100 mg
Severely irritating
2008 (HAG) ASB2012- 11436
IIA 5.2.5/10
, 1988 (MON) Z35395
Rabbit
NZW
97.76
Produced corneal opacity and
conjunctival irritation with blistering, irritation persisted in 4/5 rabbits on day 21
IIA 5.2.5/11
1997
(SYN) TOX2000-1986
Rabbit
NZW
6 ♀
95.6
100 mg
Irritant

IIA 5.2.5/12
2007 (SYN) ASB2012- 11437
Rabbit
NZW
1 ♂ 2 ♀
96.1
100 mg
Slight conjunctival redness,
conjunctival chemosis, reddening of the sclera and discharge were observed. Effects reversible and no
longer evident 7 days after treatment.
IIA 5.2.5/13
2011 (SYN) ASB2012-
11438
Rabbit
NZW
1 ♂
96.3
100 mg
Corrosive. Due to strong ocular
effects the test was stopped for humane reasons after 24 hours.
NZW = New Zealand White
Table B.6.2-25: Summary of eye irritation studies with glyphosate salts
Reference (Data owner)
Species Strain
Number and /or sex
of animal TOXs /
Purity [%]
Amount applied /
Exposure conditions
Results
Studies from the 2001 evaluation
Annex B- 5.2.6.2,
Glyphosate Monograph
1994 (MON / CHE) TOX9552340
Rabbit NZW
1 ♂, 5 ♀
IPA 62%
0.1 mL (pure) (pre-treatement with local anaesthetic)
Iris, conjunctivae: redness & chemosis within 1 h; discharge after 1 h
Slightly irritating
Annex B-
5.2.6.2,
Glyphosate Monograph
Rabbit NZW
3 ♂, 3 ♀

IPA
62
0.1 mL; ♂: eyes
unrinsed; ♀: eyes rinsed
Conjunctival redness (24 h) Slightly irritating
1989 (I.Pi.Ci.) TOX9551629
Annex B-
5.2.6.2,
Glyphosate Monograph
1987 (MON/CHE) TOX9552342
Rabbit NZW
6
NH4-
salt 90.8
0.1 g
conjunctivae: redness &
chemosis (48h); discharge in all animals within 48 h mucous membrane appearing blistered in all animals after 1 h
Slightly irritating
NZW = New Zealand White
Tier II summaries are only presented for studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation please refer to the Monograph.
Reference: IIA, 5.2.5/01
Report: 2007, Glyphosate Technical (NUP 05068): Primary Eye Irritation Study In Rabbits
Study No.: B02305 Data owner: Nufarm
Date: 2007-03-06, unpublished ASB2012-11428
Guidelines: OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004),
JMAFF guideline 2-1-5 (2005)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate technical
Identification:
NUP 05068
Description:
Solid
Lot/Batch #:
200609062
Purity:
95.1 %
Stability of test compound:
Stable under storage conditions (20 ± 5 °C), light protected; Expiry date: 2008-09-14
Vehicle and/or positive control:

None
Test animals:
Species:
Rabbit
Strain:
New Zealand White, SPF
Source:
Age:
15 weeks (male); 12 and 15 weeks (females)
Sex:
One male and 2 females
Weight at dosing:
2.969 kg (male), 2.605 kg and 3.416 kg (females)
Acclimation period:
At least five days
Diet/Food:
Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust), ad libitum
Water:
Tap water, ad libitum
Housing:
Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blooks and haysticks were provided for gnawing.
Environmental conditions:
Temperature: 17 - 23 °C
Humidity: 30 - 70 % Air changes: 10 - 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 2007-01-17 to 2007-02-26
Animal assignment and treatment:
The test was conducted using young adult New Zealand albino rabbits (1 male, 2 females). The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the left eye of the rabbits. The lids were then gently held together for about one second. The treated eyes were not rinsed after instillation. The right eye remained untreated and served as the reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after instillation. Scleral reddening and ocular discharge was also assessed. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.
Results and discussion
Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study. Body weight: All rabbits showed the expected body weight gain.
Necropsy: No necropsy was performed.
Eye observations: Very slight to slight corneal opacity were observed in all rabbits from 1 hour after

instillation up to 72 hours. No signs of iritis, corrosion or staining were observed in any animal throughout the study period. One hour after instillation slight to moderate conjunctival redness was observed in the treated eyes of all rabbits. By 24 hours the redness increased to marked in two animals and to moderate in one rabbit. After 48 hours the conjunctival redness decreased in all rabbits. Only slight redness was observed in the rabbits after 7 days. Moderate to marked chemosis of the conjunctivae was observed from 1 hour after instillation up to 24 hours. The swelling decreased with time. 72 hours after treatment slight swelling was still present in two animals. One hour after instillation two rabbits exhibited moderate ocular discharge. Moderate or marked discharge was observed in all animals at the 24-hour reading time point. This persisted at the 48-hour reading as slight or moderate in all rabbits. After 72 hour slight discharge was still present in one rabbit.

Reddening of the sclera was observed in all animals. However, one hour after instillation sclera of one animal was not assessable due to conjunctival swelling. In two animals moderate or marked reddening of the sclera was observed at this time point. After 24 hours all rabbits showed marked reddening of the sclera. This sign persisted in the rabbits as moderate or marked at the 48- and 72-hour readings. In one animal slight reddening was still present after 7 days. All rabbits were free of ocular signs by day 10 after instillation.

The group mean irritation scores (24 to 72 hours) were calculated to be 1.5 for corneal opacity, 0.0 for iris lesions, and 2.2 conjunctival redness, and 1.7 for chemosis of the conjunctiva.

The individual scores for each time point, individual mean and group mean scores (24 to 72 hours) are presented in Table B.6.2-26.

Table B.6.2-26: Results of the eye irritation test

Animal	Scoring [h]	Cornea	Iris	Conjunctiva	Sclera
		Opacity	Area	Redness	Chemosis
Rabbit 1 (male)					
	1	1	1	0	2
	24	2	2	0	3
	3	3			

3
48
2
2
0
3
2
3
72
1
1
0
2
1
2
Day 7
0
0
0
1
0
1
Day 10
0
0
0
0
0
0
Day 14
0
0
0
0
0
0
Mean (24, 48, 72 h)
1.67

0.0
2.67
2.0

Rabbit 2 (female)
1
1

1
0
1
2
2
24
2
4
0
2
3
3
48
2
4
0
2
2
3
72
2
4
0
2
1
2
Day 7
0
0
0
1
0
0
Day 10
0
0
0
0
0
0
Day 14
0
0
0
0
0

0
Mean (24, 48, 72 h)
2.0

0.0
2.0
2.0

Rabbit 3 (female)
1
1
4
0
2
3
n.a.
24
1
4
0
3
2
3
48
1
4
0
2
1
2
72
0
0
0
1
0
2
Day 7
0
0
0
1
0
0
Day 10
0

0
0
0
0
0
Day 14
0
0
0
0
0
0
Mean (24, 48, 72 h)
0.67
--
0.0
2.0
1.0

Group means
1.5

0.0
2.2
1.7
---
n.a. = not assessable due to swelling of the conjunctivae
Conclusion by the Notifiers
Based on the study results and on the and according to EU classification criteria the test substance glyphosate technical (NUP 05068) is to be classified as irritating to the eyes (Xi, R36). This corresponds to Category 2A according to the OECD Globally Harmonized System (GHS) classification criteria.

Comment by RMS:
The study is considered acceptable and the results revealed that glyphosate acid is irritant to rabbit eyes..
Reference: IIA, 5.2.5/02
Report: , 2009, Acute Eye Irritation/Corrosion Test Of Glyphosate TC In Rabbits
Data owner: Helm AG Report No.: 24878
Date: 2009-11-27, unpublished ASB2012-11429
Guidelines: OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004),
OPPTS 870.2400 (1998)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods

Test material:
Identification:
Glyphosate TC
Description:
White powder
Lot/Batch #:
2009051501
Purity:
96.4 %
Stability of test compound:
At room temperature in the dark stable until May 15, 2011.
Vehicle and/or positive control:
None
Test animals:
Species:
Rabbit
Strain:
Himalayan
Source:
Age:
Approx. 6.5 - 7.5 months
Sex:
Males
Weight at dosing:
2.5 - 2.8 kg
Acclimation period:
At least 20 days.
Diet/Food:
ssniff K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), ad libitum before and after the exposure period
Water:
Tap water, ad libitum before and after the exposure period
Housing:
Individual housing
Environmental conditions:
Temperature: 20 ± 3 °C
Humidity: 30 - 70 % Air changes: no data 12 hours light/dark cycle
Study design and methods
In life dates: 2009-10-15 to 2009-10-29
Animal assignment and treatment:
The test was conducted using three young male adult Himalayan albino rabbits. The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the right eye of the rabbits. The lids were then gently held together for about one second. 1 hour after instillation the eyes were rinsed with 20 mL NaCl solution. The left eye remained untreated and served as the

reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72 hours, as well as 4, 5, 6, 7 and 8 days after instillation. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of the study and at termination.
Results and discussion
Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study. Body weight: There were no effects on body weight noted.
Necropsy: No necropsy was performed.
Eye observations: Corneal opacity (grade 1) was observed in all animals 24 to 72 hours, in animal no. 2 until 4 days and in animal no. 1 until 7 days after instillation. The fluorescein test performed 24 hours after instillation demonstrated corneal staining in all animals. The fluorescein test performed 7 days after instillation demonstrated corneal staining only in animal no. 1.
Irritation of the iris (grade 1) was observed in all animals 24 hours, in animal no. 2 until 48 hours and in animal no. 1 until 72 hours after instillation.
Conjunctival redness (grade 1) was observed in all animals 60 minutes to 72 hours, in animal no. 1 until 4 days and in animal no. 2 until 5 days after instillation.
Chemosis (grade 1) was observed in all animals 60 minutes, in animal no. 2 until 24 hours and in animal no. 1 until 48 hours after instillation.
In addition, secretion was observed in all animals 60 minutes after instillation. There were no systemic intolerance reactions. All rabbits were free of ocular signs by Day 8 after instillation. The group mean irritation scores (24 to 72 hours) were calculated to be 1.0 for corneal opacity, 0.7 for iris lesions, and 1.0 conjunctival redness and 0.3 for chemosis of the conjunctiva.
The individual scores for each time point, individual mean and group mean scores (24 to 72 hours) are presented in Table B.6.2-27.
Table B.6.2-27: Results of the eye irritation test
Animal
Scoring [h]
Cornea
Iris
Conjunctiva
Redness
Chemosis
Rabbit 1
1
0
0
1
1
24
1
1
1
1
48
1

1
1
1
72
1
1
1
0
Day 4
1
0
1
0
Day 5
1
0
0
0
Day 6
1
0
0
0
Day 7
1
0
0
0
Mean (24, 48, 72 h)
1.0
1.0
1.0
0.7
Rabbit 2
1
0
0
1
1
24
1
1
1
1
48
1

1
1
0
72
1
0
1
0
Day 4
1
0
1
0
Day 5
0
0
1
0
Day 6
0
0
0
0
Day 7
—
—
—
—
Mean (24, 48, 72 h)
1.0
0.7
1.0
0.3
Rabbit 3
1
0
0
1
1
24
1
1
1
0
48
1

0
1
0
72
1
0
1
0
Day 4
0
0
0
0
Day 5
—
—
—
—
Day 6
—
—
—
—
Day 7
—
—
—
—
Mean (24, 48, 72 h)
1.0
0.3
1.0
0.0
Group means
1.0
0.7
1.0
0.3
Conclusion by the Notifiers
Based on the scores for cornea, iris and conjunctiva and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, glyphosate TC is not to be classified for eye irritation.
Comment by RMS:
The study is considered supplementary: Based on the study desgin, it is not possible to conclude, that the test substance is not irritating to rabbit eyes, because the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405. The results provided only additonal information.

Reference:
IIA, 5.2.5/03
Report:
1995, HR-001: Primary Eye irritation study in rabbits.
.
Report No.: IET 95-0034
Data owner: Arysta LifeScience Date: 1995-06-29, unpublished
ASB2012-11430
Guidelines:
U.S. EPA FIFRA Guideline Subdivision F
MAFF 59 NohSan No. 4200 (1985)
Deviations:
None
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Glyphosate technical
Identification:
HR-001
Description:
White crystal
Lot/Batch #:
T-941209
Purity:
97.56 %
Stability of test compound:
Not mentioned in the report
Vehicle and/or positive control:
None
Test animals:
Species:
Rabbit
Strain:
New Zealand White, Kbl:NZW
Source:
.
Age:
11 weeks
Sex:
females
Weight at dosing:
2.378 kg (Group A), 2.357 kg (Group B) and 2.426 kg (Group C)
Acclimation period:

Eleven days
Diet/Food:
Pellet Diet GC4 (Oriental Yeast Co., Ltd.)
Water:
Water filtrated and sterilized, ad libitum
Housing:
Individually in stainless steel cages.
Environmental conditions:
Temperature: 24 °C
Humidity: 52.8 – 57.9 % Air changes: 15/hour
12 hours light/dark cycle
Study design and methods:
In life dates: 1995-04-28 – 1995-05-30
Animal assignment and treatment:
12 female specific pathogen free New Zealand rabbits were given a single ocular instillation of 0.1 g of technical glyphosate. The dose was instilled in the conjunctival sac of left eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of test substance. The left eyes of animals in the eye treated groups were irrigating with water at 30 seconds (3 animals) or 2 minutes (3 animals) after application. The right eye remained untreated. All animals were observed for primary eye irritation 1, 24, 48 and 72 hours, 4, 7, 10, 13, 16, 19 and 21 days after instillation. The cornea, iris and conjunctive were examined with a hand slit-lamp during a subsequent 21-day observation period. Body weights were measured prior to application, and after the final observation.
Results and discussion
Mortality: No mortality occurred.
Clinical observations: No clinical signs of systemic toxicity were observed during the study. Body weight: All rabbits showed the expected body weight gain.
Necropsy: No necropsy was performed.
Eye observations: Results of the no eye irrigating group are summarised in the Table B.6.2-28.
Without eye irrigation
Irritation of cornea
At 1 hour after application, all animals showed score 2, the easily discernible translucent aera, details of iris slightly obscured. At 24 hours after application, one animal showed score 3, nacreous area, no details of iris visible, size of pupil barely discernible. These opacities remained until Day 21 in three animals.
Irritation of iris
At 1 hour after application, all animals showed score 1, the congestion and/or markedly deepened rugae of iris. The irritation disappeared by Day 10.
Irritation of conjunctivae
At 1 hour after application, all groups showed redness score 1, the definite hyperemia of some blood vessels. At 24 or 48 hours after application, score 2 redness of conjunctivae in all animals. These conjunctival irritations gradually began to decrease thereafter, and disappeared by Day 16.
At 1 hour after application, 4 animals showed chemosis score 2, the obvious swelling with partial eversion of lids. In addition, 2 animals showed score 3, the swelling with lids about half closed. The chemosis disappeared by Day 7.
With eye irrigation (30 seconds or 2 minutes after application)

The irridial and conjunctival irritations observed in irrigation group were almost the same as those in non-irrigating group, while the corneal irritation was slighter.
Animals in the irrigating groups showed reduced eye irritations and faster recovery as compared with animals of the non-irrigating group. Each of irrigation at 30 seconds or 2 minutes after application was effective for reduction of irritation and for recovery.
Table B.6.2-28: Results of the eye irritation without eye irrigation after application*
Animal
Scoring [h]
Cornea
Iris
Conjunctiva
Opacity
Area
Redness
Chemosis
Discharge
Rabbit 1 (female)
1
2
4
1
1
2
3
24
2
4
1
2
2
3
48
2
4
1
2
2
3
72
2
4
1
2
2
1
Day 4

1
3
1
2
1
0
Day 7
1
2
0
1
0
0
Day 10
1
1
0
1
0
0
Day 13
1
1
0
1
0
0
Day 16
1
1
0
0
0
0
Day 19
1
1
0
0
0
0
Day 21
1
1
0
0

0
0
Mean (24, 48, 72 h)
2.0

1.0
2.0
2.0

Animal
Scoring [h]
Cornea
Iris
Conjunctiva
Opacity
Area
Redness
Chemosis
Discharge
Rabbit 2 (female)
1
2
4
1
1
2
3
24
3
4
1
2
2
3
48
3
4
1
2
2
3
72
2
3
1

2
1
1
Day 4
2
2
1
2
1
0
Day 7
1
2
0
1
0
0
Day 10
1
2
0
1
0
0
Day 13
1
2
0
1
0
0
Day 16
1
2
0
0
0
0
Day 19
1
2
0
0
0
0
Day 21

1
2
0
0
0
0
Mean (24, 48, 72 h)
2.67

1.0
2.0
1.67

Rabbit 3 (female)
1
2
4
1
1
3
3
24
2
4
1
2
3
3
48
2
4
1
2
2
3
72
2
4
1
2
2
3
Day 4
2
3
1

2
1
0
Day 7
1
2
1
1
0
0
Day 10
1
2
0
1
0
0
Day 13
1
2
0
1
0
0
Day 16
1
2
0
0
0
0
Day 19
1
1
0
0
0
0
Day 21
1
1
0
0
0
0
Mean (24, 48, 72 h)

2.0

1.0
2.0
2.33

Rabbit 4 (female)
1
2
4
1
1
3
3
24
2
4
1
2
3
3
48
2
4
1
2
2
3
72
2
3
1
2
2
1
Day 4
2
2
1
2
1
1
Day 7
1
2
0

1
0
0
Day 10
1
2
0
1
0
0
Day 13
1
2
0
1
0
0
Day 16
0
0
0
0
0
0
Day 19
0
0
0
0
0
0
Day 21
0
0
0
0
0
0
Mean (24, 48, 72 h)
2.0

1.0
2.0
2.33

Rabbit 5 (female)

1
2
4
1
1
2
3
24
2
4
1
2
2
3
48
2
4
1
2
2
3
72
2
4
1
2
2
1
Day 4
1
2
1
2
1
0
Day 7
1
2
0
1
0
0
Day 10
1
1
0

1
0
0
Day 13
0
0
0
0
0
0
Day 16
0
0
0
0
0
0
Day 19
0
0
0
0
0
0
Day 21
0
0
0
0
0
0
Mean (24, 48, 72 h)
2.0

1.0
2.0
2.0

Animal
Scoring [h]
Cornea
Iris
Conjunctiva
Opacity
Area
Redness

Chemosis
Discharge
Rabbit 6 (female)
1
2
4
1
1
2
3
24
2
4
1
2
2
3
48
2
2
1
2
2
2
72
1
2
0
2
1
0
Day 4
1
1
0
1
0
0
Day 7
0
0
0
0
0
0
Day 10

0
0
0
0
0
0
Day 13
0
0
0
0
0
0
Day 16
0
0
0
0
0
0
Day 19
0
0
0
0
0
0
Day 21
0
0
0
0
0
0
Mean (24, 48, 72 h)

1.67

0.67
2.0
1.67

Group means
2.1

1.0

2.0
2.0
---
* - according to the report
Table B.6.2-29: Results of the eye irritation with eye irrigation (30 seconds after application)*
Animal
Scoring [h]
Cornea
Iris
Conjunctiva
Opacity
Area
Redness
Chemosis
Discharge
Rabbit 7 (female)
1
0
0
0
1
3
2
24
1
3
1
1
2
3
48
1
1
0
2
2
1
72
1
1
0
1
1
1
Day 4
0

0
0
1
0
0
Day 7
0
0
0
0
0
0
Mean (24, 48, 72 h)
1.0

0.33
1.33
1.67

Rabbit 8 (female)
1
0
0
0
1
2
2
24
1
2
0
1
2
2
48
1
1
0
1
1
1
72
0
0
0
1

0
0
Day 4
0
0
0
1
0
0
Day 7
0
0
0
0
0
0
Mean (24, 48, 72 h)
0.67

0.0
1.0
1.0

Rabbit 9 (female)
1
1
2
1
1
2
2
24
1
3
1
2
2
3
48
1
3
0
2
1
1
72

1
2
0
1
1
1
Day 4
0
0
0
1
0
0
Day 7
0
0
0
0
0
0
Mean (24, 48, 72 h)
1.0

0.33
1.67
1.33

Group means
0.9

0.2
1.3
1.3
---
* - according to the report
Conclusion by the Notifiers
Based on the study results and on the and according to EU classification criteria the test substance glyphosate technical (HR-001) is to be classified as irritating to the eyes (Xi, R36). This corresponds to Category 2 according to the OECD Globally Harmonized System (GHS) classification criteria.
Comment by RMS:
The study is considered acceptable. The test substance was irritating to rabbit eyes.
Reference: IIA, 5.2.5/04
Report: 2009, Expert Statement Glyphosate technical: Primary eye irritation study in rat
Data owner:
Excel
Report No.: C22897

Date: 2009-01-23, not published ASB2012-11431
Guidelines: OECD 405 (2002)
Council Regulation (EC) No 440/2008 (2008)
Deviations None
GLP: yes
Acceptability: Please see comment by RMS
Executive Summary
A pH measurement was performed with the test item in a 1% (w/w) solution in purified water before the study initiation. The pH of the test item was found to be 1.93.
According to the OECD Guidelines 405 and Council Regulation (EC) No 440/2008 B.5: Physicochemical properties and chemical reactivity – Substances exhibiting pH extremes such as ≤ 2,0 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive or irritant to the eye, then its acid reserve (buffering capacity) may also be taken into consideration.
It is assumed that the test substance item has corrosive properties; therefore, no eye irritation study in rabbits with Glyphosate Technical was performed.
According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate technical is classified for eye irritation:
- Xi R41 (Risk of serious damage to eyes);
- Category 1, H318 (Causes serious eye damage).
Conclusion by the Notifiers
The eye irritation of the test material (glyphosate technical) was concluded to be positive. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate technical is classified for eye irritation: Category 1, H318 (Causes serious eye damage).
Comment by RMS:
The study is considered acceptable. Agreed on the statement provided by the Notifiers.
Reference: IIA, 5.2.5/05
Report: 2009, Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits
Data owner: Helm AG Report No.: LPT 23914
Date: 2009-04-30, unpublished ASB2012-11432
Guidelines: OECD 405 (2002)
US EPA OPPTS 870.2400.
Deviations:
Personnel change of the head of the Quality Assurance Unit: until January 31, 2009: Dipl. Biol. S. Steuer
as of February 1, 2009: Dr. med. vet. habil. K. R. Sultan. This minor deviation did not have any effect on the validity and integrity of the scientific results obtained in this study.
GLP
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate TC
Description:

White powder
Lot/Batch #:
20080801
Purity:
98.8 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
No vehicle used
Test animals:
Species:
Rabbit
Strain:
Himalayan
Source:
Age:
Approx. 4 - 32.5 months
Sex:
Male
Weight at dosing:
Animal no. l : 4.0 kg Animal no. 2: 3.9 kg
Animal no. 3: 4.1 kg
Acclimation period:
At least 20 adaptation days
Diet/Food:
Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available ad libitum before and after the exposure period.
Water:
Tap water, ad libitum
Housing:
The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH,
16352 Schönwalde, Germany).
Environmental conditions:
Temperature: 20 ± 3 °C
Humidity: 30 - 70 % 12-hour light/dark cycle
Study design and methods
In life dates: 2009-02-02 to 2009-02-15
Animal assignment and treatment:
100 mg of the test item was administered into one eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control.The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, 2 further animals were employed 24 hours after start of the initial test.
1 hour after instillation the eyes were rinsed with 20 mL NaCl solution. The eyes were examined

ophthalmoscopically with a slit lamp prior to the administration and l , 24, 48, 72 hours and 4 days after the administration. The eye reactions were observed and registered. 24 hours after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: A single instillation of 100 mg glyphosate TC per animal into the conjunctival sac of the right eye of three rabbits caused the following changes:
Corneal opacity (grade 1) was observed in animal no. one 24 t o 72 hours and in animal no. three 24 and 48 hours after instillation.
The fluorescein test performed 24 hours after instillation revealed cornea1 staining in animal no. 1 and 3 (up to 1/4 of the surface).
Conjunctival redness (grade 1) was observed in all animals 60 minutes to 48 hours, in animal no. 1 until 72 hours after instillation.
Chemosis (grade 1) was observed in animal no. one 24 and 48 hours after instillation. In addition, secretion was observed in all animals 60 minutes after instillation.
The irises were not affected by instillation of the test item. There were no systemic intolerance reactions.
Conclusion by the Notifiers
According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions Glyphosate TC was non – irritating to eyes, hence, no labelling is required.

Comment by RMS:
The study is considered supplementary: Based on the study desgin, it is not possible to conclude, that the test substance is not irritating to rabbit eyes, because the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405. The results provided only additonal information.
Reference:
IIA, 5.2.5/06
Report:
2010, Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits
Data owner: Helm AG Report No.: LPT 24606
Date: 2010-01-06, unpublished ASB2012-11433
Guidelines:
OECD 405 (2002)
US EPA OPPTS 870.2400.
Deviations:
No deviations from the Study Plan.
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test material:

Glyphosate
Identification:
Glyphosate TC
Description:
White powder
Lot/Batch #:
20090506
Purity:
97.3 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
No vehicle was used
Test animals:
Species:
Rabbit
Strain:
Himalayan
Source:
Age:
Approx. 6 - 8 months
Sex:
Male
Weight at dosing:
At dosing
Animal no. l : 2.5 kg Animal no. 2: 2.5 kg
Animal no. 3: 2.7 kg
Acclimation period:
At least 20 adaptation days
Diet/Food:
Commercial diet, ssniffB K-H V2333 (ssniff Spezialdiäten GmbH) served as food. The food was available ad libitum before and after the exposure period.
Water:
Tap water, ad libitum
Housing:
The animals were kept singly in cages measuring 380 mm x 425 mm x 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH,
16352 Schönwalde, Germany).
Environmental conditions:
Temperature: 20 ± 3 °C
Humidity: 30 - 70 % 12-hour light/dark cycle
Study design and methods
In life dates: 2009-10-26 to 2009-11-12
Animal assignment and treatment:
100 mg of the test item were administered into one eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away

from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control. The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, 2 further animals were employed 24 hours after start of the initial test.
1 hour after instillation the eyes were rinsed with 20 mL NaCl solution. The eyes were examined ophthalmoscopically with a slit lamp prior to the administration and 1, 24, 48, 72 hours and 4 to 7 days after the administration. The eye reactions were observed and registered.
24 hours and 7 days after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Corneal opacity (grade 1) was observed in all animals 24 to 72 hours, in animal no. 1 until 4 days and in animal no. 3 until 5 days after instillation. The fluorescein test performed 24 hours after instillation revealed corneal staining in all animals (1/2 to 3/4 of the surface). Irritation of the iris (grade 1) was observed in all animals 24 and 48 hours, in animal no. 3 until 72 hours after instillation. Conjunctival redness (grade 1 or 2) was observed in all animals 60 minutes to 4 days, in animal no. 3 until 6 days after instillation. Chemosis (grade 1) was observed in all animals 60 minutes and 24 hours after instillation. In addition, secretion was observed in all animals 60 minutes and 24 hours after instillation.There were no systemic intolerance reactions.
Conclusion by the Notifiers
According to the EC-Commission directive 67/548/EEC and its subsequent amendments on the approximation of the laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances and the results obtained under the present test conditions Glyphosate TC was non - irritating to eyes, hence, no labelling is required.
Comment by RMS:
The study is considered supplementary: Based on the study desgin, it is not possible to conclude, that the test substance is not irritating to rabbit eyes, because the instilled test substance was washed out after one hour instead of 24 hours according to the current OECD Guideline 405. The results provided only additonal information.
Reference:
IIA, 5.2.5/07
Report:
2009, Glyphosate – Acute Eye Irritation Study in Rabbits
Data owner: Helm AG Report No.: 12172-08
Date: 2009-03-11, unpublished ASB2012-11434
Guidelines:
US EPA OPPTS 870.2400
Equivalent to OECD 405 (2002).
Deviations:
Humidity was in the range of 33-92 % instead of 30-70 %. This
deviation did not affect the study outcome
GLP:
yes
Acceptability:
Please see comment by RMS.
Materials and methods

Test material:
Glyphosate
Identification:
Glyphosate Tech Grade Mixed 5-Batch
Description:
White powder
Lot/Batch #:
080704-1 thru 5
Purity:
96.4 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
No vehicle was used
Test animals:
Species:
Albino rabbit
Strain:
New Zealand White
Source:
Age:
Approx. 3 months
Sex:
2 males and 1 female (nulliparous and non-pregnant)
Weight at dosing:
Males: 2.200-2.400 kg; Female: 2.300 kg
Acclimation period:
5 days
Diet/Food:
PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily
Water:
Tap water, ad libitum
Housing:
Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 - 70 %
Air changes: 10 - 12/hour 12-hour light/dark cycle
Study design and methods
In life dates: 2008-11-10 to 2008-11-27
Animal assignment and treatment:
Healthy albino rabbits were released from quarantine. Both eyes of each animal were carefully examined within 24 hours prior to treatment with a fluorescein sodium ophthalmic solution and cobalt-filtered light. Both eyes of each animal were again carefully examined just prior to treatment, but without the fluorescein sodium ophthalmic solution. Only those animals without eye defects or irritation were selected for testing. On Day 0, a dose of 0.1 mL by volume (93.2 mg) of the undiluted

test substance was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one
second to prevent loss of material. The untreated left eyes served as comparative controls. The grades of ocular reaction were recorded at 1, 24, 48 and 72 hours, and at 4, 7, 10, 14 and 17 days after treatment. The corneas of all treated eyes were examined immediately after the 24 hour observation with a fluorescein sodium ophthalmic solution. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24- hour observation.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The maximum average irritation score of 3 1.7, obtained at 24 hours after treatment, was used to rate
Glyphosate moderately irritating. Fluorescein staining was observed in two of three eyes at 24 hours after treatment and was not observed in any eyes on Day 10 after treatment.Toxicity categories are determined by the presence and duration of corneal involvement, iridic irritation, and positive conjunctival irritation. Any corneal involvement or iridic irritation with a score of 1 or more is considered positive. Any conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive.
Conclusion by the Notifiers
Based on the maximum average irritation score of 3 1.7, the test substance Glyphosate is rated moderately irritating. Since all positive effects had cleared on Day 10 after dosing, the test substance is assigned to Toxicity Category 11. No irritation was observed in any eyes on Day 17.
Comment by RMS:
The study is considered acceptable and the test substance is irritating to rabbit eyes. The Notifiers conclusion on irritating potential based on US EPA criteria: Toxicity Category II (not 11, obviously typing error) due to average irriation score of 31.7 in rabbit eyes
Reference: IIA, 5.2.5/08
Report: 2005, Eye Irritation/Corrosion Effects in rabbits (Oryctolagus cuniculus) of Glyphosate 95 TC
Data owner: Helm AG Report No.: PSL 15277
Date: 2005-04-04, unpublished ASB2012-11435
Guidelines: US EPA OPPTS 870.2400 OECD 405 (2002).
Deviations: No deviations from Study Plan.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate Acid Technical
Description:
White crystalline powder
Lot/Batch #:
040205
Purity:
97.23 %
Stability of test compound:

No data given in the report.
Vehicle and/or positive control:
No vehicle was used.
Test animals:
Species:
Rabbit
Strain:
New Zealand albino
Source:
Age:
Young adult
Sex:
Male
Weight at dosing:
No data given in the report
Acclimation period:
7 days
Diet/Food:
Pelleted Purina Rabbit Chow #5326
Water:
Tap water, ad libitum
Housing:
The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent Guide for the Care and Use of Laboratory Animals DHEW (NIH). Litter paper was placed beneath the cage and was changed at least three
times per week
Environmental conditions:
Temperature: 18-22 °C
12-hour light/dark cycle
Study design and methods:
In life dates: 2004-05-26 to 2004-06-05
Animal assignment and treatment:
A primary eye irritation test was conducted with rabbits to determine the potential for Glyphosate Acid Technical to produce irritation from a single instillation via the ocular route. Under the conditions of this study, the test substance is classified as severely irritating to the eye. Prior to use, the test substance was ground to a powder. One-tenth of a milliliter (0.06 grams) of the ground test substance was instilled into the right eye of three healthy rabbits. The left eye remained untreated and served as a control. Ocular irritation was evaluated by the method of Draize et al.. One hour after test substance instillation, all three treated eyes exhibited cornea1 opacity, iritis, and conjunctivitis. The overall incidence and severity of irritation decreased gradually over time. All animals were free of ocular irritation by Day 10 (study termination).
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: All animals appeared active and healthy. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. One hour after test substance instillation, all three treated eyes exhibited cornea1

iritis, and conjunctivitis. The overall incidence and severity of irritation decreased gradually over time. All animals were free of ocular irritation by Day 10 (study termination).
Conclusion by the Notifiers
Under the conditions of this study, Glyphosate Acid Technical is classified as severely irritating to the eye.
Comment by RMS:
The study is considered acceptable and the conclusion by the Notifiers is supported..
Reference: IIA, 5.2.5/09
Report: 2008, Acute Eye
Irritation/Corrosion Study in Rabbits with Glyphosate Technical
Data owner: Helm AG
Report No.: RF-3996.312.599.07
Date: 2008-09-12, unpublished ASB2012-11436
Guidelines: OECD 405 (2002).
Deviations: The experimental phase initiation and experimental phase conclusion dates were updated.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Glyphosate
Identification:
Glyphosate Technical
Description:
White powder
Lot/Batch #:
20070606
Purity:
98.05 %
Stability of test compound:
No data given in the report.
Vehicle and/or positive control:
No vehicle was used
Test animals:
Species:
Rabbit
Strain:
New Zealand White
Source:
Age:
18 weeks old
Sex:
One male and one female
Weight at dosing:
between 3.346 and 3.624 kg
Acclimation period:

5 to 6 days
Diet/Food:
Pelleted and autoclaved commercial diet for rabbits (Guabi, Mogiana Alimentos S.A. - Brazil)
Water:
Tap water, ad libitum
Housing:
The animals were housed individually in galvanised
steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.
Environmental conditions:
Temperature: 17 - 22 °C
Humidity: 30 - 70 % Air changes: 10 - 15/hour 12-hour light/dark cycle
Study design and methods:
In life dates: 2008-05-26 to 2008-06-17 Animal assignment and treatment:
0.1 g of the test item was applied to the eye of each animal. The test item was applied into the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. Following application, the eyelids were gently held together for about one second in order to prevent test item loss. The right eye that remained untreated was used as a negative control.
The test was performed initially using one animal for evaluation of any irritant/corrosive effect of the test item to the eye. Because some severe ocular reactions were observed in the initial test, only one additional animal was tested to confirm the response.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: The following additional ocular changes were noted to the animals during the observation period:
Rabbit #04: Blepharitis at the 48- and 72-hr time points.
Rabbit #05: Blepharitis at the 24-, 48- 72-hr, and 7- and 14-day time points; and a small raised off area on the corned surface in the right inferior quadrant at the 21-day time point.
Conclusion by the Notifiers:
Based on the "UN GHS / EU CLP Criteria for Classification (UN, 2009; EC, 2008)" the ocular Classification for Glyphosate Technical is "Category l Irreversible effects on the eye".
Comment by RMS:
The study is considered acceptable and the conclusion by the Notifiers is supported.
Reference: IIA, 5.2.5/10
Report: 1988, Primary Eye Irritation Study of
Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits
Data owner: Monsanto Monsanto Report No.: FD-88-29 Date: 1988-06-08, unpublished Z35395
Guidelines: US EPA 81-4
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test material:
Identification:
Glyphosate
Description:
White powder

Lot/Batch #:
XLI-55
Purity:
97.76 %
Stability of test compound:
Stored at room temperature
Vehicle and/or positive control:
None
Test animals:
Species:
Rabbit
Strain:
New Zealand White
Source:
Age:
Young adult
Sex:
Not specified
Weight at dosing:
Between 2-3 kg
Acclimation period:
At least five days
Diet/Food:
NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Gardners, PA, US), ad libitum
Water:
Tap water, ad libitum
Housing:
Individually in wire mesh cages
Environmental conditions:
Temperature: 20 – 23.9 ºC
Humidity: 40 – 60 % Air changes: Not specified
Light cycle: 12 hours light/dark cycle
Study design and methods
In life dates: 1988-04-11 to 1988-05-02 Animal assignment and treatment:
The test was conducted using six young adult New Zealand albino rabbits. The test substance (0.1 g) was instilled into one eye of each rabbit. The lower eyelid was pulled gently away from the eyeball to form a cup (conjunctival sac) and the test substance inserted therein. The lids were then held together for one second and released. Following scoring at 24 hours after dose administration, any residual material was rinsed from the eye with physiological saline. Treated and untreated eyes were examined at 1, 24, 48, and 72 hours, and 7, 14, and 21 days after test substance instillation. The cornea, iris, and conjunctiva were scored separately according to the Draize system. The animals were observed twice daily for mortality at least five hours apart. Body weights were obtained on study day 1 prior to dose administration and at death. At study termination, surviving animals were euthanized by intracardiac injection of sodium pentobarbital and discarded.
Results and discussion
Mortality: One rabbit was found dead 20 days after dose administration. Prior to death, this animal

exhibited anorexia, and gross necropsy revealed a clear gel-like substance in the large intestine. These findings are consistent with mucoid enteropathy, a condition occasionally noted in stock laboratory rabbits. Therefore, the death was considered spontaneous and unrelated to treatment.

Clinical observations: Not reported. Body weight: Not reported.

Necropsy: Not reported.

Eye observations: At one hour after test substance instillation, all animals exhibited conjunctival irritation (redness, swelling, blistering and discharge). Corneal opacity was noted one hour after test substance instillation in four of six (4/6) animals. Corneal opacity and conjunctival irritation were noted in all rabbits at the 24, 48 and 72 hour ad 7 day examinations. Three rabbits exhibited pannus on the cornea; two eyes (iris) had sluggish reactions to light; one rabbit had prominent vascularization of the conjunctival and another animal had a blood-like discharge. Corneal opacity persisted through study termination (day 21) in three of five (3/5) rabbits. Of the remaining two rabbits, one exhibited slight conjunctival discharge at study termination and the other rabbit's treated eye appeared normal 14 days after dose administration.

The group mean irritation scores (24 to 72 hours) were calculated to be 2.1 for corneal opacity, 0.2 for iris lesions, 2.0 for conjunctival redness, and 2.6 for conjunctival chemosis. The individual scores for each time point, individual mean and group mean scores (24 to 72 hours) are presented in Table B.6.2-30.

Table B.6.2-30: Results of the Eye Irritation Test a

Animal No.	Scoring [h]	Cornea	Iris	Conjunctivae		
		Opacity	Area	Redness	Chemosis	Discharge
Rabbit 1	1	2	1	0	2	2 b
	2	24	2	3	0	2
	2 b	2				

48
3
1
0
2
2 b
2 c
72
3 d
1
0
2
2 b
2
7 days
3 d
1
0
2
1
0
14 days
2
1
0
1
0
0
21 days
2
1
0
0
0
0
Mean
24-72 h
2.7
0.0
2.0
2.0
Rabbit 2
1
2
1
0

2
2 b
3
24
2
2
0
2
4 b
3
48
2
2
0
2
4 b
2
72
1
1
0
2
2 b
1
7 days
2
1
0
1
1
0
14 days
2
1
0
0
0
1
21 days
0
0
0
0
0
1

24-72 h
1.7
0.0
2.0
3.3
Rabbit 3
1
2
1
0
2
2 b,e
2
24
2
2
1
2
4 b,e
2
48
2
1
1
2
3 b,e
2
72
2
1
1
2
3 b,e
2
7 days
3 d
2
1
3
2 b
2
14 days
2
1
1

0
0
21 days
2
1
0
0
0
0
Mean
24-72 h
2.0
1.0
2.0
3.3
Animal No.
Scoring [h]
Cornea
Iris
Conjunctivae
Opacity
Area
Redness
Chemosis
Discharge
Rabbit 4
1
0
0
0
2
3 b
3
24
1
4
0
2
4 b
3
48
1
3
0
2
2 b

2
72
1
3
0
2
2 b
2
7 days
3
1
0
2
2
1
14 days
0
0
0
0
0
0
21 days
0
0
0
0
0
0
Mean
24-72 h
1.0
0.0
2.0
2.7
Rabbit 5
1
0
0
0
2
2 b
2
24
2
2

0
2
2 b
2
48
2
2
0
2
2 b
2
72
3
1
0
2
2 b
1
7 days
4 d
1
1
2
2
1
14 days
3 d
1
1
2
1
0
21 days
3 d
1
0
1
0
0
Mean
24-72 h
2.3
0.0
2.0
2.0
Rabbit 6

1
2
1
0
2
2 b
3
24
2
2
0
2
2 b
2
48
3
1
0
2
2 b
2
72
3
1
0
2
2 b
0
7 days
2
1
0
1
1
0
14 days
2
1
0
0
0
0
21 days
- f
-
-

-
-
-
Mean
24-72 h
2.7
0.0
2.0
2.0
Group mean
24-72 h
2.1
0.2
2.0
2.6
a Scores for treated eyes; untreated eyes appeared normal at all times.
b Mucus membrane of the eyelid appeared blistered.
c Blood-like discharge noted.
d Pannus on the cornea.
e Prominent vascularisation of the conjunctiva.
f Animal found dead 20 days after dose administration.
Conclusion by the Notifiers
Based on the study results and on the and according to EU classification criteria the test substance glyphosate is to be classified as risk of serious damage to the eyes (Xi, R41). This corresponds to Category 1 according to the OECD Globally Harmonized System (GHS) classification criteria.
Comment by RMS:
The study is considered acceptable and the conclusion by the Notifiers is supported.
Reference: IIA, 5.2.5/11
Report: 1997 Glyphosate Acid: Eye Irritation to the Rabbit
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Data owner: Syngenta Report No.: /P/5138
Date: 1997-03-18, not published TOX2000-1986
Guidelines: OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2004)
Deviations:
None.
GLP:
yes
Acceptability:
Please see comment by RMS
Materials and methods
Test Material:
Glyphosate acid
Description:
Technical, white solid

Lot/Batch number:
P24
Purity:
95.6 % w/wi
CAS#:
Not reported
Stability of test compound:
The test substance was used within the expiry date
Vehicle and/or positive
control:
None
Test Animals:
Species
Rabbit
Strain
New Zealand White albino
Age/weight at dosing
Young adult / 2951-3702 g
Source
.
Housing
Individually in aluminium sheet cages in racks suitable for
animals of this strain and the weight range expected during the course of the study.
Acclimatisation period
At least 6 days
Diet
STANRAB SQC, (Special Diet Services Limited, Stepfield,
Witham, Essex, UK) ad libitum
Water
Mains water ad libitum
Environmental conditions
Temperature: 17+2 °C
Humidity: 40-70 %
Air changes: Approximately 25/hour Photoperiod: 12 hours light / 12 hours dark
Study design and methods
In-life dates: Start: 22 May 1996 End: 6 July 1996
Animal assignment and treatment: Initially, the test substance (approximately 100mg) was applied into the conjunctival sac of the left eye of one rabbit by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were then gently held together for 1-2 seconds after which the animal was released. The other eye was untreated (control eye).
When the eye irritation potential had been fully assessed in the first animal, the test substance was applied into the test eye of the remaining five animals, as described previously.
As the initial pain reaction of the first rabbit was moderate and the irritation was less than severe, the eyes of the remaining rabbits were pre-treated with five drops of local anaesthetic (OPHTHAINE, 0.5% proparacaine hydrochloride solution) at three minute intervals between each drop.

Both eyes of each rabbit were examined within the twenty-four hours prior to dosing. The examination consisted of a visual assessment with the aid of fluorescein and only rabbits without any apparent eye defects or ocular irritation were used.
Immediately after the application of the test substance, an assessment of the initial pain reaction of the rabbit was made using a six-point scale.
The eyes were examined and the Draize scale was used to assess the grade of ocular reaction approximately one hour and 1, 2, 3, 4, 7 and 8 days after application where necessary. In addition, as an aid in the assessment of corneal damage, fluorescein staining was used at all readings from 1 day after application. A modified form of the Kay and Calandra system was used to interpret and classify the numerical scores.
Results and discussion
No deaths occurred. No systemic signs of toxicity were noted during the study.
Application into the eye caused moderate initial pain in the first animal dosed, therefore the subsequent five animals were pre-treated with the local anaesthetic OPHTHAINE prior to dosing. The group initial pain reaction was none to moderate (class 0-3 on a 0-5 scale).
Corneal effects, consisting of slight to mild opacity affecting up to the entire cornea, were seen in all animals during the first two days, persisting to day 4 in five rabbits. Slight iritis was seen in all animals during the first two days, persisting to day 3 in two rabbits. Conjunctival, effects consisting of slight to moderate redness, slight to mild chemosis and slight to severe discharge, were seen in all animals up to day 4.
Additional observations included mucoid discharge, eye closed, irregular corneal surface, convoluted eyelids, erythema of the upper and/or lower eyelids, raised corneal opacity, Harderian gland discharge and nictitating membrane partially haemorrhagic.
All signs of irritation had completely regressed in five animals 7 days after application. Slight conjunctival redness was seen in the remaining animal on day 7; the animal had completely recovered by day 8.
Table B.6.2-31: Eye irritation scores of glyphosate acid (95.6 % w/w) according to the Draize scheme
Time
Cornea
Iris
Conjunctiva
Redness
Chemosis
Animal number
17
18
19
7
8
9
17
18
19
7
8
9

17
18
19
7
8
9
17
18
19
7
8
9
after 1 hour
0
0
0
1
1
1
0
0
0
0
0
0
1
2
2
1
2
2
2
2
1
1
1
2
after 24 hours
1
1
2
2
1
2
0
1

1
1
1
1
2
2
2
2
2
2
2
2
2
2
2
after 48 hours
1
1
1
2
1
2
1
1
1
1
1
1
2
2
2
2
2
2
1
1
2
2
2
2
after 72 hours
0
1
1
2

1
2
0
0
0
0
1
1
1
2
2
2
2
2
1
1
1
2
2
2
mean scores 24-72h
1.3
0.7
1.9
1.4
after 4 days
0
1
1
1
1
1
0
0
0
0
0
0
1
1
1
2
2
2
0
1

1
1
1
2
after 7 days
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
0
1
0
0
0
0
0
0
-0
0
after 8 days
-
-
-
-
0
-
-
-
-
-
0
-
-
-
-
-

0
-
-
-
-
-
0
-
Conclusion by the Notifiers
Glyphosate acid is a moderate irritant (class 5 on a 1-8 scale) to the rabbit eye.
Comment by RMS:
The study is considered acceptable.The test substance revealed irritating properties to rabbit eyes based on Draize scheme as seen in Table B.6.2-31 [Notifiers conclusion based on modified form of Kay and Chalandra system as mentioned above].
Reference: IIA, 5.2.5/12
Report: 2007, Glyphosate Technical Material: Primary Eye Irritation Study In Rabbits
Data owner: Syngenta Report No.: B02788
Date: 2007-03-26, not published ASB2012-11437
Guidelines: OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5
(2004)
Deviations: None.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test Material:
Glyphosate Technical Material
Description:
Technical, white powder
Lot/Batch number:
0507
Purity:
96.1 % w/w Glyphosate acid
CAS#:
Stability of test compound:
Stable under storage conditions of room temperature (range of
20 oC C 5 oC), protected from light and humidity.
Vehicle and/or positive
control:
The test substance was undiluted.
Test Animals:
Species
Rabbit
Strain
New Zealand White (SPF)
Age/weight at dosing
Male: 11-12 weeks / 2640 g; Females: 14-16 weeks / 2990

and 3001 g
Source
Housing
Individually in stainless steel cages equipped with feed
hoppers and drinking water bowls.
Acclimatisation period
5/6 days
Diet
Pelleted standard Provimi Kliba 3418 rabbit maintenance diet
ad libitum.
Water
Community tap water ad libitum
Environmental conditions
Temperature: 17-23 °C
Humidity: 30-70 %
Air changes: 10-15 per hour.
Photoperiod: 12 hours light, 12 hours dark.
Study design and methods
In-life dates: Start: 27 December 2006 End: 4 January 2007
Animal assignment and treatment: On the day of treatment, 0.1 g of Glyphosate Technical Material was placed into the conjunctival sac of the left eye of each animal after gently
pulling the lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of the test substance. The right eye remained untreated and acted as the reference control. The treated eyes were not rinsed after instillation of the test substance.
As it was suspected that the test substance might produce irritancy, a single female was treated first. As neither a corrosive effect nor a severe irritant effect was observed after 1- and 24-hour examinations, the test was completed using the two remaining animals.
The ocular reaction (ie. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, at approximately 1, 24, 48 and 72 hours, as well as 7 days after instillation. Additionally, ocular discharge, reddening of the sclerae and staining of conjunctivae, sclerae and cornea by the test substance was assessed according to the scheme presented in the guideline.
The animals were observed daily throughout the study for viability, mortality and clinical signs. Bodyweights were measured at the start of acclimatisation, on the day of treatment and at termination of the observation period.
Results and discussion
No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. No abnormal findings were observed in the cornea or iris of any animal at any of the measurement intervals.
Moderate reddening of the conjunctivae was noted in all animals at the 1-hour reading and persisted in one animal as slight until the 48-hour reading and in two animals as moderate to slight until 72 hours after treatment. Slight to obvious swelling (chemosis) was observed in all three animals at the 1-hour reading and persisted as slight in one animal until the 48-hour reading and in one animal as moderate until 24 hours after instillation. Slight to moderate reddening of the sclerae was noted in all animals at the 1- and 24 hour reading and persisted as slight reddening until the 48-hour reading. Slight to moderate ocular discharge was seen in all animals at the 1-hour reading and persisted as

slight to moderate discharge in two animals at the 24-hour reading. No abnormal findings were observed in the treated eye of any animal 7 days after treatment, the end of the observation period for all animals. No staining of the treated eyes produced by the test substance was observed and no corrosion of the cornea was observed at any of the reading times.

Table B.6.2-32: Eye irritation scores of Glyphosate Technical according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004

Time
Cornea
Iris
Conjunctiva
Redness
Chemosis
Animal number
31M
32F
33F
31M
32F
33F
31M
32F
33F
31M
32F
33F
after 1 hour
0rd
0rd
0rd
0
0
0
2
2
2
1
2
2
after 24 hours
0r
0rd
0rd
0
0
0
1

2
2
0
1
2
after 48 hours
0r
0r
0r
0
0
0
1
2
2
0
0
1
after 72 hours
0
0
0
0
0
0
0
1
1
0
0
0
mean scores 24-72h
0
0
0
0
0
0
0.67
1.67
1.67
0
0.33
1
After 7 days
0

0
0
0
0
0
0
0
0
0
0
0
M = male, F = female, d = discharge, r = reddening of the sclerae.
Conclusion by the Notifiers
The instillation of Glyphosate Technical Material into the eye resulted in mild, early-onset and transient ocular changes. These effects were reversible and were no longer evident 7 days after treatment. Thus, the test item did not induce significant or irreversible damage to the rabbit eye.
Comment by RMS:
The study is considered acceptable and the test substance is irritating to eyes.
Reference: IIA, 5.2.5/13
Report: 2011, Glyphosate technical: Acute eye irritation study in rabbits
Data owner: Syngenta Report No.: 10/218-005N
Date: 2011-05-13, not published ASB2012-11438
Guidelines: OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2008)
Deviations: None.
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test Material:
Glyphosate technical
Description:
Technical, dry white powder
Lot/Batch number:
569753(BX20070911)
Purity:
96.3 % w/w Glyphosate technical
CAS#:
Not reported
Stability of test compound:
Stable under storage conditions (room temperature range <30 °C), recertification date end August 2011
Vehicle and/or positive control:
None
Test Animals:

Species
Rabbit
Strain
New Zealand White
Age/weight at dosing
Approximately 12 weeks / 3035 g
Source
Housing
Individually in metal cage
Acclimatisation period
13 days
Diet
Purina Base – Lap gr. diet (AgribrandsEurope Hungary PLC,
H-5300 Karcag, Madarasi út, Hungary) ad libitum
Water
Municipal tap water ad libitum
Environmental conditions
Temperature: 20±3 °C
Humidity: 24-64 %
Air changes: 15-20/hour
Photoperiod: 12 hours light/12 hours dark
Study design and methods
In-life dates: Start: 21 December 2011 End: 22 December 2011
Animal assignment and treatment: The primary eye irritation potential of Glyphosate Technical (96.3 % w/w glyphosate technical) was investigated according to OECD test guideline no. 405.
Approximately 1 hour before the start of the test, the eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect using a hand-held slit-lamp. The animal used in the study was free of ocular damage. Initially, a single rabbit was treated.
An amount of 0.1 g of the test material was placed into the conjunctival sac of the left eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about 1 second immediately after treatment, to prevent loss of the test material, and then released. The right eye remained untreated and was used for control purposes.
Immediately after administration of the test material, an assessment of the initial pain reaction was made according to 0-5 scale. Following review of the ocular responses produced in the first treated animal, no further animals were treated. The treated eyes were not rinsed after instillation.
The ocular reaction (i.e. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed approximately 1 and 24 hours following treatment, according to the numerical evaluation described by Draize.
The treated eye was further examined using 2 % fluorescein solution before treatment and then 24 hours after treatment.
Additionally, any other signs of eye irritation were recorded.
Results and discussion
No clinical signs of systemic toxicity were observed in the animal during the study and no mortality occurred. The body weight was considered to be within the normal range of variability.
An initial pain reaction score of 3 (on a 0-5 scale) was recorded.
Conjunctival redness, chemosis and conjunctival discharge, as well as corneal opacity, were observed

in the rabbit 1 and 24 hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, a few black points on the conjunctiva and dry surface of the eye were noted one hour after the treatment. Fluorescein staining was positive at the 24 hours observation.
Based on the symptoms, no further animals were dosed and the study was terminated after the 24 hour observation (Regulation (EC) No 440/2008).
Table B.6.2-33: Eye irritation scores of Glyphosate Technical according to the Draize scheme
Time
Cornea
Iris
Conjunctiva
Redness
Chemosis
Discharge
after 1 hour
2
0
2
3
3
after 24 hours
3
1
3
4
3
Conclusion by the Notifiers:
Under the conditions of this study, Glyphosate Technical is classified as corrosive to the eye.
Comment by RMS:
The study is considered acceptable. The conclusion by the Notifiers is supported.
B.6.2.6 Skin sensitisation
For the previous evaluation a variety of studies on glyphosate acid and two studies on glyphosate salts (IPA) regarding skin sensitising were available. For the current re-evaluation, several additional studies for skin sensitising effects on glyphosate acid were provided: 8 Magnusson-Kligman Tests and 3 Buehler Tests in guinea pigs and two Local lymph node assays in mice. No further studies on glyphosate salts were provided.
Comment by NL during peer review:
Minor comment: throughout the document the Magnusson & Kligman test for skin sensitisation is inconsequently referred to as “Magnusson & Kligman”, Magnusson- Kligman”, Magnusson and Kligman”, or “Magnusson & Kligman”.
RMS’ response: Agreement, a consistent nomenclature will be used in a revised RAR. However, this is necessary only in few cases, because the headings of the study reports should not be corrected.
Table B.6.2-34: Summary of skin sensitisation studies with glyphosate acid
Reference (Data owner)
Species Strain
Number and /or sex

of animals
Purity [%]
Exposure conditions
Test Method
Results
Studies from the 2001 evaluation
Annex B- 5.2.7.1,
Glyphosate Monograph
1994
(Herbex) TOX9500250
Guinea pig Dunkin Hartley
15 ♀
95
Induction: 1% w/v in arachis oil; challenge: 25% w/w or 50% w/w in arachis oil
MKT
Not sensitising
Annex B-
5.2.7.1,
Glyphosate Monograph
Guinea pig
English
48 (both
sexes)
≥ 95
Intradermal
induction: 5% in propylene glycol;
topical: 50% in petrolatum
MKT
Not
sensitising
1993 (Luxan)
TOX9650652
Annex B-
5.2.7.1,
Glyphosate Monograph
1991 (AGC) TOX9551796
Guinea pig
Dunkin Hartley
38 ♀
Not
stated
Intradermal
induction: 0.1% (w/v) in water; topical: 50% (w/v) in water; challenge: 25% (w/w) in water
MKT
Not
sensitising

Annex B-
5.2.7.1,
Glyphosate Monograph
Guinea pig
Dunkin Hartley
46 ♀
98.6
induction: 10% in
water; challenge: 25% in water
MKT
Not
sensitising
, 1989e (CHE) TOX9552343
Reference (Data owner)
Species Strain
Number
and /or sex of animals
Purity [%]
Exposure conditions
Test Method
Results
Studies not reviewed in the 2001 evaluation
IIA 5.2.6/01
2007 (NUF) ASB2012- 11439
Guinea pig
20 ♀/test
10
♀/control
95.1
Intradermal induction: 3% (w/v) in PEG-300; topical induction: 50% (w/v) in PEG-300;
challenge: 25% (w/v) in PEG-300
MKT
Not sensitising
IIA 5.2.6/02
2010 (HAG)
ASB2012- 11440
Guinea pig, Dunkin Hartley
15 ♀
(+ 20 for positive control)
96.4
Intradermal induction: 0.01% in water; topical induction: 50%;
challenge: 25%
MKT
Not sensitising
IIA 5.2.6/03
1995 (ALS) ASB2012- 11441

Guinea pig Hartley
60 ♀
97.56
Intradermal induction: 5% (w/v) in paraffin oil, topical induction: 25% (w/v) in white petrolatum; challenge: 25% (w/w) in white
petrolatum
MKT
Not sensitising
IIA 5.2.6/04
2009 (EXC) ASB2012- 11442
Guinea pig
15 ♂
96.66
Intradernal induction: 10% (w/w) in purified water; topical induction: 50% (w/w) in purified water; challenge: 15% (w/w) in purified
water
MKT
Not sensitising
IIA 5.2.6/05
2009 (HAG) ASB2012-
11443
Guinea pig
15 ♂
(+ 20 for positive control)
98.8
Intradermal induction: 0.01% in water, topical induction: 50%;
challenge: 50%
MKT
Not sensitising
Studies not reviewed in the 2001 evaluation
IIA 5.2.6/06
2010 (HAG) ASB2012- 11444
Guinea pig
15 ♂
(+ 20 for positive control)
97.3
Intradermal induction: 0.5% in water; topical induction: 50%;
challenge: 25%
MKT
Not sensitising
IIA 5.2.6/07
2009 (HAG) ASB2012- 11445
Guinea pig
15 ♂ 15 ♀
96.4
400 mg moistened

induction and challange
Buehler
Test
Not
sensitising
IIA 5.2.6/08
2005 (HAG) ASB2012-
11446
Guinea pig
30 ♂ ♀
97.23
70 % in water
induction and challange
Buehler
Test
Not
sensitising
IIA 5.2.6/09
Guinea pig
30 ♂
98.05
50 % inductions and
challange
Buehler
Test
Not
sensitising
2008 (HAG)
ASB2012- 11447
Reference (Data owner)
Species Strain
Number
and /or sex of animals
Purity [%]
Exposure conditions
Test Method
Results
Studies not reviewed in the 2001 evaluation
IIA 5.2,6/10
2006 (NUF) ASB2012- 11448
Guinea pig
20 ♀/test
10
♀/control
95.7
Intradermal induction: 0.195% (w/v) in isotonic saline; topical induction: 60% (w/v) in water;

challenge: 60% (w/v) & 30%
(w/v) in water
MKT
Not sensitising
IIA 5.2.6/11
1996 (SYN) TOX2000- 1987
Guinea pig
20 ♀/test
10
♀/control
95.6
Intradermal
induction: 0.1% (w/v) in water; topical induction: 75% (w/v) in water; challenge:
75% (w/v) & 30% (w/v) in water
MKT
Not
sensitising
IIA 5.2.6/12
2007 (SYN) ASB2012- 11449
Mouse CBA
4 ♀/group
96.1
Glyphosate acid dose
levels: 0, 10, 25, 45 (% w/v) Hexylcinnamaldehyd e positive control
demonstrated sensitivity of study
LLNA
Not sensitising
IIA 5.2.6/13
o, 2011 (SYN) ASB2012- 11450
Mouse CBA
4 ♀/group
96.3
Glyphosate acid dose
levels: 0, 10, 25, 50 (% w/v) Hexylcinnamaldehyd e positive control
demonstrated sensitivity of study
LLNA
Not sensitising
MKT = Magnusson Kligman Maximisation Test LLNA = Local Lymph Node Assay
Table B.6.2-35: Summary of skin sensitisation studies with glyphosate salts (provided by the Notifiers)
Reference (Data owner)
Species Strain
Number and /or sex of
animals
Purity [%]
Exposure conditions
Test Method

Result
Studies from the 2001 evaluation
Annex B.5.2.7.2,
Glyphosate Monograph
1994 (MON / CHE) TOX9552345
Guinea pig, Dunkin Hartley
38 ♀
IPA
62.2 %
Induction: 25 % w/v (injection) and 100 % (topical);
challenge: 100 %
MKT
Not sensitizing
Annex B.5.2.7.2,
Glyphosate Monograph
1984* (MON) Z35238
Guinea
pig
16
IPA
53.8 %
Induction and
challange: 100 %
Modified
Buehler Test
Not
sensitising

Description:
Technical, white solid
Lot/Batch number:
P24
Purity:
As given in report 95.6 % a.i
CAS#:
Not reported
Stability of test compound:
Not reported
Vehicle and/or positive
control:
Deionised water
Test Animals:
Species
Guinea pig
Strain
Albino Cr1 (HA) BR

Age/weight at dosing
Young adult females / 250-317 g
Source
Housing
Individually in suspended cages in racks suitable for animals
of this strain and the weight range expected during the course of the study.
Acclimatisation period
At least 6 days
Diet
RGP), supplied by Labsure, Manea, Cambridgeshire, UK ad
libitum
Water
Mains water ad libitum
Environmental conditions
Temperature: 17 ± 2 °C
Humidity: 40-70 %
Air changes: Approximately 25 changes/hour Photoperiod: 12 hours light, 12 hours dark
Study design and methods
In-life dates: Start: 25 April 1995 End: 19 May 1995
Animal assignment and treatment: In a dermal sensitisation study with glyphosate acid (95.6 % w/w a.i.) young adult, female, Albino Cr1 (HA) BR guinea pigs were tested using the method of Magnusson and Kligman. The study involved the treatment of guinea pigs using two procedures: the potential induction of an immune response and a challenge of that response.
Induction: An area approximately 5 x 5 cm on the scapular region of each animal was clipped free of hair and a row of three injections (0.05-0.1 mL each) was made on each side of the mid-line. The injections were:
i) Top: Freund's Complete Adjuvant plus deionised water in the ratio 1:1;
ii) Middle: a 0.1 % w/v preparation of the test substance in deionised water;
iii) Bottom: a 0.1 % w/v preparation of the test substance in a 1: 1 preparation of Freund's Complete Adjuvant plus deionised water.
Control animals were treated the same as the test animals, except that they were treated with deionised water in place of the test substance.
One day prior to topical induction, the application site was clipped and 0.5 mL of a 10 % w/v preparation of sodium lauryl sulphate in paraffin wax was applied in order to provoke a mild inflammatory response.
One week after intradermal injection, the scapular area was treated with a topical application of the test substance as a 75 % w/v preparation in deionised water. This preparation (0.2-
0.3 mL) was applied on filter paper (approximate size 4 cm x 2 cm) which was held in place by a piece of surgical tape. The tape was covered by a strip of adhesive bandage (approximate size 20-30 cm x 5 cm) and secured by a piece of self-adhesive PVC tape. This occlusive dressing was kept in place for approximately 2 days.
Deionised water only was applied to the filter paper for control animals.
The application sites were checked approximately 1 day after removal of the dressings.
Challenge: Two weeks after the topical inductions, an area, approximately 15 cm x 5 cm, on both flanks of all the test and control animals, was clipped free of hair. An occlusive dressing was prepared which consisted of two pieces of filter paper (approximate size 1 cm x 1.5-2.0 cm) stitched to a piece

of rubber sheeting (approximate size 12 cm x 5 cm).
A 75 % w/v preparation of the test substance in deionised water (0.05-0.1 mL) was applied to one of the pieces of filter paper and a 30 % w/v preparation in deionised water (0.05-
0.1 mL) was applied to the second piece of filter paper. The dressing was placed on the shorn flank of the guinea pig so that the 75 % w/v preparation was on the left and the 30 % w/v preparation was on the right. It was then covered with a strip of adhesive bandage (approximate size 25-40 cm x 7.5 cm) which was secured by a self-adhesive PVC tape.
After approximately 1 day, the dressings were carefully removed. Skin sites were examined approximately 1 and 2 days after removal of the dressings and any erythematous reactions were quantified and recorded, using a four-point scale.
Positive Controls: The sensitising potential of hexylcinnamaaldehyde (HCA) was assessed essentially as described above to demonstrate the sensitivity of the strain of animals used and the reliability of the experimental technique. A concentration of 0.3 % w/v HCA in corn oil was used for the intradermal injections and HCA was used undiluted for the topical induction and challenge applications.
Results and discussion
Bodyweights: There were no treatment-related effects on bodyweight during the study. Induction reactions and duration: Not reported.
Challenge reactions and duration: Following challenge of previously-induced guinea pigs with a 75 % w/v preparation of the glyphosate acid in deionised water, scattered mild redness was seen in three of the twenty test animals and one of the ten control animals. This response is considered to be due to skin irritation following topical challenge. The basis for this conclusion is that an equivalent reaction was seen in one of the ten control animals and the reaction was restricted to the 24 hour clinical observation only, which is characteristic of a mild skin irritation reaction rather than skin sensitisation.
Following challenge of previously-induced guinea pigs with a 30 % w/v preparation of the glyphosate acid in deionised water, no reaction was seen in any of the test or control animals. The net percentage response was calculated to be 0 %.
Positive control: Following challenge of previously induced guinea pigs, scattered mild redness or moderate diffuse redness was observed in 14/20 test animals. Scattered mild redness was seen in two of the ten control animals. The net % response was 50 % and, therefore, HCA was classified as a moderate skin sensitiser which demonstrated the sensitivity of the strain of animals used and the reliability of the experimental technique.
Table B.6.2-36: Number of animals with positive signs following challenge
Test flank
Challenge at 75 %
Challenge at 30 %
Scored after:
24 hours
48 hours
24 hours
48 hours
Main test – test group
3/20
0/20
0/20

0/20
Main test – negative vehicle control
1/10
0/10
0/10
0/10
Challenge at 100%
24 hours
48 hours
Positive control – test group
14/20
13/20
Positive control – vehicle control
2/10
0/10
Conclusion by the Notifiers:
Glyphosate acid is not a skin sensitiser under the conditions of the test.
Comment by RMS:
Despite reporting deficiencies the study is considered acceptable. Based on the study conditions no evidence of skin sensitising potential was observed.
Reference: IIA, 5.2.6/12
Report: 2007, Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay In The Mouse).
,
Macclesfield, Cheshire, UK Data owner: Syngenta Report No.: R61837/1004
Date: 2007-02-09, not published ASB2012-11449
Guidelines: OECD 429 (2002): OPPTS 870.2600 (2003): 2004/73/EC B.42
(2004)
Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test Material:
Glyphosate Technical Material
Description:
Technical, white solid
Lot/Batch number:
0507
Purity:
96.1 %
Stability of test compound:
Stable under storage conditions – ambient temperature in the
dark.
Vehicle and/or positive control:
The vehicle for the test substance was dimethyl sulphoxide. The vehicle for the positive control substance

(hexylcinnamaldehyde) was acetone in olive oil.
Test Animals:
Species
Mouse
Strain
CBA/Ca/Ola/Hsd
Age/weight at dosing
8-12 weeks / 16.5-20.8 g
Source
Housing
Maximum 4 per cage, in cages suitable for animals of this
strain and weight range.
Acclimatisation period
At least 5 days
Diet
Diet (RM1), supplied by Special Diets Services Limited,
Witham, Essex, UK. ad libitum
Water
Mains water supplied by an automatic system ad libitum
Environmental conditions
Temperature: 22 T 3 °C
Humidity: 30-70 %
Air changes: A minimum of 15 changes/hour Photoperiod: Artificial, 12 hours light / 12 hours dark.
Study design and methods
In-life dates: Start: 10th January 2007 End: 16th January 2007
Animal assignment and treatment: A sample of glyphosate technical material was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The assay determines the level of T lymphocyte proliferation in the lymph nodes draining the site of chemical application by measuring the amount of radiolabelled thymidine incorporated into
the dividing cells. The test substance was applied as 10, 25 or 45 % w/v preparations in dimethyl sulphoxide. Groups of four female CBA/Ca/Ola/Hsd mice were used for this study. Dose selection rationale: Approximately 25µL of a 10, 25 or 45 % w/v preparation of the test substance was used in this study as 45 % w/v was the limit of solubility.
Treatment preparation and administration: Approximately 25µL of a 10, 25 or 45 % w/v preparation of the test substance in dimethyl sulphoxide was applied, using a variable volume micro-pipette, to the dorsal surface of each ear. A vehicle control group was similarly treated using dimethyl sulphoxide alone. The procedure was repeated daily for 3 consecutive days. Three days after the third application, all the animals were injected, via the tail vein, with approximately 250µL of phosphate buffered saline (PBS) containing 20µCi of a 2.0Ci/mmol specific activity 3H-methyl thymidine. Approximately 5 hours later, the animals were humanely killed by inhalation of halothane vapour followed by cervical dislocation. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in the group, were placed in a container of PBS.
A single cell suspension was prepared by mechanical disaggregation of lymph nodes through a 200-mesh stainless steel gauze. The cell suspensions were then washed three times by centrifugation with approximately 10 ml of PBS. Approximately 3ml of 5 % w/v trichloroacetic acid (TCA) was added

and, after overnight precipitation at 4 °C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were then resuspended in approximately 1mL of TCA.
The lymph node suspensions were transferred to scintillation vials and 10 mL of scintillant (Optiphase) was added prior to n-scintillation counting using a Packard Tri-Carb 3100TR Liquid Scintillation Counter.
The reliability of the test system was assessed in a positive control study using the same method with a known sensitiser (hexylcinnamaldehyde) applied as 5 %, 10 % or 25 % w/v preparations in acetone in olive oil.
Statistics / Data Evaluation: The results are expressed as a disintegrations per minute (dpm) value per lymph node for each group. The activity of each test group is then divided by the activity of the vehicle control group to give a test:control ratio known as the stimulation index (SI), for each concentration.
The criterion for a positive response is that one or more concentrations of the test substance should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The assay is able to identify those materials that elicit responses in standard guinea pig tests for skin sensitisation. Consequently, a test substance which does not fulfil the above criterion is designated as unlikely to be a skin sensitiser.
Results and discussion
Group Mean Counts per Minute: The application of the test substance at concentrations of 10, 25 and 45 % w/v in dimethyl sulphoxide resulted in an isotope incorporation which was less than 3-fold at all concentrations. Consequently, the test substance is considered not to be a skin sensitiser under the conditions of the test.
Table B.6.2-37: Radiolabel incorporation into lymph-nodes of mice treated with glyphosate technical material
Concentration of NOA446510 (%w/v)
Number of lymph nodes assayed
Disintegrations per minute (dpm)
dpm per lymph node
Test control ratio (SI)
0 (vehicle only)
8
3912
489
N/A
10
8
2394
299
0.6
25
8
3292
412
0.8
45
8

4067
508
1.0
N/A = not applicable
In the positive control study, the application of hexylcinnamaldehyde at concentrations of 5 %, 10 % and 25 % w/v in acetone in olive oil (4:1) resulted in a greater than 3-fold increase in isotope incorporation at the 25 % w/v concentration. Therefore, hexylcinnamaldehyde was shown to be a skin sensitiser, confirming the validity of the protocol used for the study.
Table B.6.2-38: Radiolabel incorporation into lymph-nodes of mice treated with the positive control substance (hexylcinnamaldehyde)
Concentration of hexylcinnamaldehyd e (%w/v)
Number of lynph nodes assayed
Disintegrations per minute (dpm)
dpm per lymph node
Test control ratio (SI)
0 (vehicle only)
8
5939
742
N/A
5
8
10111
1264
1.7
10
8
13747
1718
2.3
25
8
38015
4752
6.4
Conclusion by the Notifiers
Glyphosate technical material is considered not to be a skin sensitiser under the conditions of the test.
Comment by RMS:
The study is considered acceptable and no evidence for skin sensitisation potential of the test substance was observed.
Reference: IIA, 5.2.6/13
Report: 2011, Glyphosate technical: Local lymph node assay in the mouse
Data owner: Syngenta Report No.: 10/218-037E
Date: 2011-04-21, not published ASB2012-11450
Guidelines: OECD 429 (2010)

Deviations: None
GLP: yes
Acceptability: Please see comment by RMS
Materials and methods
Test Material:
Glyphosate technical
Description:
Technical, dry white powder
Lot/Batch number:
569753 9 (BX20070911)
Purity:
96.3 % w/w glyphosate technical
CAS#:
Not reported
Stability of test compound:
Stable under storage conditions (room temperature range 2-
8°C), recertification date end August 2011
Vehicle and/or positive
control:
The vehicle for the test substance was propylene glycol
Test Animals:
Species
Mice
Strain
CBA/J Rj
Age/weight at dosing
9-10 weeks / 20.1-21.6 g
Source
Housing
Group housed in Type II. polypropylene/polycarbonate cages
Acclimatisation period
13 days
Diet
ssniff® SM R/M-Z+H "Autoclavable complete feed for rats
and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest
Germany ad libitum
Water
Tap water ad libitum
Environmental conditions
Temperature: 22±3 °C
Humidity: 30-70 %
Air changes: 15-20 air changes per hour Photoperiod: 12 hours light / 12 hours dark
Study design and methods
In-life dates: Start: 20 October 2010 End: 26 October 2010
A sample of glyphosate Technical (96.3 % w/w Glyphosate technical) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay, following dermal exposure when

administered topically to young adult, female CBA/J Rj mice.
Animal assignment and treatment:
Dose selection rationale: A Preliminary Irritation/Toxicity Test was performed on CBA/J Rj mice using two doses, at test item concentrations of 50 and 25 (w/v)%, respectively. This preliminary experiment was conducted in a similar experimental manner to the main study, but it was terminated on Day 6 without radioactive proliferation assay.
During the Preliminary Irritation/Toxicity Test no mortality, systemic toxicity or local irritation were observed. No treatment related effect on body weights was observed. The observations recorded in this preliminary test suggest that the formulations, the application of the material and the local effects on the animal are acceptable for a valid LLNA.
Based on the results of the preliminary experiments the following dose levels were selected for the main assay: 0 (negative control), 10, 25 and 50 w/v% Glyphosate Technical, and positive control (25 % HCA in PG). Each group comprised four mice.
Treatment and observations: Each animal was topically dosed once a day for 3 consecutive days (Days 1, 2 and 3) on the dorsal surface of each ear with 25 µL of the appropriate formulation, applied using a pipette. There was no treatment on Days 4, 5 and 6.
All animals were observed at least once daily (Days 1-6) for any clinical signs, including local irritation and systemic toxicity. Individual body weights were recorded on Day 1 (beginning of the assay) and at Day 6 (prior to 3HTdR injection).
Proliferation assay: On Day 6 each mouse was intravenously injected via the tail vein with 250 µL of sterile PBS (phosphate buffered saline) containing approximately 20 µCi of 3HTdR using a gauge 25G1" hypodermic needle with 1 mL sterile syringe. Once injected, the mice were left for 5 hours. Five hours after intravenous injection, the mice were killed by CO2 asphyxiation. The draining auricular lymph nodes were excised by making a small incision on the skin between the jaw and sternum, pulling the skin gently back towards the ears and exposing the lymph nodes. The nodes were then removed using forceps and the carcasses discarded. The nodes of mice from each test group was pooled and collected in separate Petri dishes containing a small amount (1-2 mL) of PBS to keep the nodes wet before processing.
A single cell suspension (SCS) of pooled lymph node cells (LNCs) were prepared and collected in disposable tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer using the plunger of a disposable syringe. The cell strainer was washed with PBS (up to 10 mL). Pooled LNCs were pelleted with a relative centrifugal force (RCF) of 190 x g (approximately) for 10 minutes at 4 °C. After centrifugation supernatants were discarded. Pellets were gently resuspended and 10 mL of PBS was added to the tubes. The washing step was repeated twice. This procedure was repeated for each group of pooled lymph nodes.
After the final washing step, the suspensions were centrifuged and the supernatants were removed leaving a small volume (<0.5 mL) of supernatant above each pellet. Each pellet was gently agitated before suspending the LCNs in 3 mL of 5 % TCA (trichloroacetic acid) for precipitation of macromolecules. After incubation with 5 % TCA at 2-8 °C overnight (approximately 18 hours) precipitate was recovered by centrifugation at 190 x g for 10 minutes at 4 °C), and supernatants were removed and pellets were resuspended in 1 mL of
5 % TCA solution and dispersed using an ultrasonic water bath. Each precipitate was transferred to a suitable sized scintillation vial with 10 mL of scintillation liquid and thoroughly mixed. The vials were loaded into a β-scintillation counter and 3HTdR incorporation was measured for up to 10 minutes per sample. The β-counter expressed the 3HTdR incorporation as the number of radioactive disintegrations per minute (DPM). Similarly, background radiation levels were also measured in two 1

mL aliquots of 5 % TCA. Statistics / Data evaluation: In the absence of any positive results, the statistical analysis of the data was not performed.
DPM was measured for each pooled group of nodes. The measured DPM values were corrected with the background DPM value ("DPM"). The results were expressed as "DPN" (DPM divided by the number of lymph nodes) following the industry standard for data presentation.
A stimulation index of 3 or greater is the criteria for defining a positive result.
The test item is regarded as a sensitiser if both of the following criteria are fulfilled:
• That exposure to at least one concentration resulted in an incorporation of 3HTdR at least 3-fold or greater than recorded in control mice, as indicated by the stimulation index.
• The data are compatible with a conventional dose response, although allowance must be made (especially at high topical concentrations) for either local toxicity or immunological suppression.
Results and discussion
Clinical observation: No mortality or signs of systemic toxicity were observed during the Main Study. No cutaneous reactions were observed at the site of the treatment in any treatment groups.
Body weight: No treatment related effects were observed on body weight.
Proliferation assay: Appearance of the lymph nodes was normal in the negative control group and in the test item treated groups. Larger than normal lymph nodes was observed in the positive control group.
No mortality, cutaneous reactions or signs of toxicity were observed in the positive control group. A significant lymphoproliferative response (stimulation index value of 12.2) was noted for α-Hexylcinnamaldehyde in this experiment. The results of the positive control group demonstrated the appropriate performance of the assay.
Conclusion by the Notifiers
In conclusion, under the conditions of the present assay, glyphosate technical tested in a suitable vehicle, was shown to have no skin sensitisation potential (non-sensitiser) in the Local Lymph Node Assay.
Comment by RMS:
The study is considered acceptable and no evidence for skin sensitisation potential of the test substance was observed.

B.6.3.1 Subacute studies (all routes)
B.6.3.1.1 Subacute oral studies in rats and dogs
No new sub-acute oral toxicity studies with glyphosate have been run since the previous evaluation was performed in the late 1990ies. The five studies mentioned in the old DAR were re-evaluated by the RMS. The studies by (1989, TOX9552351) and by (1991, TOX9551095, Z102035, Z102043) in rats were still considered acceptable although the latter one had the apparent deficiency that sexes were combined for reporting of hematological and clinical chemistry parameters and organ weights in the summary tables. However, raw data that may be easily found in the study report allowed separate assessment. Of a third study by (1989, TOX9552352), at least "part B" in which a limit dose of 1000 mg glyphosate/kg bw was adminstered daily for two weeks to dogs was also considered acceptable whereas part A was difficult to interpret due to a very unusual
design with increasing doses.
The pilot studies by (1978, TOX9552348) in mice and of (1989, TOX9552350) in rats are not acceptable due to serious reporting deficiencies.
The rather small number of valid subacute studies by the oral route is not considered a data gap because there is quite a lot of fully acceptable 90-day studies in all relevant species.

Studies with formulations

A study by (1982, TOX9552349) in dogs does also not completely comply to modern standards and may not be used for risk assessment of glyphosate but is of interest because higher toxicity of a formulation as compared to the active ingredient was elucidated. Strong gastrointestinal irritation became apparent when the isopropylamine (IPA) salt of glyphosate (MON 0139) was administered to Beagle dogs. Vomiting and diarrhea together with a reduction in body weight gain were observed at a dose level of 625 mg/kg bw and above. A dose of 312.5 mg/kg bw given for five days still caused a slight decrease in food consumption. Toxicity of isopropylamine alone was also tested in this experiment revealing strong irritation of gastric and esophageal mucosa at a much lower dose of 72 mg/kg bw upon

single and repeated administration suggesting that these effects were most probably not or only partly attributable to glyphosate itself.

B.6.3.1.2 Subacute inhalation studies (rat)

Only one study on subacute inhalative toxicity (14 days) in rodents (1985, TOX9551952) is available and was reviewed in the 2001 EU evaluation. The previous review concluded no treatment-related effects were observed and the NOEL (more correct NOEC) was 3.8 mg/L. However, from a todays regulatory point of view, the study must be considered unacceptable due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material.

Studies with formulations

A four-week inhalation study with the formulation MON 2139 (1983, TOX2002- 694) suggesting some effects at rather low concentrations is reported and evaluated in the Appendix on formulations containing as surfactants (see B.6.13).

B.6.3.1.3 Subacute percutaneous toxicity studies (rabbits and rats)

There was only one new study submitted for this evaluation, i.e., a 21-day dermal study on rats by (1996, ASB2012-11461) that is reported in the following in detail. A second study not mentioned in the previous DAR (2012, ASB2012-11459) was originally allocated to this section by GTF but is now reported under B.6.12 since its focus was on dermal absorption (in vitro, rabbit skin) and not on toxicity.

For details regarding the studies that were reviewed during the 2001 EU evaluation, we refer to the original DAR (1998, ASB2010-10302). Re-evaluation by the RMS revealed that the study on rats by (1993, TOX9552367) and those by (1982, TOX9552366) and (1994, TOX9650151) on rabbits may be still considered acceptable. In contrast, quality assessment of a further study in rabbits (1985, TOX9551951) was downgraded to “not acceptable” (from “supplementary” in the old DAR) because of reporting deficiencies (batch and purity lacking). The absence of statistical analysis may be explained by the very low number of animals (3 per sex and dose) but this small group size is one more reason for doubts on the reliability of this study. .

New subacute dermal toxicity study on rats (1996)

Reference: IIA, 5.3.7/04

Report: . 1996 Glyphosate Acid: 21 Day Dermal Toxicity Study In Rats

Data owner: Syngenta Report No.: /P/4985 Date: 1996-06-24

not published, ASB2012-11461

Guidelines: OECD 410 (1981): OPPTS 870.3200 (1998): 87/302/EEC B.28

(1988)

Deviations: None

GLP:

Yes

Acceptability:
See RMS comment
Dates of experimental work: 1996-01-09 to 1996-06-24
Materials and methods
Test Material:
Glyphosate acid
Description:
Technical, white solid
Lot/Batch number:
P24
Purity:
95.6 % w/w a.i
CAS#:
Not reported
Stability of test compound:
Not reported
Vehicle and/or positive
control:
Deionised water
Test Animals:
Species
Rat
Strain
Alpk:APfSD
Age/weight at dosing
6 - 8.5 weeks / males 214-249 g, females 193-227 g
Source
Housing
Individually, in cages on multiple rat racks suitable for animals of this strain and weight range expected during the
course of the study.
Acclimatisation period
At least 5 days
Diet
Diet (PCD) supplied by Special Diet Services Limited,
Witham, Essex, UK ad libitum
Water
Mains water ad libitum
Environmental conditions
Temperature: 21 ± 2 °C
Humidity: 40-70 %
Air changes: At least 15 changes/hour Photoperiod: 12 hours light/12 hours dark
Study design and methods
In-life dates: Start: 10 January 1996 End: 1 February 1996
Animal assignment: The study was divided into ten (randomised blocks), each containing one cage per treatment group. The animals were randomly allocated to groups as shown below:

Table B.6.3-1: Study design (Pinto, 1996 ASB2012-11461)

Test group	Dose level of glyphosate acid (mg/kg/day)	# male	# female
Control	0	5	5
Low	250	5	5
Mid	500	5	5
High	1000	5	5

Preparation and treatment of animal skin:

Sixteen to twenty-four hours before application of the test substance, the hair was removed with a pair of veterinary clippers from an area, approximately 10 cm x 5 cm, on the dorso- lumbar region of each animal. The rats were dosed dermally and the amount applied was calculated for each animal according to its weight at the time of dosing. The paste covered by a gauze patch (approximately 7 cm x 7 cm x 4-ply) was applied to the shorn back of each animal and was kept in contact with the skin for approximately 6 hours using an occlusive dressing. The gauze patch was covered by a patch of plastic film (7 cm x 7 cm) and was held in position using adhesive bandage (25 cm x 7.5 cm). This was secured by two pieces of PVC tape (approximately 2.5 cm x 20 cm) wrapped around the animal. The control animals were treated in a similar manner except that deionised water only was used. The rats were dosed sequentially in group order at approximately the same time each day.

At the end of each 6-hour contact period, the dressings were carefully removed. The skin, at the site of application, was cleansed using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

A total of 15 six-hour applications was made during a period of 21 days. During this time there were three two-day periods when the animals were not dosed. Following each application there was an 18-hour ‘rest’ period during which the animals were fitted with plastic collars to prevent oral contamination.

Observations:

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. Detailed clinical observations were recorded daily and after decontamination. Cage-side observations were also made as soon as possible after dosing, and towards the end of the working day.

Body weight: The body weight of each rat was recorded daily, immediately prior to application of the test substance where applicable and prior to termination on day 22.

Food consumption and test substance intake: Food consumption was recorded continuously throughout the study for each rat and calculated as a weekly mean (g food/rat/day).
Haematology and clinical chemistry: Blood was collected at termination, by cardiac puncture and the following parameters were examined:
Haemoglobin
mean cell haemoglobin concentration
Haematocrit
platelet count
red blood cell count
total white cell count
mean cell volume
differential white cell count
mean cell haemoglobin
blood cell morphology
red cell distribution width
prothrombin time
activated partial thromboplastin time
Clinical chemistry: Blood was collected at termination, by cardiac puncture and the following parameters were examined:
Urea
alkaline phosphatase activity
Creatinine
aspartate aminotransferase activity
Glucose
alanine aminotransferase activity
Albumin
gamma-glutamyl transferase activity
total protein
calcium
Cholesterol
phosphorus (as phosphate)
Triglycerides
sodium
total bilirubin
potassium
creatine kinase activity
chloride
Investigations post mortem:
Macroscopic examination: All animals were examined post mortem. This involved an external observation and an internal examination of all organs and structures.
Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:
adrenal glands
liver
Kidneys
testes

Paired organs were weighed together.
Tissue submission: The following tissues were examined in situ, removed and examined and fixed in an appropriate fixative:
gross lesions including masses
adrenal gland*
testis*
epididymis*
Kidney
treated skin
Liver
untreated skin
* Tissues marked were stored but not examined microscopically
Microscopic examination: All selected tissues (see above) processed from the control and the group receiving 1000 mg glyphosate acid/kg/day, together with macroscopic abnormalities from these groups, were examined by light microscopy.
Statistics:
Haematology, clinical chemistry, organ weights and weekly food consumption were analysed using Analysis of variance. Body weights, on initial (day 1) body weight, organ weights on final body weight were analysed using analysis of covariance. All data were analysed using SAS (1989).
Results and discussion
Mortality: There were no mortalities.
Clinical observations: There were no significant signs of toxicity at any dose level of glyphosate acid. Generally the clinical findings observed were consistent with those
commonly seen in dermal studies as a consequence of bandaging and were considered not to be related to treatment with glyphosate acid.
Bodyweight and weight gain: There were no effects due to treatment with glyphosate acid on bodyweight at any dose level.
Food consumption: There were no effects due to treatment with glyphosate acid on bodyweight at any dose level.
Haematology: A minimal statistically significant increase in haemoglobin levels was observed in females dosed at 1000 mg glyphosate acid/kg/day. A statistically significant decrease compared with control was seen in red cell distribution width in females dosed at 250 and 1000 mg glyphosate acid/kg/day. In the absence of any adverse effects on the red cell parameters, these minor changes are considered not to be of toxicological significance.
Table B.6.3-2: Intergroup comparison of selected haematology parameters (Pinto, 1996 ASB2012-11461)
Dose level of glyphosate acid (mg/kg/day)
Males
Females
Parameter
0
250
500
1000
0
250

500
1000
Haemoglobin
15.2
15.3
15.3
15.0
13.9
13.7
14.1
14.6*
Red cell distribution width
13.1
12.9
12.6
13.4
13.8
12.4**
13.0
12.6*
* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)
Blood clinical chemistry: Females dosed at 1000 mg glyphosate acid/kg/day showed a minimal but statistically significant increase in plasma urea levels, but there were no differences seen in the plasma creatinine levels. This minimal change in urea was considered not to be of toxicological significance. A minimal but statistically significant decrease in plasma triglycerides was observed in males dosed at 500 mg glyphosate acid/kg/day and as this did not form part of a dose response relationship was considered not to be treatment related.
Table B.6.3-3: Intergroup comparison of selected clinical chemistry parameters (Pinto, 1996 ASB2012-11461)
Dose level of glyphosate acid (mg/kg/day)
Males
Females
Parameter
0
250
500
1000
0
250
500
1000
plasma urea
8.4
8.2
8.5

8.1
7.6
7.7
6.9
8.6*
plasma
trigycerides
1.27
1.01
0.87*
1.27
0.70
0.66
0.69
0.76
* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
Sacrifice and pathology:
Organ weights: Testes weights were slightly but statistically significantly decreased at 500 mg glyphosate acid/kg/day, due to one animal having a very low weight recorded. There were no effects due to treatment with glyphosate acid in the other organs weighed.
Macroscopic findings: A small number of lesions were observed, none of which was related to treatment.
Microscopic findings: A small number of common spontaneous lesions were observed, none of which was related to treatment.
Conclusion by the Notifiers
There was no evidence of systemic toxicity or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg/day.
The no observed adverse effect level (NOAEL) for systemic toxicity and dermal irritation was considered to be 1000 mg glyphosate acid/kg/day in both sexes.
RMS comments:
The study is considered acceptable even though most dermal studies in rats include a treatment period of four weeks while rabbits are administered a test substance via the skin usually for 21 days. We agree with the conclusions including setting of the NOAEL for both systemic effects and dermal irritation at the limit dose of 1000 mg/kg bw.
Studies with formulations Not available.
B.6.3.2 Subchronic toxicity (oral)
B.6.3.2.1 90-day studies in rats
A total of eight valid studies of this type were used for this evaluation of which 5 had been subject to the previous one (DAR, 1998, ASB2010-10302) yet. Re-evaluation of the studies by (1987, TOX9552362), (1989; TOX9552364) and Eadie
(1989, TOX9551821) from the old DAR revealed that all these may be still considered fully acceptable even though in the latter one the actual mean dietary intake was not calculated.
In contrast, for the study by (1993; TOX9650149), former assessment as "acceptable" was changed by the RMS into "supplementary" because the batch number but no purity of the test material was given. This same holds true for a study by (1992, TOX9551096) in which reporting deficiencies were noted. Mean dietary intake was calculated in an amendment but not for the recovery group and no

information was given which of the salivary gland had been examined histologically. Apparently, no additional control group was employed for the recovery part.
Two more supplementary studies mentiond in the original DAR were not included by the RMS into the re-evalution. The study by (1981, TOX9650152, in the former DAR erroneously cited as "anonymous") may be considered as sufficiently replaced by a more recent one (1993, TOX9650149) that had been commissioned by the same company. The study by (1990, TOX9500266) was run soon after the more comprehensive by (1989, TOX9551821) in the same laboratory and the same rat strain. The same dose
levels were used and no effects were noted up to the highest dose of 7500 ppm. Thus, this study would not contribute any new information to overall assessment.
One further study (1985, TOX9551822) was briefly described in the previous DAR and regarded therein as "supplementary" but is now considered not acceptable due to major reporting deficiencies. In addition, absence of statistical analysis was noted.
In addition, three new feeding studies in rats (1996, TOX2000-1990;
1996, ASB2012-11451; 1995, ASB2012-11452) were submitted that had not been reviewed on EU level before. Subsequent to the summary table, they are reported in detail,
with comments of the RMS on bottom of the respective study. For details regarding studies reviewed during the 2001 EU evaluation, we refer to the old monograph (DAR, 1998, ASB2010-10302).
1st new subchronic study in rats (1996)
Reference: IIA, 5.3.2/01
Report: 1996 First Revision To Glyphosate Acid: 90 Day Feeding Study In Rats
Laboratory Report No.: CTL/P/1599 Data owner: Syngenta
Date: 1996-11-07, not published, TOX2000-1990
Guidelines: No guideline statement, but in accordance with OECD 408 (1998), OPPTS 870.3100 (1998), 2001/59/EC B.26 (2001)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Test material:
Identification:
Glyphosate acid
Description:
White solid
Lot/Batch #:
P15
Purity:
97.4 %
Stability of test compound:
Not reported
Vehicle:
Plain diet / none
Test animals:
Species:
Rat
Strain:

Alpk:APfSD
Source:
Age:
36-38 days
Sex:
Male and female
Weight at dosing:
♂ 98-170 g; ♀ 96-140 g
Acclimation period:
Approximately 1 week
Diet/Food:
CTI diet, ad libitum, (except during collection of urine samples)
Water:
Mains water, ad libitum, (except during collection of urine
samples)
Housing:
4/cage, sexes separately in stainless steel cages 34.0 x 37.5
x 20.3 cm giving a floor area of 1275cm2
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 36 - 60 % Air changes: ≥ 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 1996-02-25 to 1996-05-25
Animal assignment and treatment:
The study consisted of one control and three treatment groups each containing twelve male and twelve female rats.
Table B.6.3-4: Study design
Test group
Dietary concentration (ppm)
Males
Females
Control
0
12
12
Low
1000
12
12
Mid
5000
12
12
High
20000
12

The study was divided into six single-sex replicates (randomised blocks). Each replicate consisted of four cages, one per treatment group. The animals were randomly allocated to cages.
The experimental diets were made in 60 kg batches by adding the appropriate amount of glyphosate acid to the diet using dry premixes.
Samples from all dietary levels (including controls) were taken from both batches prepared for the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was determined by analysing samples from the low and high dose levels from the first batch of diet. The chemical stability of glyphosate acid in diet was determined at the highest and lowest dose levels at 1, 4, 6 and 10 weeks after preparation. Analysis was by high performance liquid chromatography (HPLC).
Mortality: Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.
Clinical observations: A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before of the beginning of the treatment period and then once a week until the end of the study.
Body weight: The bodyweight of each animal was recorded immediately before feeding of the experimental diets commenced and then on the same day, where practicable, of each subsequent week until termination. The body weight determination was done on the same day on which the detailed clinical examination was performed.
Food consumption and untilisation: Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage. The food utilisation value per cage was calculated as the bodyweight gained by the rats in the cage per 100 g of food eaten.
Ophthalmoscopic examination: The eyes of all animals from the control group and the 20000 ppm glyphosate acid dose level group were examined in the week prior to termination, using an indirect ophthalmoscope and a mydriate to dilate the pupil.
Haematology and clinical chemistry: At termination, all surviving rats were bled by cardiac puncture and the blood samples were collected both in tubes containing EDTA as anticoagulant and also in tubes containing 0.11M trisodium citrate. These samples were submitted for haematological examination and the following parameters measured: haemoglobin, haematocrit, red blood cell count, MVC, MCHC, MCH, kaolin-cephalin times, thrombocytes, leucocytes, differential white cell count, red blood cell morphology, prothrombin time.
For clinical chemistry analysis blood samples were collected by tail vein bleeding at week 4 of the study and by cardiac puncture at termination (week 13). The blood was collected in lithium heparinised tubes and the following parameters measured: glucose, urea, total protein, albumin, total cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT).
Urinalysis: Urine samples were collected over an 18 hour (approximately) period from all rats during week 13 (the week prior to termination). During urine collection, the rats were individually housed in metabolism cages and denied access to food and water. The following parameters were measured:volume, pH, specific gravity, proteins, glucose, ketones, and urobilinogen.
Sacrifice and pathology: On completion of the treatment period, all surviving animals were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, epididymides, heart, kidneys, liver, and testes. Paired organs were weight together.

Tissue samples were taken from the following organs and preserved in buffered formalin: all gross lesions, adrenals, aorta, bone marrow (femur), brain, caecum, colon, duodenum, epididymides, eyes (stored), Harderian gland (stored), heart, ileum, jejunum, kidneys, larynx (stored), liver, lungs, lymph nodes (cervical and mesenteric), mammary gland, ovaries, oviducts, pancreas, pituitary gland, prostrate, rectum, salivary glands, seminal vesicles, spinal cord, sciatic nerve, skin, spleen, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus (with cervix), voluntary muscle and nasal cavity.
Following fixation, all tissues from the control and 20000 ppm glyphosate acid groups (except those stored) were processed by standard methods, embedded in paraffin wax, sectioned at 5µm, stained with haematoxylin and eosin and examined by light microscopy. Liver, kidney, adrenals, lungs and abnormal tissues from animals fed 1000 ppm or 5000 ppm glyphosate acid were also processed to blocks and were examined histologically.
Statistics: All data were evaluated using analysis of variance (bodyweight gain from start of study, final bodyweight, haematology, clinical chemisty – blood and urine, total food consumption and utilisation, organ weights) and covariance (organ weights on terminal bodyweights) for each specified parameter using the GLM procedure in SAS (1982).
Results and discussion
Mortality: There were no mortalities.
Clinical observations: The incidence of clinical findings was low and none was unequivocally related to treatment. There was a low incidence of diarrhoea (during the second week of the study) in the group receiving 20000 ppm glyphosate acid. The faeces of both sexes at this dose level were observed to be paler than those of control or other test groups.
Body weight: No relevant differences in the mean body weight gain were noted between controls and animals given 1000 or 5000 ppm.
Body weight gain was reduced in male rats fed 20000 ppm glyphosate acid from the first week of the study. The body weights continued to diverge from control values as the study progressed, and final body weights were approximately 8 % lower than those of controls (see Table B.6.3-5).
Table B.6.3-5: Intergroup comparison of bodyweight gain – selected timepoints from start of study
Mean cumulative bodyweight gain (g)
Timepoint
Initial
Week 1
Week 2
Week 4
Week 7
Week 10
Week 13
Final weight
Dose (ppm)
Males
0
135
51.8

104.0
185.5
254.6
305.1
333.3
468.3
1000
140.3
54.3
106.3
187.5
253.4
304.1
327.0
467.3
5000
136.3
51.8
103.4
186.1
255.1
306.3
331.9
468.3
20000
134.5
45.1**
94.0*
166.9**
226.0**
272.00**
295.8**
430.3**
Females
0
121.3
26.6
47.3
81.6
112.8
130.8
143.3
264.6
1000
122.2
27.7

51.4
82.7
113.1
132.1
146.0
268.2
5000
121.3
25.9
50.2
82.9
110.0
129.5
138.4
259.8
20000
118.6
24.3
53.5*
83.3
115.1
132.7
142.5
261.1
* Statistically significant from controls, p<0.05 (Student´s t-test, 2-sided)
** Statistically significant from controls, p<0.01 (Student´s t-test, 2-sided)
Food consumption and test substance intake: The food consumption of males fed 20000 ppm glyphosate acid was reduced from the fifth week of the study compared to control values but the reduction was small and did not attain statistical significance in any week. The food utilisation efficiency of males at this dose level was reduced throughout the study. The food consumption and food utilisation efficiency of males fed 1000 or 5000 ppm glyphosate acid and of females at all dose levels were similar to those of controls.
Table B.6.3-6: Intergroup comparison of food utilisation (g growth/100 g food)– selected timepoints from start of study
Dietary concentration of glyphosate acid (ppm)
Males
Females
Weeks
0
1000
5000
20000
0
1000
5000
20000

1-4
25.15
24.85
24.99
22.89
15.73
15.42
15.78
15.91
5-8
11.25
11.09
11.36
10.31
7.49
6.74
6.71
7.29
9-13
6.45
5.76
6.05
5.88
3.08
3.95
3.10
2.96
Overall (1-13)
13.59
13.30
13.44
12.54
8.28
8.34
8.14
8.25
* Statistically significant from controls, p<0.05 (Student´s t-test, 2-sided)
Calculated mean test compound intakes are presented in the following table.
Table B.6.3-7: Overall mean test compound intake
Dietary concentration of glyphosate acid (ppm)
Males
Females
Weeks
0
1000

20000
0
1000
5000
20000
1-13
0
81.33
413.5
1612
0
90.42
446.9
1821
* Statistically significant from controls, p<0.05 (Student´s t-test, 2-sided)
Ophthalmoscopic examination: There were no test substance-related ophthalmological findings at the end of the treatment period. The small incidence of findings recorded was within the normal background incidence for rats of this age and strain.
Haematology and clinical chemistry
Haematology: There were no treatment-related effects noted in any dose group.
Blood clinical chemistry: The plasma activities of alanine transaminase (ALAT) and alkaline phosphatase (ALP) were increased in both sexes fed 20000 ppm glyphosate acid throughout the study. Plasma aspartate transaminase activity was increased in females fed 20000 ppm glyphosate acid at week 4 only.
Plasma ALT activity was also increased in males receiving 5000 ppm glyphosate acid at weeks 4 and 13 and in females at week 4 only.
The plasma ALP activities of males receiving 5000 or 1000 ppm glyphosate acid were marginally increased. These increases were not dose-related and for the 1000 ppm glyphosate acid group were attributed to the high values in 3 out of 12 males. These marginal differences from the control group are considered to be of doubtful significance and not to be treatment- related.
Plasma urea levels were marginally decreased in both sexes at week 13 and in males at week 4 in the 20000 ppm glyphosate acid group.
Males receiving glyphosate acid showed marginal reductions in plasma glucose levels at week 4 but not at week 13. Females at 20000ppm glyphosate acid showed a slight increase in this parameter at week 13 only.
Plasma cholesterol levels were unaffected by treatment with glyphosate acid. Plasma triglyceride levels were slightly reduced in males receiving 20000 ppm glyphosate acid at both weeks 4 and 13, the effect being greater at week 13.
Both males and females receiving glyphosate acid showed marginal reductions in plasma albumin and total protein. The changes were not consistent, showed no dose-response relationship and are therefore considered to be of dubious significance.
Table B.6.3-8: Intergroup comparison of selected clinical chemistry parameters
Dietary concentration of glyphosate acid (ppm)
Males
Females
Parameter

Week
0
1000
5000
20000
0
1000
5000
20000
ALAT
4
61.0
66.8
76.0**
83.8**
47.3
50.8
57.7*
73.8**
13
51.9
52.3
62.3*
65.2**
45.0
45.2
46.2
55.0**
ALP
4
273
326**
320*
411**
188
199
212
309**
13
148
159
176*
215**
91
94
99

140**
ASAT
4
62.8
67.0
69.1
68.5
56.0
57.0
57.5
64.8**
Urea
4
47.0
45.8
44.6
43.6*
45.8
45.3
46.8
44.2
13
41.9
39.9
40.0
37.7*
40.6
40.1
42.1
35.9*
Glucose
4
143
133*
132*
128**
141
146
142
136
13
191
186
208
197
182

183
183
208**
Triglycerides
4
151
145
147
136
80
75
88
84
13
153
157
144
120**
72
74
77
77
* Statistically significant from controls, p<0.05 (Student´s t-test, 2-sided)
** . Statistically significant from controls, p<0.01 (Student´s t-test, 2-sided)
Urine analysis: There were no treatment-related findings.
Necropsy
Organ weights: Absolute heart weight of top dose males was reduced compared to controls but the reduction reflected the reduced bodyweight. There were no other differences in organ weights which were considered to be related to treatment
Gross pathology: A small number of lesions were observed, none of which was related to treatment.
Histopathology: There were no histopathological findings related to treatment. The incidence of findings was low and, with one exception, of a type commonly found in rats of this strain and age. An uterine leiomyosarcoma was seen in a female fed 5000 ppm glyphosate acid. Whilst the occurrence of a malignant tumour of smooth muscle in the uterus of a young rat is unusual, this isolated finding in an intermediate dose group is considered not to be related to treatment.
Conclusion by the Notifiers
Under the experimental conditions of the study and taking into account the reduced growth (males only) and biologically insignificant changes in clinical chemistry parameters at the high dose-level, the No Observed Adverse Effect Level (NOAEL) is considered to be 5000 ppm (equivalent to 414 and 447 mg/kg bw/day for amles and females, respectively).
RMS comments
This study is considered acceptable and the conclusions, including setting of the NOAEL, are agreed with. The significant changes in clinical chemistry parameters at the mid dose level in males might be indicative of a weak effect on the liver, however, since they were not accompanied by histological lesions and/or liver weight increase, are not regarded as adverse. In addition to the study description above, it should be noted that, as in previous studies with glyphosate, urine pH was significantly

decreased in top dose males and in mid and high dose females. From the study report it seems that salivary glands were taken but neither weighed nor examined microscopically although they were a target organ in other studies.
2nd new subchronic study in rats (, 1996)
Reference: IIA, 5.3.2/03
Report: Coles, L.J., Thomas, O.N., Bartlett, A.J., Brooks, P.N. 1996, Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study In The Rat
Project No.: 434/016
Date: 1996-07-16, unpublished, ASB2012-11451
Guidelines: JMMAF 59 NohSan No. 4200
(Data from the study report is equivalent to OECD 408.)
Deviations: FOB was not conducted. Some mandatory organs were not weighed/examined.
GLP: yes
Acceptability: See RMS comment
Materials and methods
Test material:
Identification:
Technical Glyphosate
Description:
White powder
Lot/Batch #:
H95D 161 A
Purity:
95.3 %
Stability of test compound:
No data given in the report.
Vehicle:
Plain diet
Test animals:
Species:
Rats
Strain:
Sprague-Dawley (CD)
Source:
Age:
6-7 weeks
Sex:
male and female
Weight at dosing:
♂ 175 - 218 g; ♀ 145 - 195 g
Acclimation period:
7 days
Diet/Food:
Rat and Mouse SQC Ground Diet No.1 (Special Diets Services Limited, Witham, Essex, UK), ad libitum
Water:
tap water, ad libitum

Housing:
In groups of up to four by sex in polypropylene grid-floor cages.
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 1995-08-11 to 1996-01-30
Animal assignment and treatment:
In a 90 day feeding study, groups of 10 Sprague Dawley rats per sex received daily dietary doses of 0, 1000, 10000 and 50000 ppm (equivalent to mean achieved dose levels of male; 0, 79, 730 and 3706 mg/kg bw/day, female; 90, 844, 4188 mg/kg bw/day respectively) technical glyphosate in the diet.
Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.
The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for homogeneity and achieved concentration.
Clinical observations: A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded.
Body weight: Individual body weights were recorded on Day 0 (prior to treatment) and at weekly intervals thereafter. Body weights were also determined at necropsy.
Food consumption and compound intake: Food consumption was recorded once weekly for each cage group throughout the study.
Water consumption: Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.
Ophthalmoscopic examination: The eyes of all control and high dose animals were examined before administration of the test and control diets and before termination of treatment (during Week 12). Examinations included observation of the anterior structures of the eye, pupillary and corneal blink reflex and, following pupil dilation with 0.5 % Tropicamide solution ("Mydriacyl" - Alcon Laboratories Ltd., Watford, Hertfordshire, UK), detailed examination of the internal structure of each eye using a direct ophthalmoscope.
Haematology and clinical chemistry: Haematological and blood chemical investigation were performed on all animals from each test and control group at the end of the study (Day 90).
Urinalysis: Urinalytical investigations were performed on all animals during Week 12. Urine samples were collected overnight by housing the rats in metabolism cages. Animals were maintained under conditions of normal hydration during collection but without access to food.
Sacrifice and pathology: All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, gonads, heart, kidneys, liver, pituitary and spleen.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (at three levels), caecum, colon, duodenum, eyes, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary

bladder, uterus and vagina.
Statistics: Absolute and relative organ weights, haematological and blood chemical data were analysed by one way analysis of variance incorporating 'F-max' test for homogeneity of variance. Data showing heterogeneous variances were analysed using Kruskal-Wallis non- parametric analysis of variance and Mann Whitney U-Test.
The levels of probability chosen as significant were $p < 0.001$***, $p < 0.01$** and $p < 0.05$*.
Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:
1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of 1 or greater.
2. Kruskal-Wallis one way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.
The levels of probability chosen as significant were $p < 0.001$***, $p < 0.01$**, $p < 0.05$* and $p < 0.1$ (*).
Results and discussion
Mortality: No deaths occurred during the study.
Clinical observations: Animals of both sexes treated with 50,000 ppm showed soft faeces and diarrhoea from Day 4 which continued throughout the study period.
The remaining observable sign of generalised fur loss was noted in one male and two females treated with 10,000 and 1,000 ppm respectively. This is a commonly reported incidental finding in laboratory maintained rats that, in the absence of any dose-related response, is of no toxicological significance and unrelated to treatment with the test material.
Body weight: Animals of both sexes treated with 50,000 ppm showed a reduction in bodyweight gain over the first four weeks of treatment when compared with controls (see Table B.6.3-9). Female bodyweight development recovered as the study progressed and was comparable with the control group by the end of the treatment period. Male individuals showed only a partial recovery with body weight gain remaining slightly lower than the control group values during the subsequent weeks of treatment.
Body weight development was unaffected by treatment with the test material at the remaining dose levels.
Table B.6.3-9: Group mean weekly bodyweights and standard deviations (sd)
Dietary concentration
Bodyweight (g) at Day
(ppm)
0
7
14
21
28
35
42
49
56
63
70
77

84
90
Males
0
mean
206
269
315
354
382
411
444
457
488
508
523
537
536
551
sd
8
12
17
24
33
38
45
44
49
52
55
58
56
58
1000
mean
199
260
309
350
377
400
427
446
470
485

497
513
516
528
sd
11
14
19
21
24
26
30
31
32
32
35
37
36
37
10000
mean
200
257
303
338
364
393
414
429
454
470
483
494
495
506
sd
12
12
15
21
25
30
35
35
38
38

38
40
39
43
50000
mean
198
215
247
268
283
306
329
335
356
369
382
394
395
408
sd
8
8
15
21
26
31
33
38
41
43
43
44
42
44
Females
0
mean
173
197
214
232
243
256
269
276

284
291
295
306
304
307
sd
9
11
12
15
16
18
20
19
20
21
24
25
25
27
1000
mean
173
199
218
238
249
261
272
280
286
292
300
308
304
313
sd
10
13
14
16
16
17
18
19

18
18
19
21
20
20
10000
mean
166
184
201
217
226
237
246
256
262
267
272
277
276
282
sd
14
18
21
25
24
26
27
27
27
27
27
29
28
29
50000
mean
173
183
197
214
219
231
240

246
251
260
265
271
267
273
Sd
11
12
14
15
14
18
21
21
20
23
23
26
22
25

Food consumption: Animals of both sexes treated with 50,000 ppm showed a reduction in both dietary intake and food efficiency over the first four weeks of treatment when compared with controls (see Table B.6.3-10). Female food consumption and efficiency recovered as the study progressed and was comparable with control values by the end of the treatment period. Male food consumption however, remained adversely affected during the subsequent weeks of treatment. A similar prolonged effect on food efficiency was not evident during the same period as male body weight gain demonstrated a partial recovery over the corresponding weeks.

Dietary intake and food efficiency were unaffected by treatment with the test material at the remaining dose levels and were comparable with controls.

Table B.6.3-10: Group mean weekly food consumption

Dietary
concentration (ppm)

Mean food consumption (g/rat/week)

1
2
3
4
5
6
7
8
9
10

11
12
13*
Males
0
201
199
204
212
208
218
208
222
224
223
24
192
185
1000
200
205
213
211
205
210
211
217
204
219
214
191
180
(0)
(3)
(4)
(0)
(-1)
(-4)
(1)
(-2)
(-9)
(-2)
(0)
(-1)
(-3)
10000

187
193
199
204
202
198
201
205
211
211
201
185
179
(-7)
(-3)
(-2)
(-4)
(-3)
(-9)
(-3)
(-8)
(-6)
(-5)
(-6)
(-4)
(-3)
50000
122
183
178
177
183
182
168
187
189
193
188
174
171
(-39)
(-8)
(-13)
(-17)
(-12)
(-17)

(-19)
(-16)
(-16)
(-13)
(-12)
(-9)
(-8)
Females
0
140
131
171
153
149
149
152
152
151
147
155
139
128
1000
143
146
152
156
158
163
157
159
152
154
161
141
137
(2)
(+11)
(-11)
(2)
(6)
(9)
(3)
(5)
(1)
(5)

(4)
(1)
(7)
10000
123
135
142
144
143
140
143
146
143
143
142
133
131
(-12)
(3)
(-17)
(-6)
(-4)
(-6)
(-6)
(-4)
(-5)
(-3)
(-8)
(-4)
(2)
50000
128
143
131
148
167
157
148
151
151
151
161
139
139
(-9)
(9)

(-23)
(-3)
(23)
(5)
(-3)
(-1)
(0)
(3)
(4)
(0)
(9)
( ) - % change compared to control group * - Week 13 comprises six days only
Water consumption: There were no treatment-related effects on water consumption for either sex noted during the study.
Ophthalmoscopic examination: No treatment-related ocular effects for either sex noted were detected during the study.
Haematology: No treatment-related effects were detected in the haematological parameters measured.
Blood chemistry: Animals of both sexes treated with 50,000 or 10,000 ppm showed a statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase (AP) when compared with controls (see Table B.6.3-11). A statistically significant increase in inorganic phosphorus and reduction in plasma creatinine were also evident amongst animals of both sexes treated with 50,000 ppm whilst females at this dose level showed statistically significant reductions in total plasma protein and albumin in comparison with controls.
There were no further treatment-related effects.
Table B.6.3-11: Group mean blood chemical values and standard deviations (sd)
Dietary concentration
Ca2+
AP
P
Creatinine
Total protein
Albumin
(ppm)
(mmol/L)
(IU/L)
(mmol/L)
(mg/dL)
(g/dL)
(g/dL)
Males
0
mean
2.74
373
2.23

0.61
–
–
sd
0.06
101
0.22
0.03
–
–
1000
mean
2.77
404
2.22
0.62
–
–
sd
0.07
115
0.16
0.05
–
–
10000
mean
2.66*
514*
2.32
0.59
–
–
sd
0.09
106
0.28
0.04
–
–
50000
mean
2.64*
597***
2.46*
0.57*

–
–
sd
0.10
150
0.22
0.04
–
–
Dietary concentration
Ca2+
AP
P
Creatinine
Total protein
Albumin
(ppm)
(mmol/L)
(IU/L)
(mmol/L)
(mg/dL)
(g/dL)
(g/dL)
Females
0
mean
2.78
230
1.70
0.69
7.63
3.90
sd
0.11
38
0.33
0.07
0.45
0.23
1000
mean
2.76
261
1.65
0.69
7.64

3.87
sd
0.05
71
0.21
0.04
0.29
0.13
10000
mean
2.70*
408***
1.76
0.65
7.41
3.82
sd
0.07
123
0.23
0.04
0.45
0.20
50000
mean
2.56***
358**
2.12***
0.61**
6.86**
3.47***
sd
0.10
90
0.15
0.05
0.82
0.39
– no significant changes
* significantly different from control group ($p < 0.05$)
** significantly different from control group ($p < 0.01$)
*** - significantly different from control group ($p < 0.001$)
Urinalysis
Animals of both sexes treated with 50,000 ppm showed an increased level of haemoglobin in the urine when compared with controls (see Table B.6.3-12). Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated at 50,000 ppm.

This probably represents external contamination, possibly of faecal origin.
There were no treatment-related changes detected at the remaining dose levels.
Table B.6.3-12: Urine findings
Dietary concentration (ppm)
Blood (haemoglobin)
Males
Females
-
+
++
+++
-
+
++
+++
0
8
0
1
1
10
0
0
—
1000
10
0
0
0
10
0
0
—
10000
7
2
1
0
10
0
0
—
50000
1
5
2

2
4
3
3
—
– - negative
+ - ca. 5-10 + 106 ery/L; ++ - ca. 50 / 106 ery/L; +++ - ca. 250 / 106 ery/L
Organ weights
Animals of both sexes treated with 50,000 ppm showed statistically significant increases in both relative liver and kidney weight when compared with controls (see Table B.6.3-13).
There were no further direct effects of treatment.
Table B.6.3-13: Group mean organ weights and standard variations (sd)
Dietary concentration (ppm)
Relative organ weight (%)
Liver
Kidney
♂
♀
♂
♀
0
mean
2.9749
2.9734
0.5861
0.6516
sd
0.2629
0.1558
0.0575
0.0523
1000
mean
2.8868
2.9093
0.5901
0.6257
sd
0.2552
0.2146
0.0804
0.0375
10000
mean
2.8853
2.9801

0.6070
0.6454
sd
0.3758
0.1556
0.0552
0.0532
50000
mean
3.2433*
3.1989*
0.6963***
0.7180*
sd
0.2452
0.2098
0.0436
0.0707
* - significantly different from control group (p < 0.05)
*** - significantly different from control group (p < 0.001)
Necropsy
Macroscopic abnormalities were detected in the 50,000 ppm dose group with all animals showing enlarged and fluid-filled caecums whilst one female treated with 50000 ppm showed gaseous distension of the stomach at terminal kill.
There were no treatment-related macroscopic abnormalities detected at 10,000 or 1,000 ppm.
Histopathology
Treatment-related changes were observed in the caecum. Atrophy, characterised by flattening of the intestinal mucosa, was observed for five rats of both sexes dosed at 50,000 ppm (p < 0.05 for male rats) and for one male and two female rats receiving 10,000 ppm of the test material. The aetiology of this change is uncertain and may represent no more than a stretch atrophy of the mucosa resulting from caecal distension.
There were no further treatment-related changes.
Conclusion by the Notifiers
Dietary administration of the test material, technical glyphosate, to rats for a period of ninety consecutive days at dietary concentrations of up to 50,000 ppm resulted in treatment-related changes at 50,000 and 10,000 ppm. No such effects were demonstrated in the 1,000 ppm treatment group and the “No Observed Effect Level” was, therefore, considered to be 1000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females).
RMS comments
The study is considered acceptable and the conclusions drawn are supported. The data submitter/owner was not mentioned in the study description but is presumed to be Nufarm. According to the study report itself, the sponsor was Mastra Industries (Malaysia) that gave Nufarm access to this study.
The NOAEL of 79 mg/kg bw/day is agreed with. It must be kept in mind that, due to dose spacing, the margin between the low and mid dose level was very large. The LOAEL of 730 mg/kg bw/day was well above the NOAELs in other 90-day studies. Thus, despite the (relatively) low NOAEL, this study is

not suitable to prove that toxicity of glyphosate was in fact higher than previously assumed. It must be also emphasised that the top dose level of 3700 mg/kg bw/day was indeed extremely high, even for this rather non-toxic compound.
Unfortunately, it is not clear from the report whether or not salivary glands (and if, which of them) were subject to histopathology.
Organ weight changes (including higher relative testis weight in addition to liver and kidney) at the top dose level are considered secondary to reduced body weight.
3d new subchronic study in rats (1995)
Reference: IIA, 5.3.2/04
Report: (1995) HR-001 : 13-week Subchronic Oral Toxicity Study in Rats.
Laboratory Report No.: 94-0138
Data owner: Arysta LifeScience Date: 1995-07-20
not published, ASB2012-11452
Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 408 (1981)
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Materials and methods
Test material:
Glyphosate technical
Identification:
HR-001
Description:
White crystal
Lot/Batch #:
940908-1
941209
T-941209
Purity:
95.68 %
95.0 %
97.56 %
Stability of test compound:
12/12/1994
19/12/1994
26/12/1994
Vehicle:
Plain diet / none
Test animals:
Species:
Rat
Strain:

Sprague-Dawley Crj:CD
Source:
Age:
5 weeks
Sex:
Male and female
Weight at dosing:
♂ 136-150 g; ♀ 109-121 g
Acclimation period:
1 week
Diet/Food:
MF Mash (Oriental Yeast Co., Ltd.
Water:
Filtered and sterilized tap water, ad libitum
Housing:
3/cage, sexes separately in stainless steel cages 31.0 x 44.0 x 20.3 cm
Environmental conditions:
Temperature: 24 ± 2 °C
Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 1994-12-06 to 1995-03-22
Animal assignment and treatment:
The test substance was incorporated into the basal effect diet and administered on a continuous basis in the basal diet to groups of 24 Sprague-Dawley rats (12 males + 12 females) for a period of 13 weeks. Dietary concentrations were 0, 3000, 10000 and 30000 ppm.
Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level takent from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.
Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.
Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.
Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weight were recorded for all animals before necropsy.
Food consumption and utilisation
Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumptionby the number of animals per cage and by the number of days for measurement. Group

mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.
Group mean chemical intake (mg/kg/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.
Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.
Ophthalmoscopic examination
Ophthalmological examinations including observation with a haloen ophtalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13.
The following parts of the eye were inspected: eyeball, cornea, anterior chamber, pupil, iris, lens/vitreous body, fundus.
Haematology and clinical chemistry
After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomized under anesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.
The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC) and differential leukocyte count.
After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.
The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), y-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), potassium (K) and chloride (Cl).
Urinalysis
At 13 week of treatment, all surviving animals were subjected to urinalysis. Fresg urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was detmerined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semiquantitatively analyzed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.
Sacrifice and pathology
Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.
The following organs/tissues were taken: brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, pharynx, buccal mucosa of oral cavity, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

Statistics

All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologicla parameters, blood chemical parameters, and organ weights). Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

Results and discussion

Mortality:

No deaths were noted in the control and treated groups of either sex.

Clinical observations:

There were no abnormalities related to the treatment in the treated groups of either sex. In the 30 000 ppm group, one female showed a poor general condition including emaciation and decreased spontaneous motor activity. The poor general condition seemed to be caused by elongated incisor, malocclusion, or hepatorenal genetic lesions revealed by histopathology. Thus it was not considered to be treatment related.

Body weight:

In the 30 000 ppm group, body weights of males and females were slightly lower (about 5- 10 % decrease in males and 5 % in females) than those in the control throughout the treatment period. Statistically significant decreases in their body weights were sporadically observed during the treatment period (weeks 3, 4 and 11 in males and weeks 10 and 11 in females) when compared to the control.

In the 10 000 and 3 000 ppm groups, body weight changes in males and females were comparable to the control throughout the treatment period.

Food consumption and test substance intake:

In the 30 000 ppm group, males and females showed significant decreases in food consumption at week 1 which were 9 and 14 % lower than that of the control, respectively. However, their food consumption were comparable to the control at week 2 and thereafter.

In the 10 000 no significant change was observed while in the 3000 ppm group, significant changes were sporadically observed during the treatment period in females during the weeks 6 and 7. The food consumption recovered from the week 8 up to the end of the study.

The overall food consumption by males and females was comparable to the control and there were no abnormalities considered treatment related.

The overall group mean chemical intakes averages, calculated from food consumption and nominal concentrations of the test substance, through the treatment period, were:

Table B.6.3-14: Mean daily intake of glyphosate in the study by Kinoshita (1995, ASB2012-11452)

Dose (ppm)	Chemical Intake (mg/kg bw/day)	
	Male	Female
3 000	168.4	195.2
10 000	569	637
30 000	1735	

1892

Ophthalmoscopic examination

In the ophtalmological examination performed on all animals before the start of the treatment and on the animals of the control and 30 000 ppm groups at 13 weeks of treatment, no abnormalities were observed in either sex.

Haematology: Tere were no abnormalities in any group of either sex.

Blood clinical chemistry: In the 30 000 ppm group, females showed a significant increase in alkaline phosphatase (ALP) activity and a significant decrease in albumin (Alb). There were no abnormalities in males.

In the 10 000 and 3 000 ppm groups, there were no abnormalities in either sex.

Urine analysis: In the 30 000 ppm group, urine pH in males and females was significantly lower than that in the control. Urine protein showed a significant decrease in males and a decreasing trend in females. In addition, females showed a significantly higher urine volume than that of the control, but males showed a decreasing trend in urine volume as compared with the control.

In the 10 000 ppm group, urine, pH and protein in males were lower than those in the control. In females, no statistically significant change was observed in any parameter.

In the 3 000 ppm group, no statistically significant changes was observed in either sex.

Organ weights: In the 30 000 ppm group, both sexes showed significant increases in absolute and relative weights of the cecum (containing contents). In addition, females in this group also showed significant increases in relative weights of the brain and liver.

In the 10 000 ppm group, the absolute and relative weight of the cecum showed a statistically significant increase in males and increasing trend in females.

In the 3 000 ppm group, there were no abnormalities attributable to the treatment in either sex.

Gross pathology: In the 30 000 ppm group, distention of the cecum was observed in 9/12 males and 7/12 females with statistical significance. There were no other macroscopic abnormalities attributable to the treatment.

In the 10 000 ppm group, 3/12 males showed distention of the cecum, but there were no macroscopic abnormalities in females.

In the 3 000 ppm group, there were no macroscopic abnormalities attributable to the treatment in either sex.

Histopathology: Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment-related changes were not observed. One male in the 10 000 ppm group and one female in the 30 000 ppm group showed renal lesion (polycystic kidney) and hepatic lesions (bile ductal proliferation and cholangiectasis). It is generally regarded that these lesions were caused by genetic disorder and were not considered to be treatment-related.

Conclusion by the Notifiers

Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) is considered to be 3000 ppm (equivalent to 168.4 and 195.2 mg/kg bw/day for males and females, respectively).

RMS comments:

The study is considered acceptable and the proposed NOAEL is agreed with. Toxicity became apparent by the caecum findings and alterations in few clinical chemistry and urine parameters. The LOAEL of 569 mg/kg bw/day (10000 ppm) is higher than the NOAEL as established in other studies and, thus, does not contradict the previous assessment. From the study report, it became clear that submaxillary and sublingual salivary glands were histologically examined, without evidence of pathological changes. These glands were not weighed and parotid gland was not taken.

B.6.3.2.2 90-day studies in mice

There are only two acceptable studies of which one (Perry et al., 1991, TOX9552363) was already available for the previous evaluation and is described in the old DAR (1998, ASB2010-10302). A further, rather old study (Tierney and Rinehart, 1979, TOX9552360) does not comply to modern standards, is now considered not acceptable by the RMS and was deleted from current new assessment. A main deficiency of this study was that hematological and clinical chemistry evaluations were not included.

Below, the new study by Kuwahara (1995, ASB2012-11453) is described in detail and commented by the RMS.

New subchronic feeding study in mice (1995)

Reference: IIA, 5.3.2/05

Report: 1995, HR-001: 13-week Subchronic Oral Toxicity Study in Mice.

Laboratory Report No.: 94-0136

Data owner: Arysta LifeScience Date: 1995-07-24

not published, ASB2012-12453

Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985;

U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 408 (1981)

Deviations: None

GLP: Yes

Acceptability: See RMS comment

Dates of experimental work: 1994-12-15 to 1995-07-24

Materials and methods

Test material:

Glyphosate technical

Identification:

HR-001

Description:

White crystal

Lot/Batch #:

T-941209

Purity:

97.56 %

Stability of test compound:

26/12/1994

Vehicle:

Plain diet / none

Test animals:

Species:

Mouse

Strain:

Crj:CD-1

Source:

Age:

5 weeks

Sex:

Male and female

Weight at dosing:
♂ 27.3-32.7 g; ♀ 22.4-25.8 g
Acclimation period:
9 days
Diet/Food:
MF Mash (Oriental Yeast Co., Ltd.)
Water:
Filtered and sterilized tap water, ad libitum
Housing:
3/cage, sexes separately in stainless steel cages 21.5 x 33.0 x 18.0 cm
Environmental conditions:
Temperature: 24 ± 2 °C
Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle
In life dates: 1995-01-10 to 1995-04-27
Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 24 SPF ICR mice (Crj : CD-1) (12 males + 12 females) for a minimum of 90 days. Dietary concentrations were 0, 5 000, 10 000 and 50 000 ppm.
Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level takent from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.
Mortality: Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.
Clinical observations: Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.
Body weight: Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weights were recorded for all animals before necropsy.
Food consumption and utilisation: Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumptionby the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.
Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.
Group mean chemical intake (mg/kg/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.
Ophthalmoscopic examination: Ophthalmological examinations including observation with a haloen ophtalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13. The following parts of the eye were inspected: Eyeball, cornea, anterior chamber, pupil, iris.

Haematology and clinical chemistry: After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomized under anesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.
The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC).
After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.
The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), m-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P).
Urinalysis: At 13 week of treatment, all surviving animals were subjected to urinalysis. Fresh urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was determined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semiquantitatively analyzed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.
Sacrifice and pathology: Clinical pathology evaluations were also conducted. Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.
The following organs/tissues were taken and preserved: brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio- femoral joint, lymph nodes, heart, aorta, pharynx, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands,
ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.
Statistics: All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.
Results and discussion
Mortality: There were no animals found dead or had to be killed in extremis in any groups during the treatment period.
Clinical observations: There were no treatment-related abnormalities in clinical signs in the control and treated groups during the treatment period.
Body weight: In the 50 000 ppm group, mean body weights of males were lower than those of the control form week 2 to the end of the treatment period. Mean body weight of males at week 13 was 91% of that of control. Body weights of females were comparable to the control during the treatment period.

In the groups treated at 10 000 ppm or less, body weights of males and females were comparable to the controls during the treatment period.
Food consumption and test substance intake: In males of the 50 000 ppm group, a significant depression of food consumption was recorded at week 1. Average food consumption of males during the treatment period was 94 % of the control value. Food consumption of females were comparable to the control.
In the groups treated at 10 000 and 5000 ppm, food consumption of males and females was comparable to that of the controls.
The average daily chemical intakes during the treatment are shown in the following Table B.6.3-15:
Table B.6.3-15: Mean daily glyphosate intake in the study by (1995, ASB2012-11453)
Dose level (ppm)
Average chemical intake (mg/kg bw / day)
Male
Female
5 000
600.2
765.0
10 000
1221
1486
50 000
6295
7435
In the 50 000 ppm group, food efficiency of males and females was lower than that of the controls at almost all measuring points during the treatment. Average food efficiency of males and females was calculated to reach only 79 % and 88 % of the respective control value.
In the groups treated at 10 000 and 5 000 ppm, food efficiency in the treated groups of both sexes was comparable to that in the controls though some significant fluctuations were recorded sporadically.
Ophthalmoscopic examination: There were no ophtalmological abnormalities in the animals of both sexes in the highest dose group and the control group.
Haematology: In the 50 000 ppm group, females showed significant decreases in hematocrit (Ht), hemoglobin concentration (Hb) and erythrocyte count (RBC) by up to 92 % while males showed no significant differences from the control in any parameters.
There were no significant differences in any parameters between the treated groups of 10 000 ppm or less and the control of either sex..
Blood clinical chemistry: The statistically significant changes observed in the treated groups are summarized in the following table (Table B.6.3-16):
Table B.6.3-16: Alterations in clinical chemistry parameters in mice fed glyphosate for 90 days (1995, ASB2012-11453)
Parameter
Sex
Dose group (ppm)
5 000
10 000

Alkaline phosphatase (ALP)
Male
No change
No change
↗
184*
Female
No change
No change
↗
150
Glutamic pyrvic transaminase (GPT)
Female
No change
No change
↘
69
Creatine phosphokinase (CPK)
Female
↗
361
No change
↗
943
Blood urea nitrogen (BUN)
Female
No change
↗
119
No change
Inorganic phosphorus (P)
Female
No change
No change
↗
128
* The figures represent the mean percentage of change as compared to the respective control.
In the 50 000 ppm group, males and females showed a significant increase in alkaline phosphatase (ALP). In females, creatine phosphokinase (CPK) and inorganic phosphorus (P) were significantly increased, while a significant decrease in glutamic pyrvic transaminase (GPT) was noted.
In the 10 000 ppm group, females exhibited a significant increase in blood urea nitrogen (BUN). There were no significant change in any parameters in males.
In the 5 000 ppm group, females showed a significant increase in CPK, while there were no significant change in any parameters in males
Urine analysis: In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

Organ weights: In the 50 000 ppm group, males and females showed significant increases in both absolute and relative weights of the cecum. The absolute weights of the cecum of males and females were 238 % and 187 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 263 % or 195 % in males or females.
In the 10 000 ppm group, absolute and relative weights of the cecum showed increasing tendencies in males and females. The absolute weight of the cecum of males and females were 115 % and 122 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 111 % or 117 % in males or females.
In the 5 000 ppm, there were no significant changes in any organ weights of males and females.
Gross pathology: In the 50 000 ppm group, males and females showed a significant increase in incidence of distention of the cecum (12/12 in males and 10/12 in females, in contrast to 0/12 in males and females of the control group).
In the 10 000 ppm group, distention of the cecum was observed in one female. There were no significant changes in incidence of any macroscopic lesions in males.
In the 5 000 ppm group, there were no treatment-related abnormalities in males and females.
Histopathology: In the 50 000 ppm group, males showed significant increases in incidence of cystitis of the urinary bladder (4/12 as compared to 0/12 in the control group). There were no significant changes in incidence in females. Although significant increases in incidence of distention of the cecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the cecum.
In the 10 000 and 5 000 ppm groups, there were no significant differences in incidence of histopathological lesions from the control in either sex.
Conclusion by the Notifier
Under the experimental conditions of the study, the No Observed Effect Level (NOEL) is considered to be 5000 ppm (equivalent to 600.2 and 765.0 mg/kg bw/day for males and females, respectively).
RMS comments:
The study is considered acceptable. (It was noted that the study director was in fact as in a 90-day rat study from the same laboratory. performed the histopathological examinations and was apparently the report writer.)
Because of the only minor effects (slightly higher blood urea nitrogen, slight caecal distention) at the mid dose level of 10000 ppm (equal to 1221 mg/kg bw/day in males) that were not accompanied by any histopathological findings, this dose is considered the NOAEL. Target organs at the very high dose of ca 6300 mg/kg bw/d (50000 ppm) were the caecum and the bladder. Clinical chemistry findings also suggest a weak effect on the liver. This dose level was clearly toxic as additionally proven by effects on body weight gain, food consumption and efficiency and on red blood cell parameters.
Thus, the outcome of this study is in line or at least not in contradiction to the previous study by . (1991, TOX9552363) on CD mice in which no effects were observed up to the top dose level of 4500 mg/kg bw/day.
The lower urinary pH in males in all dose groups is due to acidic properties of the test subtance and cannot be considered a toxic effect.
In contrast to the publication by (1992, TOX9551954, reported below), no histological changes of the salivary glands were observed in this study as they had occurred in another strain from dietary concentrations of 6250 ppm (1065 mg/kg bw/day) onwards. Beside possible strain differences, another explanation might be that (1995, ASB2012-11453) examined the sublingual and submaxillary glands histologically but not the parotis. In the study by (1991, TOX9552363), histopathological examination of salivary glands was confined to the submaxillary.

Glyphosate was tested in the 1980ies in U.S. National Toxicology Program (NTP) for oral subchronic toxicity (Chan and Mahler, 1992, TOX9551954). The following paragraph was partly copied from the previous DAR (1998, ASB2010-10302):

Groups of ten B6C3F1 mice per sex and dose were fed glyphosate (supplied by Monsanto, approximately 99 % pure) for 13 weeks at dietary levels of 0, 3125, 6250, 12500, 25000 or 50000 ppm. Clinical pathology investigations were not performed but all animals were necropsied at study termination. Mice of the highest dose group and of the control group were subjected to complete histopathological examination. In addition, salivary glands were examined microscopically in all dose groups. There were no clinical signs of toxicity. However, one high dose female died from undetermined causes. Body weight gain was depressed at the two upper dose levels in both sexes. From the dose level of 6250 ppm onwards, a dose-related increase in occurrence and severity of cellular alteration of the parotid salivary gland was noted. This change consisted of basophilia of the acinar cells and in more severely affected glands, the cells and acini also appeared enlarged with an associated relative

reduction in the number of ducts. A dose of 3125 ppm (ca 507 mg/kg bw/day seems to represent the NOEL for substance-related effects. However, the extent of investigations performed was rather limited.

B.6.3.2.3 Oral 90-day and 1-yr studies in dogs

For the previous EU evaluation, two 90-day studies in dogs had been submitted (
1991, TOX9650150; 1985, TOX9551823) that do not comply with modern standards and were assessed now by the RMS as “not acceptable”. The same holds true for a 12-month study by (1992, TOX9650153). Therefore, these studies were excluded from current re-evaluation of glyphosate, as well as a one-year study by (1985, Z35385) that was briefly described in the 1998 DAR (ASB2010-10302) but had never been submitted as part of an EU dossier, neither in the 1990ies nor in 2012. It is available in Germany but, without effects up to the highest dose level of 500 mg/kg bw/day, this study would not alter overall assessment.

In contrast, the 12-month study of (1990, TOX9552384) is still considered acceptable from a todays point of view and is included in the respective table in Volume 1 (2.6.3) and in this chapter. It is reported in detail in the old DAR (1998, ASB2010-10302).

In addition, there is an acceptable study in which the formulation MON0139 (62.49 % IPA salt of glyphosate) had been administered for six months in gelatine capsules to Beagle dogs
, 1983, TOX9552361). This study is still included since it was found acceptable upon re-evaluation by RMS. At least, it is suitable to show that this salt proved to be of no higher toxicity in dogs than the acid.

Thus, to an even larger extent than in other fields of toxicological testing of glyphosate, evaluation of its toxicity to dogs is based on new studies that are all reported in detail below.

1st new 90-day study in dogs (2007)

Reference: IIA, 5.3.3/01

Report: 2007 Glyphosate Technical: 13-Week Toxicity Study By Oral Route (Capsule) In Beagle Dogs Laboratory Study No.: 29646 TCC Data owner: Nufarm

Date: 2007-07-15

not published, ASB2012-11454

Guidelines: OECD 409

JMMAF 12 NohSan No. 8147

Deviations: None

GLP: Yes

Acceptability: See RMS comment
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
White crystalline powder
Lot/Batch #:
H05H016A
Purity:
95.7 %
Stability of test compound:
Stable under storage conditions (< 30 °C), light protected; Expiry date: 2008-03-25
Vehicle:
Empty gelatine capsules, size 12 (Torpac, NY, US)
Test animals:
Species:
Dogs
Strain:
Beagle
Source:
Age:
Approx. 6 months
Sex
Male and female
Weight at dosing:
♂ 6.5 – 8.0 kg; ♀ 6.6 – 7.7 kg
Acclimation period:
14 days
Diet/Food:
125 C3 pelleted diet (SAFE, Villemoisson, Epinary-sur-
Orge, France), 300 g per day
(Following reduced food consumption among some animals standard tinned dog food was distributed instead or
in addition.)
Water:
tap water, ad libitum
Housing:
Individual housing in pens containing wood shavings.
Environmental conditions:
Temperature: 20 ± 5 °C
Humidity: 50 ± 20 % Air changes: 12/hour 12 hours light/dark cycle
In life dates: 2005-06-08 to 2005-09-22 Animal assignment and treatment:
In a 13-week oral toxicity study groups of four Beagle dogs per sex received daily doses of 0, 30, 300 and 1000 mg/kg bw/day glyphosate technical by capsule application. The test item capsules were prepared weekly and delivered daily to the animal room, protected from light. As the test item was

put into the capsules, no chemical analysis was performed during the study. The purity, characteristics and identification of the test item were indicated on the certificate of analysis that accompanied the test item.
Mortality: Each animal was checked for mortality or signs of morbidity twice a day during the treatment period, including weekends and public holidays.
Clinical observations: A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before of the beginning of the treatment period and then once a week until the end of the study.
Body weight: The body weight of each animal was recorded twice before group allocation, on the first day of treatment, and then once a week until the end of the study. In addition, the group 4 animals were weighed before final sacrifice on day 75.
Food consumption: The quantity of food consumed was recorded for each animal. Food intake per animal and per day was calculated for 7 days before the beginning of the treatment period and then throughout the study.
Ophthalmoscopic examination: Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.
Haematology and clinical chemistry: Haematological and blood chemical and urinalytical investigation were performed on all animals from each test and control group before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3).
Prior to blood sampling the animals were deprived of food for an overnight period of at least 14 hours.
The following parameters were determined: erythrocytes, haemoglobin, MVC, MCHC, MCH, thrombocytes, leucocytes, differential white cell count including morphology, reticulocytes, prothrombin time, activated partial thromboplastin time, sodium, potassium, chloride, calcium, inorganic phosphorous, glucose, urea, creatinine, total bilirubin, total protein, albumin, albumin/globulin ratio, total cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and gamma-glutamyl transferase (GGT).
Urinalysis: Urine samples were collected from all animals of the test and control groups before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3). During urine collection, the animals were deprived of food for an overnight period of at least 14 hours. The following parameters were assessed: appearance, colour, volume, pH, specific gravity, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen, and sediment.
Sacrifice and pathology: On completion of the treatment period (Week 11 or 13), after at least 14 hours fasting, all surviving animals (as well as moribund dogs before) were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thymus, thyroids with parathyroid and uterus.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum and femur), brain (at three levels), caecum, colon, duodenum, epididymides, oesophagus, eyes, gall bladder, heart, ileum (with Peyers patches), jejunum, kidneys, larynx, liver, lungs (with bronchi), lymph nodes (mandibular and mesenteric), mammary gland, muscle (skeletal), optic nerve, ovaries, oviducts, pancreas, pituitary gland, prostrate, rectum, salivary glands (parotid and submandibular), sciatic nerve, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, ureters, urinary bladder,

uterus (horns and cervix) and vagina.
Statistics: Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies" (OECD,
2002, ASB2013-3754), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.
Results and discussion
Mortality: Two unscheduled sacrifices (one male and one female) were necessary in animals given 1000 mg/kg bw/day:
One male was sacrificed on Day 61 on humane grounds. Vomiting was seen once in Week 7 (before dosing) and liquid faeces were noted on many occasions in Weeks 8 and 9. Prior to sacrifice, signs of poor clinical condition including thin appearance, dehydration, and pallor of lip mucosa, coldness to the touch, hypothermia (34 to 35 °C) and hypoactivity were observed. These signs were associated with a body weight loss between Weeks 7 and 9 (-34 %) and reduced food consumption from Week 7 (generally only 25 to 50 % of this animal's daily ration was consumed), followed by an absence of food intake on the day before death. Medical care (Smecta® and Lactate Ringer®) was given in order to stop the diarrhoea and rehydrate the animal.
One female was sacrificed on Day 72 for humane reasons. This animal showed liquid or soft faeces on many occasions from Week 4 and dehydration from Week 9. Vomiting was observed once in Week 10. These signs were accompanied by a body weight loss between Weeks 8 and 11 (-22 %) and decreased food consumption from Week 8 (generally only 25 to 50 % of this animal's daily ration was consumed), followed by an absence of food intake on the two days prior to sacrifice. Medical care (Smecta® and lactate Ringer®) was given in many occasions.
Clinical observations: No treatment-related clinical signs were noted in control animals or those given 30 or 300 mg/kg bw/day.
The following treatment-related clinical signs were reported in animals given 1000 mg/kg/day (excluding those killed in extremis, which are discussed separately): liquid or soft faeces on several occasions in all animals, vomiting in 2/3 females on one occasion within 30 minutes or 3 to 5 hours after treatment, thin appearance in 1/3 males and all females, dehydration in 1/3 males and 2/3 females, pallor of ears and mouth in 1/3 females.
Body weight: No relevant differences in the mean body weight gain were noted between controls and animals given 30 or 300 mg/kg bw/day during the treatment period.
Due to numerous individual body weight losses recorded from Week 4 in males and from Week 1 in females, a marked lower mean body weight was noted in animals given 1000 mg/kg/day at termination.
At the end of the treatment period this resulted in only a slight mean body weight gain in males (+4 % vs. +31 % in controls) and a mean body weight loss in females (-7 % vs. +14 % in controls) when compared to their body weight on Day 1. This effect on body weight was considered treatment-related (see Table B.6.3-17).
Table B.6.3-17: Group mean weekly bodyweights and standard deviations (sd)
Mean bodyweight and body weight change (kg)
Time point
Day 1
Week 5
Week 9
Week 11

Change
week 1-11
Week 13
Change
week 1-13
Dose
(mg/kg bw/day)
Males
0
7.4
9.0
9.5
9.7
+2.3
10.4
+3.0
30
7.2
8.5
8.9
9.1
+1.9
9.5
+2.3
300
7.3
8.5
9.0
9.2
+1.9
9.7
+2.4
1000
7.3
8.3
7.7*
7.6*
+0.3
na
na
Females
0
7.3
7.8
8.2
8.2

+1.0
8.8
+1.5
30
7.3
8.3
8.7
8.7
+1.6
9.2
+1.9
300
7.4
8.2
8.6
8.6
+1.3
9.2
+1.8
1000
7.2
7.0
6.9
6.9
-0.5
Na
na

* Statistically significant from controls ($p<0.05$). na not applicable due to premature sacrifice

Food consumption: The food consumption was not affected by the test article treatment in animals given 30 and 300 mg/kg bw/day. Reduced food consumption, varying from 25 to 75 % of the amount given, was observed on many occasions in animals given 1000 mg/kg bw/day. From Day 62, when tinned dog food was distributed instead of pelleted diet, all animals consumed their full daily ration.

Ophthalmoscopic examination: There were no ophthalmological findings at the end of the treatment period.

Heamatology and clinical chemistry: The laboratory investigations of the moribund sacrificed male showed the following changes among haematological and blood biochemical parameters when compared to pre-treatment values:

increase in leucocyte count mainly due to an increase in the neutrophil count, increase in haemoglobin level, erythrocyte count and packed cell volume, decrease in platelet count,

decrease in sodium and chloride levels, as well as an increase in potassium and inorganic phosphorus levels,

increase in glucose, protein, albumin, cholesterol, triglycerides, urea and creatinine levels. Some of the abnormalities found in the laboratory investigations (such as the increase in red blood cell parameters and in protein and albumin levels) were indicative of haemoconcentration, which was probably secondary to the dehydratation caused by the diarrhoea.

The laboratory investigations performed before sacrifice of the moribund female dog showed the

following changes among the blood biochemical parameters when compared to pre- treatment values:
decrease in sodium, potassium, chloride and inorganic phosphorus levels,
decrease in urea, protein and albumin levels and increase in total bilirubin level and alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities.
The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration).
In the following the results of the laboratory investigations of the surviving animals are summarised.
Haematology: When compared to both pre-dose and control values, no biologically relevant differences were noted in surviving animals of the test item groups in Weeks 7 and 11/13.
Blood chemistry: When compared to control values in Week 13, the following test-substance related differences were noted in animals given 1000 mg/kg bw/day in Week 11 (see Table B.6.3-18): higher alanine aminotransferase (ALAT) activity in 2/3 males and 1/3 females, lower alkaline phosphatase (ALP) activity in 3/3 females, lower protein and albumin levels in 3/3 females.
Other changes were not attributed to the test item-treatment.
Table B.6.3-18: Group mean blood chemical values and standard deviations (sd) in Week 11/13
Dose
ALAT
ALP
Total protein
Albumin
(mg/kg bw/d)
(IU/L)
(IU/L)
(g/L)
(g/L)
Males
0
mean
31
–
–
–
(Week 13)
sd
4.8
–
–
–
30
mean
34
–
–
–
(Week 13)

sd
5.32
–
–
–
300
mean
30
–
–
–
(Week 13)
sd
8.9
–
–
–
1000
mean
91
–
–
–
(Week 11)
sd
42.5
–
–
–
Females
0
mean
29
388
61
35
(Week 13)
sd
6.0
168.0
2.1
1.6
30
mean
31
281

62
34
(Week 13)
sd
10.4
91.5
2.1
1.0
300
mean
29
332
59
35
(Week 13)
sd
4.1
142.6
2.5
0.6
1000
mean
122
321
55
30
(Week 11)
sd
163.9
322.0
5.5
2.5
– - no relevant changes
Urine analysis: When compared to both pre-dose and control values, the following findings were noted at 1000 mg/kg bw/day in Week 11:
decrease in mean specific gravity in 1/3 males and 3/3 females,
increase in mean urinary volume accompanied by less marked colour of urine in 3/3 females. As these changes were only noted at the highest dose-level, they were attributed to the test item treatment.
Organ weights: Treatment-related, statistically significant effects (reduction) were restricted to the prostate.
Gross pathology: Macroscopic pathological examination of the male that was killed moribund demonstrated a reddish mucosa of the colon and rectum appeared, enlarged adrenal glands and thyroids, and reduced size of the spleen and thymus.
In the high-dose female that was killed moribund, the oesophagus, jejunum and ileum presented

many greyish/white areas and the colon mucosa showed reddish/purplish foci. The gall bladder was dilated with blackish deposits and the liver was yellowish, enlarged and firm. The kidneys were pale.

All the macroscopic changes noted in surviving animals at termination were considered to be normal variations, when compared to background data, which may be seen in untreated beagle dogs of this age, except for changes in the uterus (reduced in size) for females given 1000 mg/kg bw/day.

Histopathology: The major histopathological findings in the male dog sacrificed moribund were bilateral hyaline degeneration of the cortical tubules in the kidneys with pigment deposits, diffuse acute inflammation in the liver with pigment deposits, acute inflammation of the lamina propria of the oesophagus, bilateral hypertrophy of cortex of the adrenals, diffuse lymphoid atrophy in the spleen, acute inflammation in the lungs with alveolar spaces containing blood and increased number of adipocytes in the sternum.

The bilateral hyaline degeneration of the cortical tubules in the kidneys was considered to be test item treatment-related. However, it is not possible to determine if this lesion, which was associated with increase in urea and creatinine levels, was directly due to the test item action or the result of the dehydration caused by a severe intestinal irritation. The inflammation noted in the liver, oesophagus and lungs was considered to be test item related and was associated with change in leucocyte count. The increased number of adipocytes in the sternum seen also in the schedule killed animals was considered treatment-related. The abnormalities reported in blood electrolyte levels, glucose, triglycerides and cholesterol levels were not directly attributed to the test item treatment but were considered to be secondary to the poor clinical condition of the animal (diarrhoea, dehydration, changes in the kidneys). The modifications reported in spleen and adrenal glands were not attributed to the test item treatment, as they were non-specific changes that could be found in treated animals housed in laboratories.

At microscopic level, the major findings in the sacrificed female were bilateral vacuolation of the cortical tubules in the kidneys, macrovesicular vacuolation in the liver, diffuse hypoplasia of langerhans islet in the pancreas, severe atrophy of cortex of the thymus, increased number of adipocytes in the sternum and uterine atrophy.

The liver histopathological modification was considered to have resulted from the test item treatment and was correlated with changes in the blood biochemical parameters (i.e. urea, protein, albumin and bilirubin levels as well as liver enzyme activities). The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration). The uterine atrophy and increased number of adipocytes in the sternum, seen also in the schedule killed top dose animals, were considered treatment-related.

The atrophy noted in the thymus is a non specific change that could be found in laboratory housed animals; therefore a relationship to the test treatment was excluded. The other lesions noted (i.e. in the kidneys and pancreas) can be spontaneously observed in untreated beagle dogs of this age and sex. Therefore a relationship to the test treatment was considered unlikely.

No test-substance related histopathological changes were observed in animals of both sexes at and below 300 mg/kg bw/day.

Treatment-related changes observed in surviving animals given 1,000 mg/kg bw/day consisted of increased number of adipocytes in the sternum of 2/3 males and 3/3 females, prostate atrophy in 2/3 males and uterine atrophy in 2/3 females.

These lesions, also noted among the moribund sacrificed animals, could be related to the low body

weight of these high-dose animals caused by the test item.
All the other microscopic findings observed in the organs of both male and female animals of the high-dose group were judged to be unrelated to treatment or normal background findings.
Conclusion by the Notifiers
Under the experimental conditions of the study and taking into account the slight effects on organ weights at the mid dose-level, the No Observed Adverse Effect Level (NOAEL) is considered to be 300 mg/kg bw/day.
RMS comments:
The study is considered acceptable and the NOAEL is agreed. At the top dose level, the MTD was clearly exceeded. It was noticed that high dose effects of glyphosate administration in this study were particularly severe, much more pronounced and rather different from what was seen in other dog studies or other species. Thus, because of the clinical signs and pathological changes, its results do not fit into the toxicity profile of glyphosate as it was established in the majority of studies. In the study by (1990, TOX9552384) that is decribed in detail in the original DAR (1998, ASB2010-10302), the same high dose of 1000 mg/kg bw/day was administered also in capsules causing only minor effects. There is no explanation for this apparent difference although it is known from long-term studies in rats and mice that high-dose effects of glyphosate may differ considerably. In any case, it should be taken into consideration that this dose level is by 2000 times higher than the proposed ADI.
2nd new 90-day study in dogs (1999)
Reference: IIA, 5.3.3/02
Report: 1999 Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND
Test compound stability in experimental diet (dog feed)
Data owner: ADAMA
Study No.: 1816 AND 1817-R.FST Date: 1999-04-17 AND 1997-02-21
not published, ASB2012-11455
Guidelines: OECD 409
Deviations: Several organ weights missing: epididymis, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled (gross, lesions. Spinal cord, eyes with optic nerve, traches and mammary gland.
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
Crystalline solid
Lot/Batch #:
01.12.1997 & 01.06.97
Purity:
> 95 %
Stability of test compound:
Expiry dates: 2000-06-01 and 2000-12-01
Vehicle:

Plain diet
Test animals:
Species:
Dogs
Strain:
Beagle
Source:
Age:
6 - 8 months
Sex:
Male and female
Weight at dosing:
♂ 10.0 – 12.2 kg; ♀ 8.8 – 11.0 kg
Acclimation period:
6 days
Diet/Food:
Nutripet Pet meal (Tetragon Chemie Pvt.Ltd., Bangalore, India), was offered daily for one hour ad libitum
Water:
Deep borewell water passed through activated charcoal filter and exposed to UV rays, ad libitum
Housing:
Individual housing in floor pens.
Environmental conditions:
Temperature: 23 - 29 °C
Humidity: 40 - 70 % Air changes: no data
Natural daylight plus fluorescent light from 9 am to 5 pm
In life dates: 1998-03-18 to 1998-06-26
Animal assignment and treatment:
In a 90 day feeding study groups of four Beagle dogs per sex received daily doses of 0, 200, 2000 and 10,000 ppm Glyphosate technical in the diet (corresponding to 5.3, 53.5 and
252.6 mg/kg bw/day).
Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending. This pre-mix was then added to larger amount of basal diet and blended for 20 minutes. The feed was fortified with test compound at weekly intervals.
The stability of the test compound was examined in an additional study (No. 1817-R.FST). The homogeneity of the test material in diet was determined at start of the study. Three samples from the food fortified with the test compound were taken and analyzed.
Mortality: Each animal was checked for mortality or signs of morbidity daily during the treatment period.
Clinical observations: Each animal was daily checked for signs of toxicity. A more detailed veterinary investigation was performed before start of exposure, monthly throughout the study and before termination.
Body weight: The body weight of each animal was recorded before allocation and start of treatment, weekly throughout the study and before termination.
Food consumption: The quantity of food consumed was recorded for each animal on a weekly basis.

Ophthalmoscopic examination: Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.
Haematology and clinical chemistry: Haematological and blood chemical investigation were performed on all animals from each test and control group before the beginning of the treatment period, after 45 days of exposure and at termination from animals fasted since the last feeding.
The following parameters were determined: erythrocytes (RBC), haemoglobin (HB), hematocrit (HCT), MVC, MCHC, MCH, leucocytes (WBC), differential white cell count (Neut, Lymp,Eosi,Mono, Retic), clotting time, glucose, urea, total protein, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma- glutamyl transferase (GGT), creatinine, total bilirubin, albumin, calcium, inorganic phosphorous, total cholesterol, triglycerides, chloride, sodium, potassium.
Urinalysis: Urine was collected from all animals at termination during autopsy. Urinalyis was performed for control and high-dose group animals,
The following parameters were determined: pH, specific gravity, leucocytes, proteins, glucose, ketones, blood, and urobilinogen.
Sacrifice and pathology: On completion of the treatment period, after an overnight fasting, all surviving animals were killed and subjected to a gross pathological examination. The moribund animals were sacrificed in the same way. Any macroscopic findings were recorded. The following organ weights were determined: adrenals, kidneys, liver (with gall bladder), testes and thyroids with parathyroids.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum), brain, caecum, colon, duodenum, gall bladder, gonads, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric), oesophagus, pancreas, pituitary gland, rectum, salivary glands, sciatic nerve, spleen, stomach, thymus, thyroids with parathyroids, urinary bladder, uterus. These tissues (plus parathyroids) were microscopically investigated for all animals of the control and high dose group
Statistics: Body weights, net body weight gain, food intake, laboratory investigations (haematology and clinical chemistry values of days 0, 45 and 90), organ weights data and organ weight ratios were compared by Bartlett’s test for homogeneity of intra group Variances. When the Variances proved to be heterogeneous, the data were transformed using appropriate transformation.
The data with homogeneous intra group variances were subjected to one-way analysis of variance. Following ANOVA, when F was found to be significant, Dunnett’s pair wise comparison of means of treated groups with control mean was done individually. Following a significant difference of a test group with the control group, the Dose Response correlation was estimated including the control and all treated groups and tested by 't'-test. All analyses and comparisons are evaluated at 5 % probability level.
Results and discussion
Mortality: All animals survived until scheduled necropsy. Clinical observations: No clinical signs of toxicity were observed.
Body weight: Body weights remain essentially unaffected from treatment. A slight initial depression of body weight gain in the high dose male and female groups might be concluded (and would be in accordance with the food consumption).
Table B.6.3-19: Group mean body weights
Body weight [kg]
week
-1

1
2
3
4
5
6
7
8
9
10
11
12
13
Test
item [ppm]
Males
0
11.1
11.7
12.2
12.3
12.5
12.7
12.9
13.2
13.3
13.4
13.5
13.6
13.7
13.8
200
11.2
11.6
12.1
12.3
12.8
12.7
13.2
13.5
13.5
13.5
13.6
13.7
13.6
13.5

2000
11.1
11.7
12.1
12.0
12.6
12.7
12.9
13.2
13.4
13.4
13.3
13.3
13.2
13.2
10000
11.1
11.4
11.2
11.5
12.4
12.4
12.7
12.8
13.0
13.3
13.3
13.5
13.3
13.6
Test item
[ppm]
Females
0
10.1
10.4
10.9
10.7
11.0
11.0
11.2
11.4
11.7
11.9
11.7
12.0

11.7
11.8
200
10.2
10.7
11.1
10.9
11.3
11.4
11.5
11.7
11.8
11.5
11.6
11.6
11.6
11.4
2’000
9.8
10.2
10.3
10.2
10.8
10.9
11.0
11.2
11.4
11.3
11.4
11.5
11.6
11.5
10’000
10.0
9.9
10.1
10.1
10.6
10.6
10.7
10.9
11.2
11.3
11.3
11.3
11.3

11.2
Food consumption: The food intake of the high dose group (10,000 ppm) was significantly lower during the second week of treatment only. Except this finding the food consumption of all the treatment groups were comparable to the control group during the study period.
Table B.6.3-20: Average weekly food intake
Food consumption [g/animal/day]
week
1
2
3
4
5
6
7
8
9
10
11
12
13
Test
item [ppm]
Males
0
254
336
342
336
320
346
345
325
312
332
356
368
369
200
287
354
366
376
326
346
350
342

325
343
318
347
312
2’000
305
373
406
347
334
363
358
366
339
321
330
329
339
10’000
262
177*
380
368
342
332
330
324
328
303
341
333
330
Test
item [ppm]
Females
0
247
263
278
302
295
298
283
297
275

290
295
292
285
200
285
332
324
352
323
321
292
294
294
325
317
290
271
2’000
212
306
338
309
298
286
290
298
284
278
285
303
283
10’000
212
166*
348
327
303
261
288
294
298
262
268
262
256

The calculated mean daily test substance intake is summarised in Table B.6.3-21 below.

Table B.6.3-21: Group mean compound intake levels

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	200	5.2	5.4	5.3
3 (mid)	2000	54.2	52.8	53.5
4 (high)	10000	252.4	252.7	252.6

* based on actual food intake and body weight data

Ophthalmoscopic examination: There were no ophthalmological findings at the beginning and at the end of the treatment period.

Haematology and clinical chemistry: A significant increase in clotting time and GGT-activity was observed in both sexes at the 45-day interim bleed; however, in absence of any corresponding changes at terminal bleed or any histopathological correlate in the liver, this observation is considered to rather reflect a systemic error during determination than a real effect of the test item.

Table B.6.3-22: Summary of results for clotting time

Clotting time [s]	
[ppm]	
0	
200	
2’000	
10’000	
0	
200	
2’000	
10’000	

males
Females
Pre- exposure
bleed
145
150
147
144
154
162
149
131
45 day interim
bleed
131
153*
172*
183*
141
161*
173*
182*
90 d final
bleed
134
134
136
139
142
142
134
138
Table B.6.3-23: Summary of results for gamma-GT activity
GGT [U/L]
[ppm]
0
200
2’000
10’000
0
200
2’000
10’000
males
Females
Pre- exposure

bleed
9
10
8
7
9
7
7
11
45 day interim
bleed
13
13
16
19*
14
14
14
21*
90 d final
bleed
11
12
16
18
17
16
16
29
Total bilirubin was highered; however, in absence of a histopathological correlate on the liver, the effect was not considered adverse.
Table B.6.3-24: Summary of results for total bilirubin
Total bilirubin [µmol/L]
[ppm]
0
200
2’000
10’000
0
200
2’000
10’000
Males
females
Pre-
exposure bleed

3.71
3.99
3.71
3.14
3.67
3.51
3.96
4.02
45 day interim
bleed
5.25
5.10
5.93
5.97
5.22
5.23
6.49*
6.54*
90 d final
bleed
4.21
5.65*
5.95*
6.21*
4.00
6.57*
7.08*
7.18*
Urinalysis: All parameters were in the normal range and comparable between control and treated animals.
Organ weights: No treatment-related effects were observed.
Necropsy: No treatment-related gross pathological effects were observed.
Histopathology: There were a few incidental findings with equal distribution across control and treated groups – no relation to treatment was observed.
Conclusion by the Notifiers
Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) of glyphosate Technical in Beagle dogs is considered to be 10,000 ppm (252.6 mg/kg bw/day).
RMS comments:
The study is considered acceptable. It is agreed to consider the highest dose the NOAEL because the minor effects were indeed not adverse. The lower body weight gain at the beginning of treatment is very probably a result of impaired food consumption. Lower food intake might be due to a palatability problem or might simply result from the need of the animals to adapt to a diet with a new and perhaps strange taste. The higher bilirubin levels might be due to treatment but were not accompanied by any pathological change.
It was noted that the highest dose choosen, as compared to other studies with dietary administration to dogs, was rather low.

3d new 90-day study in dogs (1996)
Reference: IIA, 5.3.3/03
Report: 1996 HR-001: 13-week Subchronic Oral Toxicity Study in Dogs.
Laboratory Report No.: 94-0158
Data owner: Arysta LifeScience Date: 1996-09-05
not published, ASB2012-11456
Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985,
U.S. EPA 2012-11456)FIFRA Guidelines Subdivision F, 1984 OECD 409 (1981)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Test material:
Glyphosate technical
Identification:
HR-001
Description:
White crystal
Lot/Batch #:
T-940308
Purity:
94.61 %
Stability of test compound:
Not mentioned in the report
Vehicle:
Plain diet / none
Test animals:
Species:
Dog
Strain:
Beagle
Source:
Age:
♂ 5 months; ♀ 6 months
Sex:
Male and female
Weight at dosing:
♂ 27.3-32.7 g; ♀ 22.4-25.8 g
Acclimation period:
♂ 21 days; ♀50 days
Diet/Food:
Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day
Water:
Filtered and sterilized tap water, ad libitum
Housing:
Individually in stainless steel cages 83.5 x 90.0 x 80.0 cm

Environmental conditions:
Temperature: 24 ± 2 °C
Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle
In life dates: 1995-09-20 to 1996-02-08
Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 4 males and 4 females Beagle dogs for a minimum of 90 days. Dietary concentrations were 0, 1 600, 8 000 and 40000 ppm.
Homogeneity of the test substance in diet was ascertained for all dose levels using the samples taken from the top, middle and bottom portions of the mixer at the first diet preparation (before initiation of the study). The coefficient of variation of the concentrations of technical glyphosate was 2.3 % or less for all test diets and confirmed that the test substance was mixed in the basal diet at good homogeneity.
Concentrations of technical glyphosate in test diets were monitored for all batches of test diets of all dose levels during the study. The overall mean concentrations found in test diets were within a range of 94–101 % to the nominal levels and confirmed that the test substance was mixed in the test diets at acceptable concentrations.

Mortality: Mortality was expressed weekly as a ratio of the cumulative number of animals found dead or killed in extremis to the effective number of animals per dose group.
Clinical observations: Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.
Body weight: Body weights of all animals were recorded at initiation of treatment and weekly during the study. In addition, final body weight of each animal was measured before necropsy.
Food consumption and utilisation: Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:
Chemical intake (in mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.
Ophthalmoscopic examination: Prior to initiation of treatment and at week 13, all animals were subjected to ophthalmological examinations with a direct ophthalmoscope.
The following parameters were determined: Eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, and fundus.
Haematology and clinical chemistry: Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to haematological examinations. Blood samples were withdrawn with heparinised syringes from the cephalic vein of the animals following overnight starvation. A part of each sample was transferred to a cup of treated with EDTA and subjected to the haematological examination.
The following parameters were determined with a fully automated hematology analyzer: Hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC).
Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to biochemical examinations. Plasma from heparinised blood sample from haematological tests were used.
The following parameters were determined: alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), T-glutamyl transpeptidase (GGTP),

creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P).
Urinalysis: Prior to initiation of treatment and at week 13 of treatment, all animals were subjected to urinalysis.
Volume and sediments were determined on urine samples collected for 24 hours using trays. The other parameters were determined on fresh urine samples.
The following parameters were determined: specific gravity, pH, protein, glucose, ketones, accult blood, urobilinogen, bilirubin, appearance urine volume, urinary sediments.
Sacrifice and pathology: All animals were subjected to a complete necropsy and all gross findings were recorded. After 13 weeks of treatment, all animals were anesthetized and euthanized by exsanguinations from the carotid artery before necropsy. At necropsy the organs and tissues except eyes were removed and preserved in neutral-buffered 10 % formalin. The eyes were fixed in a phosphate-buffered mixed solution of formalin and glutaraldehyde for about 3 days and transferred to neutral-buffered 10 % formalin.
Weights of the following organs were recorded for all animals and the ratios to the final body weight were calculated: brain, heart, adrenals, thyroids with parathyroids, liver, ovaries, kidneys, prostate, spleen.
The following organs and tissues from all animals were histopathologically examined: brain, spinal cord, peripheral nerve, pituitary, thyroids with parathyroids, thymus, adrenals, tonsil, spleen, bone with marrow, lymph nodes, hearth, aorta, tongue, pharynx, buccal mucosa of
oral cavity, salivary glands, esophagus, stomach, liver, gallblader, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, epididymides, penis, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland, all gross lesions
Statistics: All data were evaluated using variance analysis (bodyweight, food consumption, urine specific gravity, urine volume, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.
Results and discussion
Mortality: There were no animals found dead or killed in extremis in any groups during the treatment period.
Clinical observations: Statistically significant differences in incidence of clinical signs were not observed between the control and treated goups in either sex.
Body weight: Statistically significant differences in body weights were not observed between the control and treated groups in either sex throughout the treatment.
Food consumption and test substance intake: There were no significant changes in food consumption and chemical intake in either sex of the treated groups.
The overall group mean chemical intakes (mg/kg bw/day) over the whole treatment period were calculated from food consumption, body weights, and the nominal dose levels. The results are shown in the table below:
Table B.6.3-25: Summary of compound intake
Dose level (ppm)
Overall group mean chemical intake (mg/kg bw/day)
Male

Female
1 600
39.7
39.8
8 000
198
201
40 000
1015
1014
Ophthalmoscopic examination: No ocular changes were detected in any dose groups of both sexes.
Haematology: Statistically significant changes in haematology parameters were observed in the treated groups as shown in the following table:
Table B.6.3-26: Results of haematological examinations
Dose group (ppm)
1 600
8 000
40 000
Week of treatment
0
7
13
0
7
13
0
7
13
Erythrocyte count (RBC)
Male
-
-
↗
(112)
-
-
↑ (115)
-
-
-
Mean corpuscular volume (MCV)
Male
↘
(89)
-
-

-
-
-
-
-
-
Mean corpuscular hemoglobin concentration
(MCHC)
Female
-
-
-
-
-
-
↓ (96)
-
↘
(97)
Lymphocytes (Lym)
Female
↘
(148)
-
-
-
-
-
-
-
-
Statistically evaluated by Dunnett’s multiples comparison method
Value in parenthesis means percentage of group mean value against control mean value.
Although there were statistically significant differences in some parameters in the treated groups of both sexes, no dose dependency was conceived in the changes. A significant decrease in mean corpuscular hemoglobin concentration (MCHC) observed in females of the 40 000 ppm group was considered to be incidental, because the change was also noted for the pre-treatment measurement and was not accompanied with significant abnormalities of erythrocyte count (RBC), Hematocrit (Ht), and hemoglobin (Hb).
Blood clinical chemistry: Statistically significant changes in blood biochemistry parameters were observed in the treated groups and are shown in the following table:
Table B.6.3-27: Results of clinical chemistry examinations
Dose level (ppm)
1600
8000
40000

Week of treatment
0
7
13
0
7
13
0
7
13
Glucose (Gluc)
Male
↓ (92)
-
-
-
-
-
↘
(93)
-
-
Chloride (Cl)
Male
-
-
↗
(102)
-
-
-
-
-
↗
(102)
Albumin (Alb)
Female
-
-
↗
(107)
-
-
-
-
-

Statistically evaluated by Dunnett's multiples comparison method
Value in parenthesis means percentage of group mean value against control mean value.
Although there were statistically significant differences in some parameters in the treated group of both sexes, no dose dependency was conceived in the changes. Although significant increases in chloride (Cl) were observed in males of the 1 600 and 40 000 ppm groups at week 13, the changes were considered to be incidental because of no dose dependency and their small degrees of alteration.
Urinalysis: In the 40 000 ppm group, 3 of 4 females showed decrease in urine pH at week 13, although there were no statistically significant differences between the control and treated groups of both sexes in any parameters of urinalysis.
There were no significant changes in urinalysis in males and females treated at 16 000 ppm or less.
Necropsy: There were no gross findings with statistically significant differences in incidence and relationship to the treatment in the treated groups of either sex.
Organ weights: Although a statistically significant increase was noted for the relative weight of the adrenals in females of the 1 600 ppm group, the change was considered to be incidental due to the lack of dose-dependency.
Histopathology: There were no histopathological changes related to the treatment in the treated groups of either sex. A female in the 40 000 ppm group showed cutaneous histiocytoma which is a non-specific lesion in young dogs.
Conclusion by the Notifiers
Under the experimental conditions of the study, the No Observed Effect Level (NOEL) is considered to be 40,000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively).
RMS comments:
The study is considered acceptable. The highest dose level of 40 000 ppm is considered the NOAEL because there were no adverse effects of treatment observed. The decrease in urine pH in some high dose females is most likely due to the acidic properties of the test substances and was measured in other toxicological studies before and after, too. Occurring in isolation, without concomitant signs of renal or bladder toxicity, this is not considered an adverse finding.
4th new 90-day study in dogs (1996)
Reference: IIA, 5.3.3/04
Report: 1996
First Revision To Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs
Data owner: Syngenta Report No.: /P/1802 Date: 1996-11-14
not published, TOX2000-1991
Guidelines: OECD 409 (1998): OPPTS 870.3150 (1998): 2001/59/EC B.27
(2001)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Test Material:
Glyphosate acid
Description:
Technical, white solid (passed through a 75 µm mesh)
Lot/Batch number:
D4490/1, P18

Purity:
99.1 % w/w a.i
CAS#:
Not reported
Stability of test compound:
Not reported
Vehicle:
Glyphosate acid was administered in diet.
Test Animals:
Species
Dog
Strain
Beagle
Age/weight at dosing
22 - 26 weeks
Source
Housing
Individually in indoor pens, with a floor area of 345 x 115
cm. Each pen consisted of an exercise area and separate sleeping quarters with a heated floor.
Acclimatisation period
4 – 5 weeks
Diet
Laboratory Diet A (Special Diet Services Ltd., Witham,
Essex, UK) ad libitum
Water
Mains water ad libitum
Environmental conditions
Temperature: 19 - 22 °C Humidity: Not reported
Air changes: Approximately 12 changes / hour Photoperiod: 11 hours light / 13 hours dark
In-life dates: Start: 12 August 1986 End: 19 November 1986
Animal assignment: The study consisted of one control and three treatment groups each containing 4 male and 4 female dogs. The randomisation procedure employed ensured the even distribution of animals across replicates (randomised blocks) and treatment groups, by bodyweight, placing litter mates in different treatment groups. The sexes were randomised separately.
Male dogs received 400 g and females 350 g of the appropriate diet, in the morning between 9 am and 12 noon each day. During the pre-study period, the food was removed 2-5 hours after presentation in an attempt to ensure that the dogs ate the diet rapidly. Several batches of test diets were prepared so that no one batch was fed for longer than 5 weeks.
The clinical condition and body weights of the dogs were monitored during the study, as was their biochemical and haematological status. At the end of the study the dogs were subjected to an examination post mortem. The major organs were fixed, processed and examined.
Diet preparation and analysis: All experimental diets were based on expanded, ground Laboratory Diet A.
The glyphosate acid concentration was determined for each occasion diet was mixed. The homogeneity of diets containing glyphosate acid was established by analysis of aliquots of diet taken from each mix of the low and high dose diet on the first occasion on which diets were prepared. The

stability of the low and high dose diets was determined over a 39 day period on one mix from the first occasion on which diets were prepared.
Observations: A detailed clinical examination, which included cardiac and pulmonary auscultation was made on all dogs pre-experimentally and in week 13. In the treatment period, the dogs were observed at least twice during the working day for gross clinical and behavioural abnormalities.
A daily record of faecal consistency was made during the pre-experimental and dosing periods.
Body weight: All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals, until termination.
Food consumption: Food residues were recorded daily and were then discarded. These measurements were made usually 4 hours (between 2-5 hours) after presentation of the diet during the pre-experimental period and approximately 24 hours after presentation of the diet during the dosing period.
Ophthalmoscopic examination: The eyes of all dogs were examined by indirect ophthalmoscopy pre-experimentally and in week 13.
Haematology: Jugular vein blood samples were taken before feeding from all dogs in weeks - 1, 4, 8 and 13 and the following parameters measured:
Haemoglobin
mean cell haemoglobin concentration
Haematocrit
platelet count
red blood cell count
total white cell count
mean cell volume
differential white cell count
mean cell haemoglobin
blood cell morphology
kaolin-cephalin time
prothrombin time
Bone marrow smears were taken from a femur of all dogs at necropsy, air dried, fixed in absolute methanol and stored but not examined.
Clinical chemistry: Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters assessed:.
Urea
alkaline phosphatase activity
Glucose
aspartate aminotransferase activity
Albumin
alanine aminotransferase activity
total protein
gamma-glutamyl transferase activity
Cholesterol
calcium
Triglycerides
sodium
creatine kinase activity
potassium

Urinalysis: Urine was collected by catheterisation from all dogs, once pre-experimentally and in week 13. Microscopic examination of the centrifuged deposits, from all dogs, was made pre-experimentally and in week 13 on the samples taken for biochemical analysis.
Urobilinogen
glucose
specific gravity
ketones
pH
protein
Bilirubin
blood
Macroscopic examination: At the end of the 90 day dosing period, all animals were killed and examined post mortem. This involved an external observation and an internal examination of all organs and structures.
Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:
adrenal glands
ovaries
Brain
liver
Epididymides
testes
Kidneys
thyroid glands (with parathyroids)
The left and right components of paired organs were weighed separately.
Tissue submission: The following tissues were examined in situ, removed and examined and fixed in an appropriate fixative:
gross lesions including masses
oesophagus
adrenal gland
ovary
aorta
pancreas
brain
pituitary gland
bone and bone marrow (rib)
prostate gland
caecum
rectum
colon
salivary gland
duodenum
spinal cord (lumbar)
gall bladder
skin
epididymis

spleen
eyes
sternum
femur (including stifle joint)
stomach
heart
testis
ileum
thymus
jejunum
thyroid/parathyroid gland
kidney
trachea
liver
urinary bladder
lung
uterus
lymph node - prescapular
voluntary muscle
lymph node - mesenteric
cervix
mammary gland (females only)
nerve - sciatic
Microscopic examination: All processed tissues were examined by light microscopy.
Statistics: Body weight gains from the start of the study to each week and final body weights were considered by analysis of variance, separately for males and females.
Haematology, blood and urine biochemistry data were considered, at each sampling time after the start of the study, by analysis of co-variance on pre-experimental values. Male and female data were analysed together and the results examined to determine whether differences between control and treated groups were consistent between sexes.
Organ weights at termination were considered by analysis of variance and analysis of co- variance on the last measured body weight, separately for males and females. Left and right components of paired organs were considered separately and combined to investigate for any differential effects.
All analyses allowed for the replicate design of the study and were carried out using SAS (1982).
Unbiased estimates of the treatment group means were provided by least square
means (LSMEANS option in SAS). Each treatment group was compared to the control group mean using a two-sided Student's t-test, based on the error mean square from the appropriate analysis. Where male and female data were analysed together, these comparisons were made separately.
All data were checked for atypical values and where such values were detected the analyses were repeated omitting these values to determine their influence on the conclusions.
Results and discussion
Concentration analysis results: The achieved dietary concentrations of glyphosate acid were all within ± 9 % of the target concentrations.
Homogeneity results: The homogeneity was considered to be satisfactory with all the mean values from the analysis at the different sampling points being within 6 % of the overall mean. Stability results: Over a period of 39 days, no significant change was seen in the chemical stability

50000 ppm glyphosate acid.
Mortality: There were no mortalities.
Clinical observations: The clinical observations noted were of a minor nature, often seen in studies of this duration using this strain of dog, and are considered to be unrelated to treatment with glyphosate acid.
Body weight and weight gain: Body weight gain of males given 50000 ppm glyphosate acid showed a slight depression throughout the study, but the differences were not statistically significant.
Females given 50000 ppm glyphosate acid showed slightly reduced bodyweight gains throughout the study and these were occasionally statistically significantly different from the controls.
There was no effect on growth in dogs given 2000 or 10000 ppm glyphosate acid.
Table B.6.3-28: Intergroup comparison of body weight gain (g) (selected timepoints)
Dietary concentration of glyphosate acid (ppm)
Males
Females
week
0
2000
10000
50000
0
2000
10000
50000
Initial wt
10.97
10.60
11.00
10.90
9.70
9.40
9.47
9.47
4
1.00
1.13
1.07
0.65
0.64
0.75
0.85
0.38*
9
2.07
1.92
2.07
1.65

1.31
1.42
1.52
0.97*
Final wt
13.03
13.00
13.37
12.50
11.31
11.13
11.40
10.95
* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
Food consumption and utilisation: All dogs ate all the diet presented during the dosing period. The dose received (in mg glyphosate acid/kg/day) was similar for both males and females. During the study, there was the expected decrease in the dose received, due to the increasing weight of the dogs.
One dog fed 10000 ppm glyphosate acid was given cubed diet for two days in week 5 to prevent it scooping up powdered diet and thereby allowing healing to a wound in its front paw. No glyphosate acid was received by this dog on these two days.
Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg body weight. Mean values are shown below:
Table B.6.3-29: Mean Dose Received (mg/kg bw/day)
Glyphosate acid (ppm)
2000
10000
50000
Males
68
323
1680
Females
68
334
1750
Ophthalmoscopic examination: There were no treatment-related ophthalmological findings.
Haematology: There were no differences in haematological parameters which were considered to be related to treatment.
Blood clinical chemistry: Male dogs fed 50000 ppm glyphosate acid showed slightly reduced plasma albumin and total protein concentrations, possibly representing the start of an expected effect of feeding an inert substance at a sufficiently high level to reduce the intake of nutrients. Plasma calcium levels were also minimally reduced in these animals, possibly a result of calcium sequestration which occurs with compounds structurally-related to glyphosate acid.
Female dogs given 50000 ppm glyphosate acid had slightly elevated plasma alkaline phosphatase activities throughout the study.

There were no treatment-related changes in dogs fed 2000 or 10000 ppm glyphosate acid. There were other isolated instances where results were statistically significantly different from control, but these were considered to be unrelated to treatment.

Table B.6.3-30: Intergroup comparison of clinical chemistry – selected parameters, selected weeks

Dose Level of glyphosate acid (ppm)
Males
Females
Parameter
Wk
0
2000
10000
50000
0
2000
10000
50000
Albumin
4
3.70
3.70
3.73
3.43*
3.76
3.65
3.89
3.51*
8
3.77
3.74
3.69
3.53*
3.72
3.71
3.92
3.63
13
3.92
3.97
3.77
3.66**
3.84
3.70
3.94
3.78
Total protein

4
5.57
5.42
5.34
5.14**
5.36
5.40
5.42
5.22
8
5.44
5.49
5.32
5.22*
5.32
5.30
5.52*
5.19
13
5.60
5.70
5.45
5.38
5.39
5.34
5.65*
5.30
Calcium
4
11.2
11.2
11.1
10.5**
10.9
11.1
11.2
10.7
8
11.2
11.1
10.9*
10.8**
10.9
11.0
11.2*
10.9

13
10.7
10.5
10.8
10.0**
10.4
10.3
10.6
10.4
plasma alkaline
phosphatase
4
182
190
188
193
176
181
182
220**
8
155
168
164
177
152
155
155
181*
13
149
165
160
161
140
143
145
166*

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided) Wk – week number

Urinalysis: There were no differences in urine clinical chemistry parameters, nor in urinary sediment examinations, which were considered to be related to treatment.

Organ weights: Kidney weights of males given 10000 or 50000 ppm glyphosate acid were slightly increased above control values, but the increase was not proportional to dose. There was also a small increase in liver weight at these dose levels, but in male dogs only.

Table B.6.3-31: Intergroup comparison of liver weight (g) in male dogs (adjusted for body weight)
0 ppm
2000 ppm
10000 ppm
50000 ppm
385
409
427*
436**
* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)
Thyroid weights, adjusted for bodyweight, of females given 2000 or 10000 ppm glyphosate acid were statistically significantly reduced from control values. In the absence of any dose response relationship across all groups this is considered not to be of toxicological significance.
Macroscopic findings: No macroscopic findings were observed attributable to the administration of glyphosate acid.
Microscopic findings: There was no microscopic pathology attributable to the administration of glyphosate acid.
Incidental findings included minor granulomatous/inflammatory lesions in lung, alimentary tract and lymph node associated with ascarid migration. Imperfect spermatogenesis and minimal secretory activity of the prostate were observed in several sexually immature males. Minimal cystitis manifest as infiltration of the mucosa by inflammatory cells and small haemorrhages were found in several animals and were consistent with a subclinical bacterial infection of the lower urinary tract.
Conclusion by the Notifiers

Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. The toxicological no effect level for glyphosate acid from this study was 10000 ppm in the diet, equivalent to a dose of more than 300 mg glyphosate acid/kg/day.
RMS comments:
The study is considered acceptable and the NOAEL of 10000 ppm is agreed with. It was noticed that test material of very high purity was used.
1st new one-year study in dogs (2008)
Reference: IIA, 5.3.4/01
Report: 2007 Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule)in Beagle Dogs
Data owner: Nufarm Study No.: 29647 TCC Date: 2007-07-23
not published, ASB2012-11457
Guidelines: OECD 452 (1981); JMAFF 2-1-14 (2001)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Test material:
Glyphosate technical
Identification:
Glyphosate tech
Description:

White crystalline powder
Lot/Batch #:
H05H016A
Purity:
95.7 %
Stability of test compound:
Expiry date: 2008-03-25
Vehicle:
Gelatine capsules size 12 (Torpac, New York, USA)
Test animals:
Species:
Dog
Strain:
Beagle
Source:
Age:
Approx. 6 month
Sex:
Males and females
Weight at dosing:
7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)
Acclimation period:
13 days + 20 days pre-treatment period
Diet/Food:
125 C3 pelleted diet (SAFE, Villemoisson, Epinay-sur- Orge, France), approx. 300 g per day. Due to weight loss in three animals the amount for these dogs was increased to 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.
Water:
Tap water, ad libitum
Housing:
Individually in pens containing wood shavings for bedding, except when a urine sample was required. The dogs were group-housed once a week, by sex and dose group, after the last recording of clinical signs in the afternoon, until the next morning.
Environmental conditions:
Temperature: 20 ± 5 °C
Humidity: 50 ± 20 %
Air changes: approx. 12/hour 12 hours light/dark cycle
In life dates: 2005-09-27 to 2006-10-17
Animal assignment and treatment: In a chronic oral toxicity study groups of four beagle dogs per sex received daily doses of 0, 30, 125 and 500 mg/kg bw/day glyphosate technical in gelatine capsules for 52 consecutive weeks. The dose levels were selected based on results of a 13-week oral (capsule) toxicity study in dogs. Dose formulations were prepared weekly by adding the required amount to the capsules. The dosages were calculated based on minimum nominal active substance content of 950 g/kg glyphosate in the test item. Analyses of the test item showed a glyphosate content consistently above 95 %. Thus, no adjustment was considered necessary. Since the test item was

added under GLP conditions, no additional analyses of dose formulations were deemed necessary. Administrations of dose capsules were done approximately the same daily time each day. The low and mid-dose animals received one capsule per day, the high-dose and control dogs received three capsules per day. The quantity of dosage form applied to each animal was adjusted weekly based on the most recently recorded body weight.
Clinical observations: Observations for morbidity, and mortality were made twice daily. A check for clinical signs of toxicity was made at least once daily on all animals. In addition, a detailed clinical examination was performed once before start of treatment and weekly thereafter until termination.
Body weight: Individual body weights were recorded three times before group allocation, on Day 1 (prior to treatment) and at weekly thereafter during the conduct of study and at termination.
Food consumption and compound intake: Food consumption of each animal was estimated daily by noting the difference between the amount provided and the remaining amount on the next morning. Food consumption was expressed as percentage of quantity provided. Whenever fasting was required, food was removed at the end of the day and estimation of food consumption as made at that time.
Ophthalmological examination: Ophthalmological examinations were performed on all dogs prior to start and at the end of the treatment period. Pupillary light and blink reflexes were evaluated first. Mydriasis was then induced by adding Tropicamide solution into the eyes and the appendages, optic media and fundus were examined by indirect ophthalmoscopy.
Haematology and clinical chemistry: Blood samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. The following haematological parameters were examined: haemoglobin concentration (HB), erythrocyte count (RBC), mean cell volume (MCV), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), thrombocytes (PLAT), leukocytes (WBC), differential white cell count with cell morphology, neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L), monocytes ♂, reticulocytes (RETIC), prothrombin time (PT), and activated partial thromboplastin time (APTT). The following clinical chemistry parameters were examined: alkaline phosphatase (ALP), alanine aminotransferase activity (ALAT), aspartate amino transferase (ASAT), albumin, albumin/globulin ratio, total bilirubin, glucose, urea, calcium, chloride, total cholesterol, creatinine, /-glutamyl-transferase (GGT), inorganic phosphorus, total protein, sodium, potassium, and triglycerides.
Urinalysis: Individual urine samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. Urine was collected in the presence of thymol crystals. The following examinations were made: appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen. The sediment was examined microscopically for leukocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium phosphate crystals, calcium oxalate crystals and cells.
Sacrifice and pathology: All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weight: adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary,
prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.
Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson´s fixative, and testes and epididymides which were preserved in Bouin´s fluid): adrenals, aorta, brain, caecum, colon, duodenum, oesophagus, eyes and optic nerve, epididymides, femur with articulation, gall bladder,

heart, ileum, jejunum, kidneys, larynx, liver, lungs with bronchi, mammary gland, mandibular lymph node, mesenteric lymph node, skeletal muscle, ovaries, oviducts, parathyroid, pancreas, pituitary, prostate, rectum, salivary glands (parotid and submandibular), skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum with bone marrow, stomach, sciatic nerve, testes, thymus, thyroids with parathyroid, tongue, trachea, urinary bladder, ureters, and uterus (horns and cervix).
A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.
Statistics: Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies" (OECD, 2002, ASB2013-3754), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.
Results and discussion
Mortality: No mortalities or premature sacrifices occurred during the treatment period. Clinical observations: There were no treatment-related clinical signs observed during the study period. Observed clinical signs consisted of vomiting or soft faeces, thin appearance, hyperactivity, ptyalism, skin findings (scabs and erythema, generally localised on the ear(s)) and nodules on the ears. These clinical observations were seen transiently, and were encountered with a similar incidence in both control and treated animals and/or were independent to the administered dose-level and/or are commonly noted when a test item is given by gavage and/or were already present before the beginning of the treatment period.
Body weight: There was no treatment-related effect on body weight development. The lower mean body weight recorded in high dose males at the end of the treatment period was due to the lower mean body weight gain during the first month of the study (see Table B.6.3-32). Individual body weight changes were within the range of physiological variations. In addition, such body weight changes were observed in both control and treated dogs.
Table B.6.3-32: Mean body weight and body weight changes (kg)
Males
Females
Dose level
(mg/kg bw/day)
0
30
125
500
0
30
125
500
Mean bw prior to
start (day -1)
8.2
8.3
8.3
8.3
7.4

7.4
7.6
7.4
Weeks 1 – 4
+0.6
+0.3
+0.5
+0.2*
+0.3
+0.3
+0.3
+0.3
Weeks 4 – 26
+1.4
+0.9
+1.4
+1.1
+1.2
+1.1
+1.5
+1.6
Weeks 26 – 52
+0.9
+1.4
+1.1
+0.8
+0.6
+0.2
+0.5
+1.1
Weeks 1 – 52/53
+2.8
+2.6
+2.9
+2.0
+2.1
+1.6
+2.3
+3.0
Mean bw in week
52/53
11.2
11.0
11.2
10.5
9.6

9.2
10.0
10.6
*statistically significant from control ($p < 0.05$)
The weight loss of some dogs observed in the control, and low-dose group during some periods of the study were resolved when the daily food quantity was increased. Therefore, these changes were considered not test substance related.
Food consumption
There was no treatment-related effect on food consumption noted during the study.
The reduced food consumptions noted during the study was not considered test substance related, since they occurred only on some occasions and in control and treated dogs.
Due to weight loss one male each of the low and mid dose group, and one control female received 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.
Ophthalmology: There were no ophthalmological findings observed at the end of the study period.
Haematology: There were no treatment-related effects noted in the haematological parameters.
The significant differences observed for the activated partial thromboplastin time (n), MCHC ()) and eosinophil counts ()) in the treated animals when compared to control dogs were only slight and not dose-related.
Clinical chemistry: There were no treatment-related effects noted in the clinical chemistry parameters.
The significant differences observed for the inorganic phosphorous (T), calcium ()), protein ()), glucose ()), albumin/globulin ratio (/) and AP values ()) in treated animals when compared to control dogs were only slight and not dose-related.
Urine analysis: There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.
Organ weights: There were no statistically significant differences in organ weights and organ to body weight ratios between control and treated dogs.
The statistically significant lower mean brain weight observed in males at 125 mg/kg bw/day was confined to this dose and, thus, no dose response was apparent. In addition, there were no macroscopic or histopathological findings noted in this organ. Thus, this finding is considered incidental.
Table B.6.3-33: Body/Brain weights and statistics
Dose group (mg/kg bw/day)
0
30
125
500
No of animals
4
4
4
4
Mean final body weight (g)
11165.0
10830.0

11090.0
10255.0
Mean brain weight (g)
87.41
80.06
73.96**
84.09
Mean % of bodyweight
0.78978
0.74484
0.67578
0.82550
**: DUNNETT'S TEST based on pooled variances at 1 % (**) level Assigned control group(s): 1.
Gross pathology: There were no test substance related macroscopic findings observed in any animal of all dose groups.
Histopathology: There were no test substance related microscopic findings observed in any tissue sample of any dose group.
Conclusion by the Notifiers
Based on the study results the NOEL and NOAEL in beagle dogs after 1-year oral exposure to glyphosate technical is 500 mg/kg bw/day.
RMS comments:
This study is considered acceptable. It is agreed to set the NOAEL at the highest dose level of 500 mg/kg bw/day. It can be confirmed that the alterations in clinical chemistry parameters were mostly not statitically significant and, if so, did not show a dose response. The only possible exception is a lower blood calcium level in high dose males that was observed in other studies with glyphosate, too. However, without any concomitant findings, e.g, on bones, this perhaps treatment-related effects is not considered adverse.
This study was run in the same laboratory and under similar conditions as the 90-day study by (2007, ASB2012-11454) in which severe adverse effects were seen upon treatment of Beagle dogs with glyphosate at a high dose level of 1000 mg/kg bw/day. It is clear now that these adverse reaction to treatment was in fact confined to an exaggerated dose level and that the NOAEL is higher than 300 mg/kg bw/day as established in that previous study.
2nd new one-year study in dogs (1997)
Reference: IIA, 5.3.4/02
Report: 1997
HR-001: 12-Month Oral Chronic Toxicity Study in Dogs.
Data owner: Arysta LifeScience Study No.: IET 94-0157
Date: 1997-03-20
not published, ASB2012-11458
Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985
U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 409 (1981)
Deviations: None
GLP: yes
Acceptability: See RMS comment
Materials and methods
Test material:

Glyphosate technical
Identification:
HR-001
Description:
White crystals
Lot/Batch #:
T-950308
Purity:
94.61 %
Stability of test compound:
Not mentioned in the report
Vehicle and/
or positive control:
None
Test animals:
Species:
Dog
Strain:
Beagle
Source:
Age:
5 months
Sex:
Males and females
Weight at dosing:
7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)
Acclimation period:
23 and 31 days for males and females, respectively
Diet/Food:
Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day
Water:
Tap water, ad libitum
Housing:
Individually in stainless steel cages 83.5 x 90.0 x 80.0 cm
Environmental conditions:
Temperature: 24 ± 2 °C
Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle
In life dates: 1996-03-05 to 1997-03-04
Animal assignment and treatment: Groups of 4 males and 4 females Beagle dogs received the test material by incorporating it into the basal diet at a level of 0, 1 600, 8 000 or 50 000 ppm for a period of 12 months.
Clinical observations: All animals were observed daily for clinical signs.
Body weight: Individual body weights were recorded at initiation of treatment, weekly from weeks 1 to 13, and every 4 weeks from weeks 16 to 52. In addition, final body weight was measured before necropsy.
Food consumption and compound intake: Food consumption of each animal was recorded weekly

from week 1 to 13 and every 4 weeks from week 16 to 52. Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:
[( )]
Chemical intake (mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.
Ophthalmological examination: Ophthalmological examinations were performed on all dogs prior to start of the treatment period. The following items were examined: eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, fundus.
Haematology and clinical chemistry: Blood samples were collected from all dogs prior to treatment, in weeks 25 and 52. The following haematological parameters were examined: Hematocrit, Hemoglobin concentration, Erythrocyte count, Mean corpuscular volume, Mean corpuscular hemoglobnin, Mean corpuscular hemoglobin concentration, platelet count, total leukocyte count.
All animals were subjected to blood biochemical examinations at weeks 26 and 52.
The following clinical chemistry parameters were examined alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), -glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio
(A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), Potassium (K), chloride (Cl).
Urine analysis: Prior to initiation of treatment and at weeks 25 and 51, all animals were subjected to urinalysis on the following parameters: appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen.
Sacrifice and pathology: All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weight: adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary, prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.
Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson´s fixative, and testes and epididymides which were preserved in Bouin´s fluid): brain, spinal cord, peripheral nerve, pituitary, thymus, thyroids with parathyroids, adrenals, tonsil, spleen, bone with marrow, lymph nodes, heart, aorta, tongue, buccal mucosa of oral cavity, pharynx, salivary glands, esophagus, stomach, liver with gallblader , pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, penis, epididymides, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland, all gross lesions.
A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.
Statistics: Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies" (OECD, 2002, ASB2013-3754), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.
Results and discussion
Mortality: There were no deaths in any dose groups of either sex.
Clinical observations: In the 50 000 ppm group, loose stool was observed in 3 of 4 males and 4 of 4 females. The animals in the 8 000 and 1 600 ppm groups did not show the clinical sign at all. In the control group, only one animal in each sex showed it. Most of the animals in the 50 000 ppm group

frequently showed the sign throughout the treatment period, whereas the occurrence in the suffering animals of the control group was restricted to a limited period.
For other clinical signs observed, the occurrence was sporadic in all dose groups, or the incidence was almost comparable among the dose groups.
Body weight: In the 50000 ppm groups of both sexes, retarded body weight gain became evident gradually as the study progressed. Consequently, the mean body weights in this group at termination of treatment were 6 % in males and 11 % in females lower than those in the controls. However, statistically significant differences in mean body weights were not observed throughout the treatment between the control and treated groups including the groups receiving 50000 ppm.
Food consumption: Decreased food consumption was noted for one female in the 1 600 ppm group at weeks 24, 28, and 52 and for another female in the same group at week 32. Consequently, group mean food consumption in this group was decreased at those weeks. However, food consumption in this group recorded at other weeks was comparable to that of
the controls. Moreover, the averaged group mean food consumption through the treatment period was almost coparable between the 1 600 ppm and control groups of females.
All males in all dose groups and females except the above 2 animals in the 1 600 ppm group consumed whole amount of diet offered every day.
Group mean chemical intakes were calculated from group mean values of food consumption and body weight, and the nominal dose levels. The overall group mean chemical intakes (mg/kg/day) through the whole treatment period are presented in the table below:
Table B.6.3-34: Mean test substance intake
Test substance intake (mg/kg bw/day)
Dose level (ppm)
Male
Female
1 600
34.1
37.1
8 000
182
184
50 000
1203
1259
Ophthalmology: No remarkable ocular changes were detected in animals in any dose groups at week 52.
Haematology: Statistically significant changes in haematology that were observed in treated groups are presented in the following table:
Table B.6.3-35: Results of haematological examination
Dose level (ppm)
1 600
8 000
50 000
Week of treatment
0
26

52
0
26
52
0
26
52
Sex
Female
Parameter
Hematocrit (Ht)
-
-
-
-
-
-
↓
-
↓
Hemoglobin concentration (Hb)
-
-
-
-
-
-
-
↓
↓
Erythrocyte count (RBC)
-
-
-
-
-
-
↓
-
↓
↓: P<0.05 ; - : not significant, statistically evaluated by Dunnett's multiple comparison method.
Male groups showed no significant changes in any parameters.
Females in the 50 000 ppm group showed significantly decreased values of hematocrit (Ht), hemoglobin concentration (Hb), and erythrocyte count (RBC) at week 52. Hemoglobin concentration in this group was also significantly lower at week 26. This group had already showed lower values for these 3 parameters than the controls before initiation of treatment (at week 0). In particular, the

differences from the control values in hematocrit and erythrocyte count at week 0 were statistically significant. However, the rates of deviation from the control values were, though slightly, augmented in the treatment period when compared to those at week 0.
Females in the 8 000 and 1 600 ppm groups showed no significant changes in hematological examinations
Clinical chemistry
Statistically significant changes in blood biochemistry that were observed in treated groups are presented in the table hereafter:
Table B.6.3-36: Results of clinical chemistry examination
Dose level (ppm)
1 600
8 000
50 000
Week of treatment
0
26
52
0
26
52
0
26
52
Male
Creatine phosphokinase (CPK)
-
-
-
-
-
↓
-
-
-
Female
Albumin (Alb)
-
-
-
-
-
-
-
-
↓
Calcium (Ca)

-
-
↓
-
-
-
-
-
↓
Inorganic phosphorus (P)
-
-
-
-
-
-
-
-
↓
Chloride (Cl)
-
-
-
-
-
-
-
-
↑
-
↓: P<0.05 ; ↑: P<0.01 ; - : not significant, statistically evaluated by Dunnett's multiple comparison method.
Females in the 50000 ppm group showed a significant increase in chloride (Cl) at week 26 and significant decreases in albumin (Alb), calcium (Ca), a,d inorganic phosphorous (P) at week 52. A significant decrease in calcium was also noted for females in the 1 600 ppm group at 52 weeks.
For male groups, the 8000 ppm group showed a significant decrease in creatine phosphokinase (CPK) at week 52. But this change was not observed in the 50 000 ppm group.
Urine analysis: There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.
Organ weights: Males in the 1 600 ppm group showed statistically significant increases in both absolute and relative weights of the pituitary. However, these changes were not observed in the 50 000 or 8 000 ppm groups of males.
In the 50 000 or 8 000 ppm groups, neither males nor females showed statistically significant changes in any organ weights
Gross pathology: The macroscopic lesions observed in the present study were all sporadic in nature and there were no statistically significant differences in the incidence between the control and treated groups.

Histopathology: In the 50 000 ppm group, focal pneumonia / focal granulomatous pneumonia in the lung was observed in all females. In the other female groups including the control group, the lesion was observed in only one of 4 animals each. However, the extent of the lesions was slight in all cases including those of the 50 000 ppm group. Statistically, no significant differences between the control and dose groups were found in incidence of any histological lesions, including the pulmonary lesion.
Conclusion by the Notifiers
Based on the study results the NOEL in beagle dogs after 1-year oral exposure to HR-001 is 8000 ppm (equivalent to 182 and 184 mg/kg/day for males and females, respectively).
RMS comments:
The study is considered acceptable although there was an uncertainty about the dose levels that were actually tested. In the original study summary (p. 17), dose levels of 2000, 10000, and 30000 ppm are mentioned. According to a different information on the same page and in the following part of the report, dose levels were 1600, 8000, and 50000 ppm. It is assumed that the latter is correct but this error might provoke some doubts about the quality assurance
system of the performing laboratory. The NOAEL (assumed to be 8000 ppm), however, is agreed with.
3d new one-year study in dogs (1996)
Reference: IIA, 5.3.4/03
Report: Brammer, A. 1996 Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs
Data owner: Syngenta Report No.: CTL/P/5079 Date: 1996-09-24
not published, TOX2000-1992
Guidelines: OECD 452 (1981): OPPTS 870.4100 (1998): 87/302/EEC B.30
(1988)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Test Material:
Glyphosate acid
Description:
Technical, white solid
Lot/Batch number:
P24
Purity:
As given in report 95.6 % a.i
CAS#:
If available
Stability of test compound:
Confirmed by the Sponsor
Vehicle:
The test substance was administered in the diet.
Test Animals:
Species
Dog
Strain
Beagle

Age/weight at dosing
20 – 29 weeks
Source
.
Housing
Housed by treatment group (sexes separately) in indoor pens. The pens had a sleeping platform with heated floor underneath and interlinking gates which enable the dogs to be
separated for feeding and dosing.
Acclimatisation period
4 – 5 weeks
Diet
Laboratory Diet A (Special Diet Services Ltd., Stepfield,
Witham, Essex, UK) ad libitum
Water
Mains water ad libitum
Environmental conditions
Temperature: 19 ± 2 °C
Humidity: 40-70 %
Air changes: Approximately 15 changes / hour Photoperiod: 12 hours light / 12 hours dark
In-life dates: Start: 11 April 1995 End: 12 April 1996
Animal assignment: In a chronic toxicity study, groups of four male and four female beagle dogs were fed diets containing 0 (control), 3000, 15000, or 30000 ppm glyphosate acid, for a period of at least 1 year. A randomisation procedure was used which resulted in the even distribution of dogs (16 males and 16 females) to treatment groups according to body weight ensuring that litter mates were in different groups. Each morning, male dogs received 400 g and female dogs received 350 g of their appropriate experimental diet.
Table B.6.3-37: Study design
Test group
Dietary concentration (ppm)
Dose to animal (mg/kg) Males / females
male
female
Control
0
1 – 4
5 – 8
Low
3000
90.9 / 91.1
9 – 12
13 – 16
Mid
15000
440.3 / 447.8
17 – 20
21 – 24

High
30000
906.5 / 926.2
25 – 28
29 – 32
Diet preparation and analysis: The experimental diets were made in 60 kg batches, by direct addition of glyphosate acid (allowing for purity) to ground Laboratory A diet, and mixed thoroughly. Water was then added to each batch and mixed prior to pelleting. The pellets were dried in the residual heat of an autoclave, allowed to cool and were then stored in bins at room temperature.
Samples from all dietary levels (including controls) were taken at approximately two-monthly intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in Lab diet A was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet was determined over a period of up to 10 weeks (69 days) for these same diets.
Samples were extracted with water, portions of the supernatant were diluted with water to give sample solution concentrations within the range of the calibration standards. These were derivitised using 9-fluorenylmethylchloroformate (FMOCCL) and analysed by High Performance Liquid Chromatography (HPLC).
Observations: All dogs were observed at least three times daily for clinical behavioural abnormalities (at dosing, after dosing and at the end of the working day) and, on a weekly basis, they were given a thorough examination. Individual, daily assessments of gastro- intestinal findings were made for up to 5 hours post dosing: any subsequent assessments were made on a group basis. All dogs were also given a full clinical examination by a veterinarian pre-study, during weeks 13, 26, 39 and prior to termination. The examination included cardiac and pulmonary auscultation.
Body weight: All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals until termination.
Food consumption and test substance intake: Food residues were recorded daily, approximately 4 hours after feeding and any residual food was discarded. These measurements were made for at least 2 weeks pre-study and throughout the treatment period.
Ophthalmoscopic examination: The eyes of all dogs were examined pre-study, during weeks 13, 26, 39 and prior to termination.
Haematology: Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing EDTA or trisodium citrate and the following parameters measured.
haemoglobin
mean cell haemoglobin concentration
haematocrit
platelet count
red blood cell count
total white cell count
mean cell volume
differential white cell count
mean cell haemoglobin
red cell distribution width
prothrombin time
activated partial thromboplastin time
blood cell morphology

bone marrow smears (taken but not examined)
Clinical chemistry: Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing lithium heparin and the following parameters measured.
urea
alkaline phosphatase activity
creatinine
aspartate aminotransferase activity
glucose
alanine aminotransferase activity
albumin
gamma-glutamyl transferase activity
total protein
Calcium
cholesterol
phosphorus (as phosphate)
triglycerides
Sodium
total bilirubin
Potassium
creatine kinase activity
Chloride
Urinalysis: Urine was collected by catheterisation, pre-experimentally, in week 26 and during the week prior to termination. The following parameters were measured and recorded on each urine sample:
volume
Glucose
colour (if abnormal)
Ketones
specific gravity
Protein
pH
Bilirubin
Blood
In addition, each urine sample was centrifuged and the sediment stained and examined microscopically to identify the components.
Macroscopic examination: All animals were were killed by exsanguination under terminal anaesthesia induced by intravenous administration of sodium pentobarbitone and examined post mortem.
Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:
adrenal glands
Kidney
Brain
Liver
Epididymides
Testes

thyroid glands
The left and right components of paired organs were weighed separately.
Tissue submission: The following tissues were examined in situ, removed and examined and fixed in an appropriate fixative:
gross lesions including masses
oesophagus
adrenal gland
ovary
aorta
Pancreas
brain (cerebrum, cerebellum and brainstem)
parathyroid gland
bone marrow (sternum)
pituitary gland
caecum
prostate gland
colon
Rectum
duodenum
salivary gland
epididymis
spinal cord (cervical, thoracic, lumbar)
eyes (retina, optic nerve)
Skin
femur (including stifle joint, stored not examined)
Spleen
Gall bladder
Sternum
heart
Stomach
ileum
Testis
jejunum
Thymus
kidney
thyroid gland
Larynx
Trachea
liver
urinary bladder
lung
uterus (with cervix)
lymph node - prescapular
voluntary muscle
lymph node - mesenteric
mammary gland (females only)

Microscopic examination: All processed tissues were examined by light microscopy.
Statistics: All data were evaluated using analysis of variance and / or covariance for each specified parameter using the GLM procedure in SAS (1989).
Results and discussion
Concentration analysis results: The mean achieved concentrations of glyphosate acid in analysed dietary preparation were typically within 12 % of nominal concentration.
The overall mean concentrations were within 9 % of target.
Homogeneity results: The homogeneity of glyphosate acid in diet at concentrations of 3000 ppm and 30000 ppm for a batch size of 60 kg was determined and considered satisfactory; percentage deviations from the overall mean where within 11 %.
Stability results: The chemical stability of glyphosate acid in experimental diets (determined at concentrations of 3000 ppm and 30000 ppm) when stored at room temperature, was shown to be satisfactory for 69 days. This covered the period of usage on the present study.
Mortality: None of the dogs died.
Clinical observations: There were no toxicologically significant findings. Salivation at dosing was observed in individual dogs in all treatment groups throughout the study. The apparent increased incidence in two top dose males and one female, was considered to be related to anticipation of feeding and not to treatment with glyphosate acid. There was also a low
incidence of scrotal skin reddening seen in one male in each treatment group; this was considered to be incidental to treatment with glyphosate acid.
There was no increased incidence of faecal abnormalities in dogs treated with glyphosate acid.
Bodyweight and weight gain: There was a slight bodyweight effect evident in females fed 30000 ppm glyphosate acid with a maximum reduction of 11 % (compared to controls) in week 51. These dogs showed a gradual reduction in growth rate, compared to the controls, which was consistently statistically significant from week 23 onwards. One female lost
0.6 kg during week 32 but this was related to a loss of appetite during this time. There were no effects in males at any dose level or in females at 15000 ppm but females fed 3000 ppm glyphosate acid also showed slightly poorer growth than the controls, with a maximum reduction of 8 % in week 51. However, this effect only achieved statistical significance on occasions during the study and is considered attributable to the poorer growth of two females and not an effect of glyphosate acid, since there was no effect at 15000 ppm.
Table B.6.3-38: Intergroup comparison of body weights (selected timepoints; adjusted mean values shown for weeks 2-14)
Dietary Concentration of Glyphosate acid (ppm)
Males
Females
week
0
3000
15000
30000
0
3000
15000
30000
1

11.40
11.53
11.33
11.45
9.60
9.55
9.48
9.58
8
12.66
12.40
12.48
12.37
10.74
10.40*
10.68
10.42*
16
13.35
12.97
13.28
12.95
11.46
11.03*
11.50
10.99*
32
14.19
13.69
13.93
13.69
12.28
11.63*
12.59
11.46**
53
14.57
14.24
14.24
13.85
13.10
12.25
12.94
11.76**

* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p<0.01$ (Student's t-test, 2-sided)

Food consumption: There was no effect on food consumption but 3 dogs left food on occasions which affected the group mean values:
Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg body weight. Mean values are shown below:
Table B.6.3-39: Mean Dose Received (mg/kg bw/day)
Glyphosate acid (ppm)
3000
15000
30000
Males
90.9
440.3
906.5
Females
91.1
447.8
926.2
Ophthalmoscopic examination: There was a very low incidence of corneal or lenticular opacities but these were seen in control animals as well as those fed glyphosate acid. There were no treatment related abnormalities.
Haematology: There were no differences in haematological parameters which were considered to be related to treatment.
Blood clinical chemistry: There were no toxicologically significant findings
Plasma cholesterol levels were increased slightly in the treated groups of both sexes at weeks 26 and 52 but there was no evidence of any dose relationship.
Plasma phosphorus levels were lower in the male treated groups at week 52 but this was due, in part, to slightly higher individual control values. Similarly the reduced sodium value in males fed 30000 ppm at week 52 was due solely to one male.
Various animals in all groups (including controls) showed evidence of higher plasma alanine aminotransferase, aspartate aminotransferase and creatine kinase activities throughout the study as well as pre-experimentally, but there was little evidence of any conclusive group effects.
Other statistically significant differences were minor and/or not dose related and were considered to be of no toxicological significance.
Urinalysis: There were no differences in urine clinical chemistry parameters which were considered to be related to treatment.
Organ weights: There were no treatment related effects on any organ weights. Adrenal weights were slightly raised in the male 3000 ppm group but this was exaggerated by a low value for one male in the control group.
Macroscopic findings: Several treated females showed red areas in or diffuse reddening of the urinary bladder mucosa. The incidence was not clearly related to dose and in the absence of a similar effect in males it was considered unlikely that the lesion is related to the administration of glyphosate acid.
Microscopic findings: It was considered unlikely that any of the lesions confined to the treated groups were related to the administration of glyphosate acid as they were either of low incidence or the incidence was not related to dose. The pathological no-effect level for glyphosate acid was 30000 ppm.

Conclusion by the Notifiers
Oral administration of 0, 3000, 15000 or 30000 ppm glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in bodyweight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg/day for males and 926 mg/kg/day for females. There were no other treatment related findings and the pathological no-effect level was 30000 ppm glyphosate acid.
The no-observed effect level for toxicity over 1 year for females was 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg/day). The no-observed effect level for toxicity over 1 year for males was 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg/day).
RMS comments:
The study is considered acceptable. Based on the reductions in body weight gain in high dose females, the NOAEL was the mid dose level of 15000 ppm, i.e., 447 mg/kg bw/day.
Published information
Not available for dogs.

B.6.4.1 In vitro genotoxicity testing – Bacterial assays for gene mutation
In Table B.6.4-1, the available Ames tests of acceptable quality are summarised that had been submitted either for first EU evaluation in the 1990ies or, for the first time, for this re- evaluation. The “new” studies are reported below in detail and commented by the RMS. With regard to the “old” studies from the 1998 DAR (ASB2010-10302), most of them (in fact all but two) were deleted from current evaluation because, e.g., purity or batch number were not given, the concentrations were too low as compared to other studies or if they had been assessed as “supplementary” in the previous evaluation for other reasons.
Table B.6.4-1: Summary of valid in vitro genotoxicity tests with glyphosate acid in bacteria (Ames test)

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels; batch/lot number$, purity; metabolic activation	Results
Jensen, 1991; TOX9552371; Cheminova#	Ames test	S. typhimurium TA 98, 100, 1535, 1537	- S9: 160 – 2500 µg/plate; + S9: 310 – 5000 (plate-incorpora- tion and pre-incubation test); Batch 206-JaK-25- 1, 98.6%	Negative
Shirasu et al., 1978; TOX9552368; Monsanto*#	Ames test	S. typhimurium TA 98, 100, 1535, 1537, 1538 and E. coli WP2 hcr	10 – 5000 µg/plate (plate-incorporation assay); Lot XHJ-46,	

98.4%; +/- S9
Negative (supplementary study)
Akanuma, 1995; ASB2012-
11462: Arysta
Ames test
S. typhimurium TA 98, 100, 1535, 1537 and
E. coli WP uvrA
156-5000 µg/plate (pre- incubation test);
95.68%; +/- S9
Negative (supplementary
study)
Sokolowski,
2007; ASB2012-
11463; Nufarm
Ames test
S. typhimurium TA 98,
100, 1535, 1537 and
E. coli WP uvrA
3 – 5000 µg/plate
(plate–incorporation), 33 – 5000 µg/plate (pre-
incubation test); 95.1%;
+/- S9
Negative
Reference; Study
identification; Owner
Type of study
Test organism / test system
Dose levels; batch/lot
number$, purity; metabolic activation
Results
Sokolowski, 2007; ASB2012-
11464; Nufarm
Ames test
S. typhimurium TA 98, 100, 1535, 1537 and
E. coli WP uvrA
3 – 5000 µg/plate (plate–incorporation)
33 – 5000 µg/plate (pre-
incubation test); 97.7%;
+/- S9
Negative
Sokolowski, 2007; ASB2012-
11465; Nufarm
Ames test
S. typhimurium TA 98, 100, 1535, 1537 and
E. coli WP uvrA
3 – 5000 µg/plate (plate–incorporation)

33 – 5000 µg/plate (pre-
incubation test); 95.0%;
+/- S9
Negative
Riberri do Val, 2007;
ASB2012- 11466; Helm
Ames test
S. typhimurium TA 98, 100, 102, 1535, 1537
648 – 5000 µg/plate (plate–incorporation); 98.01%; +/- S9
Negative (supplementary study)
Flügge, 2009; ASB2012- 11468; Helm
Ames test
S. typhimurium TA 98, 100, 102, 1535, 1537
31.6 – 3160 µg/plate (plate-incorporation and
pre-incubation test); 98.8%; +/- S9
Negative
Flügge, 2010; ASB2012- 11469; Helm
Ames test
S. typhimurium TA 98, 100, 102, 1535, 1537
31.6 – 3160 µg/plate (plate incorporation and
pre-incubation test); 96.4%; +/- S9
Negative
Sokolowski, 2010; ASB2012- 11470; Helm
Ames test
S. typhimurium TA 98, 100, 1535, 1537 and E. coli WP uvrA
3 – 5000 µg/plate (plate incorporation and pre- incubation test); 97.16% technical a.i. containing
0.63% glyphosine;
+/- S9
Negative
Wallner, 2010; ASB2012- 11471; Helm
Ames test
S. typhimurium TA 98, 100, 102, 1535, 1537
31.6 – 5000 µg/plate (plate incorporation and pre-incubation test);
98.2%; +/- S9
Negative
Thompson, 1996; ASB2012-
11472; Nufarm
Ames test
S. typhimurium TA 98, 100, 1535, 1537 and
E. coli WP uvrA
0 – 5000 µg/plate (plate–incorporation); 95.3%; +/- S9
Negative (supplementary study)
Callander, 1996; ASB2012-
11473; Syngenta
Ames test
S. typhimurium TA 98, 100, 1535,

E. coli WP2P uvrA
and WP2P
100 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 95.6%; +/- S9 (for pre-incubation test only with S9 mix)
Negative
Sokolowski, 2009; ASB2012-
11474; Syngenta
Ames test
S. typhimurium TA 98, 100, 1535, 1537 and
E. coli WP2 uvrA pKM 101and WP2 pKM 101
3 – 5000 µg/plate (plate- incorporation and pre- incubation assays); 96.3%; +/- S9
Negative
Schreib, 2012; ASB2014-9133;
Industria Afrasa
Ames test
S. typhimurium TA 98, 100, 102, 1535, 1537
10 – 5000 µg/plate (plate-incorporation and pre-incubation assays);
97%; +/- S9
Negative
Thompson, 2014;
ASB2014-9148;
Albaugh
Ames test
S. typhimurium TA 98, 100, 1535, 1537 and
E. coli WP2 uvrA
1.5 or 5 – 5000 µg/plate (plate-incorporation and
pre-incubation assays); 85.79%; +/- S9
Negative
$ Batch/lot numbers are only given for the “old” studies. For the “new” studies, they are mentioned in the comprehensive study descriptions below.
* Results were also published by Li & Long, 1988 (TOX9500253).
# The study was reported in the 1998 DAR (ASB2010-10302) and, accordingly, was subject to previous EU evaluation yet.
Some of the studies mentioned in Table B.6.4-1 are considered supplementary because either the plate-incorporation method or the pre-incubation method was performed but not both. This assessment is in line with current guideline requirements but does not affect validity of the results obtained with the method used. For overall assessment of this end point, the available database is sufficient.
The studies by Thompson (1995, TOX9500251), Fassio (1995, TOX9551631), Suresh (1993, TOX9551098), Jenkinson (1990, TOX9500268) and Bhide (1986, TOX9551955) as well as the stuy with the IPA salt by Wang et al. (1993, TOX9500381) as reported in the 1998 DAR (ASB2010-10302) but were not considered acceptable by the RMS upon re-evaluation. Likewise, the more recent study by Miyaji (2008; ASB2012-11467) was considered not acceptable because the highest concentration level was much too low. However, it should be noted that also these less reliable tests of lower quality did not reveal any indications of genotoxicity.
1st new Ames test (Akanuma, 1995)

Reference:
IIA, 5.4.1/01
Report:
Akanuma, M., 1995a HR-001: Reverse mutation test.
The Institute of Environmental Toxicology, Tokyo, Japan Data owner: Arysta LifeScience
Study No.: IET 94-0142 Date: 1995-04-03
not published, ASB2012-11462
Guidelines:
U.S. EPA FIFRA Guidelines, Subdivision F
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1995-02-21 to 1995-03-09
Materials and methods
Test material:
Glyphosate technical
Identification:
HR-001
Description:
Solid crystals
Lot/Batch #:
940908-1
Purity:
95.68 %
Stability of test compound:
Not mentioned in the report
Solvent used:
Sterile water
control materials:
Negative:
Sterile water
Solvent/final concentration:
Water / > 12 mg/mL
Positive: non-activation and activation
Strain
Positive controls
Without S9 (µg/plate)
With S9 (µg/plate)
TA100
AF–2 (0.01)
2-AA (1)
TA1535
NaN3 (0.5)

2-AA (2)
WP2 uvra
AF-2 (0.01)
2-AA (10)
TA98
AF-2 (0.1)
2-AA (0.5)
TA1537
9-AA (80)
2-AA (2)
AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide dissolved inDMSO; NaN : sodium azide dissolved in sterile water
3
2- AA: 2-aminoanthracene dissolved in DMSO; 9-AA: 9-aminoacridine hydrochloride dissolved in sterile water
activation:
The enzyme activity measured by mutagenicity was good. S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl2, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.
test organisms:
Escherichia coli WP2 uvrA
Salmonella typhimurium (TA100, TA1535, TA98 and TA1537)
Preliminary cytotoxicity assay:
Plate incorporation assay and pre-incubation assay: Concentrations up to 5000 Pg/plate were evaluated with and without S9 activation in strain TA1535, TA1537, TA98, TA100 and WP2 uvrA. A single plate was used, per dose, per condition.
Mutation assays:
Plate incorporation assay: 156, 313, 625, 1250, 2500 and 5000 tg/plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.
Pre-incubation assay: As above for the plate incorporation assay.
The independently repeated mutation assay was conducted using the pre-incubation modification to the standard plate incorporation test only. The pre-incubation assay was carried out as described above with the following two exceptions: 0.5 mL of buffer were added to cultures prepared for testing under non-activated conditions; prior to the addition of top agar, reaction mixtures were incubated for 20 minutes at 37 ± 1 sC.
Statistics
Results were judged without statistical analysis.
Reproducibility of results was confirmed by two independent experiments.
Results were judged positive without statistical analysis when the following criteria are all satisfied:
A two-fold or greater increase above solvent control in the mean number of revertants is observed
This increase in the number of revertants is accompanied by a dose-response relationship This increase in the number of revertants is reproducible.
Results and discussion
Analytical determinations: Not performed.
The results of the preliminary cytotoxicity assay are given in Table B.6.4-2.

Table B.6.4-2: Preliminary dose range finding test

Dose	Revertants (n° colonies/plate)					
	Base-change type			Frameshift type		
(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537	
- S9 mix						
Solvent control (H2O)	124	111	(118)	14	18	(16)
25	21	(23)	17	25	(21)	
7	5	(6)				
200	113	16	24	20	7	
500	116	15	23	10	3	
1000	116	16	20	14	4	

2000
82
10
15
5
5
5000
79
3
19
8
3
+ S9 mix
Solvent control (H2O)
83
86
(85)
11
9
(10)
21
25
(23)
29
28
(29)
6
10
(8)
200
99
11
20
28
9
500
82
7
12
30
6
1000
97
8
28
25

6
2000
96
9
18
38
7
5000
33
4
17
20
5
Positive controls
- S9 mix
Compound
AF-2
NaN3
AF-2
AF-2
9-AA
µg/plate
0.01
0.5
0.01
0.1
80
Revertants/plate
648
724
(686)
583
559
(571)
312
344
(328)
669
708
(689)
798
775
(787)
+ S9 mix
Compound
2-AA

2-AA
2-AA
2-AA
2-AA
µg/plate
1
2
10
0.5
2
Revertants/plate
640
658
(649)
371
372
(372)
610
645
(628)
285
304
(295)
71
81
(76)
( ): average
HR-001 did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.
Mutation assays
Results are shown in tables hereafter.
Table B.6.4-3: Summary data – experiment 1
Dose
Revertants (n° colonies/plate)*
Base-change type
Frameshift type
(µg/plate)
TA100
TA1535
WP2uvr A
TA98
TA1537
- S9 mix
Solvent control (H2O)
117
12

21
37
3
156
119
11
12
40
3
313
117
11
16
42
4
625
139
9
15
39
2
1250
125
9
22
43
5
Dose
Revertants (n° colonies/plate)*
Base-change type
Frameshift type
(µg/plate)
TA100
TA1535
WP2uvr A
TA98
TA1537
2500
106
3
15
38
3
5000
105
4

20
39
2
+ S9 mix
Solvent control (H2O)
78
9
21
35
7
156
83
6
19
36
9
313
77
7
19
31
5
625
99
6
19
30
8
1250
93
6
22
37
6
2500
73
7
16
39
7
5000
56
3
16
25
4

Positive controls
- S9 mix
Compound
AF-2
NaN3
AF-2
AF-2
9-AA
µg/plate
0.01
0.5
0.01
0.1
80
Revertants/plate
510
524
305
621
786
+ S9 mix
Compound
2-AA
2-AA
2-AA
2-AA
2-AA
µg/plate
1
2
10
0.5
2
Revertants/plate
606
392
522
360
75
* values are the mean of three plate
Table B.6.4-4: Summary data – experiment 2
Dose
Revertants (n° colonies/plate)*
Base-change type
Frameshift type
(µg/plate)

TA100
TA1535
WP2uvr A
TA98
TA1537
- S9 mix
Solvent control (H2O)
146
9
16
24
5
156
137
10
18
18
7
313
140
7
19
20
4
625
136
8
17
18
3
1250
136
7
15
15
3
2500
144
6
18
10
3
5000
117
10
14

9
4
+ S9 mix
Solvent control (H2O)
123
8
17
37
7
156
112
7
15
32
10
313
125
7
13
29
9
625
113
8
20
35
9
1250
107
7
14
28
9
2500
89
7
19
20
8
5000
67
4
17
17
4
Positive controls

- S9 mix
Compound
AF-2
NaN3
AF-2
AF-2
9-AA
µg/plate
0.01
0.5
0.01
0.1
80
Revertants/plate
595
527
252
742
909
+ S9 mix
Compound
2-AA
2-AA
2-AA
2-AA
2-AA
µg/plate
1
2
10
0.5
2
Revertants/plate
768
322
605
327
87
* values are the mean of three plates
Conclusion by the Notifiers
A two-fold or greater increase in the mean number of revertant colonies was not observed in any strain at any dose of HR-001 in the reverse mutation tests with or without metabolic activation. It is concluded that HR-001 is non mutagenic for bacteria under the conditions used with this experiment.
RMS comments:
The study is considered acceptable. No evidence of genotoxicity was obtained. However, it must be clarified that, according to the study report, only the pre-incubation method was used whereas the

plate-incorporation assay is not described and was apparently not performed. At least, the results given in Table B.6.4-3 and Table B.6.4-4 were obviously obtained by means of the pre-incubation method.
When the study description in the dossier was compared to the original study report, it was noted that the study director was Mie Akanuma. Erroneously, the first name had been mentioned in the dossier instead of the authors surname.
2nd new Ames test (Sokolowski, 2007)
Reference:
IIA, 5.4.1/02
Report:
Sokolowski, A. 2007 Salmonella typhimurium and Escherichia coli
Reverse mutation assay with glyphosate technical (NUP-05068) RCC Ltd., Itingen, Switzerland
Data owner: Nufarm RCC Study No.: 1061401 Date: 2007-03-16
Unpublished, ASB2012-11463
Guidelines:
The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial
Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).
Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12NohSan No.
8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.
Deviations:
None
GLP:
yes
Acceptability:
See RMS comment
Dates of experimental work: January 15, 2007 to January 25,2007
Materials and methods
Test material:
Identification:
Glyphosate technical (NUP-05068)
Description:
Crystalline powder, White
Lot/Batch #:
200609062
Purity:
95.1 %
Stability of test compound:
Not specified
Vehicle/Controls
Vehicle = water
Negative/solvent control:
Concurrent untreated and solvent controls were performed.
Positive control:

without metabolic activation: Sodium azide, NaN3
with metabolic activation: 2-aminoanthracene, 2-AA
Activation:
Phenobarbital/J3-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
Test organisms::
Histidine auxotrophic strains of Salmonella typhimurium
(TA1535, TA1537, TA1538, TA98, TA100) to
prototrophy.
Culture medium:
8 9 Merck Nutrient Broth (MERCK, 0-64293 Darmstadt)
5 9 NaCI (MERCK, 0-64293 Darmstadt)
Test concentrations::
0, 33, 100, 333, 667, 1000, or 5000 µg/plate with or
without metabolic activation.g
Study conduct:
For each strain and dose level including the controls, three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates: 100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µL 89 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL Bacteria suspension (cf. test system, pre-culture of the strains), 2000 µL overlay agar
In the pre-incubation assay 100 µL test solution, 500 µL S9 mix and S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37°C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark.
Results and discussion
The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation, with the exception of strain TA 1537, where a minor recuction in the number of revertants was observed at 5000 Iµg/plate without metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was slightly exceeded in the solvent control of strain WP2 uvrA with metabolic activation in experiment I. This minor deviation is judged to be based on biologically irrelevant fluctuations and has no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.
Conclusion by the Notifiers

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this end point.
RMS comments:
The study is considered acceptable. No evidence of a mutagenic response was obtained. The lower number of revertants in one experiment with TA 1537 might point to a weak cytotoxic effect of the test substance to this strain at a high concentration. In the past, similar observations were occasionally made with glyphosate from different sources at high concentrations (see DAR, 1998, ASB2010-10302).
3d new Ames test (Sokolowski, 2007)
Reference:
IIA, 5.4.1/03
Report:
Sokolowski, A. 2007 Salmonella typhimurium and Escherichia coli Reverse mutation assay with glyphosate technical (NUP-05070) RCC Ltd., Itingen, Switzerland
Data owner: Nufarm RCC Study No.: 1061402 Date: 2007-03-16
Unpublished, ASB2012-11464
Guidelines:
The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).
Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12NohSan No.
8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: January 15, 2007 to January 25,2007
Materials and methods
Test material:
Identification:
Glyphosate technical (NUP-05070)
Description:
Crystalline powder White
Lot/Batch #:
20060901
Purity:
97.7 %
Stability of test compound:
Not specified
Vehicle/Controls
Vehicle = water
Negative/solvent control:

Concurrent untreated and solvent controls were performed.
Positive control:
without metabolic activation: Sodium azide, NaN3
with metabolic activation: 2-aminoanthracene, 2-AA
Activation:
Phenobarbital/J3-Naphthoflavone induced rat liver S9 is
used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
Test organisms::
Histidine auxotrophic strains of Salmonella typhimurium
(TA1535, TA1537, TA1538, TA98, TA100) to
prototrophy.
Culture medium:
8 9 Merck Nutrient Broth (MERCK, 0-64293 Darmstadt)
5 9 NaCI (MERCK, 0-64293 Darmstadt)
Test concentrations::
0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or
without metabolic activation.g
Study conduct:
See Sokolowski, 2007, (ASB2012-11463) above.
Results and discussion
The plates incubated with the test item showed reduced background growth at 333 - 5000 and 2500 - 5000 in strains TA 1537 and TA 100, respectively. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. Minor toxic effects occurred at 5000 µg/plate in strain WP2 uvrA in the absence of metabolic activation in experiment I and in strain TA 98 with metabolic activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was bserved following treatment with glyphosate technical (NUP-05070) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was not quite reached in the untreated control of strain TA 1535 with and without metabolic activation in experiment II. These minor deviations (10 versus 11 colonies and 9 versus 10 colonies, respectively) are judged to be based on biologically irrelevant fluctuations in the number of colonies and have no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.
Conclusion by the Notifier
Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this end point.
RMS comments:
The study is considered acceptable. No evidence of a mutagenic response was obtained. Cytotoxic effects, if occurring, were confined to high concentrations and certain bacterial strains.
4th new Ames test (Sokolowski, 2007)
unpublished, ASB2012-11465

Guidelines:
The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).
Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline NO.2-1-19-1. >Notification 12NohSan No.
8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.
Deviations:
None
GLP:
yes
Acceptability:
See RMS comment
Dates of experimental work: January 16, 2007 to January 25,2007
Materials and methods
Test material:
Identification:
Glyphosate technical (NUP-05067)
Description:
Crystalline powder White
Lot/Batch #:
0609-1
Purity:
95 %
Stability of test compound:
Not specified
Vehicle/Controls
Vehicle = water
Negative/solvent control:
Concurrent untreated and solvent controls were performed.
Positive control:
without metabolic activation: Sodium azide, NaN3
with metabolic activation: 2-aminoanthracene, 2-AA
Activation:
Phenobarbital/J3-Naphthoflavone induced rat liver S9 is
used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar Hanlbm rats
Test organisms::
Histidine auxotrophic strains of Salmonella typhimurium
(TA1535, TA1537, TA1538, TA98, TA100) to
prototrophy.
Culture medium:
8 9 Merck Nutrient Broth (MERCK, 0-64293 Darmstadt)
5 9 NaCI (MERCK, 0-64293 Darmstadt)
Test concentrations::

0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or
without metabolic activation.g
Study conduct:
See Sokolowski, 2007a (ASB2012-11463), above.
Results and discussion
The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation, with the exception of strain TA 1537, where a minor recuction in the number of revertants was observed at 5000 Iµg/plate without metabolic
activation in experiment II. No substantial increase in revertant colony numbers of any of the five tester strains was observed follOowing treatment with Glyphosate technical (NUP- 05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. The laboratory's historical control range was slightly exceeded in the solvent control of strain WP2 uvrA with metabolic activation in experiment I. This minor deviation is judged to be based on biologically irrelevant fluctuations and has no impact on the outcome of the study. In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.
Conclusion by the Notifiers
Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.
RMS comments:
The study is considered acceptable. No evidence of a mutagenic response was obtained. Previously known evidence of cytotoxicity was confirmed but findings were confined to high concentrations and certain bacterial strains.
5th new Ames test (Ribeiro do Val, 2007,)
Reference:
IIA, 5.4.1/05
Report:
Ribeiro do Val, R. 2007 Bacterial reverse mutation test (Ames Test)
for Glifosato Téchnico Helm
TECAM Technologia Ambiental Ltda., Brazil Data owner: HAG
Report No.: 3393/2007-2.0AM-B Date: 2007-12-13
Unpublished, ASB2012-11466
Guidelines:
OECD 471
Deviations:
None
GLP:
Yes

Acceptability:
See RMS comment
Dates of experimental work: 30/11/2007 – 03/12/2007
Materials and methods
Test material:
Identification:
Glifosato Téchnico Helm
Description:
Solid
Lot/Batch #:
2007091801
Purity:
980.1 g/kg
Stability of test compound:
Stable (CIPAC MT 46, 54 °C, 14 days)
Vehicle and/
or positive control:
Test organisms/cells:
Negative control: vehicle (DMSO, 100 µL/plate) Positive controls:
Assay
Strain
Compound
S9-
TA98
2-Nitrofluorene
S9-
TA100; TA1535
Sodium azide
S9-
TA1537
ICR 191 – Acridine
S9-
TA102
Mitomycin C
S9+
TA98; TA100; TA102;
TA1535; TA1537
2-aminoanthracene
S9 = metabolic activation
Species: S. typhimurium
Strain: TA98; TA100; TA102; TA1535; TA1537
Source: Moltox Inc. (Annapolis, MD, USA) Metabolic activation system:
Test concentrations:
S9 (microsomal fraction of rat liver induced with Aroclor
1254)
Preliminary cytotoxicity test 8, 40, 200, 1000, 5000 µg/plate

Mutation assay: 648, 1080, 1800, 3000, 5000 µg/plate
Preliminary cytotoxicity assay: A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 8.0 and 5000.0 µg/plate.
Mutation study: The mutation study was performed with and without metabolic activation. Each sample was prepared by mixing 0.1 mL of test substance, 0.1 mL of a fresh bacterial suspension grown overnight, 0.5 mL S9 mix or substitution buffer (with / without metabolic activation) and 3.0 mL of top agar. Each suspension was incubated on selective agar plates for 72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 648 and 5000 µg/plate. The protein concentration of the S9 fraction was 34.9 mg/mL.
Liability check: The acceptance criteria of the assay were as follows: The presence of background lawn in the test plates.
Spontaneous revertant colonies of the negative control were in the range reported in the literature (MARON. D.M, & AMES. B.N. Revised methods for the Salmonella mutagenicity test, Mutation Research. 113: 173-215, 1983) and established in the laboratory by historical control values.
Positive controls showed mutagenic activity in all tested strains.
Results and discussion
Preliminary cytotoxicicity assay: None of the concentrations tested showed cytotoxic effects.
Mutation assays: No significant mutation rate was observed up to a concentration of 5000 µg of test item per plate.
Liability check: All acceptance criteria were met.
Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.
RMS comments:
The study is considered supplementary because acceptable although only the plate- incorporation assay was performed but not the pre-incubation method and Furthermore, E. coli strains were not included. No evidence of mutagenicity was obtained. According to the study report, some cytotoxicity occurred that became obvious by a lower number of revertants when the strains TA1537 (with metabolic activiation) and TA102 (without) were treated at the highest concentration level of 5000 µg/plate.
6th new Ames test (Miyaji, 2008)
Reference:
IIA, 5.4.1/06
Report:
Miyaji, C. K. 2008 Evaluation of the mutagenic potential of the test substance glyphosate technical by reverse mutation assay in Salmonella typhimurium (Ames Test) Bioagri Laboratorios Ltda., Brazil Data owner: HAG Report No.: RF-3996.401.391.07 Date: 2008-09-15
Unpublished, ASB2012-11467
Guidelines:
OECD 471
Deviations:
None
GLP:
yes
Acceptability:

See RMS comment
Dates of experimental work: 05/06/2008 – 30/06/2008
Materials and methods
Test material:
Identification:
GLYPHOSATE TECHNICAL
Description:
Solid
Lot/Batch #:
20070606
Purity:
980.5 g/kg
Stability of test compound:
Stable to hydrolysis at pH 3, 6 and 9 (5-35 °C)
Vehicle and/
or positive control:
Vehicle: DMSO
Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA97a	9-Aminoacridine
S9-	TA102	Cumene hydroperoxide
S9+	TA97a; TA98; TA100; TA102; TA1535	2-Aminoanthracene

S9 = metabolic activation
Test organisms/cells:
Species: S. typhimurium
Strain: TA97a; TA98; TA100; TA102; TA1535
Source: Moltox Toxicology, Inc. Metabolic activation system:
Test concentrations:
S9 (microsomal fraction of rat liver induced with Aroclor 1254)

Preliminary cytotoxicity test 0.001, 0.01, 0.1, 1.0, 2.5 mg/plate
Mutation assay: 0.001, 0.01, 0.1,0.5, 1.0 mg/plate
Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.001 and 2.5 µg/plate.
Mutation study:
The mutation study was performed with and without metabolic activation. Each sample was prepared by mixing the corresponding volume of test stock solution, of test substance, 0.1 mL of a fresh bacterial suspension grown overnight, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 0.001 and 1.0 µg/plate.
Liability check: The concentration of test item in the lowest and highest concentrated sample were determined by HPLC-UV
Results and discussion
Preliminary cytotoxicity assay: Only the highest concentration of 2.5 mg/plate showed cytotoxic effects. Therefore, the mutation assay was carried out with a maximum concentration of 1.0 mg/plate.
Mutation assays: No significant mutation rate was observed up to a concentration of 1.0 mg of test item per plate.
Liability check: The concentrations of the lowest and highest concentrations were confirmed.
Conclusion by the Notifier
The test material glyphosate technical was non-genotoxic.
RMS comments:
No evidence of mutagenicity was obtained in the plate-incorporation assay. However, since the highest concentration of 1000 µg/plate was much lower than in most other studies and since no apparent reason for not using higher dose levels was given, the study is considered not acceptable.
7th new Ames test (Flügge, 2009)
Reference:
IIA, 5.4.1/07
Report:
Flügge, C. 2009 Mutagenicity study of Glyphosate TC in the
Salmonella typhimurium Reverse Mutation Assay (in vitro)
LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany
Data owner: HAG Report No.: LPT 23916 Date: 2009-04-30
Unpublished, ASB2012-11468
Guidelines:
OECD 471
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 04/02/2009 – 27/02/2009
Materials and methods

Test material:
Identification: Glyphosate TC
Description: Solid, white powder
Lot/Batch #: 20080801
Purity: 988.0 g/kg
Stability of test compound: Stable for two years at ambient temperature Vehicle and/
or positive control: Negative control: Vehicle (aqua ad iniectabilia)
Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	9-Aminoacridine
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA102; TA1537	2-Aminoanthracene
S9+	TA100; TA1535	Cyclophosphamide

S9 = metabolic activation
Test organisms/cells:
Species: S. typhimurium
Strain:
TA98; TA100; TA102; TA1535, TA1537
Source:
Dr. Bruce N. Ames
Metabolic activation system:
S9 (microsomal fraction of rat liver induced with Aroclor 1254)
Test concentrations:
Preliminary cytotoxicity test
0.316, 1.0, 3.16, 10.0, 31.6, 100.0, 316.0, 1000.0, 3160.0,
5000.0 µg/plate
Mutation assay:
31.6, 100.0, 316.0, 1000.0, 3160.0 µg/plate

Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and / or reduction of revertants by more than 50 %.
Mutation study: Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and 2.0 mL of top agar. Each suspension was incubated on selective agar plates for 48- 72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The protein concentration of the S9 fraction was 31.55 mg/mL.
The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37 °C for 20 min prior to mixing with agar and plating as described above.
Liability check: As quality criteria the genotypes, i.e. histidine and biotin requirement (his-) (bio-), deep rough character (rfa-), UV-sensitivity (uvr B-), Ampicillin resistance (pKM 101) and Ampicillin / Tetracycline resistance (pAQ1) (only strain TA102) of the test strains were regularly confirmed.
Results and discussion
Preliminary cytotoxicity assay: Cytotoxicity was noted at concentrations of 3160.0 and 5000.0 µg/plate. Therefore, the mutation assay was carried out with a maximum concentration of 3160.0 µg/plate.
Mutation assays: No mutagenic effect was observed for glyphosate TC up to the cytotoxic concentration of 3160.0 µg/plate in the two independent experiments with and without metabolic activation.
Liability check: The genotypes of the 5 strains used were confirmed regularly.
Conclusion by the Notifier
The test material glyphosate technical was non-genotoxic.

RMS comments:
The study is considered acceptable. The highest concentration in the mutagenicity assays was choosen because there was evidence of cytotoxicity at this and above dose levels demonstrated at least for the strain TA100. This approach is reasonable and dose selection is supported.
8th new Ames test (Flügge, 2010)
Reference:
IIA, 5.4.1/08
Report:
Flügge, C. 2010 Mutagenicity study of glyphosate TC in the
Salmonella typhimurium Reverse Mutation Assay (in vitro)
LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Hamburg, Germany
Data owner: HAG Report No.: LPT 24880 Date: 2010-01-25
Unpublished, ASB2012-11469
Guidelines:
OECD 471
Deviations:
None
GLP:

Yes
Acceptability:
See RMS comment
Dates of experimental work: 15/10/2009 – 23/11/2009
Materials and methods
Test material:
Identification: Glyphosate TC
Description: Solid, white powder
Lot/Batch #: 20080801
Purity: 964.0 g/kg
Stability of test compound: Stable for two years at ambient temperature Vehicle and/
or positive control: Negative control: Vehicle (aqua ad iniectabilia)
Positive controls:

Assay	Strain	Compound
S9-	TA98	2-Nitrofluorene
S9-	TA100; TA1535	Sodium azide
S9-	TA1537	9-Aminoacridine
S9-	TA102	Methyl methane sulfonate
S9+	TA98; TA102; TA1537	2-Aminoanthracene
S9+	TA100; TA1535	Cyclophosphamide

S9 = metabolic activation
Test organisms/cells:
Species:
S. typhimurium
Strain:
TA98; TA100; TA102; TA1535, TA1537
Source:
Dr. Bruce N. Ames
Metabolic activation system:
S9 (microsomal fraction of rat liver induced with Aroclor 1254)

Test concentrations:
Preliminary cytotoxicity test
0.316, 1.0, 3.16, 10.0, 31.6, 100.0, 316.0, 1000.0, 3160.0,
5000.0 µg/plate
Mutation assay:
31.6, 100.0, 316.0, 1000.0, 3160.0 µg/plate
Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and / or reduction of revertants by more than 50 %.
Mutation study:
Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or phosphate buffer (with / without metabolic activation) and
2.0 mL of top agar. Each suspension was incubated on selective agar plates for 48-72 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The protein concentration of the S9 fraction was
26.6 mg/mL.
The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37°C for 20 min prior to mixing with agar and plating as described above.
Liability check:
As quality criteria the genotypes, i.e. histidine and biotin requirement (his-) (bio-), deep rough character (rfa-), UV-sensitivity (uvr B-), Ampicillin resistance (pKM 101) and Ampicillin / Tetracycline resistance (pAQ1) (only strain TA102) of the test strains were regularly confirmed.
Results and discussion
Preliminary cytotoxicity assay: Cytotoxicity was noted at concentrations of 3160.0 and 5000.0 µg/plate. Therefore, the mutation assay was carried out with a maximum concentration of 3160.0 µg/plate.
Mutation assays: No mutagenic effect was observed for glyphosate TC up to the cytotoxic concentration of 3160.0 µg/plate in the two independent experiments with and without metabolic activation.
Liability check: The genotypes of the 5 strains used were confirmed regularly.
Conclusion by the Notifier
The test material glyphosate technical was non-genotoxic.
RMS comments:
The study is considered acceptable. The test substance proved non-gentotoxic. The choice of the highest concentration is sufficiently explained. In addition, some precipitation was observed in the pre-test with TA100 at 5000 µg/plate.
9th new Ames test (Sokolowski, 2010)
Reference:
IIA, 5.4.1/09
Report:
Sokolowski, A. 2010 Salmonella typhimurium and Escherichia coli
Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine

Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany
Data owner: HAG Report No.: 1332300
Date: 2010-04-07
Unpublished, ASB2012-11470
Guidelines:
OECD 471
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 17/03/2010 – 22/03/2010
Materials and methods
Test material:
Identification:
Glyphosate TC (5000 mg/L) spiked with glyphosine (32 mg/L)
Description:
An aqueous solution of glyphosate technical grade active ingredient (purity 97.16 % w/w), containing 0.63 % (w/w) glyphosine in the technical grade active ingredient.
Lot/Batch #:
2009051501 (Glyphosate TC)
Purity:
971.6 g/kg
Stability of test compound:
Stable for two years at ambient temperature
Vehicle and/
or positive control:
Negative control: Vehicle (deionised water)
Positive controls:
Assay Strain Compound
S9- TA98, TA1537 4-nitro-o-phenylene-
diamine
S9- TA100; TA1535 Sodium azide
S9- WP2 uvrA Methyl methane sulfonate
S9+ TA98; TA100; 2-Aminoanthracene TA1535; TA1537,
WP2 uvrA
S9 = metabolic activation
Test organisms/cells:
Species:
S. typhimurium
Strain:
TA98; TA100; TA1535, TA1537
Species:
Escherichia coli
Strain:

WP2 uvrA
Source:
Trinova Biochem GmbH (35394 Gießen, Germany)
Metabolic activation system:
S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)
Test concentrations:
Preliminary cytotoxicity test
3; 10; 33; 100; 333; 1000; 2500; 5000 µg/plate
Mutation assay:
3; 10; 33; 100; 333; 1000; 2500; 5000 µg/plate
Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay was performed with TA100 to select concentrations for the mutation study. Concentrations of test item ranged between 0.316 and 5000.0 µg/plate. Toxicity was defined as appearance of scarce background lawn and/or reduction of revertants.
Mutation study:
Each sample was prepared by mixing 1.0 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or S9 substitution buffer (with / without metabolic activation) and
1.0 mL of top agar. Each suspension was incubated on selective agar plates for at least 48 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The protein concentration of the S9 fraction was 34.3 mg/mL.
The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37 °C for 60 min prior to mixing with agar and plating as described above.
Liability check:
The acceptance criteria of the assay were as follows:
Regular background growth in the negative and solvent control.
Spontaneous revertant colonies of the negative control were in the range of historical data. Positive controls showed mutagenic activity in all tested strains.
Results and discussion
Preliminary cytotoxicity assay: No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.
Mutation assays: No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Solution of glyphosate TC spiked with Glyphosine at any dose level, neither in the presence nor absence of metabolic activation (S9 mix).
Liability check:The acceptance criteria were met.
Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.
RMS comments:
The study is considered acceptable. No evidence of mutagenicity was obtained. The reason for glyphosine spiking of the test material is not clear but it is assumed that this substance (similar to glyphosate) may occur as an impurity in the technical active ingredient. Thus, this test might become particularly important if a certain specification needs to be assessed from a toxicological point of

view.
10th new Ames test (Wallner, 2010)
Reference:
IIA, 5.4.1/10
Report:
Wallner, B. 2010 Reverse Mutation Assay using bacteria
(Salmonella typhimurium) with Glyphosate TC
BSL Bioservice Scientific Laboratories GmbH, Planegg, Germany Data owner: Helm AG
Report No.: BSL 101268 Date: 2010-04-08
Unpublished, ASB2012-11471
Guidelines:
OECD 471
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 25/03/2010 – 06/04/2010
Materials and methods

Test material:
Identification:
Glyphosate TC
Description:
Solid.
Lot/Batch #:
200903051
Purity:
982.0 g/kg
Stability of test compound:
Stable for two years at ambient temperature
Vehicle and/
or positive control:
Negative control: Solvent controls, consisting of solvent or vehicle alone.
Positive controls:
Assay Strain Compound
S9- TA98, TA1537 4-nitro-o-phenylene-
diamine
S9- TA100; TA1535 Sodium azide
S9- TA102 Methyl methane sulfonate
S9+ TA98; TA100; 2-Aminoanthracene TA102; TA1535;
TA1537
S9 = metabolic activation
Test organisms/cells:
Species:

S. typhimurium
Strain:
TA98; TA100; TA102; TA1535, TA1537
Source:
MOLTOX, INC, NC 28607, USA
Metabolic activation system:
S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)
Test concentrations:
Preliminary cytotoxicity test
31.6, 100, 316, 1000, 2500 and 5000 µg/plate
Mutation assay:
31.6, 100, 316, 1000, 2500 and 5000 µg/plate
Preliminary cytotoxicity assay:
No preliminary cytotoxicity assay was performed.
Mutation study:
Each sample was prepared by mixing 0.1 mL of test item, 0.1 mL of a fresh bacterial suspension, 0.5 mL S9 mix or S9 substitution buffer (with / without metabolic activation) and
2.0 mL of top agar. Each suspension was incubated on selective agar plates for at least 48 hours. After incubation the revertant colonies per plate were counted. The concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The protein concentration of the S9 fraction was 33.0 mg/mL.
The mutation study was performed as two independent experiments each with and without metabolic activation. The first experiment was a plate incorporation method where all components were mixed and directly plated. The second experiment was a preincubation method, where test strain, test item and S9 mix were incubated at 37 °C for 60 min prior to mixing with agar and plating as described above.
Liability check:
The acceptance criteria of the assay were as follows:
The bacteria demonstrate their typical responses to ampicillin (TA 98, TA 100, TA 102). Regular background growth in the negative and solvent control.
Spontaneous revertant colonies of the negative control were in the range of historical data. Positive controls showed mutagenic activity in all tested strains.
Results and discussion
Preliminary cytotoxicity assay: No preliminary cytotoxicity assay was performed.
Mutation assays: In the plate incorporation test toxic effects of the test item were observed in tester strain TA 100 at a dose of 5000 µg/plate (with and without metabolic activation). In tester strain TA 1535 toxic effects of the test item were noted at doses of 2500 µg/plate and higher (with and without metabolic activation). In the preincubation test toxic effects of the test item were noted in tester strains TA 100 and TA 1535 at a dose of 5000 µg/plate (without metabolic activation).
No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with Glyphosate TC at any concentration level with or without metabolic activation.
Liability check:The acceptance criteria were met.
Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.
RMS comments:

The study is considered acceptable. Glyphosate proved non-genotoxic. Cytotoxicity was confined to very high concentrations.
11th new Ames test (Thompson, 1996)
Reference:
IIA, 5.4.1/11
Report:
Thompson, P.W. 1996 Technical glyphosate: Reverse mutation
assay “Ames test” using Salmonella typhimurium and Escherichia coli
Safepharm Laboratories, Derby, UK Data owner: Nufarm
SPL Project No.: 434/014 Date: 1996-02-20
unpublished, ASB2012-11472
Guidelines:
OECD 471 (1983)
Commission Directive (EC) 92/69/EEC (1992), Method B14 US EPA (TSCA) guidelines
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: August 19, 1995 to November 13, 1995
Materials and methods
Test material:
Identification:
Technical Glyphosate
Description:
White powder
Lot/Batch #:
H95D 161 A
Purity:
95.3 %
Stability of test compound:
No data given in the report.
Vehicle/Controls
Vehicle = sterile distilled water
Negative/solvent control:
Vehicle/solvent controls were performed.
Positive control:
Without metabolic activation:
N-ethyl-N`-nitro-N-nitrosoguanidine (ENNG):
- 2 2g/plate for WP2uvrA;
- 3 3g/plate for TA100;
- 5 5g/plate for TA1535.
9-Aminoacridine (9AA) 80 9g/plate for TA1537.
4-Nitroquinoline-1-oxide (4NQO) 0.2 4g/plate for TA98.
With metabolic activation:

2-Aminoanthracene (2AA) was used in S9 series of plates in the concentrations:
- 1 1g/plate for TA100;
- 2 2g/plate for TA1535 and TA1537;
- 10 1g/plate for WP2uvrA;
- 0.5 0g/plate for TA98.

Activation:
S9 was prepared from the livers of male Sprague-Dawley rats. Each received a single i.p. injection of Aroclor 1254 at
500 mg/kg, 5 days before S9 preparation.

Test organisms:
Salmonella typhimurium TA1535, TA 1537, TA98, and TA100
Escherichia coli WP2uvrA

Culture medium:
Top agar was prepared using 0.6 % Dicfo Bacto agar and
0.5 % sodium chloride with 5 mL of 1.0 mM histidine/1.0 mM biotin and 1.0 mM tryptophan solution added to each 100 mL of top agar.
Base agar plates were prepared using 1.2 % Oxoid Agar Technical No 3 with Vogel-Bonner Medium E and 20 mg/mL D-glucose.

Test concentrations:
0, 50, 150, 500, 1500, and 5000 0g/plate with and withput metabolic activation. In triplicate for each bacterial strain and for each concentration of test material with an without
S9-mix.

Study conduct:
For each strain and dose level including the controls, three plates were used with and without S9-mix.
The following materials were mixed in a test tube and poured onto the selective agar plates:
0.1 mL of bacterial suspension;
0.1 mL of test solution at each dose level, vehicle/solvent (negative control) or reference mutagen solution (positive control);
2 ml of molten, trace histidine/tryptophan supplemented media;
0.5 ml of buffer (for test without metabolic activation) or S9-mix (for test with metabolic activation).
Known aliquots (0.1 mL) of one of the bacterial suspensions were dispensed into sets of sterile test tubes followed by 2.0 ml of molten trace histidine/tryptophan supplemented top agar at 45 °C, 0.1 mL of the appropriately diluted test material or vehicle control or reference mutagen solution (with and without metabolic activation) and either 0.5 mL of the S9 liver microsome mix or 0.5 ml of pH 7.4 buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner agar plates (one tube per plate). Plates were incubated for approximately 48 hours at 37 °C.

Results and discussion
The plates incubated with the test item caused no visible reduction in the growth of the bacterial lawn at any dose level up to maximim recommended dose of 5000 wg/plate either with or without metabolic activation, however a decrease in the frequency of revertant colonies was observed with some bacterial strains.
No significant increase in the frequency of revertant colonies was recorded for any of the bacterial strains with any dose of the test material, either with or without metabolic activation. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

Conclusion by the Notifiers
In conclusion, the test material was found to be non-mutagenic under the conditions of this test.
RMS comments:
There was no evidence of mutagenicity obtained although there was some cytotoxicity. The study is considered supplementary since acceptable although only the plate incorporation method was applied for testing.
12th new Ames test (Callander, 1996)
B.13/B.14 (2000)
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1995-10-23 to 1996-02-16
Materials and methods
Test Material:
Glyphosate acid
Description:
Technical; white solid
Lot/Batch number:
P24
Purity:
95.6 % a.i
CAS#:
Not reported
Stability of test compound:
Confirmed by Sponsor
Control Materials:
Negative:
Dimethylsulphoxide – DMSO
Solvent control (final concentration):
Dimethylsulphoxide – DMSO (10 DL/plate)
Positive control:
Nonactivation:
Acridine mutagen ICR191 TA1537
2-Aminoanthracene TA1537, WP2 uA Daunomycin hydrochloride TA98
N-Eethyl-N´-nitro-N-nitrosoguanidine WP2P uvrA
Mitomycin C WP2P
Sodium Azide TA1535 and TA100
Activation:
2-Aminoanthracene WP2 uvrA and WP2P
TA1535,
TA1537,
TA98,
TA100,

Mammalian metabolic system: S9 derived
X
Induced
Aroclor 1254
X
Rat
X
Liver
Non-induced
X
Phenobarbitol
Mouse
Lung
None
Hamster
Other
X
Other
β-naphthoflavone
Other
The metabolic activation system (S9-mix) used in this study was prepared as a 3:7:20 mixture of S9 fraction, Sucrose-tris-EDTA buffer (250:50:1 mM) and cofactor solution.
The cofactor solution was prepared as a single stock solution Na2HPO4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and MgCl2 (12 mM), in sterile deionised water and adjusted to a final pH of 7.4.
Test organisms:
S. typhimurium strains
TA97
X
TA98
X
TA100
TA102
TA104
X
TA1535
X
TA1537
TA1538
list any others
E. coli strains
X
WP2P
(pKM101)
X
WP2P uvrA

(pKM101)
Properly maintained?
X
Yes
No
Checked for appropriate genetic markers (rfa mutation, R factor)?
X
Yes
No
Test compound concentrations used:
Nonactivated conditions: 5000, 2500, 1000, 500, 200 and 100 µg/plate
Activated conditions: 5000, 2500, 1000, 500, 200 and 100 µg/plate
For all strains triplicate plates were used for all test substance and positive control treatments. For solvent controls 5 plates were used.
Test performance
Preliminary Cytotoxicity Assay: Not performed. Type of Bacterial assay:
X standard plate test (both experiments –S9, initial experiment +S9)
pre-incubation (60 minutes) (second experiment +S9)
"Prival" modification (i.e. azo-reduction method)
spot test
other
Protocol:
Bacterial cultures were prepared from frozen stocks by incubating for 10-12 hours at 37 °C. The following materials were mixed in a test tube and poured onto the selective agar plates: 100 µL Test solution at each dose level, solvent and positive controls;
500 µL S9 mix or phosphate buffer; 100 µL Bacteria suspension;
2 mL Overlay agar containing 50 µM histidine or tryptophan as appropriate.
In this assay 100 µL aliquots of an overnight culture of each bacteria strain were stored in bijou bottles at room temperature until required (1-2 hours). 500 µL S9 mix (or buffer) was then added by dispensing syringe to the number of bijou bottles of one strain required for one dose level, followed by 0.1 mL of the appropriate concentration of the test substance solution added by micropipette. Finally, 2.0 mL top agar was added to each bijou, the force of addition was sufficient to mix the contents. The mixture was then rapidly poured onto a prepared Vogel Banner agar plate. After the agar was set the plates were incubated upside down for 64
- 68 hours at 37 °C in the dark. For each strain and dose level including the controls, three plates were used.
Following the total incubation period the plates were examined for the lack of microbial contamination and evidence that the test was valid: i.e. there should be a background lawn on the negative control plates and on the plates for (at least) the lower doses of test substance, and that the positive controlshad responded as expected.
The plates were counted using an automated colony counter (AMS 40-10) with the discrimination adjusted appropriately to permit the optimal counting of mutant colonies.
Statistical analysis: None – see Evaluation Criteria below.
Evaluation criteria: A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met:
a significant, dose-related increase in the mean number of revertants is observed;

a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) is observed at one or more concentrations
A negative result in a (valid) individual experiment is achieved when:
there is no significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance; and
in the absence of any such dose response, no increase in colony numbers is observed (at any test concentration) which exceeds 2x the concurrent solvent control.
For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain/S9 combination, then the observed effect(s) must be consistently reproducible.
Results and discussion
Mutagenicity assay: In two separate experiments, glyphosate acid did not induce any significant increases in the observed numbers of revertant colonies in the four Salmonella typhimurium strains (TA1535,TA1538, TA98, TA100) and the two Escherichia coli strains (WP2P and WP2 uvrA) in either the presence or absence of an auxiliary metabolising system (S9).
The positive controls for each experiment induced the expected responses indicating the strains were working satisfactorily in each case.
Conclusion by the Notifiers
Under the conditions of this assay, glyphosate acid gave a negative, ie non-mutagenic, response in S. typhimurium strains TA1535, TA1537, TA98 and TA100 and E. coli strains WP2P and WP2 uvrA in both the presence and absence of S9-mix, when tested to a limit dose of 5000 µg/plate.
RMS comments:
The study is considered acceptable. No evidence of mutagenicity was revealed. It should be clarified that the first experiment was performed by means of the plate incorporation method with and without metabolic activation. In the second trial, the same method was used in the absence of S9 mix. A pre-incubation assay was run with S9 mix.
13th new Ames test (Sokolowski, 2009)
Date: 2009-12-18
not published; ASB2012-11474
Guidelines:
OECD 471 (1997): OPPTS 870.5100 (1998): 2008/440/EC
B.13/B.14 (2008)
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 2009-09-15 to 2009-12-18
This study was performed to investigate the potential of Glyphosate technical (produced via the Nantong Jiangshan (glycine-route)) to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strains WP2 uvrA pKM 101 and WP2 pKM 101 over the range 3; 10; 33; 100; 333; 1000; 2500; and
5000 µg/plate (Experiment I), and 33; 100; 333; 1000; 2500; and 5000 µg/plate (Experiment II).
Materials and methods

Test Material:
Glyphosate
Description:
white solid
Lot/Batch number:
569753
Purity
96.3 % of glyphosate acid
Stability of test compound:
Not indicated by the sponsor
Control Materials:
Negative:
Concurrent untreated and solvent controls were performed
Solvent control (final concentration):
100 1L/plate
Positive control:
Nonactivation:
Sodium azide 10 µg/plate TA100, TA1535 4-nitro-o-phenylene-diamine,
50 µg/plate TA 1537, 10 µg/plate TA98
methyl methane sulfonate 3 µL/plate WP2 (pKM101), WP2 uvrA (pKM101)
Activation:
2-Aminoanthracene
2.5 µg/plate TA 1535, TA 1537, TA100, TA98
10 µg/plate WP2 (pKM101), WP2 uvrA (pKM101)
Mammalian metabolic system: S9 derived
X
Induced
Aroclor 1254
X
Rat
X
Liver
Non-induced
X
Phenobarbitol
Mouse
Lung
None
Hamster
Other
X
Other
β-naphthoflavone
Other
Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 10 % v/v in the S9 mix. Cofactors are added to the

S9 mix to reach the following concentrations in the S9 mix:
8 mM MgCl2
33 mM KCl
5 mM Glucose-6-phosphate
5 mM NADP
in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
During the experiment the S9 mix was stored in an ice bath. Test organisms:
S. typhimurium strains
TA97
X
TA98
X
TA100
TA102
TA104
X
TA1535
X
TA1537
TA1538
list any others
E. coli strains
X
WP2 (pKM101)
X
WP2 uvrA (pKM101)
Properly maintained?
X
Yes
No
Checked for appropriate genetic markers (rfa mutation, R factor)?
X
Yes
No
Test compound concentrations used
The test item was tested at the following concentrations:
Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II 33; 100; 333; 1000; 2500; and 5000 µg/plate
Test performance
Preliminary Cytotoxicity Assay Not performed.
Type of Bacterial assay
X standard plate test (pre-experiment/experiment I; –S9, +S9) X pre-incubation (60 minutes) (second experiment ; –S9, +S9)
“Prival” modification (i.e. azo-reduction method)
spot test
other

Protocol:
For each strain and dose level including the controls, three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates: 100 µLTest solution at each dose level, solvent (negative control) or reference
mutagen solution (positive control),
500 µLS9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µLBacteria suspension (cf. test system, pre-culture of the strains), 2000 µL Overlay agar
In the pre-incubation assay 100 µL test solution, 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspensions were mixed in a test tube and shaken at 37° C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates.
After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark.
* Substitution buffer: 8.5 parts of the 100 mM sodium-ortho-phosphate-buffer pH 7.4 with 1.5 parts of KCl solution 0.15 M
Statistical analysis:
None - see Evaluation Criteria below.
Evaluation criteria:
A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control is observed.
A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.
An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.
A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment.
However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.
Results and discussion
Preliminary Cytotoxicity Assay : Not performed.
Mutation assays: Glyphosate technical was assessed for its potential to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strains WP2 uvrA pKM 101 and WP2 pKM 101.
The assay was performed with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:
Pre-Experiment /Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate
The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.
No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.
No precipitation of the test item occurred up to the highest investigated dose.
No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with glyphosate technical at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing

concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

Conclusion by the Notifiers

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, glyphosate technical did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

RMS comments:

The study is considered acceptable. It could be shown that also technical glyphosate was not mutagenic when manufactured via the Nantong Jiangshan (glycine) route.

Two more tests were provided in 2014 when the revision of the original (2013) RAR was made. Despite this very late submission, they were reviewed by the RMS but described here only in brief.

B.6.4.2 In vitro genotoxicity testing – Tests for gene mutation in mammalian cells

In contrast to the numerous Ames tests in bacteria, only few gene mutation assays with glyphosate have been performed in mammalian cells. An overview on the available information is given in Table B.6.4-5

Table B.6.4-5: Summary of in vitro genotoxicty tests with glyphosate acid in mammalian cells

Reference, study identification, owner	Type of study	Test organism / test system	Dose levels, batch no., purity, metabolic activation	Results
In vitro tests for gene mutation in mammalian cells				
Studies from the 2001 evaluation				
Jensen, 1991; TOX9552372; Cheminova	Mouse lymphoma test	Mouse lymphoma cells (L5178Y)	- S9: 0.61 – 5.0 mg/mL, + S9: 0.52 – 4.2 mg/mL; 206-JaK-25- 1; 98.6%	Negative
Li, 1983*; TOX9552369; Monsanto	HGPRT assay	Chinese hamster ovary (CHO) cells	- S9: 2 – 22.5 mg/mL + S9: 5 – 22.5 (25 ??) mg/mL; Lot XHJ-64; 98.7%	Negative
New study				
Clay, 1996, TOX2000-1994; Syngenta	Mouse lymphoma test	Mouse lymphoma cells		

(L5178Y TK+/-)
+/- S9: 296 – 1000
µg/mL; P24; 95.6%
Negative
* Results were also published by Li & Long, 1988 (TOX9500253).
The previously known studies by Jensen (1991, TOX9552372) is reported in detail in the old DAR (1998, ASB2010-10302) and was confirmed to be acceptable upon re-evaluation. In this assay, there was neither evidence of gene mutation (TK locus) nor of chromosome aberrations. A similar study (Clay, 1996, TOX2000-1994) confirming the absence of gene mutation anc chromosome aberration in mouse lymphoma cells in vitro is decribed in the following in detail because it had not been submitted for previous EU evaluation. A negative HGPRT test (Li, 1983, TOX9552369) metioned in the old DAR is also considered still acceptable although it is not entirely clear from the original study report which dose level was actually the highest under activation conditions.
Mouse lymphoma assay (Clay, 1996)
Reference:
IIA, 5.4.3/01
Report:
Clay, P. 1998 Glyphosate Acid: L5178Y TK+/- Mouse Lymphoma Gene Mutation Assay
Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK
Data owner: Syngenta Report No.: CTL/P/4991 Date: 1996-05-24
not published, TOX2000-1994
Guidelines:
OECD 476 (1997): OPPTS 870.5300 (1998): 2000/32/EEC B.17
(2000)
Deviations:
The stability, homogeneity and achieved concentration of the test or
control substances in the vehicle used were not determined by
analysis and the certified purity and stability of the control substances are not available.
These deviations from the current regulatory guideline are considered not to compromise the scientific validity of the study..
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1996-01-29 to 1996-05-24
Materials and methods
Test Material:
Glyphosate acid
Description:
Technical, white solid
Lot/Batch number:
P24
Purity:
95.6 % w/w a.i
CAS#:
Not reported

Stability of test compound:
Confirmed by the Sponsor
Control Materials:
Negative:
DMSO
Solvent control
(final concentration):
1 %
Positive control:
Absence of S9 mix: Ethylmethanesulphonate (EMS),
750 µg/mL
Presence of S9 mix N-nitrosodimethylamine (NDMA),
600 µg/mL
Mammalian metabolic system: S9 derived
X
Induced
Aroclor 1254
X
Rat
X
Liver
Non-induced
X
Phenobarbitol
Mouse
Lung
None
Hamster
Other
X
Other
β-naphthoflavone
Other
X indicates those that apply
The co-factor solution was prepared as a stock solution of 75 mM NADP (disodium salt) and 1200 mM glucose-6-phosphate (monosodium salt) in RPMI 1640 culture medium with a final pH adjusted to 7.5. S9 fraction was added at 5 % (1 mL S9 added to the 20 mL cell culture) and co-factors at 1 % (200 µL to the 20 mL cell culture).
Test cells: Mammalian cells in culture
X
Mouse lymphoma L5178Y cells
V79 cells (Chinese hamster lung fibroblasts)
Chinese hamster ovary (CHO) cells
List any others
Media: RPMI 1640
Properly maintained?

X
Yes
No
Periodically checked for Mycoplasma contamination?
X
Yes
No
Periodically checked for karyotype stability?
Yes
No
Periodically “cleansed” against high spontaneous background?
Yes
No
X indicates those that apply
Locus Examined:
Thymidine kinase (TK)
Hypoxanthine-guanine-
phosphoribosyl transferase (HGPRT)
Na+/K+ ATPase
Selection agent:
Bromodeoxyuridine
(BrdU)
8-azaguanine (8-AG)
ouabain
Fluorodeoxyuridine
(FdU)
6-thioguanine (6-TG)
X
Trifluorothymidine
(TFT)
X indicates those that apply
Test compound concentrations used:
Absence of S9 mix
296, 444, 667, 1000 µg/mL
Presence of S9 mix
296, 444, 667, 1000 µg/mL
Cells were exposed to test compound, negative/solvent or positive controls for 4 hours in both the presence and absence of S9 mix.
After washing, cells were cultured for 2 days (expression period) before cell selection.
After expression, 104 cells/mL were dispensed, at 200 µL/well, into 96 well plates. The cells were cultured for 10-13 days in selection medium to determine numbers of mutants. Dilutions of the cultures to approximately 8 cells/mL were cultured for 10-13 days without selective agent to determine cloning efficiency.
Cell growth in individual microwell plates was assessed after 10-13 days using a dissecting microscope. The survival plates and viability plates were scored for the number of wells containing no cell growth (negative wells). The mutation plates were scored so that each well contained either a

small colony (considered to be associated with clastogenic effects), a large colony (considered to be associated with gene mutation effects), or no colony.
Statistical methods: None required.
Evaluation criteria: Each well of the mutation plates (those containing TFT) was scored as containing either, a small colony, a large colony or no colony according to the following criteria:
Small Colony - a small colony was one whose average diameter was less than 25 % of the diameter of the well and was usually around 15 % of the diameter of the well. A small colony should also have shown a dense clonal morphology.
Large Colony - a large colony was one whose average diameter was greater than 25 % of the diameter of the well. A large colony should also have shown less densely packed cells, especially around the edges of the colony.
Any well which contained more than one small colony was scored as a small colony. Any well which contained more than one large colony was scored as a large colony. Any well which contained a combination of large and small colonies was scored as a large colony.
An empty well was one which contained no cell growth.
Results and discussion
Preliminary toxicity assay: The maximum concentration of glyphosate acid considered appropriate for testing in the mutation experiments was estimated to be 1000 µg/mL as concentrations of 1500 µg/mL and 2000 µg/mL in the presence and absence of S9-mix were found to produce an excessive reduction in the pH of the treatment medium.Very little toxicity was seen at the concentrations tested.
Mutation assay: No significant increases in mutant frequency, compared to the solvent control cultures, were observed in cultures treated with glyphosate acid at any concentration tested in either the presence or absence of S9-mix.
The positive controls, EMS and NDMA, induced substantial increases in mutant frequency in all mutation experiments, demonstrating the activity of the S9-mix and that the assay was performing satisfactorily in being capable of detecting known mutagens.
Conclusion by the Notifiers
Glyphosate acid was not mutagenic to L5178Y TK+/- cells in the presence or absence of S9-mix.
RMS comments:
The study is considered acceptable and the conclusion is agreed with. The selection of the highest concentration of 1000 µg/mL because of pH reduction at dose levels above is reasonable. It is widely accepted that pH changes (as well as increases in osmolality) may alter the mutant frequency. However, it was noted that the resulting top dose level was much lower than in the study by Jensen (1991, TOX9552372) who did not report a decline in pH at concentrations above 1 mg/mL. This obvious difference might suggest some variability in the acidic properties of the test materials although it seems not entirely clear from the study report if Jensen (1991, TOX9552372) had in fact measured the pH after treatment.
B.6.4.3 Tests for DNA damage and repair in mammalian cells and bacteria
A direct interaction of glyphosate acid with DNA, i.e., DNA damage and repair was investigated in a small number of studies in different test systems in vitro. A UDS assay in primary rat hepatocytes by Rossberger (1994, TOX9400697) was reported in detail in the previous DAR (1998, ASB2010-10302) that is still considered acceptable. In contrast, the other studies in which this endpoint was adressed by different test methods and that had been provided for the previous EU evaluation must be considered not acceptable from a todays point of view. Thus, the UDS assay by Williams (1983, TOX9552370), a DNA repair assay with the IPA salt in E. coli by Wang (1993, TOX9500380), the SCE

assays by Wang et al. (1993, TOX9500381, again, IPA salt tested) and by Jenkinson (1990, TOX9500269) and a Rec-assay by Shirasu et al. (1980, TOX9552408) were excluded from current re-evaluation of glyphosate. In contrast, a more recent rec assay in Bacillus subtilis has been submitted and is described in detail and evaluated by the RMS below Table B.6.4-6.

Table B.6.4-6: Summary of valid in vitro tests with glyphosate acid for DNA damage and repair in mammalian cells and bacteria (provided by the Notifiers)

Reference; study identification; owner	Type of study / test system	Test organism	Dose levels; batch/lot no.; purity	Results
In vitro tests for DNA damage and repair in mammalian cells				
Previously known Rossberger, 1994; TOX9400697; ADAMA Dawley)	UDS assay	Primary rat hepatocytes	0.20 – 111.69 mM; (Sprague-F/93/032; >98%	Negative
In vitro tests for DNA damage and repair in bacteria				
New study Akanuma, 1995b; ASB2012-11477; Arysta and M45 (+/-	Rec assay	B. subtilis strains H17	+/- S9 : 7.5 – 240 µg/disk; Lot (supplementary	Negative

940908-1; 95.68%
study)
S9)
Rec assay in B. subtilis (Akanuma, 1995)
Reference:
IIA, 5.4.3/02
Report:
Akanuma, M. 1995b HR-001: DNA Repair Test (Rec-Assay). The Institute of Environmental Toxicology, Tokyo, Japan Data owner: Arysta LifeScience
Study No.: IET 94-0141 Date: 1995-03-14
not published, ASB2012-11477
Guidelines:
U.S. EPA FIFRA Guidelines, Subdivision F
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1995-02-14 to 1995-02-15
Materials and methods
Test material:
Glyphosate technical
Identification:
HR-001
Description:
Solid crystals
Lot/Batch #:
940908-1
Purity:
95.68 %
Stability of test compound:
Not mentioned in the report
Solvent used:
Sterile water
control materials:
Negative:
Kanamycin (without activation)
Solvent/final concentration:
Water / > 12 mg/mL
Positive: non-activation and activation
mitomycin C (without activation)
3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (with activation)
activation:
The enzyme activity measured by mutagenicity was good. S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9

fraction, 8 mM MgCl2, 33 mM KCl,
5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.
test organisms:
Recombination-wild (rec+) strain H17
Recombination-deficient (recE-) strain M45 of Bacillus subtilis
test concentrations
6 dose level were tested: 7.5, 15, 30, 60, 120 and
240 µg/diskwith and without S9 metabolic activation
Study conduct
DNA-damaging activity was evaluated by a DNA repair test (Rec-Assay), with Bacillus substilis strains of recombination wild (rec+) H17 and recombination-deficient (recE-) M45, at concentrations of 7.5, 15, 30, 60, 120 and 240 µg/disk with and without S9 metabolic activation. The S9 fraction for the metabolic activation was obtained from liver of male SD strain rats previously treated intraperitoneally with 30 mg/kg phenobarbital (x 1), 60 mg/kg phenobarbital (x 3) and 80 mg/kg 5,6-benzoflavone (x 1). Negative control substance, kanamycin (0.2 µg/disk) without S9, and positive control substance mitomycin C (0.01 µg/disk) without S9, and positive control substance 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (Trp-p-1, 5 µg/disk) with S9 were also tested on both strains. In addition a solvent control, sterile water (20 µL/disk) without S9, and sterile water and co-factor solution (20µl: 20 µL/disk) with S9, was included in the experiment.
Paper discs (8 mm diameter) impregnated with 20 µl of the solution of the test substance were placed on the prepared spore agar plate containing the tester organism for each test, with and without metabolic activation. Duplicate plates were used for each experimental point. Diameter of a growth inhibitory zone of each strain was measured after incubation at 37 °C for 24 hours.
Statistics
Results were judged without statistical analysis.
Evaluation Criteria
Results are judged positive when growth inhibitory zone of M45 is larger than that of H17, and the difference in diameter was 5 mm or more at one or more dose levels that caused growth inhibitory zones, or 4 mm or less in diameter in the H17 (rec+) strain.
Results and discussion
Analytical determinations: None
Mutations assays: HR-001 induced a growth inhibitory zone of 1 mm in diameter at the highest dose of 240 µg/disk in the (recE-) strain M45 with S9 system (Table hereafter). The differences of growth inhibitory zones between the strains H17 and M45 were 1 mm or less. On the other hand, HR-001 did not induce any growth inhibitory zone in either the (rec+) strain H17 with S9 system or both the strains M45 and H17 without S9 system.
The assay was considered as valid because:
-in the negative control plates treated with kanamycin, the difference of growth inhibitory zones between M45 and H17 strains was 2-3 mm
-in the positive controls of mitomycin C without S9 and Trp-p-1 with S9, growth inhibitory zone in M45 is larger than that of H17, and the difference in diameter is 19 mm and 11-12 mm, respectively.
-in the solvent control, no growth inhibitory zone was observed in either strain.
Table B.6.4-7: Results of the Rec assay
Compound
Dose (µg/disk)
S9 fraction (-)

S9 fraction (+)
Inhibitory zone* (mm)
Difference** (mm)
Inhibitory zone* (mm)
Difference** (mm)
M45
H17
M45
H17
Solvent control (H2O)
0
0
0
0
0
0
0
0
0
0
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HR-001
7.5
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120
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0
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0
240
1
0
1

0
0
0
0
0
0
0
0
0
Negative control (Kanamycin)
0.2
8
6
2
9
6
3
Positive control (Mitomycin C)
0.01
20
1
19
20
1
19
Positive control (Trp-p-1)
5
11
0
11
12
0
12
* Diameter of growth inhibitory zone subtracted the diameter of disk (8 mm)
** Diameter of growth inhibitory zone in M45 strain subtracted that in H17 strain
Conclusion by the Notifiers
Under the conditions used in this experiment, HR-001 did not have DNA-damaging activity in the bacteria.

RMS comments:
The study is considered to provide supplementary information only because the Rec assay is not a standard method for this endpoint (DNA damage and repair). Furthermore, dose selection was not explained. However, the study results are valid and it is agreed that the test compound glyphosate proved negative in this experiment, both with and without metabolic activation. This conclusion can be drawn because the evaluation criteria for a positive response were not met. The difference of growth inhibiting zone between the two strains was 1 mm at the highest concentrations

anddifferences were 0 at lower dose levels. Thus, they were below the value obtained for the negative control (kanamycin).
When the study description in the dossier was compared to the original study report, it was noted that the study director was Mie Akanuma. Erroneously, the first name had been mentioned in the dossier instead of the authors surname.
B.6.4.4 In vitro genotoxicity testing – Tests for clastogenicity in mammalian cells
As mentioned in the 1998 DAR (ASB2010-10302), a clastogenic potential of glyphosate was mainly investigated in vivo and only two in vitro studies had been submitted for the previous evaluation of which one (György et al., 1989, TOX9650157) was considered not acceptable upon re-evaluation. In contrast, a study by Van de Waart (1995, TOX9651525) is of sufficient quality and may still be used to adress this endpoint. It is considered now supplementary since the dose levels were rather low if compared to the 3 further studies that were provided for this new evaluation of glyphosate. These studies are reported in detail below Table B.6.4-8 Therefore, methodical details are not given in this table.
Table B.6.4-8: Summary of in vitro tests for chromosome aberrations with glyphosate acid
Reference (Owner)
Type of study
Test organism / test system
Dose levels*; batch/lot number; purity
Results
Study from the 2001
Van de Waart, 1995; TOX9651525;
Agrichem
Cytogenicity
Peripheral human lymphocytes (-S9: 24, 48 h exposure;
+S9: 3 h, harvest after 24 or 48 h)
- S9: 33 – 333 µg/mL
+ S9: 237 – 562 µg/mL;
Lot 22021; 96%
Negative (supplementary study)
Studies not reviewed in the 2001 evaluation
Kyomu, 1995; ASB2012-11475;
Arysta
Cytogenicity
Chinese hamster lung (CHL) cells
- S9: 62.5 – 500 µg/mL,
+ S9: 255 – 1000 µg/mL;
Lot 949908-1; 95.68%
Negative
Wright, 1996;
ASB2012-11476;
Nufarm
Cytogenicity
CHL cells
+/- S9: 312.5 - 1250
µg/mL; Lot H95D161A; 95.3%

Negative
Fox, 1998;
TOX2000-1995;
Syngenta
Cytogenicity
Human lymphocytes
- S9: 100 – 1250 µg/mL
+ S9: 100 – 1250 µg/mL;
P24, 95.6%
Negative
* Mostly, higher concentrations were included but these were the dose levels up to which metaphases were analysed.
1st new clastogenicity study in vitro (Kyomu, 1995)
Reference:
IIA, 5.4.2/01
Report:
Kyomu, M. 1995 HR-001: In vitro cytogenicity test.
The Institute of Environmental Toxicology, Tokyo, Japan Data owner: Arysta LifeScience
Study No.: IET 94-0143 Date: 1995-05-29
not published, ASB2012-11475
Guidelines:
U.S. EPA FIFRA Guidelines, Subdivision F
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1995-03-13 to 1995-05-09
Materials and methods
Test Material
Glyphosate technical
Description:
HR-001
Lot/Batch #:
940908-1
Purity:
95.68 %
Stability of test compound:
Not mentioned in the report
Solvent used:
Hank’s balances salt solution and culture medium
Control Materials
Test solvent control:
Hank’s balance salt solution
Positive solvent control:

Physiological saline (without metabolic activation)
Benzo(a)pyrene (with metabolic activation)
Positive control:
Mitomycin (without metabolic activation) DMSO (with metabolic activation)
Activation
The enzyme activity measured by mutagenicity was good.
S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.
Test organisms:
CHL cells established from the lung of Chinese hamster
Culture medium:
The growth medium was Eagle's MEM supplemented with 10 % newborn calf serum
Test concentrations:
Preliminary cytotoxicity test:
8 doses: up to 1000 µg/L for the 24-hr treatment 8 doses: up to 2000 µg/L for the 48-hr treatment
Metaphase analysis:
4 doses: 125, 250, 500 and 1000 µg/mL for the 24-hr treatment
4 doses: 62.5, 125, 250 and 500 µg/mL for the 48-hr treatment
Replicates:
Preliminary cytotoxicity test:
Duplicate
Metaphase analysis:
Duplicate
TEST PERFORMANCE
In life dates
1995-03-13 to 1995-05-09
Preliminary cytotoxicity test
CHL cells were seeded at a density of 1.0 x 105 cells with 5 mL of medium and incubated for 48 hours. In the direct method, the cultures were treated with HR-001 with the doses mentioned above during 24 and 48hours. After the treatment, relative cell growth value of each culture was measured by comparing with the staining density in the concurrent solvent control.
In the metabolic activation method, the medium was replaced with 3 mL of medium containing S9 mix and then test substance was added to the cultures.
The second growth inhibition test with the activation system was carried out with higher doses.
Duplicate cultures were used for each experimental point and their relative values were averaged.
Metaphase analysis
CHL cells were seeded at a density of 2.0 x 105 cells with 10 mL of medium and incubated for 48 hours. In the direct method, the cultures were treated with HR-001 with the doses mentioned above during 24 and 48hours.
In the metabolic activation method, the medium was replaced with 5 mL of medium containing S9 mix and then test substance was added to the cultures.
Duplicate cultures were used for each experimental point and their relative values were averaged.
Diploid metaphase cells which possessed the typical karyotype of CHL cells and polyploid metaphase cells were analysed for a structural chromosome aberration.
The following data were recorded: Number and frequency of polyploid cells

Number and frequency of each structural chromosome aberration
Number and frequency of metaphase cells with structural chromosome aberration
Only plyploid cell having 3 or more copies of haploid number of chromosomes was scored as a numerical chromosome aberration cell.
Statistics: The number of aberrant metaphases and polyploidy cells at each dose were statistically compared with those of corresponding solvent controls using a chi-square test.
Results and discussion
Preliminary cytotoxicity test: In the 24-hr and 48-hr treatments of the direct method, the doses of HR-001 which showed a reduction of the relative cell growth by 50 % or more were 1000 and 500 µg/mL, respectively. Therefore, the doses of 1000 and 500 µg/mL were chosen as the highest doses in the 24-hr and 48-hr treatments of the cytogenetics test, respectively.
In the metabolic activation method, no cell growth inhibition effect was observed at the dose of 1000 µg/mL or less, but at 2000 µg/mL or more, their cell growth was inhibited over 50 %. Therefore, the dose of 2000 µg/mL was determined to be used as the highest dose in the cytogenetics test with the metabolic activation system. It was noticed that in the both methods the color of the culture medium was turned to yellow at 500 µg/mL or more, indicating a decline of pH.
Table B.6.4-9: Preliminary growth inhibition test
Concentration (µg/ml)
Relative cell growth (%)
Direct method
24 h
Direct method
48 h
Metabolic activation method
6-18 h (1st exp.)
Metabolic activation
6-18 h (2nd exp.)
Solvent control
(Hanks)
100
100
100
100
3.9
100
100
100
-
7.8
97
101
99
-
15.6
100
101

108
-
31.3
96
110
104
-
62.5
100
106
97
-
125
102
99
103
-
250
92
82
106
-
500
74
46
112
-
1000
22
9
106
100
2000
-
-
-
22
3000
-
-
-
38
4000
-
-
-

27
5000
-
-
-
25
Metaphase analysis
It was noticed that in the both methods the color of the culture medium was turned to yellow at 500 µg/ml and above, indicating a decline of pH.
In the 24-hr and 48-hr treatments, the frequencies of the aberrant metaphases (excluding gaps) were 0.5 % and 0.0 % to 1.0 %, respectively, showing no significant increases when compared with the concurrent solvent control. The dose of 1000 µg/mL in the 48-hr treatment gave high cytotoxicity to the cells so that chromosome preparations were not obtained from the cultures treated at this dose. There was no increase in the frequencies of polyploid metaphases at any doses of HR-001 in both 24-hr and 48-hr treatments (Table B.6.4-10).
On the other hand, MMC used as a positive control caused a great increase in a frequency of aberrant metaphases that was consistent with the historical control data.
In the presence of a metabolic activation system, the frequencies of the aberrant metaphases (excluding gaps) were in the range of 0.5 % to 1.0 %, showing no significant increases when compared with the concurrent solvent control and with the solvent control, respectively. The dose of 2000 µg/ml gave high cytotoxicity to the cells so that chromosome preparations were not obtained from the cultures treated at this dose. The frequencies of polyploid cells did not significantly increase (Table B.6.4-11). The same holds true when the test compound was tested under identical conditions in the absence of metabolic activation.
On the other hand, B(a)P used as a positive control caused a remarkable increase in the frequency of aberrant metaphases in the presence of S9 mix that was consistent with the historical control data.
Table B.6.4-10: Cytogenetics test (direct method, i.e., without metabolic ativation, 24-hr and 48-hr treatments)
Treatment
Time (h)
S9 Mix
Dose (µg/ml)
Number of metaphase
Mitotic index (%)
Polyploid
Number of chromosome aberrations
Number of
aberrant metaphases
Judge
Gap g
ctb
Cte
csb
cse
Fragmen
-tation

Others
+g
-g
Jud-
ge
Untreated control
24
-
0
100
6.1
0
-
1.0
0
0
0.5
0
0
0
1.5
0.5
-
48
-
0
100
2.5
0.5
-
0.5
1.0
0
0
0
0
0
1.5
1.0
-
Sovent control (Hanks)
24
-
10%
100
6.0

0.5
0
0
0
0
0
0
0
0
0
48
-
10%
100
3.4
0.5
0
0
0
0
0
0
0
0
0
HR-001
24
-
125
100
6.7
0.5
-
0
0
0
0
0.5
0
0
0.5
0.5
-
250
100
5.9

0.5
-
1.0
0.5
0
0
0
0
0
1.5
0.5
-
500
100
5.6
0
-
1.5
0.5
0
0
0
0
0
2.0
0.5
-
1000
48
-
62.5
100
3.0
0
-
1.5
0
0
0
0
0
0
1.5
0
-
125

100
2.9
0
-
0.5
0.5
0
0
0
0
0
1.0
0.5
-
250
100
3.1
1.0
-
1.5
0.5
0
0
0.5
0
0
2.5
1.0
-
500
100
3.0
0
-
1.5
1.0
0
0
0
0
0
2.5
1.0
-
Positive control (MMC)
24

-
0.1
100
3.3
0.5
-
4.0
21.5
39.5
2.5
1.0
0
0.5
50.5
49.0
+
48
-
0.1
100
2.6
1.0
-
5.5
43.0
49.5
2.5
3.0
1.5
1.5
72.0
70.5
+
Ctb: chromatide break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; +g: including gaps;
-g: excluding gaps; MMC: mitomycin C
Table B.6.4-11: Cytogenetics test (with metabolic activation, 6-hr treatment, followed by fixation for 18 hours)
Treat-ment
Time (h)
S9
Mix
Dose (µg/ml)
Number of metaphase
Mitotic index (%)
Polyploid

Number of chromosome aberrations
Number of
aberrant metaphases
Judge
Gap
g
Ctb
Cte
csb
cse
Fragmen-
tation
Others
+g
-g
Judge
Untreated control
6
+
0
100
4.9
0
-
1.5
0.5
0
0
0
0
0
2.0
0.5
-
-
0
100
5.3
0
-
0.5
1.0
0.5
0
0
0

0
2.0
1.5
-
Sovent control (Hanks)
6
+
10%
100
6.3
0
1.5
0.5
0
0
0
0
0
1.5
0.5
-
10%
100
5.7
1.0
1.0
0
0
0
0
0
0
1.0
0
HR-001
6
+
250
100
6.7
0
-
0
1.0
0
0

0
0
0
1.0
1.0
-
500
100
5.6
0
-
1.0
1.0
0
0
0
0
0
2.0
1.0
-
1000
100
7.2
0.5
-
1.0
0.5
0
0
0
0
0
1.5
0.5
-
2000
-
250
100
5.1
0
-
1.5
1.0
0

0
0
0
0
2.0
1.0
-
500
100
4.9
0
-
0.5
0.5
0
0
0
0
0
1.0
0.5
-
1000
100
5.7
0.5
-
0.5
0.5
0
0
0
0
0
1.0
0.5
-
2000
Positive control (B(a)P)
6
+
40
100
3.8
0
-

3.0
21.0
30.0
2.0
0.5
0
0
39.5
38.5
+
-
40
100
4.7
0.5
-
0
0.5
0
0
0
0
0
0.5
0.5
-
Ctb: chromatide break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; +g: including gaps; -g: excluding gaps; B(a)P: benzo (a) pyrene
Conclusion by the Notifiers
In the direct and metabolic activation system, there was not a significant increase in the frequencies of abnormal metaphases with structural chromosome aberrations or polyploid metaphases. Based on the results obtained, it was concluded that, under the conditions of this study, the test substance HR-001 did not induce chromosome aberrations in Chinese hamster CHL cells with or without the metabolic activation system.
RMS comments:
The study is considered acceptable. No evidence of an increase in structural or numerical chromosome aberrations was obtained. With regard to the study description in the dossier, it must be clarified that metaphases were analysed up to a concentration of 500 µg/mL in the first series of experiments without metabolic activation (called above and in the study report “direct method”) and 24- or 48-hour treatment periods. In the experiments with and without activation and an exposure period of 6 hours (thereafter, cells were fixed for 18 hours), metaphases could be analysed up to a concentration of 1000 µg/mL. At concentrations above, evaluation was avoided by severe cytotoxicity.
2nd new clastogenicity study in vitro (Wright, 1996)
Reference:
IIA, 5.4.2/02

Report:
Wright, N.P. 1996 Technical glyphosate: Chromosome aberration
test in CHL cells in vitro
Safepharm Laboratories, Derby, UK Data owner: Nufarm
SPL Project No.: 434/015 Date: 1996-03-13
unpublished, ASB2012-11476
Guidelines:
not specified
Deviations:
not specified
GLP:
yes
Acceptability:
See RMS comment
Dates of experimental work: 30 August 1995 and 4 January 1996.
Materials and methods
Test material:
Identification:
TECHNICAL GLYPHOSATE
Description:
white powder
Lot/Batch #:
H95D 161A
Purity:
95.3 % w/w
Stability of test compound:
Not specified
Vehicle/Negative Controls
suspended in minimal essential culture media
Positive control:
Mitomycin C (MMC, Sigma Batch No. 104H2504)
0.05 µg/mL for cultures treated for 24 or 48 hours in the absence of metabolising enzymes.
Cyclophosphamide both with and without metabolic activation
Activation:
Lot No. Aro. S9/11/0CT/95 SPL was prepared in-house at Safepharm Laboratories on 11/0CT/95. It was prepared from the livers of male SpragueDawley rats weighing - 200 g. These had received a single ip. injection of Aroclor 1254 at 500 mglkg, up to 5 days be
Test organisms::
Hamster CHL line
Culture medium:
Eagle's Minimal Essential medium with Earle's Salts (MEM), supplemented with 10 % foetal bovine serum and antibiotics, at 37' C with 5 % CO2 in AIR
Test concentrations::
19.5 to 5000 µg/mLg
Study conduct:
A preliminary toxicity test was performed on cell cultures using 24 and 48-hour continuous exposure

times without metabolic activation and a 6-hour exposure period both with and without metabolic activation, followed by an 18-hour recovery period in treatment-free media. The dose range used was 19.5 to 5000 µg/mL. Growth inhibition was estimated by counting the number of cells at the end of the culture period on an electronic cell counter (Coulter) and expressing the cell count as a percentage of the concurrent vehicle control value. Slides were also prepared from the cells in order to check for the presence of cells in metaphase.

- Without Metabolic Activation

i) 24 hours continuous exposure to the test material prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL

ii) 48 hours continuous exposure to the test material prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL.

- With Metabolic Activation

i) 6 hours exposure to the test material and 59-mix (0.5 mL per 4.5 ml culture medium of 10 % 59 in standard co-factors). A phosphate buffered saline wash and then a further 18 hours in treatment-free media prior to cell harvest. The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL.

ii) 6 hours exposure to the test material without 59-mix. A phosphate buffered saline wash and then a further 18 hours in treatment-free media prior to cell harvest. This group acts as a 'control' for group i). The dose levels selected for assessment were 312.5, 625 and 1250 µg/mL.

After exposure, cells were harvested and scored for chromosome damage.

Results and discussion

Preliminary Toxicity Test:

In all cases except 6 hours with 59, the test material induced some evidence of cell toxicity. Microscopic assessment of the slides prepared from the treatment cultures showed metaphases present up to 5000 µg/mL in the 6-hour with and without 59-mix treatment cases. The maximum dose with metaphases present was 2500 µg/mL in the 24 and 48-hour continuous exposure treatment case. However, when a pH check was performed on culture media dosed with technical glyphosate it was observed that the pH was reduced in a'dose-related way. At the maximum two dose levels the pH was reduced by ~ 1 unit and this was considered to be unacceptable because alterations in pH have been shown to cause artefactual responses. Therefore the maximum dose level selected for the main study was 1250 µg/mL.

Chromosome Aberration Test

The test material was acidic at 2500 and 5000 µg/mL. Therefore the toxicity observed in the preliminary toxicity test was not relevant, and 1250 µg/mL was selected as the maximum dose for all treatment groups. The vehicle control cultures gave values of chromosome aberrations within the expected range. All the positive control cultures except cyclophosphamide without S9 gave highly significant increases in the frequency of cells with aberrations indicating that metabolic activation system was satisfactory and that the test method itself was operating as expected. The test material did not induce a statistically significant increase in the frequency of cells with aberrations at any dose level in any treatment group.The test material did not induce a significant increase in the numbers of polyploid cells at any dose level in any of the four treatment cases.

Conclusion by the Notifiers:

Technical glyphosate did not induce any statistically significant, dose-related increases in the frequency of cells with chromosome aberrations either in the presence or absence of a liver enzyme metabolising system or after various exposure times. Technical glyphosate is therefore considered to be non-c1astogenic to CHL cells in vitro.

RMS comments:
The study is considered acceptable. There were no indications for more frequent occurrence of chromosome aberrations. Thus, the conclusion is agreed with.
3d new clastogenicity study in vitro (Fox, 1998)
Reference:
IIA, 5.4.2/03
Report:
Fox, V. 1998 Glyphosate Acid: In Vitro Cytogenetic Assay In Human Lymphocytes
Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK
Data owner: Syngenta Report No.: CTL/P/6050 Date: 1998-10-29
not published, TOX2000-1995
Guidelines:
OECD 473 (1997): OPPTS 870.5375 (1998): 2000/32/EC B10
(2000)
Deviations:
The stability and achieved concentration of the test substance and control substances in the vehicles used were not determined by analysis.
This deviation from the current regulatory guideline is considered not to compromise the scientific validity of the study.
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1995-07-25 to 1998-10-29
Materials and methods
Test Material:
Glyphosate acid
Description:
Technical; white solid
Lot/Batch number:
P24
Purity:
95.6 % a.i
CAS#:
Not reported
Stability of test compound:
Confirmed by Sponsor
Control Materials:
Negative:
Supplemented RPMI-1640 culture medium
Solvent control (final concentration):
800 8L/mL
Positive control:
Absence of S9 mix: Mitomycin C, 0.2 Ag/mL
Presence of S9 mix: Cyclophosphamide 50 µg/mL
Mammalian metabolic system: S9 derived

X
Induced
Aroclor 1254
X
Rat
X
Liver
Non-induced
X
Phenobarbitol
Mouse
Lung
None
Hamster
Other
X
Other
β-naphthoflavone
Other
The metabolic activation system (S9-mix) used in this study was prepared as required (on each day of culture treatment) as a 1:1 mixture of S9 fraction and cofactor solution.
The cofactor solution was prepared as a single stock solution of Na2HPO4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and MgCl2 (12 mM) in sterile double deionised water and adjusted to a final pH of 7.4.
Test cells: mammalian cells in culture
V79 cells (Chinese hamster lung fibroblasts)
X
Human lymphocytes. Obtained on the days of culture initiation from healthy, non-smoking donors. Donor 1 was male and Donor 2 was female. Bothl donors had a previously established low incidence of
chromosomal aberrations in their peripheral blood lymphocytes.
Chinese hamster ovary (CHO) cells
Media: RPMI-1640 (Dutch modification)
Properly maintained?
X
Yes
No
Periodically checked for Mycoplasma contamination?
Yes
n/a
No
Periodically checked for karyotype stability?
Yes
n/a
No
Test compound concentrations used

Donor 1 – 68 hours
Donor 2 – 68 hours
Donor 2 – 92 hours
+ S9
-S9
+ S9
-S9
+ S9
-S9
100 µg/mL
750 µg/mL
1250 µg/mL
100 µg/mL
750 µg/mL
1250 µg/mL
100 µg/mL
750 µg/mL
1250 µg/mL
100 µg/mL
750 µg/mL
1250 µg/mL
1250 µg/mL
1250 µg/mL
Test performance
Cytogenetic assay: Duplicate human peripheral blood cultures were exposed to the solvent, test substance or positive control substances at appropriate concentrations in the following experiments:
A cytogenetic test using blood from Donor 1 in the presence and absence of S9-mix with a standard sampling time of 68 hours after culture initiation. Solvent and positive control cultures were included.
A second independent cytogenetic test using blood from Donor 2 in the presence and absence of S9-mix with a standard sampling time of 68 hours after culture initiation and a later sampling time of 92 hours after culture initiation. Solvent control cultures were included at both sampling times whereas the positive control cultures were only included at the 68 hour sampling time.
In both experiments a range of concentrations of glyphosate acid was used in order to define suitable concentrations for chromosomal aberration analysis.
The standard sampling time of 68 hours after culture initiation used in this study was based on a measured mean cell cycle time for cultured human peripheral blood lymphocytes of 13.5 hours in this Laboratory. The later sampling time was selected to be 24 hours after the standard sampling time.
Culture and treatment of blood samples: Cultures (10 mL) were established by the addition of 0.5 mL of whole blood to RPMI-1640 (Dutch modification) tissue culture medium supplemented with approximately 10 % foetal bovine serum (FBS), 1.0 IU/mL heparin, 100 IU/mL penicillin and 100 µg/mL streptomycin. The lymphocytes were stimulated to enter cell division by addition of phytohaemagglutinin (PHA; at 5 % v/v) and the cultures were maintained at approximately 37 °C for 48 hours with gentle daily mixing where possible.
Prior to treatment, the cultures were centrifuged and the culture medium was removed and replaced

with fresh supplemented RPMI-1640 culture medium in the solvent and positive control cultures. Approximately 48 hours after culture establishment, 8 mL aliquots of the test substance preparations were administered to duplicate cultures as appropriate to the experiment design. The positive control cultures were treated at a dosing volume of 50 µL/10 mL culture. In addition, 200 µL of a 1:1 mix of S9 and co-factor solution was added to each culture to be treated in the presence of S9-mix. Cultures treated in the presence of S9-mix were treated for a period of approximately 3 hours at 37 °C, after which the culture medium was removed following centrifugation and replaced with fresh supplemented RPMI-1640 culture medium. The cultures were re-incubated at approximately 37 °C for the remainder of the 68 hour growth period. Cultures treated in the absence of S9-mix were maintained at approximately 37 °C for the remainder of the 68 hour growth period. All cultures due for sampling at the later 92 hour sampling time received an additional culture medium change approximately 68 hours after culture initiation.
The effect of glyphosate acid on the pH and osmolality of the culture medium was investigated, using single cultures containing medium only, as changes in pH and increases in osmolality are well known to result in the production of chromosomal aberrations.
Culture harvesting: Approximately 2 hours prior to harvesting, the cultures were treated with colcemid at a final concentration of 0.4 µg/mL. Sixty-eight hours or 92 hours after culture establishment the cultures were centrifuged, the supernatant was removed and the cells were re-suspended in approximately 10 mL of 0.075 M KCl at room temperature for approximately 10 minutes.
Details of slide preparation: The cultures were centrifuged, the supernatant was removed and the remaining cells were fixed in freshly prepared methanol/glacial acetic acid fixative (3:1 v/v) added dropwise and made up to a volume of approximately 10 mL. The fixative was removed following centrifugation and replaced with freshly prepared fixative. This fixation process was repeated at least twice prior to slide preparation on clean, moist labelled microscope slides. The slides were air dried, stained in filtered Giemsa stain (10 % Gurr's R66 in buffered [pH 6.8] double deionised water) for 7 minutes, rinsed in water, air-dried and mounted with coverslips in DPX.
Slide analysis: Slides were examined to determine that they were of suitable quality and, where appropriate, the mitotic index was determined by examining 1000 lymphocytes per culture and calculating the percentage of cells in metaphase.
For each donor, both in the presence and absence of S9-mix, duplicate cultures treated with glyphosate acid at three concentrations were selected for chromosomal aberration analysis at the 68 hour sampling time along with the appropriate solvent and positive control cultures. In each case the highest concentration was selected on the basis of reduction in the pH of the culture medium and the suitability of the metaphase preparations for chromosomal aberration analysis. In addition, duplicate cultures from the Donor 2 treated with glyphosate acid at the highest concentration selected at the 68 hour sampling time in the presence and absence of S9-mix were selected for chromosomal aberration analysis at the 92 hour sampling time along with the appropriate solvent control cultures.
The slides were coded prior to analysis and one hundred cells in metaphase, where possible, were analysed from each selected culture for the incidence of structural chromosomal damage.
Evaluation criteria: The percentages of aberrant metaphases and the number of aberrations per cell were calculated for each treatment scored, both including and excluding cells with only gap-type aberrations.
The Fisher Exact Probability Test (one-sided) was used to evaluate statistically the percentage of metaphases showing aberrations (excluding cells with only gap-type aberrations). Data from each

treatment group, in the presence and absence of S9-mix, was compared with the respective solvent control group value. The data have been interpreted as follows:
No statistically significant increase in the percentage of aberrant cells (at any concentration) above concurrent solvent control values - NEGATIVE.
A statistically significant increase in the percentage of aberrant cells above concurrent solvent control values, which falls within the laboratory solvent control range - NEGATIVE.
An increase in the percentage of aberrant cells, at least at one concentration, which is substantially greater than the laboratory historical solvent control values - POSITIVE.
A statistically significant increase in the percentage of aberrant cells which is above concurrent solvent values and which is above the historical solvent control frequencies value but below that described in (c) may require further evaluation.
Significantly increased incidence of interchanges, exchange figures or re-arrangements (where none of the above criteria are met) may require further evaluation
Results and discussion
Cytogenetic assay: Small reductions in mean mitotic activity, compared to the solvent control values, were observed in cultures (37 % - Donor 1; 33 % - Donor 2) treated with the highest concentrations of glyphosate acid selected for chromosomal aberration analysis. No reductions in mitotic activity were observed for culture treated in the presence of S9-mix and harvested at the 68 hour sampling time or cultures treated in either the presence or absence of S9-mix and harvested at the 92 hour sampling time. Cultures treated with higher concentrations of glyphosate acid were considered not to be suitable for chromosomal aberration analysis due to excessive reductions in the pH of the culture medium.
Chromosomal aberration analysis: No statistically or biologically significant increases in the percentage of aberrant cells, above the solvent control values, were recorded at the 68 hour sampling time in cultures from either Donor 1 or Donor 2 treated with glyphosate acid in either the presence or absence of S9-mix.
No statistically or biologically significant increases in the percentage of aberrant cells, above the solvent control values, were recorded at the 92 hour sampling time for cultures treated with glyphosate acid in either the presence or absence of S9-mix.
The positive control materials, mitomycin C and cyclophosphamide induced statistically and biologically significant increases in the percentage of aberrant cells, compared to the solvent control cultures.
Conclusion by the Notifiers
Glyphosate acid was not clastogenic to cultured human lymphocytes treated in vitro in either the presence or absence of S9-mix.
RMS comments:
The study is considered acceptable and the conclusion is agreed with. The long duration of this study is surprising but was apparently due to the fact that the in-life phase was run early in 1996 but slides were evaluated not before 1998.
B.6.4.5 In vivo genotoxicity testing (somatic cells) – Metaphase analysis in rodent bone marrow, or micronucleus test in rodents
A number of bone marrow micronucleus tests, mostly in mice, and two cytogenetic studies in mouse bone marrow have been submitted by the different notifieres to adress this endpoint. An overview on these studies is given in Table B.6.4-12.
Three of the previously evaluated studies in mice (1994, TOX9400323;
1993, TOX9551100; 1991, TOX9552374) and one in rats (TOX9552375) that

were already mentioned in the 1998 DAR were still found by the RMS to be of acceptable quality. In contrast, the studies by (1990, TOX9500255), (1989, TOX9650159) and (1986, TOX9551957) must be regarded as not acceptable and were not taken into account for the current re-evaluation of glyphosate.

Instead, several studies of this type, mostly performed in mice and more recently, were submitted for this re-evaluation of glyphosate by the EU. All these new studies are reported in detail below Table B.6.4-12 and each was commented by the RMS. It must be emphasised that the studies by (2007, ASB2012-11480) and (2008, ASB2012- 11481) were considered not acceptable because the dose levels employed were much too low. In addition, the first one was flawed by severe toxicity at low levels and an amendment to the

Therefore, they have been

deleted from the summary table.

Table B.6.4-12: Summary of valid in vivo micronucleus assays or cytogenic studies with glyphosate in rodents

Reference, study identification, owner	Type of study	Test organism / test system, route/ treatment	Dose levels; batch/lot, purity; sampling	Results
Studies from the 2001 evaluation				
1994; TOX9400323; ADAMA	Cytogenicity in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day; batch 046, 96.8%; sampling 24 h after second dose	Negative; mitotic index ↓ at 5000 mg/kg bw
1993; TOX9551100; ADAMA	Micronucleus test in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day; batch 60, 96.8%; sampling 24 h after second dose	♂: negative ♀: weakly positive at highest dose
1991; TOX9552374; Cheminova	Micronucleus test in bone marrow	NMRI mice, single oral application	0 – 5000 mg/kg bw; 206-JaK-25-1, 98.6%; sampling after 24, 48, 72 h	

Negative
1983*; TOX9552375;
Monsanto
Cytogenicity in bone marrow
Sprague-Dawley rats, single i.p. injection
0 – 1000 mg/kg bw;
XHJ-64, 98.7%;
sampling after 6, 12,
24 h
Negative
Studies not reviewed in the 2001
2006; ASB2012- 11478;
Nufarm
Micronucleus test in bone marrow
CD-1 mice ♂; single i.p. dose
0, 150, 300, 600
mg/kg bw; H05H016A, 95.7%;
sampling after 24
and 48 h
Stat. sign. S in PCE at 600 mg/kg
bw (24 h) but within historical control; overall: negative
1999; ASB2012- 11482;
Nufarm
Micronucleus
test
Swiss albino
mice, ♂ + ♀, two i.p. injections (24 h interval)
0, 187.5, 375, 562.5
mg/kg bw; batch 037-919-113, 95.49
%; sampling 24 h after 2nd application
Negative
Reference, study identification, owner
Type of study
Test organism / test system, route/ treatment
Dose levels; batch/lot, purity; sampling
Results
1996; TOX2000- 1996;
Syngenta
Micronucleus test in bone marrow
CD-1 mice, 5
♂+ 5 ♀/dose / sampling point; single oral dose
0, 5000 mg/kg bw;
P24, 95.6%;
sampling after 24
and 48 h
Negative

2008; ASB2012- 11483;
Syngenta
Micronucleus
test in bone marrow
NMRI mice
6 ♂/dose/ sampling point; single oral dose
0, 2000 mg/kg bw,
sampling after 24
and 48 h, 500 &
1000 mg/kg bw sampling after 24 h only; Batch 20070545, 99.1%
Negative
2012;
ASB2014- 9277;
Dow
Micronucleus
test in bone marrow
Swiss albino
mice, ♂, two oral. injections (24 h interval)
0, 2000 mg/kg bw,
sampling after 24 h; Lot 20061109, 98.9 %
Negative
2012;
ASB2014- 9333;
Syngenta
Micronucleus
test in bone marrow
NMRI mice
7 ♂/ sampling point;
single oral dose
0, 2000 mg/kg bw,
sampling after 24 and 48 h; Batch 56753, 96.3 %
Negative
2009;
ASB2012- 11479; Helm
Micronucleus
test in bone marrow
CD rat, single
oral application
0, 500, 1000, 2000
mg/kg bw/day; batch 20080801, 98.8%;
sampling after 24
and 48 h
Negative
* Results were also published by Li & Long, 1988 (TOX9500253).
Mouse
1st new micronucleus test in mice (Durward, 2006)

Reference: IIA, 5.4.4/01
Report: 2006, Glyphosate Technical: Micronucleus Test In The Mouse
Data owner: Nufarm Report No.: 2060/014 Date: 2006-02-08
Unpublished; ASB2012-11478
Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 2005-06-07 - 2005-07-20
Materials and methods
Test material:
Identification:
Glyphosate Technical
Description:
White crystalline solid
Lot/Batch #:
H05H016A
Purity:
95.7 %
Stability of test compound:
At room temperature stable until March 2008.
Vehicle and/
or positive control:
PBS
Test animals:
Species:
Mouse
Strain:
CD-1
Source:
Age:
Approx. 5 - 8 weeks
Sex:
Males
Weight at dosing:
21 - 29 g
Acclimation period:
At least 7 days
Diet/Food:
Certified Rat and Mouse Diet Code 5LF2, BCM (IPS Ldt., London UK), ad libitum
Water:
Tap water, ad libitum
Housing:
In groups up to seven in solid-floor polypropylene cages with wood flake bedding.
Environmental conditions:
Temperature: 19 - 25 °C

Humidity: 30 - 70 %
Air changes: approx. 15/hour 12 hours light/dark cycle
Animal assignment and treatment:
The test was conducted using young male CD-1 mice. Groups of seven mice each were dosed via the intraperitoneal route at 150, 300 and 600 mg/kg bw.
One group from each dose level was killed by cervical dislocation 24 hours following treatment and another group dosed with test material at 600 mg/kg bw after 48 hours. In addition, three further groups of mice were included in the study; two groups (each of seven mice) were dosed via the intraperitoneal route with the vehicle alone (PBS) and a third group (five mice) was dosed orally with the positive substance cyclophosphamide. The vehicle controls were killed 24 or 48 hours following dosing and positive control group animals were killed 24 hours following dosing.
Immediately following termination both femurs were dissected from each animal, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re- suspension. The smears were air-dried, fixed in absolute methanol, stained in May- Grünwald/Giemsa, allowed to air-dry and coverslipped using mounting medium.
Results and discussion
Mortality: No mortality occurred.
Clinical observations: Clinical signs were observed in animals dosed with the test material at and above 150 mg/kg bw in both the 24 and 48-hour groups where applicable, these included as follows: hunched posture, ptosis, ataxia and lethargy.
Evaluation of bone marrow slides:
A statistically significant decrease in the percentage PCEs per 1000 erythrocytes was observed in the 24-hour 600 mg/kg bw group when compared to the control. A similar decrease was also observed in the 48-hour 600 mg/kg bw group, but the larger standard deviation resulted in no statistical significance being applied. This finding, accompanied by the presence of clinical signs, was taken to indicate that systemic absorption had occurred and exposure to the bone marrow was confirmed.
There was a small but statistically significant increase in the incidence of micronucleated PCEs in animals dosed at 600 mg/kg bw in the 24-hour group when compared to the control group. However, the response was very modest and within the historical range (see Table B.6.4-13 and Table B.6.4-14). The response seen is considered to be most likely due to a haematopoietic effect induced by the cytotoxic effect of the test material on the bone marrow rather than any genotoxic mechanism. The increased erythropoiesis caused by the test material toxicity might cause some cells to cycle more quickly than in the vehicle control animals and, therefore, there may also be less opportunity to repair spontaneously-occurring DNA damage before the final mitosis and enucleation, resulting in small increases in micronucleated cells. Therefore the response was considered to have no genotoxic significance.
The positive control group showed a marked increase in the incidence of micronucleated PCEs hence confirming the sensitivity of the system to the known clastogenic activity of cyclophosphamide under the conditions of the test.
Table B.6.4-13: Summary of results
Treatment group / sampling time
Number of PCE with micronuclei/2000 PCE
% PCE with micronuclei
% PCE / 1000
erythrocytes
Group mean

SD
Group mean
SD
Group mean
SD
Vehicle control (10 mL/kg) /
48h
2.0
2.4
0.10
0.12
36.01
4.39
Vehicle control (10 mL/kg) /
24h
1.3
1.1
0.06
0.06
38.46
4.58
Positive control (50 mg/kg) /
24h
60.6***
9.7
3.03***
0.49
51.46
4.45
Glyphosate (150 mg/kg) / 24 h
1.4
0.8
0.07
0.04
45.23
6.12
Glyphosate (300 mg/kg) / 24 h
1.1
1.1
0.06
0.05
38.57
8.69
Glyphosate (600 mg/kg) / 24 h
3.9*

1.5
0.19*
0.07
27.71**
4.95
Glyphosate (600 mg/kg) / 48 h
1.9
2.1
0.09
0.1
28.16
14.23
PCE = polychromatic erythrocytes SD = standard deviation
* : p < 0.05, ** : p < 0.01, *** : p < 0.001
Table B.6.4-14: Historical control data for relative frequency categories of micronuclei per 1000 PCE*
24-h sampling
48-h sampling
Frequency
Groups
%
Frequency
Groups
%
categories
categories
0.0 – 0.4
15
25
0.0 – 0.4
21
35
0.5 – 0.9
25
42
0.5 – 0.9
18
30
1.0 – 1.4
14
23
1.0 – 1.4
14
23
1.5 – 2.0
3
5

1.5 – 2.0
7
12
2.1 – 2.5
3
5
2.1 – 2.5
0
0
* Data from 60 studies
Conclusion by the Notifiers
The test material glyphosate technical was considered to be non-genotoxic under the conditions of the test.
RMS comments:
The study is considered acceptable. The selection of the highest dose level of 600 mg/kg bw is appropriate since the application route was intraperitoneal (for oral dosing, it might be too low). In fact, in a range-finding test, deaths were observed after i.p. application of 800 and 1000 mg/kg bw. The use of only male mice is also justified because males are known to be the more sensitive sex for micronucleus formation. The conclusion is supported. The increase in micronucleated PCE in high dose males after 24 hours (3.9/2000 = 1.95/1000) is covered by the historical controls even though it is close to its upper edge. Furthermore, no evidence of an increase was seen after 48 hours. Thus, there is no concern about clastogenicity because of this study and the conclusion of the notifiers may be agreed with.
2nd new micronucleus test in mice (, 2007)
Reference: IIA, 5.4.4/03
Report: 2007 Mammalian Erythrocyte Micronucleus Test for Glifosato Téchnico Helm
Data owner: HAG
Report No.: 3393/2007-3.0MN-B Date: 2007-12-13
Unpublished; ASB2012-11480
Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 26/11/2007 – 28/11/2007
Materials and methods
Test material:
Identification:
GLIFOSATO TECNICO HELM
Description:
Solid,
Lot/Batch #:
2007091801
Purity:
980.1 g/kg
Stability of test compound:
Stable (CIPAC MT 46, 54 °C, 14 days)

Vehicle and/
or positive control:
Vehicle: deonized water
Positive Control: Cyclophosphamide
Test animals:
Species:
Swiss mice
Source:
Age:
09 – 10 weeks
Sex:
Male
Diet/Food:
Commercial food (Biobase Biotec), ad libitum
Water:
ad libitum
Housing:
Animals were kept in groups of 6 animals in solid cages bedded with wooden chips.
Environmental conditions:
Temperature: 18 - 21 °C Humidity: ~57 %
12 hours light/dark cycle
Animal assignment and treatment:
The test was conducted using young male mice. Groups of six mice were dosed via oral route at 8.0, 15.0 and 30.0 mg/kg bw. The animals were treated twice at 0 and 24 h. Sampling was performed 24 hours after last treatment. Both femurs from each rat were dissected, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re- suspension. The smears were air-dried, fixed and stained, allowed to air-dry and coverslipped using mounting medium. 3000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. The ratio of PCE to normochromatic Erythrocytes (NCE) was determined for each animal by counting a total of 2000 erythrocytes.
Results and discussion
Mortality: No mortality occurred.
Evaluation of bone marrow slides: When animals treated with GLIFOSATO TECHNICO HELM were compared to the concurrent negative control group, no statistically significant increase in the number of micronuclei was observed at dosage of 8 or 15 mg /kg bw (see Table B.6.4-15). At 30 mg /kg bw results obtained were statistically significant when compared to the control group but they did not have biological relevance when compared to historical control data (that is however, partly based on published data and not considered robust by the RMS).
.
Table B.6.4-15: Summary of results
Treatment group / sampling time
Number of PCE with micronuclei /18000 PCE
χ^2
Vehicle control (5 mL/kg)
11
n.a.

Positive control (75 mg/kg)
347
315.4 ($p < 0.001$)
Glyphosate TC (8 mg/kg)
19
2.14 ($p = 0.144$)
Glyphosate TC (15 mg/kg)
21
3.12 ($p = 0.077$)
Glyphosate TC (30 mg/kg)
25
5.44 ($p = 0.020$)
PCE = polychromatic erythrocytes
In the third column, the frequency of micronucleated PCE in the individual groups was compared to the negative control group. This calculation was not checked or repeated by the RMS since the study is considered not acceptable.
Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.
RMS comments:
The study is considered not acceptable since it was seriously flawed (see below) and because the dose levels were much too low for any meaningful conclusion with regard to micronucleus formation, in particular when application by the oral route is taken into consideration. In the original report, some justification for dose selection is given, based on a range-finding test suggesting effects at rather low dose levels. In fact, two animals that were administered 2000 mg/kg bw, died on day 3 after having shown ataxia and prostration before. The same observations were made in 3 animals which received an oral dose of 320 mg/kg bw. They all died on day 2. Even at a dose level of 50 mg/kg bw, one out of three treated animals died on day 1. No mortality was seen at 30, 20, and 12.5 mg/kg bw. Therefore, 30 mg/kg bw was considered the MTD and was selected as the highest dose for the micronucleus assay. These findings The occurrence of deaths and clinical signs at relatively low dose levels were was obviously in contradiction to more reliable the available acute toxicity tests with glyphosate in the mouse
micronucleus assays or cytogenetic studies in mice with substance administration by the oral route described in this section (
1991;
1993 and 1994;
,
1996;
2008; see Table B.6.4-12) much higher dose levels could be used. A single
study cannot contravene or even outweigh all this data coming from a number of (independent) laboratories even though this was suggested by a comment that was provided
in the public consultation. It is much more likely that the micronucleus assay by
(2007) was seriously flawed by severe toxicity that was completely unexpected and
cannot be explained if the whole toxicological profile of glyphosate is taken into consideration. Either, strong methodical mistakes have been made when the study was conducted or the test material was not glyphosate even though it was claimed as such. Both possibilities would turn the study completely unreliable and make it unsuitable for any regulatory use. Because of this

general assessment, there is no need to discuss the weak
“increase” in micronuclei at 30 mg/kg bw that is in complete contradiction to what was seen
in the other, much more reliable studies.
3d new micronucleus test in mice (Costa, 2008)
Reference: IIA, 5.4.4/04
Report: . 2008 Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice
Data owner: HAG (original sponsor: Jingma Chemicals, Longyou
Zhejian, China)
Report No.: RF - 3996.402.395.07 Date: 2008-09-29
Unpublished; ASB2012-11481
Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 19/05/2008 – 13/08/2008
Materials and methods
Test material:
Identification:
GLYPHOSATE TECHNICAL
Description:
Solid,
Lot/Batch #:
20070606
Purity:
980.0 g/kg
Stability of test compound:
Stable to hydrolysis at pH 3, 6 and 9 (5-35 °C)
Vehicle and/
or positive control:
Vehicle: sterile corn oil
Positive Control: Cyclophosphamide
Test animals:
Species:
Swiss mice
Source:
Age:
07 – 12 weeks
Sex:
Male and Female
Weight at dosing:
approx. 30 g
Diet/Food:

Commercial food (Purina Labina, Agribrands Purina do Brsail, Ltda.)
Water:
ad libitum
Housing:
Animals were kept in groups of 5 animals by sex in solid cages bedded with sterile sawdust.
Environmental conditions:
Temperature: 20 - 24 °C
Humidity: 50 - 70 %
Air changes: approx. 10 - 15/hour 12 hours light/dark cycle
Animal assignment and treatment:
The test was conducted using young male and female mice. Groups of five male and five female mice were dosed via oral route (positive reference item was administered via intraperitoneal route) administered technical grade glyphosate by intraperitoneal injection twice after a 24 hours interval at dose levels of 15.62, 31.25 and 62.5 mg/kg bw in a final volume of 15 mL per dose. Sampling was performed after 24 hours following the second injection for all groups and after 48 hours for the vehicle control and the highest dose group. Both femurs from each rat were dissected, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed and stained, allowed to air-dry and coverslipped using mounting medium. 2000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. It is stated in the orginal report that the ratio of PCE to normochromatic erythrocytes (NCE) was
determined for each animal by counting a total of 1000 erythrocytes when the first 2000 PCE had been detected.
Results and discussion
Mortality: No mortality occurred.
Evaluation of bone marrow cells: The statistical analysis of the results pointed out that the test substance did not induce an increase in micronuclei number in polychromatic erythrocytes of the bone marrow when compared to the negative control at any evaluated concentrations. No adverse effect was observed in the ratio of polychromatic erythrocytes to normochromatics in animals treated with the test substance glyphosate technical, at any evaluated concentrations. A significant statistical increase of micronucleated cells in polychromatic erythrocytes and a slight change in the ratio of polychromatic to normochromatic erythrocytes was observed in animals treated with cyclophosphamide, as expected (see Table B.6.4-17).
Table B.6.4-16: Summary of results
Treatment group / sampling time
Male
Female
Number of PCE with micronuclei
/2000 PCE,
Group mean
PCEs/NC
Es
Number of PCE with micronuclei
/2000 PCE,
Group mean
PCEs/NC

Es
Vehicle control (15 mL/kg) / 24h
0.0
1.78197
0.0
1.77195
Positive control (27 mg/kg) / 24h
0.0
1.76831
0.0
1.79107
Glyphosate TC (15.62 mg/kg) / 24 h
0.0
1.74353
0.0
1.76047
Glyphosate TC (31.25 mg/kg) / 24 h
0.0
1.71071
0.0
1.78676
Glyphosate TC (62.5 mg/kg) / 24 h
23.0**
1.54855
12.2**
1.72844
PCE = polychromatic erythrocytes SD = standard deviation
**p<=0.01
When this section of the RAR was reviewed, this table was found to be wrong, due to a technical error. The (expected) in increase in the positiv control group had been erroneously allocated to the group receiving 62.5 mg glyphosate/kg bw in the GTF dossier and the RMS reviewers had not noticed this error when the original RAR was prepared. Furthermore, not
11481). Therefore, it was replaced now by a new one giving the appropriate allocation of test results to the individual test groups. The sampling time was always 24 hours after the second i.p. dose.
Table B.6.4-17: Summary of results
Group
Males
Females
Number of PCE with micronuclei
/2000 PCE,
Group mean
PCE/NCE
ratio
Number of PCE with micronuclei
/2000 PCE,

Group mean
PCE/NCE
ratio
Vehicle control
0.0
1.78197
0.0
1.77195
Positive control (27 mg/kg bw)
23.0**
1.54855
12.2**
1.72844
Glyphosate TC (15.62 mg/kg)
0.0
1.76831
0.0
1.79107
Glyphosate TC (31.25 mg/kg)
0.2
1.74353
0.0
1.76047
Glyphosate TC (62.5 mg/kg)
0.6
1.71071
0.0
1.78676
**p<=0.01 (Mann-Whitney test); PCE = polychromatic erythrocytes
Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.
RMS comments:
The study is considered not acceptable since the dose levels were much too low for any meaningful conclusion with regard to micronucleus formation.
Some of the information given in the dossier was apparently wrong but, unfortunately, was corrected in the revised RAR only. In the original RAR, the study was considered not acceptable since the dose levels were much too low for any meaningful conclusion with regard to micronucleus formation even though it must be taken into consideration that the exposure was via the intraperitoneal route. (Thus, the study can be hardly compared to most other micronucleus tests with glyphosate in which the test substance was administered via the oral route.) In the original report, dose selection for the"definitive test" was justified with the outcome of a preliminary test. In this range finding experiment, 3 males and 3 females per dose level received i.p. glyphosate doses of 62.5, 125, 250, 500, or 1000 mg/kg bw. The top dose level resulted in 100 % mortality and at the next lower dose level of 500 mg/kg bw, one male and two female mice died. Based on a clear decrease in the PCE/NCE ratios in both sexes, the intermediate dose of 250 mg/kg bw was found to be cytotoxic. It was recommended that 125 mg/kg bw was the most appropriate high dose to be employed in the

definitive test but, without further justification, 62.5 mg/kg bw was actually the highest dose used. That was
much lower than in other studies in which the i.p. route had been also chosen
, 1999, ASB2012-11482;
2006, ASB2012-11478).
In 2014, an amendment to this study was submitted (
2010, ASB2014-9284). In this
document, some results of testing glyphosate at dose level of 125, 250, and 375 mg/kg bw are reported. Clinical signs but no mortality were seen at all dose levels. It is not clear in which way this data is linked to the preliminary test that was performed as part of the original study since the dose levels were not exactly the same and the number of animals was different (this time 5 per sex and dose). Furthermore, in the amendment, more data on micronucleus incidences and PCE/NCE ratios at the dose levels of 15.62, 31.25, and 62.5 mg/kg bw was given, apparently based on 10 animals per sex and dose. It was confirmed that there was no clastogenic potential of the test substance. However, treatment of these animals was simply not described in the original report and the amendment cannot be considered a full study report. Taking all these deficiencies and uncertainties in the amendment as well as the use of
only very low dose levels into account, assessment of the study as “not acceptable” by the
RMS is maintained.
4th new micronucleus test in mice (, 1999)
Study No.: RF-G12.79/99 Date: 1999-12-27
unpublished, ASB2012-11482
Guidelines:
Not specified. Internal SOP M 069 - Micronucleus Test
Deviations:
Not applicable
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 28/0ctober/1999
Materials and methods
Test material:
Identification:
GLIFOSATE TECNICO NUFARM
Description:
White powder
Lot/Batch #:
3578/99
Purity:
95 %
Stability of test compound:
No data available
Vehicle and/
or positive control:
Water

Test animals:
Species:
Mouse
Strain:
Swiss albino
Source:
Age:
7-12 weeks
Sex:
Male / Female
Weight at dosing:
30.22 gg
Acclimation period:
Not specified
Diet/Food:
commercial pelleted diet (Labina, Purina)
Water:
Tap water, ad libitum
Housing:
on wood shavings, in propylene rodent cages (five of the same sex per cage) with stainless mesh lids.
Environmental conditions:
Temperature: 20-24 °C
Humidity: 50-60 %
Air changes: not specified 12-hour light/dark cycle
In life dates: 28/0ctober/1999 Animal assignment and treatment:
Three levels of the GLIFOSATE TECNICO NUFARM were tested: 187.5, 375 and
562.5 mg/kg bw. These dose levels were claimed to corresponding to 25 %, 50 % and 75 % of the (intraperioneal) LD50 for mice as determined before, with ten animals (five male and five female) per level. The animals were dosed twice with intraperitoneal injections in volumes that were adusted to be 0.45 mL per 30 g bw/animal within a 24 hours interval, and sacrificed 24 hours after the second injection. Negative control with water and positive control with cyclophosphamide (1.66 mg/mL in physiological solution, corresponding to 25 mg/kg), were
also applied with the two injections protocol. Mice were killed by cervical dislocation 24 hours after the econd dosing. From the freshly killed animal both femora were removed in total. The bones were then freed from muscle, the distal epiphyseal portion was tom off by gentle traction and the proximal end of the emur was shortened with scissors until a small opening ofthe marrow was visible. The bone marrow cells were gently flushed out with fetal calf serum. After centrifugation at 1,000 rev./min. for 5 min., the bone marrow cells were resuspended in fetal calf serum and smeared on glass slides which were air dried overnight. The following day, the smears were fixed in ethanol 70 % for 10 min. air dried and stained for 20 min. with Eosin-Methylene Blue solution. The slides were coded and observed with a 1,000X magnification objective in a Olympus microscope. The technicians were not allowed to know the corresponding coding in the slides. For each animal 1,000 polychromatic erythrocytes (PCEs) and 1,000 normochromatic erythrocytes (NCEs) were examined for the presence of micronuclei (MN). The relation PCEslNCEs were determined in the first 1,000 PCEs or NCEs enumerated. Differences in the incidence per animal of MNPCEs and MNNCEs per 1000 cells and the relation PCEslNCEs were compared using the Kruskal Wallis test for independent

samples. All the tests were compared to the negative control. The criteria for a positive response was the detection of a reproducible and statistically significant (p :0; 0.05) positive response for at least one dose level and the increase in the number of micronuclei to be at least twice the vehicle control. The test is considered valid only if the number of micronuclei in the vehicle control stays within the historic value of the laboratory.

Results and discussion

Mortality: There were no mortalities during the study.

Clinical observations: No systemic or local signs of toxicity were observed during the study period.

Body weight: No significant changes observed.

Necropsy: No macroscopic changes of significance were noted. Bone marrow evaluations

Table B.6.4-18: Summary of results

Micronuclei in 1000

Number of

Group

PCE

NCE.

PCE

NCE

PCE/NEC

ratio

Vehicle

0.6

0

879

997.7

0.9151

187.5 mg/Kg

0.3

0.1

779.2

978.1

0.81341

375 mglKg

0.6

0.3

871.7

948.4

0.9348

562.5 mg/Kg

0.5

0.3

832.8

987.8

0.8513

Cyclophospha-

mide

4.8*
2.0*
648.9
1029.5
0.6296
* p= 0.05, Kruskal Wallis test
Conclusion by the Notifiers
Presence of micronuclei in PCEs and NCEs were similar to control animals. Animals treated with the positive control cyclophosphamide showed a significant increase in micronuclei. Therefore, under the test conditions, the GLIFOSATE TECNICO NUFARM did not have mutagenic activity in mice.
Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.
RMS comments:
The study is considered acceptable and the conclusion is agreed with. The real dates for the experimental phase were from 28 October to 10 December 1999.
new micronucleus test in mice , 1996)
Reference: IIA, 5.4.4/06
Report: 1996 Glyphosate Acid: Mouse Bone Marrow Micronucleus Test
Data owner: Syngenta Report No.: /P/4954 Date: 1996-03-21
not published, TOX2000-1996
Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000)
Deviations: The stability, homogeneity and achieved concentration of the test and control substances in the vehicles used were not determined by analysis. The certified purity and stability of the control substances are not available.
The above deviations from the current regulatory guideline are considered not to compromise the scientific validity of the study
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: 1995-10-04 to 1996-03-21
Materials and methods
Test Material:
Glyphosate acid
Description:
Technical, white solid
Lot/Batch number:
P24
Purity:
95.6 % w/w a.i
CAS#:
Not reported
Stability of test compound:
Confirmed for the duration of the study.
Control Materials:
Negative control
(if not vehicle) :
N/A

Final Volume: N/A
Route: N/A
Vehicle:
Physiological
saline
Final Volume:
20mL/kg
Route: oral
Positive control :
Cyclo-
phosphamide
Final Doses: 65 mg/kg
Route: oral
Test Animals:
Species
Mouse
Strain
CD-1
Age/weight at dosing
6-7 weeks / 22.8-37.6 g
Source
Housing
Up to 5/cage
Test Animals:
Acclimatisation period
At least 5 days
Diet
CT1 (supplied by Special Diets Services, Stepfield, Witham,
Essex, UK ad libitum
Water
Mains water ad libitum
Environmental conditions
Temperature: 19-23 °C
Humidity: 40-70 % Air changes: 15/hour
Photoperiod: 12hours dark/12 hours light
Test compound administration:
Dose Levels
Final Volume
Route
Preliminary:
Main Study:
5000 mg/kg
5000 mg/kg
20 mL/kg
20 mL/kg
oral

oral
Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethalities or severe toxicity observed over a four-day observation period following a single oral dose of 5000 mg/kg.
Micronucleus Test: Male and female mice were weighed and given a single oral dose and sacrificed 24 or 48 hours after dosing as shown in the table below:
Table B.6.4-19: Experimental Design
Treatment
Dose
Number of Animals /Time of kill
24 hours
48 hours
Glyphosate acid
5000 mg/kg
5 male and 5 female
5 male and 5 female
Vehicle control
10 mL/kg
5 male and 5 female
5 male and 5 female
Positive control
(cyclophoshamide)
65 mg/kg
5 male and 5 female
Slide Preparation: All animals were killed by over-exposure to halothane followed by cervical dislocation.
Femurs were removed and stripped clean of muscle. The iliac end of the femur was removed and a fine paint brush was rinsed in saline, wiped to remove the excess and wetted with a solution of albumin (6 % w/v in physiological saline). This was then dipped into the marrow canal and two smears were painted on an appropriately labelled clean, dry microscope slide. This procedure was repeated to give four smears of marrow per slide.
The slides were allowed to air dry and were stained with polychrome methylene blue and eosin using an automatic staining machine.
Slide Analyses: Slides were coded and scored blind. Two thousand immature polychromatic erythrocytes were examined for the presence of micronuclei for each animal. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of immature to mature erythrocytes in a sample of 1000 erythrocytes.
Statistics: The incidence of micronucleated polychromatic erythrocytes and percentage polychromatic erythrocytes in the erythrocyte sample, were considered by analysis of variance at 24 and 48 hours, separately for males and females.
Analyses were carried out using the GLM procedure in SAS (1989). Each treatment group mean was compared with the control group mean at the corresponding sampling time using a one-sided Student's t-test, based on the error mean square in the analysis.
Results and discussion
Preliminary toxicity assay: As no clinical signs or lethalities were observed over a four day

observation period, at the limit dose level of 5000 mg/kg, this was selected to represent the maximum tolerated dose for both males and females.
Micronucleus test: No adverse reactions to treatment were observed for either males or females dosed with glyphosate acid at the limit dose of 5000 mg/kg.
No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were observed in either males or females at either sampling time investigated.
No statistically significant differences in the percentage of polychromatic erythrocytes, between the vehicle control and glyphosate acid treated animals, were observed in either males or females at either sampling time investigated.
The test system positive control, cyclophosphamide, induced statistically and biologically significant increases in the frequency of micronucleated polychromatic erythrocytes in both male and female mice at the 24 hour sampling time.
Conclusion by the Notifiers
Glyphosate acid, under the conditions of test, was not clastogenic in the mouse micronucleus test.
RMS comments:
The study is considered acceptable and the conclusion is agreed with.
new micronucleus test in mice (2008)
Reference: IIA, 5.4.4/07
Report: 2008 Glyphosate Technical – Micronucleus Assay in Bone Marrow Cells of the Mouse
Data owner: Syngenta Report No.: 1158500
Date: 2008-06-09
not published, ASB2012-11483
Guidelines: OECD 474 (1997): EPA OPPTS 870.5395 (1998): 2000/32/EEC
B.12 (2000)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 2008-02-11 to 2008-06-09
Materials and methods
Test Material:
Glyphosate technical
Description:
solid, white
Lot/Batch number:
20070545
Purity:
99.1 % w/w glyphosate (estimated error ± 0.3 %)
CAS#:
1071-83-6
Stability of test compound:
not available
Control Materials:
Negative control
(if not vehicle) :
N/A

Final Volume: N/A
Route: N/A
Vehicle:
0.5 % CMC
Final Volume: 20 mL/kg
Route: oral
Positive control :
Cyclophosphamide
Final Doses: 40 mg/kg
Route: oral
Test Animals:
Species
mouse
Strain
NMRI
Age/weight at dosing
7-8 weeks
Source
Housing
1/cage
Acclimatisation period
At least 5 days
Diet
ad libitum
Water
tap water ad libitum
Environmental conditions
Temperature: 19-25 °C
Humidity: 30-70 % Air changes: 15/hour
Photoperiod: 12hours dark/12 hours light
Test compound administration:
Dose Levels
Final Volume
Route
Preliminary: Main Study:
2000 mg/kg b.w.
500, 1000, 2000 mg/kg
b.w.
20 mL/kg b.w.
20 mL/kg b.w.
oral oral
Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethalities or severe toxicity observed over a two-day observation period following a single oral dose.
Micronucleus Test:
Table B.6.4-20: Experimental Design
Treatment

Dose
Number of Animals /Time of kill
24 hours
48 hours
Vehicle control
10 ml/kg
6*
6*
Positive control
40 mg/kg
6*
Test substance
2000 mg/kg
6*
6*
Test substance
1000 mg/kg
6*
Test substance
500 mg/kg
6*
*: the 6th animal was used as a reserve.
Slide Preparation: All animals designated for bone marrow smears were killed by over- exposure to CO2 followed by bleeding.
The animals were sacrificed using CO2 followed by bleeding. The femora were removed, the epiphyses were cut off and the marrow was flushed out with foetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald (Merck, D-64293 Darmstadt)/Giemsa (Merck, D-64293 Darmstadt). Cover slips were mounted with EUKITT (Kindler, D-79110 Freiburg). At least one slide was made from each bone marrow sample.
Slide Analysis: Slides were coded and scored blind. Two thousand immature erythrocytes were examined for the presence of micronuclei for each animal. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of immature to mature erythrocytes and expressed in immature erythrocytes per 2000 erythrocytes.
Results and discussion
Preliminary toxicity assay: In a pre-experiment 4 animals (2 males, 2 females) received orally a single dose of 2000 mg/kg b.w. glyphosate technical formulated in 0.5 % CMC. The volume administered was 20 mL/kg b.w..
The animals treated with 2000 mg/kg b.w. did not express any toxic reactions.
Micronucleus test: In the main experiment for the highest dose group 12 males received orally a single dose of 2000 mg/kg b.w. glyphosate technical formulated in 0.5 % CMC. For the mid and low doses 6 males per group received orally a single dose of 1000 or 500 mg/kg b.w. Glyphosate Technical formulated in 0.5 % CMC. The volume administered was 20 mL/kg b.w.
Neither the test item treated animals nor those treated with the vehicle control (0.5 %

expressed any toxic reactions.
The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control, indicating that glyphosate technical did not have any cytotoxic properties in the bone marrow.
In comparison to the corresponding vehicle controls there was no biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after treatment with Glyphosate Technical were near to the value of the vehicle control group and within the historical vehicle control range.
Conclusion by the Notifiers
In conclusion, it can be stated that during the study described and under the experimental conditions reported, the test item did not induce micronuclei as determined by the micronucleus test in the bone marrow cells of the mouse.
RMS comments:
The study is considered acceptable and the conclusion is agreed with. It seems that technical material from a different (Chinese) source and of rather high purity was tested.
The following two studies have been provided to the RMS in 2014 and were not part of the original dossier of the GTF. Here, they are described in brief:
7th new micronucleus test in mice (
2012)
An in vivo bone marrow micronucleus assay was conducted on behalf of DowAgroSciences
(DAS study no. 120709) in a laboratory of the
The study (
2012; ASB2014-9277;
study no. 485-1-06-4696) was performed
according to OECD Guideline 474, was GLP-compliant (certified in 2011 by the Dutch Food and Consumer Product Safety Authority) and audited by an internal QAU. The study may be considered acceptable.
Male Swiss albino mice (6 per group) were administered either glyphosate (Lot no. 20061109 of Chinese production, purity 98.9 %) at a dose level of 2000 mg/kg bw/day or the vehicle (vegetable oil) by oral gavage (dose volume 10 mL/kg bw) on two consecutive days. A third (positive control) group of same size received a single intraperitoneal injection of 1 mg mitomycin C/kg bw on day 2 of the study. All animals were sacrificed and slides from femur bone marrow prepared 24 hours following the last treatment. In each animal, at least 2000 polychromatic erythrocytes were scored for the presence of micronuclei.
The mice tolerated the treatment without mortality or exhibiting clinical signs. The ratio of polychromatic to total erythrocytes was nearly the same in the vehicle control and glyphosate-treated groups but depressed in the animals that had received the positive control substance.
Treatment with glyphosate did not induce micronuclei in any of the animals (0 % group mean). In the vehicle control group, a mean percentage of 0.033 % of micronucleated polychromatic erythrocytes was noted whereas injection of mitomycin C had caused the expected and statistically significant increase in such cells (2.492 %).
Thus, glyphosate proved negative for clastogenicity under the conditions of this assay.
8th new micronucleus test in mice (
2012)

An in vivo bone marrow micronucleus assay was conducted on behalf of Syngenta by
. The GLP-compliant study (
2012;
ASB2014-9333; Report no. 1479200) was performed according to OECD Guideline and audited by an internal QAU. The study may be considered acceptable.
Male NMRI mice (7 per sampling time in the test and 5 in the negative control groups) were obtained from
The animals were
administered by oral gavage a single dose (dosing volume 20 mL/kg bw) of technical grade glyphosate (batch no. 56753, purity 96.3 %) at a dose level of 2000 mg/kg bw/day or of the vehicle (1 % CMC). A third (positive control) group of 5 mice received a single oral dose of 40 mg cyclophosphamide/kg bw in a dosing volume of 10 mL/kg bw. The animals from the test and negative control groups were sacrificed and slides from femur bone marrow prepared
24 hours or 48 hours following treatment. In the positive control group, sampling was performed only 24 hours after dosing. In each animal, 2000 polychromatic erythrocytes were
scored for the presence of micronuclei.
After glyphosate treatment, the animals did not exhibit any clinical signs. The ratio of polychromatic to total erythrocytes was nearly the same in all groups. Treatment with glyphosate did not induce an increase in micronucleus incidence in the polychromatic erythrocytes (0.114 % group mean after 24 h and 0.057 % after 48 h). In the vehicle control group, similar mean percentages of 0.160 % (24 h) and 0.070 % (48 h) were observed. In contrast, 24 h after administration of cyclophosphamide, a mean incidence of 2.010 % was noted proving the validity of the test.
Glyphosate proved negative for clastogenicity under the conditions of this assay.
Rat
1st new micronucleus test in rats (2009)
Reference: IIA, 5.4.4/02
Report: 2009 Micronucleus test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration
Data owner: HAG Report No.: LPT 23917 Date: 2009-05-18
Unpublished; ASB2012-11479
Guidelines: OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 04/02/2009 – 06/03/2009
Materials and methods
Test material:
Identification:
Glyphosate TC
Description:
Solid, white powder
Lot/Batch #:
20080801
Purity:
988.0 g/kg
Stability of test compound:

Stable for two years at ambient temperature
Vehicle and/
or positive control:
Vehicle: 0.8 % hydroxypropylmethylcellulose Positive Control: Cyclophosphamide
Test animals:
Species:
Rat
Strain:
CD
Source:
Age:
Males: 32 – 33 days
Females: 33 – 34 days
Sex:
Male and Female
Weight at dosing:
Males: 106 – 132 g
Females: 88 – 111 g
Diet/Food:
Commercial ssniff® R/M-H V1534, feeding was discontinued approx. 16 hours befor administration
Water:
Tap water, ad libitum
Housing:
Animals were kept in groups of 2 – 3 animals by sex in solid cages with wood flake bedding.
Environmental conditions:
Temperature: 19 - 25 °C
Humidity: 30 - 70 %
Air changes: approx. 15/hour 12 hours light/dark cycle
Animal assignment and treatment:
The test was conducted using young male and female CD rats. Groups of five male and five female rats were dosed via oral rout (positive reference item was administered via intraperitoneal route) at 500, 1000 and 2000 mg/kg bw. Sampling was performed after 24 hours for all groups and after 48 hours for the vehicle control and the highest dose group. Both femurs from each rat were dissected, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed in absolute methanol, stained in Mayers Haemaleum and eosin, allowed to air-dry and coverslipped using mounting medium. 2000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. The ratio of PCE to normochromatic Erythrocytes (NCE) was determined for each animal by counting a total of 1000 erythrocytes.
Results and discussion
Mortality: No mortality occurred.
Clinical observations: No signs of systemic toxicity were noted after administration of glyphosate TC up to the highest reasonable dose level of 2000 mg glyphosate TC/kg b.w. until 48 hours after administration (the last sampling time point).
Evaluation of bone marrow slides:
No test item-related increase of micronucleated polychromatic erythrocytes was observed in the

treated groups as compared to the corresponding vehicle control group (see Table B.6.4-21) at the two sampling times. The positive reference item group which received cyclophosphamide (27 mg/kg b.w., i.p.) exhibited a significant increase in the number of micronucleated polychromatic erythrocytes. Historical control data is shown in Table B.6.4-22.

Table B.6.4-21: Summary of results

Treatment group / sampling time	Number of PCE with micronuclei/2000 PCE (male animals)		Number of PCE with micronuclei/2000 PCE (female animals)	
	Group mean	SD	Group mean	SD
Vehicle control (20 mL/kg) / 48h	2.0	1.9	2.2	1.3
Vehicle control (20 mL/kg) / 24h	1.6	1.1	1.8	0.4
Positive control (27 mg/kg) / 24h	30.2	10.5	24.0	4.9
Glyphosate TC (500 mg/kg) / 24 h	1.0	1.2	1.2	1.3
Glyphosate TC (1000 mg/kg) / 24 h	0.8	0.4	1.6	0.9
Glyphosate TC (2000 mg/kg) / 24 h	1.2	0.8	0.8	0.8
Glyphosate TC (2000 mg/kg) / 48 h	1.6			

0.9
0.8
0.8
PCE = polychromatic erythrocytes SD = standard deviation
Table B.6.4-22: Historical control data
Sex
Group mean ratio PCE/- NCE#1
Group mean frequency of
micronucleated PCE (per 1000) #1
Animals (%) with 0, 1 or more micronucleated PCE (per 1000) #2
0
1
2
3
4
5
>6
Males
Mean
Range
0.87
0.26 – 2.94
1.97
0.4 – 5.7
11.3
34.7
30.0
10.7
6.7
4.0
2.7
Females
Mean
Range
0.76
0.32 – 1.47
1.86
0.4 – 4.7
14.0
30.0
21.3
18.7
7.3
5.3
3.3
#1 Average of group means from the most recent background data. Data from 24, 48 and 72 hour

samplings are combined.
#2 Individual animal profile based on the above experiments; data from 300 animals. m male
f female
PCE polychromatic erythrocytes NCE normochromatic erythrocytes
Conclusion by the Notifiers
The test material glyphosate technical was non-genotoxic.
RMS comments:
The study is considered acceptable and the conclusion is agreed with. Thus, absence of clastogenicity in vivo was also confirmed in the rat.
B.6.4.6 In vivo studies in germ cells
For the previous EU glyphosate evaluation, dominant lethal assays in rats and mice had been submitted in which genotoxic effects on germ cells had been examined in. The studies by (1992, TOX9551102) in rats and by (1980, TOX9552377) in mice may
be still considered acceptable. No genotoxcic effect of glyphosate on germinal tissues was found up to the highest dose levels. No new data became available since then.
Table B.6.4-23: In vivo germ cell genotoxicty testing of glyphosate acid in rats (provided by the Notifiers)
Reference; study identificaion; owner
Type of study
Test organism / test system
Dose levels; batch, purity
Results
Studies from the 2001 evaluation
1992; TOX9551102; (ADM)
DLT
Wistar rats, single oral dose, 10 successive
one-week mating periods (1:1 sex ratio)
0, 200, 1000, 5000 mg/kg
bw/day; batch 60, 96.8 %
Negative
, 1980;
TOX9552377;
Monsanto
DLT
CD-1 mice, single oral
dose; each treated male mated with a total of 16 females over a period of 8 weeks
0, 200, 800, 2000 mg/kg bw/day;
XHJ-64, 98.7 %
Negative
DLT = dominant lethal test
B.6.4.7 Genotoxicity of formulations (taking into account published data that were released before 2000)
In contrast to studies with the active ingredient that were, with one possible exception (1993, TOX9551100), negative, findings obtained with formulations were contradictory. This concern has been addressed by the RMS during previous EU evaluation yet (Addendum of 02 August 2000 to the DAR on glyphosate; ASB2013-2748). Because this information is considered relevant also for current

re-evaluation, the descriptions and evaluations of studies and published information on mutagenicity of formulations from this addendum have been transferred into this RAR. Afterwards (see B.6.4.8), scientific publications are addressed that have been published since 2000, i.e., after the addendum had been prepared.

Amended copy from the 2000 addendum (ASB2013-2748)

(current study identification by the RMS was amended and enumeration of tables was adjusted, two additional studies are reported)

I. Original studies

A total of eight mutagenicity studies using four different glyphosate formulations was made available to the Rapporteur by the companies Monsanto and Cheminova. For each of these formulations, an Ames test and a mouse bone marrow micronucleus test were submitted. The studies are reliable since they were performed at least to a large extent in compliance with current OECD guidelines (Guideline 471 for bacterial reverse mutation tests and Guideline 474 for mammalian erythrocyte micronucleus tests) under GLP-like conditions. They are all scientifically valid and may be used for risk assessment although the studies with the formulation Glifos are considered of limited value for this purpose only. Both test systems are widely accepted for mutagenicity testing of chemicals and respective data for glyphosate active ingredient are available allowing a direct comparison between the active substance and some of its formulations. Unfortunately, these data do not refer to those formulations for which acute toxicity studies have been submitted for purposes of EU re-evaluation of glyphosate.

The studies on RodeoR were submitted as part of the joint dossier of Monsanto and Cheminova since this formulation is considered representative for the glyphosate IPA salt without any further chemicals contained. The six other study reports were kindly provided by Monsanto on request for purposes of this addendum and were not part of the original EU submission.

Following a short characterization of the products investigated, test conditions and results are summarized in Table B.6.4-24 (in vitro testing) and Table B.6.4-25 (in vivo studies). The individual studies are briefly listed below.

Brief description of formulations tested:

RodeoR is a formulation containing 54 % glyphosate IPA salt and water but no surfactants. According to information obtained by Monsanto, it is especially intended for aquatic use. The studies have been performed and data submitted to facilitate the assessment of genotoxicity of the IPA salt since in most mutagenicity studies the test material was glyphosate acid.

The RoundupR formulation tested by Monsanto (MON 2139) is made of 31 % glyphosate (acid equivalents), (MON 0818, i.e., a surfactant), and water.

The third tested Monsanto product DirectD (MON 14445) contains 72% glyphosate acid equivalents formulated as ammonium salt with also a (Ethomeen T25, C20-C25 surfactant. According to the Rapporteurs database, it is the only glyphosate
ammonium salt tested for mutagenicity.

The product called Glifos in Brazil (in Europe Glyphos) is a formulation of glyphosate manufactured by Cheminova. As indicated by the test facility , it contains the IPA salt at a concentration of 360 g/L. According to the German national registration data files, the product is made of the IPA salt, the by-product Berol 907, and water.

Overview on mutagenicity studies:

Table B.6.4-24: Genotoxicity studies on herbicidal formulations containing glyphosate

- In vitro testing in bacteria (Ames test)

Study type

Test material
Test system
Dose range/ Test conditions
Result
Reference
Ames test
RodeoR (containing
IPA salt and water only)
S. typhimurium strains TA 98, 100, 1535, 1537
50 - 5000 µg/plate;
-/+ S9
Negative; no signs of cytotoxicity
Kier et al., 1992 TOX9552373
Ames test
MON 2139
(Roundup(containing IPA salt, a
S. typhimurium strains TA 98, 100, 1535, 1537
5 - 500 µg/plate (-
S9)/ 15 - 1500
µg/plate (+S9)
Negative; cytotoxic at the maximum dose levels, occasionally also at lower concentrations
Kier et al., 1992 TOX1999-239
surfactant and
water)
Ames test
MON 14445t
(Direct(, containing ammonium salt, a
S. typhimurium strains TA 98, 100, 1535, 1537
5 - 500 µg/plate (-
S9)/ 15 - 1500
µg/plate (+S9)
Negative; cytotoxic at the maximum dose levels, occasionally also at lower concentrations
Kier et al., 1992 TOX1999-320
surfactant and
water)
Ames test
Glifos formulation (IPA salt,
Berol 907 and water)
S. typhimurium strains TA 97a, 98, 100 and 1535
1, 10, 100, 1000,
5000 µg/plate;
-/+ S9
Negative; cytotoxic at the two upper concentrations
Vargas, 1996* TOX1999-884
* study of limited value for risk assessment only In all trials, the solvent was distilled water.
Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992, TOX9552373): Ames/Salmonella

mutagenicity assay of Rodeom. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91184, Sponsor Project no. ML-91-441. Dates of experimental work: 26 November 1991 - 30 December 1991. GLP: yes (self-certification of the laboratory). A respective statement of the Quality Assurance Unit (QAU) is included. The study is considered acceptable.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992, TOX1999-239): Ames/Salmonella mutagenicity assay of MON 2139 (ROUNDUPm herbicide formulation).

Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91183, Project no. ML-91-440, Report no. MSL-11729. Dates of experimental work: 26 November 1991 - 06 January 1992. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Kier, L.D.; Stegeman, S.D.; Costello, J.G. and Schermes, S. (1992, TOX1999-320): Ames/Salmonella mutagenicity assay of MON 14445 (DIRECTm herbicide formulation). Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; EHL study no. 91185, Project no. ML-91-442, Report no. MSL-11731. Dates of experimental work: 26 November 1991 - 30 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.

Vargas, A.A.T. (1996, TOX1999-884): The Salmonella typhimurium reverse mutation by GLIFOS. BioAgri (Biotecnologia Agricola Ltda.), Piracicaba, Sao Paulo, Brazil on behalf of Cheminova; BioAgri Report G.1.1 - 050/96. Dates of experimental work: 12 October 1996 -
23 December 1996. GLP: No. However, a QAU statement is included. The study is considered of limited value for risk assessment only since a legal statement on GLP compliance is lacking and since there were some minor reporting deficiencies in particular regarding the negative (absolute and solvent) and positive control values.

Table B.6.4-25: Genotoxicity studies on herbicidal formulations containing glyphosate

- In vivo experiments (micronucleus test)

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Micro- nucleus test	RodeoR for- mulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single i.p. administration	0-850-1700-3400 mg/kg bw; sampling after 24, 48 and 72 h	Negative for chromosome aberrations; overt toxicity (clinical signs, bwN, death) at the upper dosages	1992 TOX9552376
Micro- nucleus test	RoundupR formulation in 0.9% saline	CD-1 mice (m/f), bone marrow, single			

i.p. administration
0-140-280-555
mg/kg bw; sampling after 24,
48 and 72 h
Negative (no chromosome aberrations); toxic to mice at 555 mg/kg bw with some deaths occurring, cytotoxic to the bone marrow (PCE/NCE ratio/ at 48-h sampling) at this
top dose level
1992
TOX1999-242
Micro- nucleus test
DirectD formulation in 0.9% saline
CD-1 mice (m/f), bone marrow, single
i.p. administration
0-91-183-365
mg/kg bw; sampling after 24,
48 and 72 h
Negative for chromosome aberrations; signs of general toxicity at the top and, although less pronounced, mid dose
level
1992
TOX1999-322
Micro- nucleus test
Glifos formulation in distilled water
Swiss albino mice (m/f), two i.p. injections with 24-h inverval
0-68-137-206
mg/kg bw; sampling at 24 h after the second dose
Negative. No indications of cytotoxic effects to the bone marrow. No information regarding general toxicity in the
main study.
, 1996* TOX1999-253
m/f male and female mice used
* study of limited value for risk assessment only
(1992, TOX9552376): Mouse micronucleus study of RODEO(herbicide formulation. Monsanto
on behalf of Monsanto; EHL study nos. 91201 (toxicity range-finding study,
not tabulated above) and 91205 (micronucleus test), Sponsor study nos. ML-91-435/ML-91-
438. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.
study of ROUNDUPs herbicide formulation.
(1992, TOX1999-242): Mouse micronucleus
on behalf of Monsanto; EHL study nos. 91200 (toxicity range-finding study, not tabulated above) and 91204 (micronucleus test), Sponsor study nos. ML-91-434/ML-91-
437. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.
study of DIRECTs herbicide formulation.

(1992, TOX1999-322): Mouse micronucleus
on behalf of Monsanto; EHL study nos. 91202 (two toxicity range-finding experiments, not tabulated above) and 91206 (micronucleus test), Sponsor study nos. ML-91- 436/ML-91-439. Dates of experimental work: 13 November 1991 - 26 December 1991. GLP: yes (self-certification of the laboratory). A respective QAU statement is included. The study is considered acceptable.
(1996, TOX1999-253): A micronucleus study in mice for the product GLIFOS. BioAgri (Biotecnologia Agricola Ltda.), Piracicaba, Sao Paulo, Brazil on behalf of Cheminova; BioAgri Report G.1.2 - 060/96. Dates of experimental work: 08 October 1996 - 19 November 1996. Dose levels were chosen on the basis of a preliminary toxicity test (LD50 determination) described in the study report. GLP: No. However, a QAU statement is included. The study is considered of limited value for risk assessment only since a legal statement on GLP compliance is lacking and since there was no information regarding general health effects of treatment to the animals. Therefore, it is not clear whether the highest possible dose was actually reached.
Assessment:
Four glyphosate formulations were tested for mutagenicity in the reverse mutation assay in bacteria as well as in vivo by means of the mouse bone marrow micronucleus test. Unequivocally, all these products proved negative in both test systems. Thus, it can be concluded that the formulations Rodeoe, Roundup, (MON 2139), Direct and Glifos containing either the IPA or the ammonium salt of glyphosate, alone or in combination with different surfactants, do not cause point (gene) mutations in various Salmonella typhimurium strains and are devoid of a clastogenic potential in vivo.
However, when the studies of the same type (Ames test and Micronucleus test) for the active substance and the formulations are compared, it becomes obvious that the highest concentrations or dosages to be tested were generally lower with the formulations except Rodeox. This is apparently due to a higher degree of cytotoxicity as well as of general mammalian toxicity related to the formulations containing other ingredients than glyphosate salt and water.
To facilitate direct comparison of the Ames tests, the respective table from the monograph is reproduced here once more. (Remark, 2013: Deleted by the RMS since a few of these studies that were used for previous EU evaluation are now considered not acceptable. The interested reader is kindly referred to Table B.6.4-1)
It is clearly to be seen that much higher concentrations of the active substance could be tested without causing significant cytotoxicity. According to the literature (Chan and Mahler, 1992, TOX9551954), even concentrations up to 10,000 µg/plate have been reached. With the formulations described above, only Rodeor which is made of glyphosate IPA salt and water could be succesfully tested at such high concentrations. In contrast, strong cytotoxicity avoided meaningful evaluation of mutagenicity of the three other formulations at least at the highest of the selected concentrations and was still to be seen at much lower dose levels. Therefore, it can be assumed that cytotoxicity is due to the surfactants contained but not to glyphosate or its salts. The effects appeared more pronounced with Roundup and Direct than with Glifos suggesting a particularly high cytotoxic activity of tallowamine surfactants.
Regarding the micronucleus tests, a similar pattern becomes apparent. A number of micronucleus studies with glyphosate a.i. in mice is available. However, all these experiments were performed using the oral route. General and cytotoxicity (i.e. bone marrow effects) were confined to very high doses of 4000 or 5000 mg/kg bw corresponding to the known low acute oral toxicity of this compound. The only miconucleus test using the i.p. route (as with the formulations) was performed in rats. The highest dose of 1000 mg glyphosate a.i./kg bw did not cause clastogenicity (1983; TOX9552369, also published by Li and Long, 1988, TOX9500253). As shown by (1992, TOX9552373),

the IPA salt when dissolved in water (Rodeob formulation) can be given intraperitoneally to mice at a similar dose level (850 mg/kg bw) without causing neither toxicity nor clastogenic effects. Toxicity was confined to higher dosages (1700 and 3400 mg/kg bw) but genotoxicity was not observed. In contrast, toxicity of the other formulations containing surfactants was much higher although, again, no evidence of a clastogenic effect was obtained 1992, TOX1999-242 and TOX1999-322; 1996, TOX1999-253).

II. Published literature (before 2000)

During the past few years, a number of studies was published dealing with possible mutagenic effects of glyphosate formulations in different test systems. Scientific assessment of these data is very difficult for at least two reasons.

One main deficiency is the lack of precise description of the test material. Usually, source, composition and/or purity neither of the formulations nor, if tested, of the active ingredient are not stated at all or, at least, not sufficiently reported in the publications. It should be also taken into consideration that different formulations may be marketed in different countries under the same trademark, e.g. Roundups. Further confusion comes from the fact that sometimes by-products in formulations (e.g. surfactants) were replaced by others but the name of the product was not changed. On request, data on the ingredients were submitted by the manufacturer Monsanto but even this information was not sufficient to clarify all uncertainties about the test substances. However, on the basis of the information available so far, it can be be stated that the Roundup products used in the different published studies were not identical. Thus, it is questionable whether results obtained with one product will apply to others containing different non-active ingredients in different concentrations.

A second point of concern is the frequent use of less validated test systems with no proven relevance of the findings for human health risk assessment even if such systems may be well accepted to predict special environmental hazards. With regard to health effects, there are no current guidelines for these test methods and there is no actual experience how to assess positive findings in such test systems. For other test methods used, OECD guidelines do exist but the experiments were not carried out in compliance with these recommendations.

To facilitate presentation of data, it was decided to start with those experiments for which, in principle, widely agreed guidelines are available. Because of the large background database, the SCE assays were also included here. In the subsequent part of this section, investigations in test systems less frequently used for examination of plant protection products and with no guidelines existing are reported. As a result of this approach, one and the same publication may be referred to repeatedly on different sites.

It should be mentioned that in some publications also experiments are reported which were carried out with glyphosate active substance (i.e., the acid or one of its salts) being the test material. These data were not included in the monograph since the respective publications, for different reasons, were considered unacceptable for evaluation purposes (for justification, see description of experimental conditions below) in particular when the current OECD criteria for assessment of published data were applied. However, the findings are reported in this addendum since a direct comparison between active ingredient and formulation data may be of particular interest.

Although various test systems measuring different endpoints were used, it was tried to summarize the available studies in Table B.6.4-26 (see next pages) to facilitate general overview before the individual publications were discussed in greater detail below. For practical reasons, in particular to facilitate direct comparison, the studies were divided into sections according to the test systems and methods and the experiments separately tabulated.

Table B.6.4-26: Overview on published studies on mutagenicity of glyphosate, its salts and formulations

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
Ames test in S.typhimurium strains TA98 and TA100 (+/- S9 mix)	Roundup (48% glyphosate IPA; polyoxyethylene surfactant)	0 - 1440 µg/plate (calculated as glyphosate IPA salt)	Equivocal. Occasional increase in mutation rate but no clear dose response. Marked cytotoxicity from 360 or 720 µg/plate onwards.	Study not acceptable for evaluation purposes due to serious deficiencies. Reliable assessment avoided by cytotoxicity.	Rank et al., 1993 Z82234
Micronucleus test in mouse bone marrow; single i.p. administration; sampling after 24 or 48 h	Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA; surfactant)	0, 100, 150, 200 mg/kg bw (glyphosate IPA); 0, 133, 200 mg/kg bw (Roundup, calculated as IPA salt)	Negative. Indication of dose- related bone marrow cytotoxicity with the Roundup formulation but not with glyphosate IPA.	Supplementary study confirming previous results.	Rank et al., 1993 Z82234
Micronucleus test in mouse bone marrow; two i.p. administrations with a 24-h interval between; sampling after 6 and 24 h after the final dose	Glyphosate a.i. (99.9% pure) and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 mg/kg bw (2X150 mg/kg bw/d) for glyphosate; 0, 450 mg/kg bw (2x225 mg/kg bw/d) for Roundup	Weakly positive for glyposate after 24 h and for Roundup at both sampling times. Some evidence of bone marrow cytotoxicity of Roundup.	Supplementary study (methodical deficiencies) revealing an increase in micronucleus frequency, data in contrast to previous results.	Bolognesi et al., 1997 Z59299
SCE assay in human lymphocytes	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0 - 6 mg/ml for glyphosate; 0, 0.1, 0.33 mg/ml for Roundup	Positive for glyphosate from 1 mg/ml onwards and for Roundup at both concentrations. With		

Roundup, complete cytotoxicity at concentrations >0.33 mg/ml.
Insufficient data. In addition, a positive result in this assay is of equivocal biological significance against the background of more
appropriate mutagenicity studies.
Bolognesi et al., 1997 Z59299
SCE assay in human lymphocytes
Roundup (not specified)
0, 250, 2500, 25000 µg/ml
Weakly positive at the low and mid dose level (for one of two donors). Cytotoxic at the high dose.
see comment above
Vigfusson and Vyse, 1980 TOX9700576 /
ASB2012-12044
Alkaline elution assay for DNA single-strand breaks and formation of alkali labile sites in DNA obtained from liver and kidneys of mice following single i.p.administration
Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)
0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw; sampling after 4 and 24 h
Weakly positive after 4 h in both organs suggesting possible transient DNA damage.
Supplementary study (methodical deficiencies). Biological significance equivocal. Results in contrast to the negative outcome of the
UDS assay. Effects might be also due to toxicity.
Bolognesi et al., 1997 Z59299
Test method/ test system
Test material
Dose levels/ Dose range
Results
Remarks
Reference
Investigations for oxida-tive damage in liver and kidney of
i.p. treated mice by measuring the number of 8-OHdG (hydroxydesoxyguanosine)
adducts
Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)
0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw (single
i.p. administra-tion); sampling after 4 and 24 h
Evidence of stimulation of oxidative metabolism in the liver (only glyphosate) or kidney (only Roundup) after 24 h.
Finding not indicative of mutagenicity but could indicate one possible mechanism of toxicity.
Bolognesi et al., 1997 Z59299
Measuring of DNA adducts by means of 32P-postla-beling technique in the liver and kidney of mice following single i.p. administration
Glyphosate IPA salt and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)
0, 130, 270 mg/kg bw
(glyphosate IPA); 0, 400, 500, 600 mg/kg bw (Roundup)
Weak dose-related increase in adducts with Roundup; no adducts seen with the IPA salt alone and in

the control group.
Indication of possible DNA damage, however, biolo-gical significance of this finding equivocal. Further characterization of ad-ducts needed. Toxicity not adressed. However, non-mutagenic toxic effects can also cause DNA adducts.
Peluso et al., 1998 TOX1999-318
Table B.6.4-25: Overview on published studies on mutagenicity of glyphosate, its salts and formulations (continued)
Test method/ test system
Test material
Dose levels/ Dose range
Results
Remarks
Reference
Comet assay for single-strand DNA breaks in tadpole erythrocytes
Roundup (41% glyphosate IPA; surfactant)
0-1.69-6.75-27-108 mg/l water
Dose-related effect on DNA at
6.75 and 27 mg/l; completely lethal at 108 mg/l.
Impact of this formulation on tadpole DNA under environmental conditions indicated. Effect could be also due to toxicity. No relevance for human health risk
evaluation.
Clements et al., 1997 Z101728
Test for lethal mutations in Drosophila melanogaster after treatment of larvae
Roundup (assumed to contain 41% glyphosate IPA and
Not specified but indicated to be around LC50 concentration.
Positive.
Not predictive for mutagenicity in mammals. Concentrations used were expected to exhibit high toxicity making evaluation of
results very difficult.
Kale et al., 1995 Z73986
surfactant);
Pondmaster (probably made
from 41% glyphosate IPA; alkyle sulfate surfactant)
Anaphase-telophase allium test for chromosome aberrations in onion root cells
Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA;
surfactant)
0-720-1440-2880 µg/l (for Roundup calculated as IPA salt)
Roundup: increase in chro- mosome aberrations at the two upper levels indicating rather polyploidy than clastogenicity, no clear dose response Glyphosate IPA: negative
Effects in plant cells not predictive for mutagenicity in mammals.
Testing a herbicide for genotoxic effects in plants generally doubtful since
cytotoxicity may be expected.
Rank et al., 1993 Z82234
Chromosome aberration (CA) and Sister chromatid exchange
(SCE) in human lymphocytes
Glyphosate (purity ≥ 98 %)

0-5.0-8.5-17.0-51.0 µM
Increase in CA and SCE frequency
Increase of SCE not dose related in highest dose group
Lioi et al, 1998a ASB2013-9836
CA and SCE in bovine
lymphocytes
Glyphosate (purity ≥ 98 %)
0-17-85-170 µM
Increase in CA and SCE
frequency
Increase of SCE not dose
related in highest dose group
Lioi et al, 1998b
ASB2013-9837
a.e. acid equivalents
Studies in test systems for which guidelines exist and/or much experience is available (i.e. Ames test, micronucleus test, SCE assay)
(1993, Z82234) studied the mutagenic potential of the herbicide Roundup and of glyphosate isopropylamine salt in different test systems in vitro as well as in vivo. An Ames test (plate incorporation test) was performed with Roundup only in the Salmonella typhimurium strains TA 98 and TA 100 with and without S-9 mix for metabolic activation. Evidence of mutagenicity was confined to the strain TA 98 under non-activation conditions as indicated by a slight but significant increase in the mean number of revertants at a concentration level of 360 µg/plate (calculated as IPA salt) which was also confirmed in the repeat experiment. With activation, however, no increase in mutation rate was seen up to this dose level. From the next higher concentration (720 µg/plate) onwards, cytotoxicity became apparent with and without metabolic activation avoiding meaningful evaluation. The study authors also reported a positive result for TA 100 in the presence of S9 mix at a concentation of 720 µg/plate but already the next lower dose of 360 µg had markedly reduced the number of revertants as compared to the control suggesting a cytotoxic effect. Furthermore, a dose response was lacking. Thus, the marked increase in mutation frequency at 720 µg/plate is not reliable. In the second experiment, this dose level was not included. Without activation, concentrations from 720 µg/plate onwards appeared cytotoxic. At lower concentrations, no increase in mutation rate was noted with strain TA 100.
In a micronucleus test in mouse bone marrow erythrocytes following single i.p. administration, Roundup as well as the IPA salt (i.e., a 1:1 mixture of glyphosate technical and isoproplyamine) proved negative up to the highest dose of 200 mg/kg bw. However, with Roundup but not with the glyphosate IPA salt alone, there was evidence of bone marrow cytotoxicity at this top dose level as indicated by a significantly lower percentage of polychromatic erythrocytes.
Comment: According to the publication and to further information submitted by Monsanto, it is assumed that the Roundup formulation used was made of 48 % IPA salt,
surfactant, and water. The study design of the Ames test does not comply with current guideline requirements, e.g. the plate number scored was inconsistent throughout the study. The data obtained are so controversial that a reliable interpretation is not possible. Unfortunately, a complete confirmatory experiment was not performed since repeated testing was confined to the dose of 360 µg/plate and an additional concentration of 180 µg/plate was included. A more extensive study by (1992, TOX1999-242, see above in section I) using four S. typhimurium strains including also TA 98

and TA 100 failed to elicit any indications of mutagenicity. This latter trial was conducted in compliance with OECD guideline 471 requirements and is of higher reliability, therefore. Of course, the Roundup formulations tested by Rank and her group and by were not identical but similar since both contained only the active substance formulated as IPA salt,
surfactant, and water. The cytotoxicity of Roundup was described by both groups but the respective concentrations were different.
The design of the micronucleus test was also not in compliance with guideline requirements. A direct comparison between results obtained with the IPA salt and Roundup is not feasible since not exactly the same dose levels were used and since there was a difference in sampling time (24 and 48 h post dosing for the IPA experiment versus only at 24 h after administration of Roundup). The negative outcome of previous micronucleus studies with the IPA salt (Rodeoo formulation, 1992, TOX9552376) and with a similar Roundup formulation in mice (1992, TOX1999-242) was confirmed. The reported weak
bone marrow cytotoxicity occurring already after single i.p. administration of 200 mg Roundup/kg bw (amount calculated as the IPA salt to facilitate comparison) may be considered a possible formulation-related effect when the observations in other micronucleus studies (see section I) are taken into consideration.
In contrast, (1997, Z59299) reported positive results from a micronucleus test in mouse bone marrow erythrocytes. Either glyphosate a.i. (declared as 99.9 % pure) or a Roundup formulation were administered to Swiss mice once daily by the i.p. route on two consecutive days. Cell samples were harvested at 6 and 24 hours following the final dose. A weak positive effect was observed at total dose levels of 300 mg/kg bw (2 x 150 mg/kg bw/day) after 24 hours for glyphosate and of 450 mg/kg bw (2 x 225 mg/kg bw/day) at both sampling times for Roundup.
Further data in this publication indicated for high purity glyphosate a significant and dose- dependent increase in SCE frequency in human lymphocyte cultures obtained from two female donors from a concentration of 1000 µg/mL onwards. For Roundup, this effect became apparent even at lower concentrations of 100 and 330 µg/mL.
Comment: The outcome of the micronucleus test with glyphosate a.i. is at least surprising since much higher doses of this compound had been tested before and did not reveal indications of clastogenicity (see section B.5.4.2.1 in the monograph). A direct comparison is not possible since the only available test using the i.p. route in which the highest dose of 1000 mg/kg bw proved negative (1983, TOX9552369) was performed in rats. The respective study by (1993, Z82234, see above) was conducted in mice but the test material was glyphosate IPA salt and the dose administered was probably too low for meaningful evaluation. However, a number of well-performed micronucleus tests with oral administration to mice is available. Even when the low oral absorption rate of glyphosate (about 30%) is taken into account, the dose levels (up to 5000 mg/kg bw nominal) are much higher than those given by Bolognesi and her co-workers but no convincing evidence of a potential to cause chromosome aberrations in vivo was obtained. It should be emphasized that the increase in the incidence of micronucleated polychromatic erythrocytes as reported in this publication was rather weak only. The test was not performed according to the current OECD guideline. In particular, the number of animals used (three male mice per dose group) was too low since a group size of at least five is recommended. A dose response cannot be assessed since only one dose level was included. The basis for statistical comparison is questionable since it is not clear when the six control animals were sacrificed because only one group mean value was indicated. Due to these deficiencies, this isolated positive finding is not considered to provide sufficient evidence to contravene the previously obtained negative results regarding the active substance.

The same methodical shortcomings apply to the experiment with the Roundup fomulation. The formulation tested is reported to contain 30.4 % glyphosate acid equivalents. The a.i. is formulated as the IPA salt. Alkyl sulfate surfactant (MON 8080) is also contained (source of information: Monsanto). The weak positive response is in contrast to the beforementioned GLP-like study by (1992, TOX1999-242) in which Roundupb proved negative. However, these two Roundup formulations were not identical since the glyphosate concentrations were nearly the same but the surfactants contained were different making a direct comparison of the study results difficult. Little is known on mutagenicity of alkyle sulfate itself, however, MON 8080 proved negative in the Ames test but was clearly cytotoxic at relatively low concentrations (see section III of this addendum). Some evidence of bone marrow cytotoxicity was obtained with both Roundup products as indicated by a decrease in the ratio between polychromatic and normochromatic erythrocytes. Cytotoxicity could have
also an impact on chromosome aberration frequency. An overall, unequivocal conclusion from the experiment of Bolognesi and her group cannot be drawn, however an actual clastogenic response is not very likely. Even if a positive result could be confirmed, it would not be applicable to products containing other surfactants.
A higher SCE frequency is not considered to provide evidence of mutagenicity against the large number of studies in which glyphosate proved clearly negative. The two other studies of this type which have been submitted for purposes of toxicological evaluation of glyphosate (1990, TOX9500269 and 1993, TOX9500381, the latter using the IPA salt) did not reveal an increase in sister chromatid exchange frequency but, unfortunately, did not include the high concentrations as tested by the group of Bolognesi (see section B.5.4.1.3 of the monograph). Apart from general doubts about biological significance of a positive result in an SCE assay, some methodical deficiencies became obviously in this publication. For statistical reasons, the number of only two subjects to be included in the study appears too low for meaningful evaluation. Furthermore, the data from two experiments were pooled for the two donors and individual values were not given. Therefore, a possible influence of interindividual variation could not be sufficiently assessed by the reviewer. As shown below, this variation may well reach a considerable level. Again, the positive result obtained with Roundup at least might be also due to cytotoxicity of the formulation avoiding further testing at dose levels exceeding 330 µg/ml since no mitotic cells were present any more.
(1980, TOX9700576) also reported a weak but statistically significant increase in SCE frequency in human lymphocytes obtained from two donors when the cultures were exposed to Roundup (not specified) at concentrations of 250 and 2500 µg/mL. At the next higher concentration of 25000 µg/mL, the test substance was absolutely cytotoxic.
Comment: The reported increase is doubtful since a dose response was seen in the cultures from one of the two donors only. Furthermore, this increase in SCE frequency over the control was weak only and the statistically increased values in the cultures provided from donor 1 were below the control value from donor 2. Furthermore, possible cytotoxicity was not adressed in this paper. Generally, the SCE assay is not accepted to provide convincing evidence of mutagenicity but is rather a screening test. For clarification, the study authors themselves recommended further mutagenicity tests to be conducted.
. (1998a, ASB2013-9836 and 1998b, ASB2013-9837) reported an increase in CA and SCE frequency in human lymphocytes of 3 donors in concetrations between 5 an 51 µM and in bovine lymphocytes between 17 and 170 µM.
Comment: The results are questionable because a number of well performed and validated studies in vitro im mammalian cells and in vivo in mammals did not register comparable effects even in dose

levels more than 10 times higher than the doses used in the studies described by (1998a and 1998b, ASB2013-9836 and 1998b, ASB2013-9837). A replication would be needed to confirm such aberrant results.

Other test systems (Comet assay in tadpole erythrocytes, tests for DNA adducts in rats and mice, Drosophila melanogaster, plant cells)

Clements et al. (1997, Z101728) investigated the genotoxicity of selected herbicides in Rana catesbeiana (bullfrog) tadpoles using the single-cell gel DNA electrophoresis test (`Comet´ assay). After a previous study had shown a higher amount of DNA damage in bullfrog tadpoles inhabiting small bodies of water in agricultural areas as compared to non-agricultural regions, the impact of Roundup and some other commonly used herbicides on the DNA of tadpole erythrocytes was investigated in this test system under alkaline conditions. This modification allows the detection of single-stranded DNA breaks which are indicated by an increase in length:width ratio of the DNA mass following electrophoresis. DNA was obtained from tadpole erythrocytes (nucleated cells in amphibians) after the animals had been exposed to different concentrations of Roundup in the surrounding water for 24 hours. Whereas the low dose of 1.69 mg/L did not cause evidence of DNA damage, a clear and dose-dependent effect became apparent at the following concentrations of 6.75 mg/L and 27 mg/L. At 27 mg/L, the effect level caused by the positive control substance methylmethanesulphonate (MMS) was already approached. The intended top dose level of 108 mg/L could not be evaluated since all tadpoles died during the exposure period. According to the study authors, the concentrations tested were well below the recommended application levels suggesting an environmental mutagenic hazard in particular for organisms living in small adjacent bodies of water that are usually the first to be affected by pesticide runoff.

Comment: Generally, information on genotoxic effects of pesticides under natural conditions is scarce and, thus, this test system may provide important information regarding environmental effects.

In this special case, however, it appears equivocal whether the observed impact on the DNA was indicative of a true mutagenic effect or rather caused by cytotoxicity. It is known that a positive response in the Comet assay may be not only the result of direct interaction with cellular DNA but can be also mediated by toxic and other effects causing apoptosis or necrosis. Cytotoxicity is not addressed in the publication because it is not directly measured in this test system. A certain degree of general toxicity can be assumed since the highest dose was completely lethal to the tadpoles. This effect could be well in line with the toxicity of certain glyphosate formulations to aquatic organisms as reported in the monograph. The Roundup product tested by Clements et al. was made of 41 % glyphosate IPA salt and MON 0818, i.e. the surfactant which is already known to cause toxic effects in different test systems in vitro as well as in vivo. Of course, although there is some evidence of a cytotoxic mechanism behind the positive result in the Comet assay, a direct impact of the test compound on the DNA cannot be completely excluded.

At this time, it is not clear whether a positive result of this test obtained in tadpole erythrocytes, even if it was actually due to mutagenicity, would be of any relevance to human beings exposed. In particular, this is doubtful when the strong body of evidence that neither glyphosate nor its formulations are mutagenic as coming from many studies in various test systems is taken into consideration. Thus, the outcome of the Comet assay should be rather used for environmental hazard evaluation only. Again, the application of results obtained with one formulation to others must be critically regarded.

A possible impact on the DNA was also investigated by (1997, Z59299) in further experiments. A transient but significant effect towards DNA damage in liver and kidney was noted in the alkaline

elution assay after glyphosate (300 mg/kg bw) or Roundup (900 mg/kg bw) had been administered once by the i.p. route to mice. This assay may indicate the induction of DNA single-strand breaks and alkali labile sites. A test for DNA oxidative damage suggested glyphosate and the formulation Roundup to stimulate oxidative metabolism in the liver (glyphosate) or in the kidney (Roundup) at 24 hours after application.
In a subsequent study from the same institute (1998, TOX1999-318), a low incidence of DNA adducts was found by means of the very sensitive 32P-postlabeling technique in the liver and kidney of mice following single intraperitoneal administration of Roundup. All tested concentrations (400, 500 and 600 mg Roundup/kg bw, corresponding to
122, 152, and 182 mg glyphosate salt/kg bw) caused DNA adducts in both organs. A dose response was to be seen. In contrast, treatment with the vehicle (i.e., a DMSO/olive oil mixture) and with doses of 130 and 270 mg glyphosate IPA salt/kg bw did not result in DNA adduct formation.
Comment: The data from the tests for DNA damage and stimulation of oxidative metabolism (., 1997, Z59299) are hardly to interpret since the results are given in summary figures only which are based on pooled individual data. There are reporting inconsistencies, e.g. it is not clear how many animals were actually used for testing. A positive control substance was not included. Taking into account that glyphosate proved negative in the UDS assay which is generally accepted to indicate a more frequent occurrence of DNA damage and repair (see section B.5.4.1.3 in the monograph), the published findings are not considered to provide convincing evidence of an interaction with the DNA. Positive results in the alkaline elution assay may also occur as a result of toxic but not-mutagenic effects. Stimulation of oxidative metabolism is not a sign of mutagenicity but may elucidate a possible mechanism behind toxic effects.
The results of (1998, TOX1999-318) and his group suggest a direct effect on the DNA. It has been shown that the observed effects were related to administration of the formulation only but not to glyphosate IPA salt. Biological significance of the results is equivocal. Generally, it is questionable whether findings after i.p. administration can be applied to more realistic exposure conditions. Of course, the occurrence of such effects also after oral intake would be much more relevant for human health evaluation. Furthermore, some deficiencies of this study make a definitive assessment difficult. It is rather equivocal what a low incidence of DNA adducts per animal as compared to no adducts in the control group actually means since a positive control substance was not included. The degree of variation between the animals is not known because only mean values for the groups comprizing of 3 to 6 mice were reported and individual values are not given but would be helpful for interpretation of the results. Another point of concern is the lacking information on toxicity. At least with Roundup, one could expect marked general toxicity when the observations reported from the micronucleus tests (see section I of this addendum) and from the acute intraperitonal toxicity studies (see section B.5.2.4 in the monograph) were taken into account. It is known that DNA adducts may be formed not only as a result of direct interaction of cellular DNA with chemicals but also occur naturally or can be even related to a treatment-dependent increase in endogenous metabolites. Thus, further characterisation of these adducts and clarification of their nature would be desirable.
(1995, Z73986) examined nine agricultural chemicals in the sex-linked recessive lethal test in Drosphila melanogaster for their ability to cause genotoxic damage to the germ cells leading to lethal mutations in the subsequent generations. The group of test compounds included two insecticides and seven herbicides among those were the glyphosate formulations Roundup and Pondmaster. Unlike the generally used method of feeding the test substance to adult males only, larvae were treated in this experiment. This modification was expected by the study scientists to improve the sensitivity of the test system. All products tested proved positive.

Comment: This test system is not considered predictive for mutagenicity in mammals. Generally, tests in Drosophila are considered helpful for screening purposes. For glyphosate, however, a large database on the basis of much more reliable test systems does exist. Furthermore, since lethal changes in spermatogonia and spermatocytes were the relevant endpoint, it appears difficult to distinguish between mutagenicity and general toxicity. The
dose level tested was not specified but it is stated in the publication that concentrations around the LC50 were used. At such a high dosage, some toxicity must be expected.
An anaphase-telophase allium test in onion root cells was conducted by (1993, Z82234) to detect a possible induction of chromosome aberrations. The exposure period was
24 hours. In this plant system, a significant increase in the occurrence of chromosome aberrations was noted at the two upper dose levels when Roundup was tested. However, there was no dose response, since the total incidence of aberrations at 1440 µg/L was twice that seen at 2880 µg/L. The authors attributed this lack of a clear dose response to cytotoxicity, however, mitotic index was not dramatically reduced (24.2 in the mean at 2880 µg/L versus
28.2 at 1440 µg/L). According to the investigators, the type and pattern of aberrations suggest rather spindle disturbances than clastogenicity in particular when compared to the effects caused by the positive control substance MMS. In contrast, the glyphosate IPA salt did not increase the frequency of chromosome aberrations in this experiment.
Comment: The Roundup product tested was made of the IPA surfactant, and water (for details see description of the Ames test and the micronucleus test portions of this study above). The more pronounced effect of the formulation as compared to the IPA salt could be explained by an improved uptake by the onion root cells as mediated by the surfactant. However, genotoxic or aneugenic effects in a plant system are generally not accepted to be indicative of mutagenicity in mammals. For glyphosate and its formulations, a number of well-performed studies in mammals is available for this purpose. Generally, it appears questionable whether a herbicide should be tested for mutagenicity in a plant cell system since at least a certain degree of cytotoxicity must be expected.
Assessment
In the whole, the published data are not sufficient to provide convincing evidence of mutagenic effects caused by glyphosate or its formulations. Of cours, the effects observed in different test systems cannot be totally ignored. Looking for an explanation, the data obtained in the mutagenicity studies with formulations (see section I) must be also considered. Taking all the findings together, the effects reported in the literature appear rather due to cytotoxic properties of the formulations than to a genotoxic mode of action. The same conclusion was also reached by the Danish EPA in an assessment (Rasmussen, 1997, ASB2013-9671) which was made available to the Rapporteur. It has been already known before, that cytotoxicity is much more pronounced with glyphosate formulations than with the active substance and, therefore, is probably due to by-products or impurities. In particular, surfactants are the agents to be suspected for causing such effects.
There are even data suggesting the possiblity of a direct interaction of glyphosate formulations with cellular DNA in some test systems. This is evidenced by a higher frequency of DNA adducts in mouse liver and kidneys following i.p. administration (Peluso et al., 1998, TOX1999-318) as well as from the Comet assay in tadpole erythrocytes (
1997, Z101728). Since glyphosate active ingredient is apparently devoid of a DNA damaging potential (see monograph), these effects, if occurring, can be certainly assumed to be related to co-formulants. Damage to the DNA is not essentially indicative of mutagenicity but could also result from cytotoxicity. Irrespective of the origin of these effects on DNA level, they appear to be confined to very special exposure situations only and not to represent a health hazard to human beings.

III. Mutagenicity testing of surfactants

On the basis of the extensive mutagenicity database for glyphosate a.i. and formulations, the conclusion can be drawn that neither the active substance nor the formulations so far investigated are genotoxic. A certain potential of some formulations to cause damage to the DNA, however, cannot be excluded and might be related to the marked cytotoxic acitivity of these products. Data suggest that cytotoxicity is rather due to certain by-products used as surfactants than to the active ingredient. Thus, it is of particular interest to look at mutagenicity tests performed with such chemicals which are contained in glyphosate formulations mostly with the intention to improve the uptake of the herbicide glyphosate by the target plants. For three different surfactants, studies on mutagenicity of surfactants have been submitted. The studies are summarised in Table B.6.4-27 and briefly listed below.

Table B.6.4-27: Mutagenicity studies with surfactants contained in glyphosate formulations

Study type	Test material	Test system	Dose range/ Test conditions	Result	Reference
Ames test	MON 8080	dissolved in distilled water	S. typhimurium strains TA 98, 100, 1535, and 1537; plate incor- poration and spot test# performed	0.003 - 3.0 µL/plate (+/-S9) in the plate incorporation test	Negative up to 0.9 µl/ plate with and without activation; cytotoxicity occurring at this dose, complete toxicity at 3 µL/plate avoiding counting of revertants	Flowers, 1981 TOX1999-319
Ames test	MON 0818	dissolved in DMSO	S. typhimurium strains TA 98, 100, 1535, 1537; plate incorpora-tion test with/ without metabolic activation	Lowest concentra-tions: 0.3 or 1 µg/plate, diffe-rent maximum amounts per plate reached for the strains, i.e. TA98:300µg (-S9) 1000µg (+S9); TA100 and TA1535: 100µg (+/-S9); TA1537:100µg(-S9) 300µg (+S9)	Negative. Cytotoxic effects occurring at the maximum dose levels avoiding evaluation and occasionally also at lower concentrations. (Mutagenicity data for TA 1535 (+S9) not given probably due to excessive toxicity.)	Stegeman and Li, 1990 TOX1999-241
Ames test	Dodigen 4022	dissolved in distilled water	S. typhimurium strains TA 98, 100, 1535, 1537, 138; E.coli strain			

WP2uvrA; plate incorporation test
4 µg/plate -
10000 µg/plate (+/-S9)
Negative for mutagenicity. No cytotoxic effects observed.
Stammberger and Mayer, 1992 TOX1999-324
Cytogenetic study for chromosom e aberrations in vitro
Dodigen 4022
dissolved in cell culture medium
Chinese hamster V79 cells
0-600-3000-6000
µg/mL; (+/-S9); 4 h exposure,
sampling at 7, 18 and 28 h after start of treatment
Negative for clastogenicity and polyploidy. Reversible inhibition of cell cycle (mitotic indexN) after 7 h at the highest dose (+/- S9). Cell survival rate/ at 3000 µg/ml
and above (only without activation).
1992,TOX199
9-325
Study type
Test material
Test system
Dose range/ Test conditions
Result
Reference
Micronu- cleus test
MON 0818
dissolved in corn oil
CD 1-mice (m/f), bone marrow erythrocytes
0 and 100 mg/kg bw; single i.p. injection; evaluation at 24 and 48 h after
dosing
Negative.
Also, no indications neither of general toxicity nor of bone marrow cytotoxicity to be observed.
1998* TOX1999-240
The spot test did not provide indications of a mutagenic response, however, does not allow quantitative assessment. This variation of the Ames test is no longer in use in routine genetic toxicology. Therefore, the data are not shown here.
* supplementary study
Flowers, L.J. (1981, TOX1999-319): Ames/Salmonella mutagenicity assay of MON 8080. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; Project no. ML-80-294/800281; Report no. MSL 1538. Dates of experimental work: 31 Oktober 1980 - 28 November 1980. GLP: No. When the study was performed, GLP was not compulsory. However, a Quality Assurance Audit statement is included. The study is considered acceptable.
Stegeman, S.D. and Li, A.P. (1990, TOX1999-241): Ames/Salmonella mutagenicity assay of MON 0818. Monsanto Environmental Health Laboratory, St. Louis, U.S.A. on behalf of Monsanto; Project no. EHL 89178/ML-89-461; Report no. MSL-10625. Dates of experimental work: 28 November 1989 - 29 January 1990. GLP: Yes (self-certification of the laboratory). A respective Quality Assurance Audit statement is included. The study is considered acceptable.

Stammberger, I. and Mayer, D. (1992, TOX1999-324): Dodigen 4022: Study of the mutagenic potential in strains of Salmonella typhimurium (Ames test) and Escherichia coli. Pharma Development Central Toxicology, Hoechst AG, Frankfurt/Main, Germany; Study no. 92.0336, Report no. 92.0467. Dates of experimental work: 03 June 1992 - 19 June 1992. GLP: Yes. The study is considered acceptable.
Stammberger, I. (1992, TOX1999-325): Dodigen 4022: Chromosome aberrations in vitro in V79 chinese hamster cells. Pharma Development Central Toxicology, Hoechst AG, Frankfurt/Main, Germany; Study no. 92.0337, Report no. 92.1024. Dates of experimental work: 22 July 1992 - 03 November 1992. GLP: Yes. The study is considered acceptable.
(1998, TOX1999-240): Mouse micronucleus screening assay
of MON-0818. on behalf of
Monsanto; Project no. EHL 89182/ML-89-463. Dates of experimental work: 06 November 1980 - 05 February 1990. GLP: Not stated in the report. The study is considered supplementary only since it was not in compliance with OECD recommendations for tests of this type. In particular, the only dose level used was too low for definitive assessment.
Furthermore, the notifier Monsanto submitted to the Rapporteur published data suggesting that also the sorbitol ester surfactants Tween 20 and Tween 80 proved negative in either the mouse lymphoma test or in the Ames test and the mouse micronucleus test, respectively. However, since these co-formulants were not contained in the glyphosate formulations for which mutagenic effects had been reported, the respective data were not reviewed in detail.
Assessment
The available studies clearly show a lack of mutagenicity of the tested surfactants in the limited number of test systems used confirming the negative outcome of respective studies with glyphosate formulations. In contrast, marked cytotoxicity was caused in the Ames test by the surfactant MON 0118 as well as by the alkyle sulfate surfactant MON 8080 suggesting that cytotoxicity observed in mutagenicity testing of formulations are mainly due these surfactants. This assumption is supported by the result of an Ames test using the surfactant-free Rodeor formulation (1992, TOX9552373) with no signs of cytotoxicity occurring. The more recently introduced surfactant Dodigen 4022 proved non- cytotoxic in the Ames test and caused cytotoxic effects in V79 cells at very high concentrations only.
It is widely accepted that cytotoxicity of a compound can result in positive results in mutagenicity assays and it is often difficult clearly to distinguish between true substance- related genotoxic effects and “mutagenicity“ mediated by excessive cytotoxicity. A close relation between cytotoxicity and mutagenicity became apparent also in the chromosome aberration test with Dodigen 4022 (1992, TOX1999-325). The markedly reduced mitiotc index at the first sampling time indicating an adverse effect at least of high doses (only the top dose concentration of 6000 µg/mL and the solvent control were assessed after 7 hours) was accompanied by a slight increase in chromosome aberration frequencies including and excluding gaps in the absence as well as in the presence of the metabolically activating S9 mix. However, at the later sampling times (18 and 28 h following substance application), the mitotic index had normalised again and there was no increase in the incidence of chromosome aberrations any more. Therefore, the test substance was considered negative in this test system. This example provides further evidence that suspected mutagenic effects of formulations as reported in section II might be readily due to cytotoxicity.
These results are in line with data suggesting a higher toxicity and irritancy of certain surfactants as compared to the active substance and to formulations as reported in chapter
B.5.11 in the monograph. It can be also assumed that specific adverse effects of surfactants might

have significantly contributed to the rather unexpected mammalian toxicity of some glyphosate formulations. Despite the low general toxicity of glyphosate technical, a number of poisoning incidents in humans sometimes resulting in death was reported in particular from asian countries (see chapter B.5.9 in the monograph).

Severe intoxication was mainly characterised by a decrease in blood pressure and further cardiovascular symptoms followed by pulmonary dysfunction and renal failure and by signs of irritation in the gastointestinal tract. Pathophyiology of poisoning is assumed to include irritation or corrosion of the intestinal mucosa resulting in electrolyte imbalances, hypovolemic shock and disturbances in the cardiovascular system. The respiratory signs, as well as renal symptoms, are considered secondary to this mechanism beeing caused either by pulmonary edema related to disturbed circulation or by aspiration pneumonia following emesis (, 1987, Z35531; see also monograph, chapter B.5.9). There is evidence that the first step, i.e. damage to gut mucosa, might be primarily caused by
surfactants due to their irritating properties.

Of course, the clinical reports on human posionings with glyphosate formulations are often difficult to interpret since most of the severe intoxications were attempts of suicide. In such cases, also the frequent concomitant intake of drugs and alcohol should be considered.

However, the hypothesis of surfactant effects being involved is further supported by mechanistic and pharmacological studies (see section B.5.8.2.3 in the monograph) suggesting
that the acutely toxic effects may be caused by the surfactant alone, too, and that toxicity may be even enhanced when complete Roundup formulations were tested.

Furthermore, according to the information available to the Rapporteur, the cases of severe or even fatal intoxication were related to the ingestion of glyphosate products containing surfactant. Sawada and Nagai (1987, Z35531) reported two cases of human
poisonings with surfactants causing clinical signs resembling very much those observed after ingestion of large amounts of Roundup.

A possible potentiation of toxicity of glyphosate IPA salt and POEA in animals was reported by (1991, Z80636) who tested the acute oral toxicity of Roundup formulations in rats. Using the intratracheal route of administration being of clinical relevance in cases of aspiration, the same authors observed a marked toxic effect of Roundup and of POEA alone to the lungs but this was much less pronounced with Polysorbate-80, i.e. another non-ionic surfactant.

Mucosal irritation in the respiratory tract caused by surfactant may be also behind the much lower threshold level for adverse effects of a Roundup formulation as compared to glyphosate a.i. upon subacute inhalative exposure (see section B.5.3.3.2 in the monograph, also reported by WHO/IPCS in 1994, TOX9500301).

A statement of the notifier Monsanto was submitted to the Rapporteur in October, 1998. In this paper, it is suggested that the toxic and cytotoxic effects of polyoxyethylenamine (POEA) were responsible for the observed adverse effects on health and environment. Since it is an important objective to use environmentally safe and less toxic products, the polyoxyethylen surfactants were replaced at least in some Monsanto products by others. The
company stated that this decision was mainly based on the eye irritation potential and the aquatic toxicity related to the formerly used substances. Accordingly, in the formulations for which toxicological data have been submitted as part of the joint dossier of Monsanto and Cheminova, surfactants of this type are not contained any more. Indeed, cytotoxicity of other surfactants, e.g. Dodigen 4022, and their potential to cause acutely toxic or irritating effects are much lower as compared to POEA.

Thus, it can be expected that the replacement of toxic and irritating surfactants like POEA by other and less critical substances may reduce the risk of death or severe health effects following intentional or accidental ingestion of glyphosate products as well as the severity of eye or respiratory tract irritation.
Recently, the notifier Monsanto provided a new assessment explaining that POEA is a group of chemicals not all capable of causing adverse effects. It is suggested that only particular substances belonging to this group might be responsible for the toxic effects described in this addendum. However, since this is clearly relevant for assessment of formulations but not for health evaluation of the active ingredient, this item hould be considered on a Member state level.

B.6.5.1 Long-term toxicity and carcinogenicity in the rat
B.6.5.1.1 New studies in rats 1st study: 1996
Reference: IIA, 5.5.1/01
Report: (1996)
Glyphosate Acid: One Year Dietary Toxicity Study in Rats
Data owner: Syngenta Study No.: CTL/P/5143 Date: 1996-10-02
not published TOX2000-1998
Guidelines: OECD 452, US EPA 83-1 Deviations: Several organ weights not determined GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 1995-04-03 - 1996-06-03
Materials and methods
Test material:
Identification:
Glyphosate acid
Description:
White solid
Lot/Batch #:
P24
Purity:
95.6 %
Stability of test compound:
At least 1 year when stored at RT.
Vehicle and/
or positive control:
Diet
Test animals:
Species:
Rat
Strain:
Wistar (Alpk:APfSD)
Source:
Age:
22-24 days (on delivery)
Sex:
Males and females
Weight at dosing:

Males: 150.5 – 151.5 g (mean values); females: 126.7 –
133.3 g (mean values)
Acclimation period:
At least 10 days.
Diet/Food:
CT1 diet (Special Diet services Ltd., Essex, UK), ad libitum
Water:
Mains drinking water, ad libitum
Housing:
Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage.
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: at least 15/hour 12 hours light/dark cycle
In life dates: 1995-04-03 to 1996-06-03 Animal assignment and treatment:
In a chronic toxicity study groups of 24 Wistar-derived rats per sex received daily dietary doses of 0, 2,000, 8,000 and 20,000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females).
Test diets were prepared in either 30 or 60 kg batches by mixing the appropriate amount of the test substance with the basal diet. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20000 ppm.
Observations
Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study. The eyes of the control and high dose group were additionally examined one week to termination.
Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 14 and every two weeks thereafter until termination.
Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13, once in Week 16 and every fourth week thereafter.
Haematology and clinical chemistry
Blood was collected from 12 animals per sex and group at Week 14, 27 and at termination (Week 53). The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), ,-glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.
Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses (except for Week 52) at Week 13, 26 and 52. The following parameters were determined: Volume, colour, appearance, specific gravity, pH, glucose, ketones, protein, urobilinogen and blood.
Sacrifice and pathology

Necropsy was conducted on all animals except for Rats 38 and 149-152, which were killed during Week 6/7 due to a sexing error. The following organ weights were determined from all animals surviving to scheduled termination: Adrenals, brain, epididymides, kidneys, liver and testes.
Tissue samples were taken from the following organs: Adrenals, aorta, bone & bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eye, gross lesions, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, nasopharyngeal cavity, sciatic nerve, oesophagus, oral cavity, ovary, pancreas, pituitary, prostrate, rectum, salivary glands, seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and voluntary muscle.
Statistics
All data were evaluated using analysis of variance and covariance for each specified parameter using the GLM procedure in SAS (1989). Differences from control were tested statistically by comparing each treatment group least- squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis. All statistical tests were two sided.
Results and discussion
Analysis of dose formulations
The mean achieved concentrations of glyphosate acid in each dietary preparation were within 8 % of the nominal concentration and the overall mean concentrations were within 4 % of nominal.
The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20000 ppm was satisfactory; percentage deviations were within 7 % of the overall mean.
The stability tests determined at 2000 and 20000 ppm showed that the test substance is stable for at least 61 days when stored at room temperature.
Mortality
There were no treatment-related death.
Clinical observations
There was a small increase in the number of animals in the 20000 ppm group which had urinary staining (wet or dry). All other clinical observations were of a type and incidence expected for rats of this strain.
Body weight
Body weights of rats receiving 20000 ppm glyphosate acid were lower than those of controls throughout the study. Bodyweights in the intermediate group were slightly reduced throughout the study. The difference from control was not statistically significant in males and was statistically significant in females only from Week 46. As the pattern of the effect was similar to that of the high dose rats for both sexes this minor difference in bodyweight is considered to be related to administration of glyphosate acid.
There was no effect on bodyweight in rats receiving 2000 ppm glyphosate acid.
Food consumption and compound intake
Food consumption was generally lower in rats receiving 20000 ppm than in controls. The difference was most marked at the start of the study. Food consumption was generally slightly lower than controls in rats receiving 8000 ppm glyphosate acid. There was no effect on food consumption in rats receiving 2000 ppm.
The group mean achieved doses are summarised below.
Table B.6.5-1: Group mean achieved dose levels
Dose

Dietary concentration (ppm)
Mean achieved dose level (mg/kg bw/day)
Males
Females
1 (control)
0
2 (low)
2000
141
167
3 (mid)
8000
560
671
4 (high)
20000
1409
1664
The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.
Ophthalmoscopy
There were no treatment-related effects observed.
Haematology and clinical chemistry Haematology
A number of statistically significant differences from control were identified but there was no evidence of a relationship to dose and the differences were small and not seen consistently at all the time points and therefore were considered to be unrelated to glyphosate acid administration.
Clinical chemistry
Plasma cholesterol and plasma triglycerides were marginally reduced in males receiving 20000 or 8000 ppm at Weeks 14 and 27.
Moreover, there was a treatment- and dose-related increase in plasma ALP activity throughout the study. For rats receiving 2000 ppm glyphosate acid the increase was marginal and was statistically significant only for females at Week 14. The increase in the activity of plasma ALP in animals at all dose levels was compound-related but as there was no accompanying pathological change in either the liver or bone this is considered not to be of toxicological significance.
All other differences from control were small and/or were not dose-related and are considered to be incidental to administration of glyphosate acid.
Table B.6.5-2: Clinical chemistry findings
Dose group (ppm)
0
2000
8000
20000
♂
♀
♂

♀
♂
♀
♂
♀
Alkaline Phosphatase
(IU/L)
Week 14
248
161
281
201*
342**
227**
429**
292**
Week 27
221
135
250
171
306**
200**
412**
254**
Week 53
232
87
258
100
291**
114
379**
160**
* $p < 0.05$; ** $p < 0.01$
Urinanalysis
There were no consistent treatment- and dose-related effects seen in the any urinary parameters.
Necropsy
Gross pathology
There were no treatment-related macroscopic effects.
Organ weights
There were no treatment- and dose-related effects on organ weights when corrected for bodyweight.
Histopathology
An increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland were seen in both sexes receiving 20000 ppm glyphosate acid. This change was considered to be

related to treatment and consequently the salivary glands of the 8000 ppm dose group were examined. The examples of focal parotid basophilia seen at this dose were all of minimal severity and the incidence was comparable to that in the control group.
All other observed differences in the incidence of findings are considered to be unrelated to the treatment with glyphosate acid in view of the spontaneous incidence in this strain. No treatment-related neoplasms were found.
Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 12 month is 8000 ppm (corresponding to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females).
RMS comment:
The study is considered acceptable. In addition to the effects described, food utilization was less efficient at the top dose level. A certain increase in basophilia of acinar cells of the parotid salivary gland was still noted in mid dose females although the incidence in males was indeed comparable to the control group (see additional Table B.6.5-3).
Table B.6.5-3: Incidence of focal basophilia in parotid salivary gland of rats treated with glyphosate acid for one year
Dose group (ppm)
0
2000
8000
20000
♂
♀
♂
♀
♂
♀
♂
♀
Focal basophilia
Minimal
2
2
0
0
3
6
10
8
Slight
0
0
0
0
0
0

3
5
Moderate
0
0
0
0
0
0
0
2
Based on this histopathological alteration, the lower body weight even at the mid dose level towards the end of the study, a lower food consumption and higher AP acitivity at the two upper dose levels, the NOAEL in this chronic study is rather seen at 2000 ppm (equal to a mean daily intake of 141 or 167 mg/kg bw in male or female rats) instead of the next higher dose as suggested by GTF. There was no evidence of carcinogenicity but duration of treatment was too short for a definitive conclusion.
2nd study: 1997
Reference: IIA, 5.5.2/02
Report: (1997)
HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats
Data owner: Arysta Life Sciences Study No.: 94-0150
Date: 1997-07-01
not published,
ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012- 11487
Guidelines: OECD 453 (1981), JMAFF 59 NohSan 3850 (1984), US-EPA (1989)
Deviations: None
GLP:
yes
Acceptability:
See RMS comment
Dates of experimental work: 1994-12-19 - 1996-12-25
Materials and methods
Test material:
Identification:
Glyphosate technical, Code: HR-001
Description:
White crystal
Lot/Batch #:
T-941209; T-950308
Purity:
97.56 %; 94.61 %
Stability of test compound:
No data given the report.
Vehicle and/
or positive control:

Diet
Test animals:
Species:
Rat
Strain:
Sprague-Dawley (Crj:CD)
Source:
Age:
5 weeks (males), 6 weeks (females)
Sex:
Males and females
Weight at dosing:
65 – 85 g
Acclimation period:
At least one week
Diet/Food:
MF Mash (Oriental Yeast Co., Ltd, Japan), ad libitum
Water:
Well water treated with sand and charcoal filter, HCl and UV rays, ad libitum
Housing:
In groups of ten animals of the same sex in wire-mesh stainless steel cages during the acclimatisation period. During the study males were housed in groups of 5 per cage until week 72, in groups of ≤3 until week 78 and
individually thereafter. Females were housed in groups of five until week 78, and individually thereafter.
Environmental conditions:
Temperature: 24 ± 2 °C
Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 1994-12-19 to 1996-12-25 Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats/sex/group received daily dietary doses of 0, 3000, 10000 and 30000 ppm (equivalent to mean achieved dose levels of 0, 104, 354 and 1127 mg/kg bw/day in males and 0, 115, 393 and 1247 mg/kg bw/day in females) HR-001. In addition, 30 rats/sex/group were included for interim sacrifices at 26, 52 and 78 weeks.
Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet. This pre-mix was then added to a larger amount of basal diet and blended by a blending machine.
The stability of the test substance in food was previously determined in a 4-week dose-range finding study in mice. Homogeneity analyses were performed on samples of each dose level of the first diet preparation. Analyses for achieved concentrations were done for each dose level in monthly intervals.
Observations
Rats of all groups were examined for toxic signs and pre-terminal deaths once a day. In addition a detailed veterinary examination was made at least once per week. Ophthalmic examination was done at the start of the study and at termination.

Body weight

Individual body weights were recorded at weekly intervals until the end of Week 13 and every 4 weeks thereafter and before necropsy, except for dead or moribund satellite animals, which were discarded without body weight determination.

Food consumption and compound intake

Food consumption was measured for a period of three consecutive days weekly from Week 1 to 13 and every four weeks from Week 16 to 104. Mean individual food consumption, group mean food consumption and group compound intake were calculated.

Haematology and clinical chemistry

Blood samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in week 104. Before sampling animals were fasted overnight. The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, alkaline phosphatise, glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT),
m-glutamyl-transpeptidase, creatine phosphokinase, creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis

Individual urine samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in Week 104. The following measurements were made: density, volume, appearance, pH, protein, glucose, occult blood, ketones, urobilinogen, sediments.

Sacrifice and pathology

Necropsy and histopathological examinations were carried out on all tissues collected at interim and terminal sacrifice. The following organ weights were determined from all animals: adrenals, brain, caecum, kidneys, liver and testis.

Tissue samples were taken from the following organs: adrenals, aorta, bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons and medulla oblongata), caecum, colon, duodenum, epididymides, eyes, gross lesions, Harderian glands, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands (submaxillary and
sublingual), sciatic nerve, seminal vesicles and coagulating glands, skeletal muscle, skin (females only), spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (horns and cervix) and vagina.

Statistics

Statistical significance of the difference between the control group and the treated groups was estimated at 5 % and 1 % levels of probability.

The data of body weight (main group only), food consumption, urine specific gravity, urine volume, haematological parameters, blood biochemical parameters, and organ weights were evaluated by Bartlett’s test for equality of variance. When group variances were homogeneous, a parametric analysis of variance of a one-way layout type was conducted to determine if any statistical differences existed among groups. When the analysis of variance was significant, Dunnett's (when sample size of each group was equal) or Scheffé’s (when sample size of each group was different) multiple comparison test was applied to evaluate differences between the treated and the control groups. When the group variances were heterogeneous, the data were analyzed by Kruskal-Wallis

non-parametric analysis of variance. When significant, Dunnett type (when sample size of each group was equal) or Scheffé type (when sample size of each group was different) mean rank sum test was applied to determine if any significant differences existed between the treated and the control groups. The data of urinalysis except for specific gravity and urine volume were assessed by Mann-Whitney's U test.

Mortality was analyzed by Life table analysis.

The data of clinical sign (main group only), ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability test.

Results and discussion

Analysis of dose formulations

The coefficient of variation for the homogeneity of the test substance for each dose level was 2.2% and less. Hence, the results indicated a good homogeneity.

Analyses for concentrations showed that the diet preparations achieved 97 - 98% of the target concentration. Thus, the concentrations of the test substance in the test diets were within acceptable limits.

Mortality

In the high dose group neither sex showed an increase in mortality, although mortality in males was lower than the control during the last half of the treatment period with statistical significance in most of the weeks. In all other groups mortality was comparable to control. The final mortality is given in Table B.6.5-4:

Table B.6.5-4: Final mortality at termination of treatment (%)

Dose group (ppm)*	0	3000	10000	30000
Sex				
Male	32/50 (64)	30/50 (60)	32/50 (64)	21/50 (42)
Female	35/50 (70)	31/50 (62)	34/50 (68)	36/50 (72)

* number of mortalities / total number of rats/group (% mortality)

Clinical observations

In the high dose group, significant increases in incidence of bradypnea, mass, and soiled fur were observed in males when compared to the control. Analysis of location of each mass showed that the ones in the tail were present in 27 males, which was apparently high in
incidence compared to 11 of the control. The incidences of mass in other locations were comparable to the control. With respect to soiled fur, the sign was located at the external genital or perianal region. Males in this group also showed significant decreases in incidence of tactile hair loss, wound, and hair loss. In females, a significant increase in incidence of wetted fur was observed; the sign was

mainly seen in the external genital region. Besides the signs mentioned above, loose stool was observed in all cages of this group from Week 24 in males and Week 23 in females until the end of the treatment. Animals showing loose stool could not be identified because of group housing, therefore the sign is only described here in the text but not included in Table B.6.5-5.
In the mid dose group, the incidence of tactile hair loss was significantly decreased in males and significantly increased in females when compared to the respective control.
In the low dose group, significant increases in incidence of decreased spontaneous motor activity, bradypnea, and soiled fur and a significant decrease in incidence of tactile hair loss were observed in males. Analysis of location of the soiled fur demonstrated predominant occurrences of the sign in the external genital region and foreleg. Females in this group showed significant increases in incidence of ptosis and tactile hair loss.
Table B.6.5-5: Statistically significant changes in clinical signs
Dose group (ppm)
0
3000
10000
30000
Parameters
♂
♀
♂
♀
♂
♀
♂
♀
Decreased spontaneous motor activity
9
23
19*
22
9
20
13
26
Bradypnea
3
7
10*
14
4
6
11*
12
Ptosis
7

4
6
12*
4
6
6
6
Tactile hair loss
5
1
0*
17**
0*
9**
0*
4
Integument
Wound
7
2
4
2
6
2
0**
1
Mass
22
37
26
36
21
38
37**
43
Hair loss
12
16
7
13
15
21
3*
25
Soiled fur
10

16
20*
17
12
11
21*
18
Wetted fur
9
5
7
5
7
5
16
15*
* p < 0.05; ** p < 0.01
Body weight
In the high dose group, body weights were lower than the control throughout the treatment period; significant decreases in their body weights were observed during Weeks 1 to 80 in males and at Week 7 and during Weeks 9 to 60 in females. The final group mean body weights of males and females at termination of the treatment period were both 93% of the respective control.
In the mid dose group, males showed a decreased body weight gain during the first few weeks of treatment with a statistically significant difference from the control at Week 6. Their retarded growth persisted throughout the treatment period, and the final group mean body weight at termination of treatment was 95 % of the control. Body weight change in females was comparable to the control throughout the treatment period.
In the low dose group, body weights of both sexes were comparable to the control except for a significant increase in females at Week 16.
Food consumption and compound intake
In the high dose group, consistent with the decreasing body weight or decreasing body weight trends, food consumption showed a decreasing trend in males during the first few weeks.
In the other groups, food consumption in males and females was comparable to the respective control.
The group mean achieved doses are summarised below.
Table B.6.5-6: Group mean achieved dose levels in the main groups
Dose group
Dietary
concentration (ppm)
Mean achieved dose level (mg/kg bw/day)
Males
Females
1 (control)
0
2 (low)
3000

104
115
3 (mid)
10000
354
393
4 (high)
30000
1127
1247
The results show a higher test material intake for females when compared to males for each dose level.
Ophthalmologic examinations: No abnormalities were observed.
Haematology and clinical chemistry: Haematological and blood biochemical analyses did not demonstrate apparent toxicity of the test substance in either sex or group.
Statistically significant changes in haematology and blood chemistry are displayed in Table B.6.5-7 and Table B.6.5-8.
Table B.6.5-7: Statistically significant changes in haematology
Dose group (ppm)
3000
10000
30000
Parameters
♂
♀
♂
♀
♂
♀
Haematocrit
108a
99
111
84
131*
96
Platelet count
91
105
88
115
66**
104
a Figures represent values in the treated groups when the corresponding control is 100.
* $p < 0.05$; ** $p < 0.01$
Table B.6.5-8: Statistically significant changes in blood chemistry

Dose group (ppm)
3000
10000
30000
Parameters
♂
♀
♂
♀
♂
♀
Alkaline phosphatase
Week 52
129a
127
145
118
136
214**
Week 78
185*
303
154
106
171
116
Glutamic pyruvic transaminase Week 52
94
91
148
75
67*
66
Creatinine
Week 26
102
95
99
91*
97
89**
Total protein
Week 52
100
101
100

96*
99
96
Albumin
Week 26
100
92*
100
99
103
95
Globulin
Week 26
98
102
95
95
95
95*
Week 52
103
101
101
93*
100
99
Glucose
Week 26
101
104
107
99
97
87**
Total bilirubin
Week 26
100
80**
94
96
106
88
Chloride
Week 104
97*
100

97*
100
98
101
a Figures represent values in the treated groups when the corresponding control is 100.
* p < 0.05; ** p < 0.01
Urine analysis
Urinalysis did not demonstrate apparent toxicity of the test substance in either sex or group. Statistically significant changes in urinalysis parameters are displayed in Table B.6.5-9.
Table B.6.5-9: Statistically significant changes in urinalysis
Dose group (ppm)
3000
10000
30000
Parameters
♂
♀
♂
♀
♂
♀
pH Week 26
↓*
↓**
↓
Week 52
↓*
↓**
↓**
Week 78
↓
↓*
↓
Week 104
↓
↓**
↓
Protein
↓*
Volume
↑*
Appearance
Dark*
Dark*
Dark*
* p < 0.05; ** p < 0.01

Metabolism of glyphosate after absorption from the intestine is minimal. Thus, most of the glyphosate is excreted via urine as the unchanged parent compound. In the urine glyphosate dissociates into the free acid, which can lead to a reduction of the urinary pH. Therefore, the reduced urinary pH might be of no toxicological significance.
Necropsy
In the high dose group significant increases in incidence of distension of the caecum were observed in both sexes, accompanied by soiled fur in the perianal region in males. Moreover, significant increases in absolute and relative weights of the caecum in both sexes in the high and mid dose group were seen, but not associated with histopathological abnormalities.
The incidences of thickened areas in the skin of the tail, corresponding to the tail mass in the clinical observations, were significantly increased in the mid and high dose group. The lesion was histopathologically diagnosed as follicular hyperkeratosis and/or folliculitis/follicular abscess. An increased incidence of hair loss was also observed in high-dosed females, but it lacked corresponding histopathological changes.
All changes regarding neoplastic lesions were not statistically significant.
From this, it is concluded that the test compound at the doses tested does not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.
Conclusion by the Notifiers
Based on the slight body weight effects, and necropsy findings without correlating histopathology at the mid-dose the NOAEL in rats after chronic exposure to HR-001 for 24 month is 3000 ppm (corresponding to 104 mg/kg bw/day for males and 115 mg/kg bw/day for females). It is concluded that HR-001 is not carcinogenic in rats.
RMS comment:
The study is acceptable. We agree with the evaluation by the notifier and support the NOAEL of 104 mg/kg bw/day.
3d study: 1997
Reference: IIA, 5.5.2/07
Report: (1997)
Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat
Study No.: 1231
Date: 1997-02-15
not published, ASB2012-11489
Guidelines: OECD 453 (1981)
Deviations: Organ weights were not determined for all animals; weights of heart, spleen and (para)thyroids are missing
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: 1994-06-09 - 1996-06-12
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
No data given in the report.
Lot/Batch #:

No data given in the report.
Purity:
No data given in the report.
Stability of test compound:
No data given in the report.
Vehicle and/
or positive control:
Diet
Test animals:
Species:
Rat
Strain:
Sprague-Dawley
Source:
Age:
Approx. 6 weeks
Sex:
Males and females
Weight at dosing:
Males: 70.0 – 93.2 g, females: 70.0 – 90.6 g
Acclimation period:
One week
Diet/Food:
Powdered rat feed (Lipton India Ltd, India), ad libitum
Water:
Filtered pure water, ad libitum
Housing:
Initially in groups of five in polypropylene cages, in groups of three from Week 24 to 52 and in groups of two from Week 53 to termination.
Environmental conditions:
Temperature: 22 - 25 °C
Humidity: 50 - 70 % Air changes: 10 - 15/hour 12 hours light/dark cycle
In life dates: 1994-06-09 to 1996-06-12
Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague Dawley rats per sex received daily dietary doses of 0, 3000, 15000 and 25000 ppm (equivalent to mean achieved dose levels of 0, 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0, 0.21, 1.06 and 1.74 g/kg bw/day (females)) Glyphosate technical for two years. In addition, for the control and each dose group 20 rats per sex were included for interim sacrifice in Week 52 to study non- neoplastic histopathological changes (chronic toxicity study). Selected dose levels were the same except for the highest dose which was 30000 ppm. Here the dietary doses correspond to 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for
3000, 15000 and 30000 ppm, respectively
Test diets were prepared weekly by mixing appropriate amounts of the test substance with the basal diet. The stability and homogeneity of the test substance in food was determined in- house stability study at all dose levels before the start of dosing. Analyses for achieved concentrations were

performed monthly during the study period.
Clinical observations
Rats were examined for toxic signs once and pre-terminal deaths twice a day. Ophthalmic examination was done at the start of the study, at interim sacrifice and at termination in the control and high dose group
Body weight
Individual body weights were recorded on Day 0, at weekly intervals thereafter until the end of Week 13 and every 4 weeks thereafter until termination.
Food consumption and compound intake
Food consumption was recorded once weekly for each group from Week 1 to Week 13 and subsequently in Week 25, 38, 51, 65, 78, 92 and 104.
Haematology and clinical chemistry Haematology
Individual blood samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Haemoglobin, erythrocyte count, PCV, thrombocytes, total leukocyte count and differential leukocyte count.
Blood chemistry
Individual plasma samples were collected from 10 rats/sex/group of the main groups at 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Total serum proteins, albumin, ALT, AST, GGTP, SAP, blood urea nitrogen and blood glucose.
Urinalysis
Individual urine samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. The following measurements were made: Specific gravity, volume, appearance, pH, protein, glucose, occult blood, ketones, microscopy of sediments.
Sacrifice and pathology
Necropsy was performed on all animals at scheduled termination.
The following organ weights were determined from 10 rats per sex per main group and on all animals of the satellite groups: adrenals, brain, gonads, kidneys and liver.
Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups.
Tissue samples were taken from the following organs of all animals: adrenals, aorta, body cavities, brain, caecum, colon, duodenum, epididymis, eyes (both), femur, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric and mandibular), mammary gland, oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.
Statistics
Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier. Animals withdrawn from study during the interval (those taken for moribund sacrifice) are taken into consideration by giving enough weightage.
The incidence of neoplasms was analysed by Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.
In addition to these tests the Fisher exact test for pairwise comparisons and the Cochran Armitage

linear trend test for dose response trends were carried out. All reported P-values for the tumour incidence analysis are one-sided.
The biochemical, haematological and organ weight data was analyzed for significance using Student 't' test or Cochran 't' test.
Results and discussion
Analysis of dose formulations
Analyses for concentrations showed that the diet preparations recovered 86.1 - 98.3 % of the target concentration. Thus, the concentrations of the test substance in the test diets were within acceptable limits.
Analyses for homogeneity recovered 87.5 - 90.0 % for 3000 ppm, 91.7 - 93.0 % for 15000 ppm, 94.3 - 95.1 % for 25000 ppm and 91.8 - 92.6 % for 30000 ppm. Hence, the results indicated a good homogeneity.
Moreover, stability analyses showed that recovery one month after diet preparation ranged between 87.5 and 95.0 %.
Mortality
No treatment-related clinical signs or deaths were observed in the satellite groups, e.g. the chronic toxicity study.
In the carcinogenicity study, e.g. after 104 weeks, male animals of the high dose group exhibited slight but statistically insignificant higher mortalities.
The numbers of pre-terminal deaths in the main group are displayed in Table B.6.5-10.
Table B.6.5-10: Cumulated mortalities after 104-week dietary exposure to Glyphosate technical*

Dose group (ppm)	0	3000	15000	25000
Sex				
Male	16/50	17/50 (2)	18/50 (4)	23/50 (14)
Female	19/50	20/50 (2)	20/50 (2)	25/50 (12)

* Values in parentheses indicate increases in mortality compared to control in percent.
Clinical observations
No significant toxic signs were observed in treated or control groups.
Body weight
Significantly reduced body weight gain that lasted throughout study until Week 104 was observed in males receiving the highest dose. In all other groups body weight gain was comparable to the control at termination.
Food consumption and compound intake
There were no treatment-related effects on food consumption for either sex or group noted during

the study.
The results show a higher test material intake for females when compared to males for each dose level. The mean intake in the chronic toxicity study for each dose group is 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively.
The mean intake in the carcinogenicity study for each dose group is 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0.21, 1.06 and 1.74 g/kg bw/day (females) for 3000, 15000 and 25000 ppm, respectively.
The group mean achieved doses are summarised below.
Table B.6.5-11: Group mean achieved dose levels
Dose group
Dietary concentration (ppm)
Mean achieved dose level (mg/kg bw/day)*
Males
Females
Chronic toxicity study (52 weeks)
low
3000
0.18
0.24
mid
15000
0.92
1.13
high
30000
1.92
2.54
Carcinogenicity study (104 weeks)
low
3000
0.15
0.21
mid
15000
0.78
1.06
high
25000
1.29
1.74
* Calculations were done with values from Week 13 (chronic) and Week 25 (carcinogenicity)
Ophthalmological examinations
Ophthalmological examinations revealed no abnormalities.
Laboratory investigation
Haematological examination did not reveal any abnormalities attributable to the treatment.

Regarding the clinical chemical investigations, a significant increase in the alkaline phosphatase level was only seen in the high dose of the carcinogenicity study at study termination (see Table B.6.5-12). Other significant changes observed in haematological, and biochemical parameters were within the range of the historical control data and hence appear to be of no biological significance.

Table B.6.5-12: Statistically significant changes in blood chemistry

Dose group (ppm)	0	3000	15000	25000
Parameters	♂	♀	♂	♀
	♂	♀	♂	♀
Alkaline phosphatase				
Month 6				
	25.58	24.96	24.97	25.25
	24.85	25. 2	23.07	25.11
Month 12				
	25.64	19.04	25.96	25.35*
	27.64	28.3*	22.88	22.88*
Month 18				
	27.7	24.47	25.94	28.42
	28.73	27.71	26.68	

25.28
Month 24
26.04
24.87
26.75
26.95*
28.42*
25.75
47.71*
53.86*
* $p < 0.05$
Urinanalysis
Urinalysis did not reveal any abnormalities attributable to the treatment.
Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed during the study period.
Organ weights
Significant and dose-dependent effects in the chronic toxicity study were found in both sexes of the high-dose group. In males, weights of kidneys, brain and testes were increased. In females, in addition to kidneys and brain, the liver weight was increased as well.
In the carcinogenicity study which lasted 52 weeks longer, significant and dose-dependent effects in males consisted of increased weight of brain and testes in the mid and high dose group. Effects on the kidneys were not observed, perhaps due to the lower dose level in the highest group compared to the chronic toxicity study, e.g. 25000 ppm to 30000 ppm, respectively.
In females, significant and dose-dependent effects after 24 months occurred only in kidneys. Like for male animals, this increase could be due to the different high dose levels.
Histopathology
Histopathological changes were found at all dose levels including control, hence it is concluded that these are no treatment-related effects.
Neoplastic changes
There were no treatment-related neoplasms observed.
Conclusion by the Notifiers
Based on the mild toxic effects on body weight gain and the increased organ weights without histopathological changes the NOAEL in rats after chronic exposure to Glyphosate technical for 24 month is 25,000 ppm (corresponding to 1290 mg/kg bw/day for males and 1740 mg/kg bw/day for females). It is concluded that Glyphosate technical is not carcinogenic in rats.
RMS comment:
This study is not acceptable because no core information on the test substance such as batch number or purity was given and, thus, it is not clear what was in fact tested. Even if this data would be available, the study might be considered at best supplementary because of its many additional deficiencies. No storage conditions or expiry date for the test substance was given. Mean daily dietary intake of the test substance was not given but apparently calculated by GTF afterwards. In addition, there were some deviations from OECD guideline 453, in
particular with regard to frequency of observations. Even more important, the reported total incidence of neoplasia was surprisingly low and especially the very low frequency of malignant tumours (one female control animal affected by an mammary gland adenocarcinoma among 400

animals on study) might produce doubts about suitability (i.e., sufficient vulnerability) of the rat strain used. Neither the NOAEL nor an LOAEL were proposed in the study report. In contrast to GTF opinion, the NOAEL is seen by the RMS at 3000 ppm (ca 150 mg/kg bw/day). This might be considered very conservative because it is based mainly on changes in clinical chemistry parameters without concomitant liver pathology. However, it must be taken into account that the number of animals that were subject to histopathological examination and of which organs were weighed was lower than usually required. Provided that is can be shown that glyphosate of a certain batch and precisely determined purity was tested, it would be of interest to get historical control data for tumour incidences in this rat strain and laboratory to have a better chance to assess reliability of the results with regard to carcinogenicity. In addition, the study owner should be mentioned.
4thstudy: 2001
Reference: IIA, 5.5.2/03
Report: (2001)
Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats
Data owner: Syngenta Study No.: CTL/PR1111 Date: 2001-03-15
not published, ASB2012-11488
Guidelines: OECD 453 (1981), EEC B.33 (1988), MITI (1992), US OPTTS
870.4300 (1998)
Deviations: None
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: 1998-04-07 - 2000-10-16
Materials and methods
Test material:
Identification:
Glyphosate acid (technical material)
Description:
White solid
Lot/Batch #:
P30
Purity:
97.6 % w/w
Stability of test compound:
At least 2 years when stored at -20 °C.
Vehicle and/
or positive control:
Diet
Test animals:
Species:
Rat
Strain:
Wistar (Alpk:APfSD)
Source:
Age:
3 weeks (on delivery)
Sex:

Males and females
Weight at dosing:
Males: 155.0 – 156.6 g (mean values); females: 136.0 –
138.4 g (mean values)
Acclimation period:
At least 10 days.
Diet/Food:
CT1 diet (Special Diet services Ltd., Essex, UK), ad libitum
Water:
Mains drinking water, ad libitum
Housing:
Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 30 – 70 %
Air changes: at least 15/hour 12 hours light/dark cycle
In life dates: 1998-04-07 to 2000-05-07
Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 52 Wistar-derived rats per sex received daily dietary doses of 0, 2,000, 6,000 and 20000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females).
A further twelve animals per sex were added to each group and were designated for interim kill after one year to study chronic toxicity and non-neoplastic histopathological changes.
Test diets were prepared in 60 kg batches by mixing a known amount of the test substance with 1 kg of basal diet. This pre-mix was then added to the remainder of the 60 kg batch of basal diet and mixed thoroughly. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20000 ppm.
Clinical observations
Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study, at Week 52 and prior to termination. A functional observational battery including motor activity was conducted in Week 52 in animals allocated to the chronic toxicity assessment of the study.
Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 15 and every two weeks thereafter until termination.
Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 14, once in week 16 and every fourth week thereafter.
Haematology and clinical chemistry
Blood was collected from 13 animals per sex and group at Week 14, 27, 53, 79 and at termination. Different animals were used for the tail vein haematology and clinical chemistry samples.
The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count,

reticulocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), ,-glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis

Individual urine samples were collected from the same animals as those used for haematology analyses at Week 13, 26, 52, 78 and prior to termination. The following parameters were determined: volume, abnormal colour and appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, and blood.

Sacrifice and pathology

Necropsy was conducted on all animals. The following organ weights were determined from all animals surviving to scheduled termination: adrenals, brain, gonads, heart, kidneys, liver and spleen. Tissue samples were taken from the following organs: adrenals, aorta, bone & bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eyes (retina, optic nerve), gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, lachrymal gland, larynx, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, muscle, oesophagus, ovary, pancreas, pharynx, pituitary, prostrate, rectum, salivary glands (submandibular, parotid), seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.

Statistics

All data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter using the MIXED procedure in SAS (1996). Kaplan-Meier survival estimates were calculated separately for each sex and treatment group.

The overall incidence of each tumour type was considered by comparing each treated group and the control group using Fisher's Exact Test. In addition, a test for trend with group number was performed using the Cochran-Armitage Test. Analyses were carried out for all animals, intercurrent deaths and at terminal kill.

Results and discussion

Analysis of dose formulations

The mean achieved concentrations of glyphosate acid in each dietary preparation were within 10 % of the nominal concentration and the overall mean concentrations were within 1 % of nominal.

The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20,000 ppm was satisfactory; percentage deviations were within 2 % of the overall mean for the 20000 ppm group and within 4-9 % of the overall mean for the 2000 ppm group.

The stability tests determined at 2000 and 20000 ppm showed that the test substance stability was satisfactory at room temperature and when stored at -20°C for at least 45 days which covered the period of use in the current study.

Mortality

The male groups were terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25% (criteria for termination of the study). Statistically significantly better survival was observed in males fed 20000 ppm than in the other groups (p
= 0.02). A statistically significant overall trend was also observed for males (p = 0.03).

The female groups survived to scheduled termination and there were no significant differences in mortality between the groups.

The survival rates are displayed in Table B.6.5-13.

Table B.6.5-13: Survival rates during up to 104-week dietary exposure to glyphosate technical

Dose group (ppm)	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 1								
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Week 13								
	0.98	1.00	1.00	1.00	0.98	0.98	1.00	1.00
Week 26								
	0.95	1.00	1.00	1.00	0.98	0.98	1.00	1.00
Week 39								
	0.94	1.00	1.00	1.00				

0.97
0.98
1.00
1.00
Week 52
0.91
1.00
0.97
1.00
0.97
0.98
0.98
0.98
Week 56
0.89
1.00
0.93
1.00
0.93
0.98
0.98
0.98
Week 60
0.87
1.00
0.92
1.00
0.91
0.97
0.98
0.97
Week 64
0.87
1.00
0.90
0.98
0.91
0.95
0.98
0.97
Week 68
0.87
0.94
0.88
0.96
0.87

0.95
0.98
0.95
Week 72
0.85
0.94
0.84
0.96
0.85
0.93
0.97
0.91
Week 76
0.81
0.94
0.80
0.92
0.82
0.89
0.97
0.91
Week 80
0.73
0.88
0.78
0.87
0.72
0.89
0.89
0.83
Week 84
0.69
0.85
0.67
0.83
0.63
0.89
0.85
0.83
Week 88
0.64
0.81
0.57
0.81
0.59
0.83

0.77
0.81
Week 92
0.56
0.79
0.50
0.81
0.53
0.81
0.71
0.80
Week 96
0.50
0.73
0.46
0.73
0.53
0.77
0.66
0.72
Week 100
0.40
0.69
0.44
0.63
0.42
0.77
0.56
0.66
Week 104
—*
0.62
—*
0.56
—*
0.77
—*
0.57
* Terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25% (criteria for termination of the study).
Clinical observations
At 20000 ppm there was a treatment related increase in the incidence of red-brown staining of tray papers, particularly in males.
There were no other treatment related clinical observations.
There were also no treatment-related effects noted in the functional observational battery.

Body weight

The body weights of the animals fed 20000 ppm glyphosate acid were statistically significantly lower than controls throughout the study. The maximum reduction from control values was approximately 5 % for males and 8 % for females.

There were no treatment related effects in animals fed 2000 or 6000 ppm glyphosate acid.

Food consumption and compound intake

Food consumption was lower throughout the first year of the study in animals fed 20000 ppm glyphosate acid. In females the difference was statistically significant over the first 11 weeks (with a maximum reduction of approximately 5%) and again in weeks 40-56 (with a maximum reduction of 6%). In males, the difference was statistically significant over most of the first 6 months with a maximum reduction of 6%.

The group mean achieved doses are summarised below.

Table B.6.5-14: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	2000	121	145
3 (mid)	6000	361	437
4 (high)	20000	1214	1498

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.

Ophthalmoscopy

There were no treatment-related effects observed.

Haematology and clinical chemistry Haematology

Minor variations from control values were obtained for most parameters but showed no consistency and were confined to intermediate time points and/or dose groups and were considered not to be treatment-related. An increased haemoglobin concentration and decreased platelet count was seen in all female treated groups at the interim kill but, in the absence of any apparent dose-response or effects at other time points, these variations from mean control values are considered not to be treatment-related (see Table B.6.5-15).

Table B.6.5-15: Haemoglobin and platelet count

Dose group (ppm)
0
2000
6000
20000
♂
♀
♂
♀
♂
♀
♂
♀
Haemoglobin (g/dL)
Week 14
15.9
15.7
16.0
15.5
16.0
15.9
15.8
15.0*
Week 27
15.5
15.7
15.8
15.8
15.8
15.7
15.7
15.6
Interim Kill
14.7
14.4
14.4
15.1**
14.3
14.9*
14.4
15.0*
Week 53
16.1
15.9
15.7*
15.9

15.5**
15.9
15.9
15.8
Week 79
15.9
15.9
15.2
15.8
15.5
16.0
15.4
15.5
Week 105
13.3
14.3
12.9
14.1
13.1
13.8
13.6
14.2
Platelet count (P 109/L)
Week 14
885
911
897
877
892
910
847
948
Week 27
903
909
871
868
917
858
880
830*
Interim Kill
889
821
895
761*

888
740**
860
764*
Week 53
911
842
977
794
911
754
865
814
Week 79
963
854
993
796
950
817
935
855
Week 105
1015
780
980
783
988
750
877
846
* p < 0.05; ** p < 0.01

Clinical chemistry

In rats fed 20000 ppm glyphosate acid, increases in plasma alkaline phosphatase were present until Week 79 (Table B.6.5-16). Increases in alanine aminotransferase activities were present consistently in males until Week 79 and in females in Weeks 14, 79 and 105. Increased total bilirubin was also present in these males throughout the study and increased plasma aspartate aminotransferase activity was present in males at the interim kill. Plasma triglycerides and cholesterol levels were reduced (from Weeks 14-53 and Weeks 53 onwards, respectively) in males.

In animals fed 6000 ppm, there were small increases in alkaline phosphatase activity over the first year of the study and variable increases in plasma alanine aminotransferase activity at intermediate time points throughout the study.

Plasma creatinine values were lower in all treated female groups at Week 27 and in females receiving 6000 and 20000 ppm at Week 14, but in the absence of any effects later in the study, this is considered to be of no toxicological significance.

Other minor variations from mean control values were confined to intermediate dose groups or time

points and/or showed no dose response, and so were considered not to be treatment- related.

Table B.6.5-16: Clinical chemical findings

Dose group (ppm)

0

2000

6000

20000

♂

♀

♂

♀

♂

♀

♂

♀

Alkaline Phosphatase

(IU/L)

Week 14

234

156

246

177

284**

245**

387**

266**

Week 27

196

121

219

136

239**

166**

327**

203**

Interim Kill

230

82

244

102

269

123*

306**

144**

Week 53

231

92
249
117*
277**
152**
357**
172**
Week 79
208
114
254*
131
244
181**
353**
178**
Week 105
184
144
205
129
218
158
280
173
Alanine Aminotransferase
(IU/L)
Dose group (ppm)
0
2000
6000
20000
♂
♀
♂
♀
♂
♀
♂
♀
Week 14
94.9
81.9
103.5
92.5
121.8**

103.9*
143.4**
104.7*
Week 27
91.8
99.5
95.9
113.8
116.8
132.7*
125.9*
101.8
Interim Kill
77.6
83.4
84.0
82.8
97.7
113.2*
123.3**
95.9
Week 53
84.2
90.1
99.8
108.2
103.5
121.5*
133.8*
114.0
Week 79
69.2
90.0
81.2
97.2
102.4**
110.6
105.9**
116.0*
Week 105
64.1
83.5
58.6
78.6
63.9
78.9

82.7
108.2**
Total Bilirubin
(µmol/L)
Week 14
1.23
2.00
1.23
1.92
1.46
2.00
1.85**
2.46*
Week 27
2.08
2.31
2.31
2.08
2.31
2.08
2.62**
2.23
Interim Kill
2.09
2.50
1.91
2.42
2.18
2.58
2.67**
2.64
Week 53
2.62
2.54
2.46
2.31
2.92
2.46
3.46**
3.15**
Week 79
2.46
2.92
2.92
2.31
2.85

2.38
3.15**
3.08
Week 105
1.75
1.19
2.29
1.04
1.67
1.77
2.54
1.40
Aspartate Aminotransferase
(IU/L)
Week 14
107.9
104.5
113.5
112.6
129.2
124.0
148.0*
114.3
Week 27
110.5
156.8
114.8
185.5
138.0
208.4
141.3
148.3
Interim Kill
90.0
117.8
91.5
109.0
110.4
149.3
132.0*
131.5
Week 53
111.8
151.9
124.8
194.4

130.2
219.1*
160.7
214.8*
Week 79
88.2
156.0
102.7
129.2
130.0
177.7
112.2
197.0
Week 105
75.8
130.7
81.4
102.8
78.4
121.8
92.8
168.5
Plasma Triglycerides
(mmol/L)
Week 14
1.33
1.03
1.48
096
1.43
0.96
1.11*
0.94
Week 27
1.40
1.18
1.42
1.22
1.38
0.95*
1.14*
1.09
Interim Kill
1.65
1.00
2.07

1.13
2.09
1.07
1.45
0.99
Week 53
1.53
1.62
1.55
1.75
1.50
1.39
1.15*
1.39
Week 79
1.90
2.15
1.96
2.77
1.67
2.26
1.42
2.31
Week 105
1.83
3.26
1.81
3.58
1.94
3.02
1.67
2.82
Cholesterol
(mmol/L)
Week 14
2.40
2.66
2.51
2.62
2.48
2.80
2.54
2.71
Week 27
2.92
3.19

3.02
3.24
3.18
3.13
2.98
3.15
Interim Kill
4.74
2.69
5.05
2.95
4.83
2.98
3.89*
3.01
Week 53
5.03
3.56
4.57
3.49
5.15
3.45
4.06**
3.66
Week 79
6.87
4.26
6.30
4.64
5.81*
3.92
5.20**
3.96
Week 105
6.76
4.44
7.22
4.54
7.79
4.13
5.72*
4.11
Plasma Creatinine
(µmol/L)
Week 14
58.5

61.4
59.9
59.6
57.2
59.0*
56.8
58.6**
Week 27
60.8
62.7
61.2
60.3*
59.4
60.5*
58.4*
58.2**
Interim Kill
55.8
53.6
58.0
51.8
56.5
52.3
56.6
50.9
Week 53
61.0
58.8
61.5
59.5
62.5
58.1
60.5
58.2
Week 79
80.7
62.7
85.9
59.2
86.2
62.8
66.4
61.8
Week 105
79.1
50.9

80.8
51.4
79.2
53.5
66.2
50.7
* p < 0.05; ** p < 0.01
Urinanalysis
Urinary pH was lower throughout the study in males fed 20000 ppm glyphosate acid (Table B.6.5-17). Moreover, in the same dose group an increased incidence and severity of blood/red blood cells was present in males and, to a lesser extent, in females.
There were no other treatment related findings in the urinalysis.
Table B.6.5-17: Urinalytical findings
Dose group (ppm)
0
2000
6000
20000
♂
♀
♂
♀
♂
♀
♂
♀
Urine pH
Week 13
6.85
6.00
6.77
6.00
6.92
6.08
6.31**
5.85
Week 26
6.77
5.77
6.69
5.85
6.69
6.00
6.15**
5.77
Week 52

6.85
6.15
6.85
6.23
6.85
6.31
6.15**
5.92
Week 78
6.54
6.38
6.28
6.77
6.15
6.46
5.69**
6.00
Week 98
6.08
—
6.00
—
6.00
—
5.85
—
Week 104
—
6.00
—
6.08
—
6.15
—
6.00
** p < 0.01; NEG: negative, +: very few (1 or 2); ++: few; +++: many
Necropsy
Gross pathology
Treatment-related macroscopic findings were seen in males fed 20000 ppm and/or 6000 ppm in the kidneys, liver, prostate and testes. These findings consisted of a minor increase in incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes.
Additional findings were not considered to be treatment related.
Organ weights
Significant lower relative adrenal gland weight was noted at the interim kill in females fed 20000 ppm and 6000 ppm glyphosate acid. Furthermore, the liver weight was significantly lower at the

interim kill in males fed 20000 ppm glyphosate acid.
There were no other significant and dose-related effects on organ weights.
Histopathology
A minor increase in the incidence but not severity of proliferative cholangitis in the liver was present in males fed 20000 ppm glyphosate acid at interim and terminal kill (see Table B.6.5-18) .
Moreover, in males fed 20000 ppm glyphosate acid an increased incidence of hepatitis and periodontal inflammation was observed. The incidence of prostatitis was higher than the control group in all treated males and there was a decrease in the incidence of tubular degeneration of the testis in males fed 20000 ppm glyphosate acid. The incidence of prostatitis was within historical background levels in all treated groups but, as the control value in this study was low, the relationship to treatment at the high dose level cannot be entirely dismissed.
The main changes in interim and terminal kill males and, to a lesser extent, females fed 20000 ppm glyphosate acid, were observed in the kidney. These changes consisted of slight increased incidence of papillary necrosis with varying degrees of mineralisation of the papilla and/or transitional cell hyperplasia. There was also a very small increased incidence of papillary mineralisation only (males and females fed 20000 ppm glyphosate acid) and transitional cell hyperplasia alone (20000 ppm males only).
All other observed differences in the incidence of findings either fall within the historical background level or are considered to be unrelated to the treatment with glyphosate acid.
Table B.6.5-18: Summary of histopathological findings
Dietary concentration of glyphosate (ppm)
Males (n=64)
Females (n=64)
Finding
Historical
Control
0
2000
6000
20000
Historical
Control
0
2000
6000
20000
Liver
Proliferative cholangitis
-
56
57
55
64
-
55
58

59
61
Hepatitis
4.7 [2 - 8]
8
6
9
13
-
6
7
4
6
Kidney
Papillary necrosis
0.4 [0 – 2]
0
1
0
14
-
0
1
2
5
Transitional cell
hyperplasia
-
2
3
0
5
-
3
1
0
1
Prostate
Prostatitis
23.4
[13 – 35]
13
22
23
37
-

-
-
-
-
Testis
Unilateral tubular degeneration
-
18
13
18
5
-
-
-
-
-
Periodontal
inflammation
-
25
27
23
42
-
18
24
32
28
n = number of animals per group Historical control (mean and [range])
Neoplastic changes
There was no evidence of carcinogenicity and no differences between the groups in tumour incidence.
Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 24 month is 6000 ppm (corresponding to 361 mg/kg bw/day in males and 437 mg/kg bw/day in females). It is concluded that glyphosate technical is not carcinogenic in rats.
RMS comment:
The study is considered acceptable. We agree with the description of the study and its findings and support the conclusions including the NOAEL. It was surprising that the salivary gland findings reported by Milburn (1996, TOX2000-1998) were not confirmed although the study was run in the same laboratory employing rats of the same strain. No further remarks.
5th study: . (2009)
Reference: IIA, 5.5.2/08
Report: (2009)
Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat
Study No.: 2060-0012

Date: 2009-04-23, amended 2009-05-08 not published, ASB2012-11490
Guidelines: OECD 453 (1981), JMAFF Guideline 2-1-16 (2005), US OPTTS 870.4300 (1996)
Deviations: None
GLP: yes
Acceptability:
See RMS comment
Dates of experimental work: 2005-09-01 - 2008-03-19
Materials and methods
Test material:
Identification:
Glyphosate Technical
Description:
White crystalline solid
Lot/Batch #:
H05H016A
Purity:
95.7 % w/w
Stability of test compound:
No data
Vehicle :
Diet
Test animals:
Species:
Rat
Strain:
Wistar Han Crl:WI
Source:
Age:
5 – 6 weeks
Sex:
Males and females
Weight at dosing:
Males: 112 – 183 g, females: 98 – 150 g
Acclimation period:
At least ten days
Diet/Food:
Rat and Mouse SQC Ground Diet No.1 (BCM IPS Ltd., London, UK), ad libitum
Water:
Mains drinking water, ad libitum
Housing:
Initially in groups of three per sex in polypropylene solid- floor cages.
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: at least 15/hour 12 hours light/dark cycle

In life dates: 2005-09-01 to 2007-08-31 Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 51 Wistar rats per sex received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 95.0, 316.9 and 1229.7 mg/kg bw/day) Glyphosate technical. To ensure that a received dose of 1000 mg/kg bw/day overall was achieved, the highest dose level was progressively increased to 24000 ppm.
In addition, three satellite groups with 15 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes. The satellite control group with 12 rats per sex served as veterinary control. The animals were to be used for investigations should any health problems have developed with study animals. No such problems occurred and therefore the observations of these animals have not been included in the report.
Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet for 19 minutes at a constant speed. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.
The stability and homogeneity of the test substance in the diet was determined in an in-house stability study. The homogeneity and achieved concentrations of the test substance preparations was determined at monthly intervals until Week 26, and in 3-month intervals thereafter.
Clinical observations
Rats were examined for toxic signs, ill-health or behavioural changes once and for pre- terminal deaths twice a day. A routine clinical observation session including veterinary examination was made weekly, including palpation for new or existing masses. Ophthalmic examination was done at the start of the study in all satellite animals and at Week 50 in ten satellite animals per sex of the control and high dose group. Prior to treatment and at weekly intervals thereafter all satellite animals were observed for behavioural toxicity.
Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13 and every four weeks thereafter until termination as well at terminal kill.
Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently for one week in each four weeks until termination.
Water consumption
Water intake was observed daily, for each cage group, by visual inspection.
Haematology and clinical chemistry
Haematological examinations were performed on ten animals per sex from the satellite and main groups at 3, 6 and 12 months. Further haematological investigations were performed on 20 animals per sex from the main groups at 18 and 24 months. The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, prothrombin time, and activated partial thromboplastin time.
Blood chemical investigations were performed on ten animals per sex from the satellite groups at 6 and 12 months and from the main groups at 18 and 24 months. The following parameters were determined: urea, glucose, total protein, albumin, albumin/globulin ratio, sodium, potassium, chloride, calcium, inorganic phosphorus, ASAT, ALAT, alkaline phosphatise, creatinine, total cholesterol, total bilirubin, and cholinesterase.
Urinalysis
Urinalytical investigations were performed on ten animals per sex from satellite groups at 3, 6 and 12

months and from main groups at 18 and 24 months. The following measurements were made: specific gravity, volume, pH, protein, glucose, ketones, blood, urobilinogen, reducing substances and microscopic examination of sediment.

Sacrifice and pathology

Necropsy was conducted for all animals surviving until study termination (main groups: 104 weeks; satellite groups: 52 weeks) as well for all animals found dead or killed in extremis.

The following organ weights were determined from 10 rats per sex and main group and from all satellite animals: adrenals, brain, gonads, heart, kidneys, liver, spleen and thymus.

Tissue samples were taken from the following organs: adrenals, aorta (thoraic), bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions including palpable masses, head (pharynx, nasopharynx, paranasal sinuses), heart, Harderian gland, ileum (incl. Peyer's patches), jejunum, kidneys, liver, lungs (with bronchi), lymph nodes (cervical and mesenteric), mammary gland, muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands (submaxillary), sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina. A detailed histopathological examination was performed on all sampled tissues of the control and high- dose animals. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.

Histopathological examination was initially carried out on all tissues collected from control and high dose groups; all pre-terminally dead and moribund sacrificed rats and on all lesions and palpable masses of the terminally sacrificed rats from the low and mid dose groups.

Since there were no indications of treatment-related bone marrow changes, examination was subsequently extended to the remaining treatment groups.

Statistics

Where appropriate quantitative data was analysed by the ProvantisTM Tables and Statistics Module. For each variable, the most suitable transformation of the data was found; the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANCOVA and Bartletts's test. The transformed data was analysed to find the lowest treatment level that shows a significant effect, using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response is found, but the data shows non- homogeneity of means, the data will be analysed by a stepwise Dunnett (parametric) or Steel (non-parametric) test to determine significant differences from the control group. Finally, if required, pair-wise tests are performed using the Student t-test (parametric) or the Mann- Whitney U test (non-parametric).

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes.

1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of I or greater.
2. Kruskal-Wallis one way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

Results and discussion

Analysis of dose formulations

Stability assessment demonstrated that the test material preparations in the diet were stable for at least six weeks.

Analyses for achieved concentrations showed that the diet preparations were within an acceptable range. On one occasion the achieved concentrations of the low, mid and high-dose group were 79%,

83%, and 87%, respectively. At week 2 the concentration in the mid dose group was 112%. However, these isolated deviations from the nominal range were still considered to be acceptable.

Mortality

No significant treatment-related effects on mortality were observed during the study. The numbers of pre-terminal deaths in the main group are displayed below:

Table B.6.5-19: Cumulated mortalities after 104-week dietary exposure to glyphosate technical

Dose group (ppm)
Sex
0
1500
5000
15000-24000
Male
12
14
13
6
Female
14
17
15
12

Clinical observations

No significant treatment-related clinical observations occurred during the study.

There were no treatment-related effects on behavioural assessments, functional performance tests or sensory reactivity assessments observed.

Body weight

There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

Food consumption and compound intake

There were no treatment-related effects on food consumption or food efficiency for either sex noted during the study.

The group mean achieved doses are summarised below.

Table B.6.5-20: Group mean achieved dose levels

Dose group
Dietary concentration (ppm)
Mean achieved dose level (mg/kg bw/day)
Males
Females
Overall mean
1 (control)
0
2 (low)
1500
85.5
104.5

95.0
3 (mid)
5000
285.2
348.6
316.9
4 (high)
15000
Week 1-11
1077.4
1381.9
1229.7
17000
Week 12-15
19000
Week 16-26
21000
Week 27-39
24000
Week 40-104
The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 95.0, 316.9 and 1229.7 mg/kg bw/day for 1500, 5000, and 15000-24000 ppm, respectively. The mean intake values represent the combination of satellite and main group values.
Water consumption
There were no treatment-related effects on water consumption during the study.
Ophthalmoscopy
There were no treatment-related effects observed.
Haematology and clinical chemistry Haematology
All variations were considered to be incidental and unrelated to treatment because of the lack of either a true dose response, a consistent change throughout the study, a lack of progression of change with time and/or lack of concomitant effect in both sexes.
Clinical chemistry
At the highest dose level there was an increase in alkaline phosphatase activity for satellite group males and females compared with controls at 6 and 12 months. Main group males were also affected at 18 months. Values for all alkaline phosphatase activity values are presented as follows:
Table B.6.5-21: Alkaline phosphatase activity (IU/L)
Dose level
Control
Low
Intermediate
High
Timepoint
♂
♀
♂

♀
♂
♀
♂
♀
Month 6 (Satellite)
87.8
49.6
94.5
62.9
103.4
62.0
128.5**
91.9**
Month 12 (Satellite)
87.7
46.1
96.5
59.7
116.3*
58.1
140.2**
91.3**
Month 18 (Main)
93.3
65.7
110.5
55.8
110.9
70.9
125.0*
92.7
Month 24 (Main)
107.2
66.0
98.8
58.5
101.0
81.7
111.9
86.8
* $p < 0.05$; ** $p < 0.01$
The magnitude of the effect does not appear to increase with age plus the lack of a consistent effect for females does suggest this to be of limited toxicological importance.
At the 18 month evaluation there was an increase in plasma electrolytes for both sexes. Sodium and chloride values for males and females and potassium values for males only were increased compared

with controls. Female calcium levels were lower than controls. These elevations/decrements were also observed at lower dose levels but were not seen in a dose related trend. In addition at the 12 month evaluation for satellite females a lower sodium value was seen for females. Values for all calcium and chloride values are presented as follows:

Table B.6.5-22: Calcium and chloride values (mmol/L)

Dose level
Control
Low
Intermediate
High
Timepoint
♂
♀
♂
♀
♂
♀
♂
♀
Calcium
Month 6 (Satellite)
2.587
3.693
2.701
3.752
2.617
3.637
2.508
3.604*
Month 12 (Satellite)
2.530
2.602
2.543
2.587
2.458
2.475
2.514
2.483
Month 18 (Main)
2.231
2.775
2.523
2.645*
2.656
2.554**
2.598

2.468**
Month 24 (Main)
2.431
2.293
2.487
2.396
2.511
2.288
2.297
2.347
Chloride
Month 6 (Satellite)
107.7
105.8
107.1
106.1
107.0
106.1
108.5
106.7
Month 12 (Satellite)
105.6
103.9
105.1
104.8
104.3
104.7
105.9
104.2
Month 18 (Main)
103.3
101.8
105.8**
104.2**
105.8**
106.4**
107.6**
107.8**
Month 24 (Main)
104.5
103.4
104.4
103.1
104.3
102.2
105.4

102.8
* p < 0.05; ** p < 0.01
At intermediate level similar findings to the highest dose level were seen for plasma electrolytes at the 18 month evaluation. A slight increase in alkaline phosphatase activity was seen for satellite group males at 12 months. At the low dose level there was a similar effect on
the plasma electrolytes for both sexes at the 18 month evaluation of main group animals. Whilst these observations were seen at the highest dose level, the lack of dose response or the effect being limited to one sex does make the toxicological significance questionable.
All other differences were isolated in their finding and are therefore not toxicologically relevant.
Urinanalysis
There were no treatment-related effects observed.

Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed during the study period.
Organ weights
No effects on organ weight values were observed.
Histopathology
Adipose infiltration of the bone marrow was seen for the majority of animals examined, with both sexes being more or less equally affected in terms of incidence and severity. However, greater effects were seen among male rats dosed at the highest level and this attained statistical significance for terminal kill animals. This data indicates the possibility of myeloid hypoplasia as a consequence of treatment. However, given the normal variability of this condition and the influence of other pathological conditions upon marrow cellularity in ageing rats, the effect was not altogether convincing but cannot be dismissed. A similar effect was not seen among male rats in the remaining treatment groups but among premature deaths for animals of both sexes at the intermediate level and only low-dosed females. However, the variable duration of exposure and significant background pathology for premature death animals further negates this as an effect of treatment upon marrow cellularity for female rats.
Moreover, at the highest dose level there was a significant difference in the site of mineral deposition within the kidneys compared with controls. Pelvic mineralisation was commonly seen in both sexes and was more prevalent among female rats; however corticomedullary mineralisation was seen in female rats only. Nephrocalcinosis in rats is generally considered to be related to diet and hormonal status. There was a lower incidence of pelvic/papillary deposition and an increase in the corticomedullary deposition. At the same time there was a reduction in the incidence of renal pelvic hyperplasia in both sexes; which is considered to be a consequence of the decreased mineral deposition.
The effects on pelvic and corticomedullary mineralisation, and hyperplasia of the pelvic/papillary epithelium were confined to high dose animals with no indication of a similar effect at any other treatment level for either sex.
No other treatment-related changes were observed.
Neoplastic changes
No significant effects associated with tumour development were observed.
Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 24000 ppm (corresponding to 1229.7 mg/kg bw/day for combined sexes). It is concluded

that Glyphosate technical is not carcinogenic in rats.
RMS comment:
The study is acceptable. In principle, the conclusions are agreed with but the NOAEL is seen rather at the mid dose level of 5000 ppm (equal to 285 mg/kg bw/day in males) because of minor effects that were observed at the highest dose level. These effects comprised a by up to 9 % lower body weight gain in male rats, a transient increase in alkaline phosphatase activity (confirming the same findings in many other studies with glyphosate), fatty infiltration of the bone marrow that might be indicative of hypoplasia and the kidney findings of equivocal relevance. In addition, there were some histological skin changes that resemble those reported by (1997, ASB2012-11484, ASB2012-11485, ASB2012-11486, ASB2012-
11487). They comprised a slightly higher incidence of areas of necrosis or giant cell reaction to keratin and a non-significant increase in keratoacanthoma in high dose males (occurring in 6/51 animals as compared to 2/51 in the control group, 3/51 in the low and 0/51 in the mid dose groups; incidence in females always 0). However, the assessment of glyphosate as non- carcinogenic in this study is not doubted.
B.6.5.1.2 Previously known long-term studies in rats (already subject to EU evaluation)
1st study: 1981
Reference: IIA, 5.5.2/05
Report: (1981)
A Lifetime Feeding Study
of Glyphosate (ROUNDUP Technical) in Rats
Data owner: Monsanto Study/Project No.: 77-2062
Date: 1981-09-18
not published, TOX2000-595
Guidelines: Not stated; In general accordance with OECD 453 (1981)
Deviations: None
GLP: no
Acceptability: See RMS comment
Dates of experimental work: In-life: 1978-07-12 to 1980-09-04
Materials and methods
Test material:
Identification:
Glyphosate acid (Round-up technical material)
Description:
Fine White powder
Lot/Batch #:
XHJ-64
Purity:
98.7 % w/w
Stability of test compound:
At least 45 days when stored at -20 °C.
Vehicle and/
or positive control:
Diet
Test animals:
Species:

Rat
Strain:
Sprague-Dawley CD
Source:
Age:
28 days (on delivery), 41 days at initiation of delivery
Sex:
Males and females
Weight at dosing:
Males: 155.0 – 156.6 g (mean values); females: 136.0 –
138.4 g (mean values)
Acclimation period:
12 days.
Diet/Food:
Standard laboratory diet (Purina Lab Chow) ad libitum.
Freshly prepared weekly
Water:
Mains automated water system (Elizabethtown Water Company), ad libitum
Housing:
Individually in elevated stainless steel cages.
Environmental conditions:
Temperature: Monitored but values are not stated Humidity: not stated
Air changes: not stated 12 hours light/dark cycle
In life dates: 12-07-1978 to 04-09-1980
Animal assignment and treatment:
In a chronic toxicity and carcinogenic study, groups of 50 male and 50 female Sprague- Dawley rats were administered in the diet glyphosate (Round-up technical) at concentrations of 0, 30, 100 and 300 ppm for the first week and at concentrations of 0 (control) 3.05, 10.30
and 31.49 mg/kg bw/day for the males, and 0 (control), 3.37, 11.22 and 34.02 mg/kg bw/day for the females for 26 months. Males received treatment for 775 - 776 days and females 784 - 785 days before termination.
A 0.5 oz. sample of the test substance was taken at weeks 1, 11, 16, 24, 36, 48, 60, 72, 84 and 96 and submitted to the sponsor for analysis. Samples of control feed and feed for each dose level were collected for analysis.
Observations
Rats were examined for mortality and signs of toxicity twice daily. Detailed physical and clinical examinations were performed weekly and included palpations for tissue masses. Ophthalmic examination was done in all animals at the start of the study, at Week 52 and prior to termination. A functional observational battery including motor activity was conducted in Week 52 in animals allocated to the chronic toxicity assessment of the study.
Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 14 and every two weeks thereafter until termination.
Food consumption and compound intake
Food consumption was recorded pre-test, once weekly for from Week 1 to Week 14 and every second week thereafter until termination.

Water consumption

Water consumption was investigated during the 18 and 24 months of treatment over 2-three day periods in 10 animals/sex/dose group.

Laboratory investigations

Haematology and clinical chemistry investigations were performed on 10 males and 10 females of each dose group during Months 4, 8, 12, 18 and 24 months. Blood was obtained via venipuncture of the orbital sinus (retrobulbar venous plexus) under light ether anaesthesia. Animals were selected randomly; the same animals were used at all intervals when feasible. Rats were fasted overnight prior to blood collections and were not dosed until after samples were collected.

Haematological parameters investigated included haematocrit, haemoglobin, erythrocyte count, platelet count, total leukocyte count and differential leukocyte count. Clinical chemistry parameters were alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic acid dehydrogenase, blood urea nitrogen, fasting glucose, albumin, globulin, albumin/globulin ratio, total bilirubin and direct bilirubin, triglycerides, cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride. However as a result of a technician error, potassium was not evaluated at months 8 and 12 and inorganic phosphorus was evaluated at months 8 and 12 only.

Urinalysis was performed during the same months as for haematology and clinical chemistry except at 8 months and the parameters reported included gross appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, and blood and microscopic analysis.

Sacrifice and pathology

Necropsy was conducted on all animals which died prematurely or were killed at termination as scheduled. Organ weights were determined for all animals surviving to scheduled termination and included the adrenals, brain, gonads, heart, thyroid, kidneys, liver, pituitary and spleen.

Samples from organs and tissues including the adrenals, aorta, blood smears, bone & bone marrow (costochondral junction), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eyes (retina, optic nerve), gross lesions including palpable masses, Harderian gland, heart, intestines (Including the caecum, colon, duodenum, ileum and jejunum), kidneys, lachrymal gland, larynx, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, muscle, oesophagus, ovary, pancreas, pharynx, pituitary, prostrate, rectum, salivary glands (submandibular, parotid), seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and vagina.

Statistics

Parameters analyzed statistically were bodyweight, food consumption, haematology and clinical chemistry values, terminal organ and body weights, organ/body weight ratios and organ/brain weight ratios.

Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control. A statistical test for trend in the dose levels was also performed. In the parametric case (i.e. equal variance) standard regression techniques with a test for trend and lack of fit were used. In the

nonparametric case Jonckheere's test for monotonic trend was used. The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

Results and discussion

Analysis of dose formulations

Results of diet analyses were not reported.

Mortality and clinical signs

There was no significant difference between the control and treated both sexes with regard to the survival rate during the course of this study. Survival was approximately 80-90% through Month 20 of the study for all groups. Thereafter, significant reductions in the number of surviving animals occurred in all groups in roughly an equivalent fashion, culminating in the termination of the study at Month 26. At this time, survival had decreased to 30% in the low dose males and the high dose females, requiring that the study be terminated to insure a sufficient number of animals at the terminal necropsy. At 24 months, survival levels equalled or exceeded 50%, which is comparable to historical control data for rats of this strain.

The survival rates are displayed in Table B.6.5-23.

There were no treatment-related signs of toxicity and physical findings observed including alopecia, excessive lacrimation, nasal discharge and rales were present in all groups without a treatment-related trend.

Table B.6.5-23: Survival rates during up to 26-month dietary exposure to glyphosate technical (%)

Dose group (mg/kg bw/day)
Males
Females
0
3.05
10.30
31.49
0
3.37
11.22
34.02
Month 20
74
86
86
96
92
88
88
76
Month 24
44
56
46
66
52

62
64
48
Month 26
30
52
32
52
36
46
60
30
Functional observations
A functional observational battery of tests was not performed. It is not considered to affect the validity of this study.
Body weight
There were no statistically significant differences in mean body weights in males. During part of the growth period, a slight but consistent trend toward reduced body weights in the treated males was evident. The maximum decrease was approximately 6% in high dose males. Thereafter, this difference decreased resulting in little difference in mean body weights between groups at termination. Because this effect was slight and not evident at termination of the study and did not affect survival, it is not considered to be toxicologically significant. In females, no statistically significant difference in mean body weights was observed in treated
animals compared to the controls up to Month 19 of the study. However, for the following 2 months, the treated groups showed statistically significant reductions in mean body weights as compared to the control, although not in a dose-related fashion. The magnitude of the reduction ranged between 10-15% with the greatest difference evident in the low and mid- dose group. Thereafter, the treated females gained weight relative to the control group resulting in nearly identical group mean body weights at termination. The absence of a dose- response relationship in this observation suggests the finding was most likely due to biological variation which is evident from the standard deviation of the mean body weights for all dose groups. The body weight changes are noted to have occurred well after the main growth phase in both sexes and lacked a dose- response relationship in females. The top dose was only statistically significantly reduced compared with controls during weeks 92 and 94 when body weight reduction was approximately 11.5% and 11% respectively; however at the next measurement during Week 96 body weight reduction compared with controls was only 5.7 %.
Food consumption and compound intake
Occasional statistically significant differences were noted in the treated animals of both sexes relative to their respective controls. However, these changes in mean food consumption values were slight and occurred sporadically and showed no treatment-relationship.
The target concentrations for treatment as administered in the first were 0 (control), 30, 100 and 300 ppm which corresponded to approximate compound intakes of 0, 3.05, 10.30 and
31.49 mg/kg bw/day for males and 0, 3.37, 11.22 and 34.02 mg/kg bw/day.
Water consumption
There were no treatment-related effects observed.
Laboratory investigation Haematology
Haematology data did not indicate any toxicologically significant differences in the findings

sexes for any of the parameters evaluated. All mean data were within the normal physiological range for the laboratory rat. The few statistically significant differences noted appeared to be due to random variation as no consistent treatment-related pattern was evident. Thus, haematological parameters were unaffected by the treatment of glyphosate.

Clinical chemistry

Clinical chemistry parameters for both males and females were within the normal physiological range and did not deviate significantly in a treatment-related manner from controls. Occasional statistically significant differences were noted, but these appear to be due to random fluctuation, as no treatment-related pattern emerged.

Urinalysis

Urinalysis parameters did not show any significant differences were between treated and control groups. Occasional values outside the normal range were found; however, these values occurred sporadically exhibiting no consistent pattern..

Necropsy

Gross pathology

There were no treatment-related gross pathological findings.

Organ weights

There were no statistically significant differences noted in the terminal organ weights, organ/body weight ratios and organ/brain weight ratios of the treated animals compared with their respective controls.

Histopathology Neoplastic changes

The most common tumours were found in the pituitary in both sexes of both control and treated animals

In the females, mammary gland tumours were the next most common neoplasm found. In general, the incidence of all neoplasms observed in the treated and control animals were to a similar degree, or occurred at low incidence such that a treatment-related association could not be made (Table B.6.5-24).

Table B.6.5-24: Summary of critical tumour findings in 26-month dietary study with glyphosate technical

Dose group (mg/kg bw/day)

Males

Females

Dose Groups

0

3.05

10.3

31.49

0

3.37

11.22

34.02

Pituitary tumours

Adenomas

16/48

19/49

20/48

18/47
34/48
29/48
31/50
26/49
Carcinomas
3/48
2/49
3/48
1/47
8/48
7/48
5/48
12/49
Combined
19/48
21/49
23/48
19/47
42/48
36/48
36/50
38/49
However, the incidence of interstitial cell tumours of the testes in male rats in both the scheduled terminal sacrifice animals as well as for all animals suggested a possible treatment- related finding and was presented along with the most recent historical control data at the time of the study for comparison. It was noted that at 12 months the incidence of interstitial tumours was near zero however in animals aged 24-29 months at necropsy, the incidence increased to approximately 10%. The historical control data for chronic toxicity and carcinogenicity from 5 studies terminated at 24-29 months showed background levels of interstitial cell tumours comparable to that found at the highest dose in the study. The incidence of interstitial cell hyperplasia did not provide evidence of a preneoplastic lesion.

Table B.6.5-25: Summary of the interstitial cell tumour findings in the testes of rats after 26-month dietary exposure to glyphosate technical

Dose group (mg/kg bw/day)*
Parameter
0
3.05
10.3
31.49
Interstitial cell tumour
Terminal sacrifice
0/15
(0%)
2/26
(7.7%)

1/16
(6.3%)
4/26
(15.4%)
All Animals
0/50
(0%)
3/50
(6%)
1/50
(2%)
6/50
(12%)
Interstitial cell hyperplasia
Terminal sacrifice
1/15
(6.7%)
1/26
(3.8%)
0/16
(0%)
0/26
(0%)
All Animals
1/50
(2%)
1/50
(2%)
1/50
(2%)
0/50
(0%)
*number of animals affected / total number of animals examined ( ): Percentage
Table B.6.5-26: Summary of the concurrent historical control data for interstitial cell tumours in the testes of in chronic toxicity studies
Parameter
Study
1
2
3
4
5
Terminal
Sacrifice
4/65
(6.2%)

3/11
(27.3%)
3/26
(11.5%)
3/24
(12.5%)
3/40
(7.5%)
All Animals
4/116
(3.4%)
5/75
(6.6%)
4/113
(3.5%)
6/113
(5.3%)
5/118
(4,2%)
*number of animals affected / total number of animals examined ( ): Percentage
The investigators noted that at terminal sacrifice, the incidence in the present study was 15.4% (4/26), while the range in control animals from 5 contemporary studies (historical controls) was 6.2% (4/65) to 27.3% (3/11) with an overall mean value of 9.6% (16/166). When all animals on test are included, the incidence for the Group IV males was 12% (6/50) compared to a range of 3.4% (4/116) to 6.7% (5/75) with a mean of 4.5% (24/535). Therefore, this comparison suggests an incidence of this tumour in the Group I males which is slightly lower (0%), and an incidence in the Group IV males which is slightly higher than recent historical control data. Although an effect on the incidence of this tumour due to the administration of the test substance cannot be ruled out, the data suggests that the incidence in treated rats is within the normal biological variation observed for tumours at this site in this strain of rat.
Conclusion by the Notifier
Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 24 month is 31.5 mg/kg bw/day in males and 34.0 mg/kg bw/day in females. It is concluded that glyphosate technical is not carcinogenic in rats. This old study, initiated before the establishment of regulatory testing guidelines, no longer meets current testing guideline criteria due to the low doses employed. Therefore, this study type was repeated by Monsanto with higher doses, in accordance with subsequent regulatory test guidelines.
RMS comment:
It is agreed that this study does not comply to modern standards and should not taken into consideration for evaluation of glyphosate any longer although the NOAEL is agreed with. In particular, the dose levels selected were much too low (in particular to assess carcinogenicity) and there were serious reporting deficiencies.
2nd study: , 1990
Reference: IIA, 5.5.2/06
Report: (1990)
Chronic study of glyphosate administered in feed to Albino rats Monsanto Agricultural Company, St.

Louis, Missouri, USA Data owner: Monsanto
Project No.: ML-87-148 Date: 1990-09-26
not published, TOX9300244
Guidelines: US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982); in general accordance with OECD 453
Deviations:
(From OECD 453: only 10 rats/sex for interim sacrifice; overall survival at termination was below 50%)
GLP:
yes
Acceptability:
See RMS comment
Dates of experimental work: 1987-08-05 – 1989-08-10
Materials and methods
Test material:
Identification:
Glyphosate
Description:
White solid
Lot/Batch #:
XLH-264
Purity:
96.5 %
Stability of test compound:
Guaranteed for the study period. Confirmed by analysis.
Vehicle and/
or positive control:
Diet
Test animals:
Species:
Albino Rat
Strain:
Sprague-Dawley (CD)
Source:
Age:
Approx. 8 weeks (at start of study)
Sex:
Males and females
Weight at dosing:
Males: approx. 284 g; females: approx. 221 g
Acclimation period:
29 days
Diet/Food:
Purina Mills certified Rodent Chow #5002 (Purina Mills),
ad libitum
Water:

Mains drinking water, ad libitum
Housing:
In stainless steel cages with wire mesh bottoms suspended over paper bedding
Environmental conditions:
Animal housing & husbandry were in accordance with the provisions of ´Guide to the Care and Use of Laboratory Animal´; USPHS-NIH Publ. No. 85-23
Temperature: 17.8 – 21.1 °C
Humidity: 40 – 70% Air changes: not specified 12 hours light/dark cycle
In life dates: 1987-08-05 to 1989-08-10 Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 2,000, 8000 and 20000 ppm glyphosate (equivalent to mean achieved dose levels of 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females) for 24 months.
A further ten animals per sex were added to each group and were designated for interim kill after 12 month to study chronic toxicity and non-neoplastic histopathological changes.
Test diets were prepared in approximately weekly intervals by mixing a known amount of the test substance with basal diet. The stability of the dietary formulations were determined by analysis of samples of the low- and high-dose levels after storage at room temperature for 7 and 14 days, and frozen after storage for 35 days. The homogeneity of the test substance in the diet was determined for the low- and high-dose level preparations in the first and 88th week of the study. Analyses for achieved concentrations were done for all dose levels for the first six weeks, and for at least one dose level in weekly intervals thereafter. The stability of the neat test substance was verified by analysis before the start of the study, during month 8, 14 and 21, and after termination.
Clinical observations
All rats were examined for mortality and clinical signs of toxicity twice daily. Detailed clinical observations were conducted weekly. An ophthalmic examination was done in all animals before the start of the study, and prior to termination.
Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13 and every four weeks thereafter until termination.
Food consumption and compound intake
Food consumption was recorded at weekly intervals for the first 13 weeks, and every fourth week thereafter.
Haematology and clinical chemistry
Blood was collected from 10 fasted animals per sex and group at Months 6, 12, 18, and at termination. The following parameters were measured: haematocrit, haemoglobin, total erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, total bilirubin, direct bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.
Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses at Month 6, 12, 18 and prior to termination. Sampling was done over a period of about 18-hours via metabolism trays. The following parameters were determined: appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, urobilinogen and blood. In case that blood and / or protein in

excess of the control urine samples were found, the sediment was examined for the presence of bacteria, epithelial cells, erythrocytes, leukocytes, casts or abnormal crystals.

Sacrifice and pathology

A gross necropsy was conducted on all surviving animals at scheduled sacrifice after 12 and 24 month. The following organ weights were determined: brain, kidneys, liver, and testes with epididymides.

Tissue samples were taken from the following organs and subjected to a histopathological examination: adrenals, aorta, bone & bone marrow, brain, caecum, colon, duodenum, eyes, gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung (with main stem bronchi), lymph nodes (mesenteric and submandibular), muscle, nasal turbinates, oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, sciatic nerve,
seminal vesicles, skin (with mammary tissue), spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary salivary gland, testes with epididymis, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (corpus and cervix).

Statistics

Dunnett's Multiple Comparison Test (two-tailed) was used for body weights, cumulative body weight changes, food consumption, absolute leukocyte counts, reticulocyte counts, urine pH, urine specific gravity and clinical chemistry data obtained at Months 6, 12 and 18. Fisher´s exact test (one-tailed) was used for incidence of selected ocular lesions, as well as in combination with Bonferroni inequality procedure for incidences of non-neoplastic (at $p \leq 0.01$) and neoplastic lesions (at $p \leq 0.01$ and ≤ 0.05). EHL decision tree analysis was used for evaluation of terminal haematology, clinical chemistry, body weight, absolute and relative organ weight data and organ to brain weight ratios. Depending on the results either parametric (Dunnett´s Test and linear regression) or nonparametric (Kruskal-Wallis, Jonckheere´s and / or Mann-Whitney Tests) were applied. Mortality data were analysed by SAS lifetable procedure, and Peto Analysis was used for evaluation of histopathological data.

Results and discussion

Analysis of dose formulations

The stability analyses proved the neat test substance to be stable throughout the study period. The stability and homogeneity of glyphosate in diet at concentrations of 2000 and 20000 ppm was satisfactory. The mean achieved concentrations of glyphosate in each dietary preparation were 95 % of the nominal concentration.

Mortality

There were no statistically significant differences in the group survival rates. The percentage of survival in each of the dose groups are summarised below.

Table B.6.5-27: Percentage survival at termination after 24-month dietary exposure to glyphosate

Dose group (ppm)

Sex

0

2000

8000

20000

Male

29

38

34

34
Female
44
44
34
36
Clinical observations
There were no treatment-related clinical sings noted except the ophthalmological findings (see below).
Body weight
There were no effects on body weight noted in males of any dose group. In high-dose females body weights were statistically significant reduced from Week 7 through approximately the 20th month. During this time, absolute body weights gradually decreased to 14 % below the control value. The maximum difference in body weights was observed at 20th month. At this time-point the cumulative body weight gain in high-dose females was 23 % lower as compared to controls
There were no treatment-related effects in females fed 2000 or 8000 ppm glyphosate.
Food consumption and compound intake
There were no statistically significant decreases in food consumption in any group of either sex during the study period. However, significant increased food consumption was noted frequently in high-dose males, and on some occasions in low-dose males. The group mean achieved doses are summarised below.
Table B.6.5-28: Group mean achieved dose levels
Dose group
Dietary concentration
(ppm)
Mean achieved dose level (mg/kg bw/day)
Males
Females
1 (control)
0
0
0
2 (low)
2000
89
113
3 (mid)
8000
362
457
4 (high)
20000
940
1183
Ophthalmoscopy
There were no treatment-related ocular effects observed in females of any

males of the low-, and mid-dose group. In high-dose males a statistically increased incidence ($p \leq 0.05$) of cataractous lens changes were observed at the ophthalmic examination prior to termination. However, the observed incidence of 25% was within the historical control range of 0-33 %. A second independent ophthalmic examination also performed prior to termination confirmed a statistically significant increase ($p \leq 0.05$) in the incidence of cataractous lens changes in high-dose males (1/14 (control) compared to 8/19 (high dose)). The results are summarised in the table below.

Table B.6.5-29: Incidences of cataract and lens fibre degeneration in males observed during ophthalmic examinations

Dose group (ppm in diet)*	0	2000	8000	20000
1st examination	0/15	1/22	3/18	5/20**
2nd examination	0/14	2/22	3/17	8/19**

* number of rats affected / number of rats examined

** statistically significant from control ($p \leq 0.05$)

The histopathological examination confirmed a slightly, but not statistically, increased incidence of degenerative lens changes (i.e. cataract and/or lens fibre degeneration) in high- dose males (see Table B.6.5-30 below).

Table B.6.5-30: Histopathological confirmed incidences of cataract and lens fibre degeneration in males

Dose group (ppm in diet)*	0	2000	8000	20000
Terminal sacrifice	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

* Number of rats affected / number of rats examined

Due to the small number of rats examined ophthalmologically and affected at termination, the results are difficult to interpret. Nevertheless, the occurrence of degenerative lens changes in high-dose males appears to be exacerbated by treatment.

Haematology and clinical chemistry

Haematology and clinical chemistry evaluations noted various changes in the examined parameters. However, the changes were not consistently noted at more than one time point, were within historical control ranges, small in magnitude, and/or did not occur in a dose- related manner. Therefore, they were considered to be either unrelated to treatment or toxicologically insignificant. The statistically increased alkaline phosphatase level observed in high-dose females at termination was mostly due to an extremely high value for one animal. However, this finding is in line with observation made in other long-term studies in rats.

Urinanalysis

Urine specific gravity was statistically significant increased at the Month 6 examination. The observed statistically significant decreased urinary pH at 6, 18 and 24 months might be related to the renal excretion of glyphosate, which is an acid.

Necropsy

Gross pathology

There were no treatment-related gross pathological findings observed at necropsy.

Organ weights

At interim kill after 12 months relative liver weights were slightly, but statistically significant increased in high-dose males. At terminal sacrifice absolute liver weights, as well as liver to brain weight ratios were also statistically increased in high-dose males. There were no other significant and dose-related effects on organ weights.

Histopathology

Non-neoplastic lesions

Apart from the eye findings mentioned above histopathological examination showed only one other lesion that reached statistical significance. This was an increased incidence of inflammation of the stomach squamous mucosa in females fed 8,000 ppm glyphosate (see Table B.6.5-31).

Table B.6.5-31: Incidence of inflammation and hyperplasia of the stomach squamous mucosa

Dose group (ppm in diet)*
0
2000
8000
20000
Males
Inflammation
2/58
3/58
5/59
7/59
Hyperplasia
3/58
3/58
4/59
7/59
Females

Inflammation
0/59
3/60
9/60**
6/59
Hyperplasia
2/59
3/60
7/60
6/59
* Number of rats affected / number of rats examined
** statistically significant at p≤ 0.01 (Fisher exact test with Bonferroni inequality)
Although the incidence of this lesion in mid-dose females (15 %) was slightly outside the historical control range (0 – 13.3 %) for the laboratory, there was no dose-related trend across all groups of females, and there was also no significance difference in male rats. Therefore, this finding is considered to be incidental and not related to treatment with glyphosate.
Neoplastic lesions
The only statistically significant difference in neoplastic lesions was an increased incidence of pancreatic islet cell adenomas observed in low-dose males (see Table B.6.5-32). The incidence (14%) in low-dose males was outside the historical control range (1.8 – 8.5 %) for this laboratory, but was in the historical control range (≥ 17 %) observed in reports from other laboratories. In addition, there was no dose-related trend for this finding in the male groups, as indicated by the lack of statistical significance in the Peto trend test. Due to the lack of a dose-related proliferative effect (hyperplasia) and or progression (carcinoma) of this lesion, and as such effects were not observed in females, this finding was not considered to be treatment-related.
Table B.6.5-32: Incidence of pancreatic islet cell findings
Finding
Sex
Dose group (ppm in diet)*
0
2000
8000
20000
Hyperplasia
Males
2/58
0/57
4/60
2/59
Females
4/60
1/60
1/60
0/59
Adenoma
Males

1/58
8/57**
5/60
7/59
Females
5/60
1/60
4/60
0/59
Carcinoma
Males
1/58
0/57
0/60
0/59
Females
0/60
0/60
0/60
0/59
* Number of rats affected / number of rats examined
** Statistically significant at p≤ 0.01 (Fisher exact test with Bonferroni inequality)
Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate for 24 month is 8000 ppm (corresponding to 362 mg/kg bw/day in males and 457 mg/kg bw/day in females). It is concluded that glyphosate is not carcinogenic in rats.
RMS comment:
The study is considered acceptable. However, based on stomach mucosal irritation (although not strictly dose-related) at the two upper dose levesl, a NOAEL of 2000 ppm (89 mg/kg bw/day) was established. Further toxic effects on body weight, liver and eyes (cataracts) were apparently confined to the top dose level.
3d study: 1993
Guidelines:
US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982)
Deviations:
None
GLP:
yes
Acceptability:
See RMS comment.
Dates of experimental work: 1990-02-16 to 1992-03-09
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:

White powder
Lot/Batch #:
229-Jak-5-1; 229-Jak-142-6
Purity:
98.9 %; 98.7 %
Stability of test compound:
At least two years at ambient temperature in the dark
Vehicle and/
or positive control:
Diet
Test animals:
Species:
Rat
Strain:
Sprague-Dawley
Source:
Age:
Approx. 4 weeks upon arrival at testing facility
Sex:
Males and females
Weight at dosing:
Males: 85 ± 5 g, females: 60 ± 5 g
Acclimation period:
14 days
Diet/Food:
SQC Expanded (Fine Ground) Rat and Mouse Maintenance Diet No. 1 (Special Diet Services Limited, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
In groups of five per sex in suspended polypropylene cages with stainless steel wire grid tops and bottoms
Environmental conditions:
Temperature: 20 ± 2 °C
Humidity: 55 ± 10 % Air changes: 15 – 20 / hour 12 hours light/dark cycle
In life dates: 1990-02-16 to 1992-03-09 Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 10, 100, 300 or 1000 mg/kg bw/day glyphosate technical. An additional five groups with 35 rats per sex receiving daily dietary doses of 0, 10, 100, 300, or 1000 mg/kg bw/day were included for the toxicity study. Fifteen rats per sex and per dose of the toxicity study were scheduled for interim sacrifice after 12 months. The dose levels were selected based on the results of a 13-week dietary toxicity study in rats.
Test diets were prepared once per week for the first 13 weeks and at least once every two weeks thereafter by direct admixture of the test substance to the plain diet and mixing for 20 minutes. Analyses for achieved concentrations of the test substance in the diet were conducted from formulated diets at approximately fortnight intervals for the first 12 weeks and in intervals of 2

month thereafter.
The stability and homogeneity of the test substance in the diet was determined prior to the start of the study.
Clinical observations
A check for mortality was made twice daily on all animals throughout the study. In addition, all animals were examined for clinical signs during each day. A detailed clinical examination and check for palpable masses were done once each week on every animal. An ophthalmoscopic examination was conducted on 20 rats per sex of each group of the oncogenicity study before the start of the study and on 20 rats per sex of the control and high- dose group of the oncogenicity study at weeks 24 and 50. In addition, an opthalmoscopic examination was conducted on all control and high-dose rats of the oncogenicity and toxicity study at week 102.
Body weight
Individual body weights were recorded for each animal before dosing, at weekly intervals until the end of week 13 and approximately every 4 weeks thereafter until termination.
Food and water consumption and compound intake
Food consumption was recorded once weekly for each cage group starting one week before treatment until Week 13 and subsequently every 4 weeks until termination. Water consumption was monitored by visual inspection throughout the study period.
Achieved dosages were calculated from nominal dietary concentration, taking into account actual food consumption and body weight data.
Haematology and clinical chemistry
Individual blood samples for haematology and clinical chemistry evaluations were collected from the orbital sinus of 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point.
Individual blood smears for differential blood counts were taken from the tail vein after approximately 52, 78, and 103 weeks of dosing from all surviving animals of the oncogenicity study.
Haematology
The following parameters were measured: Haemoglobin, haematocrit, total erythrocyte count total leukocyte count, differential leukocyte count, platelets, and clotting time. Absolute indices were calculated.
Differential blood counts were evaluated with blood smear samples from all control and high- dose animals of the oncogenicity study at weeks 53 and 79. In addition, differential blood cell counts were evaluated on all surviving animals of the oncogenicity study at week 104.
Blood chemistry
The following parameters were measured: Total proteins, albumin, albumin-globulin ratio, ALT, AST, ALP, blood urea nitrogen, blood glucose, sodium, potassium, chloride,
cholesterol, creatinine, calcium, phosphate, total bilirubin, plasma cholinesterase, creatinie phosphokinase and red blood cell cholinesterase.
Brain cholinesterase activity determination
Brain cholinesterase activity was determined from 10 rats per sex from each dose group at the week 52 and 104 necropsies. Approximately 0.5 g of brain was removed at the week 52 and 104 necropsies and stored at -20°C until analysis.
Urinalysis
Individual urine samples were collected from 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point. Samples were collected over a period of 4 hours of food and water

deprivation in metabolism cages. The following measurements were made: volume, specific gravity, pH, urobilinogen, bilirubin, blood pigments, protein, glucose, ketones, microscopy of sediments.

Sacrifice and pathology

At interim kill after 52 weeks 15 rats per sex from each toxicity study group were sacrificed and necropsied. All remaining toxicity study and surviving oncogenicity study animals were killed and necropsied after 104 weeks. All pre-terminally dead and moribund sacrificed rats were also necropsied.

The following organs were weighed from all interim kill animals of the toxicity study and from 10 rats per sex per group of the oncogenicicty study: adrenals, brain, heart, kidneys, liver, lungs, ovaries (with fallopian tubes), parotid salivary glands, pituitary, prostrate, sublingual and submaxillary salivary glands (weighed together), spleen, testes including epididymides, thymus and uterus.

The following organs were collected: adrenals, aortic arch, any abnormal tissue, bladder, bone and bone marrow (sternum and rib), brain, ears, eyes, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric and submandibular), muscle (thigh), nasal cavity (oncogenicity study only), oesophagus, optic nerve, ovaries (with fallopian tubes), pancreas, parotid salivary glands, pituitary, prostrate, sciatic nerve, seminal vesicles, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach (glandular and non-glandular), sublingual salivary glands, submaxillary salivary glands, testes with epididymes, thymus, thyroid/parathyroid, tongue, trachea, uterus and vagina.

A detailed histopathological examination was performed on all tissues collected from the control and high-dose animals at interim kill, all oncogenicity study animals, and all animals that died or were killed in extremis. In addition, a histopathological examination of the liver, kidneys and lungs was performed on all other toxicity study animals at interim kill and all oncogenicicty study animals. Furthermore, the salivary glands of all low- and mid-dose animals at interim kill and the oncogenicity study were examined.

Statistics

Haematology, clinical chemistry, organ weight and body weight data were analysed for homogeneity of variance using the F-max test. If the group variances appeared homogeneous a parametric ANOVA was used and pair wise comparisons made via Student's t-test using Fisher´s F-protected LSD. If the variances were heterogeneous log or square root, transformations were used. If the variances remained heterogeneous a non-parametric test (e.g., Kruskal-Wallis ANOVA) was used. Organ weights were also analysed conditional on body weight (i.e., ANOVA). Differences in survival between the control and test substance

groups from the oncogenicity study were assessed graphically using Kaplan-Meier plots and tested formally using the Gehan-Wilcoxon test. Because no notable survival differences were evident, histological lesion incidences were analysed using Fisher Exact test.

Results and discussion

Analysis of dose formulations

Analyses for achieved concentrations showed that the diet preparations of all dose groups were within an acceptable degree of accuracy (± 10%).

Mortality

There were 336 pre-terminal deaths throughout the study. There was no evidence to suggest that any of these deaths were treatment related. There were also no significant treatment- related effects on the survival times in the oncogenicity study.

The numbers of pre-terminal deaths are summarised in Table B.6.5-33 below.

Table B.6.5-33: Cumulated mortalities after 104-week dietary exposure to glyphosate technical

Dose group (mg/kg bw/day)*
Sex
0
10
100
300
1000
Male
27/85
32/85
25/85
26/85
26/85
Female
42/85
41/85
42/85
40/85
35/85
*number of dead / total number
Clinical observations
The only notable clinical sign was pale faeces, from weeks 16-104, the majority or all the cages of animals (males and females) in the 300 and 1000 mg/kg /day dose groups had pale faeces. However, this clinical sign was not considered to be toxicologically significant. There were no other notable clinical signs considered to be treatment related.
Opthalmoscopy examinations demonstrated no inter-group differences.
Body weight
The high-dose group males and females had statistically significant reductions in body weight throughout the study. Reductions started at week one of dosing and were still apparent at week 104. The high-dose group males displayed the greatest reduction in body weights and body weight gains. The mean body weight gain data are summarised in Table B.6.5-34 below.
Table B.6.5-34: Body weight development (mean values) after 52 and 104-week dietary exposure to glyphosate technical – oncogenicity study
Dose group (mg/kg bw/day)
0
10
100
300
1000
♂
♀
♂
♀
♂
♀
♂

♀
♂
♀
Weight gain (g)
(0-52 weeks)
514
265
498
285
523
270
500
274
450
243
% of control
--
--
97
108
102
102
97
103
88
92
Weight gain (g)
0-104 weeks
635
376
609
445
644
391
623
405
549
333
% of control
--
--
96
118
101
104
98

108
86
89
Food and water consumption and compound intake
There were no treatment-related effects on food and water consumption for either sex noted during the study.
The overall group mean achieved doses are summarised in Table B.6.5-35 below.
Table B.6.5-35: Group mean achieved dose levels – oncogenicity study
Dose group
Nominal dose (mg/kg bw/day)
Mean achieved dose level (mg/kg bw/day)
Mean achieved dose level (% of nominal)

Males
Females
Males
Females
1 (control)
0
--
--
--
--
2 (low)
10
10
10
100
100
3 (mid I)
100
101
103
101
103
4 (mid II)
300
306
311
102
104
5 (high)
1000
1007
1018
101

Over the entire study duration the mean achieved dosages in all groups were close to the nominal.
Haematology and clinical chemistry Haematology
Haemoglobin, haematocrit and mean corpuscular haemoglobin were occasionally increased in 100 and 1000 mg/kg bw/day dose group males. Haemoglobin was also increased in males from the 300 mg/kg bw/day dose group and females from the 1000 mg/kg bw/day group. Females of the 1000 mg/kg bw/day dose group also had increased levels of mean corpuscular haemoglobin.
The haematological changes were not considered to be treatment related due to the lack of a clear dose–response relationship. In addition, the differences observed were rather small and no consistent trend became obvious throughout the study. In the absence of any histopathological change these small increases are not considered to be of toxicological significance (see Table B.6.5-36).
Clinical chemistry
Clinical chemistry measurements showed significant increased alkaline phosphatase levels in males at 1000 mg/kg bw/day and in females at 100, 300 and 1000 mg/kg bw/day. Although the increases were of small magnitude they were consistent and might be treatment-related. However, in the absence of any histopathological changes these small changes are not considered to be of toxicological significance (see Table B.6.5-37). All other changes in clinical chemistry parameters were not considered to be treatment-related.
Table B.6.5-36: Haematology findings (group mean values)
Dose group (mg/kg bw/day)
0
10
100
300
1000
♂
♀
♂
♀
♂
♀
♂
♀
♂
♀
Haemoglobin
(g/dL)
Week 14/15
15.6
15.5
15.8
15.0*
16.2
15.0*
16.2

15.5
16.2
15.9
Week 25/26
15.3
15.2
15.5
14.9
16.1***
14.9
15.9*
15.4
16.4***
15.6
Week 51/52
15.3
14.7
15.5
14.6
15.9
14.5
15.4
14.7
15.6
15.3*
Week 78/79
15.1
14.1
14.3
13.8
15.7
14.4
14.6
14.4
15.4
15.1
Week 102/103
14.0
12.1
13.1
13.6
14.3
13.1
13.8
13.3
14.6

12.9
Haematocrit
(L/L)
Week 14/15
0.397
0.396
0.405
0.386
0.406
0.387
0.407
0.395
0.411
0.407
Week 25/26
0.388
0.392
0.389
0.389
0.409**
0.384
0.399
0.398
0.409**
0.403
Week 51/52
0.406
0.394
0.415
0.388
0.415
0.386
0.410
0.392
0.414
0.408
Week 78/79
0.405
0.382
0.386
0.375
0.415
0.382
0.392
0.387
0.411

0.406
Week 102/103
0.392
0.343
0.365
0.381
0.394
0.367
0.387
0.369
0.401
0.363
MCH (pg)
Week 14/15
21.3
22.6
21.1
22.5
21.7
22.4
21.9
22.4
21.8
22.8
Week 25/26
21.2
22.4
21.4
22.4
21.9
22.4
21.9
22.2
22.0
22.8
Week 51/52
20.2
22.1
20.1
22.3
21.1*
22.1
20.8
22.2
20.9*
22.7

Week 78/79
20.1
22.3
19.7
22.4
20.8*
22.4
20.6
23.0
20.9*
23.1**
Week 102/103
20.4
22.3
20.1
22.3
20.1
22.0
20.9
22.6
20.6
22.7
WBC (x 10-
9/L)
Week 14/15
14.0
12.0
14.5
13.3
13.4
12.0
13.7
11.1
14.2
12.0
Week 25/26
13.4
8.8
13.2
10.3
11.8
9.9
12.2
8.9
12.7
10.5

Week 51/52
12.8
7.9
13.7
9.1
11.7
7.7
12.9
7.4
12.4
8.8
Week 78/79
12.4
7.7
13.6
7.3
10.9
8.1
13.6
6.8
10.6
7.0
Week 102/103
10.5
10.1
12.2
7.1*
10.3
6.4**
11.6
7.3*
9.5
8.4
Lymphocytes
(x 10-9/L)
Week 14/15
11.7
10.8
12.6
11.9
12.0
10.9
11.8
9.2
12.2
10.7

Week 25/26
10.7
7.1
10.8
8.2
9.6
8.1
10.1
7.4
10.3
8.6
Week 51/52
10.9
6.5
11.0
7.4
9.7
6.6
10.8
6.0
10.3
7.5
Week 78/79
10.0
5.7
10.3
5.6
8.7
6.4
10.1
4.8
8.5
5.6
Week 102/103
7.6
5.7
8.0
4.8
7.3
4.3**
7.8
4.7*
6.7
5.6
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
Table B.6.5-37: Clinical chemistry findings (group mean values)

Dose group (mg/kg bw/day)
0
10
100
300
1000
♂
♀
♂
♀
♂
♀
♂
♀
♂
♀
ALP (IU/L)
Week 14
287
182
329
158
320
213
334
223
461***
244*
Week 25
251
148
272
152
267
201*
306
227**
367**
225**
Week 51
308
144
293
143
310
190*

353
195*
403
221**
Week 78
258
124
286
139
284
172
351*
207**
414***
186*
Week 102
212
190
265
161
287*
193
267
228
365***
286*
* p < 0.05; ** p < 0.01; *** p < 0.001
Urine analysis
Urinary pH was slightly reduced in males at 1000 mg/kg bw/day. This change was consistent with that found in a previously conducted 13-week toxicity study with glyphosate.
Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed at the interim and terminal kill necropsies.
Organ weights
At the interim kill (week 52) absolute liver weights were reduced in males and females at doses of 100 mg/kg bw/day and above. However, for males this finding was not confirmed by the sensitive means of covariance analysis, i.e., with correction for final body weight. Absolute adrenal weights were reduced in males at 300 and 1000 mg/kg bw/day. However, this finding was also not confirmed by the sensitive means of covariance analysis, i.e., with correction for final body weight.
At the terminal kill (week 104) no statistical significant decrease in liver and adrenal weights was noted in any dose group. Absolute kidney weight was reduced in males at 100 and 1000 mg/kg bw/day after 104 weeks, but a clear dose relationship was lacking.
At 52 weeks parotid salivary gland weight was increased in males at 100, 300 and 1000 mg/kg bw/day. Combined sublingual and submaxillary gland weight was increased in high-dose males and females. However, salivary gland weights were not affected at week 104 at any dose level.

Histopathology
The most notable histological finding was seen in the salivary glands where cellular alteration was seen in submaxillary and parotid salivary glands in males and females at 300 and 1000 mg/kg bw/day at week 52, and in both sexes at 100, 300 and 1000 mg/kg bw/day at week 104. These changes followed a dose-related pattern and are considered to be treatment related; however, these cellular alterations are similar to those seen occasionally in other subchronic or long-term dietary studies and are considered an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and are of no adverse consequence.
Another histopathological finding was a decreased incidence of nephropathy in males at 100, 300 and 1000 mg/kg bw/day at interim kill. This finding was also noted in high-dose males at 104 weeks, but with reduced severity. Nephropathy is a common finding in old rats and as the incidence is decreased this finding is not considered as toxicologically significant.
In addition, the decreased incidence of urothelial hyperplasia in high-dose females at week 52 and 104, as well as in females at 300 mg/kg bw/day at week 104, is also not considered to be of toxicological significance.
Neoplastic changes
Neoplastic lesions were seen in all dose groups, however there was no dose relationship in the incidence of any individual tumour or in the incidence of animals with tumours.
It is concluded that the test compound at dose levels up to and including 1000 mg/kg bw/day produced no carcinogenic effect.
Conclusion by the Notifiers
Based on the study results and the lack of toxicological significance of the salivary gland findings, as well as a slight increase of plasma alkaline phosphatise observed at 300 mg/kg bw/day, the NOAEL in rats after chronic exposure to glyphosate technical for 104 weeks is considered to be 300 mg/kg bw/day. It is concluded that glyphosate technical is not carcinogenic in rats.
RMS comment:
The study is considered acceptable. However, the NOAEL is rather 100 mg/kg bw/day because histological alterations of the salivary glands were clearly more pronounced at the two upper dose levels and were accompanied by a clear increase in parotid gland weight after 1 year. The AP increase at these dose levels were in parallel to a liver weight at least after one year. Therefore, 300 mg/kg bw/day is considered the LOAEL. Effects at the NOAEL were still be seen but minor in nature. This evaluation is in line with the most recent one of WHO/FAO (JMPR, 2004, ASB2008-6266).
4th study: 1996
Reference: IIA, 5.5.2/01
Report: (1996)
Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats
Data owner: ADAMA Agan Ltd
Study No.: 886.C.C-R Date: 1996-07-18 GLP:
not published, TOX9651587
Guidelines: OECD 453 (1981)
Deviations: Individual animals exceed the 20% range in body weight; organ weights were not determined for all animals; weights of heart, spleen and (para)thyroids are missing
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: 1992-03-04 - 1994-03-04
Materials and methods

Test material:
Identification:
Glyphosate technical
Description:
White odourless crystals
Lot/Batch #:
60; 046
Purity:
96.8 %; 96.8 %
Stability of test compound:
More than two years at ambient temperature
Vehicle:
Diet
Test animals:
Species:
Rat
Strain:
Wistar
Source:
Age:
6 weeks
Sex:
Males and females
Weight at dosing:
Males: 90 – 179 g, females: 80 – 151 g
Acclimation period:
At least one week
Diet/Food:
Standard "Gold Mohur" (M/S Lipton India Ltd, India), ad libitum
Water:
Deep bore well water treated with charcoal filter and UV
rays, ad libitum
Housing:
Initially in groups of five per sex in polypropylene cages
and in groups of three from Week 12 onwards.
Environmental conditions:
Temperature: 19 -25 °C
Humidity: 40 - 70% Air changes: not reported 12 hours light/dark cycle
In life dates: 1992-03-04 to 1994-03-04
Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Wistar rats per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 7.4, 73.9 and 740.6 mg/kg bw/day for 24 months respectively) glyphosate technical. In additional one vehicle control with ten rats per sex and one high dose group with 20 rats per sex were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes.
Test diets were prepared fortnightly by mixing a known amount of the test substance with a small

amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 20 minutes.
The stability of the test substance in food was determined in an in-house stability study at 2000 and 20000 ppm.
Observations
Veterinary examination was made before and after grouping and at the end of each month of experimental schedule. Rats were examined for toxic signs and pre-terminal deaths once a day. Ophthalmic examination was done at the start of the study and at termination.
Body weight
Individual body weights were recorded before dosing, at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.
Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination.
Haematology and clinical chemistry Haematology
Individual blood samples were collected from 20 rats/sex/group at 3, 6, 12, 18 and 24 months. The following parameters were measured: Haemoglobin, haematocrit, erythrocyte count, clotting time and total leukocyte count and differential leukocyte count.
Blood chemistry
At the scheduled intervals of 6, 12, 18 and 24 months, blood collected from 10 rats/sex/group was subjected to clinical chemistry analysis. The following parameters were measured: Total proteins, albumin, ALT, AST, GGT, ALP, blood urea nitrogen and blood glucose.
Urinalysis
Individual urine samples were collected from 10 rats/sex/group at 3, 6, 12, 18 and 24 months. The following measurements were made: Volume, appearance, pH, nitrite, urobilinogen, bilirubin, erythrocytes, protein, glucose, ketones, microscopy of sediments.
Sacrifice and pathology
Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups.
The following organ weights were determined from 10 rats per sex per group: adrenals, brain, gonads, kidneys and liver.
Tissue samples were taken from the following organs: adrenals, aorta (main group animals), bone & bone marrow (sternum and femur incl. joint), brain, caecum, colon, duodenum, epididymides (main group animals), eyes (with optic nerve), heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric, mandibular and mediastinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, tumour/mass, urinary bladder and uterus.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.
Statistics
Using specific computer programs, body weight, net body weight gain, food consumption, haematology, clinical chemistry and organ weight data of different groups were compared by Bartlett's test for homogeneity of intra group variances. When the variances

heterogeneous, the data were transformed using appropriate transformation. The data with homogeneous intra group variances were subjected to one-way analysis of variance (ANOVA - Snedecor and Cochran). When 'F' value was significant, Dunnett's pair wise comparison (Scheffe) of means of treated groups with control mean was done individually.
Net food intake (g/kg bw/d) and test compound intake (mg/kg bw/d) was calculated for the whole study period using calculated means and food intake was statistically analysed by the procedure given above. Incidence of gross, histopathological changes of mass(es) and incidence of benign and malignant neoplasia in the treatment groups were statistically compared with control group by Z-test wherever it was applicable/necessary. The incidence of
neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.
When a significant difference to the control was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t' test.
Results and discussion
Analysis of dose formulations
Analyses for achieved concentrations showed that the diet preparations of the control, low, mid- and high dose group were within an acceptable range. The mean achieved concentrations of the test substance of eight batches of the prepared test substance diets were 0.0, 99.1 ± 4.7,
995.3 ± 36.8 and 9993.1 ± 277.5 ppm, for the control, low, mid and high dose group, respectively.
Mortality
There were no treatment-related deaths observed during the study.
The numbers of pre-terminal deaths in the carcinogenicity study groups are displayed in Table B.6.5-38.
Table B.6.5-38: Cumulated mortalities after 104-week dietary exposure to glyphosate technical
Dose group (ppm)
Sex
0
100
1000
10000
Male
30
30
32
21
Female
26
24
17
29
Clinical observations:
There were no treatment-related clinical signs of toxicity observed during the study.
Body weight:
There were no treatment-related effects on male and female overall body weight gain during the conduct of study.
Food consumption and compound intake

There were no treatment-related effects on food consumption for either sex noted during the study. The group mean achieved doses are summarised below.

Table B.6.5-39: Group mean achieved dose levels in the main groups

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)		
		Males	Females	Overall mean
1 (control)	0			
2 (low)	100	6.3	8.6	7.4
3 (mid)	1000	59.4	88.5	73.9
4 (high)	10000	595.2	886.0	740.6

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 7.4, 73.9 and 740.6 mg/kg bw/day for 100, 1000, and 10,000 ppm, respectively.

Haematology and clinical chemistry

The following significant dose related changes of the blood chemistry parameters were seen at the high dose:

decrease in GGT level at 12 months in male rats decrease in Albumin level at 6 months in female rats increase in AP (alkaline phosphatase) level at 6 months in female rats

No other dose or treatment related significant changes were observed in haematological, and biochemical parameters. These changes observed were only temporal and were not consistently seen at all sampling periods throughout the study. The dose related changes were also within the range of the historical control data and hence appear to be of no biological significance.

Table B.6.5-40: Statistically significant changes in blood chemistry

Parameters	Timepoint	Dose group (ppm)
		0
		100
		1000
		10000

♂
♀
♂
♀
♂
♀
♂
♀
Albumin (g/dL)
6 month
4.0
3.7
3.9
3.7
4.0
3.7
3.9
3.5*
Alkaline phosphatase (U/L)
6 month
213
133
251
146
227
153
185
235*
GGT (U/L)
12 month
8.3
5.8
8.3
7.7
8.4
6.3
5.1*
5.3
* $p < 0.05$;
Urinanalysis
There were no treatment-related findings.
Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed during the study period.
Organ weights
There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

None of the significant microscopic changes, both increased and decreased incidences (in liver, spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) observed have shown dose relationship, hence appeared to be incidental and not related to the treatment with the test compound.

Neoplastic changes

The historical data on neoplasm incidence for the test species indicates that the incidences of various tumours observed in the present study are within the range. The types of tumours seen were also comparable to the historical records.

No statistically significant inter group difference between the control and low, mid and high dose treatment groups has been recorded in respect of the number of rats with neoplasms, number of malignant neoplasms and incidence of metastasis either sexwise or for combined sex.

Table B.6.5-41: Summary of neoplastic histopathological findings

Dose group (ppm)
Males
Females
0
100
1000
10000
0
100
1000
10000
Findings for dead and moribund sacrificed animals
Cholangiocarcinoma
0/30
2/30
2/32
2/21
1/26
0/23
0/17
0/29
Hepatocellular adenoma
9/30
9/30
6/32
6/21
2/26
8/23
3/17
5/29
Hepatocellular carcinoma
12/30
12/30

9/32
5/21
4/26
4/23
2/17
5/29
Intrahepatic bile duct adenoma
1/30
1/30
0/32
0/21
0/26
0/23
0/17
0/29
Histiocytic sarcoma
2/30
0/30
2/32
1/21
1/26
0/23
0/17
0/29
Fibrosarcoma
0/30
1/30
0/32
0/21
0/26
0/23
0/17
0/29
Findings for animals sacrificed at termination
Cholangiocarcinoma
1/20
1/20
0/16
1/29
0/24
0/25
0/32
0/21
Hepatocellular adenoma
15/20
13/20

4/20
15/20
16/24
10/25
16/32
8/21
Hepatocellular carcinoma
9/20
16/20
9/16
19/29
6/24
11/25
12/32
4/21
Intrahepatic bile duct adenoma
1/20
0/20
0/16
0/29
6/24
11/25
12/32
4/21
Histiocytic sarcoma
0/20
1/20
1/16
0/29
0/24
1/25
0/32
0/21
Benign mixed intra-hepatic bile
duct adenoma
0/20
0/20
1/16
0/29
0/24
0/25
0/32
0/21
Incidentally, the number of benign tumours in the low and mid dose group males and combined sex was lower and higher in the mid dose group females. There was no dose- response relationship and the significances were considered incidental.

The different liver tumours observed in the dead and moribund sacrificed and terminally sacrificed rats included hepatocellular adenoma, intrahepatic bile duct adenomas, cholangiocarcinoma, hepatocellular carcinoma, histiocytic sarcoma, fibrosarcoma and lymphosarcoma. Of these, hepatocellular adenomas and carcinomas occurred more frequently, as often observed in ageing rats. The occurrence of these tumours appeared to be incidental and not compound-related as their frequency of occurrence was not dose dependent. No reasons could be ascribed for the decrease in the number of benign tumours in the low and mid dose group males and for combined sex and for an increase seen in the mid group dose females (see Table B.6.5-41).
From this, it is concluded that the test compound at the doses tested does not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.
Conclusion by the Notifiers
Based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 595 mg/kg bw/day for males, and 886 mg/kg bw/day for females (740 mg/kg bw/day for combined). It is concluded that glyphosate technical is not carcinogenic in rats.
RMS comment:
The study is considered acceptable. However, it should be amended that AP activity was increased in female rats not only at 6-month sampling but also after 12 and 18 months achieving statistical significance for the two upper dose levels (unfortunately not shown in Table B.6.5-6 but in the EU DAR). Because this increase that was observed in other studies with glyphosate, too, was not accompanied by further liver findings, it is not considered adverse. However, there was a slight increase in cataract frequency with 3, 4, 2, and 7 males and 1, 4, 5, and 4 females being affected in the control and the treatment groups at study termination. Since a similar finding was reported by (1990, TOX9300244), a treatment-related and adverse effect of glyphosate administration cannot be excluded. Accordingly, the NOAEL was set at 1000 ppm, i.e., ca 60 mg/kg bw/day.
The RMS is not aware of any further long-term studies with the active ingredient, apart from an earlier IBT study (1974, Z35230) on rats in which only low dose levels were employed with some minor liver effects occurring at the highest dose of 300 ppm after 2 years. This study was mentioned by (1981, TOX2000-595) but was not submitted for EU evaluation.

B.6.5.2 Carcinogenicity in the mouse
The carcinogenicity studies by (1983, TOX9552381) and by
(1993, TOX9552382) were reported in detail in the original DAR (1998, ASB2010- 10302). They have been both re-evaluated by the RMS and were confirmed as acceptable. Core information, NOAELs/LOAELs and the outcome of re-evaluation are mentioned in Vol. 1 (2.6.5).
Two more long-term studies in mice had been submitted for previous EU evaluation (
1988, TOX9551831; , 1982/1992, TOX9650154) but were considered not acceptable for reliable assessment of carcinogenicity. Thus, they were not taken into consideration for this re-evaluation. A brief description may be found in the original DAR (1998, ASB2010-10302).
In the following, descriptions of the new studies (i.e., those not previously used for EU evaluation) are given. Reporting of these studies is based on the GTF dossier. Comments by the RMS and additional information, mainly historical control data from the performing laboratories but also from the literature, may be found below the individual studies. A summary view on carcinogenicity studies in the mouse is presented in Vol. 1 (2.6.5).
1st new long-term study in mice (2001)
Reference: KIIA 5.5.3

Report: (2001)
Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice
Data owner: ADAMA Agan Ltd
Study No.: Toxi: 1559.CARCI-M Date: 2001-10-10
not published, ASB2012-11491
Guidelines: OECD 451 (1981)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 1997-12-18 - 1999-06-29
Materials and methods
Test material:
Glyphosate technical
Identification:
Glyphosate
Description:
Solid white, odourless crystals
Lot/Batch #:
01/06/97
Purity:
> 95 % (w/w)
Stability of test compound:
Expiry: December 1999
Vehicle and/
or positive control:
Diet
Test animals:
Species:
Mouse
Strain:
Swiss albino, HsdOla: MF1
Source:
Age:
6 weeks
Sex:
Males and females
Weight at dosing:
Males: 25 – 47 g, females: 21 – 26 g
Acclimation period:
5 days
Diet/Food:
Ssniff rat/mouse powder food maintenance meal – low in germs (M/s Ssniff Spezialdiäten, D-59494 Soest, Germany), ad libitum
Water:
Well water passed through activated charcoal filter and
exposed to UV rays, ad libitum

Housing:
In groups of five per sex in polypropylene mouse cages with stainless steel top grill and staem sterilized clean
paddy husk bedding.
Environmental conditions:
Temperature: 19 - 25 °C
Humidity: 30 - 70 % Air changes: 12 - 15/hour
12 hours light/dark cycle
In life dates: 1997-12-23 to 1999-06-29 Animal assignment and treatment:
In a carcinogenicity feeding study groups of 50 Swiss albino mice per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 14.5, 149.7 and 1453 mg/kg bw/day for males, and 0, 15.0, 151.2 and 1466.8 mg/kg bw/day for females) glyphosate technical in diet for 18 month. The dose levels were chosen based on results of a 50-day pre-study in mice. Test diets were prepared prior to start of treatment and then in intervals ranging from 10 to 23 days. Diets were prepared in quantities of 10, 12 or 15 kg. For preparation of 12 kg diet mixtures 1.2 g, 12 g and 120 g for the low-, mid- and high-dose group, respectively, of the test substance was mixed with approximately with
0.5 kg basal diet and blended for 3 minutes. This pre-mix was then mixed manually with approximately 0.5 kg food and then added in portions to the remaining bulk amount of food (approximately 11.0 kg) and blended in a stainless steel ribbon mixer for 20 minutes.
The homogeneity of the test material in diet was determined at beginning of treatment, and at 12 and 18 month. Analyses for achieved concentration were done at three and six month of
the study. The stability of glyphosate technical in the diet was determined prior to start of the study for the 100 and 10000 ppm dose levels.
Clinical observations
A detailed veterinary examination of all mice was done before and after grouping and monthly thereafter. A check for clinical signs of toxicity, appearance, behaviour, and neurological changes and mortality was made once daily on all mice. For mice with observed tumours a separate record was maintained with details of the tumour development.
Ophthalmological examination
Ophthalmological examinations were performed on all mice prior to start of treatment at 6, 12 and 18 month of the study. Mydriasis was induced before examination by adding 1% Tropicamide solution into the eyes. All other grossly visible eye findings were recorded also at the daily observations.
Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.
Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from week 1 to week 13 and subsequently at weeks 26, 39, 52, 65 and 68. Food efficiency and compound intake was calculated from the recorded food consumption data.
Haematology
Blood smear samples were collected at 9 month and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.
Sacrifice and pathology

All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately or preserved in 10% buffered neutral formalin until necropsy.
All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: adrenals, kidneys, liver and gall bladder, ovaries, and testes.
Tissue samples were taken from each mice from the following organs and preserved in 10% buffered neutral formalin: adrenals, bone & bone marrow (sternum and femur (incl. joint)), brain (incl. cerebrum, cerebellum pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), heart, jejunum, kidneys, larynx, liver and gall bladder, lungs, lymph nodes (mandibular, mesenteric, and superficial inguinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and all lesions and tumours/masses.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of gross lesions and masses from all mice were examined microscopically.
Statistics
Body weight, body weight gain, food consumption and differential leukocyte counts of different groups were compared by Bartlett´s test for homogeneity of intra group variances. Heterogeneous data were transformed using log transformation. Data with homogeneous intra group variances were subjected to one-way analysis of variance using ANOVA. When “F” values were significant, Dunnetts pair wise comparison of means of treated groups with control means was done individually.
Incidence of gross lesions and non-neoplastic histopathological changes and incidences of benign and malignant neoplasms in the test substance groups were statistically compared with control group by Z-test where necessary. The incidence of neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto´s incidental tumour analysis. When a significant difference over the control group was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t-test. All analyses and comparisons were evaluated at the 5 % level and statistically significant differences ($p \leq 0.05$) were indicated
Results and discussion
Analysis of dose formulations
Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss of 8.37 % at the 100 ppm level and 6.99 % at the 10000 ppm level, when stored at room temperature in PE bags inside stainless steel drums.
Analyses for homogeneity at the start and at 12 and 18 month of treatment indicated that the dose preparations were homogeneous. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 10% of the nominal concentration for all diet samples. The overall mean achieved concentrations were
94.0 ± 1.66, 949.5 ± 15.84 and 950.7 ± 142.28 as compared to the nominal concentrations of 100, 1000 and 10000 ppm, respectively.
Mortality
The cumulated pre-terminal deaths (including moribund sacrifice) are summarised in Table B.6.5-42 below.
Table B.6.5-42: Cumulated mortalities after 78-week dietary exposure to glyphosate technical
Historical control#

Dose group (ppm)**
Sex
Min- max*
Mean ± SD
0
100
1000
10000
Male
11/50 – 27/50
18 ± 5
22 (6)
20 (6)
22 (8)
27 (8)
Female
12/50 – 20/50
16 ± 3
16 (7)
16 (7)
20 (2)
20 (3)
Combined sex
12/100 –
47/100
17 ± 4
38 (13)
36 (13)
42 (10)
47 (11)
Derived from the control groups of 9 studies performed in the timeframe embracing the study summarised here
* Number of dead animals / total number of animals
** Total number of animals per group = 50 () number of animals killed in extremis
The percentage of survival in each of the dose groups are summarised below.
Table B.6.5-43: Percentage survival at termination after 18-month dietary exposure to glyphosate technical
Dose group (ppm)
Sex
0
100
1000
10000
Male
56
60

56
46
Female
68
68
60
60
Combined
62
64
58
53
The survival percentage was slightly decreased at the high dose level but the decrease did not attain statistical significance.
As can be seen from the historical control data, the mortality in the high-dose group is still within the historical control range even though at the upper end.
Clinical observations
There were no significant treatment-related clinical signs of toxicity observed.
Body weight
There were no significant treatment-related effects on male and female body weight and overall body weight gain during the conduct of study.
In males, incidences of slightly decreased body weights in week 10 at 100 ppm and in months 7 and 8 at 1000 ppm were considered incidental, since no effects on body weights were observed in the high-dose group.
In females, decreased net body weight gain was observed in month 18 at 100 ppm only. Therefore, this finding was also considered as incidental.
Food consumption and compound intake
There were no treatment-related effects on food consumption for either sex noted during the study.
The observed slightly lower food consumption observed in males in week 1 at 100 ppm and in weeks 1 and 7 at 10000 ppm was considered incidental since the changes were minimal and the effects was not consistent during the remaining parts of the study period.
In females, lower food consumptions were observed in week 2 for all dose levels and in week 26 at 10000 ppm. Higher food consumption occurred in week 11 at 100 ppm and in weeks 3 and 4 at 10000 ppm. These findings were also considered incidental since the changes were minimal and food consumption during the remaining parts of the study was comparable with the control group.
The calculated mean daily test substance intake is summarised in Table B.6.5-44 below.
Table B.6.5-44: Group mean daily compound intake levels
Dose group
Dietary concentration (ppm)
Mean daily test substance intake (mg/kg bw/day)*
Males
Females
Combined
1 (control)
0
0.0

0.0
0.0
2 (low)
100
14.5
15.0
14.7
3 (mid)
1000
149.7
151.2
150.5
4 (high)
10000
1453.8
1466.8
1460.3
* based on actual food intake and body weight data

Haematology

Differential leukocyte counts at 9 and 18 month

There were no significance treatment-related changes in the white blood cell counts for either sex at both 9 and 18 month. Slightly higher neutrophile counts and slightly lower lymphocyte counts in high dose males at 9 month were within the historical control ranges. The slightly higher eosinophil counts, higher neutrophil and monocyte counts, as well as slightly lower lymphocyte counts observed in high dose females at 18 month were comparable with historical control values and therefore considered incidental.

Differential leukocyte counts of moribund sacrificed mice

Although the differential leukocyte count data were not statistically analysed, they appeared to be within the range of biological variation.

Ophthalmological examination

There were no treatment-related findings observed at the ophthalmological examinations performed at 6, 12 and 18 month of treatment.

Gross pathology

There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.

In animals found dead or sacrificed moribund across control and all dose levels the incidence of enlargement of superficial inguinal lymph nodes and thymus in mid dose females and in the high dose for combined sexes was statistically significantly increased. These enlargements were associated with neoplasms of the hemolymphoreticular system. Other changes included enlargement of various lymph nodes, and thymus, both associated with neoplasms of the hemolymphoreticular system, enlargement of the spleen, associated with neoplasia and amyloidosis and increased extramedullary haematopoiesis. The low incidence of observed liver enlargements was associated with neoplasia and amyloidosis. However, none of these findings were dose-dependent.

In mice sacrificed at termination the following changes were observed: Kidney surface rough/uneven in high dose males, discoloration / enlargement of mesenteric lymph nodes in high dose females and discoloration in high dose combined sex, and enlargement of spleens in both sexes combined at the

high dose were significantly higher than in control mice. Since none of these changes showed a dose-dependency, and the corresponding histopathological changes were not significantly higher in these groups, the findings were considered incidental.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

There were no treatment-related histopathological findings observed in any dose group of either sex.

In mice found dead or sacrificed moribund during the study period the following significant histopathological changes were seen (Table B.6.5-45): Cystic glands of the stomach were significantly increased in high dose males and for both sexes combined. However, the incidence of these findings was similar to historical control data and did not show a dose dependency. Therefore, these finding was considered incidental. Increased haematopoiesis was seen in the bone (femur) of high dose males, mid- and high-dose animals combined sex. Cell debris in tubules of epididymides was increased in mid dose males and the incidence of
sub-capsular cell hyperplasia was increased in adrenals of low dose males. In addition, the incidence of kidney nephropathy in mid-dose females, as well as the incidence of lymphocyte infiltration of epididymides in mid dose males was significant decreased. All these findings were also observed at lower doses and/or were not dose dependent. Thus, these findings were also considered incidental. Furthermore, it is necessary to consider the frequency of this finding in animals surviving to scheduled termination.

In mice sacrificed at termination the following more frequent observed changes were observed (Table B.6.5-45):

Cystic glands of the stomach were significantly increased in low-, mid- and high-dose males but without a dose-response.

Degenerative heart changes were higher in high-dose males and females, and significant higher when sexes were combined. Since the incidences were similar or slightly higher than in the historical controls, and since no dose-dependency was observed, this finding is considered incidental.

In mandibular lymph nodes lymphoid hyperplasia was significantly increase in low and mid- dose males and combined sex, whereas the incidence was significantly lower in high dose females.

In addition, extramedullary haematopoiesis was significantly increased in these lymph nodes at the mid-dose level in combined sex. In spleen extramedullary haematopoiesis was significantly increased in females and combined sex at the low dose level. In the absence of any dose-relation these findings, as well as several not statistically significant changes considered incidental.

Table B.6.5-45: Summary of non-neoplastic histopathological findings for dead and moribund animals

Dietary concentration of glyphosate (ppm)

Males

Females

Combined sex

Finding

0

100

1000

10000

0

100

1000

10000
0
100
1000
10000
Number examined
22
20
22
27
16
16
20
20
38
36
42
47
Stomach
Cystic glands (n)
8
8
9
16
1
4
5
6
9
12
14
22+
Kidney
Nephropathy (n)
9
7
10
12
5
1
1
3
14
8
11
15

Bone (femur)
Increased haematopoises (n)
1
1
8+
5
0
1
2
3
1
2
10+
8+
Epididymes
Cell debris in tubules (n)
0
1
4
0
--
--
--
--
--
--
--
--
Epididymes
Lymphocyte infiltration (n)
4
1
0
3
--
--
--
--
--
--
--
--

Heart
Degenerative changes (n)
11

14
13
16
4
2
4
1
15
16
17
17
Adrenals
sub-capsular cell hyperplasia (n)
3
8+
7
10
12
11
13
15
15
19
20
25
Mandibular LN
extramedullary haematopoiesis (n)
3
2
5
3
1
1
1
2
4
3
6
5
n = number of animals affected; LN = lymph node
+ significantly increased; -- not examined/determined
The number of malignant lymphoma was slightly elevated in the high dose group compared to control. This tumour of the hemolymphoreticular system is one of the most common tumours of mice accounting for the highest percentage of spontaneous tumours in this species. Therefore, the observed tumour incidence is considered incidental and not treatment-related. In addition, the incidences of this tumour varied with sex and fate (i.e. pre-terminal and terminal deaths).

Table B.6.5-46: Summary of non-neoplastic and neoplastic histopathological findings at termination
Dietary concentration of glyphosate (ppm)
Males
Females
Combined sex
Finding
0
100
100
0
1000
0
0
100
1000
1000
0
0
100
1000
10000
Stomach(N)
28
30
28
23
34
-
-
30
62
64
58
53
Cystic glands (n)
9
19+
22+
17+
22
-
-
19
31
-
-

36
Kidney(N)
28
6
4
23
34
2
1
30
62
64
58
53
Nephropathy (n)
7
4
3
6
5
2
0
2
12
6
3
8
Bone (femur) (N)
28
-
-
23
34
2
1
30
62
--
--
53
Increased
haematopoises (n)
1
-
--
0

1
0
0
2
Epididymes (N)
28
1
-
23
--
--
--
--
--
--
--
--
Lymphocyte infiltration
(n)
0
0
--
1
--
--
--
--
--
--
--
--
Mandibular LN (N)
28
30
28
23
34
33
28
30
62
64
58
53
Extramedullary
haematopoiesis (n)

5
7
9
9
3
9
7
4
8
16
16+
13
Heart (N)
28
2
--
23
34
--
-
30
62
--
--
53
Degenerative changes
(n)
14
1
--
17
2
--
-
6
16
--
--
23+
Adrenals (N)
28
--
--
23
34
-

-
29
62
--
--
53
sub-capsular cell
hyperplasia (n)
15
--
--
13
27
--
--
22
42
--
--
35
Hemolymphoreticular
system (N)
28
30
28
23
34
34
30
30
62
64
58
53
malignant lymphoma
(n)
1
3
3
6
9
10
6
13
10
13

9
19
N = number examined; n = number of animals affected; LN = lymph node
+ significantly increased; -- not examined/determined
Table B.6.5-47: Incidences of malignant lymphoma and comparison with historical control
Dietary concentration of glyphosate (ppm)
Males
Females
♂
♀
0
100
1000
10000
0
100
1000
10000
Dead & moribund
Number examined
75
77
22
20
22
27
16
16
20
20
Number affected
20
49
9
12
13
13
9
10
13
12
Percentage affected
26.7
63.6
41.0
60.0+

59.0+
48.0
56.0
63.0
65.0
60.0
Mean %
26
61.8
--
--
--
--
--
--
--
--
Range %
0-44
0-100
--
--
--
--
--
--
--
--
--
--
--
--
--
--
--
--
Terminal sacrifice
Number examined
175
175
28
3028
23
34
34
30

30
28
Number affected
26
50
1
3
3
6+
9
10
6
13
Percentage affected
14.9
28.9
3.6
10.0
10.7
26.1+
26.5
29.4
20.0
43.3+
Mean %
14.8
28.8
--
--
--
--
--
--
--
--
Range %
8-24
20-43
--
--
--
--
--
--
--
--

--
--
--
--
--
--
--
--
All fates
Number examined
250
250
50
50
50
50
50
50
50
50
Number affected
46
99
10
15
16
19+
18
20
19
25
Percentage affected
18.4
39.6
20.0
30.0
32.0
38.0+
36.0
40.0
38.0
50.0+
Mean %
18.4
41.6
--

--
--
--
--
--
--
--
Range %
6-30
14-58
--
--
--
--
--
--
--
--
+ significantly increased; -- not examined/determined
Conclusion by the Notifiers

Based on mortality at the upper limit of the historical control range, the NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is conservatively set at 1000 ppm, corresponding to 149.7 mg/kg bw/day for males, 151.2 mg/kg bw/day for females, and
150.5 mg/kg bw/day for both sexes combined. It is concluded that Glyphosate is not carcinogenic in mice.
RMS comments
The study is considered acceptable and the NOAEL of 150 mg/kg bw/day is agreed but should be based on different considerations. In fact, mortality was slightly higher in males receiving the high dose (54% as compared to 44 % in the control) and in females which were fed the mid and high dose (40% in both vs. 32 % in the control) but these differences were not that convincing. As shown in Table B.6.5-42, mortality was ut the upper edge of the historical control range. Based on additional information that can be obtained from the original study (occurrence of first pre-terminal deaths in the individual groups, slopes for cumulative mortality curves, mean survival for the animals that were found dead pre-terminally or were sacrificed moribund, investigations on causes of deaths, so far performed), it is not likely that this slightly lower survival rate in these few groups was due to glyphosate feeding.
In contrast, two pathological findings should be given more attention.
There was a more frequent occurrence of cystic glands of the stomach in male mice that becomes more clear when the incidences in animals found dead or moribund and those sacrificed at scheduled termination are combined (Table B.6.5-48). Indeed, the difference between the treated groups is not large and, taking into account the large dose spacing, a clear dose response may be doubted but, according to the study author, the incidence was
higher than the historical control data. Unfortunately, this historical control data was not given. In contrast, no increase became apparent in females.
Table B.6.5-48: Stomach cystic glands in the study by Kumar (2001, ASB2012-11491) in Swiss albino

mice, total incidence
Sex
Males
Females
Dose
0
100
1000
10000
0
100
1000
10000
No.
examined
50
50
50
50
50
16
20
50
Animals with cystic
stomach glands
17
27
31
33
23
4
5
25
Based on this table and the statistical significance mentioned in Table B.6.5-47 for male animals, there was no NOEL in this study because it cannot be excluded that this finding was due to treatment. The clinical relevance of cystic glands of the stomach is not clear. In any case, there was no increase in severity (always minimal to mild) and, more important, the cysts formation did not progress to any other pathological lesion, even at a dose level that was 100 times higher than the lowest. Thus, this finding should not be taken into account when the NOAEL for this study is set.
As can be seen in Table B.6.5-46 and Table B.5.6-47, an increase in malignant lymphoma was noted in both the male and female groups receiving the highest dose. The incidence was statistically significantly elevated as compared to the actual control groups in this study, was above the mean values of the (relatively small) historical control and, for males, outside the historical control range. Even though malignant lymphoma is a common tumour in mice (accounting for 54.6% of all tumours in this study), it cannot be completely excluded that the higher incidence in the top dose groups were somehow related to treatment. The RMS conclusion is that there was limited evidence of a

carcinogenic potential of glyphosate in this mouse strain at the very high dose level of 10000 ppm (about 1460 mg/kg bw/day for sexes combined) in this study, with male animals being more affected. The NOAEL should be set at the mid dose level of ca 150 mg/kg bw/day confirming a previous figure established by (1983, TOX9552381) even though effects at higher dose levels were completely different.

The issue of malignant lymphoma was discussed in length on the PRAS 125 expert meeting held in February, 2015, in Parma. On request of this meeting, the RMS collected additional data from the literature to substantiate the claim of a high background incidence of this tumour type in mice in general and in particular in the Swiss mouse. In fact, a few articles could be identified and are briefly reported in the following. Even though some of them were rather old, they clearly demonstrate a frequent, however variable, occurrence of malignant lymphoma. For historical data on lymphoma incidence in CD-1 mice, see the RMS comments below the descriptions of the next two studies.

similar experiment, the incidence in males was lower (5.5%) but, this time, accounted for 36.3% in females. This latter information may be considered the first published evidence of a remarkable sex difference in the frequency of this tumour type and a higher vulnerability of female mice as it was nearly consistently reported thereafter.

More than 10 years later, Sher (1974, Z22020) published a review on spontaneous tumour incidences in various non-inbred mouse strains, based on scientific articles that had been released between 1960 and 1974. For Swiss random-bred strains, lymphomas and leukemias were mentioned to occur as the most common tumours. However, again, extremely variable incidences ranging from 0 to 21.4% were reported in long term studies for untreated males, depending on strain and source. In female Swiss mice, the incidences varied even between 0 and 36.4%. The maximum incidence had been noted in minimally inbred Carworth CF-1 mice (not related to Swiss mouse strains) with 53% in females.

Roe and Tucker (1974, ASB2015-2534) reported an incidence of 22.5 to 27.5% of (not further specified) lymphoreticular neoplasms in male Swiss mice (n=80) if fed ad libitum but a much lower tumour rate when diet was restricted.

Tucker (1979, Z83266) found 18% of male Swiss albino mice (Alderley Part strain) and 28% of the females with lymphoma, nearly all of them malignant. Her analysis was based on 50 males and 50 females fed ad libitum from weaning for their lifespan with the last, very few surviving animals killed after 3 years.

A large colony of (minimally inbred)”Swiss-derived” Icr:Ha(ICR) mouse had a 15% incidence of lymphoma in total with an approximate 2:1 ratio between females and males (precise percentages not given). In addition, 5% of the mice had developed leukemia (Eaton et al., 1980, ASB2015-2537). Only lung tumours occurred more frequently (23%). With regard to Swiss mice in general, the authors emphasised that ”… differences occur between colonies and even within a colony with the passage of time so that contradictory results may be obtained using ‘Swiss’ stock from different sources. For example, the incidence of spontaneous neoplasia, although seldom reported in detail, varies with source and age.” According to a more recent article (Taddesse-Heath et al., 2000, ASB2015-2535), a much higher incidence of hematopoietic neoplasia of 58% was observed in a colony of CFW Swiss mice in the USA. Lymphoma (mostly of B-cell origin) accounted for 85% of these cases giving a total incidence of nearly 50%. The authors ascribed these tumours mainly to ”infectious expression of murine leukemia viruses”. It is not known to which extent such a latent infection might have contributed to lymphoma incidences reported earlier or even in the studies described in this RAR. A possible etiologic role of oncogenic viruses had been suspected by Roe and Tucker (1974, ASB2015-2534) yet who complained that many scientists performing long-term studies

would often ignore this problem.
2nd new long-term study in mice (2009)
Reference: IIA, 5.5.3/02
Report: (2009b), Glyphosate
technical: Dietary Carcinogenicity Study in the Mouse
SPL Project No.: 2060-0011 Data owner: Nufarm
Date: 2009-04-22
not published, ASB2012-11492
Guidelines: OECD 451 (1981), JMAFF guideline 2-1-15 (2005), US-EPA
OPPTS 870.4200 (1996)
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 2005-10-10 - 2007-11-19
Materials and methods
Test material:
Glyphosate technical
Identification:
Glyphosate
Description:
White crystalline solid
Lot/Batch #:
H05H016A
Purity:
95.7 %
Stability of test compound:
Expiry: 2008-03-25
Vehicle and/
or positive control:
Diet
Test animals:
Species:
Mouse
Strain:
CD-1, Crl:CD-1 (ICR) BR
Source:
Age:
Approx. 5 – 6 weeks
Sex:
Males and females
Weight at dosing:
Males: 22 – 32 g, females: 18 – 28 g
Acclimation period:

At least ten days
Diet/Food:
Rat and Mouse SQC Ground diet No. 1, Special Diet Services Limited, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
Initially in groups of three per sex in polypropylene solid- floor cages.
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: at least 15/hour 12 hours light/dark cycle
In life dates: 2005-10-10 to 2007-11-19 Animal assignment and treatment:
In a carcinogenicity feeding study groups of 51 CD-1 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm (equivalent to mean achieved dose levels of 0, 84.7,
266.8 and 945.6 mg/kg bw/day) Glyphosate technical in diet. Additional 12 mice per sex, designated for veterinary controls, were housed and maintained alongside treated animals. Ten animals per sex from each group were set aside for an interim kill (toxicity assessment), which was carried out on the survivors after 39 weeks of dosing. The remaining 50 mice per sex and dose-level were dosed for a maximum of 79 weeks (carcinogenicity assessment).
Test diets were prepared prior to start of treatment and then weekly by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.
The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for achieved concentration monthly for the first six months and then every three months thereafter.
Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all mice and recorded weekly. Observations for morbidity, and mortality were made twice daily. Additional unscheduled examinations were performed on animals that showed ill- health.
All surviving animals were palpated weekly for size, position and appearance of new or existing masses.
Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. Body weights were also determined before sacrifice. Body weight data were reported only until Week 77.
Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination. Food consumption data were reported only until Week 77. Food efficiency and compound intake was calculated from the recorded food consumption data.
Water consumption
Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.
Haematology
Blood smear samples were collected after 12 months and at termination from all animals, and from mice that were killed in extremis. Differential white cell counts were performed on all control and

high-dose animals and on the animals killed in extremis.
Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, and all animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, spleen, and testes.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (incl. cerebrum, cerebellum and pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions incl. palpable masses, head (incl. pharynx, nasopharynx and paranasal sinuses), heart, Harderian and lacrimal glands, ileum, jejunum, kidneys, larynx, liver and gall bladder, lungs (with bronchi), mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord
(cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of the liver, lungs and kidneys, as well as gross macroscopic lesions and palpable masses from low and intermediate dose groups at termination were examined microscopically.
Statistics
All data were summarised in tabular form and analysed by computerised analysis using ProvantisTM Tables and Statistics Module. For each variable the of variance incorporating Student´s t-test and F-test. For each variable the most suitable transformation of data was found, the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANOVA and Bartlett´s test. The lowest treatment-related significant effects were determined using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no response is found, but the data showed non-homogeneity of means, data were further analysed by a stepwise Dunnet (parametric) or Steel (non-parametric) test to determine significant differences from control. If required, pair-wise tests are performed using Students t-test (parametric) or the Mann-Whitney U test (non-parametric)
The levels of probability chosen as significant were $p < 0.01$** and $p < 0.05$*.
Histopathology data were analysed using Chi squared analysis (differences in the incidence of lesions occurring with an overall frequency of 1 or greater) and the Kruskal-Wallis one-way non-parametric analysis of variance (comparison of severity grades).
The levels of probability chosen as significant were $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.1$.
Results and discussion
Analysis of dose formulations
Analyses for homogeneity and stability indicated that the dose preparations were homogeneous and stable for at least six weeks. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 5 % of the nominal concentration for all but 1 sample (500 ppm –level), which was + 10 % of the nominal concentration.
The group mean achieved doses are summarised below.
Table B.6.5-49: Group mean achieved dose levels
Dose group
Dietary concentration

(ppm)
Achieved dose level (mg/kg bw/day)*
Males
Females
Overall mean
Mean
Range
Mean
Range
1 (control)
0
2 (low)
500
71.4
33 – 104
97.9
55 – 155
84.7
3 (mid)
1500
234.2
101 – 365
299.5
176 – 466
266.8
4 (high)
5000
810
461 - 1143
1081.2
610 - 1728
945.6
* based on actual food intake and body weight data

The results show a higher test material intake for females when compared to males for each dose level. Highest intakes were achieved within the first few treatment weeks, with subsequent decline thereafter. The mean intake for each dose group (sexes combined) is therefore 84.7, 266.8 and 945.6 mg/kg bw/day for 500, 1500, and 5000 ppm, respectively.

Mortality

No treatment-related effects on the deaths occurred during the study, as well as no treatment-related effects on the time of death. From three male mice that were killed in extremis, examination results suggest that the morbidity of these animals was due to fighting between cage mates.

Table B.6.5-50: Cumulated mortalities after 78-week dietary exposure to Glyphosate technical

Dose group (ppm)
Sex
0
500

1500
5000
Male
12 (6)
10 (8)
12 (6)
16 (6)
Female
14 (10)
13 (7)
13 (10)
11 (8)
( ): number of animals killed in extremis
The percentage of survival in each of the dose groups are summarised below.
Table B.6.5-51: Percentage survival at termination after 78-week dietary exposure to glyphosate technical
Dose group (ppm)
Sex
0
500
1500
5000
Male
76
80
76
69
Female
73
75
75
78
Clinical observations
There were no significant treatment-related clinical signs of toxicity observed.
There were no trends in the proportion of palpable masses observed during the study period. A significant proportion observed showed evidence for regression before the animal reached the point of death or termination. Based on the results (see Table B.6.5-52) no treatment- related effect on the development of palpable masses is seen for either sex. The slight increase in the mean number of masses per animal for high-dose females and mid-dose males was considered a coincidence. The median time to appearance of palpable masses was comparable for all dose groups of either sex.
Table B.6.5-52: Group summary of palpable masses
Dose
Total number of animals in group
Number of animals with palpable masses
Total number of masses per group
Mean number of masses per animal

Median time (weeks) to appearance of masses

♂
♀
♂
♀
♂
♀
♂
♀
♂
♀
0
51
51
28
23
45
38
0.88
0.75
42.00
45.75
500
51
51
32
28
49
49
0.96
0.96
42.00
46.08
1500
51
51
39
23
60
38
1.20
0.75
42.43
44.83
5000

51
51
25
23
49
51
0.96
1.00
41.67
42.50
Body weight

2nd new long-term study in mice (2009)
Reference: IIA, 5.5.3/02
Report: (2009b), Glyphosate
technical: Dietary Carcinogenicity Study in the Mouse
SPL Project No.: 2060-0011 Data owner: Nufarm
Date: 2009-04-22
not published, ASB2012-11492
Guidelines: OECD 451 (1981), JMAFF guideline 2-1-15 (2005), US-EPA
OPPTS 870.4200 (1996)
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 2005-10-10 - 2007-11-19
Materials and methods
Test material:
Glyphosate technical
Identification:
Glyphosate
Description:
White crystalline solid
Lot/Batch #:
H05H016A
Purity:
95.7 %
Stability of test compound:
Expiry: 2008-03-25
Vehicle and/
or positive control:
Diet
Test animals:
Species:

Mouse
Strain:
CD-1, Crl:CD-1 (ICR) BR
Source:
Age:
Approx. 5 – 6 weeks
Sex:
Males and females
Weight at dosing:
Males: 22 – 32 g, females: 18 – 28 g
Acclimation period:
At least ten days
Diet/Food:
Rat and Mouse SQC Ground diet No. 1, Special Diet Services Limited, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
Initially in groups of three per sex in polypropylene solid- floor cages.
Environmental conditions:
Temperature: 21 ± 2 °C
Humidity: 55 ± 15 %
Air changes: at least 15/hour 12 hours light/dark cycle
In life dates: 2005-10-10 to 2007-11-19 Animal assignment and treatment:
In a carcinogenicity feeding study groups of 51 CD-1 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm (equivalent to mean achieved dose levels of 0, 84.7,
266.8 and 945.6 mg/kg bw/day) Glyphosate technical in diet. Additional 12 mice per sex, designated for veterinary controls, were housed and maintained alongside treated animals. Ten animals per sex from each group were set aside for an interim kill (toxicity assessment), which was carried out on the survivors after 39 weeks of dosing. The remaining 50 mice per sex and dose-level were dosed for a maximum of 79 weeks (carcinogenicity assessment).
Test diets were prepared prior to start of treatment and then weekly by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.
The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for achieved concentration monthly for the first six months and then every three months thereafter.
Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all mice and recorded weekly. Observations for morbidity, and mortality were made twice daily. Additional unscheduled examinations were performed on animals that showed ill- health.
All surviving animals were palpated weekly for size, position and appearance of new or existing masses.
Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. Body weights were also determined before sacrifice. Body weight data were reported only until Week 77.

Food consumption and compound intake

Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination. Food consumption data were reported only until Week 77. Food efficiency and compound intake was calculated from the recorded food consumption data.

Water consumption

Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Haematology

Blood smear samples were collected after 12 months and at termination from all animals, and from mice that were killed in extremis. Differential white cell counts were performed on all control and high-dose animals and on the animals killed in extremis.

Sacrifice and pathology

All animals that died or were killed in extremis during the conduct of the study, and all animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined from 10 mice per sex per group: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, spleen, and testes.

Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (incl. cerebrum, cerebellum and pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions incl. palpable masses, head (incl. pharynx, nasopharynx and paranasal sinuses), heart, Harderian and lacrimal glands, ileum, jejunum, kidneys, larynx, liver and gall bladder, lungs (with bronchi), mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord
(cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of the liver, lungs and kidneys, as well as gross macroscopic lesions and palpable masses from low and intermediate dose groups at termination were examined microscopically.

Statistics

All data were summarised in tabular form and analysed by computerised analysis using ProvantisTM Tables and Statistics Module. For each variable the of variance incorporating Student´s t-test and F-test. For each variable the most suitable transformation of data was found, the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANOVA and Bartlett´s test. The lowest treatment-related significant effects were determined using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no response is found, but the data showed non-homogeneity of means, data were further analysed by a stepwise Dunnet (parametric) or Steel (non-parametric) test to determine significant differences from control. If required, pair-wise tests are performed using Students t-test (parametric) or the Mann-Whitney U test (non-parametric) The levels of probability chosen as significant were $p < 0.01$** and $p < 0.05$*.

Histopathology data were analysed using Chi squared analysis (differences in the incidence of lesions occurring with an overall frequency of 1 or greater) and the Kruskal-Wallis one-way non-parametric analysis of variance (comparison of severity grades).

The levels of probability chosen as significant were $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.1$.

Results and discussion

Analysis of dose formulations

Analyses for homogeneity and stability indicated that the dose preparations were homogeneous and stable for at least six weeks. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 5 % of the nominal concentration for all but 1 sample (500 ppm –level), which was + 10 % of the nominal concentration.

The group mean achieved doses are summarised below.

Table B.6.5-49: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Achieved dose level (mg/kg bw/day)*				
		Males		Females		Overall mean
		Mean	Range	Mean	Range	
1 (control)	0					
2 (low)	500	71.4	33 – 104	97.9	55 – 155	84.7
3 (mid)	1500	234.2	101 – 365	299.5	176 – 466	266.8
4 (high)	5000	810	461 - 1143	1081.2	610 - 1728	945.6

* based on actual food intake and body weight data

The results show a higher test material intake for females when compared to males for each dose level. Highest intakes were achieved within the first few treatment weeks, with subsequent decline

thereafter. The mean intake for each dose group (sexes combined) is therefore 84.7, 266.8 and 945.6 mg/kg bw/day for 500, 1500, and 5000 ppm, respectively.

Mortality

No treatment-related effects on the deaths occurred during the study, as well as no treatment-related effects on the time of death. From three male mice that were killed in extremis, examination results suggest that the morbidity of these animals was due to fighting between cage mates.

Table B.6.5-50: Cumulated mortalities after 78-week dietary exposure to Glyphosate technical

Dose group (ppm)	0	500	1500	5000
Sex				
Male	12 (6)	10 (8)	12 (6)	16 (6)
Female	14 (10)	13 (7)	13 (10)	11 (8)

( ): number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table B.6.5-51: Percentage survival at termination after 78-week dietary exposure to glyphosate technical

Dose group (ppm)	0	500	1500	5000
Sex				
Male	76	80	76	69
Female	73	75	75	78

Clinical observations

There were no significant treatment-related clinical signs of toxicity observed.

There were no trends in the proportion of palpable masses observed during the study period. A

significant proportion observed showed evidence for regression before the animal reached the point of death or termination. Based on the results (see Table B.6.5-52) no treatment- related effect on the development of palpable masses is seen for either sex. The slight increase in the mean number of masses per animal for high-dose females and mid-dose males was considered a coincidence. The median time to appearance of palpable masses was comparable for all dose groups of either sex.

Table B.6.5-52: Group summary of palpable masses

Dose										
Total number of animals in group	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	0		51		51		28		23	
Number of animals with palpable masses			45		38		0.88		0.75	
Total number of masses per group			42.00		45.75		500			
			51		51		32			
Mean number of masses per animal			28		49		49			
			0.96		0.96					
Median time (weeks) to appearance of masses			42.00		46.08		1500			

51
51
39
23
60
38
1.20
0.75
42.43
44.83
5000
51
51
25
23
49
51
0.96
1.00
41.67
42.50
Body weight

Body weight
There were no treatment-related effects on male and female overall body weight gain during the conduct of study.
Food consumption and compound intake
There were no treatment-related effects on food consumption for either sex noted during the study.
Water consumption
There were no treatment-related effects on water consumption for either sex noted during the study.
Haematology
There were no significance differences in the proportions of white blood cell counts for either sex at both 12 and 18 month.
Necropsy
Gross pathology
There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.
Organ weights
There were no treatment-related findings observed in organ weights or relative organ weights.
Histopathology
There were no treatment-related histopathological findings observed in any dose group of either sex.
Conclusion by the Notifiers
Based on the study results the NOEL and NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is 810 mg/kg bw/day for males, and 1081 mg/kg bw/day for females. It is concluded that Glyphosate technical is not carcinogenic in mice.

RMS comments
The study is considered acceptable and setting of the NOAEL at the highest dose level of 5000 ppm (eqivalent to 810 mg/kg bw/day in males and 1081 mg/kg bw/day in females) is supported. Indeed, there was no evidence for carcinogenicity up to this dose level and the very comprehensive ranges of tissues that were examined histologically does not suggest an i
t s i a
differentiation between the two testes of the animals when effects were reported.
Survival and growth of the animals were not affected. However, the dose levels choosen, although sufficiently high for a study of this type, were much lower than in other long-term studies with glyphosate in mice.
It was noted that histological examination of salivary glands covered submaxillary, sublingual and parotid glands. However, no lesions similar to those found by
(1992, TOX9551954, see B.6.3.2) in another mouse strain following administration of glyphosate ober 90 days at higher doses were reported.
There was no increase in malignant lymphoma.
There was a weak increase in malignant lymphoma incidence in male mice at the top dose level. The actual numbers of affected animals were 0, 1, 2, and 5 in the control, low, mid and high dose groups (n=51 in each of them). In females, the respective figures were 11/51, 8/51, 10/51 and, again, 11/51. Thus, no evidence of any change in lymphoma frequency was seen in female mice in this study. Even in males, the difference was not statistically significant but a possible effect might be suspected and should be clarified because of the increase in
malignant lymphoma in the study by
(2001, ASB2012-11491, “1st new study”, see
above) and because of a weakly higher incidence in the study by
(1997, ASB2012-
11493, ”3d new study”, see below). On request of the RMS, the GTF submitted historical
control data for malignant lymphoma from the performing laboratory (
2015;
ASB2015-2531)
February, 2015.
but, unfortunately, only after the PRAS
Therefore, the following data was not
125 meeting that was
subject to peer review
held
by
in
the
regulatory agencies of the MS.
There are more sources to support, based on historical control data, remarkable differences in the occurrence of malignant lymphoma in CD-1 mice. According to information obtained from the ”Registry of Industrial Toxicology Animal-data” (RITA) database (Fraunhofer ITEM Institute, Hannover, Germany; http://reni.item.fraunhofer.de/reni,) and made available to the RMS only very recently by the GTF, male CD-1 mice had a mean incidence of 3.4% (of
470 animals in total) in the control groups from nine 18-/19-month long-term studies performed between 1994 and 1998. In the individual studies, incidences ranged from 0 up to 12%. In female

mice, the mean control incidence was much higher (16.9% in a total of 350 examined animals). In line with that, actual study incidences in female mice varied between 4 and 32% (Anonym, 2015, ASB2015-2532).
11492), in their glyphosate study], Giknis and Clifford (2010, ASB2015-2529) reported data from a total of 13 (males) or 14 studies (females) with a duration between 78 and 104 weeks that had been performed between 2002 and 2006 by
. (Also this
data was submitted by GTF following PRAS 125 meeting.) In males, malignant lymphoma was more rarely seen than in females since tumours of this type were found in the control groups in 8 out of 13 studies only with a minimum study incidence of 1/75 and a maximum
one
of
5/49
closely
resembling
that
one
at
the
top
dose
level
of
the
(2009,
ASB2012-11492) study with glyphosate. In female CD-1 mice, malignant lymphoma was observed in all but one of the 14 studies, even though with an extremely variable study incidence ranging from 2/60 up to 22/50.
Based on their retrospective analysis of 20 long-term studies for carcinogenicity (Huntingdon Life Sciences, U.K., 1990-2002) Son and Gopinath (2004, ASB2015-2533) described lymphoma as the most common tumour in young control CD-1 mice. This result was based on an analysis of premature deaths in these studies. In a total of 101 fatalities occurring up to week 50 of treatment in all these studies among male animals, lymphoma was found in 23 cases. In the 190 males which died between weeks 50 and 80 before scheduled termination, 36 were diagnosed with lymphoma. Among females, there were 68 premature deaths up to week 50 of which 19 had lymphoma suggesting a slightly higher rate than in males (28% vs. 23%). Between weeks 50 and 80, there were 211 deaths and, among them, 61 with lymphoma (ca 29% vs. 19% in males). It was noted that lymphoma incidence in the Huntingdon colony was similar in females as in the ICR mouse (Giknes and Clifford, 2010, ASB2015-2529) or in CD-1 mice included in the RITA database (Anonym, 2015, ASB2015-2532) whereas a more frequent occurrence of this tumour type was noted in males. However, this might be due to a
different focus of the analysis. In the RITA database and in the review from
all animals on study were considered. In contrast, Son and Gopinath (2004,
ASB2015-2533) looked only at the premature deaths to which malignant lymphoma might have contributed to a rather large extent.
3d new long-term study in mice (1997)

Reference: IIA, 5.5.3/03
Report: (1997)
HR-001: 18-Month Oral Oncogenicity Study in Mice.
Data owner: Arysta LifeScience Study No.: 94-0151
Date: 1997-06-18
Not published, ASB2012-11493
Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985
U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 451 (1981).
Deviations: None
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: 1995-02-21 to 1996-09-06
Materials and methods
Test material:
Glyphosate technical
Identification:
HR-001
Description:
Solid crystals
Lot/Batch #:
T-941209 T-950308
Purity:
97.56 % 94.61 %
Stability of test compound:
Not mentioned in the report
Vehicle and/
or positive control:
Diet
Test animals:
Species:
Mouse
Strain:
SPF ICR (Crj:CD-1)
Source:
Age:
5 weeks
Sex:
Males and females
Weight at dosing:
Males: 15 – 25 g, females: 14 – 23 g
Acclimation period:
9 days in males; 7 days in females
Diet/Food:
Certified diet MF Mash (Oriental Yeast Co., Ltd.), ad libitum
Water:
Filtered and sterilized water, ad libitum

Housing:
In groups of four per sex in aluminium cages with wiremesh floors
Environmental conditions:
Temperature: 24 ± 2°C Humidity: 55 ± 15% Air changes: 15/hour
12 hours light/dark cycle
In life dates: 1995-02-21 to 1996-09-06
Animal assignment and treatment:
Groups of 50 males and 50 females Specific –Pathogen-Free (SPF) ICR (Crj : CD-1) mice received the test material by incorporating it into the basal diet at a level of 0, 1 600, 8 000 or 40 000 ppm for a period of 18 months.
Clinical observations
All animals were conducted a cage-side observation daily for clinical signs and their deaths during the study. In addition, a detailed examination including palpation of the body was performed at least once a week. Moribund animals showing marked delibility were euthanized by exsanguinations under deep ether anesthesia and necropsied when an unfavourable prognosis was predicted. Dead animals were taken from the cage as soon as possible after discovery o minimize the loss of tissues by cannibalism and necropsied.
Mortality was expressed as ratios of cumulative number of animals found dead or killed in extremis to effective number of animal group.
Body weight
Individual body weights were recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 76. Body weights were also measured at week 78, at the end of treatment, and used for calculation of relative organ weights. Group mean body weights were calculated at each measurement.
Food consumption and compound intake
Food consumption by each cage was recorded for a period of 3 or 4 consecutive days weekly during the first 13 weeks and every 4 weeks from week 16 to 76. Food efficiency and compound intake was calculated from the recorded food consumption data.
Haematology
Blood smear samples were collected at week 52 and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.
Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately.
All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: brain, adrenals, kidneys, spleen, liver and gall bladder, ovaries, and testes.
Tissue samples were taken from each mice from the following organs and preserved in 10% buffered neutral formalin: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, esophagus, stomach, liver with gallblader , pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, pharynx, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.
A detailed histopathological examination was performed on all sampled tissues of the control and

high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of gross lesions and masses from all mice were examined microscopically. The following tissues were examined: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, esophagus, stomach, liver with gallblader , pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions

Statistics

Body weight, food consumption and organ weights were evaluated by Bartlett´s test for homogeneity of intra group variances. When group variances were homogenous, a parametric analysis of variance of a one way layout type was conducted to determine if any statistical differences exist among groups. When the analysis of variance was significant, Dunnett's or Scheffe's multiple comparison test was applied. When the group variance were heterogeneous, the data were evaluated by Kruskal-Wallis non-parametric analysis of variance. When significant Dunnett type mean rank test or Scheffe's type mean rank test was applied.

Mortality was assessed by a life table analysis.

Urinalysis were analyzed by Mann-Whitney's U test to compare data between the treatment groups and the controls.

Mann-Whitney's U test was used to analyze difference of the differential leukocyte counts between the high dose groups and the controls. For comparison of the data from all groups, Dunnett's and Scheffe's multiple comparison test was applied. The data from males killed in extremis during the treatment were examined by Mann-Whitney's U test.

Fisher's exact probability test was used to analyze the data of clinical signs and incidences of gross lesions at necropsy and histopathological lesions.

Results and discussion Analysis of dose formulations

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss 8.37 % Homogeneity of the test substance in diet was analysed on the samples taken from the top, middle, and bottom portion of the mixer. The coefficient of variation for each test diet was within 5.2 % or less. The results indicated that homogeneity of the test substance in diet was satisfactory in each test diet.

In order to verify concentration of the test substance in test diets, every batch of test diet was analysed during the treatment period. Mean concentration of the test substance in test diet at a nominal level of 1 600, 8 000 or 40 000 ppm was 1 561± 86.7, 7 790±394.4 or 38 783±1 655.0 (mean + standard deviation) ppm, respectively. The values were within 97-98 % of the target concentrations and satisfied the acceptable limit of concentration for test substance.

Mortality

No significant differences were noted for mortality between the treated groups and the respective control of either sex. Cumulative mortality of each group of either sex is shown in the following table:

Table B.6.5-53: Final mortality at termination of treatment (%)

Dose group (ppm)

Male

Female

0

24/50 (48)

18/50 (36)

1 600
16/50 (32)
14/50 (28)
8 000
23/50 (46)
10/50 (20)
40 000
21/50 (42)
15/50 (30)
Clinical observations
Statistically significant changes in clinical signs observed in the treated groups of either sex are shown in the following table:
Table B.6.5-54: Statistically significant changes in clinical signs:
Male
Female
Dose group (ppm)
0
1 600
8 000
40 000
0
1 600
8 000
40 000
Number of animals examined
50
50
50
50
50
50
50
50
Perinasal region : tactile hair loss
0
3
3
6*
5
13*
9
8
Anus : mass(es)
0
0
0

8**
0
0
0
0
Integument :
Wound
22
16
20
6*
3
0
0
0
Erosion/Ulcer
9
5
12
8
16
4**
1**
2**
Swelling
16
6*
13
9
6
2
0
1
Mass(es)
15
13
13
10
13
11
9
4*
Pale-colored skin
2
3
6

10*
4
2
6
6
Hair loss
11
12
21*
12
22
23
18
14
Wetted fur
11
9
7
4*
1
1
1
1
* : p<0.05 ; **, p<0.01 (Fisher's exact probability test).
In the 40 000 ppm group, males showed increased incidences of tactile hair loss, pale-colored skin, and mass(es) of anus and decreases of wound and wetted fur. In females of this group decreased incidences were observed in ulcer/erosion and mass(es) of skin. Although, in addition to these signs, loose stool was observed in the cages of both sexes beginning at week 21 in males and 20 in females, the group housing failed to identify which animal excreted the loose stool.
In the 8 000 ppm group, males showed an increased incidence in hair loss of the skin and females represented decreases in ulcer/erosion and swelling of the skin.
In the 1 600 ppm group, males showed a decrease in swelling of the skin and females represented an increase in tactile hair loss as well as a decrease in ulcer/erosion of the skin.
None of the observed effects seems to be dose-related. Whatever the dose tested, females were more sensitive to erosion/ulcer of the integument than males.
Body weight
In the 40 000 ppm group, males and females showed retarded growth during the treatment manifesting significantly lowered weights at weeks 16 to 36 in males and at weeks 6 and thereafter in females compared to the respective control. At the end of treatment, mean average weights were 93 % and 86 % of the respective control in males and females, respectively.
In the 8 000 ppm group, females showed significantly decreased weights at week 6 and weeks 9 to 24 compared to the control and the final mean average weight was 92% of the control at the end of the treatment, while growth rate in males was comparable to the control.
In the 1 600 ppm group, males and females showed similar growth curves to the controls during the treatment period.
Effects on the body weight were more important in females than in males. These effects were

durable in the 40 000 ppm female group whereas they were stopped at week 36 in the male group of the same treatment dose. Sporadic effects were observed in the 8000 ppm female group. No significant effects were seen in the 1600 ppm male and female groups.

Food consumption and compound intake

In the 40 000 ppm group, males showed significant depressions in food consumption at weeks 1 and 68, revealing an overall group mean food consumption at 94% of the control during the treatment period. Females in this group also showed significantly decreased food consumption at weeks 1, 4, 8, 12, 20, 28, 40, 48 and 68, revealing an overall group mean food consumption at 93% of the control during the treatment period.

In the 8 000 ppm group, females showed significantly lowered food consumption at weeks 28, 40, and 68 compared to the control manifesting an overall group mean food consumption at 96 % of the control. Whereas, food consumption in males was comparable to the control during the treatment period.

No statistically significant effects was observed in the 1600 ppm group either in males or females.

The food consumption depressions were more important in female than in males. They were not time-related

Overall average chemical intake in each treated group of either sex was calculated from food consumption and nominal concentration as shown in the following table:

Table B.6.5-55: Calculated test substance intake in mg/kg bw/day:

Dose level (mg/kg bw/day)
Dose level (ppm)
Male
Female
1 600
165.0
153.2
8 000
838.1
786.8
40 000
4348
4116

Haematology

Statistically significant changes in differential leucocyte counts observed in the treated group of either sex are shown in the following table.

Table B.6.5-56: Statistically significant changes in haematology parameters:

Parameter
Sex
Fate of animals a
Dose group (ppm)
1600
8000
40000
Lymphocytes
Males
Ke

NDb
172
Females
tk
c163
Neutrophil (segmented)
Males
ke
ND
81
Numbers in the above table show values in the treated groups when the corresponding value in the control group is 100.
a: ke, killed in extremis; tk, terminal kill b: ND, not determined
c: Dunnett's or Scheffe's multiple comparison test
cc: Mann-Whitney's U test

In the 40000 ppm group, males killed in extremis during the treatment period showed an increase of lymphocytes in differential leukocyte counts and a decrease of neutrophil (segmented form). In females of this group, differential count of lymphocytes was significantly increased at week 78. There were no significant differences in differential leukocyte counts at other intervals of examination in the 40000 ppm group of both sexes, males killed in extremis in the 8000 ppm group, and females at week 78 in the 8000 and 1600 ppm groups compared to the controls. No significant treatment-related effects were conceived in morphology of the leukocytes.
Necropsy
Gross pathology
Statistically significant changes in incidence of macroscopic lesions observed in the treated groups of either sex are shown in the following table.
Table B.6.5-57: Statistically significant changes in macroscopic lesions:
Sex
Male
Female
Dose group (ppm)
0
1600
8000
40000
0
1600
8000
40000
78tk (N=)
(26)
(34)
(27)
(29)
(32)

(36)
(40)
(35)
External appearance: Loss of tactile
hair
0
0
1
5*
4
8
8
0*
Soiled fur on external genital
region
9
7
2*
6
0
0
0
0
Spleen: Swelling
5
1*
4
2
7
2
3
3
Lung: Mass(es)
4
12
11*
9
8
6
18
8
Cecum: Distention
0
0
0
11**

0
0
0
16**
Kidney: Cyst(s)
4
4
2
0*
2
0
4
1
Uterus: Cyst(s)
-
-
-
-
6
2
2
0*
Skin: Loss of hair
1
4
7*
6
8
11
16
5
Ke/fd (N=)
(24)
(16)
(23)
(21)
(18)
(14)
(10)
(15)
Lymph nodes (mesenteric):
Swelling
0
2
0
5*

1
2
1
4
Lymph nodes (others): Swelling
5
2
4
9
0
3
4*
4*
Kidney: Coarse surface
4
2
1
1
6
3
0*
4
Skin: Loss of hair
5
4
7
4
11
5
2*
4
Wound
6
2
3
0*
0
0
0
0
Ulcer/Erosion
6
3
4
6
5

3
0
0
All (N=)
(50)
(50)
(50)
(50)
(50)
(50)
(50)
(50)
External appearance: Loss of tactile
hair
0
0
1
6*
5
11
9
3
Sex
Male
Female
Dose group (ppm)
0
1600
8000
40000
0
1600
8000
40000
Lymph nodes (cervical): Swelling
5
3
6
9
12
6
4*
7
Lymph nodes (mesenteric):
Swelling
0

2
0
6*
3
2
1
5
Spleen: Swelling
16
4**
12
14
17
8*
8*
10
Lung: Mass(es)
9
14
17
15
10
8
20*
11
Cecum: Distention
0
0
0
14**
0
0
0
18**
Anus: Anal prolapse
0
0
0
5*
0
0
0
0
Kidney: Pale in color
6
2

4
2
7
4
1*
4
Coarse surface
6
2
2
1
7
4
0**
5
Testis: Atrophy
5
2
5
0*
-
-
-
-
Uterus: Cyst(s)
-
-
-
-
6
2
2
0*
Eye: Opacity
1
1
5
2
5
1
0*
0*
Auricle: Partial amputation
6
2
1

0*
4
2
0
1
Skin: Loss of hair
6
8
14*
10
19
16
18
9*
Wound
9
3
3
1*
0
0
0
0
Ulcer/Erosion
7
4
9
6
8
3
1*
0**
Swelling
7
1*
3
1*
3
0
0
0
Tk: Terminal kill
Ke/fd: Killed in extremis or found dead All: All animals examined
(N=): Number of animals examined
*, p<0.05 (Fisher's exact probability test); **, p<0.01
In the 40000 ppm group, males and females showed significant increases in icidence of distention of

the cecum at terminal kill after 78 weeks of treatment. Significant increases in incidence of the lesion were also noted in all animals examined recording 28 % (14/50) in males and 36 % (18/50) in females. Distended cecum was filled with loose stool-like materials. In addition, males showed an increase in loss of tactile hair and a decrease of cyct(s) in the kidney in thosenecropsied at terminal kill, and an increase of swelling in the lymph nodes (mesenteric) and a decrease of wound in the skin in those killed in extremis or foud dead during the treatment period when compared to the controls. Among these, significant differences in incidence were also noted in all animals examined for increases in loss of tactile hair and swelling of the lymph nodes (mesenteric) and a decrease in wound in the skin. Moreover, significant differences in incidence were also noted in all animals examined for an increase in anal prolapse of the anus and decreases in atrophy of the testis, partial amputation of the auricle, and swelling of the skin. Females showed decreases in loss of tactile hair and cyst(s) of the uterus in those necropsied at terminal kill, and an increase in swelling of the lymph nodes (others) and a decrease in ulcer/erosion of the skin in those killed in extremis or found dead during the treatment period. Among these, significant differences in incidence were noted in all animals examined for decreases in cyst(s) of the uterus and ulcer/erosion of the skin. Moreover, significant differences in incidence were also noted in all animals examined for decreases in opacity of the eye and loss of hair of the skin.

In the 8000 ppm group, males showed increases in mass(es) of the lung and loss of hair of skin and a decrease in soiled fur on external genital region in those necropsied at terminal kill when compared to the control. An increased incidence was also noted in all animals examined for loss of hair of the skin. Females killed in extremis or found dead during the treatment period in this group showed an increase in swelling of the lymph nodes (others) and decreases in coarse surface of the kidney and loss of hair of the skin. Moreover, significant differences in incidence were noted in all animals for an increase in mass(es) of the lung and decreases in
swelling of the lymph nodes (cervical) and spleen, pale in color and coarse surface of the kidney, opacity of the eye, and ulcer/erosion of the skin.

In the 1600 ppm group, males showed decreased incidences in swelling of the spleen in those necropsied at terminal kill and in all animals examined and in swelling of the skin in all animals examined, while females disclosed a decreased incidence in swelling of the spleen in all animals examined.

Organ weights

In the 40000 ppm group, males and females showed significant increases in absolute and relative weights of the cecum. The percentages of the values to those of the respective control were 173 % in males and 187% in females for absolute weight, respectively, and 174 % and 212 % for relative weight, respectively. In females, relative weight of the kidney was also increased significantly at a level of 111 % of the control.

Histopathology Neoplastic lesions

The table below shows neoplastic lesions in the treated groups of aither sex with statistically significant differences in incidence from those of the controls.

Table B.6.5-58: Statistically significant changes in histopathology findings:

Sex

Male

Female

Dose group (ppm)

0

1600

8000
40000
0
1600
8000
40000
78tk (N=)
(26)
(34)
(27)
(29)
(32)
(36)
(40)
(35)
Hematopoietic & Lymphatic system:
General: Malignant lymphoma
0
0
1
5*
4
8
8
0*
Tk: Terminal kill
(N=): Number of animals examined
*, $p<0.05$ (Fisher's exact probability test)
As to neoplatic lesions, the incidence of malignant lymphoma was significantly decreased in females of the 1600 ppm group necropsied at terminal kill compared to the control. Neither increases in incidence nor nearly occurrences compared to the controls were noted for neoplatic lesions in the treated groups of both sexes.
Non-neoplatic lesions
Statistically significant changes in incidence of non-neoplastic lesions observed in the treated groups of either sex are shown in the following table.
Statistically significant changes in incidence of non-neoplastic lesions:
Table B.6.5-59: Statistically significant changes in non-neoplastic lesions:
Sex
Male
Female
Dose group (ppm)
0
1600
8000
40000
0

1600
8000
40000
78tk (N=)
(26)
(34)
(27)
(29)
(32)
(36)
(40)
(35)
Spleen: Increased extramedullary hematopoiesis
5
2
4
3
6
5
1*
4
Liver: Micro-granuloma
1
5
5
4
15
16
14
7*
Kidney: Cortical cyct(s)
9
6
9
0*
2
1
5
0
Tibio-femoral joint: Proliferation of
cartilageous tissue
14
17
11
15
18

14
11*
15
Ke/fd (N=)
(24)
(16)
(23)
(21)
(18)
(14)
(10)
(15)
Sex
Male
Female
Dose group (ppm)
0
1600
8000
40000
0
1600
8000
40000
78tk (N=)
(26)
(34)
(27)
(29)
(32)
(36)
(40)
(35)
Bone marrow (femur): Increased
hematopoiesis
6
3
7
6
7
1*
1
2
Lymph nodes (cervical): Plasma cell hyperplasia
6
1

5
4
5
3
0
0*
Spleen: Amyloid deposition
2
3
2
0
8
3
0*
1*
Small intestine: Amyloid deposition
1
1
1
0
5
0*
0
2
Liver: Amyloid deposition
3
3
2
0
10
3
0**
1**
Thyroid: Amyloid deposition
2
2
2
0
8
1*
0*
2
Parathyroid: Amyloid deposition
1
1
2

0
7
1
0*
2
Skin: Wound
9
5
9
4
9
5
1*
3
All (N=)
(50)
(50)
(50)
(50)
(50)
(50)
(50)
(50)
Bone marrow (femur): Increased hematopoiesis
9
3
10
10
9
2*
2*
2*
Bone marrow (sternum): Increased hematopoiesis
9
3
9
10
9
3
2*
2*
Bone marrow (Vertebra): Increased hematopoiesis
9

3
10
10
9
3
2*
2*
Lymph nodes (cervical): Plasma cell hyperplasia
6
2
8
5
8
3
1*
0*
Lymph nodes (mesenteric): Myeloid cell aggregation
5
0*
3
2
4
1
1
1
Spleen: Increased extramedullary hematopoiesis
20
7*
14
14
13
10
5*
9
Amyloid deposition
3
3
4
0
10
3*
0**
1**

Lung: Alveolar epithelial cell hyperplasia
0
5*
1
1
3
4
5
5
Small intestine: Amyloid deposition
2
1
1
0
8
0**
0**
3
Liver: Micro-granuloma
1
6
5
5
16
16
14
7*
Amyloid deposition
5
3
4
0*
12
3*
0**
1**
Kidney: Cortical cyct(s)
10
8
13
2*
5
1
5
0*
Glomerular amyloidosis

1
1
2
0
7
2
0**
2
Uterus: Amyloid deposition
-
-
-
-
6
0*
0*
1
Thyroid: Amyloid deposition
3
2
4
0
11
1**
0**
2**
Parathyroidc: Amyloid deposition
2
1
4
0
10
1**
0**
2*
Eye: Cataract
4
5
5
5
5
2
0*
2
Skin: Skin subacutaneous abscess
3

1
2
5
5
1
0*
1
Tk: Terminal kill
Ke/fd: Killed in extremis or found dead All: All animals examined
(N=): Number of animals examined
*, p<0.05; **, p<0.01 (Fisher's exact probability test)

c: The number animals examined in the control, 1600, 8000 or 40000 ppm groups were 46, 48, 48 or 46 in males
and 48, 48, 50 or 49 in females, repscetively.
In the 40000 ppm group, males showed significant decreases in incidence of amyloid deposition in the liver in all animals examined and cyst(s) in the kidney in those necropsied at terminal kill and in all animals examined, when compared to the control. In these males, erosion/ulcer in the anus was observed in a total of 8 animals including 6 cases killed in extremis or found dead during the treatment period and 2 cases necropsied at terminal kill. There was even a large abscess in one case. Among these, regressive hyperplasia of mucous eithelium of the large intestine was seen in 2 cases with severe lesions in the anus. However, as the histopathological examinations were carried out only on the anus which were observed macroscopic lesions, the incidence of erosion/ulcer in the anus was not assessed by a statistical method. In females of this group, statistical significant decreases in incidence were
noted in all animals examined as follows; increase hematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), cyst(s) in the kidney, micro-granuloma in the liver, and amyloid deposition in the spleen, liver, thyroid, and parathyroid. Among these, significant decreases in incidence were also noted for micro- granuloma in the liver in those necropsied at terminal kill and plasma cell hyperplasia in the lymph nodes (cervical) and amyloid deposition in the spleen and liver in those killed in extremis or found dead during the treatment period.
In the 8000 ppm group, although males did not show any non-neoplastic lesions with statistically significant differences in incidence from the control, females disclosed significant decreases in incidence of proliferation of cartilageous tissue in the tibio-femoral joint in those necropsied at terminal kill, wound in the skin in those killed in extremis or found dead during the treatment period, and subcutaneous abscess in the skin in all animals examined. In addition, significant decreases in incidence, when compared to the control, were observed in all animals examined as follows; increase hematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), extramedullary hematopoiesis in the spleen, amyloid deposition in the spleen, small intestine, liver, kidney (glomerular amyloidosis), uterus, thyroid, and parathyroid, and cataract in the eye. Among these, the incidences of extramedullary hematopoiesis in the spleen in those necropsied at terminal kill and amyloid deposition in the spleen, liver, thyroid, and parathyroid in those killed in extremis or found dead during the treatment period were also decreased significantly.
In the 1600 ppm group, males in all animals examined showed a significant increase in incidence of alveolar epithelial cell hyperplasia in the lung and decreases in incidence of myeloid cell aggregation

in the lymph nodes (mesentery) and extramedullary hematopoiesis in the spleen. In females of this group, the incidences in all animals examined were decreased significantly in increased hematopoiesis in bone marrow (femur) and amyloid deposition in the spleen, small intestine, liver, uterus, thyroid, and parathyroid. Among these, significantly decreased incidences were also noted for increased hematopoiesis in bone marrow (femur) and amyloid deposition in the small intestine and thyroid in those killed in extremis or found dead during the treatment period

Conclusion by the Notifiers

Based on the results, no oncogenic potential was observed in glyphosate after treated to mice at a dietary level of as high as 40 000 ppm for a period of 18 months (78 weeks).

RMS comments

The study is considered acceptable. Based on the effects in female mice on food consumption and body weight gain at the mid dose level of 8000 ppm, the lowest dose of 1600 ppm (ca. 153 mg/kg bw/day) is considered the NOAEL in this study. In contrast, the masses in lung mentioned in the dossier were not dose-related and there was no convincing evidence of lymph node swelling.

Male mice appeared less vulnerable.

There was an apparent error with regard to carcinogenicity:

If compared to the original study report (Text table 6, p. 48), the figures given by GTF in Table B.6.5-60 (6.5-58 in the dossier) are wrong. The precise figures are as follows:

Table B.6.5-60: Incidence of malignant lymphoma at terminal sacrifice in the study by (1997, ASB2012-11493), revised

Sex
Male
Female
Dose group (ppm)
0
1600
8000
40000
0
1600
8000
40000
78 Tk (N=)
(26)
(34)
(27)
(29)
(32)
(36)
(40)
(35)
Hematopoietic & Lymphatic system:
General: Malignant lymphoma
0
0
0

0
1
0
5*
2
4
4
8
0*
8
5
0*
3
Tk: Terminal kill
(N=): Number of animals examined
* p<0.05 (Fisher's exact probability test)
If these figures are used, the paragraph that is written below in the original text becomes clear.
Total incidence of malignant lymphoma (including animals that were prematurely found dead or had to be killed in extremis) is given in the following Table B.6.5-61 that was introduced by the RMS.
Table B.6.5-61: Total incidence of malignant lymphoma in the study by (1997)
Sex
Male
Female
Dose group (ppm)
0
1600
8000
40000
0
1600
8000
40000
No. examined
50
50
50
50
50
50
50
50
Hematopoietic & Lymphatic system:
General: Malignant lymphoma
2
2
0

6
6
4
8
7
The slight increase in high dose males was not statistically significant. Unfortunately, no historical control data for malignant lymphoma from the performing laboratory was provided. On request, the GTF submitted historical control data for malignant lymphoma from the performing laboratory (Kitazawa, 2013; ASB2014-9146). A total of 9 long-term studies (no information on actual duration provided) in the same mouse strain was covered that had been performed or at least terminated (perhaps commenced before) between 1993 and 1998, i.e., exactly the time in which the study under review was conducted. In male mice, the total incidence of malignant lymphoma in control groups varied considerably, ranging from ca 4 (actually 3.58) to ca 19 % (19.23). In fact, 8 of 9 studies had a control incidence below 12 % (6 % or lower) as observed now at the top dose level but, in principle, this incidence fell into the historical control range. Thus, the conclusion is that the higher incidence at the exaggerated dose level of 40,000 ppm as compared to the control group is a chance findings and cannot be used to support the assumption of a carcinogenic effects of glyphosate in mice that is based on the results of the study by (2001, ASB2012-11491).
In female control groups, malignant lymphoma incidence was between 8 and 27 % and, thus, the actual incidences in the control and treated groups were well covered.
Furthermore, it was noted that the study director was actually Mika Kinoshita. The report writer (Kayoko Sugimoto) was as a pathologist involved in histopathological examination.

B.6.6.1 Two generation reproductive toxicity in the rat
The reproductive toxicity of glyphosate was tested in a variety of multi-generation studies in rats. For the previous EU evaluation, a total of 8 studies in rats had been submitted of which four were still considered acceptable or, in case of a single one-generation study, at least supplementary upon re-evaluation. The studies by (1981, TOX9552385), by (1985, TOX9650161) and by (both 1988, TOX9551832 and TOX9551965), however, were deleted from current evaluation due to major deficiencies and/or because the dose levels were much too low and therefore one could not expect the occurrence of any toxic effects.
Three new studies were provided in the GTF dossier and were submitted either for the first time for this evaluation or had been subject to JMPR evaluation (JMPR, 2004, ASB2008- 6266) yet.
Reference: IIA, 5.6.1/01
Report: (2007)
Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat
Data owner: Nufarm
SPL project no.: 2060/0013
Date:2007-10-31 (amended 2008-04-08 and 2008-08-08)
not published ASB2012-11494
Guidelines: OECD 416 (2001), JMAFF 2-1-17 (2001), US-EPA OPPTS 870.3800 (1998)
Deviations:
None

GLP:
yes
Acceptability:
See RMS comment
Dates of experimental work: 2005-11-18 to 2006-11-06
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
White crystalline solid
Lot/Batch #:
H05H016A
Purity:
95.7 % (w/w)
Stability of test compound:
Not reported
Vehicle and/
or positive control:
Plain diet
Test animals:
Species:
Rat
Strain:
Sprague-Dawley Crl:CD (SD) IGS BR
Source:
Age:
Approximately 8 weeks
Sex:
Males and females
Weight at dosing:
Males: 138 – 257 g; females: 140 – 195 g
Acclimation period:
At least 14 days
Diet/Food:
Rodent PMI 5002 (certified) diet (BCM IPS Limited, UK),
ad libitum
Water:
Tap water, ad libitum
Housing:
Initially in groups of up to four in polypropylene cages with stainless steel grid floors and tops, suspended over polypropylene trays lined with absorbent paper. During mating animals were house one male : one female. Mated females were housed individually during gestation and lactation in polypropylene cages with solid floors and stainless steel lids, furnished with softwood flakes.
Environmental conditions:

Temperature: 21 ± 2°C Humidity: 55 ± 15%
Air changes: at least 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 2005-11-18 to 2006-11-06
Animal assignment and treatment:
In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females) glyphosate technical in diet. The dose levels were chosen based on results of a previously conducted study. After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. At weaning of offspring from the F0 mating phase, groups of twenty-four male and twenty-four female offspring from each dose group were selected to form the F1 generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 post partum, followed by the termination of all F0 male dose groups. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups.
Diet preparation and analyses
For preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet at a constant speed for 19 minutes in a Hobart QE200 mixer. This pre- mix was then added to larger amount of basal diet and blended for further 30 minutes in a Hobart H800 mixer.
The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration weekly for the first four weeks of the study and monthly thereafter.
Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.
Body weight
Individual body weights were recorded for F0 males on Day 1 (prior to treatment) and at weekly intervals for F0 and F1 males until termination. F0 and F1 females were weighed daily until mating was evident. Bodyweights for females showing evidence of mating were recorded on Days 0, 7, 14 and 21 post coitum. Females with live litters were weighed on Days 1, 4, 7, 14 and 21 post partum.
Food consumption and compound intake
During the maturation period, weekly food consumption was recorded for each cage of adults. For females showing evidence of mating, food consumption was recorded for the periods covering Days 0 - 7, 7 - 14 and 14 – 21 post coitum. For females with live litters, food consumption was recorded for the period covering Days 1 - 4, 4 - 7, 7 - 14, 14 - 21 post partum.
Food conversion efficiency (the ratio of bodyweight change / dietary intake) was calculated retrospectively for males for both the pre-mating and post-mating phases of the study. For females, food conversion efficiency was only calculated for the pre-mating phases of the study. Due to offspring growth, milk production and weaning, food efficiency could not be accurately calculated for the gestation and lactation phases of the study.
Water consumption
Water intake was observed daily by visual inspection of water bottles for any overt change.
Reproduction parameters Oestrus cycle

Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous.

Pregnancy and parturition

Pregnant females were observed at approximately 0830, 1230 and 1630 hours daily, and at approximately 0830 and 1230 hours on weekends and public holidays. In addition, the females were observed around the period of expected parturition. The date of mating, date and time of start and end of parturition and duration of gestation was recorded.

Litter data

The following litter data were recorded:

The number of offspring born, the number of offspring alive recorded daily and reported on Day 1, 4, 7, 14, 21 post partum. On Days 1, 4 and 21, the sex of individual offspring was recorded. The clinical condition of offspring during lactation, as well as individual offspring and total litter weights were recorded after birth on Day 1, 4, 7, 14.

Physical and sexual development

All live offspring were observed for the detachment and unfolding of pinna, incisor eruption and eyelid separation and assessed for reflexological response to stimuli by assessing surface righting reflex on Day 1 post partum and air righting reflex on Day 17 post partum. Pupillary reflex and auditory startle response were performed on Day 21 post partum.

All selected F1 offspring were observed for sexual development and the bodyweight for each individual animal at the time of sexual maturation was recorded. In addition, the ano-genital distance was recorded for all F2 generation offspring on day 1 post partum

Sacrifice and pathology

All surviving adult females and surviving offspring, except offspring selected to form the F1 generation, as well as surviving males were sacrificed on Day 21 post partum.

All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For females the uterine implantation sites were counted. In addition, the corpora lutea of all ovaries from pregnant females were counted at necropsy.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the study sampled, weighed and preserved, except for the thyroids, which were weight after fixation:

adrenals, brain, left cauda epididymis, epididymides, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, thymus, thyroid glands, and uterus (with cervix and oviducts).

The following organs from one male and one female offspring from the F0 and F1 pairings were weighed: brain, spleen, thymus, and uterus.

The following tissues were preserved from all F0 males and females from each dose group in 10 % buffered formalin, except for the right epididymis, right testis, which were fixed in Bouins fluid and 70% IMS: adrenals, coagulating gland, right epididymis, ovaries, right testis, pituitary, prostrate, seminal vesicles, Uterus (with oviducts) and cervix, vagina and all gross lesions.

A detailed histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on animals that died or were killed in extremis.

During the histopathological examination there were indications of treatment-related changes in the adrenal glands for the F1 animals. Thus, the microscopic examination was subsequently extended to include similarly prepared sections of adrenals from the F1 animals from the 5000 and 1500 ppm dose groups.

Semen assessment

At necropsy of adult F0 and F1 males at least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the testis and cauda epididymis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number

From ten control and ten high dose females of the F1 generation slides of the ovaries were prepared and analysed for visible oocytes. The identified oocytes were classified as small, medium or large follicles.

Statistics

Organ weight (absolute and relative to terminal bodyweight), weekly bodyweight gain, litter weights and offspring bodyweights were assessed for dose response relationships by linear regression analysis, followed by one way analysis of variance (ANOVA) incorporating Levene's test for homogeneity of variance. Where variances were shown to be homogenous, pair wise comparisons were conducted using Dennett's test. Where Levene's test showed unequal variances the data were analysed using non-parametric methods: Kruskal-Wallis ANOVA and Mann-Whitney 'U' test.

The non-parametric methods were also used to analyse implantation loss, offspring sex ratio and developmental landmarks and reflexological responses.

Probability values (p) are presented as follows: $p < 0.001$ ***

$p < 0.01$ ** $p < 0.05$ *

$p \geq 0.05$ (not significant)

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:

1. Chi-squared analysis for differences in the incidence of lesions occurring with an overall frequency of one or greater.
2. Kruskal-Wallis one-way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

Probability values (p) were calculated as follows: $p < 0.001$ +++ --- ***

$p < 0.01$ ++ -- ** $p < 0.05$ + - *

$p < 0.1$ (+) (-) (*)

$p \geq 0.1$ N.S. (not significant)

(+)-signs indicate positive differences from the control group, and (-)-signs indicate negative differences. * refer to overall differences between group variation which is non-directional.

Results and discussion

Analysis of dose formulations

Stability analyses indicated that the dose preparations at nominal concentrations of 1500, 5000 and 15000 ppm were stable for at least six weeks at ambient temperature.

Analyses for homogeneity at the start of treatment indicated that the dose preparations were homogeneous.

Analyses for achieved concentration performed on ten separate occasions demonstrated that the prepared dietary admixture concentrations given to the animals were in the range of 83 to 102% of the nominal concentration.

Test compound intake

The group mean achieved dosages are summarised in Table B.6.6-1 below.

Table B.6.6-1: Group mean achieved dose levels

Group

Dietary concentration
Estimated dose level
Mean achieved dose level
(ppm)
(mg/kg
bw/day)
(mg/kg bw/day)
Males
Females
Maturation
Gestation
Lactation
Control
0
0
0
0
0
0
Low
1500
75
104
126
108
252
Intermediate
5000
250
351
423
358
808
High
15000
750
1063
1273
1109
2520
Mortality
There were no test substance related mortalities.
Four unscheduled deaths occurred during the study. In the F0 generation one male of the low dose group and one female of the mid dose group was killed on humane reasons on Days 87 and 103, respectively. The male exhibited a mass of about 3 x 4 cm on the lower jar. The female was in extremis following a suspected prolonged parturition. One high dose female was found dead on Day

97 possibly due to complications during parturition.
In the F1 generation one control female was killed on Day 99 following severe clinical signs (pallor of the extremities, lethargy, pilo-erection, hunched posture and staining around the ano-genital region); however the aetiology of the signs was not established.
Clinical observations
No treatment-related clinical signs of toxicity were noted. Clinical signs observed in control and treated animals of the F0 and F1 generation are summarised in Table B.6.6-2 and Table B.6.6-3 below. These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.
Table B.6.6-2: Observed clinical signs in F0 generation
Clinical sign
Number of rats affected in dose group*
Control
(0 ppm)
Low
(1500 ppm)
Mid
(5000ppm)
High
(15000 ppm)
♂
♀
♂
♀
♂
♀
♂
♀
Abrasion to dorsal region
2/28
0/28
0/28
0/28
0/28
0/28
1/28
0/28
Generalised fur loss
5/28
5/28
3/28
5/28
2/28
6/28
2/28

3/28
Red/brown staining around snout
4/28
0/28
4/28
0/28
1/28
3/28
5/28
0/28
Red/brown staining of fur
1/28
0/28
1/28
0/28
2/28
2/28
1/28
0/28
Red/brown staining around eyes
1/28
0/28
1/28
1/28
0/28
0/28
3/28
0/28
Swollen face (due to overgrowth
tooth)
1/28
0/28
0/28
0/28
0/28
0/28
0/28
0/28
Cranial abrasion
0/28
0/28
0/28
0/28
0/28
0/28
2/28

0/28
Red stained urine
0/28
0/28
0/28
0/28
0/28
0/28
1/28
0/28
Facial scab formation
1/28
0/28
0/28
0/28
1/28
0/28
0/28
0/28
Scab formation
1/28
0/28
1/28
0/28
1/28
0/28
0/28
0/28
Large mass under lower jar
0/28
0/28
1/28
0/28
0/28
0/28
0/28
0/28
Mass on dorsal region
0/28
0/28
0/28
0/28
1/28
0/28
0/28
0/28

Scab formation around right eye
0/28
0/28
0/28
0/28
1/28
0/28
0/28
0/28
Physical injury to tail apex
0/28
0/28
0/28
1/28
0/28
0/28
0/28
0/28
Stained fur on head
0/28
0/28
0/28
0/28
0/28
0/28
0/28
1/28
Red swollen ears
0/28
/28
0/28
0/28
0/28
1/28
0/28
1/28
Blood seen without evidence of
offspring born
0/28
1/28
0/28
0/28
028
0/28
0/28
0/28

Blood around vagina (suspected prolonged parturition, killed in extremis)
0/28
0/28
0/28
0/28
0/28
1/28
0/28
0/28
Pilo-erection
0/28
0/28
0/28
0/28
0/28
0/28
0/28
1/28
Exophthalmia
0/28
/28
0/28
0/28
0/28
0/28
0/28
1/28
* x/y: number affected / total number of animals in group
Table B.6.6-3: Observed clinical signs in F1 generation
Clinical sign
Number of rats affected in dose group*
Control
(0 ppm)
Low
(1500 ppm)
Mid
(5000ppm)
High
(15000 ppm)
♂
♀
♂
♀
♂
♀

♂
♀
Generalised fur loss
3/28
4/28
0/28
2/28
0/28
6/28
0/28
4/28
Red/brown staining around eyes
2/28
1/28
0/28
0/28
1/28
0/28
0/28
0/28
Red/brown staining of fur
0/28
1/28
2/28
0/28
0/28
2/28
1/28
1/28
Red/brown staining around snout
1/28
7/28
1/28
0/28
4/28
7/28
1/28
4/28
Scabbing and fur loss around eye
0/28
/28
0/28
/28
0/28
/28
1/28

/28
Protruding sternum
0/28
2/28
0/28
3/28
0/28
3/28
0/28
0/28
Lethargy
0/28
1/28
0/28
0/28
0/28
0/28
0/28
0/28
Hunched posture
0/28
1/28
0/28
0/28
0/28
0/28
0/28
0/28
Staining around ano-genital region
1/28
0/28
0/28
0/28
0/28
0/28
0/28
0/28
Pallor of extremities
1/28
0/28
0/28
0/28
0/28
0/28
0/28
0/28

* x/y: number affected / total number of animals in group

Body weight

No adverse effect of body weight change was evident for treated animals in comparison to controls throughout the treatment period for both the F0 and F1 generations except for post- partum females treated with 15000 ppm (see Table B.6.6-4). During the final week of lactation, both the F0 and F1 generations showed statistically significant less body weight loss in comparison to controls ($p < 0.001$ and $p < 0.01$ respectively).

Table B.6.6-4: Body weight changes during lactation (Group mean values)

Dietary concentration (ppm)
No. of animals
Body weight Change (g) at Day
4
7
14
21
F0 Generation
0 (Control)
26
mean
15
22
0
-23
sd
14
9
15
10
1500
27
mean
16
16
3
-26
sd
9
9
13
13
5000
26
mean
16
18
1

-23
sd
14
13
11
11
15000
26
mean
18
18
1
-8***
sd
11
12
14
14
F1 Generation
0 (Control)
26
mean
14
9
9
-16
sd
11
13
14
13
1500
27
mean
14
16
3
-21
sd
7
11
9
17
5000
26
mean

17
10
5
-17
sd
12
10
13
13
15000
26
mean
16
11
10
-4**
sd
9
9
12
13
sd - standard deviation
** - significantly different from control group $p < 0.01$
*** - significantly different from control group $p < 0.001$

Water consumption

Daily visual inspection of water bottles showed no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations, when compared to their concurrent controls.

Reproductive parameters Oestrus cycle

There were no toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation

There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

Litter data

Size and Viability

No overt differences in litter size and viability were detected. The mean numbers of corpora lutea and subsequent number of implantations did not indicate any adverse effect of dietary exposure and pre and post implantation loss for treated animals were essentially similar to controls. There were no toxicologically significant differences in sex ratio for both F0 - F1 and F1 - F2 litters.

Growth and Development

No adverse effects on mean offspring bodyweights, bodyweight change or development were detected for male and female offspring in comparison to their controls.

Clinical signs

No clinically observable signs of toxicity were observed for offspring from treated animals.

Pathology Necropsy

There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1

animals, nor in the offspring.

Organ weights

F0 females treated with 15000 ppm displayed statistically significant increases in liver weights, both absolute and relative to terminal body weight ($p < 0.001$). An increase in liver weights was also noted for F1 females treated with 15000 ppm (absolute: $p < 0.05$, relative: $p < 0.01$). In the absence of any histopathological changes in the liver, and as increased liver weights without histopathological changes were also noted in another repeated dose toxicity study this finding is considered as an adaptive response rather than an adverse effect. Furthermore, F0 females treated with 15000 ppm displayed an increase in kidney weights, both absolute ($p < 0.001$) and relative to terminal body weight ($p < 0.01$) (see Table B.6.6-5). No such observations were detected for males treated with 15000 ppm from either generation.

Table B.6.6-5: Liver and kidney weights (relative and absolute) of females (Group mean values)

Dietary concentration (ppm)	No. of animals	Organ weight (g)			
		Liver		Kidney	
		Absolute	Relative	Absolute	Relative
F0 Generation					
0 (Control)	26	mean	15.0328	4.3103	2.4315	0.6977
		sd	1.0493	0.2864	0.1706	0.0548
1500	27	mean	15.1465	4.3027	2.5395	0.7233
		sd	1.4948	0.3435	0.1602	

5000
27
mean
15.8791
4.3570
2.5654*
0.7062
sd
1.7649
0.2810
0.2361
0.0592
15000
26
mean
16.9704***
4.6806***
2.7096***
0.7490**
sd
1.7620
0.2977
0.2203
0.0521
F1 Generation
0 (Control)
22
mean
16.4887
4.5970
2.6792
0.7483
sd
2.0275
0.4038
0.4137
0.1070
1500
23
mean
16.3848
4.6047
2.5777
0.7257
sd
1.7744

0.2858
0.2776
0.0647
5000
24
mean
17.2591
4.6543
2.8124
0.7585
sd
2.0969
0.3628
0.5326
0.1229
15000
23
mean
18.0724*
4.9591**
2.7660
0.7578
sd
1.2434
0.3130
0.2616
0.0517
sd - standard deviation
* - significantly different from control group $p < 0.05$
** - significantly different from control group $p < 0.01$
*** - significantly different from control group $p < 0.001$

There were no toxicologically significant intergroup differences detected for the brain, spleen or thymus for offspring of either sex from either generation. Furthermore, there were no differences in uterus weights for treated females from either generation when compared to controls.

Sperm assessment

There were no toxicologically significant effects on the concentration, motility or morphology of samples of sperm from treated F0 and F1 generation males when compared to their controls. Furthermore, no abnormal sperm were detected in the control and treated males from either generation.

Oocyte assessment

There were no toxicologically significant differences in follicle numbers for F1 females treated with 15000 ppm when compared to controls.

Histopathology

No treatment-related changes were detected in the F0 generation animals.

In the F1 generation cortical vacuolation of the adrenal glands was observed with a lower incidence and with generally lower grades of severity among males treated with 15000 ppm ($p < 0.05$), 5000

ppm ($p < 0.05 - 0.01$), and 1500 ppm ($p < 0.1 - 0.05$) when compared to controls. The group distribution of incidence and of severity grades may also suggest a consequence of treatment. However, the absence of a dose-related response, may suggest that a higher than normal background incidence of the condition among control male rats may have contributed to the effect on this occasion.

Table B.6.6-6: Incidence of adrenal cortical vacuolation in males at terminal kill

Historical control data
Dietary concentration (ppm)
0
1500
5000
15000
Generation
--
F0
F1
F0
F1
F0
F1
F0
F1
Animals examined
234
28
24
27
24
28
24
28
24
adrenal cortical vacuolation
Absent
153
20
7
--
14
--
16
16
14
Present
81

8
17
--
10*
--
8**
12
10***
Minimal
57
6
10
--
6
--
6
8
7
Slight
23
2
7
--
4
--
2
4
2
moderate
1
0
0
--
0
--
0
0
1
% present
34.6
28.6
71%
--
24
--

42.9
42
* - significantly different from control group $p < 0.1 – p < 0.05$
** - significantly different from control group $p < 0.01 – p < 0.05$
*** - significantly different from control group $p < 0.05$
All remaining morphological changes were those commonly observed in laboratory maintained rats of the age and strain employed and, since there were no differences in incidence or severity between control and treatment groups, all were considered to be without toxicological significance.
Conclusion by the Notifiers
The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 15000 ppm for two successive generations resulted in possible treatment-related changes at 15000 ppm. The effects however were considered not to represent an adverse health effect, therefore the NOAEL was considered to be 15000 ppm (equivalent to 1063 and 1634 mg/kg bw/day, for males and females, respectively) for adult toxicity for both the F0 and F1 generations.
The NOAEL for reproductive and developmental toxicity, for both generations and offspring was considered to be 15000 ppm.
Comment by RMS:
The study is considered acceptable. The NOAEL for parental, reproductive and offspring toxicity is considered to be 5000 ppm (approx. equivalent to 351 mg/kg bw/d) instead the proposal by the Notifiers being 15000 ppm (> 1000 mg/kg bw/d): At highest dose level of 15000 ppm increased organ weights in liver (F0 & F1 females) and kidneys (F0 females) were observed. The Notifiers stated that there is no toxicological concern regarding the significant increased liver weights due to the absence of any histopathological changes in the liver. In fact, in the present study no evidence for histopathological examination of the liver was given.
At this high dose level a significant decrease in homogenisation resistant spermatids (HRS, cauda epididymis) was counted in F0 males (Control: 399.9 million/gram; 15000 ppm: 309.0 million/gram**). No remarkable effects were seen at lower dose levels.
Furthermore, in F1 male offsprings sexual maturation (preputial separation) was delayed at 15000 ppm without any additional developmental retardation (e.g. body weight, please see Table B.6.6-7 below). The authors of the study considered this finding in F1 males (45.9 d versus control 43.0 d) to be unrelated to treatment, because no effects on sexual maturation were evident for females and there were no differences in mating performance. Sperm changes and histopathological examinations did not reveal any changes in the testis or epididymes. Although, the later onset of preputial separation in male offsprings at 15000 ppm had obviously no impact on reproductive performance in week 29, a treatment related effect on sexual maturation at parental toxic dose cannot be excluded.
All in all, the NOAEL of 5000 ppm (ca. 351 mg/kg bw/d) is considered to be more appropriate regarding parental, reproductive and offspring toxicity.
Table B.6.6-7: Sexual Maturation (balano preputial separation) of F1-males (provided by RMS)
Diet concentration (ppm)
Number of animals
Age (Days) at completion
Body weight (g) at attainment
0 (Control)
24

mean
43.0
210
sd
2.3
23
1500
24
mean
43.3
216
sd
1.6
22
5000
24
mean
43.5
219
sd
2.3
22
15000
24
mean
45.9**
230**
3.1
28
** p<0.01
Reference: IIA, 5.6.1/02
Report: (2000)
Glyphosate acid: Multigeneration reproduction toxicity study in rats
Data owner: Syngenta, Monsanto Report No.: /P/6332 Date:2000-06-16
not published TOX2000-2000
Guidelines: OECD 416, Annex V 67/548/EEC, 9.ATP 87/302/EEC OJEC, L133, 47-50 (1988), US-EPA OPPTS 870.3800 (1998)
Deviations: None
GLP: Yes
Acceptability: See RMS comment.
Dates of experimental work: 1998-09-01 to 1999-12-10
Materials and methods
Test material:
Identification:
Glyphosate acid, technical
Description:

White solid
Lot/Batch #:
Y04707/082
Purity:
97.6% (w/w)
Stability of test compound:
At least 10 years at ambient temperature
Vehicle and/
or positive control:
Plain diet
Test animals:
Species:
Rat
Strain:
Alpk:APfSD (Wistar-derived)
Source:
Age:
At least 5 weeks old
Sex:
Males and females
Weight at dosing:
Males: approx. 160 g; females: approx. 140 g
Acclimation period:
At least 14 days
Diet/Food:
CT1 diet (Special Diet Services Ltd., Witham, Essex, UK),
ad libitum
Water:
Tap water, ad libitum
Housing:
Rats were house in pairs (same sex) in multiple rat racks (with rats of the same group in adjacent cages). During mating animals were house one male : one female. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were house in pairs until
termination. Males were housed up to four per cage after being used for mating.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 50 ± 20 %
Air changes: at least 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: not reported
Animal assignment and treatment:
In a two-generation reproduction study groups of 26 Alpk:APfSD rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate acid in diet. The dose levels were chosen based on results of a previously conducted chronic toxicity study.
After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one

male: one female basis, to produce the F1 litters. On Day 29 post partum, groups of twenty-six male and twenty-six female offspring from each dose group of the F0 generation were selected to form the F1 generation. F0 males were terminated after the completion of littering and females were terminated on or soon after Day 29 of lactation. Unselected offspring were terminated at Day 29 post partum. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned off on Day 29 post partum and terminated thereafter.

Diet preparation and analyses

For preparation of diet mixtures (60 kg) a known amount of the test substance was mixed with a small amount of basal diet in a mortar using a pestle. Further milled diet was added to give a pre-mix of 1000 g. Each pre-mix was grounded at a constant speed for 15 min with an automatic pestle and mortar. This pre-mix was then added to a larger amount of basal diet and blended for further 6 minutes in a Pharma Matrix Blender Model PMA 150S (TK Fielder). Control diet was treated in the same way but without addition of the test substance. The stability and homogeneity of the test material in diet were determined in the lowest and the highest dose. Dietary admixtures were analysed for achieved concentration at a 2 month interval.

Clinical observations

A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.

Body weight

Individual body weights were recorded for F0 adults immediately prior to treatment and weekly thereafter throughout the pre-mating period. F0 males were weighed weekly thereafter until termination. Successfully mated F0 females were weighed on Day 1, 5, 8, 15 and 22 of gestation and on Day 1, 5, 8, 15, 22 and 29 post partum. Initial body weights for the F1 adults were recorded at selection on Day 29 post partum and weekly thereafter throughout the pre- mating period. F1 males were weighed weekly thereafter until termination. Successfully mated F1 females were weighed on Day 1, 5, 8, 15 and 22 of gestation and on Day 1, 5, 8, 15, 22 and 29 post partum. All rats were weighed at termination.

Food consumption and compound intake

Food consumption for each cage was recorded throughout the pre-mating period and calculated on a weekly basis. Food utilisation was calculated as the bodyweight gained by the
rats in the cage per 100 g of food eaten. Food consumption was also recorded for females during gestation and lactation and calculated on a weekly basis.

Reproduction parameters Oestrus cycle

Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous. A vaginal smear was also taken and examined from all F0 and F1 females at termination.

Reproductive performance

The success of mating (production of viable litter) was established. Length of gestation was measured in days from the date of the positive smear to the date of birth. Pre-coital interval was measured as the number of days from the date of pairing to the date of the positive smear.

Litter data

The following litter data were recorded:

The number of offspring born and the number of offspring alive were counted within 24 h after parturition and thereafter on Day 5, 8, 15, 22 and 29 post partum. The sex and the litter weight was also recorded at these times. Any clinical findings were recorded. Litters were examined for dead or moribund pups at least once daily.

Physical and sexual development

All selected F1 offspring were observed for sexual development and the bodyweight for each individual animal at the time of sexual maturation was recorded.

Sacrifice and pathology

All surviving adult females and surviving offspring, except offspring selected to form the F1 generation, were sacrificed on Day 29 post partum. Males were sacrificed at completion of the littering. All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For F0 and F1 females the uterine implantation sites were counted.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the study were sampled, weighed and preserved:

adrenal gland, brain, left and right epididymides and caudae, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, uterus (with cervix and oviducts).

The following organs from one male and one female offspring from the F1 pairings were weighed: brain, spleen and thymus.

The following tissues were preserved from all F0 males and females from each dose group in 10% buffered formalin, except for the left epididymis, left testis, which were fixed in Bouins fixative: adrenals, brain, coagulating gland, left epididymis, ovary, left testis, pituitary, prostrate, seminal vesicle, uterus (with oviducts) and cervix, vagina and all gross lesions.

Beside all pups killed in extremis (age 18-29 days) 3 male and 3 female per F2-litter were given a macroscopic examination at termination on Day 29 post partum. One of the 3 pups/sex/litter was used for organ weight determination as described above. Following tissues were stored from these pups: brain, spleen, thymus, salivary gland. Abnormal tissue from all these pups were taken and fixed as described earlier.

The reproductive organs from animals suspected of reduced fertility were processed for histopathological examination.

Semen assessment

At necropsy of adult F0 and F1 males sperm were taken from the right distal cauda epididymis. At least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the right testis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number

Primordial and small growing follicles were quantified in the left ovary of all F1 females from the control and high dose groups. Quantification was done using five 5 µm thick sections cut from the central third of each ovary and taken at least 100 µm apart and as evenly spaced as possible.

Statistics

One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: analyses of variance (ANOVA), analyses of covariance, ANOVA followed by analyses of covariance, as well as ANOVA following the double arcsine transformation of Freeman and Tukey (1950), or ANOVA following a square root formation, or Fisher's Exact Test.

All analyses were carried out in SAS (1996). For Fisher's Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance, with the exception of pup organ weights, allowed for the replicate structure of the study design.

Least-squares means for each group were calculated using the LSMEAN Option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each

treatment group least-squares mean and the control group least- squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student‘s t-test, based on the error mean square in the analysis.
All statistical tests were two sided.
Results and discussion
Analysis of dose formulations
The chemical stability of glyphosate acid in the diet at nominal concentrations of 1,000 and 10,000 ppm was consistent for at least 6 weeks (at room temperature). Homogeneity of the test substance in the dietary mixture was satisfactory, percentage deviations from the overall mean were within 4 %. The mean achieved concentrations of glyphosate acid in the preparations were within 9 % of the nominal concentrations and the overall mean concentrations were within 3 % of the nominal concentrations.
Test compound intake
The group mean achieved dosages are summarised in Table B.6.6-8 below.
Table B.6.6-8: Group mean achieved dose levels F0 and F1-generation
Group
Dietary concentration
Mean achieved dose level
(ppm)
(mg/kg bw/day)
Males*
Females
Maturation
Gestation
Lactation
Control
0
0
0
0
0
108.0
Low
1000
[99.4 / F0]
[116.5 / F1]
113.9
90.4
227.9
322.2
Intermediate
3000
[292.6 / F0]
[351.8 / F1]
346.8

277.9
752.4
1072.9
High
10000
[984.7 / F0]
[1161.0 / F1]
1136.2
910.9
2424.8
* Values of achieved dose levels, separated in F0 and F1 were inserted by the RMS
Mortality
There were no test substance related mortalities.
Seven unscheduled deaths occurred during the study. In the F0 generation one control male was killed for humane reasons during week 9 because it was found to have a ruptured eyeball. In the low level dose group one female was killed for humane reasons during week 14 having failed to litter on time, dead foetuses were present in the uterus. In the intermediate level dose group one female was killed in week 14 on gestation day 23 due to difficulties with parturition. In the high-level dose group one female with an imperforate vagina and one male having a subcutaneous mass were killed in week 15 and 18, respectively.
In the F1 generation two control animals were killed in extremis. One male due to an accidental injury in week 2 and one female in week 15 due to difficulties with parturition (one dead foetus present in uterus).
Clinical observations
No treatment-related clinical signs of toxicity were noted.
During the pre-mating period, annular constrictions were visible on the tails of the F0 and F1 male and female rats. Almost all males and approximately half of the females, in all groups, were affected. Scaly tail was also observed in some of the animals. These findings were considered incidental to the administration of glyphosate acid in the diet. Other recorded changes in clinical condition were either isolated occurrences or of an incidence comparable with that of the control group
These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.
Body weight
There was no effect of glyphosate acid on body weight adjusted for initial weight for the F0 rats, males and females, during the pre-mating period. For the F1 males given 10000 ppm, body weight was slightly lower at week 1, in comparison with the control group. Thereafter, body weights adjusted for initial weight remained lower than the controls for the duration of the pre-mating period and were statistically significant different from week 2 through to week 8 (see Table B.6.6-9). There was no effect of 10000 ppm on the body weight of the Fl females and no effect of 3000 or 1000 ppm on the body weight of the F1 males or the F1 females (see Table B.6.6-9). There was no effect of glyphosate acid on body weight adjusted for initial weight for either the F0 or F1 rats during gestation or lactation.
Table B.6.6-9: Body weight during the pre-mating period-F1 generation (Group mean values)
F1 generation
body weight (g)
Control

Low
Mid
High
(0 ppm)
(1,000 ppm)
(3,000ppm)
(10,000 ppm)
Week
♂
(n=25)
♀
(n=26)
♂
(n=26)
♀
(n=26)
♂
(n=26)
♀
(n=26)
♂
(n=26)
♀
(n=26)
1
80.2
74
81.1
75.2
78.1
74.2
75.3
73.4
2
130.1
115.4
132.3
115.7
128.6
114.7
127.6*
115.2
3
188.5
152.6
190.7

154.7
186.5
151.2
183.3*
152.3
4
246.2
178.3
247.6
180.2
242.8
176.5
237.3**
179.4
5
300.3
201
304.1
202.7
296.5
199.7
289.5**
202.1
6
345
219.8
347.5
224.1
334.5
217.2
328.7**
218.4
7
377.2
231.7
382.4
237.1
369
228.3
360.5**
234.4
8
403.6
241.9
410.1
245.1

395.3
237.2
387.0*
245.6
9
425
250.3
433.3
253.6
416.3
245.1
411.8
252.5
10
443.4
259.7
453.1
263.8
435.1
251.7
431.6
258.1
11
461.7
265.7
471.3
271.2
455.5
258.8
449.7
266.9
* - significantly different from control group $p < 0.05$
** - significantly different from control group $p < 0.01$

Food consumption

There was no effect of glyphosate acid on food consumption for the F0 generation, all F1 females and F1 males of the low and intermediate level dose group during the pre-mating period. Only F1 males of the high-level dose group showed significantly lower food consumption throughout the pre-mating period. There was no effect of glyphosate acid on food utilisation for the F0 generation, all F1 females and F1 males of the low and intermediate dose group during the pre-mating period. Food utilisation was slightly higher for F1 males given 10000 ppm glyphosate acid, the difference from control being statistically significant for weeks 5-8 only. There was no effect of glyphosate acid on food consumption for either the F0 or F1 rats during gestation or lactation.

Reproductive parameters Oestrus cycle

There were no consistent toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation

There were no treatment-related effects on pre-coital interval, mating performance,

length for both F0 and F1 generation animals.

Litter data

Size and Viability

No overt effects of glyphosate acid on pup survival or on litter size during lactation were detected. In both generations the incidence of whole litter losses was low and similar across all groups. Glyphosate acid treatment did not affect the percentage of post-implantation loss. The proportion of F1A and F2A pups born live was slightly higher in the glyphosate acid groupsthan in the control group. There was no effect of glyphosate acid on litter size at birth or during the time of lactation for either the F1A or F2A pups. The proportion of litters with all pups surviving and the proportion of pups surviving during lactation were also unaffected by the treatment. An increased proportion of litters with all pups surviving noted for the F1A

litters in the 10000 ppm group in comparison with the control group were not present for the F2A litters since the F2A controls showed an improvement over the F1A controls. Sex distribution within the litters was not altered by the administration of glyphosate acid.

Growth and Development

There was no effect of glyphosate acid on pup weight at birth for the F1A or F2A pups. Thereafter, the bodyweights of the F1A pups in the 10000 ppm group were lower in comparison with the control group The differences from control were statistically significant for males from day 8 through to day 29 and for females, from day 5 through to day 29. A similar effect was neither observed for the F2A pups in the 10000 ppm group nor for the F1A pups of the low and intermediate dose level groups. There was no effect of glyphosate acid on total litter weight of either generation. Also the day of age when preputial separation or vaginal opening occurred in the F1 parents was unaffected by treatment.

Clinical signs

No clinically observable signs of toxicity were noted for offspring from treated animals.

Pathology Necropsy

No macroscopic findings that could be attributed to the treatment with glyphosate acid were observed in any animal of the F0 and F1 generation.

The incidence of unilateral pelvic dilatation was slightly higher (9/69) in F2A females in the 10,000 ppm group compared with the other groups. Unilateral pelvic dilatation is a very common spontaneous change in the Alpk:APfSD strain of rat. There was no increase in incidence in the F0 or F1 adults or in the F1A pups and, as an isolated observation, it is considered incidental to treatment with glyphosate acid.

Organ weights

The treatment of rats with glyphosate acid did not affect the weight of the adrenal glands, brain, right cauda epididymis, epididymides, kidney, liver, ovary, pituitary gland, prostate gland, spleen, seminal vesicles, testes or uterus. For the F0 males given 10000 ppm glyphosate acid, liver and kidney weights adjusted for bodyweight were statistically significantly greater than in the control group. Similar changes were not observed in the F1 males given 10000 ppm glyphosate acid. Absolute and relative values were comparable with the control group (see Table B.6.6-10). The weight changes seen in the liver and kidney of the F0 males were therefore considered not to be treatment related. For the F0 males given 3000 or 10000 ppm glyphosate acid, brain weight adjusted for bodyweight was statistically significantly greater than in the control group. Absolute values were comparable with the control group (see Table B.6.6-10). Similar changes were not observed in the F1 animals. The weight changes seen in the brain of the F0 males were therefore considered to be incidental to treatment.

Table B.6.6-10: Liver, kidney and brain weights (relative and absolute) of males (Group mean values)
Dietary concentration (ppm)
No. of animals
Organ weight (g)
Liver
Kidney
Brain
Absolute
Relative
Absolute
Relative
Absolute
Relative
F0 Generation
0 (Control)
25
mean
19.3
3.4
3.20
0.57
2.11
0.38
sd
2.6
0.2
0.38
0.04
0.09
0.03
1000
26
mean
19.1
3.5
3.17
0.58
2.12
0.39
sd
2.3
0.2
0.36
0.04
0.08
0.03

3000
26
mean
18.7
3.5
3.11
0.58
2.12
0.40
sd
1.9
0.2
0.27
0.03
0.07
0.03
10000
25
mean
19.7
3.6
3.23
0.59
2.13
0.40
sd
2.7
0.2
0.38
0.04
0.07
0.05
F1 Generation
0 (Control)
25
mean
21.4
3.7
3.42
0.6
2.11
0.37
sd
2
0.3
0.31

0.05
0.07
0.02
1000
26
mean
21.4
3.7
3.45
0.59
2.12
0.37
sd
3.3
0.4
0.37
0.04
0.07
0.02
3000
26
mean
20.1
3.6
3.32
0.6
2.1
0.38
sd
2.6
0.3
0.31
0.04
0.07
0.03
10000
26
mean
19.7*
3.6
3.36
0.62
2.1
0.39
sd
2.3

0.3
0.28
0.04
0.07
0.03
sd - standard deviation
* - significantly different from control group $p < 0.05$
There was no effect of glyphosate acid on brain, spleen or thymus weight.
For the F1A female pups in the 10000 ppm group absolute thymus weight was statistically significantly lower than in the control group. There was no effect of glyphosate acid on the thymus weight of the F2A pups. The observation in the F1A females is therefore considered incidental to treatment with glyphosate acid.
Sperm assessment
In F0 and F1 males no effect of glyphosate acid on the number of sperm, sperm motility parameters or sperm morphology was observed.
Oocyte assessment
There was no effect of 10000 ppm glyphosate acid on the number of primordial and small growing follicles in the left ovary of the F1 parent animals.
Histopathology
No treatment-related changes were detected in the F0 and F1 generations.
Conclusion by the Notifiers
The oral administration of glyphosate acid to rats by dietary admixture at a maximum dose level of 10,000 ppm for two successive generations of the Alpk:APfSD rat resulted in possible treatment-related changes at 10,000 ppm, where a reduction in the bodyweight of the F1A pups in the 10000 ppm group with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the pre-mating period was observed. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 3000 ppm (equivalent to 322
and 459 mg/kg bw/day for males and females, respectively) for maternal and offspring for both the F0 and F1 generations.
Comment by RMS:
The study is considered acceptable and the evaluation is confirmed. Addtionally, up to 10000 ppm over to successive generations no effect on the sexuality and fertility was observed in males and females.
Furthermore, the NOAEL for parental and offspring toxicity was considered to be 3000 ppm, which has to be converted into 293 mg/kg bw/d (mean daily intake of glyphosate during pre- mating phase in F0 males.
Comment by GTF on the first draft of the RAR (July 2013):
The GTF suggested that the appropriate NOAEL value should be based on 3000 ppm in F1 males, 352 mg/kg/day, and not on the achieved dose in the parental males of 293 mg/kg/day as proposed by the RMS.
RMS comment (August 2013):
The NOAEL 3000 ppm (293 mg/kg bw/d) was set for both offspring and parents (F1) on the basis of a reduction in body weight of F1 pups and a subsequent reduction in body weight of in F1 males. The dose of 352 mg/kg bw/d was only achieved in F1 males during pre-mating period (please refer to Table B.6.6-8).
Reference: IIA, 5.6.1/03

Report: (1997)
HR-001: A two-generation reproduction study in rats
Data owner: Arysta Life Sciences Study No.: IET 96-0031
Date: 1997-06-19
not published ASB2012-11495
Guidelines: OECD 416 (1981), US-EPA FIFREA Guidelines Subdivision F (1984), Japan MAFF Guideline 59 NohSan No. 4200 (1985)
Deviations: None
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: 1996-04-16 to 1997-03-31
Materials and methods
Test material:
Identification:
Glyphosate technical, Code: HR-001
Description:
White crystal
Lot/Batch #:
T-950308
Purity:
94.61 % (w/w)
Stability of test compound:
Not reported
Vehicle and/
or positive control:
Plain diet
Test animals:
Species:
Rat
Strain:
Sprague-Dawley; Crj:CD (SD)
Source:
Age:
5 weeks
Sex:
Males and females
Weight at dosing:
Males: 132 - 148 g; females: 112 - 126 g
Acclimation period:
7 days
Diet/Food:
Certified pulverized feed (MF Mash, Oriental Yeast Co., Ltd), ad libitum
Water:
Filtered, sterilized well water, ad libitum
Housing:
During acclimatisation in groups of five per sex in suspended wire-mesh stainless steel cages). During

pre- mating, and mating periods animals were housed in groups o 3/sex/cage. During mating one male and one female were housed in aluminium cages with wire-mesh floors and fronts. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were house in pairs until termination. Males were housed up to four per
cage after being used for mating.
Environmental conditions:
Temperature: 22 ± 2 °C
Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 1996-04-16 - 1997-03-31
Animal assignment and treatment:
In a two-generation reproduction study groups of 24 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1200, 6000 and 30000 ppm HR-001 in diet. The dose levels were chosen based on results of a preliminary reproductive study in Crj:CD (SD) rats..
After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. The day of proved copulation was designated Day 0 of gestation. Copulated females were placed individually into breeding boxes with nestle material. The day of completed parturition was designated Day 0 of lactation. On Day 4 post partum, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on Day 21 of lactation and all F0 parental animals were sacrificed. Groups of 24 male and 24 female offspring from each dose group of the F0 generation were selected to form the F1 parents. Unselected offspring were sacrificed and subjected to a gross necropsy.
The offspring selected for the F1 generation were dosed for 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned on Day 21 of lactation and terminated together with F1 parental animals. F1 parental rats which failed to produce F2 offspring (10 males and 10 females with normal external genitalia and oestrus cycle) were mated with untreated rats of the same strain and sacrificed thereafter for fertility assessment (reproductive performance).
Diet preparation and analyses
Diets were prepared monthly during the pre-mating period, and biweekly during the breeding period. For each dose level a specified amount of the test substance was mixed with a small amount of basal diet in a mortar. This pre-mix was stirred into the remaining part of the diet. The diets were stored at about 4 °C in the dark. Analyses for homogeneity were done for each dose level of the first diet preparation. Analyses for achieved concentration were done for all prepared diets.
Clinical observations
A check for clinical signs of toxicity and mortality was made once daily on all F0 and F1 parental animals. A detailed physical examination was performed on males prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. Females were examined prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.
Body weight
Individual body weights F0 and F1 males adults were determined prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. F0 and F1 females were weighed prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.

Food consumption and compound intake

Food consumption for each cage was recorded and daily food consumption was calculated. Determination of food consumption was made on a weekly basis during the pre-mating period for males and females and during the breeding period for males. In addition, for females total food consumption was determined at the following intervals: Day 0-7, 7-14, 14-20 of gestation and of days 0-7, 7-14 and 14-21 of lactation.

Compound intakes in parental animals were calculated during the pre-mating periods for each sex on a weekly basis.

Reproduction parameters Oestrus cycle

The oestrus cycle was checked daily by microscopically examination of vaginal smears. Examinations were done for each female for one week prior to mating until copulation was confirmed.

Reproductive performance

Mating indices for males and females were calculated separately after copulation was confirmed. In addition, fertility and gestation indices, the length of gestation, as well as the number of implantation sites were determined.

Sperm assessment

An assessment of motility and morphology of epididymal sperm was done at necropsy for 10 males per group, which were selected for the organ weight measurement, as well as for males that failed to impregnate females.

Litter data

Total number of live and dead pups, and the number of males and females per litter were determined on Day 0 of lactation. The sex ratio was calculated for each group. Viability indices, were determined for each litter on lactation days 0, 4 and 21. Body weights were determined on lactation days 0, 4, 7, 14 and 21.

A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. A detailed physical examination was done on lactation days 0, 4, 7, 14 and 21.

Sacrifice and pathology

All surviving parental F0 and F1 males and females were sacrificed on Day 21 post partum and subjected to a gross pathological examination. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. The following organs and tissues were preserved: adrenals, aorta, brain, caecum, colon, duodenum, epididymis, eyes, gross lesions, head (incl. nasal cavity, paranasal sinuses, buccal mucosa and ears), heart, ileum, jejunum, kidneys, larynx, liver, lung, mammary gland, oesophagus, ovaries, pancreas, pharynx, pituitary, prostrate, rectum, seminal vesicles, spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus (cornua and cervix) and vagina.

F1 and F2 pups that were not selected on Day 4 of lactation were also killed and necropsied on that day. In addition, F1 weanlings that were not selected for parental animals of the F1 generation and all F2 weanlings were necropsied at 22-26 and 21-26 days of their age, respectively. The same organs, as described above, were preserved from one animal per sex per litter of the F1 and F2 weanlings necropsied.

The following organs weights of 10 F0 and F1 males and females from each dose group that were sacrificed at the end of the study, as well as from pairs of parental animals that failed to mate: adrenal gland, brain, epididymides, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), testes, uterus.

A histopathological examination was performed on the reproductive organs and pituitary of the control and high dose F0 and F1 parental animals that survived until scheduled termination. A

histopathological examination of the reproductive organs and pituitary in the low and mid-dose group was only performed on pairs of animals that had failed to produce offspring. In addition, a histopathological examination was performed on organs with significant weight change, and on all organs with gross pathological changes.

Statistics

One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Bartlett´s test for equality of variance (p=0.05) followed by parametric analyses of variance in one-way classification (p=0.05) or Dunnett´s t-test or Scheffé ´s multiple comparison test (p=0.05, 0.01 or 0.001); or Bartlett´s test followed by Kruskal-Wallis test (P=0.05) and Dunnett-type mean rank test or Scheffé-type mean rank test ((p=0.05, 0.01 or 0.001). Fisher´s exact probability test ((p=0.05, 0.01 or 0.001) and Mann- Whitney´s U-test (p=0.05 or 0.01) were also used.

Results and discussion

Analysis of dose formulations

Based on the results of the dose-finding study the chemical stability of the test substance in the was given for 5 weeks (at room temperature) in sealed plastic bags in the dark, and for at least 2 weeks after being released from the plastic bags.

Homogeneity of the test substance in the dietary mixtures was satisfactory, percentage deviations from the overall mean were within 4%. The mean achieved concentrations of HR- 001 in the diet preparations were in the range of 90 – 105 % of the nominal and therefore acceptable.

Mortality

F0 and F1 males

Seven unscheduled deaths occurred during the study. In the F0 generation one control male was killed for humane reasons during week 9 because it was found to have a ruptured eyeball. In the low level dose group one female was killed for humane reasons during week 14 having failed to litter on time, dead foetuses were present in the uterus. In the intermediate level dose group one female was killed in week 14 on gestation day 23 due to difficulties with parturition. In the high-level dose group one female with an imperforate vagina and one male having a subcutaneous mass were killed in week 15 and 18, respectively.

In the F1 generation two control animals were killed in extremis. One male due to an accidental injury in week 2 and one female in week 15 due to difficulties with parturition (one dead foetus present in uterus).

F0 and F1 females

There were no mortalities observed during the study period.

Clinical observations F0 and F1 males

There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups.

At 30000 ppm F0 and F1 parental males exhibited loose stool with incidences during the pre- mating growth and breeding periods of 3/24 and 2/24 for the F0 generation, and of 13/24 and 0/24 for the F1 generation, respectively, with a significant difference in the value for the pre- mating growth period of the F1 generation. Since this finding was not observed in other groups including control, defecation of loose stool was considered to be treatment-related.

Statistically significant differences were also observed in the incidence of hair loss during the breeding period for F0 males in all test substance groups. However, the occurrence of this change in the treated groups was rather lower than controls, and was considered to be incidental.

During the study period, one F0 male and one F1 male in the control group and one F1 male in the 6000 ppm group showed malocclusion of the incisors, respiratory wheezing, and red sebum. The

aforementioned one F1 male of the 6000 ppm group also showed distension of the abdomen. These animals were euthanatised within several days after discovery due to unfavourable prognosis. Necropsy noted a fracture of the facial bones in all cases, suggesting that the alterations were caused by an accident in the cage. Accident malocclusion of incisors was also observed in one F1 male in the 1200 ppm group. However, test substance treatment of this animal was continued until termination of the study because its condition was improved.

F0 and F1 females

There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups.

In F0 and F1 parental females, loose stool was also observed at 30000 ppm. The incidences during the pre-mating growth period and the lactation and post-weaning period were 1/24 and 6/24 for the F0 generation, and 4/24 and 2/24 for the F1 generation, respectively, with a significant difference in the value for the lactation and post-weaning period of the F0 generation.

Bodyweight

F0 and F1 males

Mean body weights of F0 and F1 males in the 30000 ppm group were consistently lower than those in the control group from treatment week 1 to the day of necropsy, and the differences from controls at treatment weeks 1-12 and 14 for the F0 generation, and treatment weeks 1-6 for the F1 generation were statistically significant. In the 1200 and 6000 ppm groups, mean body weights of F0 and F1 parental males were comparable to the controls throughout the study.

F0 and F1 females

There were no significant differences in mean body weights of F0 females in any treatment group when compared to control. In F1 females in the 30000 ppm group, mean body weight on lactation day 0 was significantly higher than that in the control group. In the 1200 and 6000 ppm groups, mean body weights of F1 parental females were comparable to the controls throughout the study.

Food consumption and test compound intake F0 and F1 males

In F0 males, mean food consumption at treatment week 13 in the 1200 ppm group was significantly higher than that in the control group. Since there was no such increase observed in the mid- and high-dose groups throughout the study, this change was not thought to be treatment-related.

In F1 males in the 30000 ppm group, mean food consumption at treatment week 4 was significantly lower than that in the control group, but the values on the other treatment weeks in this dose group were comparable to the controls. In the 1200 and 6000 ppm groups, mean food consumption of F1 males was comparable to the controls throughout the study.

F0 and F1 females

In F0 females, the values on treatment weeks 2-4 in the 30000 ppm group were significantly higher than the controls. Inversely, the value on lactation days 7-14 in this dose group was significantly lower than those in the control group. So it was unclear these changes were treatment-related or not. In the 1200 and 6000 ppm groups, mean food consumption of F0 females was comparable to the controls throughout the study.

In F1 females in the 1200 and 6000 groups, mean food consumption on lactation days 14-21 were significantly higher than those in the control group. However, these changes were thought to be incidental because no such increase was observed in the highest dose group. In the 30000 ppm group, mean food consumption of F1 females was comparable to the controls throughout the study.

The group mean achieved dosages are summarised in Table B.6.6-11 below.

Table B.6.6-11: Group mean achieved dose levels F0 and F1-generation

Group

Dietary concentration

Mean achieved dose level
(ppm)
(mg/kg bw/day)
Males
Females
F0
F1
F0
F1
Control
0
0
0
0
0
Low
1200
83.6
91.7
96.9
104.8
Intermediate
6000
417
458
485
530
High
30000
2150
2411
2532
2760

Reproductive parameters F0 males and females

Reproductive performance of F0 parental animals was not adversely affected by test substance treatment, and no significant differences were observed in such parameters as percentage of females having normal oestrous cycle, mating index, fertility index, gestation index, duration of gestation, number of implantation sites , and number, motility and morphology of epididymal sperm between the control group and the treated groups.

F1 males and females

In F1 parental animals, reproductive parameters in the treated groups were also comparable to the controls with the exception of gestation index and number of implantation sites, on which some biases were occasionally observed.

The significant higher number of implantation sites at 1200 ppm when compared to control was considered to be unrelated to treatment, since there was no increase noted at 6000 and 30000 ppm. A similar bias was also found in the fertility index. The fertility indices in the control, 1200, 6000 and

30000 ppm groups were 95.8 (23/24), 95.8 (23/24), 87.5 (21/24) and 79.2% (23/24), respectively, with somewhat low values in the 2 higher dose groups. However, these decreases were considered to be incidental because the differences between the control and treated groups were not statistically significant, and because, as described below, normal reproduction results were obtained in the F1 parental animals, which had failed to produce offspring in this study, after remating with untreated animals.

Among the total of ten F1 females mated with untreated males, only one female in the 30000 ppm group did not undergo pregnancy. Histopathological of this female showed no abnormalities in the reproductive organs and pituitary. So the cause of infertility of this female was not known. The other nine F1 females were proved to have normal reproductive performance. One F1 male in each of the 1200, 6000 and 30000 ppm groups could not successfully impregnate untreated females mated. These 3 males had histopathological

abnormalities in the testes and epididymides, and abnormalities in the sperm parameters, as a cause of infertility. However, the other 7 males were proved to have normal reproductive performance. Thus, the majority of F1 males and females which had failed to produce offspring were proved to have normal reproductive performance.

Litter data

Number of pups delivered

Mean number of F1 and F2 pups delivered in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Sex ratio

Sex ratios of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Viability index

The viability indices of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Body weights F1 pups

There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls. F1 pups of both sexes in the 30000 ppm group, showed significantly higher mean body weights on lactation day 0 than the controls. However, mean body weights on days 14 and 21 were significantly decreased when compared controls.

F2 pups

There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls during the lactation period. In F2 pups in the 30000 ppm group, mean body weights of both sexes on day 21 of lactation were significantly lower than those in the control group.

Clinical signs

There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group.

During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.

Pathology Necropsy

F0 and F1 generation

Necropsy of parental animals of both sexes noted several findings in all groups including the control group. Among these alterations, the incidences of distension of the caecum in F0 and F1 males and females of the 30000 ppm group were significantly higher than those of the controls, and were considered treatment-related. Statistically significant differences from controls were also found in

the incidences of hair loss in F0 males of the 1200, 6000 and 30000 ppm groups. However, the values were rather lower than controls and were considered to be incidental. Other findings were low in their incidences and considered not treatment- related.

F1 and F2 pups

Necropsy of stillbirths found on lactation days 0, pups found dead during lactation days 1-4, and pups killed to reduce the litter size on lactation day 4 demonstrated no treatment-related abnormalities in any of the F1 and F2 pups.

During days 5-21 of lactation, only 2 F1 pups in the 1200 ppm group were found dead. Necropsy of these dead pups were not performed due to advanced autolysis.

Necropsy of F1 and F2 weanlings in the 30000 ppm group noted distension of the caecum, suggesting a treatment-related occurrence. In the 1200 and 6000 ppm groups, no treatment- related abnormalities were observed in any of the F1 and F2 weanlings.

Organ weights F0 and F1 males:

There were no effects in the absolute and relative organ weights in F0 and F1 males of the low- and mid-dose groups. At 30000 ppm relative weights of the liver and kidneys of F0 and F1 males were significantly higher than the control values. These increases were considered treatment-related. In F1 males in the high-dose group, there was also a significant decrease noted in the absolute and relative weights of the prostate. Besides these changes, the relative brain weight of F0 males in the 30000 ppm group was significantly higher than the control value. However, this finding was considered to be the change associated with the low body weights in this group.

F0 and F1 females

In F0 females, the absolute and relative weights of all organs were comparable between the control and treated groups. In F1 females in the 30000 ppm group, the absolute and relative weights of the liver and kidneys were significantly higher than the controls, and these increases were considered treatment-related.

Significantly higher-than-control value was also observed in the absolute kidney weight in the 6000 ppm group. However, this increase was not considered treatment-related because statistical significance in the difference between the control and 6000 ppm groups disappeared when all F1 females were subjected to the weighing of the kidneys fixed in 10 % neutral buffered formalin. The significant lower relative ovarian weight observed in F1 females in the 1200 ppm group was considered to be an incidental finding because no such decrease was observed in the mid- and high-dose groups.

Histopathology

F0 and F1 generations

In all F0 and F1 males and females in the 30000 ppm group, histopathological examinations of the reproductive organs and pituitaries did not indicate any treatment-related alterations.

No treatment-related histopathological alterations were also evident in the following organs in which significant weight changes were detected: kidneys of F1 females in the 6000 ppm group; kidneys of F0 males and F1 males and females in the 30000 ppm group; and liver of F1 males and females in the 30000 ppm group.

Conclusion by the Notifiers

The oral administration of HR-001 to rats by dietary admixture at a maximum dose level of 30000 ppm for two successive generations of Sprague-Dawley rat resulted in maternal toxicity at 30000 ppm. Thus, the NOAEL for maternal toxicity is 6000 ppm, equivalent to 417

- 458 mg/kg bw/day and 485 - 530 mg/kg bw/day for males and females, respectively.

The NOAEL for reproduction is 30000 ppm, since the reproductive performance was not affected in

any dose group. Based on the body weight effects and increased incidences of caecum distension the NOAEL for offspring is considered to be 6000 ppm.
Comment by RMS:
The study is considered acceptable. Parental toxicity was observed at highest dose of 30000 ppm (> 2000 mg/kg bw/d) only and consisted of loose stool (F0/F1, m/f), reduced body weight (F0/F1, m) caecum distension (F0/F1, m/f), increased liver and kidney weights (F0/F1, m/f), decreased prostate weight (F1). Histopathological alterations were not detected.
Lower gestation indices were observed at mid and high dose level, however without statistical significance. Indeed, most of the F1 animals were proved to have normal reproductive performance after re-mating with untreated animals, but this is not in accordance with current test guidelines: re-mating should be performed with treated males of the same dose group. The NOAEL for reproductie toxicity is considered 6000 ppm (417 mg/kg bw/d) based on lower gestation indices of F1 females at high dose level.
Reproductive toxicity was not observed up to the highest dose level, despite lower gestation indices of F1 females at mid dose of 6000 ppm (530 mg/kg bw/d) and high dose level. This finding was considered not to be treatment related, because most of the F1 animals which failed to produce offspring were proved to have normal reproductive performance after re- mating with untreated animals. Offspring toxicity was observed at highest dose level only and confined to reduced body weight and caecum distension in both sexes. Sexual maturation (preputial separation, vaginal opening) was not examined in this study. Based on the results the NOAEL for parental and offspring toxicity was considered to be 6000 ppm and for reproductive toxicity to be 30000 ppm (>2000 mg/kg bw/d).
Reference: IIA, 5.6.1/04
Report: (1993)
Two Generation Reproduction Study in Wistar Rats.
Data owner: ADAMA Agan Ltd
Study No.: TOXI 885-RP-G2 Date: 1993-08-27
GLP: yes
not published TOX9300009
Guidelines: OECD 416 (1983)
Deviations: None
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: May 1991 - April 1992 (not further specified)
Materials and methods
Test material:
Identification:
Glyphosate technical
Chemical name
N-(Phosphonomethyl) glycine
Description:

Odourless, white crystal
Batch #:
60
Purity:

96.8 %
Date of receipt
11/9/1990
Stability of test compound:
More than two years at ambient temperature
Vehicle and/
or positive control:
Plain diet
Test animals:
Species:
Rat
Strain:
Wistar rats (Random bred)
Source:
Age at start of treatment (F0):
8 weeks
Sex:
Males and females
Mean body weight at initiation of dosing:
Males: 160 - 190 g; females: 141 - 160 g
Acclimation period:
7 days
Diet/Food:
Standard "Gold Mohur" brand powdered rat feed manufactured by M/s Lipton India Limited, Bangalore,
India
Water:
Deep bore well water passed through activated charcoal filter and exposed to UV rays (Aquaguard on-line water filter cum-purifier manufactured by M/s Eureka Forbes Limited, Bombay, India) was provided in glass bottles ad
libitum
Housing:
Groups of five/three rats of same sex per cage depending on the size of the animals were accommodated in standard polypropylene rat cages (size: L 430 x W 270x H 150 mm) with stainless steel top grill; bedding material (paddy husk)
was changed three times per week.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 40-70 % Air changes: 10-15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: May 1991 to April 1992 (not further specified)
Animal assignment and treatment:
In a two-generation reproduction study groups of 30 Wistar rats per sex of the F0 generation received daily dietary doses of 0, 100, 1000 and 10000 ppm glyphosate technical in diet.
After at least 8 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. The day of proved copulation (vaginal smear)

was designated Day 0 of gestation. On Day 4 post partum, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on Day 21 of lactation and all F0 parental animals were
sacrificed. Groups of 30 male and 30 female offspring from each dose group of the F0 generation were selected to form the F1 parents.
The offspring selected for the F1 generation were paired within each dose group to produce the F2 litters. F2 litters were weaned on Day 21 of lactation and terminated together with F1 parental animals.
Diet preparation and analyses
The required quantities of test compound were weighed and mixed manually with 1.0 kg of powdered rat feed to prepare the premix. The premixes were added to the bulk of remaining quantities of feed and mixed in ribbon mixer. Prepared feed bulks were sampled at different intervals for assaying test compound concentration in experimental diet.
Clinical observations
All animals were observed daily throughout the study and any visible clinical signs were recorded with details on type, severity, time of onset and duration. Any animal found dead or sacrificed in extremis was necropsied and macroscopically abnormal tissues were retained.
Body weight
Males were weighed weekly until termination. Females were weighed weekly during pre- mating, on Gestational Days 0, 6, 13, and 20 and on Days 1, 4, 7, 14 and 21 of lactation.
Offspring were weighed sex-wise as litters on Days 1, 4, 7, 14 and 21 post partum.
Food consumption and compound intake
Food consumption for each cage of males was recorded weekly until termination. Food consumption of females was recorded weekly during pre-mating and at the following intervals: Days 0-6, 6-13, 13-20 of gestation and Days 1-4, 4-7, 7-14 and 14-21 of lactation.
Reproduction parameters Reproductive performance
The following reproductive indices were recorded: Male and female fertility index, fecundity index, mean number of implantations, parturition percentage, percentage mortality of pregnant dams, percentage of live pups born, in females the pre-coital interval (time elapsed between initial pairing and detection of mating) and duration of gestation.
Litter data
Total number of live and dead pups, viability indices (mean viable litter size on day 0, live birth index), litter weight, individual sex and observations on individual pups (if any) were determined within 24 hours after birth. Survival indices were determined on Days 2, 4, 7, 14
and 21 of lactation. Body weights were determined on Lactation Days 0, 4, 7, 14 and 21. A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. On Day 4 post partum, offsprings were culled to reduce litter size to eight.
Sacrifice and pathology
All surviving parental F0 and F1 males and females and the non-selected weanlings from F1 and all F2 weanlings were sacrificed and subjected to a gross pathological examination. Tissue collection was done for parent generation only. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible.
The following organs and tissues were preserved from all F0 and F1 parents of all groups: Ovaries, uterus, vagina, testes, epididymides, seminal vesicles, prostate, coagulation glands, pituitary, adrenals, liver and kidneys. The organs were examined for gross pathological changes and those found abnormal were examined histopathologically.

Females failing to get mated within 21 days and females failing to produce a viable litter by Day 25 post coitum were necropsied and any macroscopically abnormal tissue was retained for histopathological examination. The presence of corpora lutea, implantations and resorptions was examined in females which had failed to produce a viable litter.
On Day 4 post partum, offsprings were culled to reduce litter size to eight, where possible; culled offspring or found dead were necropsied. All F2 pups were sacrificed at weaning.
Statistics
One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Dunnett´s t-test (for body weight, food consumption, litter number, litter weight, gestation and lactation period), Z Test (for mating performance, fertility index, gestation index, live birth index, viability index, lactation index, pups survival data, number of dead pups at birth, survival indices, number littered) and t/r test (for dose-response relationship).
Results and discussion
Analysis of dose formulations
In-house stability study for glyphosate technical was carried out at 0, 2000 and 20000 ppm. Chemical stability was given for 30 days at room temperature with a loss of less than 7 % at 0, 2000 and 20000 ppm levels in experimental diet when stored in polyethylene lined stainless steel drums.
The mean achieved concentrations of glyphosate in the diet preparations were analysed; the achieved concentrations were in the range of 96-100 % of the nominal and therefore acceptable.
Mortality
F0 and F1 males
There were no deaths in male animals.
F0 females
In the females there were three deaths, two in the low dose group, (one dystokia and one suppurative pneumonia) and one in the high dose group (cause of death not ascertained).
F1 females
One dam in low dose group died of dystokia and no other mortalities were seen.
Clinical observations F0 generation
Nasal discharge and snuffling and cannibalism were seen in all groups. No other treatment related changes in clinical signs were observed.
F1 generation
The incidence of clinical signs was low and not treatment or dose related.
Body weight F0 males
Initial body weight of treatment groups was higher compared to the control group and this trend continued during the entire treatment period. The absolute weight gain (difference between initial and terminal) during entire treatment period was similar to control group in low and high dose while in mid dose it was slightly higher.
F0 females
No significant treatment related differences were noted between treated and control groups.
F1 males
Mid dose group body weight (both initial and subsequent weeks) was more than control. In high dose group initial body weight (Week 0) was higher than control but at Week 2 and 3 it was less. However in this group the body weight tended to be higher (not significant) during last seven weeks.
F1 females
The body weight of all treatment groups at selection (Week 0) was higher than in the control group and continued to be significantly higher than in the control group for up to Week 10 in mid and high

dose groups. Body weights of the high dose group dams on Days 0, 6 and 13 of gestation period were significantly higher compared to controls but the body weight gain was statistically not significantly different. Another incidental significant finding was higher body weight (Gestational Day 0-20) of mid dose group dams compared to controls. Absolute body weight of mid dose group on Lactation Days 1 and 4 and that of high dose group during all periods of lactation was significantly higher than in control group. The mid dose group had lost body weight during Days 7-14, 14-21 and 1-21 of lactation period as compared to control.

Food consumption and test compound intake F0 parents

Mean food consumption of males was comparable to the controls throughout the study. High dose female animals tended to consume significantly more food than controls during gestation. During lactation low and mid-dose females consumed significantly less than controls, especially for the Periods 7-14 and 14-21. High dose females consumed significantly more food for Lactation Days 4-7 as compared to controls.

F1 males

Treatment groups did not show consistent and dose related changes as compared to control group. However initially (Weeks 0-2) mid and high dose groups consumed significantly less feed and later on a few occasions mid dose group showed increased consumption.

F1 females

Treatment group dams did not show treatment and dose related consistent difference from control group; on a few occasions the treatment groups showed both increased/decreased food consumption over control. During gestation there was no statistically significant inter group difference in feed consumption between control and treatments during gestation period. Low dose dams consumed significantly less food than controls during different lactation periods (except for Day 7 and Period 7-14). Mid and high dose group dams did not show any treatment and dose related changes over control except for an incidental finding of increased and decreased feed consumption on Day 7, 14 and Period 7-14 and 14-21 respectively in mid dose group.

Reproductive parameters

Reproductive performance parameters of F0 parental animals such as female fertility index, number of implantations, gestation index, duration of gestation, live birth index, and duration of gestation were not significantly different between treated and control groups. Male fertility index was significantly higher in low and high dose groups over control.

F0 generation

On Day 1 of lactation, mean litter size was significantly less than control in low and mid dose groups and the mean viable litter size at birth was significantly less in low dose group; the number of live pups on Day 1 was significantly lower in the mid-dose group.

F1 generation

Reproductive performance parameters of F1 parental animals such as male and female fertility index, fecundity index, parturition percentage and mortality of pregnant dams was not different between treatment and control groups. The incidence of dams not littered tended to be higher in the mid-dose group compared to controls. A significantly decreased number of implantations was observed in low and mid dose groups; the percentage of live pups born was significantly reduced in the in mid dose group and significantly increased in the high dose group.

Table B.6.6-12: Reproductive parameters of F0- and F1-generation

Group 1 - control

Group 2

Group 3

Group 4
0 ppm
100 ppm
1000 ppm
10000 ppm
F0
F1
F0
F1
F0
F1
F0
F1
Number of dams
in group
30
30
30
30
30
30
30
30
Number of dams
littered
29
26
26
27
28
22
30
28
Mean litter size
11.3
11.7
9.8*
10.4
9.9*
10.9
10.4
11.9
Mean viable litter
size at birth
11.0
11.7

9.7*
10.4
9.9
10.9
9.9
11.9
Number of pups
alive on day 1
320
305
253
281
276*
239
296
334
Mean number of
implantations
12.1
13.4
11.2
11.6*
11.0
12.0*
12.3
12.9
Percentage of live
pups born [%]
87.9
87.6
83.5
86.5
86.5
79.7*
80.0*
92.8**
*significantly decreased; **significantly increased
Litter data
Number of pups delivered
Mean number of F1 and F2 pups delivered and mean litter sizes in the 100, 1000 and 10000 ppm groups were comparable to the controls.
Sex ratio
Sex ratios of F1 and F2 pups in the 100, 1000 and 10000 ppm groups were comparable to the controls.
Viability index F1 pups
In the low dose group the pup survival index for Days 4, 14 and 21 was significantly lower than in

controls. In the mid dose group the live birth index and Day 14 survival index were higher and Day 4 survival index was lower compared to controls. In the high dose group on Day 14 and 21 survival index was higher than in controls. Dose response relationship was not seen in these parameters.
F2 pups
There were no statistically significant inter group differences between control and treatment groups in parameters of F2 litters at first observation including incidence of external abnormalities in pups. The mean number of pups (combined and individual sex) during different periods of lactation did not show statistically significant differences compared to control group.
The group mean values of pup survival data parameters like: live birth index, 24 hours survival index and survival index for Days 4, 7, 14 and 21 did not show any significant inter group difference between control and treatment groups.
Body weights F1 pups
Mean litter weight of combined sex and female pups in treatment groups were significantly more than control group on Day 1 and 4, respectively. On Day 7 combined sex litter weight and male pup weight was significantly less than control in low dose group while in high dose group it was more than control group. On Day 21 the mean body weight of complete litter and individual sex pups of mid dose group were more than control group. None of these showed any apparent dose response relationship.
F2 pups
Combined sex litter weight on day one and that of female pups of all treatment groups was higher than in controls; in addition combined sex litter weight in low and mid dose groups and that of male and female in mid dose group was higher than control on Day 4. In high dose group the male pup body weight on Day 14 and 21 was lower than control. None of these parameters showed any dose response relations.
Clinical signs
There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group. During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.
Pathology Necropsy
F0 generation
The gross pathological lesions seen were consolidated lungs with ecchymoses, chronic liver changes, kidneys with cysts and dilated pelvis, and hypoplastic testes (1 in the control group, 2 in the mid-dose and 1 in the high-dose group). The incidence was low and did not appear to be compound or dose related.
F1 generation
The gross pathological lesions seen were consolidated and collapsed lungs with emphysema, hydronephrotic kidneys, and unilateral hypoplastic testes. The lesions observed were few and appeared to be incidentally. A single incidence of unilateral testicular hypoplasia was observed in each of the three treatment groups, hydronephrosis was seen in two animals in the high dose group.
F1 pups
A higher incidence of emaciated pups was recorded for the mid and high dose groups compared to controls. A low incidence of minor developmental abnormalities like Kinky tail, rudimentary tail, kidney hydro-nephrosis and dilated pelvis occurred without dose-response relation.
F2 pups
A higher incidence of emaciation has been observed in pups of high dose group. Occasional not

treatment and dose related incidence of hydronephrosis and dilated pelvis in kidney have been recorded.
Histopathology F0 generation
Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one control animal, two mid dose and one high dose animal. The control and high dose animals showed degenerative changes in the seminiferous tubules while the mid dose group were normal. These changes appeared to be incidental and not compound related.
F1 generation
Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one animal in each of the three treatment groups; the testes in the low and mid dose groups showed unilateral degenerative changes and giant cell formation in the seminiferous tubules and focal chronic inflammation. The testes in the high dose were normal though unequal in size. The changes appeared to be incidental and not compound related.
Conclusion by the Notifiers
The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations of Wistar-Dawley rats resulted in no maternal toxicity.
The NOAEL for reproduction is considered to be 10000 ppm, since the reproductive performance was not affected in a dose-related manner. The NOAEL for offspring is 10000 ppm, since no treatment-related effects on offspring could be observed.
Comment by RMS (Re-evaluation):
The previous evaluation in 2001 regarding the NOAELs is confirmed. However, this study is now considered supplementary, because an effect dose was lacking. The highest dose of 10000 ppm is considered to be the NOAEL for parental, reproductive and offspring toxicitiy. This dietary level would correspond to a mean daily compound intake of 700-800 mg/kg bw/d. [The mean daily intake was not reported for all dieatry levels, but for the low level of 100 ppm a corresponding average value of 7.7 mg/kg bw/d was given in the original report].
Reference: IIA, 5.6.1/05
Report: (1981)
A three generation reproduction study in rats with glyphosate
Data owner: Monsanto
Study No.: 77-2063; BDN 77-417
Date: 1981-03-31
not published TOX9552385
Guidelines: None (pre-guideline)
Deviations: Not applicable
GLP: no, pre-GLP
Acceptability: See RMS comment
Dates of experimental work: 1978-06-13 to 1980-04-09
Materials and methods
Test material:
Identification:
Glyphosate
Description:
Fine white powder
Lot #:
XHJ-64

Purity:
considered 100 % active ingredient for dosing preparations;
Stability of test compound:
Not reported
Vehicle and/
or positive control:
Plain diet
Test animals:
Species:
Rat
Strain:
CD® (Sprague-Dawley derived)
Source:
Age at treatment initiation:
43 days
Sex:
Males and females
Mean weight at initiation of dosing:
Males: 139.9 - 144.3 g; females: 118.0 - 119.2 g
Acclimation period:
7 days
Diet/Food:
Standard laboratory diet (Purina Lab Chow® 5001), ad libitum
Water:
Automated watering system (Elizabethtown Water Company), ad libitum
Housing:
Individually (except during mating and lactation), in elevated stainless steel wire mesh cages; nesting material: Litter Kleen® hardwood shavings added to cages on Day 19 of gestation and changed when wet or soiled through
Day 14 of lactation
Environmental conditions:
12 hours light/dark cycle
No details on temperature and humidity reported
Study design and methods:
In life dates: 1978-06-14 - 1980-04-09
Table B.6.6-13: Study design
Group
Dose level (mg/kg bw/day)
No. of adults initially assigned to mate
F0, F1, F2
No. of matings per generation
F0, F1, F2
Gross post- mortem examination
Histopathology of
F0, F1 and F2 parents, F3b weanlings

Females
Male
Female
1 Control
(plain diet)
0
12
24
2
All
10
10
2
3
12
24
2
none
none
3
10
12
24
2
none
none
4
30
12
24
2
10
10
Animal assignment and treatment:
In a three generation reproduction study groups of 12 male and 24 female CD rats received beginning 63 days prior to mating of the F0 generation daily dietary doses of 0, 3, 10 and 30 mg glyphosate/kg bw in diet. Diet samples were taken at four week intervals for analysis of achieved test substance concentrations.
Mating: One male and two females of equivalent dose levels were caged together nightly until a sign of mating (sperm and/or copulation plug in the vagina) was observed or until 15 days had elapsed with no evidence of mating. The day on which evidence of mating was observed was defined as Day 0 of gestation.
In this study, the first litters (F1a, F2a and F3a) from each mating were raised to weaning and discarded. Rats produced by the second matings (F1b and F2b) were selected to become parents of succeeding generations or to be subjected to complete gross necropsy (F3b).
Diet preparation and analyses

Diets were prepared weekly during the study and were adjusted on the basis of body weight and food consumption.

Clinical observations

A check for clinical signs of toxicity and mortality was made twice daily. A detailed physical examination was performed on adult generations at weekly intervals throughout the study.

Body weight

Body weights of all animals were determined weekly during growth and rest periods of all generations. Pregnant females were weighed on Days 0, 6, 15 and 20 of gestation and lactating females were weighed on Days 0, 4, 14 and 21 of lactation.

Food consumption and compound intake

Food consumption was recorded weekly during growth and rest periods of all generations. Test substance intake was calculated from individual body weight and food consumption data and reported as a group mean value for weekly intervals during the growth and rest periods of all generations.

Reproduction parameters

The day on which evidence of mating was observed was designated as Day 0 of gestation; the day of delivery was designated as Day 0 of lactation.

Mating indices, pregnancy rates, length of gestation and male fertility indices were recorded.

Litter data

Pups of all generations were examined daily for general appearance and mortality. On Days 0, 4, 14, and 21 they were counted to record the number of live and dead pups. Body weights were determined on Days 0, 4, 14, and 21 as a litter and on Day 21 individually.

Total number of live and dead pups, and the number of males and females per litter were determined on Day 0 of lactation. The sex ratio was calculated for each group on Days 0 and 21 of lactation. Viability indices, were determined for each litter on Lactation Days 0, 4 and 21.

Sacrifice and pathology

Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. All adult males and females were sacrificed after pup selection of the last Fb litter (F0, F1) and after last F3b litter weaned (F2) by lethal exposure to ether. Pups that were found dead or stillborn pups were weighed and given a gross post-mortem examination including internal sex determination, presence of milk in stomach. F1a, F2a, F3a and F3b animals were sacrificed at weaning, given a gross post- mortem examination and abnormal tissues were saved. F1b and F2b animals which were not selected as future parents were sacrificed after ensuing selection of parental animals, given a gross post-mortem examination and abnormal tissues were saved.

The following organs and tissues were preserved from all parents (F0, F1, F2) and from 10/sex/group of the F3b weanlings: adrenals, aorta, bone and bone marrow (sternal), brain, colon, duodenum, eyes with optic nerve and Harderian gland, gonads (ovaries and testes), heart, ileum, kidney (2), liver (2 sections), lung with main stem bronchi, lymph nodes (mesenteric), mammary gland (right inguinal), pancreas, pituitary, salivary gland, skeletal muscle (biceps femoris with right sciatic nerve), skin, spinal cord, spleen, stomach, thyroid/parathyroid, urinary bladder, uterus/prostate, gross lesions, tissue masses, thymus.

Microscopic examination of histological sections of these tissues were done for 10 male and 10 female animals from control and high-dose groups of F0, F1 and F2 parents and of F3b offsprings.

The following organs were weighed from all parents sacrificed after weaning of the second litters and from eighty F3b weanlings (10 males and 10 females per group): adrenals, gonads, kidneys, brain,

spleen, liver, heart and pituitary.
All pups of the second litter of the F2 parents (F3b) were necropsied at weaning and specified tissues were preserved for selected animals in each group.
Statistics
Body weights, body weight gain, maternal body weights, food consumption, number of offspring, offspring body weights, terminal body weights and organ weight data (absolute and relative), offspring survival, litter survival, pup viability index at birth, mating indices, pregnancy rates and male fertility indices data were compared to the control. Statistically significant differences were evaluated using several methods including Dunnett's test, ANOVA, Barlett's test, Kruskal-Wallis test and Fisher Exact Test.
Results and discussion Analysis of dose formulations Not reported.
Mortality
F0 adults (2 dead females in mid-dose group)
In the F0 generation, no unscheduled mortality occurred in the control, low- or high-dose groups. One female of the mid-dose group died during on Lactation Day 20 of first litter having 13 live pups at time of death. A second female of the mid-dose group died on Lactation Day 7 of second litter; this female delivered eight pups - seven live and one dead - and all pups were dead at time of death. No mid-dose F0 male died.
F1 adults (1 dead female in mid-dose group, 1 dead female in high-dose group)
In the F1 generation, no unscheduled mortality occurred in the control or low-dose groups. In the mid dose group one female was killed in a moribund condition during the post-mating period for the second litter. This female had mated during the first mating but did not deliver a litter; during the second mating this female had not mated. No other mortality occurred in the mid-dose group. In the high dose group one female died due to an accident (animal was caught in the feeder jar). A second high-dose female died on Day 21 of gestation for the second litter; the uterus of this female contained 15 term foetuses. No other mortality occurred in the high-dose group.
F2 adults (1 dead female in low-dose group, 1 dead male in mid-dose group)
In the F2 generation, no unscheduled mortality occurred in the control or high-dose groups. In the low-dose group one female died during the F3a lactation period. This female delivered a litter containing only dead pups (13 pups) and died the day after parturition. No other mortality occurred in the low-dose group. In the mid-dose group one male was killed in a moribund condition during the period between mating of the first and second litters. This male had mated and impregnated both females during the first mating period. No other mortality occurred in the mid dose group.
Clinical observations
Clinical observation data were similar between the control and treated groups for each generation interval throughout the study. No adverse treatment effects were indicated.
Body weight
Mean body weight data during the growth and rest periods were comparable between the control and treated groups for each generation, throughout the study. Likewise, mean weight gain during the growth periods were comparable between these same groups for both sexes
throughout all generations. No treatment effect on body weight data during the growth and rest periods was evident.
Food consumption and test compound intake
Mean food consumption data were considered comparable between the control and treated groups (both sexes) during the growth and rest periods for each generation, throughout the study. No adverse effect of treatment on food consumption was evident throughout the study. Mean weekly

test substance intake values ranged from 2.8 to 3.3 mg/kg bw/day for the low- dose group, from 9.5 to 11.2 mg/kg bw/day for the mid-dose group and from 27.7 to
33.1 mg/kg bw/day for the high dose group for all generations including both genders.
Reproductive parameters
Male and female mating indices and male fertility indices during both mating intervals of the F0 generation were considered comparable between the control and treated groups. During the second mating interval of the F0, pregnancy rates were lower than control in each of the treated groups; however, no indication of a dose-relationship was evident as the lowest pregnancy rate was seen in the mid-dose group. This reduction in pregnancy rate for the mid- dose group was not statistically significant. In the absence of a dose-response relationship the reduction in pregnancy rate during this mating interval (F1b) in the treated groups was not considered treatment-related.
In the F1 generation, mating indices (males and females) for both litter intervals were comparable between the control and treated groups. It is note-worthy that for both mating intervals of this generation, mating indices for control and some treated groups were lower than normally encountered in multi-generation studies. The reason for the poorer mating performance in this generation was unclear but no treatment effect was indicated since mating indices were lowest in the control group. Pregnancy and male fertility indices for the first mating interval of the F1 were comparable between the control and treated groups. During the second litter interval, pregnancy rates were lower than those seen for the first interval in control and treated groups. The lowest pregnancy rate was seen in the high-dose group; however, this difference from the control value was not statistically significant. Pregnancy rates for the low- and mid-dose groups, during the second mating interval, were considered comparable to control. Male fertility indices for this same mating interval were considered comparable between the control and treated groups.
In the F2 generation mating indices for the treated groups were lower than control for each mating interval. During the first mating interval of the F2 generation, the female mating indices were lower than control in each of the treated groups; however, only in the high-dose group was this difference from control statistically significant. The female mating index for the control group at this interval was 100 % which is higher than normally encountered. The female mating indices observed for the control group in this study have shown considerable variability ranging from 70.9 to 100 % The poor mating performance for the treated groups during the first mating interval is attributed to two males in each treatment group that did not mate either female in their mating unit (each mating unit was comprised of one male and two females).
During the second mating interval of the F2 generation, male mating performance improved in the mid- and high-dose groups as both mid-dose males and one of two high-dose males that did not mate during the first mating interval, mated and impregnated at least one female. Male mating indices for the low-dose group remained unchanged as the same two males that did not
mate during the first interval, failed to mate during the second interval. Pregnancy and fertility indices for the treated groups were comparable to control for both litter intervals of the F2 generation.
Mean gestation length was comparable between the control and treated groups for each pregnancy interval in each generation. Over the entire study no consistent, dose-related effect was seen in mating, fertility or pregnancy indices to indicate an adverse effect of treatment.
Litter data Litter size
Mean litter size data on Day 21 of lactation (weaning) was comparable between the control and treated groups for each litter interval throughout the study.
Sex ratio

Pup sex distributions ratios at Day 0 and 21 were generally comparable between the control and treated groups for each litter interval for each generation. No adverse treatment effect on sex distribution data was evident.

Viability index

The mean numbers of live, dead and total pups at birth and pup viability at birth for each pregnancy interval, were comparable between the control and treated groups for each generation. The litter survival indices were comparable between the control and treated groups for each lactation interval in the F0, F1 and F2 generation. In the F0 generation, postnatal survival indices for Days 0-4 and 4-21 were comparable between the control and treated groups for the first lactation interval (F1a). For the second litter interval of the F0, postnatal survival indices for the Day 0-4 interval were comparable between the control and treated groups. During the Day 4-21 interval, survival indices were significantly lower than control in each treatment group. The increase in pup mortality during this interval (i.e. Days 4-21) was attributed to high pup mortality within one or more litters at each treatment level. In the low-dose group the lower pup survival was attributed to one female that experienced complete litter mortality (litter contained 14 live pups at Day 4). In the mid-dose group, one female died on Day 7 of lactation and all seven pups in her litter died during the Day 4-7 lactation interval. Additionally, three mid-dose litters lost five or more pups from their litters during the Day 4-21 lactation interval. In the high-dose group, one female lost nine of l2 pups during the Day 4-21 lactation interval.

In the F1 and F2 generations postnatal survival indices for Days 0-4 and 4-21 during both litter intervals were considered comparable between the control and treated groups. Some statistically significant differences in these indices were observed between the control and treated groups; however, no trend was evident through successive generations to indicate an adverse effect of treatment.

Body weights Maternal body weights

Mean body weight data during the gestation and lactation intervals and mean weight change during these same periods were comparable between the control and treated group for each pregnancy interval from each generation throughout the study. No treatment effect was indicated in gestation - lactation body weight data throughout the study.

Offspring body weights

Mean pup body weight data during each litter interval for each generation were comparable between the control and treated groups. No adverse effects of treatment on pup weight data was evident.

Adult animals (F0, F1 and F2)

Mean terminal body weight data were comparable between the control and treated groups for both males and females throughout the study.

Pathology Necropsy

F0, F1 and F2 generations

Gross necropsy of parental animals of both sexes did not indicate any adverse effect of treatment.

F1, F2 and F3 offspring

Gross post-mortem observations of offspring at weaning (F1a, F2a, F3a, F3b) or post- weaning (F1b, F2b) did not demonstrate an adverse effect of treatment. Likewise, evaluation of dead pups recovered at birth and during the 21-day lactation period did not note a treatment-related effect.

Organ weights

F0, F1 and F2 generations

Mean organ weight data (absolute and relative to body weights or brain weights) were comparable

between the control and treated groups for both males and females from the F0 and F1 generations. Some statistically significant differences were noted between control and treated groups both in mean organ weight data and in the relative weight data; however, no trends were evident within dose levels or through these generations.
In the F2 generation, mean organ weight data (absolute and relative) for the males were comparable between the control and treated groups. In the F2 female group, mean liver/body weight ratios were significantly lower than control in each of the treated groups; however, no clear dose-relationship was apparent. Mean liver/brain weight ratios for the treated F2 females were lower than control; however, these differences from control values were not statistically significant. Mean spleen weights (absolute and relative to brain and body weights) were significantly higher than the control value in the F2 mid-dose female group; however, mean spleen weight data for the low- and high-dose F2 females were comparable to control values. In the absence of an effect on spleen weight in the high-dose Fg female group, the change seen in spleen weight data for the mid-dose females was considered spurious and not biologically meaningful. Other mean organ weight data (absolute and relative to body weight or brain
weight) for the treated F2 female groups were considered comparable to control data.
F3b offspring
Mean organ weight data (absolute and relative to body weights or brain weights) were comparable between the control and treated groups for both males and females. No treatment- related effect was evident in organ weight data for the F3b offspring.
Histopathology
In total 160 male and female rats (40 adults of each generation F0, F1 and F2 and 40 weanlings of F3b) were examined microscopically. No microscopic findings were considered treatment related. Proliferative tissue changes diagnosed as neoplasms were few. The microscopic tissue alterations, neoplastic and non-neoplastic, were indicative of common incidental histological findings.
Conclusion by the Notifiers
The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 30 mg/kg bw/day for three successive generations of CD rats resulted in no treatment-related signs of toxicity in parental animals. The NOAEL for reproduction is 30 mg/kg bw/day, since the reproductive performance was not affected in any dose group. The NOAEL for offspring is 30 mg/kg bw/day, since no adverse effects on offspring were observed.
Comment by RMS (Re-evaluation):
This study is now considered not acceptable due to the selected dose levels that were much too low. Accordingly, an effect dose was not reached (Evaluation in 2001 not confirmed).
Reference:
IIA, 5.6.1/06
Report:
(1992)
The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat.
Data owner: Cheminova Project no.: CHV 47/911129 Date: 1992-05-14
not published TOX9552389
Guidelines:
OECD 416 (1983), US-EPA FIFRA 83-4 (1982)
Deviations:
None

GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1990-03-29 - 1991-03-22
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
White solid
Lot/Batch #:
206-Jak-119-1
Purity:
99.2 %
Stability of test compound:
Stable during the treatment period.
Vehicle and/
or positive control:
Plain diet
Test animals:
Species:
Rat
Strain:
Sprague-Dawley Crl:CD (SD) BR VAF/Plus
Source:
Age:
Approximately 6 weeks
Sex:
Males and females
Weight at dosing:
Males: 143 – 201 g; females: 106 – 175 g
Acclimation period:
At least 15 days
Diet/Food:
Biosure Laboratory Animal Diet No.2, ad libitum
Water:
Tap water, ad libitum
Housing:
During pre-mating periods, animals were housed in groups of four in metal cages with wire mesh front, floor and top. During the first week of F1A and contingency animals of F2B animals were housed in plastic cages.
During mating animals were housed on an 1:1 basis in plastic cages where females stayed after mating for breeding. Males were re-housed in former metal cages.
Environmental conditions:
Temperature: 23 ± 4 °C

Humidity: 45 ± 24 % Air changes: not reported 12 hours light/dark cycle
Study design and methods:
In life dates: 1990-03-14 to 1991-03-22
Animal assignment and treatment:
In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate technical. The dose levels were chosen based on results of a previously conducted study. After at least 70 days of treatment pairing of animals within each dose group was undertaken on a 1:1 basis to produce the F1 litters. At Day 21 post partum of offspring from the F0 mating phase, groups of 24 male and 24 female offspring from each dose group were selected to form the F1A generation. The remaining pups were sacrificed. Approximately 10 days following the weaning of all F1A pups, F0 males and females were re-mated. At Day 21 post partum all F1B pups were sacrificed. F0 males and females were terminated shortly after weaning of F1B pups.
The selected F1A animals were dosed from approximately Week 4 of age for at least 84 days and then mated on a 1:1 basis (sibling pairings were avoided). On Day 4 post partum F2A litters were standardized to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 post partum all F2A pups were sacrificed. Approximately 10 days following the weaning of all F2A pups, F1 males and females were re-mated. On Day 4 post partum F2B litters were standardized to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 post partum all F2B pups were sacrificed. F1 males and females were terminated shortly after weaning of F2B pups.
Diet preparation and analyses
For the weekly preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 7 minutes in a rotary double-cone-blender.
The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration throughout the study.
Clinical observations
A check for clinical signs or ill health was made once daily and recorded daily for the first week of treatment and on a weekly basis thereafter. Rats showing marked signs of ill health or reaction to treatment were killed and subjected to necropsy.
Body weight
Individual body weights were recorded at the start of each generation (F0: Week 6 of age; F1A: Week 4 of age) and subsequent at weekly intervals. Females were weighed daily during mating and continued until parturition. Weights were reported for Days 0, 7, 14, 17 and 20 of pregnancy. Females with live litters were weighed on Days 0, 7, 14 and 21 post partum.
Food consumption and compound intake
Food consumption was recorded on a weekly basis from allocation throughout the first pre- mating phase of each generation. During this period food conversion ratios and achieved intake (mg/kg bw/day) were calculated.
Water consumption
Water intake was observed daily during the initial and final two weeks of the first pre-mating period for each generation and from allocation for the F0 generation.
Reproduction parameters
Vaginal smears were taken daily during the 20-day mating period to examine the oestrus cycle and median pre-coital time. Additionally, date of mating and duration of gestation was recorded.
Litter data

The number of offspring born and the number of offspring alive were recorded daily. Pups were weighed on Days 0 and 4 and all litters containing more than eight pups were culled to eight retaining, where possible, ideally 4 pups per sex. The remaining pups were also weighed on Days 8, 12, 16 and 21. Dead and culled young were subjected to necropsy.

Sacrifice and pathology

All adult animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded.

The following organs were weighed of adults: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostrate (with seminal vesicles and coagulating gland), testes (with epididymides), thymus.

The following tissues were preserved from all adults: adrenals, aorta, bone (femur and joint), bone marrow (sternum), brain, cranial vault (for lachrymal glands, teeth, nasal turbinates, inner ear), caecum, colon, duodenum, eyes, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (cervical/mesenteric), mammary gland, macroscopically abnormal tissues,
oesophagus, ovaries*, pancreas, pituitary*, prostate with seminal vesicles (with coagulating gland)*, rectum, salivary gland, sciatic nerve, skeletal muscle, skin, spinal column (vertebral column), spleen, stomach, testes (with epididymides)*, thymus, thyroids (with parathyroids), tongue, trachea (with larynx and pharynx), urinary bladder, uterus (with cervix)* and vagina*.

Histology of the reproductive tract was restricted to adults of the control and high-dose group and any apparently infertile animals at the lower dietary concentrations and confined to tissues marked with an asterisk (*).

Statistics

Two tailed significance tests were performed on adult parameters (water consumption, food consumption, bodyweight, organ weights) and litter data. Evaluation of other parameters were found not to be useful. Significances at 1 % and 5 % were reported.

Results and discussion

Analysis of dose formulations

Stability analyses indicated that the dose preparations at nominal concentrations of 500 and 30000 ppm were stable for up to 18 days during storage under animal room conditions.

Analyses for homogeneity at nominal concentrations of 500 and 30000 ppm indicated that the dose preparations were homogeneous.

Analyses for achieved concentration performed at 4-5 weekly intervals demonstrated that the prepared dietary admixture concentrations given to the animals were within ± 15 % of the nominal concentration in all groups.

Test compound intake

The group mean intakes of glyphosate are summarised in Table B.6.6-14 and Table B.6.6-15 below.

Table B.6.6-14: Group mean achieved intakes of glyphosate - F0 generation

Group

Dietary concentration

Mean intakes (Week 1 - 10)

(ppm)

(mg/kg bw/day)

Males

Females

Control

0

0

0
Low
1000
66.4
75.3
Intermediate
3000
196.8
226.0
High
10000
668.1
752.3
Table B.6.6-15: Group mean achieved intakes of glyphosate - F1 generation
Group
Dietary concentration
Mean intakes (Week 5 - 16)
(ppm)
(mg/kg bw/day)
Males
Females
Control
0
0
0
Low
1000
76.1
82.1
Intermediate
3000
230.2
244.9
High
10000
771.3
841.1
Mortality
There were no test substance related mortalities.
Four unscheduled deaths occurred during each generation.
In the F0 generation one female of the low-dose group and one male of the high dose group were killed for humane reasons during Week 15 and 23, respectively. The female exhibited pilo-erection and thin appearance and the necropsy noted thickened forestomach, invaginated stomach and abnormal contents in the gastro-intestinal tract. The male was unable to use hind limbs, exhibiting aberrations of brain and spinal cord at necropsy. Another male of the high- dose group died during Week 3, with effects on pancreas and liver noted at necropsy. One control male was sacrificed during

Week 16 following poor condition, however, the aetiology of the signs was not established.
In the F1 generation one female of the low-dose group was killed following a procedural error. In the mid-dose group one male died during Week 34 but autolytic changes precluded a valid necropsy. Moreover, one male and one female died and were sacrificed, respectively, during Week 23. Necropsies failed to identify a specific cause of death.
Clinical observations
No treatment-related clinical signs of toxicity were noted. General signs were observed in occasional animals from both generations and were not related to treatment.
Body weight
No adverse effect of bodyweight change was evident for treated animals in comparison to controls for both generations.
However, absolute mean body weights in high-dose F1 males were slightly lower as compared to control. In addition it was noted that during the first mate of each generation, body weight gains during the initial stages of pregnancy tended to be slightly lower than controls at all dietary levels. Since no consistent dose-response was apparent these effects cannot conclusively be attributed to treatment.
Food and water consumption
Apart from a slightly higher but not statistically significant food consumption of high-dose F1 females during the second half of the pre-mating period, there were no marked intergroup differences in food consumption of males or females.
Apart from a slight increase among high-dose F1 females (attaining statistical significance in Week 16), no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations when compared to their concurrent controls.
Reproductive parameters
There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.
Litter data
Size and Viability
No overt differences in litter viability were detected.
In the high-dose group total litter size at birth was consistently, but not significantly, lower than controls across all four matings and remained lower than controls at Day 4 in three of the four matings. Since the mean litter size at birth within each mating, was not always the lowest litter size recorded, this finding could not be clearly attributed to treatment.
Growth and Development
No adverse effects on mean offspring bodyweights, bodyweight change or development were detected for male and female offspring in comparison to their controls.
Clinical signs
No clinically observable signs of toxicity were observed for offspring from treated animals.
Pathology Necropsy
There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1 animals, or offspring.
Organ weights
There were no overt or statistically significant treatment-related changes in any organ weights analysed in either generation.
Histopathology
No treatment-related changes in tissues associated with the reproductive tract were detected in the

F0 or F1 generation animals.
Examination of two previously identified target organs, the parotid and submaxillary salivary glands, was initially performed only in the control and high-dose groups. Due to effects seen in the parotid gland, examination was extended to the remaining treatment groups. For the submaxillary gland, examination was extended to only the F0 and F1 females in the low- and mid dose group. The findings are summarised in Table B.6.6-16.
Table B.6.6-16: Incidence of salivary gland findings
Observation
Dietary concentration (ppm)
Males
Females
0
1000
3000
10000
0
1000
3000
10000
F0 Generation
Animals examined
27
28
28
26
28
27
28
28
Hypertrophy of acinar cells with prominent
granular cytoplasm (minimal)
parotid
2
2
3
12
0
2
5
17
submaxillary
0
-
-
0
0

1
4
14
F1 Generation
Animals examined
24
24
23
23
24
23
24
23
Hypertrophy of acinar cells with prominent
granular cytoplasm (minimal)
parotid
1
0
4
11
0
0
4
9
submaxillary
0
-
-
0
0
0
0
3
- = not examined
Treatment-related minimal changes were apparent in the parotid salivary gland of both F0 and F1 males and females in the mid- and high-dose groups and the submaxillary salivary gland of the F0 females in the mid- and high-dose groups and F1 females in the high-dose group. This finding is similar to those seen occasionally in other subchronic and long-term dietary
studies and is considered to be an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and is not considered to be adverse. There were no effects on the salivary glands noted in the low-dose group.
Conclusion by the Notifiers
The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations resulted in minimal effects consisting of increased food and water consumption of F1 females, possibly reduced bodyweights of F1 males and minimal histological changes in the target organ (salivary glands) in F0 and F1

findings associated with treatment at 3000 ppm were minimal histopathological changes of the salivary glands in F0 and F1 adults. No effects were apparent at 1000 ppm. Thus, the parental reproductive and offspring NOAELs are considered to be 10000 ppm, corresponding to 668 and 752 mg/kg bw/day in males and females, respectively.
Comment by RMS (Re-evaluation):
The study is considered acceptable. According to the study report, balano preputial cleavage was delayed in F1 males at all dose levels (42.5; 42.2; 42.6 d versus 39.9 d in controls). However, the calculated mean value for control animals seems to be reported incorrectly and should be calculated to be 41.8 d. Based on the revised mean value for preputial separation in control animals, no definite difference between the treatment groups was observed.
In conclusion, no evidence of reproductive effects was observed and therefore, the NOAEL on reproductive toxicity is considered 10000 ppm (668 mg/kg bw/d). The NOAEL for parental and offspring toxicity is calculated to be 3000 ppm (197 mg/kg bw/d) based on increased food and water consumption in F1 females, lower body weight of F1 males and an increased incidence of cellular alteration of the parotid (males and females) and submaxillary (females) salivary gland in both F0 and F1 adults at 10000 ppm (Evaluation in 2001 confirmed).
Reference: IIA, 5.6.1/07
Report: (1990)
Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats
Data owner: Monsanto Report No.: MSL-10387
Project No.: ML-88-106/EHL 88038 Date: 1990-08-27
not published ASB2012-11496
Guidelines: Not stated, but in general accordance with OECD 416 (1983)
Deviations: Yes: no data on food efficiency; no details on fertility indices, number of live births and post-implantation loss, number of pups with grossly visible abnormalities,
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: 1988-10-24 to 1989-10-13
Materials and methods
Test material:
Identification:
Glyphosate (Identification code: T880068)
Description:
White powder
Lot/Batch #:
XLI-203
Purity:
97.67 %
Stability of test compound:
Not reported
Vehicle and/
or positive control:
Plain diet
Test animals:
Species:
Albino Rat

Strain:
Sprague-Dawley
Source:
Age:
Approximately 7 weeks (F0 adults)
Sex:
Males and females
Weight at study start (F0):
Males: 165 – 207.6 g; Females: 135.6 – 162.7 g
Acclimation period:
No data
Diet/Food:
Purina Mills Certified RODENT CHOW No. 5002, ad libitum
Water:
St. Louis public water, ad libitum
Housing:
Housing for premating and gestation (day 0 through 13): individual suspended stainless steel cages over paper bedding; during mating females were housed in the male´s cages
Housing for gestation and lactation (from day 14 of gestation through lactation): females housed in double wide cages with solid bottoms and wood shavings for bedding
Environmental conditions:
Temperature: 18 - 26 °C
Humidity: 40 - 70 % 12 hours light/dark cycle
Study design and methods:
Animal assignment and treatment:
In a two-generation reproduction study groups of 30 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 2000, 10000 and 30000 ppm (corresponding to 132-140, 666-711, 1983-2230 mg/kg bw for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females (calculated from F0 and F1a adults)) glyphosate in the diet. After 11 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis in a male´s cage for 7 days, to produce the F1a litters. If there was no evidence of mating after 7 days (copulatory plug, or vaginal smear), the female was co- housed with a male having recorded copulatory activity for additional 7 days, or until copulatory evidence was found. For F0 and F1 generation, gestation day 0 was set on the day on which copulatory evidence was found and lactation day 0 the day on which delivery of pups was completed.
At weaning of offspring from the F0 mating phase, groups of 30 males and 30 females offspring from each dose group were selected to form the F1 generation and the mating procedure for F1a adults was conducted in the same way except modifications to exclude sibling matings. The remaining surviving F0 females and unselected offspring were terminated at Day 21 post partum. F0 males were killed at completion of mating phase. The offspring selected for the F1a generation were dosed for at approximately 14 weeks and then mated to produce the F2a and F2b litters (a second mating of the F1 generation was performed due to reduced litter sizes in pups from F0 of the 30000 ppm dose group). At weaning of the F2 litters all surviving adults and their offspring were killed, whereas F1 males were sacrificed after completion of mating phase.
Diet preparation and analyses
Approximately each week (except in one week when diets were prepared twice the same week and

not during the following week) a known amount of glyphosate was mixed with the diet for 10 minutes in a HOBART HCM-450 mixing machine to achieve a batch size of 18 kilograms at each dose level.
The stability and homogeneity of the test substance in the diet were determined by liquid chromatography of duplicate samples from top, middle and bottom of mixer from the lowest and highest dietary levels stored in an open container at ambient temperature for 6 and 14 days or when frozen in a closed container for 35 days.
Clinical observations
A detailed observation for signs of toxicity was performed once weekly for the adult animals and for the offspring on days of weight measurement.
Body weight
Adult male animals of the F0 and F1a generation were individually weighted once weekly. The same was done for the female animals until copulation was confirmed, then females were weighted on days 0, 7, 14, and 21 of gestation and lactation.
Offspring was weighted on days 0, 4 (pre- and post-culling), 14 and 21 of lactation (except F1a males approximately two weeks prior to sacrifice and F1a females for approximately three weeks prior to mating for the F2b generation).
Food consumption and compound intake
Food consumption was recorded weekly for F0 and F1a adult males, except during mating, and also weekly for adult F0 and F1a female animals until mating. After confirmed copulation, the maternal food consumption was monitored for days 0-7, 7-14 and 14-21 of gestation and lactation, but it was not determined for females approximately three weeks prior to mating for the F2b generation and generally not for female animals that did not became pregnant.
Food conversion efficiency was not calculated.
Water consumption
No data on water consumption was given in the report. Reproduction parameters
Pregnancy and parturition
Data on total paired females, females with confirmed copulation/total paired, pregnant/total paired, pregnant/ confirmed copulation was monitored as well as precoital (for pregnant animals) and gestational length in days. For males, the following items of interest were given: males with confirmed copulation/total paired, males impregnating females/total paired and males impregnating females/confirmed copulation.
Litter data
The following litter data were recorded: Litter size, dead pups/litter, mean pup weight (on day 0, 4 (pre-/post-cull), 14, 21) and survival (%).
Physical and sexual development
No details on physical and sexual development of the offspring was reported.
Sacrifice and pathology
All adult animals, which died or were sacrificed in moribund condition were subjected to a gross necropsy and selected tissues were sampled. Pups found dead or culled pups also underwent gross pathology, but no tissues were saved. No organ weights were determined.
All F1a weanlings, that were not selected for mating, F2a and F2b weanling pups as well as females which had littered on or after 21 of lactation were sacrificed as scheduled. Non- pregnant adult females were killed at least 5 days after last expected parturition date and adult males after completion of the mating phase.
External and internal cavities of the dead animals were opened and the organs were examined in

place and then removed. Hollow organs were opened and examined. The following organs of F0 and F1a males and females from each dose group that were sacrificed at the end of the study sampled, were weighed: ovaries and testes with epididymides. When present, the following organs from the F0 and F1a adults (unscheduled deaths and scheduled sacrifice) were retained: kidneys, ovaries, prostate, seminal vesicle, skin/mammary gland, testes, epididymis, uterus/vagina and gross lesions (pituitary retained for F1a adults only). Tissues from the F1a weanlings were saved at the discretion of the necropsist. From the F2a and F2b weanlings, which where sacrificed at schedule, the kidneys of 1 pup per sex and litter were saved.
A histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on one F2b weanling/sex/litter (selected at random) as well as on all retained tissues from unscheduled adult deaths. For preparation, fixed tissues were washed, dehydrated, embedded in paraffin, sectioned, stained with haematoxylin and eosin and examinated under light microscopy.
Statistics
Dunnett´s multiple comparison test (two-tailed) was used to detect statistically significant differences in adult body weights and food consumption between treated animals and their respective control.
Terminal body weights, maternal body weights and food consumption during gestation and lactation, pup weights, precoital length, gestational length, litter size, dead pups/litter, pup survival, absolute organ weights and organ/body weight ratios were evaluated by decision- tree statistical analyses procedures which, depending on the results of tests for normality and homogeneity of variance [Bartlett's Test], were chosed either parametric [Dunnett's Test and Linear Regression] or nonparametric [Kruskal-Wallis, Donckheere's and/or Mann-Whitney Tests] routines to detect differences and analyzed for trend.
The uncorrected Chi-Square test was used to examine fertility indices, e.g. females/males with confirmed copulation/total paired, pregnant/confirmed copulation (females) and males impregnating females/total paired as well as males impregnating females/confirmed copulation.
Fisher´s Exact test with Bonferroni Inequality Procedure was used for statistical analysis of microscopic lesions.
Other statistical routines used for some data included: Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample (group) means could be considered as an estimate of a common population, and Grubb's Test to detect outliers.
Results and discussion
Analysis of dose formulations
The analysis of the test substance stability conducted over the time span of the study indicated that the test material was stable in the diet and homogeneity was adequate for study use. The stability of the test material in the diet was demonstrated at the low and high dose level, stored in an open container at ambient temperature for 6 and 14 days, or when frozen in a closed container for 35 days.
Analysis for achieved concentrations, demonstrated that the test substance-levels in the prepared diet were in the range of 95 to 96.7 % of the nominal concentration.
Test compound intake
The group mean achieved dosages are summarised in the table below.
Table B.6.6-17: Group mean compound intake levels during pre-mating periods of F0 and F1
Dose group
Dietary concentration

(ppm)
Mean daily test substance intake (mg/kg bw/day)*
F0
F1
Males
Females
Males
Females
control
0
0
0
0
0
low
2000
132
160
140
163
mid
10000
666
777
711
804
high
30000
1983
2322
2320
2536
* based on actual food intake and body weight data; values were calculated in the report
Mortality
There were no treatment-related mortalities.
One female of the F0 generation died early in the study. This animal was never mated and at necropsy changes in bladder in kidneys were observed. Two male animals of the 2000 and 30000 ppm dose groups (F1 generation) died. Necropsy of these animals noted thymus and respiratory changes. One female animal of the F1 generation (2000 ppm) was sacrificed in extremis and another female (same generation, same dose group) died. Kidney changes and retained foetus; pups in uterus and stomach changes, respectively, were observed in these two females.
Concerning the offspring, dead pup counts at day 0 and survival of all F1a, F2a and F2b treated pups were not adversely affected when compared to the controls.
Clinical observations
The only clinical signs that were related to the test substance were soft stool in the animals of the 30000 ppm dose group. Other clinical signs, such as red ocular discharge / laboured respiration /

overgrown teeth / piloerection / abrasions / emaciated and dehydrated
appearance / misuse of limbs / focal loss of hair / swollen feed, occurred sporadically and were not considered to be treatment-related.

Body weight

At the highest exposure level of 30000 ppm, reduced body weights were observed in both sexes and in F0 and F1 generation. In the F0 generation, body weights gradually decreased within time to approximately 8 % less than controls prior to mating. F0/F1 weaning animals were lighter in weight as their corresponding controls and maintained that weight difference (approx. 10 % less than control) until the end of the study (see Table B.6.6-18).

No test-substance related body weight effects were observed in the adult animals of the 2000 and 10000 ppm dose groups prior to mating.

During gestation and lactation, maternal body weights in the highest dose group tended to remain lower than in controls, but the animals showed a rather greater body weight gain than the controls during gestation and lactation so that by the end of lactation, body weights were approximately the same as those of the controls (see Table B.6.6-19 and Table B.6.6-20).

Terminal body weights were significantly decreased for both sexes at the highest exposure level (see Table B.6.6-18).

Table B.6.6-18: Mean group body weights

Dietary concentration
(ppm)
No. of animals
Mean group body weight (g) at Day
0
72
T#
0
72
T#
F0 Generation
Males
Females
0 (Control)
30
mean
187.9
494.6
549.56
150.5
276.7
296.31
sd
11.65
34.86
46.76
6.86
23.85

23.63
2000
30
mean
188.1
497.6
550.19
150.5
272.6
290.64
sd
11.35
49.87
80.72
7.03
22.86

19.50
10000
30
mean
188.1
484.4
539
150.2
273
290.71
sd
11.57
42.13
58.13
7.04
27.92
25.35
30000
30
mean
188
455.8**
503.51**
150.3
253.8**
265.91
sd
11.56
46.46

45.66
7.06
18.46
15.44
F1 Generation
129
219
T#
128
219
T#
0 (Control)
30
mean
118.3
534.7
625.04
99.8
285.8
316.21
sd
26.11
38.84
53.11
17.44
27.63
37.37
2000
30
mean
115.2
540.3
632.14
96.7
282.1
313.74
sd
16.2
44.9
74.57
11.47
24.5
30.53
10000
30
mean

114.8
514.1
590.98
97.1
275.9
312.36
sd
17.42
58.31
70.06
14.18
20.55
26.71
30000
30
mean
104.9*
483.4**
543.40**
88.8*
253.7**
284.72**
sd
19.79
41.32
58.12
16.32
19.56
18.04
*: Dunnett´s test (two-tailed) indicates statistically significant difference (p<0.05)
**: Dunnett´s test (two-tailed) indicates statistically significant difference (p<0.01)
#T: Termination
Table B.6.6-19: Mean maternal body weights during gestation
Dietary concentration (ppm)
No. of animals
Mean group body weight (g) at Day (Gestation)
0
7
14
21
F0 Generation
0 (Control)
24
mean
274
301.83

324.41
398.26
Sd
24.26
24.58
22.85
26.12
2000
29
mean
272.72
297.33
319.90
392.86
Sd
20.52
21.71
19.84
24.28
10000
28
mean
271.80
299.22
323.43
395.08
Sd
24.12
26.40
28.44
25.87
30000
28
mean
255.05**
282.44**
305.83**
375**
Sd
16.49
16.27
17.44
24.70
F1 Generation (First Mating)
0
7

14
21
0 (Control)
24
mean
285.29
308.95
328.70
392.56
Sd
25.48
26.58
29.18
36.19
2000
29
mean
278.65
304.40
324.15
383.45
Sd
23.42
23.48
25.06
28.18
10000
28
mean
268.89*
297.23
319.08
382.71
Sd
19.24
18.81
19.27
21.77
30000
28
mean
251.30**
276.28**
299.48**
360.46**
Sd

17.42
18.92
19.29
33.31
F1 Generation (Second Mating)
0
7
14
21
0 (Control)
24
mean
324.22
340.99
363.44
428.99
Sd
23.11
27.81
27.98
36.87
2000
29
mean
315.21
338.27
360.35
426.88
Sd
26.06
28.67
28.39
33.67
10000
28
mean
305.27*
333.66
357.50
428.51
Sd
20.26
22.45
24.49
26.17
30000

28
mean
281.46**
308.92**
330.95**
393.67**
Sd
17.79
22.19
22.36
34.88
Table B.6.6-20: Mean maternal body weights during lactation
Dietary concentration (ppm)
No. of animals
Mean group body weight (g) at Day (Lactation)
0
7
14
21
F0 Generation
0 (Control)
24
mean
299.96
319.59
317.33
313.39
Sd
23.21
23.58
28.96
20.01
2000
29
mean
297.48
317.91
314.53
313.96
Sd
21.10
18.66
25.22
16.63
10000
28

mean
298.78
315.15
312.41
319.10
Sd
20.81
22.04
22.94
18.61
30000
28
mean
285.84*
307.64
304.75
316.68
Sd
13.91
12.48
20.68
15.43
F1 Generation (First Mating)
0
7
14
21
0 (Control)
24
mean
299.29
313.60
337.68
313.49
Sd
27.02
26.12
25.31
21.38
2000
29
mean
295.16
308.28
332.10
314.69

Sd
23.58
22.56
23.92
23.95
10000
28
mean
296.63
310.80
328.29
313.14
Sd
19.01
18.64
18.33
14.06
30000
28
Mean
277.91**
289.88**
315.88**
306.15
Sd
17.89
17.23
17.47
20.18
F1 Generation (Second Mating)
0
7
14
21
0 (Control)
24
mean
342.78
343.21
353.34
337.16
Sd
32.46
27.11
21.15

2000
29
mean
340.16
336.62
348.40
331.96
Sd
28.54
16.11
25.89
20.67
10000
28
mean
333.80
342.41
352.70
334.56
Sd
23.35
26.93
20.43
13.82
30000
28
mean
312.39**
324.09*
337.08
329.95
Sd
23.73
20.50
19.09
18.41
Food consumption
Overall, food intake was not notably affected during the study.
All animals of the 30000 ppm dose group consumed about 1 to 2 grams/day less than controls. This effect was mostly pronounced in the first week of exposure and also observed in the F0 dams. Subsequent dams (F1 first and second matings) tended to eat similar or larger amounts of the diet than controls.
No effects on food consumption were observed in the animals of the 2000 and 10000 ppm dose groups.
Reproductive parameters
Mating Performance, Fertility, Gestation and Lactation

No effects on mating and fertility rates were observed in the F0 and F1a dams when compared to controls and no effects were observed on precoital length at any treatment level.

Litter data

Size and Viability

Day 0 dead pup counts among treated groups were comparable to the control group for all three litters of pups (F1a, F2a and F2b generation).

A slight reduction in the average litter size was observed in the F0 dams of the 30000 ppm dose group. This effect was less pronounced in animals after the first F1 mating. Although the difference was not statistically significant and not accompanied by an increase in dead pups/litter, a treatment-related effect could not be excluded. Therefore a second mating of the F1a adults was performed. In the resulting F2b generation, no dose-related decrease in litter size was observed.

Growth and Development

Birth weights and initial growth rate for pups from the treated dams compared well to the ones of the control, except the pups of the 30000 ppm dose group had reduced body weights on day 21 of lactation (more than 10 % difference to controls). The effect was earlier pronounced in the F1 matings (day 14). This effect was reasoned by the titrated uptake of the test substance-containing diet at the end of lactation.

In the mid dose group, slight and transient decreases in the body weights of the pups were observed. They were not evident in both sexes from all generations and therefore regarded of questionable toxicological significance.

Table B.6.6-21: Mean pup weights

Dietary concentration (ppm)

No. of animals

Mean group body weight (g) at Day

0

21

0

21

Males

Females

F0 Generation

0 (Control)

24

mean

6.28

53.39

6.96

50.80

Sd

0.49

3.90

0.52

4.39

2000

29

mean

6.27
51.82
6.91
49.47
Sd
0.48
5.26
0.48
5.05
10000
28
mean
6.43
50.42*
6.15
49.16
Sd
0.47
3.66
0.50
3.12
30000
27
mean
6.47
46.30**
6.12
44.99**
Sd
0.62
4.09
0.59
4.34
F1 Generation (First Mating)
0 (Control)
28
mean
6.33
55.11
5.95
51.93
Sd
0.60
5.64
0.55

2000
23
mean
6.20
52.47
5.90
51.42
Sd
0.76
9.15
0.70
4.08
10000
22
mean
6.32
51.53*
5.98
48.49*
Sd
0.74
7.35
0.64
5.93
30000
25
mean
6.50
47.29**
6.05
44.41**
Sd
0.84
4.62
0.74
4.90
F1 Generation (Second Mating)
0 (Control)
16
mean
6.48
55.03
6.04
49.35
Sd
0.75

6.38
0.63
10.96
2000
18
mean
6.17
52.74
5.86
50.73
Sd
0.74
6.12
0.83
5.91
10000
17
mean
6.36
52.29
5.92
49.48
Sd
0.52
3.35
0.47
2.52
30000
23
mean
6.51
44.43**
6.04
43.10**
Sd
0.63
6.86
0.55
3.81

Clinical signs

No clinical signs were observed in the offspring of treated animals.

Pathology Necropsy

There were no toxicologically significant macroscopic gross lesions attributed to the test chemical administration.

Organ weights

There were no statistically significant organ weight changes, except a slight increase in testes

weight ratios in F1a adults of the 30,000 ppm dose group. This effect was attributed to their lower terminal body weight.

Histopathology

No treatment-related changes were detected.

Conclusion by the Notifiers

The oral administration of glyphosate to rats via diet at a dose levels of 2000, 10000 and 30000 ppm for two successive generations resulted in possible treatment-related changes at the maximum dose of 30000 ppm. A high incidence of soft stools in adults was accompanied by consistent reduction of body weights of adults and pups at this dose level. Decreases in body weights of the pups obviously occurred at the end of lactation, with the beginning of consuming the test substance-containing diet. Furthermore, slightly but not statistically significant reduced average litter size was noted in F0 dams of the 30000 ppm dose group at first mating.

Therefore the NOAEL was considered to be 10000 ppm for adult toxicity for both the F0 and F1 generations (corresponding to 666-711 mg/kg bw for males and 777-804 mg/kg bw/day for females). The NOAEL for reproductive toxicity, for both generations and offspring was considered to be 30000 ppm.

The NOAEL for developmental toxicity, for both generations and offspring was considered to be 10000 ppm.

Comment by RMS (Re-evaluation):

The study is considered acceptable. In contrast to the Notifiers, the mid dose of 10000 ppm is considered the NOAEL for parental, offspring and reproductive toxicity. This assessment is in accordance with the evaluation in the previous DAR (2001, ASB2010-10302) and based on reduced body weight gain and soft stools in high dose adults, decreased pup body weight gain and equivocally reduced litter size at highest dose level of 30000 ppm. The intermediate dietary concentration of 10000 ppm was calculated to be 722 for male rats and 757 mg/kg bw/d for females.

B.6.6.10 Teratogenicity test by the oral route in the rat

For the previous EU evaluation, a total of 5 studies in rats were reported in the DAR of which four were still considered acceptable or at least supplementary and may be used for current evaluation. The previous assessment of the studies by . (1991, TOX9552393) and by (1980, TOX9552392) and of the study (1981, TOX9650160, Author's name perhaps but not verified) were confirmed by the RMS upon re- evaluation. However, during re-evaluation the study by (TOX9551834) was considered not acceptable due to several reporting deficiencies and therefore not reported in the present RAR.

In addition, two new studies in rats have been performed and were provided in the GTF dossier. These studies were submitted either for the first time for this evaluation or had been subject to JMPR evaluation in 2004 yet. They were considered acceptable by the RMS without restrictions.

Reference: IIA, 5.6.10/01

Report: (2002)

Amendment 001 to Glyphosate Acid: Developmental Toxicity Study in the Rat

Data owner: Syngenta Report No.: CTL/P/4819 Date: 2002-11-20

GLP:

not published ASB2012-10080

Guidelines: OECD 414 (2001): OPPTS 870.3700 (1998): 2004/73/EC B.31

(2004)

Deviations: None

GLP: yes

Acceptability: See RMS comment

Dates of experimental work: 1995-05-17 to 1996-03-26
Materials and methods
Test Material:
Glyphosate acid
Description:
Technical, white solid
Lot/Batch number:
P24
Purity:
95.6 % w/wa.i
CAS#:
Not reported
Stability of test compound:
Confirmed
Vehicle and/or positive
control:
Deionised water.
Test Animals:
Species
Rat
Strain
Alpk:APfSD Wistar-derived
Age/weight on arrival
Approximately 11 weeks / 210 – 303 g
Source
Housing
Individually
Acclimatisation period
Not applicable
Diet
CT1 diet (Special Diet Services, Witham, Essex, UK) ad
libitum
Water
Mains water ad libitum
Environmental conditions
Temperature: 21 ± 2 °C
Humidity: 40 - 70 %
Air changes: 25 – 30 changes / hour Photoperiod: 12 hours light / 12 hours dark
Study design and methods
In-life dates: Start: Not reported End: Not reported (QA audits conducted between May 1995 and March 1996)
Mating procedure: Virgin female rats were paired overnight (at the Breeding Unit) with males of the same strain. On the following morning, vaginal smears from these females were examined for the presence of sperm. The day when spermatozoa were detected was designated day 1 of gestation and, on this same day, successfully mated females were delivered to the experimental unit at CTL.
Animal assignment: A total of 96 mated females was supplied over a two week period. Twelve female

rats were supplied on each of eight days. The study was divided into twenty four replicates (randomised blocks) with each replicate containing one rat from each group. Animals were randomly assigned to test groups as shown in the following table.

Table B.6.6-22: Animal numbers and treatment groups

Dose level of Glyphosate acid (mg/kg bw/day)
0 (control)
250
500
1000
1 - 24
25 – 48
49 - 72
73 - 96

Dose selection rationale: The dose. levels selected for this study were based on a dose range finding study in the pregnant rat. The highest dose level of 1000 mg/kg/day is the limit dose for this type of study.

Dose preparation and analysis: Glyphosate acid was administered in deionised water and the concentration was adjusted to give a constant volume of 1 mL/100 g bodyweight for each dose level. An appropriate amount of deionised water was added to a weighed amount of test substance (adjusted for purity) to provide each preparation. One preparation per concentration (ie 25, 50 and 100 mg/mL) was made. Each preparation was thoroughly mixed before being subdivided into aliquots. The control substance was also dispensed into aliquots. The aliquots were stored at room temperature and fresh aliquots were used for each day of the study.

A sample of each preparation was analysed prior to the start of dosing to verify the achieved concentrations of glyphosate acid in deionised water. Samples of the lowest concentration of dosing formulation was analysed to confirm the homogeneity of glyphosate acid in deionised water. The homogeneity of the 100 mg/mL formulation was not determined as part of this study and the data have been obtained from a preliminary study (Moxon ME, 1995, Study cited but not submitted) for which the method of preparation of the dosing formulations was the same. The chemical stability of glyphosate acid in deionised water was determined by re- analysis of the lowest and highest concentrations of dosing formulation after an interval of 26 days.

Concentration analysis results: The achieved concentrations of glyphosate acid in deionised water were within 5 % of nominal concentrations.

Homogeneity results: The homogeneity of glyphosate acid in deionised water at concentrations of 25 mg/mL and 100 mg/mL was within 5 % of the overall mean.

Stability results: The stability of the 25 mg/mL and the 100 mg/mL formulations was satisfactory over a period of 26 days which exceeded the period of use in this study.

Dosage administration: All animals were dosed once daily from days 7 – 16 (inclusive) of gestation with 1 mL of dosing formulation per 100 g bodyweight using a disposable syringe and a plastic nelaton catheter. The volume given to each animal was adjusted daily according to body weight. Control animals received the appropriate volume of deionised water. Dosing was performed in group order with all animals receiving the same dose level being dosed sequentially.

Observations:

Maternal observations: All animals were observed on arrival to ensure that they were physically normal externally and were subsequently observed at least twice each day. Any changes in behaviour

or clinical condition were recorded daily during the study.
Bodyweight: The bodyweight of each animal was recorded on arrival and on days 4, 7-16 (inclusive) and on days 19 and 22 of gestation.
Food consumption: The amount of food consumed by each animal over three day periods was measured by giving a weighed quantity of food contained in a glass jar on days 1, 4, 7, 10, 13, 16 and 19 and calculating the amount consumed from the residue on days 4, 7, 10, 13, 16, 19 and 22, respectively.
Terminal investigations: One rat requiring euthanasia was killed by over-exposure to halothane Ph. Eur. vapour and given a macroscopic examination post mortem.
On day 22 of gestation the animals were killed by over-exposure to halothane-Ph. Eur. vapour and a macroscopic examination post mortem was performed. The uterus from any animal without clear evidence of implantation was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred.
For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries and uterus were then examined and the following data recorded:-
Number of corpora lutea in each ovary
Number and position of implantations subdivided into:
a) live foetuses
b) early intra-uterine deaths (decidual or placental tissue only)
c) late intra-uterine deaths (embryonic/foetal tissue plus placental tissue) Individual foetal weights
The implantations were assigned letters of the alphabet to identify their position in utero starting at the ovarian end of the left horn and ending at the ovarian end of the right horn. In addition, each foetus was weighed and individually identified within the litter by means of a cardboard tag. After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 mL of 200 mg/mL pentobarbitone sodium solution.
Percentage pre-implantation loss and percentage post-implantation loss were calculated.
% pre-implantation loss = number of corpora lutea - number of implantations x 100
number of corpora lutea
% post-implantation loss = number of implantations - number of live foetuses x 100
number of implantations
Foetal observations: An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral abnormalities, sexed, eviscerated and fixed in 70 % industrial methylated spirits. After approximately 24 hours the head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70 % industrial methylated spirits for subsequent processing and staining with Alizarin Red S. The stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed. The individual bones of the manus and pes were assessed and the result converted to a six point scale. The observations were classified as major (permanent structural or functional deviations considered likely to be incompatible with survival or rarely seen) or minor defects or variants (small, generally transient deviations considered compatible with survival). The difference between the minor defect and variant classification is the frequency of occurrence in the control population of rats of this strain.
Statistical analyses: Data relating to animals which were non-pregnant, totally resorbed their litters or died intercurrently were excluded from the statistical analysis.

Maternal body weight during the dosing and post-dosing periods was considered by analysis of covariance on initial (day 7) body weight.
Maternal food consumption during the dosing and post-dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean manus and pes scores per litter were considered by analysis of variance.
Maternal-performance data (excluding the animal with undetermined pregnancy status), the proportion of foetuses with each individual manus and pes score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher's Exact Test.
Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external/visceral defects, minor external/visceral defects, external/visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:
All analyses were carried out in SAS (1989). For Fisher's Exact Test the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis.
All statistical tests were two-sided.
Results
Maternal toxicity:
Mortality and clinical signs: One control animal was killed on day 7 as a result of being misdosed. Excess watery fluid in the thoracic cavity and dark red areas on the surface of the lung lobes were observed at examination post mortem. The pregnancy status of the animal was not determined.
There were no changes in the clinical condition of the animals given glyphosate acid which were considered to be treatment-related.
Body weight: There was no effect of glyphosate acid on maternal body weight.
Table B.6.6-23: Intergroup comparison of maternal body weight (g) (selected timepoints, adjusted means for days 8 and 22)
Dose level of glyphosate acid (mg/kg/day)
day
0 (control)
250
500
1000
1
255.6
255.5
253.5
252.8
8
288.2
288.1

288.0
287.5
22
406.4
410.1
411.1
408.6
Food consumption: There was no adverse effect of glyphosate acid on maternal food consumption. The amount of food consumed by the animals given 1000 mg glyphosate acid/kg/day was marginally lower during the dosing period but differences from the controls were not statistically significant.
Table B.6.6-24: Intergroup comparison of food consumption (g/day) (selected timepoints)
Dose level of glyphosate acid (mg/kg/day)
day
0 (control)
250
500
1000
1-4
23.9
24.6
24.6
23.2
13-16
33.2
33.4
33.7
31.9
19226
29.5
31.6*
30.5
30.5
* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
Sacrifice and pathology:
Gross pathology: There were no macroscopic findings which were considered to be related to the administration of glyphosate acid.
Developmental Toxicity: There was-no effect of glyphosate acid on the number, growth or survival of the foetuses in utero.
Table B.6.6-25: Intergroup comparison of maternal performance
Observation
Glyphosate acid (mg/kg/day)
0 (control)
250
500
1000
Animals Assigned (Mated)

24
24
24
24
Animals Pregnant
22
24
23
24
#pregnancy status not determined (intercurrent death)
1
0
0
0
Gravid uterus weight (g)
89.7
87.2
91.3
89.9
#Intercurrent deaths
0
0
0
0
#aborted
0
0
0
0
totally resorbed at termination
0
0
0
1
Corpora Lutea/Dam
15.8
15.7
15.5
15.5
Implantations/Dam
14.4
12.9*
14.1
13.6
Total # Litters (viable)

22
24
23
23
Live Foetuses/Dam
12.9
12.4
13.1
12.9
Early (Proportion of litters affected)
8.7
3.4**
6.2
5.5
Late (Proportion of litters affected)
1.3
0.5
1.6
0.3
Litter Weight (g)
62.4
61.2
64.3
63.6
Mean Foetal Weight (g)
4.86
5.02
4.95
4.96
Sex Ratio (% Males per litter)
51.9
54.1
53.3
51.0
Preimplantation Loss (%)
8.7
18.0**
8.8
12.0
Postimplantation Loss (%)
9.9
4.0**
7.8
5.8*
* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, $p<0.01$ (Student's t-test, 2-sided)

Major defects: The incidence of foetuses with major defects was 1/284, 1/297, 1/301 and 2/296 in the control and 250, 500 and 1000 mg glyphosate acid/kg/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The defects were dissimilar in type and of single incidence.
Minor defect: The proportion of foetuses with minor external/visceral defects and the proportion of foetuses with minor skeletal defects were similar for all groups. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.
Variants: The proportion of foetuses with external/visceral variants and the proportion of foetuses with skeletal variants were lower in the glyphosate acid treated groups than in the control group. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.
Manus and pes assessment: There was no effect of glyphosate acid on the ossification of the manus or pes.
Conclusion by the Notifiers
The dose level of 1000 mg glyphosate acid/kg/day was the no observed effect level in this study for both maternal and developmental effects.
Comment by RMS:
The study is considered acceptable. The conclusion is agreed.
Reference: IIA, 5.6.10/02
Report: (1995)
HR-001: Teratogenicity Study in Rats.
Data owner: Arysta LifeScience Study No.: IET 94-0152
Date: 1995-07-21
not published ASB2012-11497
Guidelines: Japan MAFF Guidelines 59 NohSan No.4200, 1985
U.S. EPA FIFRA Guidelines Subdivision F, 1984
Deviations: None
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: 1995-03-23 to 1995-06-26
Materials and methods
Test material:
Glyphosate technical
Identification:
HR-001
Description:
Solid crystals
Lot/Batch #:
940908
Purity:
95.68 %
Stability of test compound:
Not mentioned in the report
Vehicle and/
or positive control:
Diet
Test animals:

Species:
Rat
Strain:
SPF Crj:CD (SD)
Source:
Age:
13 weeks
Sex:
Males and females
Weight at dosing:
Males: 380 – 450 g, females: 267 – 322 g
Acclimation period:
11 days
Diet/Food:
Certified diet MF Mash (Oriental Yeast Co., Ltd.), ad libitum
Water:
Filtered and sterilized water, ad libitum
Housing:
By pair in aluminium cages with wire-mesh floors for mating period; Individually for copulated females in aluminium cages with wire-mesh floors.
Environmental conditions:
Temperature: 24 ± 2 °C
Humidity: 55 ± 10 % Air changes: 12/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 1995-03-23 to 1995-06-26
Animal assignment and treatment:
Vaginal smears were taken from females for microscopic examination. Females showing proestrus or estrus vaginal smears were paired overnight with males on a 1:1 basis. The females were examined next morning for the presence of vaginal plugs and sperm in vaginal smears and considered to copulate when vaginal plugs and/or sperm were observed. These mating procedures were repeated for 4 consecutive days.
Four test groups were set. The test substance was administered orally with a stomach tube to 10 copulated Crj:CD (SD) female rats per group at dose levels of 0, 300, 1000 mg/kg/day from day 6 to 15 of gestation.
Clinical observations
Each female was observed for clinical signs and mortality at least once daily during the pre- dosing and post-dosing periods and at least twice daily during the dosing period.
Body weight
Individual body weights were recorded on days 0, 6-15 (daily during the dosing period) and 20 of gestation. Adjusted body weight gains were calculated by subtracting the gravid uterine weight from the body weight value on day 20 of gestation.
Sacrifice and pathology
All surviving females were euthanized by overdosage of ether inhalation and cesarian section was performed on day 20 of gestation. Each female was necropsied. The ovaries and uterus were removed and the gravid uterine weight and numbers of copora lutea and implants were recorded. Then the uterus was opened and the numbers of live and dead foetuses were recorded with their

positions in the uterine horns. Resorbed embryos or dead foetuses were classified into implantation sites, placental remnants or macerated foetuses (including dead foetuses) according to developmental stage in which resorptions or deaths occurred. When no uterine implants were grossly apparent, the uterus was stained with 10 % ammonium sulphide solution to detect very early resorptions. The weights of each live foetus and of each placenta were determined and recorded. Live foetuses were sexed and were euthanized by an intraperitoneal injection of pentobarbital sodium solution for examination of external abnormalities. The eyes were examined for alterations after removing the palpebral skin. Then the foetuses were examined for visceral and skeletal abnormalities.

Statistics

Variance analysis using Bartlett's test was evaluated for body weights, adjusted body weights, body weight gains and food consumption of maternal rats, numbers of corpora lutea, implants and live foetuses, and weights of gravid uteri, foetuses and placentas.

Results and discussion

Clinical observations

During the pre-dosing period, clinical observation revealed no abnormalities in any groups. During the dosing period, no abnormalities were observed in maternal rats of the control group. In the 30 and 300 mg/kg groups, one or two maternal rats had hair loss or scabs on the skin which have been usually observed in the historical control rats. In the 1 000 mg/kg group, 20 out of 22 pregnant females showed slightly loose stool and the increase in its incidence was statistically significant. During the post-dosing period, slightly loose stool was also observed on the following day of last dosing (day 16 of gestation) in 9 out of 20 females that showed this finding during the dosing period in the 1 000 mg/kg group. Another finding observed during this period was hair loss in 1-2 maternal rats in each treated group.

No deaths occurred during the study in any groups.

Body weight

No significant differences were found in the mean body weights and the mean adjusted body weights of maternal rats between the control groups and any of the treated group.

No significant differences were found in the mean body weight gains of maternal rats between the control group and any of the treated groups.

Food consumption and compound intake

No significant differences were found in the mean food consumption of maternal rats between the control group and any of the 30 and 300 mg/kg groups. In the 1 000 mg/kg group, lower and higher values were observed in the mean food consumption at intervals of days 6-9 of gestation (early dosing period) and days 15-20 of gestation (post-dosing period), respectively, and the differences from the corresponding controls were statistically significant.

Necropsy:

Gross pathology at cesarian section

Gross pathological examination of maternal rats at cesarean section revealed several findings such as hair loss and pelvic dilatation in the kidney in 1-2 animals in all groups including the control group. These findings were not considered to be due to test substance treatment.

Ovaries and uterus

Out of 24 copulated females, 23, 24, 24 and 22 were proved to be pregnant in the control, 30 mg/kg, 300 mg/kg and 1 000 mg/kg groups, respectively.

No significant differences were found in the mean gravid uterine weights and the mean numbers of corpora lutea and implants between the control group and any of the treated group.

Foetuses
Number of live foetuses and percent incidences of resorptions and foetal deaths
There were no significant differences in the mean number of live foetuses and the mean percent incidence of resorptions and foetal deaths between the control group and any of the treated groups.
Sex ratio, fetal body weights and placental weights.
There were no significant differences in the foetal sex ratio, the mean foetal body weights and the mean placental weights between the control group and any of the treated group.
Findings in external, visceral and skeletal examination
External malformations observed were short tail in a foetus of the 30 mg/kg group and microphthalmia in a foetus of the 1 000 mg/kg group.
Visceral examination revealed two types of malformations: right aortic arch in a foetus of the 300 mg/kg group and ventricular septal defects in a foetus of each of the 300 and 1 000 mg/kg groups.
Visceral variations were observed in all groups including the control group. The types and number in fetuses were thymic remnant in the neck, dilatation of the renal pelvis and left umbilical artery in 16-26, 1-2 and 0-3, respectively.
Skeletal examination revealed three types of malformations: splitting of the ossification centers of the thoracic vertebral bodies in 2, 1 and 2 foetuses in the control, 300 mg/kg and 1 000 mg/kg groups, respectively, asymmetry of the sternebrae with sterno-costal joint displacement in a foetus of the 300 mg/kg group, and fusion of the sternebrae in a foetus of the 300 mg/kg group, and fusion of the sternebrae in a foetus of the 1 000 mg/kg group.
Skeletal variations were observed in all groups including the control group. The types and the number in foetuses were cervical ribs shortening of the 13th ribs, lumber ribs, sacralization of the lumber vertebra and asymmetry and/or splitting of the sternebrae in 0-1, 0-1, 1-11, 0-1 and 3-5, respectively
Conclusion by the Notifiers
Based on these results, no observable effect level and minimal toxic level in the teratogenicity study with technical glyphosate in SD rats were established as follows.
Maternal rats
Foetal rat
No observable effect level
300 mg/kg/day
1000 mg/kg/day
Minimal toxic level
1000 mg/kg/day
-
It is also concluded that the highest dose level of 1000 mg/kg/day of HR-001 is not teratogenic to SD rat foetuses.
Comment by RMS:
The study is considered acceptable. The evaluation regarding maternal toxicity is agreed, which was confined to loose stool at highest dose level. The NOAEL for developmental toxicity is not supported due to the slight increase in skeletal variations at highest dose level: lumbar ribs were observed in 11 fetuses out of 7 litters compared to only 4 fetuses out of 2 litters in control animals. Teratogenic effects were not observed. Based on findings in dams and foetuses, the NOAEL for both maternal and developmental toxicity is considered to be 300 mg/kg bw/d.
B.6.6.11 Teratogenicity test by the oral route in the rabbit
Five developmental studies in rabbits had been submitted for the previous EU evaluation of which

four may be still used following re-evaluation by the RMS. For the current re- evaluation the feeding study in rabbits by (1981, TOX9650160) was considered not acceptable due to serious reporting deficiencies and therefore not reported in the present RAR.
In addition, three new studies in rabbits were provided in the GTF dossier. These studies were submitted either for the first time for this evaluation or had been subject to JMPR evaluation in 2004 yet and were all considered acceptable by the RMS.
Reference: IIA, 5.6.11/01
Report: (1995)
HR-001: A Teratogenicity Study in Rabbits
Data owner: Arysta Life Sciences Study no.: IET 94-0153
Date: 1995-07-21
not published ASB2012-11498
Guidelines: OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3
(1984)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 1995-03-31 to 1995-06-09
Materials and methods
Test material:
Identification:
Glyphosate technical, Code: HR-001
Description:
White crystal
Lot/Batch #:
T-941209
Purity:
97.56 %
Stability of test compound:
Not reported
Vehicle and/
or positive control:
0.5 % carboxymethylcellulose
Test animals:
Species:
Rabbit
Strain:
Japanese White rabbits Kbl:JW, SPF
Source:
Age:
18 weeks (females); 5-50 month (males)
Sex:
Males and females
Weight at dosing:
3.32 – 4.08 kg
Acclimation period:

10 days
Diet/Food:
GC4 (Oriental Yeast Co., Ltd.), ad libitum (females) /
120 g/day (males)
Water:
Tap water, ad libitum
Housing:
Individually in aluminium cages with wire-mesh floors.
Environmental conditions:
Temperature: 22 ± 2 °C
Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 1995-03-31 to 1995-06-09
Animal assignment and treatment:
In a teratogenicity study groups of 18 Japanese White female rabbits received doses of 0, 10, 100 and 300 mg/kg bw/day test substance in carboxymethylcellulose by gavage from Gestation Day 6-18 after artificial insemination performed on 12 or 16 females each day for 5 consecutive days. The dose levels were chosen based on results of a preliminary teratogenicity study.
Diet preparation and analyses
For each dose level, dosing solutions were prepared two times during the study by suspending the test substance in purified water with the aid of 0.5 % sodium carboxymethylcellulose. For each dose level dosing solutions were analyzed for concentration of the test substance before use.
Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.
Body weight
Individual body weights were recorded on Day 0, 6-18, 24 and 27 of gestation. Body weight gains were calculated by subtracting the body weight value on Day 0 of gestation from each value determined on Days 6 through 27 of gestation. Adjusted weights were also calculated by subtracting the gravid uterine weight from the body weight on Day 27 of gestation.
Food consumption
Food consumption of females was determined on alternate days from Day 0 to Day 26 of gestation and on Days 26- 27 of gestation. In each interval, daily food consumption (g/rabbit/day) was calculated for each female by dividing values of total food consumption by the number of days.
Sacrifice and pathology
Females were euthanatised by an injection of an overdose of a pentobarbital sodium solution into the auricular vein on Day 27 of gestation and subjected to caesarean sectioning.
The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. When uterine implants were not grossly apparent, the uteri were stained with 10 % ammonium sulfide solution to detect very early resorptions. After examination of the ovaries and conceptuses, each female was necropsied.
Developmental parameters
Live foetuses and their placentas were individually weighed. Live foetuses were uniquely identified by litters. Then they were euthanatized by an intraperitoneal injection of a pentobarbital sodium

solution and examined for external abnormalities. The eyes were examined for alterations after removing the palpebral skin. The sex of the foetuses was determined by observation of the gonads. After these examinations, each foetus was examined for visceral abnormalities. Then the thoracic and abdominal organs were removed and preserved in 10 % neutral- buffered formalin along with the ovaries and placentas. The remaining skeletons were fixed in 70 % isopropanol, stained with alizarin red S and cleared in 70 % glycerin for examination of skeletal abnormalities. After examination, skeletal specimens were stored.

Statistics

The following statistical tests were used to estimate significance of differences between the control group and the treated groups. The data on body weights, adjusted body weights, body weight gains, and food consumption of maternal rabbits, numbers of corpora lutea, implants, and live foetuses, and weights of gravid uteri, foetuses and placentas were evaluated as follows: Equality of variances was first evaluated by Bartlett's test. When group variances were homogeneous, a parametric analysis of variance in one-way classifications was used to determine if any statistical differences exist among groups. If the analysis of variance was significant, Dunnett's t-test or Scheffé's multiple comparison test was performed to detect any statistically significant differences between the treated groups and their corresponding controls. When Bartlett's test indicated that the variances were not homogeneous, Kruskal- Wallis test was used for detecting any statistical differences among groups and if significant, Dunnett-type mean rank test or Scheffé-type mean rank test was performed to detect statistical differences between the treated groups and their corresponding controls. Fisher's exact probability test was used for the data on the incidences of clinical and gross pathological findings in maternal rabbits, incidences of maternal rabbits having foetuses with malformations and variations, incidences of foetal malformations and variations, and foetal sex ratio, and Mann-Whitney's U-test for the data on the percent incidences of resorptions and foetal deaths.

Results and discussion

Analysis of dose formulations

The test substance was detected at levels of 95-105 % of the target concentrations in each dosing solution.

Food consumption

Mean food consumption in the treated groups was comparable to that in the control group throughout the study period.

Mortality

One rabbit in the high dose group died on Day 20 of gestation without showing any clinical signs.

Clinical observations

During the treatment period one animal each showed hair loss (forelimb) and scab on the auricle, respectively in low and mid dose group, respectively (see Table B.6.6-26). In the high dose group four animals showed loose stool and two showed soiled fur in the perianal region that was considered to be an alteration caused by defecation of loose stool. The incidence of loose stool was significantly high when compared with the control.

During the post-dosing period, two and one animal in the control group showed loose stool and red material on the tray, respectively. In the low dose group, hair loss (forelimb) was found in one animal and loose stool in another. Besides these findings, one dam aborted on Day 20 of gestation, and another one prematurely delivered on Day 27 of gestation. In the mid dose group only one animal showed hair loss in the lower abdominal region. In the high dose

group, two animals out of four, that had shown loose stool during the dosing period, still showed this alteration, and one animal out of these two aborted on Day 26 of gestation. Although loose stool

disappeared from the two other dams, the first prematurely delivered on Day 27 of gestation and the second had hair loss (dorsal region).
Considering the results of the preliminary study, defecation of loose stool and subsequent abortion or premature delivery observed in the highest dose group were considered to be related to test substance treatment.
Table B.6.6-26: Observed clinical signs during the dosing period
Clinical sign
Number of rabbits affected in dose group#
Control
(0 mg/kg/day)
Low
(10 mg/kg/day)
Mid
(100 mg/kg/day)
High
(300 mg/kg/day)
No abnormalities detected
18/18 (0)
16/17 (1)
15/16 (2)
13/17 (0)
Hair loss
0/18 (0)
1/17 (0)
0/16 (0)
0/17 (0)
Scab on the auricle
0/18 (0)
0/17 (0)
1/16 (0)
0/17 (0)
Soiled fur in the perianal
region
0/18 (0)
0/17 (0)
0/16 (0)
2/17 (0)
Loose stool
0/18 (0)
0/17 (0)
0/16 (0)
4/17 (1)*
x/y: number affected / total number of animals in group
* Significantly different from control at $p < 0.05$.
Figures in parentheses represent the number of animals having no grossly observable conceptus. These animals were excluded from statistical evaluation.

Body weight

Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, although differences from controls were not statistically significant, the mean values on Days 16-24 of gestation were somewhat lower than those in the control group.

Pathology Necropsy

Necropsy of maternal animals aborted, prematurely delivered or found dead on the study noted no abnormalities in the rabbits in the low dose group. In the high dose group, the aborted rabbit had yellow-coloured adipose tissue, a hair bolus in the stomach, watery contents in the large intestine and accentuated lobular pattern in the liver. The prematurely delivered rabbit in the high dose group had soiled fur in the perianal region, erosion in the stomach, a hair bolus in the stomach, and watery contents in the caecum. In the dead rabbit, pale liver and ascites (red) in the abdominal cavity were found; however, the cause of death was not known.

Gross pathological findings observed in animals which survived to termination of the study were: hair loss in the lower abdominal or dorsal region in one animal in each of the mid and high dose groups; hair bolus in the stomach in one animal each of the control and low dose groups. The occurrence of these gross pathological findings was low, and considered to be unrelated to test substance treatment.

Observations on the ovary and uterus

In the control, low, mid and high dose groups, 18, 16, 18, and 15 females, respectively, survived to termination of the study and were proven to be pregnant. However, one, two and one females in the low, mid and high dose group, respectively, had no grossly observable conceptus while implantation sites were detected by uterine staining with a 10 % ammonium
sulfide solution, indicating very early resorptions;. all data from these females were excluded from subsequent calculations.

Examinations of uterine contents demonstrated no abnormalities in all groups including the control. Mean gravid uterine weights and mean numbers of corpora lutea and implants were comparable between the control and the treated groups.

Developmental parameters Number and viability of foetuses

No statistically significant differences were noted in the mean number of live foetuses and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

Sex ratio, foetal body weights and placental weights

No statistically significant differences were noted in the sex ratios, mean foetal body weights, mean placental weights, mean number of live foetuses, and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

External, visceral and skeletal examination

No statistically significant differences were noted in the incidences of maternal animals having foetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the number of litters with malformations was significantly higher than that in the control group (see Table B.6.6-27). This increased malformation rate was due to an increase in skeletal malformations, as no external or visceral malformations were noted in foetuses from the high dose group. This was considered to be a sporadic alteration rather than the test substance treatment-related alteration because the types of skeletal malformations observed were inconsistent. Further, a dose-response in the number of foetuses showing skeletal malformations was not evident across dose groups.

With regard to variations, the incidence of total no. of litters with skeletal variations in the 100 mg/kg bw/day group was significantly higher than that in the control group. This high value was due to a significantly high incidence (87.5 % of litters, 27.3 % of the foetuses) of lumbar ribs in this dose group when compared with the control (72.2 % of litters, 16.4 % of foetuses). The total litter incidence for skeletal variations in the 100 mg/kg/day group was 100 %. However, the increased incidence of lumbar ribs in the 100 mg/kg/day group was considered to be a sporadic alteration because the value was within the historical control range (8.1-35.0 % of examined foetuses), and because no such increase was observed in the 300 mg/kg bw/day group (13.4 %).

Table B.6.6-27: Incidence of fetal malformations and variations in rabbits treated with HR-001

Foetal findings
Dose level (mg/kg bw/day)
0
10
100
300
Malformations
No. of litters examined
18
15
16
14
No. of foetuses examined
140
130
150
112
No of litters with anomalous foetuses
1
3
3
5*
Percentage of litters with malformations (%)
5.5
20.0
18.8
35.7
Skeletal malformations
Fusion of the frontal/parietal bones
0
1
0
2
Fissure of the parietal bone
0
0
3

0
Hypoplasia of the interparietal bone
0
1
0
0
Splitting of the parietal bones
0
0
3
1
Shortening of the nasal/frontal/mandibular bones
0
0
1
0
Hemivertebra
1
0
0
2
Unilateral ossification centre of the thoracic/lumbar vertebral bodies
0
1
0
0
Bifurcation of the ribs
1
0
0
0
Sternal cleft
0
0
1
0
Splitting of the sternebrae with sternocostal joint displacement
0
2
0
0
Total no. of foetuses with skeletal malformations
1
4
6
5

Percentage of foetuses with skeletal malformations (%)
0.7
3.1
4.0
4.5
Total no. of litters with skeletal malformations
1
3
2
5
Percentage of litters with skeletal malformations (%)
5.6
20.0
12.5
29.4
Variations
No. of litters examined
18
15
16
14
No. of foetuses examined
140
130
150
112
No of litters with anomalous foetuses
16
14
16
8*
Percentage of litters with variations (%)
88.9
93.3
100
57.1
Skeletal variations
No. of foetuses examined
140
130
150
112
27 presacral vertebrae
4
1
4

3
27 presacral vertebrae with 13th ribs
12
9
15
12
Cervical ribs
1
3
1
1
Lumbar ribs
23
19
41*
15
Extra ossification centre anterior to the 1st sternebra with costal cartilage joining
0
0
0
1
Total no. of foetuses with skeletal variations
40
32
61*
31
Total no. of litters with skeletal variations
16
12
16
8
Percentage of litters with skeletal variations (%)
88.9
80
100
57.1
* Significantly different from control at $p < 0.05$.

Conclusion by the Notifiers

The oral administration of HR-001 to artificially inseminated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 300 mg/kg bw/day. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 100 mg/kg bw/day for maternal toxicity. The NOAEL for offspring was considered to be 300 mg/kg bw/day.

Comment by RMS:

The study is considered acceptable. The NOAEL for both maternal and developmental toxicity in this study conducted with artificial inseminated rabbits are agreed. No developmental toxic effects were

observed.
Reference: IIA, 5.6.11/02
Report: (1996)
Glyphosate technical: Oral gavage teratology study in the rabbit
Data owner: Nufarm SPL project no.: 434/020 Date: 1996-07-04
GLP: yes
not published ASB2012-11499
Guidelines: OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations: None
GLP:
Acceptability: See RMS comment
Dates of experimental work: 1995-10-13 - 1995-12-12
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
White powder
Lot/Batch #:
H95D161A
Purity:
95.3 %
Stability of test compound:
not reported
Vehicle and/
or positive control:
1 % carboxymethyl cellulose
Test animals:
Species:
Rabbit
Strain:
New Zealand White
Source:
Age:
17 - 19 weeks
Sex:
Females (time-mated)
Weight at dosing:
2.2 - 4.1 kg
Acclimation period:
At least 4 days
Diet/Food:
SQC Standard Rabbit Diet (SDS Ltd., Witham, Essex, UK), ad libitum
Water:
Tap water, ad libitum

Housing:
Individually in stainless steel cages with grid floor
Environmental conditions:
Temperature: 20 ± 3 °C
Humidity: 50 ± 20 % Air changes: 15/hour 12 hours light/dark cycle
Study design and methods:
In life dates: 1995-10-13 - 1995-12-12
Animal assignment and treatment in the preliminary study:
Twenty-four time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated Day 0 of gestation. The females were delivered to Safepharm Laboratories Ltd. at or before Day 3 of gestation and were allocated randomised to treatment groups. Groups of 6 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1 % carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation Day 7-19. The dose levels were chosen based on results of a preliminary dose finding study with 6 female nulliparous rabbits, where administration of
500 or 1000 mg/kg bw resulted in toxicity signs (scours, fluid filled caecum, stomach ulceration, body weight loss, reduced food consumption). Based on these findings dose levels of ≥ 500 mg/kg bw were considered to be too high for a prolonged study.
Animal assignment and treatment in the main study:
Seventy-two time-mated females were supplied as described for the preliminary study (see above). Groups of 18 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1 % carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation Day 7-19.
Dose formulation and analysis
For each dose level, the test material was suspended daily in 1% carboxymethyl cellulose by weighing the required amount into a glass jar and adding vehicle to make the appropriate final volume. Homogeneity was assured by mixing the formulation with a homogeniser. The concentration, stability and homogeneity of the test material were analysed. The formulation was stable for at least 1 h.
Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.
Body weight
Individual body weights were recorded on Day 3, 7, 10, 13, 16, 19, 22, 25 and 29 of gestation.
Food consumption
Food consumption of females was recorded on Days 3 to 7, Days 7 to 10, Days 10-13, Days 13-16, Days 16-19, Days 19-22, Days 22-25 and Days 25-29 of gestation.
Sacrifice and pathology
Females were euthanatized by an i.v. injection of an overdose of sodium pentobarbitone into the auricular vein on Day 29 of gestation, examined for macroscopic abnormalities and subjected to caesarean sectioning. The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. After examination of the ovaries and conceptuses, each female was necropsied.
Developmental parameters
The foetuses were killed by intrathoracic injection of sodium pentobarbitone. All foetuses were

dissected and examined for visceral abnormalities macroscopically. The heads of alternate foetuses were removed and identified using an indelible marker and placed in Bouin's fixative. After a minimum of 14 days, the heads were transferred to 90 % industrial methylated spirits (IMS) in distilled water and examined for visceral anomalies under a low power binocular microscope. All foetuses were identified using colour coded wires and placed in 70 % IMS in distilled water. The foetuses were eviscerated, processed and the skeletons stained with alizarin red.The foetuses were examined for skeletal development and anomalies.

Statistics in the main study

Female bodyweight change (relative to Day 7 of gestation) and food consumption were analysed statistically by one-way analysis of variance with the Bonferroni multiple comparison test followed by pair wise analysis of control values against treated group values using Students 't' test where appropriate.

All foetal parameters, skeletal development, group incidence of specific visceral and skeletal anomalies were analysed statistically by Kruskall-Wallis non parametric analysis of variance followed by pair wise analysis of control values against treated values using the Mann- Whitney U - test where appropriate.

Results and discussion

Analysis of dose formulations

The test substance was detected at the levels of 81-102% of the target concentrations in each dosing solution.

Food consumption

In the preliminary study, significantly reduced food consumption was observed while administering in the high dose level of 400 mg/kg/day (Days 7 to19 of gestation). This observation was confirmed in the main study. At the high dose level, there was a reduction in food consumption during the dosing period compared to controls (Days 10 to 13, $p < 0.05$; Days 13 to 19, $p < 0.01$). No other significant changes were observed in the remaining groups during the main study.

Mortality

In the preliminary study, two does were killed in extremis in the high dose group, one had aborted foetuses and the other was bleeding from the vagina. No mortalities occurred at any dose up to 400 mg/kg/day in the preliminary study.

In the main study, two rabbits were found dead or moribund at the high dose level. One female was found dead prior to dosing on Day 19 of treatment. One female was killed in extremis on Day 20 of treatment. Clinical observations noted at this time included hunched posture, lethargy, ptosis, hypothermia and blood on the litter tray. At the intermediate dose level, one female was found dead after dosing on Day 16 of treatment. Necropsy findings of reddened lungs, a fluid filled thorax and test material in thoracic cavity are consistent with mal-dosing. At the low dose level, no mortalities occurred. One female was found dead two minutes after dosing in the control group. Necropsy findings of blood in thorax, inflated appearance of lungs and a large area of congestion on the right caudal lobe are consistent with mal-dosing.

Clinical Observations

In both the preliminary and the main study, the clinical signs were in general the same. There was a toxicologically significant increase in the incidence of clinical observations, particularly scours, reduced faecal output and diarrhoea at the high dose level (400 mg/kg bw/day). Observations of lethargy, ptosis, hunched posture, hypothermia and blood on tray were noted for one animal of the main study killed in extremis.

At 200 mg/kg bw/day, vaginal bleeding and blood on tray were noted for one animal of the main

study. Scours were also noted in animals at 200 and 50 mg/kg bw/day as well as in the control group, but the incidence and duration were not as severe as at the high dose level (see Table B.6.6-28). No other treatment-related observations were evident.
Thus, for the findings observed at doses below 400 mg/kg bw/day, a clear dose-response could not be established.
Table B.6.6-28: Observed clinical signs during the dosing period
Clinical sign
Number of rabbits affected in dose group#
Control
(0 mg/kg/day)
Low
(50 mg/kg/day)
Intermediate
(200 mg/kg/day)
High
(400 mg/kg/day)
Scours
5/14 (4)
10/18 (0)
7/16 (2)
16/16 (2)
Reduced faecal output
0/14 (4)
1/18 (0)
2/16 (2)
2/16 (2)
Diarrhoea
0/14 (4)
1/18 (0)
0/16 (2)
10/16 (2)
Diuresis
0/14 (4)
0/18 (0)
1/16 (2)
0/16 (2)
Blood on tray
0/14 (4)
0/18 (0)
1/16 (2)
1/16 (2)
Noisy respiration
0/14 (4)
0/18 (0)
1/16 (2)
1/16 (2)

Lethargy
0/14 (4)
0/18 (0)
0/16 (2)
1/16 (2)
Ptosis
0/14 (4)
0/18 (0)
0/16 (2)
1/16 (2)
Hunched posture
0/14 (4)
0/18 (0)
0/16 (2)
1/16 (2)
Hypthermia
0/14 (4)
0/18 (0)
0/16 (2)
1/16 (2)
Anal staining
0/14 (4)
0/18 (0)
0/16 (2)
1/16 (2)
Subdued behaviour
0/14 (4)
0/18 (0)
0/16 (2)
1/16 (2)
Vaginal bleeding
0/14 (4)
0/18 (0)
1/16 (2)
0/16 (2)
x/y: number affected / total number of animals in group
Figures in parentheses represent the number of animals having no grossly observable conceptus.
Body weight
In the preliminary, study a toxicologically significant decrease in body weight gain from Day 13 to 19 post coitum was evident at the high and intermediate dose levels.
Likewise a reduction in group mean body weight gain from Days 9 to 29 post coitum was observed in the high dose level group during the main study. The difference in group mean bodyweight change compared to controls was statistically significant ($P<0.05$ to 0.01) from Days 13 to 29 post coitum. Also in the intermediate dose level group a slight reduction (although not statistically significant) in group mean body weight gain from Day 9 to Day 29 post coitum was noted. In the low dose level group body weight gain was comparable to controls throughout the study period (see Table B.6.6-

29).
Table B.6.6-29: Mean body weight gain during gestation
Dose level (mg/kg bw)
No. of animals
Body weight change (g) at Day (relative to Day 7)
10
13
16
19
22
25
29
0 (Control)
14
29
95
202
260
314
375
409
50
18
12
75
158
223
278
325
395
200
15
-11
54
143
198
263
309
294
400
15
-33
-45*
11**
21**
96**

153**
250*
* Significantly different from control at p < 0.05.
** Significantly different from control at p < 0.01.
Pathology Necropsy
The macroscopic necropsy findings of the two does of the high-level dose group that died or were killed in extremis included fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, duodenum congested and colon, rectum and appendix gas distended. These findings indicate that the test material may affect the gastrointestinal tract. The animal killed in extremis at this level also had both uterine horns containing blood and dead foetuses in the uterus. This may be a result of maternal toxicity. All other necropsy findings were not treatment-related.
Observations on the ovary and uterus
No treatment related effects were evident in both the preliminary and the main study.
In the control, low, intermediate and high dose level groups 14, 18, 16, and 16 females, respectively, survived to termination of the main study and were proven to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.
Developmental parameters Number and viability of foetuses
The litter size at caesarean section was comparable in all treatment groups. In the high dose level group, there were slight, but not statistically significant, increases in late foetal deaths and post implantation loss, mainly due to one animal that had nine late deaths, resulting in a post implantation loss of 69.2 %. This was therefore considered not to be a treatment-related effect. At 200 mg/kg bw/day, there were statistically significant increases ($p<0.05$) in total foetal deaths and post implantation loss. These increases were caused by a slight, but not statistically significant, rise in early foetal deaths. As at this dose level, there was no rise in late foetal deaths, as seen at the high level; the effect on early foetal deaths was considered not to be treatment-related.
Foetal body weights
No statistically significant differences were noted in the mean foetal body weights between the control group and the treated groups. Mean total litter weights were comparable in all treatment groups.
External, visceral and skeletal examination
At the high dose level, there was one litter with one foetus with major malformations. This foetus was found to have spina bifida and clubbed and malrotated hind limbs. At the intermediate dose level, two foetuses of two different litters had major malformations. One
foetus had retinal infolding and a haemorrhage in the retinal layer, the other acephaly, small kinked tail, bilateral forelimb flexure, interrupted aorta and an intraventricular septal defect. At skeletal examination, this foetus was found to have multiple rib and vertebral column abnormalities. At the low dose level, three foetuses of two different litters had major abnormalities. In one litter, one foetus had forked ribs with a displaced vertebral centrum. In another litter, one foetus had a small eye with retinal infolding and aphakia. A second foetus from this litter had nostrils close together, and a thin nasal septum not attached at posterior pole near the front of the nasal passages. In the control group, there were two foetuses from two different litters with major abnormalities. One foetus had gastroschisis and the other foetus had an extra vertebral arch resulting in scoliosis. These findings were considered to be within the range of normal variation for this species. There were no treatment-related effects on the degree of skeletal development.
Table B.6.6-30: Incidence of foetal malformations and variations in rabbits treated with glyphosate

acid
Foetal findings
Dose level (mg/kg bw/day)
0
50
200
400
No. of litters examined
14
18
15
15
No. of foetuses examined
128
157
119
134
Skeletal malformations
Total no. of foetuses with skeletal malformations
1
0
1
0
Total no. of litters with skeletal malformations
1
0
1
0
Percentage of litters with skeletal malformations (%)
7.1
0.0
6.7
0.0
Skeletal variations
Total no. of foetuses with skeletal variations
43
48
39
49
Total no. of litters with skeletal variations
13
18
15
15
Percentage of litters with skeletal variations (%)
92.8

100
100
100
External and visceral findings
No. of litters examined
14
18
15
15
No. of foetuses examined
128
157
119
134
No of litters with anomalous foetuses
2
5
2
3
Percentage of litters with anomalous foetuses (%)
14.3
27.8
13.3
20
No. of litters with major malformations
2
2
2
1
Percentage of litters with malformed foetuses (%)
14.3
11.1
13.3
6.7
Conclusion by the Notifiers
The oral administration of glyphosate technical to pregnant rabbits by gavage from gestation Day 7-19 resulted maternal toxicity at 400 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 200 mg/kg bw/day for maternal toxicity. The 'No Observed Adverse Effect Level' (NOAEL) for developmental toxicity was considered to be 400 mg/kg bw/day.
Comment by RMS:
The study is considered acceptable. The NOAEL of 200 mg/kg bw/d for maternal toxicity is not supported. The NOAEL is considered to be 50 mg/kg bw/d due to slight reduction in body weight gain at 200 mg/kg bw/d.
The NOAEL of 400 mg/kg bw/d for developmental toxicity is neither supported. The NOAEL is considered to be 50 mg/kg bw/d due to significantly increased post-implantation loss at 200 mg/kg

bw/d. The statement, that this increase was caused by a slight rise in early foetal deaths and not in late foetal deaths, as seen at the high dose level and therefore considered not to be treatment-related, cannot be followed, because there is no information given
regarding the mechanism behind this foetal deaths. Due to some reporting deficiencies, it remains unclear, whether the heart was part of visceral examination.
Comment by GTF on the first draft of the RAR (July 2013):
1. The GTF believes the developmental NOAEL in is 400 mg/kg/day, with no evidence to test substance related dose-response, citing the published expert review: Kimmel et al., 2013, (ASB2013-3462) Evaluation of developmental toxicity studies of glyphosate with attention to cardiovascular development, Crit Rev Toxicol, 2013; 43(2): 79– 95.
2. Regarding (1996, ASB2012-11499) the RMS notes that "it remains unclear whether the heart was part of visceral examination". The study sponsor contacted the contract laboratory and the response is noted….The contract laboratory Head of reprotox at the contract notes Reprotox "The examination of the heart is conducted on the fresh fetus for rabbit developmental toxicity studies. This is mentioned in our standard operating procedure". This is reflected the draft RAR Volume 3, B.6.6.11, noting "All foetuses were dissected and examined for visceral abnormalities macroscopically"……..
RMS comment (August 2013):
1. As already stated, the NOAEL is considered to be 50 mg/kg bw/d due to significantly increased post-implantation loss at 200 mg/kg bw/d. The statement, that this increase was caused by a slight rise in early foetal deaths and not in late foetal deaths, as seen at the high dose level and therefore considered not to be treatment-related, cannot be followed, because there is no information given regarding the mechanism behind this foetal deaths (please refer to the additionally inserted table below. Considering the individual animal data: at low dose level of 50 mg/kg bw/d 4/18 pregnant animals revealed post implantation losses, which was comparable to control animals (4/14 animals). Whereas at the mid dose of 200 mg/kg bw/d 10/15 animals were affected and at 400 mg/kg bw/d 9/15 animals. Currently, RMS does not see the need to change the assessment.
[Remark: The published expert review by Kimmel et al. (2013, ASB2013-3462) was already available and considered when preparing the first draft of the RAR, please refer to B.6.6.12- Published data]

Table B.6.6-31: Group Mean Litter Data
2: The additional information is acceptable, but was not given in the study report.
Reference: IIA, 5.6.11/03
Report: (1996)
Glyphosate acid: Developmental toxicity study in the rabbit
Data owner: Syngenta Report No.: CTL/P/5009 Date: 1996-07-02
not published TOX2000-2002
Guidelines: OECD 414 (1981), EEC B.31 (1988),US-EPA 83-3
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 1996-01-01 to 1996-02-09
Materials and methods
Test material:

Identification:
Glyphosate acid
Description:
White solid
Lot/Batch #:
Y04704/034
Purity:
95.6 %
Stability of test compound:
The stability of the test substance was confirmed for the study period.
Vehicle and/
or positive control:
Deionised water
Test animals:
Species:
Rabbit
Strain:
New Zealand White
Source:
Age:
Not reported
Sex:
Females (time-mated)
Weight at dosing:
approximately 3.8 kg
Acclimation period:
At least 4 days
Diet/Food:
Harlan Teklad 9603TRB rabbit diet, ad libitum
Water:
Tap water, ad libitum
Housing:
Individually in mobile rabbit units
Environmental conditions:
Temperature: 17 ± 2 °C
Humidity: 55 ± 15 % Air changes: 25-30/hour 12 hours light/dark cycle
Study design and methods
In life dates: 1996-01-01 to 1996-02-09
Animal assignment and treatment:
Eighty time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated Day 1 of gestation. The females were delivered to CTL at or before Day 3 of gestation and were allocated randomised to treatment groups. Groups of 20 time-mated New Zealand white female rabbits received 0, 100, 175 or 300 mg/kg bw/day test substance by gavage (2 mL/kg bw) from gestation Day 8-20. The dose levels were chosen based on results of a preliminary dose finding.
Dose formulation and analysis

For each dose level an appropriate amount of deionised water was added to a weighed amount of glyphosate acid (adjusted for purity). Each preparation was thoroughly mixed and subdivided into aliquots. Fresh aliquots were used for each day of the study. Two preparations were made per concentration (i.e. 0, 50, 87.5 and 150 mg/mL). The dosing preparations were stored at room temperature.
Representative samples of each dosing preparation were analysed prior to being used for dosing to verify the achieved concentration of glyphosate acid in the vehicle. Samples were taken for the determination of homogeneity at 50 and l50 mg glyphosate acid/mL (low and high dose levels).
The chemical stability of glyphosate acid in the vehicle was determined by re-analysis of the lowest and highest concentrations of the dosing preparations after an interval of 40 days.
Dose formulations were shaken prior to dosing, and during dosing as required.
Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.
Body weight
Individual body weights were recorded on arrival, on Day 4, prior to dosing on Days 8 to 20 and on Days 23, 26 and 30 of gestation.
Food consumption
Food consumption of females was recorded on Days 4-8, Days 8-11, Days 11-14, Days 14-17, Days 17-20, Days 20-23, Days 23-26 and Days 26-30 of gestation.
Sacrifice and pathology
All rabbits at scheduled termination on day 30 and any requiring euthanasia during the study were killed by an overdose of 200 mg/mL sodium pentobarbitone solution given as i.v. injection. All animals were subjected to an examination post mortem. This involved an external observation and an examination of the thoracic and abdominal viscera. The pregnancy status of each animal was determined. Where there was no clear evidence of implantation, the uterus was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred. For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries, uterus and contents where then examined. Number of corpora lutea, number and position of implantations, number of live foetuses, foetus weight and early and late intrauterine deaths were determined for each sacrificed doe.
Developmental parameters
After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 mL of 200 mg/mL pentobarbitone sodium solution. An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral abnormalities, sexed, eviscerated and fixed in 70 % industrial methylated spirits. After approximately 24 h the head of each foetus was cut along the fronto- parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70 % industrial methylated spirits for subsequent processing and staining with Alizarin Red S. The remaining stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed.
Statistics
Data relating to those animals which were non-pregnant and animals that died intercurrently were excluded from the statistical analysis. Maternal bodyweight during the dosing and post dosing periods was considered by analysis of covariance on initial (Day 8) bodyweight. Maternal food consumption during the dosing and post dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean

manus and pes scores per litter were considered by ANOVA. Maternal performance data, the proportion of foetuses with each individual manus and pes score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher‘s Exact Test. Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external visceral defects, minor external/visceral defects, external visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:

1) Percentages were analysed by ANOVA following double arcsine transformations,

2) the proportion of foetuses and, with the exception of male foetuses, the proportion of litters affected were considered by Fisher‘ s Exact Test.

All analyses were carried out in SAS (1989). For Fisher‘s Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student‘s t-test, based on the error mean square in the analysis.

All statistical tests were two sided.

Results and discussion

Analysis of dose formulations

The concentrations of glyphosate acid in the dosing formulations were within 12 % of the target concentrations. The homogeneity and stability of the test substance in the dosing formulations was satisfactory.

Food consumption

During the dosing period, does receiving 175 or 300 mg/kg bw/day showed reduced food consumption compared to the controls.

Mortality

The incidence of intercurrent deaths was 1, 2, 2 and 2 in the control, 100, 175 and 300 mg/kg/ bw/day groups, respectively.

In the post-dosing period, one doe in the control group showed weight loss, reduced food consumption, signs of diarrhoea, mucus in the faeces, few faeces and staining in the genital area. This animal aborted on Day 30. Changes in the stomach and caecum were observed post mortem.

In the low dose level group, one doe showed slight loss of bodyweight and reduced food consumption between Days 4 and 8 (i.e. prior to the onset of dosing) and this response continued into the dosing period, until the animal aborted its litter on Day 19. Examination post mortem noted the presence of a mass in the right inguinal region of the abdominal cavity. A second animal in this group aborted its litter on Day 25 having shown weight loss and reduced food consumption from Day 14.

At the intermediate dose level, one doe was killed for humane reasons on Day 23 having shown loss of bodyweight and reduced food consumption from Day 4 on. By Day 23, the animal had become thin and subdued and all uterine implantations were found to be dead. A second animal in this group aborted its litter on Day 22 having shown slight weight loss from Day 14 and reduced food consumption from Day 4.

At the high dose level, two animals aborted their litters on Days 24 and 23, respectively. Both animals showed a reduction in food consumption from Day 11 and bodyweight loss from Day 11/13. A hair-

like substance was found in the stomachs of both animals at examination post mortem.

Clinical Observations

In the high dose level group, there was an increased incidence of animals producing few faeces, with signs of diarrhoea or with staining in the genital area, in comparison with the control group. The production of few faeces and signs of diarrhoea were also of increased incidence in does of the intermediate dose group. There were no clinical effects observed in rabbits treated at a dose level of 100 mg/kg bw/day (see Table B.6.6-32).

Table B.6.6-32: Observed clinical signs during the dosing period

Clinical sign
Number of rabbits affected in dose group
Control
Low
Intermediate
High
(0 mg/kg/day)
(100 mg/kg/day)
(175 mg/kg/day)
(300 mg/kg/day)
Blood on tray
0
2
2
1
Cold
0
0
1
0
Dry sores 1 or more areas
0
1
0
0
Ears torn
0
2
1
1
Eye opaque
0
1
0
0
Few faeces on tray
3
3

9
9
Mucus in faeces
1
0
0
0
No faeces on tray
0
1
2
3
Scabs in 1 or more areas
4
6
3
3
Signs of diarrhoea
4
5
11
19
Staining in genital area
2
2
3
11
Subdued behaviour
0
0
1
0
Thin
0
0
1
2
Urine coloured
0
1
1
0
Wet sores in 1 or more areas
2
0
1

0
Body weight
Administration of 300 mg/kg bw/day was associated with a reduction in maternal body weight gain. The statistical different observed body weight development at 175 mg/kg bw/day was due to differences in body weights at the begin o the study. All animals except one of the high dose group showed signs of recovery in the post-dosing period. The reduction in food consumption was therefore accompanied by a corresponding reduction in body weight. In the low dose level group, body weight gain was comparable to controls throughout the study period (see Table B.6.6-33).
Table B.6.6-33: Mean body weight development (in g) during gestation
Dose level in mg/kg bw/day
0 (Control)
100
175
300
Animals per group
17
18
17
17
Day of gestation
8
3924
3771
3822
3815
9
3845
3837
3834
3823
10
3857
3863
3856
3830
11
3885
3873
3874
3854
12
3894
3879
3877
3856
13

3917
3905
3902
3880
14
3942
3932
3930
3875
15
3975
3982
3939
3896
16
4020
4031
3959
3907*
17
4049
4053
3982
3923*
18
4063
4051
3990
3914**
19
4085
4061
4005
3927**
20
4088
4059
3995
3926**
23
4177
4118
4049*
3951**
26
4236

4210
4169
4093*
30
4313
4294
4256
4183
* Significantly different from control at $p < 0.05$.
** Significantly different from control at $p < 0.01$.
Pathology Necropsy
There were no macroscopic findings that were considered to be related to the administration of glyphosate acid.
Observations on the ovary and uterus

No treatment related effects were evident in the study.
In the control, low, intermediate and high dose level groups 17, 18, 17, and 17 females, respectively, survived to termination of the main study and were proven to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.
Developmental parameters Number and viability of foetuses
The proportion of foetuses that were male was statistically significantly increased in the intermediate dose level group, in comparison with the control group. In the absence of a dose- related trend, this finding was considered incidental to the administration of glyphosate acid.
There was no adverse effect of glyphosate acid on the number or survival of the foetuses in utero.
Foetal body weights
There was a statistically significant reduction in mean foetal weight in the high dose level group, in comparison with the control group. This difference was considered attributable to two litters for which the mean pup weight was particularly low.
External, visceral and skeletal examination
The number of foetuses with major defects was 3/143 (2/17 litters), 1/147 (1/18 litters), 0/135 (0/17 litters) and 2/144 (2/17 litters) in the control, 100, 175 and 300 mg/kg bw/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The proportion of foetuses with minor external visceral defects was similar for all groups, including the control. There were no significant differences in litter incidences for minor external/visceral defects noted. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid (see Table B.6.6-34 and Table B.6.6-35).
The proportion of foetuses with minor skeletal defects was statistically significantly increased in the 100 and 300 mg/kg bw/day groups, in comparison with the control group, but not in the 175 mg/kg bw/day group. Evaluation of the specific defects noted an increased incidence of foetuses in the high dose level group with partially ossified transverse processes on the 7th cervical vertebra (8 foetuses in 2 litters), unossified transverse processes on the 7th lumbar vertebra (14 foetuses in 4 litters) or partially ossified 6th sternebra (16 foetuses in 7 litters). None of the specific minor defects were statistically significantly increased in the low or intermediate dose level groups. None of the foetuses were found to have an external/visceral variant.
The proportion of foetuses with skeletal variants was statistically significantly increased

dose level group, in comparison with the control group. Evaluation of the specific variants noted a slight, but not statistically significant, increase in the incidence of foetuses in this group with partially ossified odontoids (62 foetuses in 15 litters) or with 27 pre-sacral vertebrae (37 foetuses in 12 litters). The slightly higher mean manus score observed in the high dose level group, in comparison with the control group, was due to a slight reduction in ossification as shown by the increase in incidence of foetuses scoring 4 or 5. A similar response was apparent from the pes scores.

Table B.6.6-34: Summary of the type and incidence of major defects

Major foetal defects	Number of foetuses affected in dose group*			
	Control	Low	Intermediate	High
	(0 mg/kg/day)	(100 mg/kg/day)	(175 mg/kg/day)	(300 mg/kg/day)
Heart single ventricle, ventricle walls thickened, aorta enlarged, pulmonary artery reduced	0/143	1/147	0/135	1/144
Encephalocoele (gross malformation of the skull)	0/143	0/147	0/135	1/144
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, aorta enlarged, persistent truncus arteriosus	1/143	0/147	0/135	0/144
Shortened upper and lower jaws, cleft lip, cleft palate, nares absent, forepaws flexed (right extremely, left slight)	1/143	0/147	0/135	0/144
Reduced number of lumbar vertebrae (25 pre-sacral vertebrae)	1/143	0/147		

0/135
0/144
* number affected / total number
Table B.6.6-35: Summary of the type and incidence of major defects (litter incidences)
Major foetal defects
Number of litters affected in dose group*
Control
Low
Intermediate
High
(0 mg/kg/day)
(100 mg/kg/day)
(175 mg/kg/day)
(300 mg/kg/day)
Heart single ventricle
0/17
1/18
0/17
1/18
aorta enlarged
1/17
1/18
0/17
1/18
pulmonary artery reduced
0/17
1/18
0/17
1/18
Encephalocoele (gross
malformation of the skull)
0/17
0/18
0/17
1/18
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, Shortened upper and lower jaws, cleft lip, cleft palate,
nares absent
1/17
0/18
0/17
0/18
persistent truncus arteriosus
1/17
0/18
0/17

0/18
forepaws flexed (right extremely,
left slight)
1/17
0/18
0/17
0/18
Reduced number of lumbar
vertebrae, 25 pre-sacral vertebrae
1/17
0/18
0/17
0/18
* number affected / total number
Table B.6.6-36: Incidence of foetal malformations and variations in rabbits treated with glyphosate acid
Foetal findings
Dose level (mg/kg bw/day)
0
100
175
300
No. of litters examined
17
18
17
17
No. of foetuses examined
143
147
135
144
Skeletal malformations
Total no. of foetuses with major defects
3
0
0
1
Total no. of litters with major defects
2
0
0
1
Percentage of litters with major defects (%)
11.8
0.0

0.0
5.9
Total no. of foetuses with minor defects
58
82*
59
79*
Total no. of litters with minor defects
16
18
16
17
Percentage of litters with minor defects (%)
94.1
100
94.1
100
Skeletal variations
Total no. of foetuses affected
119
129
116
132*
Total no. of litters affected
17
18
17
17
Percentage of litters affected (%)
100
100
100
100
External and visceral findings
No. of foetuses with major defects
2
1
0
2
No of litters with foetuses with major defects
2
1
0
2
Percentage of litters with foetuses with major defects (%)
11.8

5.6
0.0
11.8
No. of foetuses with minor defects
12
7
9
11
No of litters with foetuses with minor defects
8
5
8
7
Percentage of litters with foetuses with minor defects (%)
47.1
27.8
47.1
41.2
* Statistically significant from control ($p < 0.05$)
Conclusion by the Notifiers
The oral administration of glyphosate acid to time-mated rabbits by gavage at a maximum dose level of 300 mg/kg bw/day from Gestation Day 8-20 resulted maternal toxicity at 175 and 300 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore the 'No Observed Effect Level' (NOEL) was considered to be 100 mg/kg bw/day for maternal toxicity. The 'No Observed Effect Level' (NOEL) for developmental toxicity was considered to be 175 mg/kg bw/day.
Comment by RMS
The study is considered acceptable. The NOAEL for both maternal and developmental toxicity are agreed. Maternal toxicity comprised clinical signs, reduced food consumption and body weight gain at 175 mg/kg bw/d and above. Developmental toxicity consisted of reduced foetal body weight and reduced ossification at 300 mg/kg bw/d.
Reference:
IIA, 5.6.11/04
Report:
. (1980)
Technical Glyphosate: Teratology study in rabbits
Monsanto Report No.: IR-79-016 Date: 1980-02-29
not published TOX9552392
Guidelines:
Not stated. (pre-guideline; satisfies in general the requirements of OECD 414 (1981), but not of OECD 414 (2001))
Deviations:
Not applicable
GLP:
no (pre-GLP study)
Acceptability:

See RMS comment
Dates of experimental work: 1979-04-10 to 1979-05-11
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
White powder
Lot/Batch #:
XHJ-64
Purity:
98.7 %
Stability of test compound:
Not reported
Vehicle and/
or positive control:
0.5 % aqueous Methocel®
Test animals:
Species:
Rabbit
Strain:
Dutch Belted
Source:
Age:
Approx. 7 month
Sex:
Females
Weight at dosing:
2.533 – 3.234 kg
Acclimation period:
At least 30 days
Diet/Food:
Purina Rabbit Chow Checkers 5301, ad libitum
Water:
Tap water, ad libitum
Housing:
Individually in suspended wire mesh cages
Environmental conditions:
Temperature: Exact values not reported Humidity: Exact values not reported Air changes: Exact values not reported Light controlled
Study design and methods
In life dates: 1979-04-10 to 1979-05-11
Animal assignment and treatment:
Sixty-four female Dutch Belted rabbits were artificially inseminated and randomly assigned to treatment groups of 16 animals. The day of insemination was designated Day 0 of gestation. The rabbits received daily doses of 0, 75, 175 or 350 mg/kg bw/day test substance by gavage (1 mL/kg

bw) from gestation Day 6 to 27. Individual doses based on individual body weights determined on gestation Day 6.

Dose formulation

For each dose level an appropriate amount of grounded technical glyphosate was suspended in 0.5 % aqueous Methocel® solution and homogenised. The dose solutions were prepared daily.

Clinical observations

A check for mortality or behavioural changes was made once daily prior to treatment. During the treatment and post-treatment period all rabbits were observed once daily for clinical signs of toxicity, mortality or behavioural changes.

Body weight

Individual body weights of dams were recorded on gestation Days 0, 6, 12, 18, 24 and 28. These time points for body weight determination differ from the requirements of the current OECD guideline 414 (2001) (i.e., body weights should be determined on gestation day 0 and at 3-day intervals thereafter). Although the time-intervals were longer than required, the time points for body weight determination are considered to be sufficient to evaluate the body weight development of the pregnant animals.

Sacrifice and pathology Dams

All rabbits at scheduled termination on day 28 were sacrificed, the uterus was excised and weight and the foetuses were removed. The number and location of viable foetuses, early and late resorptions, the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs were examined for gross pathological changes.

Rabbits that died during the study were necropsied to determine the cause of death.

Foetuses

All foetuses were weight, sexed and examined for external malformation and variations, as well as for visceral malformations and variations. The carcasses were then fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for skeletal examination.

Statistics

All statistical analyses compared the treatment groups to the control group with a level of significance at $p < 0.05$. Foetal sex distribution and number of litters with malformations were analysed using the Chi-square test with Yates correction and/or Fisher´s exact probability test. Ther number of early and late resorption and post-implantation losses were compared by the Mann-Whitney U-test.

Mean numbers of viable fetuses, total implantations, corpora lutea and mean fetal body weights were compared by ANOVA (one-way), Bartlett´s test for homogeneity and appropriate t-test.

Results and discussion

Mortality:

There was an increased incidence of mortalities in the high dose group (see Table B.6.6-37).

Table B.6.6-37: Mortalities of dams

Control	Low	Intermediate	High
(0 mg/kg/day)	(75 mg/kg/day)	(175 mg/kg/day)	(350 mg/kg/day)

Spontaneous deaths*
0/16
1/16
2/16
10/17
Time of death (gestation day)
--
26
22, 25
3 to 21
% mortality
0.0
6.3
12.5
58.8
Sacrificed after abortion
2
0
1
1
Sacrificed on gestation day
22
--
27
23
* dead animals / total animals in group
For five of the rabbits that died spontaneously, the cause of death was attributed to pneumonia, respiratory disease, enteritis or gastroenteritis. For one rabbit of the mid-dose group and the other 7 rabbits of the high dose group, the cause of death could not be determined.
The mortality rates in the intermediate- and especially in the high-dose groups were greater than 10 %, which exceeds the OECD guideline 414 (2001) suggestion of no more than approximately 10 % maternal mortality.
Clinical Observations
Clinical signs consisting of soft stool and diarrhea were noted in all dose groups during the treatment period. In the 175 mg/kg bw/day dose group, the incidence of this finding was slightly increased when compared with the control group. At 350 mg/kg bw/day, either soft stool, diarrhea or both were observed in each animal at least once during the treatment period. Also in the high dose group, there was an increased incidence of animals with nasal discharge in comparison with the control group.
Body weight
There were no treatment-related effects on maternal body weights and body weight gain.
Pathology Necropsy
There were no macroscopic findings in dams that were considered to be related to the administration of glyphosate technical.
Observations on the ovary and uterus
No treatment-related effects were evident in the study.

Table B.6.6-38: Maternal observations

Historical control	Control	Low	Intermediate	High
	(0 mg/kg/day)	(75 mg/kg/day)	(175 mg/kg/day)	(350 mg/kg/day)
Surviving dams at caesarean section*	27/28	14/16	15/16	13/16	6/17
Pregnant rabbits	24/28	12/16	15/16	11/16	6/17
Non-pregnant rabbits	3/28	2/16	0/16	2/16	0/17
Abortions	1/28	2/16	0/16	1/16	1/16

* number of surviving animals / total animals in group

Developmental parameters

There were no statistically significant differences in the mean numbers of early or late resorptions, total implantations, corpora lutea, foetal body weights or foetal sex ratio in any of the test substance groups when compared to control. The number of viable foetuses was slightly, but statistically significantly, increased in the low-dose group at 75 mg/kg bw/day. However, this finding was considered incidental and not related to the test substance.

The mean foetal body weights were slightly decreased in the test substance groups as compared to control. However, the mean foetal body weights in all test substance groups were comparable to the historical control data (i.e. 30.9 g) (see table below).

Table B.6.6-39: Mean litter data at caesarean section

Historical control
Control
Low
Intermediate
High
(0 mg/kg/day)
(75 mg/kg/day)
(175
mg/kg/day)
(350
mg/kg/day)
Pregnant dams#
24
12
15
11
6
Viable foetuses/dam
6.7
5.3 ± 2.73
7.6* ± 1.84
5.9 ± 2.77
6.3 ± 2.25
Post implantation loss/dam##
0.8
0.7 ± 0.89
0.4 ± 0.63
0.2 ± 0.40
0.8 ± 1.33
Total implantations /dam##
7.5
5.9 ± 2.39
8.0 ± 1.81
6.1 ± 2.84
7.2 ± 2.93
Corpora lutea/dam##
10.1
9.0 ± 2.13
10.1 ± 1.64
10.5 ± 3.45
8.5 ± 1.87
Foetal sex distribution
(males/females) #
83/77
28/35
53/61

32/33
17/21
Mean foetal body weight (g) ##
30.9
33.4 ± 7.27
30.9 ± 4.43
29.9 ± 7.21
29.3 ± 4.82
Total number
Number ± SD; historical control without SD
* Statistically significant difference compared to control ($p < 0.05$)
It should be noted that, in all dose groups, the number of pregnant dams were less than the number of pregnant dams required by the current OECD guideline 414 (2001); i.e., 16. Therefore, the evaluation of the developmental parameters may be limited.
Skeletal and visceral examination
The percentages of foetuses with skeletal malformations were 0.0, 2.6, 3.1 and 5.3 in the control, 75, 175 and 350 mg/kg bw/day groups, respectively. Although malformations were observed in the test substance groups, neither the type nor incidence of the malformations provided evidence for an adverse effect of glyphosate acid. There were no visceral malformations observed in any of the dose groups including control. There were no statistically significance differences in the variation observed in the test substance group when compared to the control group (see Table B.6.6-40).
Table B.6.6-40: Summary of foetal malformations and variations
Hist. contr.
Control
Low
Intermediate
High
(0 mg/kg/day)
(75 mg/kg/day)
(175 mg/kg/day)
(350 mg/kg/day)
Number of litters examined
12
15
11
6
%
x/y
%
x/y
%
x/y
%
x/y
%
Skeletal malformations

0/63
0.0
3/114
2.6
2/65
3.1
2/38
5.3
Exencephaly
--
0/63
0.0
0/114
0.0
1/65
(1/11)
1.5
0/38
0.0
Acrania
--
0/63
0.0
0/114
0.0
0/65
0.0
1/38
(1/6)
2.6
Scoliosis with associated rib
anomalies
0.6
0/63
0.0
2/114
(2/15)
1.8
0/65
0.0
0/38
0.0
T1 rib absent
--
0/63
0.0

0/114
0.0
1/65
(1/11)
1.5
0/38
0.0
Carpal flexure
0.6
0/63
0.0
0/114
0.0
0/65
0.0
1/38
(1/6)
2.6
Fused cervical vertebral centra
0.6
0/63
0.0
1/114 (1/15)
0.9
0/65
0.0
0/38
0.0
Visceral malformation
--
0/63
0.0
0/114
0.0
0/65
0.0
0/38
0.0
Total malformations
0/63
0.0
3/114
2.6
2/65
3.1
2/38

5.3
Variations
27 presceral vertebrae
8.7
6/63
(5/12)
9.5
7/114
(3/15)
6.1
9/65
(4/11)
13.8
7/38
(5/6)
18.4
13th rudimentary rib(s)
3.7
5/63
(3/12)
7.9
14/114
(6/15)
12.3
3/65
(3/11)
4.6
3/38
(3/6)
7.9
13th full rib(s)
8.1
3/63
(3/12)
4.8
10/114
(4/15)
8.8
5/65
(2/11)
7.7
6/38
(3/6)
15.8
Hyoid arches bent
--

--
--
2/114
(1/15)
1.8
1/65
(1/11)
1.5
--
--
Hyoid body unossified
--
6/63 (2/12)
9.5
2/114 (2/15)
1.8
6/65 (3/11)
9.2
--
--
Parietals reduced in ossification
0.6
1/63
(1/12)
1.6
--
--
1/65
(1/11)
1.5
--
--
Sternebrae #5 and/or #6
unossified
5.6
6/63
(3/12)
9.5
13/114
(7/15)
11.4
13/65
(5/11)
20.0
4/38
(2/6)

10.5
Pubis unossified
--
4/63
(1/12)
6.3
1/114
(1/15)
0.9
4/65
(1/11)
6.2
--
--
Talus unossified
--
3/63
(1/12)
4.8
--
--
5/65
(3/11)
7.7
--
--
Extra ossification center, cervical
area
--
--
--
--
--
1/65
(1/11)
1.5
--
--
Major vessel variations
8.7
11/63
(6/12)
17.5
14/114
(8/15)
12.3

14/65
(5/11)
21.5
6/38
(4/6)
15.8
x/y: number of foetuses affected / total number of foetuses examined (a/b): number of litters affected / total number of litters
Conclusion by the Notifiers
The oral administration of glyphosate acid to pregnant rabbits by gavage from Gestation Day 6-27 resulted maternal toxicity at ≥175 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore the NOAEL was considered to be 75 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 350 mg/kg bw/day.
Comment by RMS (Re-evaluation):
The study is considered supplementary, because the highest dose level revealed only 6 does with litters for examination due to the high maternal mortality. The NOAEL of 75 mg/kg bw/d for maternal toxicity is agreed. However, the previously established NOAEL for developmental toxicity of 350 mg/kg bw/day was lowered to 175 mg/kg bw/day because of the low number of available foetuses in the high dose group avoiding meaningful evaluation.
Comment by GTF on the first draft of the RAR (July 2013):
(1980, TOX9552390) is considered a supplementary study based on high mater- nal mortality and therefore an insufficient number of litters to thoroughly evaluate potential developmental effects at 350 mg/kg/day. However, the study data are still robust and appli- cable for establishing valid NOAEL values from the mid and low doses of 175 and 75 mg/kg/day, respectively.
Further explanation:
While a dose-response curve cannot be confirmed for adverse developmental endpoints, due to high maternal morality and thus insufficient number of litters at the high dose, a dose- response is evident for maternal mortality and toxicity. Therefore this study may be considered robust for establishing the maternal toxicity NOAEL of 75 mg/kg/day. Similarly, the mid and low doses clearly demonstrated no adverse effects to offspring, and thus the offspring NOAEL of 175 mg/kg/day in this study is unequivocal, especially as this value was also the developmental NOAEL in the Moxon study.
RMS comment (August 2013):
What is the idea behind this comment? It is only a summary of the conclusion/comment given by the RMS in the Draft RAR.
Reference:
IIA, 5.6.11/05
Report:
(1991)
The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations)
Data owner: Cheminova
Study/Project No.: CHV 45 & 39 & 40/901303 Date: 1991-10-14
not published TOX9552391
Guidelines:
OECD 414, US EPA 83-3

Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment.
Dates of experimental work: 1989-12-14 to 1990-03-02
Materials and methods
Test material:
Identification:
Glyphosate acid
Description:
White solid
Lot/Batch #:
206-JAK-25-1
Purity:
98.6 %
Stability of test compound:
Stable over the duration of the study
Vehicle and/
or positive control:
1 % methylcellulose
Test animals:
Species:
Rabbit
Strain:
New Zealand White
Source:
Age:
11-24 weeks (on delivery)
Sex:
Female
Weight at dosing:
Females: 3582 – 3709 g (mean values)
Acclimation period:
7 days
Diet/Food:
SQC Standard rabbit diet (Special Diet Services Ltd., Essex, UK), ad libitum
Water:
Mains drinking water, ad libitum
Housing:
Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage
Environmental conditions:
Temperature: 19 ± 1 °C
Humidity: 49 ± 15 % Air changes: Not recorded

Natural lighting supplemented with artificial lighting from 07 – 21:00 hours
Study design and methods:
In life dates: 14 -12-1989 – 02-03-1990
Animal assignment and treatment:
In a developmental toxicity study, groups of 16 - 20 time-mated female New Zealand White rabbits were administered glyphosate in 1% methylcellulose (dose volume 5 mL/kg) once daily by gavage at dose levels of 0 (vehicle control), 50, 150 or 450 mg/kg bw/day from Day 7 to Day 19 of pregnancy. Dose volumes were calculated for individual animals on day 7 and adjusted according to body weight on Days 9, 11 and 15. The day of mating was considered as Day 0. Dose levels were based on the findings of a preliminary study.
Dosing formulations were prepared daily and administered within 3 hours of preparation.
Observations
All animals were regularly handled and observed daily for overt changes or signs of reaction to treatment. Animals that died or were killed for animal welfare reasons were weighed and subjected to post-mortem examination.
Body weight
Individual body weights were recorded Days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation.
Food consumption and compound intake
Food consumption was recorded on days of weighing throughout gestation.
Sacrifice and pathology
On day 29 of pregnancy all surviving does were subjected to post-mortem examinations for congenital abnormalities and gross pathological changes in maternal organs.
The ovaries and uteri were examined to determine the number of corpora lutea, the number and distribution of live young, the number and distribution of embryonic and /foetal deaths, individual foetal weight and foetal abnormalities. Embryonic/foetal deaths were classified as Early, Late or Abortions.
Litter parameters
Live young were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were killed by intrathoracic injection of pentobarbitone sodium then weighed and dissected for examination of visceral abnormalities. Where appropriate, suspected abnormalities were further examined by alternative procedures such as microdissection and histopathology to clarify initial observations. Pups were fixed in industrial methylated spirit, the heads sliced along the line of the frontoparietal suture and the brain examined for abnormalities before clearing and staining by the modified Dawson technique of the carcasses for skeletal examination. Structural changes were presented as malformations, anomalies or variants.
Statistics
Two-tailed tests for significance were performed on litter data only, and significance at 1 % and 5 % were reported. Mean values of litter size, pre and post-implantation loss, litter weight, mean foetal weight and the incidence of anomalous offspring were analysed by the Kruskal-Wallis test. Intergroup comparisons were made by the non-parametric equivalent of the Williams' test following a significant h-statistic. Where 75 % of the values for a given variable consisted of one value, a Fisher's exact test was used.
Results and discussion
Analysis of dose formulations
The analysis of the dosing formulations taken at the first dosing showed the mid- and high- dose group to be within 6 % of the nominal dose whilst the low-dose group was 19 % below the nominal

dose; however, a renalysis on Day 19 showed the concentration to be 5 % above the nominal dose.
Food consumption
During the dosing period, females receiving 150 and 450 mg/kg bw/day showed reduced food consumption compared to the controls. A slight reduction was evident from Days 11 – 19 at 150 mg/kg bw/day (approximately 12 % compared with controls) and throughout the treatment period for the 450 mg/kg bw/day dose group (6-17 % during Days 7-19) (see Table B.6.6-41).
Table B.6.6-41: Summary of mean food consumption (g/rabbit/day)
Dose Group (mg/kg bw/day)
0 (control)
50
150
450
Mated females
19
19
16
20
No. of animals included in
assessment
18
12
15
13
Food consumption
(g/rabbit/day) during
Days 1-6
142
143
141
152
Days 7-8
143
154
150
135
Days 9-10
146
148
148
132
Days 11-14
153
149
134
129
Days 15-19

148
151
131
123
Days 20-23
142
154
149
149
Days 24-28
131
143
153
166
Mortality
There was one death in the 450 mg/kg bw/day dose group on Day 20 following signs of abortion on Day 19 and signs of gastrointestinal disturbance, manifested as soft/liquid faeces, severe reduction in food consumption and bodyweight loss from the onset of treatment. Two other deaths (a broken hind leg and an incidence of congenital abnormality) were unrelated to the treatment and were eliminated from the study assessment.
Clinical Observations
Clinical signs included a dose-related increase in the number of females showing soft/liquid faeces (gastrointestinal disturbances) and signs of lack of appetite (off feed/reduction in food consumption) at 150 and 450 mg/kg bw/day (see Table B.6.6-41).
Table B.6.6-42: Summary of relevant clinical signs in does
Parameter
Dose Group (mg/kg bw/day)
0 (control)
50
150
450
Mated females
19
19
16
20
Not pregnant
0
6
1
5
Number of does with live
young or litters at Day 29
18
12
15

13
Clinical signs#
Off-feed
8
6
10
9
Reduced faecal output
9
8
11
12
Soft/liquid faeces
0
2
5
13
Only animals with live young included
Body weight
A slight reduction in body weight gain was noted from Day 11 of pregnancy to termination of treatment in the 150 and 450 mg/kg bw/day dose groups, which coincided with the reduction in food consumption during the same period (see Table B.6.6-42).
Table B.6.6-43: Summary of body weight data (group means)
Parameter
Dose Group (mg/kg bw/day)
0 (control)
50
150
450
Mated females
19
19
16
20
No of animals included in
assessment
18
12
15
13
Bodyweights (g) at
Day 1
3538
3524
3568
3658

Day 7
3582
3604
3624
3709
Day 9
3589
3639
3637
3732
Day 11
3601
3653
3661
3743
Day 15
3742
3804
3779
3833
Day 20
3770
3831
3775
3835
Day 24
3844
3927
3849
3965
Day 29
3999
4084
3975
4103

Pathology Necropsy

Gross examination of does at post-mortem did not identify any treatment-related effects.

Observations on the ovary and uterus

A total of 18, 12, 15 and 13 pregnant females survived to termination and 163, 104, 112 and 95 foetuses were recorded for the 0 (control), 50, 150 and 450 mg/kg bw/day dose groups respectively. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss was recorded for one female of the 450 mg/kg bw/day dose group which aborted on Day 19 and died and also for one female at 50 mg/kg bw/day. One female at 150 mg/kg bw/day aborted 1/9 foetuses.

There were no significant intergroup differences in the numbers of corpora lutea, implantations, pre-implantation loss, foetal sex ratios or foetal weights (see Table B.6.6-44). There was a statistically

significant increase in embryo/fetal death and post-implantation loss at all exposure levels. The study investigators questioned the biological significance of these findings for several reasons: 1) No dose-response pattern was evident, 2) the control value was at the lower end of the historical control range, while those of the exposed groups were at the higher end, and 3) the values in all groups were within or slightly above the historical control range. The latter two statements are supported by the historical control data provided in the study report (page 32) (see Table B.6.6-44). Although embryo/foetal death was within the historical control range, post-implantation loss was above the historical control values in the high-dose group, and both of these parameters were statistically significant ($p<0.01$) at the high dose.

Table B.6.6-44: Summary of the maternal and litter parameters (group mean values)

Parameter
Dose Group (mg/kg bw/day)
Historical control range
(mean value)
0(control)

50
150
450
No. of mated females
19
19
16
20
--
No. not pregnant
0
6
1
5
--
No. of does with live young or
litters at Day 29
18
12
15
13
--
Corpora lutea
11.5
12.4
11.7
11.3
9.0 – 12.9 (11.2)
Implantations
9.7

10.5
9.0
9.2
7.0 – 11.1 (9.5)
Pre-implantation loss
14.6
15.4
23.4
18.8
2.3 – 26.1 (15.1)
Early embryonic deaths
0.4
0.9
0.9
0.5
0.3 – 1.1 (0.6)
Late embryonic deaths
0.2
0.9
0.5
1.3**
0.1 – 1.3 (0.7)
Abortions
0.0
0.0
0.1
0.0#
0.0 – 0.1 (0)
Total embryonic deaths
0.6
1.8*
1.5*
1.8**
0.6 – 2.0 (1.2)
Post-implantation loss (%)
5.7
19.5*
15.3*
21.0**
6.5 – 17.5 (12.9)
Live young
9.1
8.7
7.5
7.3
6.1 – 9.5 (8.3)

Litter weight (g)
389.5
370.6
320.5
315.0
281.9 – 402.2
(352.9)
Mean foetal weight (g)
43.9
43.3
44.0
44.5
41.4 – 47.6 (44.1)
Sex (% males)
55.3
55.8
57.6
53.8
--
* Statistically significant by Kruskal –Wallis 'H' test P < 0.05
** Statistically significant by Kruskal –Wallis 'H' test P < 0.01
# Fisher exact test follow-up by intergroup comparison with control was not statistically significant P > 0.05
Developmental parameters Number and viability of foetuses
There were 18, 12, 15 and 13 viable litters at 0, 50, 150 and 450 mg/kg bw/day, respectively. The concurrent control showed low mean values for embryonic deaths and post implantation losses when compared with historical control values. When compared with these historical data as noted above, mean values in the treated groups were within the expected range; therefore, it was concluded that no adverse effect on foetal survival was attributed to glyphosate.
Foetal body weights
There was a dose-related reduction in mean foetal weight on a litter basis in all treated groups (not statistically significant) compared with the control; however, the mean individual foetal weight was not affected.
External, visceral and skeletal examination
Malformations were slightly increased in the 150 and 450 mg/kg bw/day dose groups compared to controls and appeared to be associated with an apparent increase in malformations of the thoracic region. However, neither the incidence nor the percentage of malformed foetuses was outside the historical control range and the values were not statistically different from concurrent control values. Several of the cardiovascular malformations that were observed, particularly in the high-dose group, occurred in the same animals and are related to a single morphogenetic mechanism (i.e., displacement of the developing aorticopulmonary septum), which is likely to adjust during the first two weeks of postnatal life. These related findings, which often cluster together, included dilated/narrow aorta and narrow/dilated pulmonary artery; interventricular septal defect; and disproportionately sized right and left ventricles. These findings were observed (often in clusters) in the historical control data that were provided by the conducting laboratory.
Individual presentation of these malformations in tables when the malformations occurred together

in the same foetus and are due to the same mechanisms and artificially inflates the sense that there is a much stronger cardiac effect than is actually present.
The cardiac malformation observed with greatest frequency in this study was interventricular septal defect. The number of foetuses and litters with ventricular septal defects were 1, 1, 1 and 4 in the 0, 50, 150 and 450 mg/kg bw/day dose groups, respectively. Comparison of the historical control data shows that the heart findings (when presented on a percent individual and/or litter incidence basis) were slightly outside of the historical background range from 13 studies conducted during the same period. However, the disparity in values is a consequence of the small numbers of litters in the study report. If the data are displayed as a fraction (rather than a percentage), then the number of litters affected were 1/18, 1/12, 1/15, and 4/13 in the 0, 50, 150, and 450 mg/kg/day dose groups, respectively. The historical control range is 0/19 – 3/13. Thus, the findings at the high dose are barely outside of the historical control range. Further, they were observed in conjunction with clear signs of maternal toxicity (reduced food consumption, body weight gains and increased clinical signs).
The other cardiovascular finding found in this study not related to the morphogenetic mechanism involving formation of the spiral septum is retroesophageal right subclavian artery. This finding was also observed regularly throughout the historical period. It is not uncommon and is oftentimes an inconsequential anatomical difference in vascular arrangement. At autopsy this condition is found in 0.5 – 2.0 % of subjects.
The malformations of the cranial region, the lumbar and the lumbar/sacral regions did not show any treatment-related trend and are considered to be incidental. The incidences of anomalies and variants did not suggest any treatment relationship. The incidence of foetuses with reduced ossification did not show any dose-relationship; however, lower foetal weights were observed for the 450 mg/kg bw/day dose group with reduced ossification.
The observed foetal malformations and anomalies are summarised in the following (Table B.6.6-45).
Table B.6.6-45: Summary of foetal parameters
Parameter
Dose Group (mg/kg bw/day)
Historical control range or x/y /
(mean)
0(control)
50
150
450
Number of does with live young or litters at
Day 29
18
12
15
13
--
Mean foetal weight (g)
43.9
43.3
44.0
44.5
41.4 – 47.6

Sex (% males)
55.3
55.8
57.6
53.8
--
Malformations
--
Total number of foetuses examined
163
104
112
95
1511
No. of malformed foetuses
3
3
5
6
51
%
1.9
5.8
4.3
5.9 (F)
0.7 – 5.9 (3.8)
Number of Affected Litters
3
3
3
5
43/188
%
16.67
25
20
38.5
22.9
Thoracic region malformations
--
No. of foetuses with interventricular septal
defect
1
1
1
4

10/1511
%
0.6
1.0
0.9
4.2
0.66
Litter incidence
1
1
1
4
10/188
%
5.56
8.3
6.67
30.8
5.32
Foetuses with enlarged left, reduced right ventricles
0
0
0
2
2/1511
%
0.0
0.0
0.0
2.1
0.13
Litter incidence
0
0
0
2
2/188
%
0
0
0
15.4
1.10
Foetuses with retro-oesophageal right subclavian artery

0
0
3
2
7/1511
%
0.0
0.0
2.7
2.1
0.46
Litter incidence
0
0
1
1
7/188
%
0
0
6.6
7.6
3.72
Foetuses with narrow/dilated aortic
arch/pulmonary trunk/arterial trunk
1
1
1
3
8/1511
%
0.6
1.0
0.9
3.2
0.52
Litter incidence
1
1
1
3
8/188
%
5.56
8.3
6.67

23.1
4.25
Anomalies
--
Total number of foetuses examined#
160
101
107
89
--
No. of foetuses with gross/visceral anomalies
9
14
14
6
--
%
6.4
19.5
12.9
9.6 (K)
--
No. of foetuses with skeletal anomalies
21
13
14
11
--
%
11.7
17.7
12.5
10.1
(K)
--
No. of foetuses with reduced ossification
7
4
5
4
--
%
4.4
4.0
4.7
4.5

--
Mean foetal weight of foetuses with reduced
ossification (g)
37.9
43.6
37.7
26.1
--
* number affected / total number examined
# Malformed foetuses are excluded
(F) Fisher's exact test applied, not statistically significant (P > 0.05)
(K) Kruskal-Wallis 'H' statistic, not significant (P > 0.05)
-- no data
Conclusion by the Notifiers
Glyphosate was not teratogenic in this developmental toxicity study in rabbits. The NOAEL for maternal toxicity was 50 mg/kg bw/day based on clinical signs of toxicity including reduced feed consumption and body weight gain and soft/liquid faeces during the dosing
period. The NOAEL for foetotoxicity was 150 mg/kg bw/day based on statistically significantly increased embryo/foetal deaths and post-implantation loss. The NOAEL for teratogenicity was 450 mg/kg bw/day.
Comment by RMS (Re-evaluation):
The study is considered acceptable. In this study there was a significant increase in embryonic death and post-implantation loss in treated groups compared to controls, however without a clear dose-relationship. Regarding the post-implantation losss, values for the low and high dose groups are outside the historical control range.
The cardiac malformation observed with greatest frequency in this study was the interventricular septal defect. At 450 mg/kg bw/d this effect was outside the historical control range (4.2 % compared to 0.66 % in historical controls). Taken into account the high post- implantation loss at the same dose level, the incidence of additionally cardiac malformation may be covered, therefore. At mid dose level foetuses with an higher incidende of retro- oesophageal right subclavian artery were reported. However, this effect has to be considered equivocal, because no clear-dose relationship could be established.
In conclusion, the NOAEL for maternal toxicity is considered 50 mg/kg bw/d based on slightly restricted inappetence, slightly reduced body weight gain and soft/liquid faeces at 150 mg/kg bw/d. The NOAEL for developmental toxicity is considered to be 150 mg/kg bw/d based on the post-implantation loss, late embryonic death and an increase in cardiac malformations at 450 mg/kg bw/d.
Reference: IIA, 5.6.11/06
Report: (1993)
Teratogenicity study in rabbits – Test compound: Glyphosate technical (FSG 03090 H/05 March 1990)
Data owner: ADAMA Agan Ltd
Study No.: TOXI: 884-TER-RB
Date: 1993-04-17, amended 1994-06-18 not published
TOX9551106
Guidelines: OECD 414 (1981)
Deviations: None

GLP:
Acceptability: See RMS comment
Dates of experimental work: 1991-12-24 to 1992-03-06
Materials and methods
Test material:
Identification:
Glyphosate acid
Description:
Odourless white crystals
Lot/Batch #:
60
Purity:
96.8 %
Stability of test compound:
Stable over 2 years at ambient temperature.
Vehicle and/
or positive control:
0.5 % w/v carboxymethylcellulose
Test animals:
Species:
Rabbit
Strain:
Female New Zealand White
Source:
Age:
Approximately 6 months and above (at the start of study)
Sex:
Males and females
Weight at dosing:
Females: >2500 g (mean values)
Acclimation period:
At least 10 days.
Diet/Food:
Pelleted rabbit diet, supplied by M/S Lipton India Ltd, Bangalore (composition and feed analysis reports were provided) was provided ad libitum
Water:
Protected deep bore well drinking water, treated via
activated charcoal filter and UV in Aquaguard on-line water filter-cum-purifier provided ad libitum
Housing:
Individually in 3-tier all aluminium cages with wire mesh
bottom and common self-draining litter trays.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 40 – 70 % Air changes: 10 – 15/h
Natural lighting supplemented with fluorescent lighting 12 hours light/dark cycle
Study design and methods:

In life dates: not reported
Animal assignment and treatment:
In a developmental toxicity study, groups of presumed mated female New Zealand White rabbits were administered once daily by gavage, glyphosate (batch no.: 60, purity 96.8 %) in 0.5 % carboxymethylcellulose (dose volume 2 ml/kg) at dose levels of 0 (vehicle control), 20, 100 or 500 mg/kg bw/day from Day 6 to Day 18 of pregnancy. Dosages for individual animals were calculated from day 6 and adjusted daily according to body weight. Dose levels were based on the findings of preliminary studies.
In a preliminary dose-range finding study, one male rabbit/dose group was administered by gavage glyphosate technical dissolved in 0.5 % carboxymethylcellulose (dose volume 2 mL/kg bw) at dose levels of 0 (control), 10, 20, 50, 500 or 1000 mg/kg bw/day for 13 days. Doses of ≥ 500 mg/kg bw/day resulted in loss in body weight and in feed intake and the 1000 mg/kg bw/day test animal died on Day 9 of treatment.
In a second dose-range finding study, one pregnant rabbit was administered 500 mg/kg bw/day glyphosate from day 6 to 18 of gestation and the findings compared with that of 20 historical control animals. Caesarean section and terminal necropsy was performed on day 28. There were no signs of toxicity from the treatment; body weight gain was greater (26 % more than the historical control mean) but notable apparent treatment-related changes were substantial reduction in feed intake (34 % of historical control mean) and reduced litter size in the test female (4) compared with the historical control mean (7).
Observations
All animals were observed twice daily for onset and duration of signs of toxicity and for mortality. All animals in the experiment that died, were killed moribund or killed at termination were subjected to post-mortem gross pathological examination. Tissues with gross lesions were preserved for histopathological examination as necessary.
Body weight
Individual body weights were recorded on Days 0, daily from Days 6 – 18 and on Day 28 of gestation.
Food consumption and compound intake
Food consumption was recorded on days of weighing throughout gestation.
Sacrifice and pathology
On day 28 of pregnancy, all surviving dams were subjected to post-mortem examinations and pups were delivered by Caesarean section. The ovaries and uteri were excised and weighed and maternal and foetal data were recorded. The maternal data determined were pregnant/non- pregnant, uterine weight, the number of corpora lutea, the number of implantations, the number of embryonic and foetal resorptions. The foetal data recorded were the number of dead/abnormal/live foetuses, individual foetal weight and sex.
Litter parameters
All the foetuses were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were euthanised with ether and visceral organs examined by a modified Wilson technique. Skeletal assessments were performed after appropriate preparation including staining in Alizarin Red. Structural changes were presented as variants, minor and major malformations.
Statistics
Statistical methods employed included the following. Maternal body weight and weight gain, feed intake, number of corpora lutea, number of implantations and mean foetal weight were analyzed by Bartlett‘s test followed by ANOVA and Dunnett’s test. Day ‘0’and absolute body weight data were

compared by the Paired Student's ´t` test. The number and percent embryonic resorptions and foetal resorptions, the number of dead foetuses, the number of abnormal foetuses and percentage pre-implantation and post-implantation loss by Mann Whitney test. Litter size was by Student 't' test. The sex ratio, number of dams with any resorptions, number of dams with all resorptions and incidence of malformations were analysed by Chi-square test.
The statistical analysis and comparison of individual treatment groups with control value were done at 5 % probability level and the results were designated as significantly higher (+) / lower (-) than control value at $P \leq 0.05$.
Results and discussion Analysis of dose formulations Not reported.
Food consumption
During the dosing period, feed consumption was significantly reduced (31 %) in females receiving the 500 mg/kg bw/day dose compared to the controls (see Table B.6.6-46). Feed consumption during the post-treatment period did not show significant intergroup differences.
Table B.6.6-46: Summary of food consumption
Parameter
Historical
positive control#
Dose Group (mg/kg bw/day)
0 (control)
20
100
500
Food consumption (g/rabbit/day)
No of dams included in assessment
7
20
13
12
6
Day 0 – 6
(Pre-treatment)
105
114
88*
125
118
Day 6 – 19
(Treatment)
70*
103
109
102
71*
Day 19 – 28
(Post treatment)
129

109
135
107
105
Day 0 - 28
96
107
113
108
92
Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw (treatment: Day 6-18; post-treatment: Day 18-28)
* Significantly lower than controls by Dunnett's test $P \leq 0.05$
Mortality
The four and eight deaths observed in the mid- and high-dose group were considered to be treatment-related by the study director (see Table B.6.6-47 below). However, the two confirmed misdosings in the control, the absence of signs of toxicity at 100 mg/kg bw and the absence of mortality in this dose range in the considerably high number of parallel studies shed serious doubt on a relation to treatment at this dose level. Further, various findings at gross necropsy were noted in the lungs and trachea for the 100 and 500 mg/kg/day dose groups; these findings suggest possible gavage errors, which could be responsible for some of the deaths observed at these doses and and are not appropriately discussed in the report.
Table B.6.6-47: Summary of mortality in dams
Parameter
Dose Group (mg/kg bw/day)
0 (control)
20
100
500
Mated females
26
17
16
15
Dead during treatment
1*
0
4
5
Died post-treatment
1*
0
0
3
Total number of deaths
2

0
4
8
% mortality
7.7
0.0
25.0
53.3
* Animal died due to wrong gavaging
Clinical observations
Signs of toxicity were observed at the 500 mg/kg bw/day dose group and were predominantly gastrointestinal effects, which included soft stool/liquid faeces and soft stool with mucus. Further signs of toxicity were rales, weakness, dyspnoea and ocular discharge.
Table B.6.6-48: Summary of relevant clinical signs in dams
Parameter / clinical sign
Dose Group (mg/kg bw/day)
0 (control)
20
100
500
Mated females
26
17
16
15
Pregnant at termination
20
13
12
6
Rales
1
0
0
3
Soft stool with mucus
0
0
0
2
Soft stool/liquid faeces
0
0
1
12

0
0
0
2
Ocular discharge
0
0
0
1
Dyspnoea
0
0
0
1
Body weight
No treatment-related and dose-related significant changes were observed in maternal body weight and body weight gain between the control, low- and mid-dose groups. In the high-dose group, initial body weight and body weights at the different time intervals were significantly lower than in the control group.
Table B.6.6-49: Summary of maternal body weight data
Parameter
Historical positive
control#
Dose Group (mg/kg bw/day)
0 (control)
20
100
500
Number of dams pregnant at
termination
20
13
12
6
Mean body weights (kg)
Day 0
3.1
2.8
3.0
2.6*
Day 6
3.2
3.0
3.0
2.8*
Day 18

3.2
3.1
3.1
2.8
Day 28
3.3
3.3
3.3
3.0*
Day 28 (body weight – uterine
weight)
2.7*
3.0
3.0
2.9
2.7
Mean body weight gain (kg)
Day 0 – 6
(Pre-treatment)
0.2
0.1
0.2
0.0
0.1
Day 6 – 18
(Treatment)
-0.1
0.1
0.1
0.1
0.0
Day 18 – 28
(Post treatment)
0.2
0.1
0.2
0.2
0.2
Day 0 – 28
gestation)
(Throughout
0.3
0.2
0.5**
0.3
0.3

Treatment with acetylsalicylic acid (ASA) at 200 mg/kg bw/day
* Significantly lower than controls by Dunnett's test P ≤ 0.05
** Significantly higher than controls by Dunnett's test P ≤ 0.05

Pathology Necropsy

Gross examination of dams at post-mortem did not identify any treatment-related effects. However, various findings were noted in the lungs and trachea for the 100 and 500 mg/kg/day dose groups which suggest possible gavage errors and issues with animal husbandry.

Observations on the ovary and uterus

A total of 20, 13, 12 and 6 pregnant females survived to termination and 134, 80, 78 and 28 foetuses were recorded for the 0 (control), 20, 100 and 500 mg/kg bw/day dose groups, respectively, and were included in the assessment. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss (complete resorptions) was recorded for one female in the 500 mg/kg bw/day dose group; otherwise, the incidence of dams with any resorptions did not show any treatment-related differences.

There were no significant intergroup differences in the mean numbers of corpora lutea, pre-implantation and post-implantation losses and resorptions (embryonic and foetal) (see Table B.6.6-50).

Table B.6.6-50: Summary of maternal observations

Parameter
Historical positive control#
Dose group (mg/kg bw/day)
0 (control)
20
100
500
Mated females
12
26
17
16
15
Total number of deaths
4
2
0
4
8
Pregnant at termination
7
20
13
12
6
Mean number of corpora lutea
9

11
10
10
9
Mean number of implantations
8
8
8
9
6
Total number of embryonic resorptions
(%)
6 (11)
10 (7)
11 (11)
11 (11)
9 (24)
Total number of foetal resorptions (%)
2 (4)
8 (5)
7 (7)
13 (13)
1(3)
Total number of pre-implantation loss (%)
10 (19)
72 (48)
28 (29)
20 (20)
14 (37)
Total number of post-implantation loss
(%)
8 (15)
18 (12)
18 (18)
24 (24)
10 (26)
Number of dams with any resorptions (%)
2 (29)
12 (60)
11 (85)
9 (75)
2 (33)
Dams with complete resorptions (%)
1 (14)
0 (0)
0 (0)

0 (0)
1 (17)
Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw
Developmental parameters Number and viability of foetuses
Because of the large number of maternal deaths at 500 mg/kg/day (and thus, the reduced number of total litters), the total number of foetuses was substantially less in this dose group compared to the other dose groups. However, the mean litter size, the mean numbers of abnormal, dead or live foetuses and the sex ratios of foetuses did not show any significant treatment-related differences. Glyphosate also did not cause an increase in the number of foetal deaths in utero (see Table B.6.6-51).
Foetal body weights
Although foetal body weights in the 20 and 100 mg/kg/day dose groups were reported to be significantly different from control, the weights were increased, the changes were less than 10 % of control values and no dose-response across treatment groups was evident. Thus, the foetal body weight differences observed in these two dose groups are biologically inconsequential with respect to adverse effects (see Table B.6.6-51).
Table B.6.6-51: Mean litter data at caesarean section
Historical
positive control#
Dose group (mg/kg bw/day)
0
20
100
500
Mated females
12
26
17
16
15
Total number of deaths
4
2
0
4
8
Pregnant at termination
7
20
13
12
6
Number of litters
6
20
13

12
5
Total number of foetuses
46
134
80
78
28
Mean litter size
8
7
6
7
6
Abnormal foetuses (%)
0 (0)
1 (1)
2 (3)
0
0
Dead foetuses (%)
0 (0)
0 (0)
1 (1)
1 (1)
0 (0)
Post-implantation loss (%)
8 (15)
18 (12)
18 (18)
24 (24)
10 (26)
Number of live foetuses
46
133
77
77
28
Mean weight of live foetuses (g ±
SD)
29 ± 1.4
32 ± 5.3
35 ± 3.7*
35 ± 2.4*
33 ± 4.9
Sex ratio (Male : Female)

1 : 1.3
1 : 0.7
1 : 1.2
1 : 1.2
1 : 1.8
Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw SD = standard deviation
* Significantly higher than controls by Dunnett's test P ≤ 0.05
External, visceral and skeletal examination
The incidence of major external malformations did not identify any treatment-related differences; further, none of the external malformations occurred in the highest dose group.
Visceral examination noted no significant treatment-related incidences of minor malformations or variants. Major visceral malformations primarily affected the heart, but occurred in single incidences and showed no dose-response (see Table B.6.6-52). The exception was dilated heart, which was reported in four foetuses of 3 litters in the 20 mg/kg bw/day dose group, 4 foetuses (3 + 1) from 2 litters of the 100 mg/kg bw/day dose group and all foetuses (4) of one litter and one foetus of another litter at the 500 mg/kg bw/day (Statistically significant P≤ 0.05). The terminology used to describe the heart malformations in this study is different than that typically employed in teratology research (e.g., dilated heart, seal-shaped heart). Consequently, what is meant by the description "dilated heart" is not well defined and not documented with photographs or retained tissue sections or slides.How this malformation might relate to others reported in the heart (i.e., dilated left or right ventricle, seal-shaped heart, cardiomegaly) is not clear. Further, because too few foetuses were available for examination in the high dose group, it cannot be determined whether these defects exhibited a true dose-related increase. It is important to note, however, that only 2 litters exhibited major visceral malformations in the high dose group. Additionally, these findings were found in the presence of extensive maternal toxicity, evidenced by reduced food consumption and body weight gains in the few animals that survived this dose level, clinical signs, and substantial deaths.
Major, minor and skeletal malformations did not show any clear treatment-related findings and appeared to be incidental
Table B.6.6-52: Summary of relevant external, visceral and skeletal findings (litter data)
Foetal findings
HC
#
Dose level (mg/kg bw/day)
Data
0
20
100
500
No. of litters examined
6
20
13
12
5
No. of foetuses examined
46

133
79
77
28
Minor external malformations
Percentage of small foetuses (%)
--
0
0
1.3
0
Litter incidence (%)
--
0
0
8.3
0
Major external malformations
Percentage of foetuses with upper cleft palate (%)
0
0.8
2.5
0
0
Litter incidence (%)
0
5
15
0
0
Percentage of foetuses with forelimb arthrogryposis
--
0
0
1.3
0
Litter incidence (%)
--
0
0
8.3
0
Percentage of foetuses with multiple malformations
0
0.8
2.5

0
0
Litter incidence (%)
0
5
15
0
0
Percentage of foetuses with major malformations (%)
0
1.5
2.5
1.3
0
Litter incidence (%)
--
10
7.7
8.3
0
Major visceral malformations
Percentage of foetuses with dilated heart (%)
--
0
5.1
5.2
17.9
Litter incidence (%)
--
0
23.1
16.7
40.0
Percentage of foetuses with anencephaly (%)
0
0.8
0
0
0
Litter incidence (%)
0
5.0
0
0
0
Percentage of foetuses with heart–seal shaped (%)

0
0.8
0
0
0
Litter incidence (%)
0
5.0
0
0
0
Percentage of foetuses with cardiomegaly & sealed heart (%)
--
0
0
1.3
0
Litter incidence (%)
--
0
0
8.3
0
Percentage of foetuses with dilated ventricle (left) (%)
--
0
0
1.3
0
Litter incidence (%)
--
0
0
8.3
0
Percentage of foetuses with dilated ventricle (right) (%)
--
0
0
0
3.6
Litter incidence (%)
--
0
0
0

20
Percentage of foetuses with persistent truncus arteriousus (%)
--
0.8
0
0
0
Litter incidence (%)
--
5.0
0
0
0
Percentage of foetuses with gallbladder absent (%)
--
0
0
0
3.6
Litter incidence (%)
--
0
0
0
20
Percentage of foetuses with liver (median) haematoma (%)
--
0
0
0
3.6
Litter incidence (%)
--
0
0
0
20
Minor skeletal malformations
No. of foetuses with extra 13th rib
0
1
2
1
Percentage of foetuses with extra 13th rib
8.7**
0

1.3
2.6
3.6*
Litter incidence (%)
--
0
7.7
16.7
20
Major skeletal malformations
Percentage of foetuses major malformations (%)
10.9
8.3
6.3
0*
3.6
Litter incidence (%)
50
20
23.1
0
20
Historical positive control data (--: no data available)
* Significantly different from control at $p < 0.05$.
** Significantly different from control by Contingency test ($P \leq 0.05$)
Conclusion by the Notifiers
Glyphosate technical was not considered to be teratogenic in this developmental toxicity study in rabbits. The incidence of one visceral effect, dilated heart, was increased at the highest test dose and was present at lower dose levels, but there were too few foetuses present in the high dose group to corroborate a dose-response relationship. Further, foetal findings at the highest test dose were observed in the presence of extensive maternal toxicity that exceeded guideline recommendations for a high dose. The NOAEL for maternal toxicity was
100 mg/kg bw/day based on mortalities at dose levels of ≥ 100 mg/kg bw/day. Mortality and clinical signs of toxicity including reduced feed consumption and soft faeces and reduced bodyweight gain during the dosing period one incidence of complete resorptions at the 500 mg/kg bw/day dose level. The NOAEL for foetotoxicity and teratogenicity was 100 mg/kg bw/day based on occurrence of general signs of secondary toxicity (incomplete ossification and similar) at the high dose.
Comment by RMS (Re-evaluation):
The study is considered supplementary due to several weaknesses including a small number of litters for examination (low pregnancy rate at all dose levels, lethality in mid and high dose dams) and reporting deficiencies. The percentage of foetuses with ‘dilated heart’ was significantly increased at all dose levels. However, the absolute number of affected foetuses and litters is quite small and did not show a marked difference between the treated groups. Furthermore, the diagnosis ‘dilated heart’ was not defined in this study report and neither criteria used to this diagnosis nor measurements of the heart were provided.
The NOAEL for maternal toxicity is still considered 20 mg/kg bw/d, because it cannot be excluded,

that the intercurrent four deaths at 100 mg/kg bw/d were substance-related.
Therefore, the previous evaluation in 2001 is confirmed: the maternal NOAEL is considered 20 mg/kg bw/d and the developmental NOAEL 100 mg/kg bw/d.
Comment by GTF on the first draft of the RAR (July 2013):
The GTF seriously questions whether the findings reported in the Suresh study were directly related to treatment. The GTF respectfully suggests that the additional safety margin for risk assessment (disregarding the 75 mg/kg/day NOAEL) as proposed by the RMS, is not neces- sary.
Further explanation:
Whilst an increase in mortality was observed in the Suresh study, it is uncertain if the mortalities observed were directly related to treatment, given that clinical signs of rales and dyspnoea were observed and that various findings at gross necropsy were noted in the lungs and trachea for animals in the 100 and 500 mg/kg/day dose groups. These findings suggest possible gavage errors, may well be responsible for some of the deaths observed at these doses. These findings, along with the confirmed gavage traumas in the control group call into question the technical competency of the dosing procedure in the conducting laboratory. This would likely have caused additional stress to the animals during the dosing procedure and rabbits are highly susceptible to stress induced mortality.
RMS comment (August 2013):
Not agreed. According to the study report, one gavage error was reported in the control group, but not at 100 and 500 mg/kg bw/d. Assuming technical incompetency as the main reason for lethality at mid and high dose level, one would expect the same observations at lowest dose level. Furthermore, at highest dose level 3 out of 8 animals died post-treatment, which can not be related to administration procedure, definitely.
Reference: IIA, 5.6.11/07
Report: (1989)
Rabbit Teratology Study with Glyphosate Technical
Data owner: Excel
Study no.: IIT Project No. 1086 Date: 1989-11-03
not published TOX9551960
Guidelines: OECD 414 (1981)
Deviations: no uterine weight, no maternal necropsy findings
GLP: no
Acceptability: See RMS comment
Dates of experimental work: 1989-07-03 to 1989-11-02
Materials and methods
Test material:
Identification:
Glyphosate technical
Description:
White amorphous powder
Lot #:
38
Purity:
95 %
Stability of test compound:
Not reported
Vehicle and/

or positive control:
0.1 % gum acacia in water
Test animals:
Species:
Rabbit
Strain:
New Zealand White
Source:
Age:
24 - 28 weeks
Sex:
Females
Weight at dosing:
1.50 – 2.00 kg
Acclimation period:
6 days
Diet/Food:
Pelleted rabbit feed supplied by Lipton India Ltd., Bangalore, India
Water:
Tap water, ad libitum, supplied in polypropylene bottles by
Maharashtra Industrial Development Corpn., New Bombay
Housing:
Individually in stainless steel cages equipped with food and
water dispensers and stainless steel grate at bottom
Environmental conditions:
Temperature: 20 ± 3 °C
Humidity: 30 to 70 % Air changes: not reported 12 hours light/dark cycle
Study design and methods:
In life dates: 1989-07-03 to 1989-11-02
Animal assignment and treatment:
In a teratogenicity study groups of 15 New Zealand White female rabbits received doses of 0, 125, 250 and 500 mg/kg bw/day test substance in 0.1 % gum acacia in water by gavage from Gestation Day 6-18 after successful mating with adult vigorous males. The day of mating was taken as the 1st day of pregnancy.
Diet preparation and analyses
For each dose level, dosing solutions were prepared in 0.1 % gum acacia in water as vehicle.
Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made twice daily (before and after dosing) during the dosing period.
Body weight
Individual body weights were recorded on Days 0, 6, 12, 18, 23, and 29 (at necropsy). Changes in body weight were calculated and recorded as group maternal weight changes for the periods of Days 0-6 (pre-exposure), 6-12, 12-18, 18-23, 23-29 and 18-29 (post-exposure observation period).
Food consumption
Food consumption was recorded on Days 0, 6, 12, 18, 23, and 29 (at necropsy).
Sacrifice and pathology

Females were euthanatized by carbon dioxide asphyxiation on Day 29 of gestation and examined for any abnormalities that would affect pregnancy.
The ovaries and uteri were removed, the uteri were weighed, and the ovaries were examined for the number of corpora lutea and uteri for the number and position of implants and dead or live foetuses. Uteri from non-gravid females were placed in 10% ammonium sulfide solution for detection of early resorptions.
Developmental parameters
Each rabbit foetus was removed from the uterus and was killed by injection of pentobarbitone. All live foetuses were weighed and examined for external malformations including cleft palate and variations. All live foetuses were examined for thoracic and visceral abnormalities, and each foetus was sexed. Following visceral examination, all foetuses were eviscerated and processed for skeletal staining with Alizarin Red SAll foetuses were decapitated and heads were fixed in Bouin's solution for examination of craniofacial structures.
Statistics Not reported.
Results and discussion
Analysis of dose formulations
The analytical purity of test substance was stated to be 95 %.
Food consumption
Mean food consumption in the low and mid dose groups was comparable to that in the control group throughout the study period. Significantly lower food consumption (~17 % lower mean food consumption compared to control, low or mid dose group) was observed in the high dose group starting with the day of treatment throughout the rest of the observation period.
Mortality
None of the rabbits died during the study period.
Clinical observations
No toxic symptoms were observed in any of the animals during the study.
Body weight
Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, the mean maternal weight increase was lower for each of the observation periods between Days 12-29 compared to controls, but no stastical comparison was provided in the report.
Pathology Necropsy
No abnormalities that could affect pregnancy were reported at maternal necropsy.
Observations on the ovary and uterus
Two animals of the high dose group aborted (see Table B.6.6-53).
Table B.6.6-53: Gestational parameters in rabbits treated with glyphosate
Gestational parameter
Dose level (mg/kg bw/day)
0
125
250
500
No. of pregnant females
15
15
15

15
No. of early deliveries
0
0
0
0
No. of abortions
0
0
0
2
No. of females with no live foetuses
0
0
0
2
No. nonpregnant at termination
2
1
1
3
No. of litters
13
14
14
12
Mean no. of corpora lutea per doe
10.0
10.1
10.3
9.8
Mean no. of total implants per litter
9.0
9.3
9.4
8.5
Mean % pre-implantation loss
21.3
14.9
14.7
13.1
Mean no. of viable implants per litter
7.3
8.0
8.0
5.2

Mean no. of non-viable implants per litter
0.07
0.13
0.27
1.4
Mean no. of early resorptions per litter
1.7
1.1
1.0
1.9
Sex ratio (% males)
44.4
49.2
49.7
50.1
Mean foetal body weight per litter
40.6
47.1
47.5
48.7
Developmental parameters Number and viability of foetuses
The mean number of viable implants (foetuses) per litter was lower in the high dose group, and accordingly, the mean number of non-viable implants (foetuses) per litter was greater in the high dose group (see Table B.6.6-53), but no statistical comparisons were provided in the report.
Sex ratio, foetal body weights and placental weights
No differences were noted in the sex ratios, mean foetal body weights, mean number of corpora lutea per dose, mean number of total implants per litter, mean percentage of pre- implantation loss, and mean number of early resorptions between the control and the treated groups. In the high dose group, two dams had no live foetuses due to abortions (see Table B.6.6-53). However, stasticial analyses were provided in the report.
External, visceral and skeletal examination
No difference was noted in the incidences of maternal animals having foetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the incidences of external, visceral and skeletal malformations were higher than that in the control group (see Table B.6.6-54). With regard to the heart malformations, 0, 1, 1, and 2 interventricular septal defects were observed in the 0,
125, 250, and 500 mg/kg bw/day dose groups.
A similar pattern was seen in the variations observed externally, viscerally and skeletally; in the high dose group, the total number of observed variations was higher than those of the control, low or mid dose groups. The increase in malformations and variations observed in the high dose group occurred in the presence of maternal toxicity (reduced food consumption and body weight gains). Further, this was at a dose (500 mg/kg bw/day) that caused significant toxicity, including mortality, in another rabbit developmental study. However, stasticial analyses were provided in the report.
Table B.6.6-54: Incidence of foetal malformations and variations in rabbits treated with glyphosate
Foetal findings
Dose level (mg/kg bw/day)

0
125
250
500
Malformations
No. of litters examined
13
14
14
12
No. of foetuses examined
109
113
120
78
No of litters with malformations
3
6
10
12
% of litters with malformations
23.08
42.86
71.43
100
No. of foetuses with malformations
3
6
10
20
% of foetuses with malformations
2.75
5.31
8.33
25.64
Number of foetuses (litters) with external malformations
Tail abnormal
1 (1)
1 (1)
2 (2)
3 (2)
Low-set ears
0 (0)
1 (1)
1 (1)
2 (1)

Total external malformations
1
2
3
3
Total external malformations (%)
0.92
1.77
2.50
3.85
Number of foetuses (litters)with visceral malformations
Ventricular septal defect
0 (0)
1 (1)
1 (1)
2 (2)
Postcaval lung lobe absent
0 (0)
1 (1)
2 (2)
4 (3)
Kidney(s) absent
1 (1)
2 (2)
2 (2)
6 (4)
Total visceral malformations
1
4
5
12
Total visceral malformations (%)
0.92
3.54
4.17
15.38
Number of foetuses (litters) with skeletal malformations
Rudimentary rib (no. 14)
1 (1)
0 (0)
2 (2)
5 (2)
Total skeletal malformations
1
0
2

5
Total skeletal malformations (%)
0.92
0.00
1.67
6.41
Variations
No. of foetuses examined
109
113
120
78
Total no. of observed variations
26
30
49
93
Number of foetuses (litters) with external variations
Tail blunt tipped
1 (1)
0 (0)
3 (2)
5 (4)
Number of foetuses (litters) with visceral variations
Irregular rugae on palate
0 (0)
2 (1)
3 (2)
2 (2)
Lateral ventricles of cerebrum dilated
0 (0)
2 (2)
2 (2)
6 (4)
Right ventricle small than normal
1 (1)
3 (2)
3 (2)
5 (3)
Globular heart
2 (2)
0 (0)
3 (2)
5 (4)
Incomplete separation of lung lobes
1 (1)

2 (1)
2 (1)
4 (2)
Parietal foetal atelectasis
0 (0)
1 (1)
1 (1)
1 (1)
Liver irregular shape
0 (0)
2 (1)
2 (2)
6 (4)
Kidney(s) globular shape
0 (0)
0 (0)
2 (1)
5 (3)
Foetal findings
Dose level (mg/kg bw/day)
0
125
250
500
Number of foetuses (litters) with skeletal variations
Cervical centra 1-3 and/or 4 bilobed
1 (1)
0 (0)
1 (1)
2 (2)
Anterior arch of the atlas poorly ossified
2 (1)
2 (1)
1 (1)
4 (2)
Anterior arch of the atlas split
0 (0)
0 (0)
2 (1)
3 (1)
Extra thoracic centrum and arch
1 (1)
3 (2)
2 (1)
5 (3)
Thoracic centrum only one ossification centre

1 (1)
0 (0)
1 (1)
3 (2)
Thoracic centra fused
2 (1)
1 (1)
1 (1)
2 (1)
Extra ribs on thoracic centra and arch 13 bilateral
1 (1)
0 (0)
3 (2)
5 (4)
Sternebra 6 poorly ossified
2 (1)
1 (1)
2 (1)
4 (2)
Sternebra(e) split
2 (1)
2 (1)
1 (1)
5 (3)
Sternebra(e) unossified
3 (2)
1 (1)
3 (2)
6 (4)
Pubis, poorly ossified
3 (2)
2 (2)
3 (1)
4 (3)
Some ossification in knee area
1 (1)
0 (0)
3 (2)
4 (3)
Skull bones poorly ossified
1 (1)
3 (2)
2 (1)
2 (2)
Frontal, hole in bone
0 (0)

1 (1)
2 (2)
2 (2)
Reduced number of caudal segments
1 (1)
2 (2)
1 (1)
3 (2)
Conclusion by the Notifiers
The oral administration of glyphosate to mated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 500 mg/kg bw/day. Therefore the NOAEL for reprotoxic and non-reprotoxic effects was considered to be 250 mg/kg bw/day. Considering the significantly reduced food consumption and gain in body weight at 500 mg/kg bw/day, the maternal NOAEL is 250 mg/kg bw/day.
Comment by RMS (Re-evaluation):
The study is considered supplementary due to serious reporting deficiencies (e.g. no individual data, no statistical analysis, no uterine weights, no results of maternal necropsy). The previous NOAEL for maternal and developmental toxicity is still considered to be 250 mg/kg bw/d based on reduced food consumption and body weight gain at 500 mg/kg bw/d in does
Developmental effects were visible as foetolethality and several malformations (external, visceral, skeletal) at high dose levels: The previous evaluation did not mentioned the external malformation in rabbits which are now reported in the present RAR (abnormal tails). Total number of foetuses per litter with malformations was higher in the groups receiving the mid and high dose lever, but without statistical significance. However, it remains unclear, whether statistical analysis of the data had been performed at all. Ventricular septal defects were noted in 2 out of 78 foetuses in the high dose group (control incidence 0/109). The higher number of further visceral malformations at the top dose level was due to absent kidneys and postcaval lung lobes. Because no individual data are provided it is not identifiable, whether the malformations described were confined to single foetuses or if the foetuses were multiple malformed.

Acute neurotoxicity study in rats (Horner, 1996)
Reference: IIA, 5.7.1/01
Report: 1996
Glyphosate acid: Acute neurotoxicity study in rats
Data owner: Syngenta Report No. CTL/P/4866 Date: 1996-03-01
Unpublished, ASB2012-11500
Guidelines: No guideline stated in the report but in general compliance with OECD 424 (1997).
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Materials and methods
Test material:
Identification:
Glyphosate acid
Description:
White solid

Lot/Batch #:
Y04707/034
Purity:
95.6% w/w
Stability of test compound:
The test substance was shown to be stable for the period of use.
Vehicle:
Deionised water
Test animals:
Species:
Rats
Strain:
Alpk:APfSD (Wistar-derived)
Source:
Age:
At least 28 days
Sex:
Males and females
Weight at dosing:
♂ 171.4 – 175.0 g; ♀ 144.6 – 148.7 g
Acclimation period:
Approx. 2 weeks
Diet/Food:
CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK), ad libitum, except 24 h prior dosing
Water:
Tap water, ad libitum
Housing:
In groups of five, separated by sex, in multiple rats racks.
Environmental conditions:
Temperature: 19 – 23 °C
Humidity: 40 – 70 % Air changes: 25 – 30/hour 12 hours light/dark cycle
In life dates: Not reported. The study was conducted during May and June 1995.
Animal assignment and treatment
In an acute neurotoxicity study groups of ten male and ten female Alpk:APfSD (Wistar derived) rats were administered with a single oral dose of 0, 500, 1000 and 2000 mg/kg bw glyphosate acid by gavage.
Dosing Formulation Analysis
Verification of the achieved concentrations was done with samples of each preparation. Homogeneity was determined with samples from the low to high dose levels. The chemical stability of glyphosate acid in water was also determined for all dose formulations over a period of 10 days.
Clinical observations
Clinical observations were made prior to administration and daily thereafter. Any abnormalities together with the observation of no abnormality detected were recorded.
Body weight
The body weight of each rat was recorded on Days -7 and -1, immediately before dosing (Day 1),

approximately 6 hours after dosing (Day 1) and on Days 8 and 15.

Food consumption

Food consumption for each cage of rats was recorded throughout the study and calculated on a weekly basis.

Functional Observational Battery

Prior to the start of treatment (Week -1) and on Day 1, 8 and 15, all animals were observed for signs of functional/behavioural toxicity. Detailed clinical assessments and functional performance tests were performed together with an assessment of sensory reactivity to different stimuli. Locomoter activity was also assessed at these time points.

Sacrifice and pathology

At scheduled termination, 5 rats/sex/group designated for neuropathology were sacrificed. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve. Neuropathological examination was performed on control and highest dose group animals only.

Statistics

Analyses of variance and covariance were carried out using the GLM procedure in SAS (1989). Least-squares means for each group were calculated using LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squared mean. Differences from control were tested statistically by comparing each treatment group least- squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

Results and discussion

Dosing formulation analysis: The achieved concentrations of glyphosate acid in water were within 3 % of the nominal levels. The homogeneity was considered acceptable, with a deviation from the overall mean values of approximately ± 8 %. The chemical stability was considered satisfactory.

Mortality and clinical observations:

Two females receiving 2000 mg/kg bw glyphosate acid showed subdued behaviour, decreased activity, hunched posture, sides pinched in, tip-toe gait and hypothermia on the day of administration. One of these animals died on the subsequent day. The other one together with an additional female which showed diarrhoea on the day of administration regained full recovery the subsequent day.

One female receiving 500 mg/kg bw, was found dead approximately 6 h after administration. In the absence of any treatment-related clinical signs prior to death, and because no deaths were observed at the intermediate dose level of 1000 mg/kg bw, the death of this animal was considered not to be treatment related.

Distension of the abdomen was recorded for several males from all treated groups on the day of administration. However, in the absence of any dose relationship, this was not considered to be treatment-related.

Body weight:

No treatment-related effects were observed.

Food consumption

During Week 1, mean food consumption was lower in animals receiving 2000 mg/kg bw glyphosate acid compared to controls, although the difference did attain statistical significance only in females (see Table B.6.7-1). There was no evidence of treatment-related effects in animals receiving 500

1000 mg/kg bw.
Table B.6.7-1: Intergroup comparison of food consumption (g/rat/day) during Week 1
Dose level of glyphosate (mg/kg bw)
0 (control)
Mean ± SD
500
Mean ± SD
1000
Mean ± SD
2000
Mean ± SD
Males
29.9 ± 0.7
29.0 ± 0.1
30.1 ± 0.4
28.4 ± 0.2
Females
22.4 ± 1.0
22.2 ± 0.2
22.8 ± 0.3
20.6* ± 0.3
* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)
Functional observation battery: Examinations of the functional observational battery did not identify any conclusive treatment- and dose-related effects
Necropsy: No macroscopic findings were detected.
Histopathology: No microscopic findings were considered to be treatment-related.
Conclusion by the Notifiers
Based on the study results the NOAEL for acute neurotoxicity, following single oral administration of glyphosate acid is 2000 mg/kg bw.
RMS comments:
The study is considered acceptable. Its evaluation as provided in the dossier is agreed with. There was no evidence of specific neurotoxicity up to the highest single dose of 2000 mg/kg bw. Clinical signs indicative of acute toxicity occurred in few animals at this dose and included one death. Thus, the NOAEL for systemic toxicity was 1000 mg/kg.
Subchronic neurotoxicity study in rats (1996)
Reference: IIA, 5.7.4/01
Report: 1996, Glyphosate acid: Subchronic neurotoxicity study in rats
Data owner: Syngenta Report No.: CTL/P/4867 Date: 1996-03-11
unpublished, ASB2012-11501
Guidelines: Study was pre-guideline, but satisfies in general the requirements of OECD 424 (1997)
Deviations: None
GLP:
yes
Acceptability:
See RMS comment

Dates of experimental work: 1995-04-25 to August 1995
Materials and methods
Test material:
Identification:
Glyphosate acid (technical)
Description:
White solid
Lot/Batch #:
P24
Purity:
95.6 %
Stability of test compound:
Confirmed for the study period
Vehicle:
Plain diet
Test animals:
Species:
Rats
Strain:
Alpk:APfSD
Source:
Age:
At least 6 weeks
Sex:
male and female
Weight at dosing:
♂ 215.0 – 218.6 g (mean); ♀ 173.5 – 178.8 g (mean)
Acclimation period:
Approximately 2 weeks
Diet/Food:
CT1 diet (Special Diet Services Limited, Witham, Essex, UK), ad libitum (except up to 24 hours prior to dosing)
Water:
Tap water, ad libitum
Housing:
Four per cage per sex in stainless steel cages (26.5 x 50.0 x 20.7cm)
Environmental conditions:
Temperature: 19-23 °C
Humidity: 40-70 % Air changes: 25-30/hour
Photoperiod: 12 hours light/dark cycle
In life dates: 1995-05-09 to August 1995 Animal assignment and treatment:
In a subchronic neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar- derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks. (equivalent to mean achieved dose levels of 0, 155.5, 617.1 and 1546.5 mg/kg bw/day for males, and 0, 166.3, 672.1 and 1630.6 mg/kg bw/day for females) glyphosate technical.
All diets were based on CT1 diet supplied by Special Diets Services Limited, Stepfield, Witham, Essex,

UK. The experimental diets were prepared in 30 kg batches by direct addition of the test substance to 30 kg of CT1 diet and mixing thoroughly. The diets were stored at room temperature until required for use.
Samples from all dietary levels (including controls) were taken at intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet, under the conditions of storage used on this study, was determined for 2000 ppm and 20000 ppm diets prepared for use on a concurrent 1 year feeding study in the rat in the same laboratory.
Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded. A detailed physical examination was performed on each rat prior to start of treatment, and at weekly intervals thereafter.
Functional observational battery (FOB)
Prior to the start of treatment and during Weeks -1, 5, 9 and 14, all animals were observed for signs of functional/behavioural toxicity. The assessment involved observations in the home cage and/or while the rat was moving freely in a standard arena followed by manipulative/in hand tests
Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. The examinations included quantitative assessments of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hind limb grip strength). The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour; reactivity to stimuli; changes in level of arousal; sensorimotor responses; alterations in respiration.
Locomotor activity
Locomotor activity was monitored by an automated activity recording apparatus. All animals were tested at weeks -1, 5, 9 and 14. Each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimise disturbances.
Body weight
Individual body weights were recorded in week -1, immediately prior to treatment), at weekly intervals thereafter, and at necropsy.
Food consumption and compound intake
Food consumption was recorded as required for each cage group throughout the study and calculated on a weekly basis. Food utilisation and compound intake were calculated.
Water consumption Not reported.
Ophthalmoscopic examination
Not performed. However, ophthalmological data are available from other repeated dose studies.
Sacrifice and pathology
At the scheduled termination, all main study animals not required for neuropathology, were killed by overexposure to rising concentrations of carbon dioxide gas and were discarded without examination.
At termination, the six rats/sex/group designated for neuropathology were deeply anaesthetised with intraperitoneal sodium pentobarbitone and killed by whole body perfusion fixation with

modified Karnovsky's solution. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve.
Brain weight, brain length and brain width were determined.
Submitted tissues were processed as follows: brain (seven levels including the cerebral cortex, the hippocampus, the cerebellum, the pons and medulla), dorsal root ganglia and spinal roots from cervical and lumbar regions of the cord after decalcification, and gastrocnemius muscle from rats receiving either control diet or diet containing 20000 ppm glyphosate acid were routinely processed, paraffin wax embedded and 5µm thick sections were cut and then stained with haematoxylin and eosin. Sections of brain and cord were in the transverse plane.
The Gasserian ganglion, sciatic nerve, spinal cord (cervical and lumbar portions), sural and tibial nerve from control and high dose group rats were processed and then embedded in Araldite. Semi-thin sections were cut and then stained with toluidine blue. For bilateral tissues only the left was processed. All tissues were sectioned in the transverse plane except the sciatic nerve which was sectioned in both the transverse and the longitudinal plane.
Neuropathological examination was performed on control and highest dose group animals only. All sections were examined by light microscopy.
Statistics
All data were evaluated using analysis of variance and/or nalysis of covariance for each specified parameter using the GLM procedure in SAS (1989).
The levels of probability chosen as significant different from control were $p < 0.01$** and $p < 0.05$* (Student's t-test, two-sided).
Results and discussion
Dosing formulation analysis: The achieved mean concentrations of Glyphosate acid in diet were within 4 % of the nominal levels, with individual values being within 15 % of nominal. There were considered acceptable. The homogeneity of the low- and high-dose diets was considered acceptable, with a deviation from the overall mean values of ± 4 %. The chemical stability was considered satisfactory.
Mortality: No deaths occurred during the study.
Clinical observations: There were no treatment-related clinical signs of toxicity. Functional observational battery (FOB)
There were no clinical signs that could be attributed to administration of glyphosate acid.
There was an apparent increase in the incidence of miosis and decreased pupil response to light in males receiving 20000 ppm. However, as these signs were seen for several of these males pre-experimentally and were also present at a similar incidence in females with no obvious relationship to treatment, this was considered to be incidental and unrelated to administration of glyphosate acid.
Landing Foot Splay Measurements
There was no evidence of any treatment-related effect on landing foot splay.
Time to Tail-Flick
There was no evidence of any treatment-related effect on time to tail-flick.
Grip Strength Measurements
There was no evidence of any treatment-related effect on forelimb or hind limb grip strength.
Motor activity
There was no evidence of any treatment-related effect on locomotor activity.
During week 5, slightly reduced locomotor activity was recorded on occasions for females receiving

20000 ppm. However, in the absence of any treatment-related effects on motor activity for these animals at other time points during the study, this is considered to be incidental and unrelated to administration of glyphosate acid.

Table B.6.7-2: Selected motor activity findings

Dietary concentration (ppm)
Males
Females
Week
Assessment period (min)
0
2000
8000
20000
0
2000
8000
20000
5
1-50
388.7
472.1
335.6
384.4
441.2
379.3
457.8
359.3
9
1-50
304.7
413.4*
298.4
327.3
512.3
488.9
555.1
557.0
14
1-50
299.4
395.1
292.2
372.8
553.0
512.7
569.3

514.7
* Statistically significant difference from control group mean at the 5% level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1% level (Student's t-test, 2-sided)
Body weight: Group mean bodyweight for males receiving 20000 ppm was statistically significantly lower than that of controls throughout the study. At week 14, group mean bodyweight for these animals was 92.8 % that of controls, equating to a reduction in bodyweight gain of approximately 12 %.
Group mean body weight for males receiving 8000 ppm was also marginally lower than that of controls from weeks 6 to 14. However, these differences did not attain statistical significance and were considered too small to be of biological importance.
For males receiving 2000 ppm, and for females at all dose levels, mean body weight was essentially similar to that of concurrent controls throughout the study.
Table B.6.7-3: Intergroup comparison of body weights (g)
Dietary concentration (ppm)
Males
Females
Week
0
2000
8000
20000
0
2000
8000
20000
1
216.0
217.0
218.6
215.0
173.5
178.8
175.6
175.3
2
263.5
264.7
264.9
254.6**
192.7
200.6
196.1
194.3
4

338.2
340.7
339.6
323.7*
214.3
228.3**
224.9**
219.2
8
440.7
440.1
429.1
405.8**
253.6
262.1
260.4
255.4
14
534.7
532.8
526.5
496.1**
285.1
291.5
287.9
281.0
* Statistically significant difference from control group mean at the 5 % level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1 % level (Student's t-test, 2-sided)
Food consumption and compound intake: There were no effects on food consumption. The efficiency of food utilisation for males receiving 20000 ppm was statistically significantly lower than that of concurrent controls during weeks 1 to 8. There were no changes in the efficiency of food utilisation for males receiving 2000 or 8000 ppm or for females from all treated groups.
Table B.6.7-4: Intergroup comparison of food utilisation (g growth/100 g food)
Dietary concentration (ppm)
Males
Females
Week
0
2000
8000
20000
0
2000
8000

20000
1-4
18.13
17.16
16.94
16.28*
9.42
9.73
9.36
9.61
5-8
11.52
10.69
10.35
9.93*
5.99
5.55
5.39
5.70
1-13
12.00
11.45
11.38
10.87**
6.08
6.03
6.06
5.96

* Statistically significant difference from control group mean at the 5 % level (Student's t-test, 2-sided)

** Statistically significant difference from control group mean at the 1 % level (Student's t-test, 2-sided)

The mean doses received for males and females respectively were 155.5, 617.1, 1546.5 and 166.3, 672.1, 1630.6 mg glyphosate acid/kg/day at dose levels of 2000, 8000 and 20000 ppm, respectively

Brain measurements: There was no evidence of any effects on brain weight, length or width.

Necropsy: There were no macroscopic findings that were considered to be attributable to treatment.

Histopathology: There were no microscopic findings in the peripheral or central nervous system that were considered to be attributable to treatment.

Conclusion by the Notifiers

Dietary administration of glyphosate acid to rats for a period of ninety consecutive days at dietary concentrations of up to 20000 ppm produced evidence of toxicity in the form of reduced growth and reductions in food utilisation for males. Comprehensive histopathological evaluation of the nervous system showed no evidence of any changes in the peripheral or central nervous system which could be attributed to administration of glyphosate acid.

The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm, corresponding to 1546.5 / 1630.6 mg/kg

bw/day for males and females, respectively.
RMS comments:
The study is considered acceptable. The conclusion is agreed with. No evidence of a neurotoxic potential was obtained up to the the highest dose of 20000 ppm. The NOAEL for systemic toxicity was 8000 ppm (617 mg/kg bw/day) in males, based on lower body weight (gain) and impaired food utilisation, and 20000 ppm (1631 mg/kg bw/day) in females, i.e., no effects were noted at the top dose level.
Delayed neurotoxicity study in chicken (1996)
Reference: Not applicable since study was not part of the dossier
Report: 1996, Glyphosate Acid: Acute delayed neurotoxicity study in the domestic hen.
Data owner: Syngenta (study was not part of the dossier but submitted to RMS for other purposes before)
Report No.: CTL/C/3122 Date: 1996-08-23
unpublished, ASB2013-9828
Guidelines: U.S. EPA Subdivision F 81-7
Deviations: None
GLP: Yes
Acceptability: The study is considered acceptable by RMS.
Materials and methods
Test material:
Identification:
Glyphosate acid (technical)
Description:
White solid
Lot/Batch #:
P24
Purity:
95.6 %
Stability of test compound:
Confirmed for the study period
Vehicle:
Distilled water
Positive control substance:
Identification
Tri-ortho-cresylphosphate (TOCP)
Lot/Batch:
143-41C
Purity:
99.0 %
Expiry date:
November 1997
Supplier:
Chem Services Inc.
Vehicle:
Corn oil
Test animals:

Species:
Chicken
Strain:
Lohmann Brown (a hybrid brown laying strain)
Source:
Age:
Approx. 12 months
Sex:
Female
Weight at dosing:
1927 – 2215 g (range)
Acclimation period:
Approximately 2 weeks
Diet/Food:
HRC layer ration in pellet form (Parker Bros. Ltd., Lark Mills, Suffolk, UK), ad libitum (except overnight starvation prior to dosing)
Water:
Tap water, ad libitum
Housing:
Floor pens (galvanised steel, concrete floor) measuring 1.8
x 1.4 m with up to (not further specified) hen from the same group
Environmental conditions:
Temperature: 15-17 °C Humidity: 79 % (mean)
Air changes: not given in the report but ventilation considered “adequate” by study author
Photoperiod: 12 hours light/dark cycle
In life dates: 1996-01-09 to 1996-02-14
Animal assignment and treatment:
20 hen were administered glyphosate acid as a single dose of 2000 mg/kg bw by oral gavage. 12 birds were employed as positive controls and received a single dose of 1000 mg TOCP/kg bw. The negative control group consisted also of 12 hen and received once distilled water also by gavage. The same volume of 10 mL/kg bw was applied to all chicken. Treatment was followed by an observation period of 21 or 22 days.
Clinical observations
A check for mortality, clinical signs of toxicity, ill health and behavioural changes was made twice daily on all animals.
Ataxia assessment
Following treatment, hen were examined daily for signs of (delayed) ataxia.
Body weight
Individual body weights were recorded weekly.
Food consumption and compound intake No information given.
Water consumption Not reported.
Clinical pathology
3 pre-determined chicken from each group were sacrificed 48 hours after dosing to determine brain cholinesterase, brain neuropathy target esterase and lumbar spinal cord neuropathy target esterase (NTE) activities
Sacrifice and pathology

At the scheduled termination, 6 hen from each group were selected for necropsy and histopathological examinations. Whereas in the negative control and glyphosate-treated groups the first six birds in numerical order (because of the absence of clinical signs) were employed, care was taken in the TOCP-treated group to include all animals that had shown clinical ataxia. The remaining hen from all three groups were killed and discarded.

At termination, after perfusion through the heart with fixative, head and spinal column (with brain and spinal cord exposed but left in place) and dissected sciatic nerves (including tibial branches) from the six hen/group designated for neuropathology were taken and stored. The following tissues were used to take samples for histological examination: brain (forebrain, mid and hindbrain), spinal cord (upper and lower cervical, mid-thoracic and lumbo-sacral parts), sciatic nerve (proximal and distal, above knee), tibial nerve. One transverse and two longitudinal sections were performed at each level.

Statistics

Apparently, no statistical analysis was necessary since the results were quite clear and number of animals limited.

Results and discussion

Mortality: There were two unscheduled deaths during the study.

• In the test group receiving glyphosate acid, one bird was found dead on day 10 after dosing. This hen had not exhibited any signs of toxicity prior to death. The cause of death was apparently not elucidated but if the singularity of this case (1/20) and the absence of clinical signs is taken into consideration, the opinion of the study author can be agreed with that mortality was unrelated to treatment.

• In the positive (TOCP) control group, one bird had been severely pecked by other hen and was sacrificed on humane grounds, apparently during the acclimation period yet.

Clinical observations: In the glyphosate-treated and negative control groups, there were no was a common finding (see below). In addition, one more bird in this group was hurt by other hen by pecking and had to be treated by applying Stockholm tar to the wounds.

Ataxia assessment

Ataxia was confined to the positive control group receiving 1000 mg TOCP/kg bw. 5 of 11 hen were affected. Signs occurred for the first time between post-observation days 11 and 21 and the severity of ataxia was variable.

Body weight

Group mean body weight increased in the glyphosate-treated and negative control groups but weight loss was observed in the positive controls receiving TOCP.

Clinical chemistry

In line with ataxia observations, NTE levels in brain and spinal cord were clearly reduced in the positive control group (by 84% for brain and by 78% for spinal cord as compared to negative control group) but no effect was seen in the group receiving glyphosate. A very low reduction of brain cholinesterase (6% less than in negative control) was seen in the hen that had received glyphosate. In the positive control group, the decrease in brain cholinesterase activity accounted for 19%. In the study report, the lack of statistical significance for the latter findings is emphasised but it is doubted if meaningful statistical analysis was possible with
only three birds per group under investigation. Taking into account the very low difference to negative control birds and the fact that glyphosate is not known to inhibit cholinesterases, a treatment-related effects is not likely.

Necropsy: There were no macroscopic findings that were attributable to treatment.

Histopathology: The evaluation of histological findings is complicated by the fact that axonal degeneration in the spinal cord and peripheral nerves were observed in all three groups in nearly all birds suggesting high background incidence. In the TOCP-treated group, the cerebellum was also affected in five out of 6 animals (as compared to only one bird in the glyphosate group). Furthermore, axonal degeneration in general was more severe in the positive control group.
Conclusion (RMS)
Based mainly on the occurrence of ataxia in the positive control group but not in chicken receiving glyphosate and the related reduction of NTE activity after dosing with TOCP but not with glyphosate, there is no potential of glyphosate to cause delayed neuropathy.

B.6.8.1 Toxicity of the metabolite AMPA
Table B.6.8-1: Summary of toxicological studies with AMPA
Reference; study identification, owner
Type of study; Species,
strain
AMPA
purity [%]
Exposure conditions / test method;
dose levels
Results
1993; TOX9552395;
Cheminova
Acute oral toxicity Rat, SD
99.2
Limit test (m/f)
LD50 > 5000 mg/kg bw; some general and gastrointestinal clinical signs observed
Acute oral toxicity;
Mouse, ICR
99.33
Limit test (m/f)
LD50 > 5000 mg/kg bw, no evidence of toxicity
, 1988; TOX9500044;
Syngenta
Acute oral
toxicity Rat, Wistar
> 99
Limit test (m/f)
LD50 > 5000 mg/kg bw, diarrhea
and some general signs of intoxication
, 2002*; ASB2012-
11503; ADAMA
Acute dermal toxicity
Rat, CD
98.0
Limit test (m/f)
LD50 > 2000 mg/kg bw, no evidence of toxicity

1993; TOX9552396;
Cheminova
Acute dermal toxicity
Rat, SD
99.2
Limit test (m/f)
LD50 > 2000 mg/kg bw, no evidence of toxicity
1993; TOX9300374;
Cheminova
Skin sensitisation Guinea pig (Female Dunkin-
Hartley)
99.2
Maximisation test (M&K)
Not sensitising
2002*, ASB2012-
11506; ADAMA
Skin sensitisation Guinea pig (Male Dunkin-
Hartley)
98.0
Maximisation test (M&K)
Not sensitising
1993; TOX9300349;
Cheminova
4-week oral toxicity, Rat, SD
99.2
Gavage; 0, 10,
100, 350, 1000
mg/kg bw/d (m/f)
NOAEL = 100 mg/kg bw/day, based on higher kidney wt in m and lower bw gain in f
1993;
TOX9300377;
Cheminova
13-week oral
toxicity, Rat, SD
99.2
Gavage; 0, 10,
100, 1000
mg/kg bw/d (m/f)
NOAEL = 1000 mg/kg bw/day
Reference; study identification, owner
Type of study; Species,
strain
AMPA
purity [%]
Exposure conditions / test method;
dose levels

Results
1979; TOX9552401;
Monsanto
90-day oral toxicity, Rat, CD
99.96
Feeding (adjusted for dose levels); 0, 400, 1200,
4800 mg/kg bw/d (m/f)
NOAEL = 400 mg/kg bw/day, based on bw gain↓, urothelial hyper-plasia (bladder) and gastro-intestinal clinical signs; at top dose level in addition mortality following blood collection (f), food consumption↓, clinical chemistry findings (LDH
activity↑) and hyper-plasia of renal pelvis epithelium
, 1991; TOX9552406;
Monsanto
90-day oral toxicity, Beagle dog
87.8
Capsule; 8.8,
26.3, 87,8, 263
mg/kg bw/d when adjusted for purity (m/f)
NOAEL = 263 mg/kg bw/day; no treatment-related findings
, 1993; TOX9300378;
Cheminova
Genotoxicity in bacteria; S. typhimurium TA100, TA98, TA1535, and TA1537
99.2
Ames test, ±S9 mix for metabolic activation, 310-
5000 µg/plate, plate incorpo- ration and pre-
incubation assay
Negative
1993; TOX9300380;
Cheminova
Genotoxicity in mammali- an cells, Mouse lymphoma (L5178Y)
cells
99.2
Mouse lymphoma assay, ±S9 mix for metabolic activation, 310-
5000 µg/mL
Negative
Callander, 1988; TOX9500043;
Syngenta
Genotoxicity in bacteria; S. typhimurium TA100, TA98, TA1535, TA1537, TA1538, and
E. coli (WP2
uvrA)
> 99
Ames test, ±S9 mix for metabolic activation, 1.6 -
5000 µg/plate, plate incorpo- ration and (only with S9 mix) pre-incubation
assay
Negative
Akanuma, 1996*,

ASB2012-11507;
Arysta
Genotoxicity
in bacteria;
S.
typhimurium TA100, TA98, TA1535,
TA1537 and
E. coli (WP2
uvrA)
99.33
Ames test, ±S9
mix for metabolic activation, 313
- 5000 µg/plate, pre-incubation assay
Negative (supplementary study)
Reference; study identification, owner
Type of study; Species,
strain
AMPA
purity [%]
Exposure conditions / test method;
dose levels
Results
Nesslany, 2002*; ASB2012-11508;
Arysta
Genotoxicity in vitro (DNA
damage and repair); primary rat
hepatocytes
99.9
UDS in rat hepatocytes, 0.625-10 mM
Negative
Bakke, 1991; TOX9552409;
Monsanto
Genotoxicity in vitro (DNA
damage and repair); primary rat
hepatocytes
94.38
UDS in rat hepatocytes, 5 –
5000 µg/mL
Negative up to 2500 µg/mL, meaningful evaluation of higher concentrations not possible due to cytotoxicity
1993; TOX9300379;
Cheminova
Genotoxicity (clastogenici- ty) in vivo, Mouse, NMRI
99.2
Micronucleus test in bone marrow, 5000 mg/kg bw, single oral
exposure (m/f)

Negative
, 1993; TOX9552413;
Monsanto
Genotoxicity
(clastogenici- ty) in vivo, Mouse, CD-1
94.38
Micronucleus
test in bone marrow, 100,
500, 1000
mg/kg bw, single i.p.
injection (m/f)
Negative
1992;
TOX9300348;
Cheminova
Develop-
mental toxicity Rat, SD
99.2
0, 100, 350,
1000 mg/kg bw/d by
gavage, gesta- tion days 6-16
NOAEL = 1000 mg/kg bw/day
(maternal and developmental)
1991;
TOX9552414 ;
Monsanto
Develop-
mental toxicity Rat, SD
94.38
0, 150, 400,
1000 mg/kg bw/d by ga- vage, gestation days 6-15
Maternal NOAEL 150 mg/kg
bw/day, based on clinical signs, bw gain/food consumption↓; Developmental NOAEL 400 mg/kg
bw/d, based on mean
fetal wt↓
* Study previously not evaluated by the EU
1996)
Reference:
IIA, 5.8/01
Report:
1996 AMPA: Acute Oral Toxicity Study In Mice.
Report No.: IET 96-0075 Date: 1996-11-11
not published, ASB2012-11502
Guidelines:
OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
Deviations:

None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1996-09-24 to 1996-10-08
Materials and methods
Test material:
Identification:
AMPA
Description:
White powder
Lot/Batch #:
A-960719
Purity:
99.33 %
Stability of test compound:
Stable for 1 year at RT.
Vehicle:
1 % carboxymethyl-cellulose (CMC)
Test animals:
Species:
Mice
Strain:
ICR (Crj:CD-1), SPF
Source:
Age:
6 weeks
Sex:
Male and females
Weight at dosing:
♂ 30.5 – 34.6 g; ♀ 22.9 – 24.8 g
Acclimation period:
7 days
Diet/Food:
Pellet Diet MF (Oriental Yeast Co., Japan), ad libitum
except for approx. 3 h before and after dosing
Water:
Tap water, ad libitum
Housing:
Aluminium cages with wire-mesh floors in groups of 5 animals/sex/cage.
Environmental conditions:
Temperature: 23 ± 3°C Humidity: 55 ± 15% Air changes: 12/hour
12-hour light/dark cycle
Animal assignment and treatment:
A group of five fasted mice per sex received the test material at a dose level of 5000 mg/kg bw by

oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made 1, 3 and 6 h after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanized under ether anaesthesia and subjected to gross necropsy.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: No body weight losses were recorded in any animal 7 and 14 days after the administration.
Necropsy: The gross necropsy conducted at termination of the study noted no observable abnormalities.
Conclusion by the Notifiers
The oral LD50 of the test material (AMPA) was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute oral toxicity.
RMS comments:
The study is considered acceptable. The conclusion is agreed with.
Acute oral toxicity in the rat (Leah, 1988)
Reference: IIA, 5.8/02
Report: . 1988
Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat.
Data owner: Syngenta Report No.: CTL/P/2266 Date: 1988-08-10
GLP:
not published, TOX9500044
Guidelines: Not stated, but method is in accordance with OECD 401.
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: No date given in the report.
Materials and methods
Test material:
Identification:
Aminomethyl Phosphonic Acid (AMPA)
Description:
White solid
Lot/Batch #:
Y06384/001/001 (CTL reference)
Purity:
100 % (assumed)
Stability of test compound:
No data given in the report.
Vehicle:
0.5 % (w/v) aqueous polysorbate 80
Test animals:
Species:

Rat
Strain:
Wistar (Alpk:APfSD), SPF
Source:
Age:
Approx. 8-9 weeks
Sex:
Male and females
Weight at dosing:
♂ 280 – 312 g; ♀ 204 – 214 g
Acclimation period:
At least 6 days
Diet/Food:
Porton Combined Diet (Special Diets Services Ltd.), ad libitum except for approx. 24 h before dosing
Water:
Tap water, ad libitum
Housing:
Suspended stainless steel/polycarbonate cages with stainless steel mesh floors in groups of max. 5 animals/sex/cage.
Environmental conditions:
Temperature: 15 – 24 °C
Humidity: 50 ± 10 % Air changes: 20 – 30/hour 12-hour light/dark cycle
Animal assignment and treatment:
A group of five fasted rats per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 10 mL/kg bw. One animal was accidentally killed (by mis-dosing) on Day 1 and another animal was therefore substituted, but was dosed one day later.
Observations for mortality and clinical/behavioural signs of toxicity were made once 30-90 minutes, 4 and 6 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded one day prior to dosing, the day of dosing (Day 1) and on Days 3, 5 or 6, 8 and 15. On Day 15 after dosing, each animal was euthanized under ether anaesthesia and subjected to gross necropsy.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: Signs of slight toxicity (diarrhoea, chromodacryorrhea, piloerection, stains around nose, ungroomed appearance, signs of urinary incontinence) were seen in the animals, but these did not persist and all animals had recovered by Day 3 or 4.
Body weight: All animals lost weight initially due to the pre-dose fast, but all then gained weight and had exceeded their initial bodyweight by Day 6. Moreover, one male lost weight between Day 6 and 8 and one further male and three females between Day 8 and 15. The reason was unclear as there were no associated clinical abnormalities, nor were there any abnormalities at necropsy.
Necropsy: The gross necropsy conducted at termination of the study noted no observable abnormalities.
Conclusion by the Notifiers
The oral LD50 of the test material (AMPA) was estimated to be greater than 5000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute oral toxicity.

RMS comments:
This study is considered acceptable and its conclusion is agreeed with. It should be noted that it was already evaluated by the RMS (and later on by the EU) when submitted as part of the toxicological data package for glyphosate-trimesium (DAR, 1998, ASB2010-10493)
Acute dermal toxicity in the rat (Leuschner, 2002)
Reference: IIA, 5.8/03
Report: 2002
Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration – LIMIT TEST
Data owner: ADAMA Agan Ltd
Report No.: 16168/02 Date: 2002-12-03
not published, ASB2012-11503
Guidelines: OECD 402 (1987), EEC B.3 (1992)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 2002-10-21 to 2002-11-01
Materials and methods
Test material:
Identification:
AMPA (Aminomethyl Phosphonic Acid)
Description:
White solid powder
Lot/Batch #:
FA005563
Purity:
98.0 %
Stability of test compound:
Stable until 2004-12-03 at RT.
Vehicle:
0.5 % aqueous hydroxypropylmethyl cellulose gel
Test animals:
Species:
Rat
Strain:
CD / Crl:CD
Source:
Age:
20 - 22 days
Sex:
Male and females
Weight at dosing:
♂ 214 – 238 g; ♀ 213 – 223 g
Acclimation period:
At least 5 days.
Diet/Food:

ssniff R/M-H V1530 (ssniff Spezialdiäten GmbH, Soest, Germany), ad libitum except for approx. 16 h before dosing
Water:
Tap water, ad libitum
Housing:
Individually in MAKROLON cages (type III) with granulated textured wood as bedding.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 55 ± 15 % Air changes: not reported 12-hour light/dark cycle
Animal assignment and treatment:
The acute dermal toxicity of AMPA was tested on five male and five female CD rats. One day before treatment the administration site was clipped free of hair. A single dose of 2000 mg/kg bw test substance prepared as suspension in 0.5 % aqueous hydroxypropylmethyl cellulose gel was applied uniformly over an area of about 10 % of the total body surface. The dosing volume was 10 mL/kg bw. The application site was covered with an occlusive dressing for 24 hours. After removal of the dressing, possible residual substance was removed. All animals were observed for overt signs of toxicity or behavioural changes before and immediately, 5, 15, 30 and 60 minutes, as well as 3, 6 and 24 h after administration and subsequently once daily for 14 days. Individual body weights were recorded before administration and on Days 7 and 14. All surviving animals were killed at the end of the 14-day observation period.
Results and discussion
Mortality: There were no mortalities during the study.
Clinical observations: No clinical signs were observed during the study.
Body weight: No body weight losses were recorded in any animal 7 and 14 days after the administration.
Necropsy: The gross necropsy conducted at termination of the study noted no observable abnormalities.
Conclusion by the Notifiers
The dermal LD50 of the test material (AMPA) was estimated to be greater than 2000 mg/kg bw. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for acute dermal toxicity.
RMS comments:
The study is considered acceptable and the conclusion is agreed with.
Skin sensitisation in the guinea pig by M&K test (2002)
Reference: IIA, 5.8/04
Report: 2002 Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitisation Test in Guinea Pigs according to Magnusson And Kligman (Maximisation Test)
Data owner: ADAMA Agan Ltd
Report No.: 16169/02 Date: 2002-12-03
Unpublished, ASB2012-11506
Guidelines: OECD 406 (1992); EEC B.6 (1996)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 2002-10-12 to 2002-11-26
Materials and methods

Test material:
Identification:
AMPA
Description:
White solid powder
Lot/Batch #:
FA005563
Purity:
98.0 %
Stability of test compound:
At room temperature stable until December 31, 2004.
Vehicle:
Purified water
Test animals:
Species:
Guinea pig
Strain:
Dunkin Hartley
Source:
Age:
22 days
Sex:
Male
Weight at dosing:
252 - 307 g; positive control group: 228 - 341 g
Acclimation period:
At least 5 days.
Diet/Food:
ssniff Ms-H (ssniff Spezialdiäten GmbH, Soest, Germany),
ad libitum
Water:
Tap water, ad libitum
Housing:
In pairs in Makrolon cages (type IV) with granulated textured wood bedding.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 55 ± 15 % Air changes: no data 12 hours light/dark cycle
Animal assignment and treatment:
AMPA was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Male Dunkin Hartley guinea pigs, young adults with body weights ranging from 228 to 341 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals. The main study was performed in 10 test animals, 5 control animals and 20 positive control animals.
The induction phase consisted of an intradermal injection at Day 0 and an epidermal application on Day 7. On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin of the shoulder region at a concentration of 5% either in purified water or in a 1:1 (v/v) mixture of Freund´s

Complete Adjuvant and purified water.
On Day 6 the skin was shaved and coated with 0.5 mL sodium laurylsulfate 10% in vaseline in order to induce a local irritation. On Day 7 the test substance was topically applied at a concentration of 50 % to the clipped and shaved skin of the shoulder region and covered with an occlusive dressing, which was left in place for 48 hours.
The challenge was conducted on Day 21 by an occlusive patch at a concentration of 50% which was applied to the clipped and shaved left flank of each animal for 24 h. The clipped and shaved right flank of each animal was treated in the same way with the vehicle only. 24
and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale.
The animals of the positive control group were treated with a 2 % benzocaine solution intracutaneously in the induction phase and with a 5 % solution topically in the induction phase and at challenge.
Body weights were determined at the first day of treatment of the main study and at termination.
Mortality and clinical signs were recorded daily during the study period.
Evaluation criteria for classification as a potential skin sensitizer:
At the 24-hour and/or 48-hour reading, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.
Results and discussion
Mortality: No deaths occurred.
Clinical observations: No signs of systemic toxicity were observed. Body weight: All animals showed the expected gain in body weight.
Skin reactions: No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group.
Animals treated with the positive control benzocaine in 40% ethanolic 0.9% NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).
Conclusion by the Notifiers
Based on the study results and according to the EU and OECD Globally Harmonized System (GHS) classification criteria, AMPA is not to be classified for skin sensitization.
RMS comments:
The study is considered acceptable. AMPA was not a skin sensitiser in the M&K test.
1st “new” mutagenicity (Ames) test in bacteria (Callander, 1988)
Dates of experimental work: 1988-03-01 to 1988-09-21
Materials and methods
Test Material:
Aminomethyl phosphonic acid
Description:
Crop metabolite of, and an impurity in, ICIA0224, white
solid
Lot/Batch number:
48F-3893
Purity:
>99 % a.i
CAS#:
Not reported
Stability of test compound:

Confirmed by Sponsor
Control Materials:
Negative:
Water
Solvent control (final concentration):
Dimethylsulphoxide – DMSO (10 DL/plate)
Positive control:
Nonactivation:
Acridine mutagen ICR191 TA1537
2-Aminoanthracene TA1537, WP2 uA Daunomycin hydrochloride TA98
4-Nitro-o-phenylene diamine TA1538
N-Methyl-N´-nitro-N-nitrosoguanidine TA1535, TA100, WP2 uA
Activation:
2-Aminoanthracene TA1535, TA1537, TA1538, TA98, TA100, WP2 uA
Acridine mutagen ICR191 TA1537
2-Aminothracene TA1535, TA1537, TA1538, TA98, TA100, WP2 uA
N-Methyl-N´-nitro-N-nitrosoguanidine WP2 uA
Mammalian metabolic system: S9 derived
X
Induced
X
Aroclor 1254
X
Rat
X
Liver
Non-induced
Phenobarbitol
Mouse
Lung
None
Hamster
Other
Other
β-naphthoflavone
Other
The metabolic activation system (S9-mix) used in this study was prepared as a 3:7:20 mixture of S9 fraction, Sucrose-tris-EDTA buffer (250:50:1 mM) and cofactor solution.
The cofactor solution was prepared in bulk as follows: Na2HPO4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and MgCl2 (12 mM).
Test organisms:
S. typhimurium strains
TA97
X
TA98
X

TA100
TA102
TA104
X
TA1535
X
TA1537
X
TA1538
list any others
E. coli strains
WP2
(pKM101)
X
WP2 uvrA
(pKM101)
Properly maintained?
X
Yes
No
Checked for appropriate genetic markers (rfa mutation, R factor)?
X
Yes
No
Test compound concentrations used:
Nonactivated conditions: 5000, 1000, 200, 40, 8 and 1.6 µg/plate
Activated conditions: 5000, 1000, 200, 40, 8 and 1.6 µg/plate
For all strains triplicate plates were used for all test substance and positive control treatments. For solvent controls 5 plates were used.
Study design and methods
Preliminary Cytotoxicity Assay: Not performed. Type of Bacterial assay:
X standard plate test (both experiments –S9, initial experiment +S9)
pre-incubation (60 minutes) (second experiment +S9)
"Prival" modification (i.e. azo-reduction method)
spot test
other
Protocol:
Bacterial cultures were prepared from frozen stocks by incubating for 10-12 hours at 37 °C. The following materials were mixed in a test tube and poured onto the selective agar plates: 100 µL Test solution at each dose level, solvent and positive controls;
500 µL S9 mix or phosphate buffer; 100 µL Bacteria suspension;
2 mL Overlay agar containing 50 µM histidine or tryptophan as appropriate.
In this assay 100 µL aliquots of an overnight culture of each bacteria strain were stored in bijou bottles at room temperature until required (1-2 hours). 500 µL S9 mix (or Co- factor/Buffer mix) was then added by dispensing syringe to the number of bijou bottles of one strain required for one dose level, followed by 0.1 mL of the appropriate concentration of the test substance solution added by

micropipette. Finally, 2.0 mL top agar was added to each bijou, the force of addition was sufficient to mix the contents. The mixture was then rapidly poured onto a prepared Vogel Banner agar plate. After the agar was set the plates were incubated upside down for 64 - 68 hours at 37 °C in the dark. For each strain and dose level including the controls, three plates were used.
Following the total incubation period the plates were examined for the lack of microbial contamination and evidence that the test was valid: i.e. there should be a background lawn on the negative control plates and on the plates for (at least) the lower doses of test substance, and that the positive controls should show at least a two-fold increase in average reversion frequency rate and there should be a dose-response relationship.
The plates were counted using an automated colony counter (AMS 40-10) with the discrimination adjusted appropriately to permit the optimal counting of mutant colonies.
Statistical analysis: None – see Evaluation Criteria below.
Evaluation criteria: A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met:
a significant, dose-related increase in the mean number of revertants is observed;
a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) is observed at one or more concentrations
A negative result in a (valid) individual experiment is achieved when:
there is no significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance; and
in the absence of any such dose response, no increase in colony numbers is observed (at any test concentration) which exceeds 2x the concurrent solvent control.
For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain/S9 combination, then the observed effect(s) must be consistently reproducible.
Results
Mutagenicity assay: In two separate experiments, aminomethyl phosphonic acid did not induce any significant increases in the observed numbers of revertant colonies in Salmonella typhimurium strains TA1535,TA1538, TA98, TA100 and Escherichia coli WP2 uvrA pKM101 in either the presence or absence of an auxiliary metabolising system (S9).
In the first experiment, slight responses were observed in strain TA1537, reaching maxima of 1.9 x and 2.0 x background in the presence and absence of S9 respectively. These responses were only of limited dose-relationships, and were of limited statistical significance in both cases. In two further experiments, no significant increases in colony numbers were observed either with or without S9. This lack of reproducibility indicates that the observed effects in the first experiment are not due to compound-induced mutations.
The positive controls for each experiment induced the expected responses indicating the strains were working satisfactorily in each case.
Conclusion by the Notifiers
Under the conditions of this assay, aminomethyl phosphonic acid gave an unequivocal negative, ie non-mutagenic, response, when tested to a limit dose of 5000 μg/plate.
RMS comments:
The study is considered acceptable. No evidence of genotoxicity of AMPA in bacteria was obtained. The slight increase in revertant numbers in one strain in the first experiment was rather weak and was sufficiently contravened by subsequent trials in which the test material proved clearly negative. It should be noted that this study was already evaluated by the RMS (and later on by the EU) when

submitted as part of the toxicological data package for glyphosate-trimesium (DAR, 1998, ASB2010-10493)
2nd new mutagenicity (Ames) test in bacteria (Akanuma, 1996)
Reference:
IIA, 5.8/06
Report:
Akanuma M. 1996 AMPA Reverse mutation test.
The Institute of Environmental Toxicology, Tokyo, Japan Data owner: Arysta LifeScience
Study No.: IET 96-0076 Date: 1996-12-09
not published, ASB2012-11507
Guidelines:
U.S. EPA FIFRA Guidelines, Subdivision F OECD guidelines 471, 472 (1983)
Japan MAFF guidelines 59 NohSan N° 4200 (1985)
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1996-09-09 to 1996-10-11
Materials and methods
Test material:
AMPA
Identification:
AMPA
Description:
White powder
Lot/Batch #:
A-960719
Purity:
99.33 %
Stability of test compound:
Stable for 1 year at room temperature
Solvent used:
Sterile water
2. control materials:
Negative:
Sterile water
Solvent/final concentration:
Water / 50 mg/mL
Positive: non-activation and activation
Strain
Positive controls
Without S9 (µg/plate)
With S9 (µg/plate)
TA100

AF – 2 (0.01)
2-AA (1)
TA1535
NaN3 (0.5)
2-AA (2)
WP2 uvra
AF-2 (0.01)
2-AA (10)
TA98
AF-2 (0.1)
2-AA (0.5)
TA1537
9-AA (80)
2-AA (2)
AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide dissolved inDMSO; NaN : sodium azide dissolved in sterile water
3
2-AA: 2-aminoanthracene dissolved in DMSO; 9-AA: 9-aminoacridine hydrochloride dissolved in sterile water
activation:
The enzyme activity measured by mutagenicity was good. S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor. The component of S9 mix were 10 % (v/v) S9 fraction, 8 mM MgCl2, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADH, 4 mM NADPH and 100 mM sodium phosphate buffer.
test organisms:
Escherichia coli WP2 uvrA
Salmonella typhimurium (TA100, TA1535, TA98 and TA1537)
test concentrations:
Preliminary cytotoxicity assay: One preliminary assay was performed:
Plate incorporation assay: Concentrations up to 5000 Pg/plate were evaluated with and without S9 activation in strain TA1535, TA1537, TA98, TA100 and WP2 uvrA. A single plate was used, per dose, per condition.
Pre-incubation assay: As above.
Mutation assays:
Plate incorporation assay: 156, 313, 625, 1250, 2500 and 5000 Pg/plate were evaluated in triplicate in the presence and absence of S9 activation; all test strains were used.
Pre-incubation assay: As above for the plate incorporation assay.
The independently repeated mutation assay was conducted using the pre-incubation modification to the standard plate incorporation test. The pre-incubation assay was carried out as described above with the following two exceptions: 0.5 mL of buffer were added to cultures prepared for testing under non-activated conditions; prior to the addition of top agar, reaction mixtures were incubated for 20 minutes at 37 ± 11C.
Statistics
Results were judged without statistical analysis.
Evaluation Criteria
The test items were carried out twice. Reproducibility of results was confirmed by two independent

experiments. Results were judged positive without statistical analysis when the following criteria are all satisfied:
A two-fold or greater increase above solvent control in the mean number of revertants is observed This increase in the number of revertants is accompanied by a dose-response relationship This increase in the number of revertants is reproducible.
Results and discussion
Analytical determinations: None
Table B.6.8-2: Results of the preliminary dose range finding test
Concentration
Revertants (n° colonies/plate)
(µg/plate)
TA100
TA1535
WP2 uvr A
TA98
TA1537
- S9 mix
Solvent Control (H2O)
150*
8*
20*
24*
7*
200
131
11
21
12
8
500
152
10
19
22
6
1000
131
10
15
20
5
2000
154
3
23
24

10
5000
140
11
20
18
11
+ S9 mix
Solvent Control (H2O)
106*
6*
28*
25*
10*
200
92
7
19
16
16
500
116
1
20
24
15
1000
124
5
25
17
12
2000
102
7
24
30
8
5000
128
10
29
26
11
Positive Control
- S9

Mix
Compound
AF-2
NaN3
AF-2
AF-2
9-AA
µg/plate
0.01
0.5
0.01
0.1
80
Revertants/plate
666*
673*
201*
658*
540*
+ S9
Mix
Compound
2-AA
2-AA
2-AA
2-AA
2-AA
µg/plate
1
2
10
0.5
2
Revertants/plate
450*
233*
415*
456*
84*
AMPA did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.
Mutation assays: Results are shown in the following tables.
Table B.6.8-3: Reverse mutation tests without metabolic activation– Experiment 1
Concentration (µg/plate)
Revertants (n° colonies/plate) *
TA100

TA1535
WP2 uvr A
TA98
TA1537
- S9 mix
Solvent Control
(H2O)
101±17
11±1
17±4
18±3
4±3
313
84±7
10±5
17±6
14±2
5±3
625
91±14
8±6
17±7
16±4
4±2
1250
97±17
8±3
14±2
13±2
4±2
2500
91±7
9±1
15±8
15±2
6±2
5000
100±4
7±3
16±4
16±5
3±1
Positive Control (- S9)
Compound
AF-2
NaN3

AF-2
AF-2
9-AA
µg/plate
0.01
0.5
0.01
0.1
80
Revertants/plate
619±57
619±45
160±22
667±60
710±73
* : Average ± SD
Table B.6.8-4: Reverse mutation tests with metabolic activation– Experiment 1
Concentration (µg/plate)
Revertants (n° colonies/plate) *
TA100
TA1535
WP2 uvr A
TA98
TA1537
+ S9 mix
Solvent Control
(H2O)
105±11
10±4
19±1
30±5
10±1
313
105±5
12±2
16±4
28±5
9±3
625
92±6
6±1
16±1
28±7
13±2
1250
90±3

6±1
16±2
25±7
11±3
2500
83±9
9±4
20±4
25±8
10±3
5000
93±10
10±4
24±6
32±10
7±1
Positive Control
(+S9)
Compound
2-AA
2-AA
2-AA
2-AA
2-AA
µg/plate
1
2
10
0.5
2
Revertants/plate
529±33
184±5
384±20
407±11
94±2
* : Average ± SD
Table B.6.8-5: Reverse mutation tests without metabolic activation– Experiment 2
Concentration (µg/plate)
Revertants (n° colonies/plate) *
TA100
TA1535
WP2 uvr A
TA98
TA1537
- S9 mix

Solvent Control
(H2O)
120±3
9±3
15±3
18±4
3±2
313
136±9
4±1
18±3
14±4
4±3
625
124±16
5±2
16±3
13±3
3±2
1250
107±11
6±4
12±4
15±2
3±3
2500
96±6
9±4
12±3
16±6
4±0
5000
117±2
7±3
20±5
13±2
3±2
Positive Control (- S9)
Compound
AF-2
NaN3
AF-2
AF-2
9-AA
µg/plate

0.5
0.01
0.1
80
Revertants/plate
668±27
696±20
182±16
650±8
698±53
* : Average ± SD
Table B.6.8-6: Reverse mutation tests with metabolic activation– Experiment 2
Concentration (µg/plate)
Revertants (n° colonies/plate) *
TA100
TA1535
WP2 uvr A
TA98
TA1537
+ S9 mix
Solvent Control
(H2O)
95±3
8±2
17±3
28±5
7±2
313
112±14
8±3
17±4
21±6
10±5
625
84±5
7±4
16±5
21±5
7±3
1250
106±8
7±2
17±4
28±9
7±1
2500

97±4
11±3
16±2
21±1
6±1
5000
115±12
9±5
22±2
22±3
6±5
Positive Control (+S9)
Compound
2-AA
2-AA
2-AA
2-AA
2-AA
µg/plate
1
2
10
0.5
2
Revertants/plate
584±56
169±28
461±8
334±14
82±4
* : Average ± SD
Conclusion by the Notifiers
A two-fold or greater increase in the mean number of revertant colonies was not observed in any strain at any dose of AMPA in the reverse mutation tests with or without metabolic activation. It is concluded that AMPA is non mutagenic for bacteria under the conditions used with this experiment.
RMS comments:
In contrast to the description in the dossier, it seems that testing was performed by means of the pre-incubation method only. Therefore, the study is considered supplementary only because a plate-incorporation test was not performed.
When the study description in the dossier was compared to the original study report, it was noted that the study director was Mie Akanuma. Erroneously, the first name had been mentioned in the dossier instead of the authors surname.
UDS asssay for DNA damage and repair in vitro (Nesslany, 2002)
Reference:
IIA, 5.8/07
Report:

Nesslany, F. 2002 Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes in vitro procedure with AMPA (Amino methyl phosphonic acid).
The Institute of Environmental Toxicology, Tokyo, Japan Data owner: Arysta LifeScience
Study No.: IPL-R-02025 Date: 2002-09-10
not published, ASB2012-11508
Guidelines:
OECD guideline n° 482
Deviations:
None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 2002-04-29 to 2002-07-02
Materials and methods
Test material:
AMPA
Identification:
AMPA
Description:
White crystalline powder
Lot/Batch #:
020404
Purity:
99.9 %
Stability of test compound:
Not mentioned in the report
Solvent used:
William's E medium Gibco
2. control materials:
Negative:
/
Solvent/final concentration:
See above
Positive:
2-acetamidofluprene
activation:
None
test organisms:
Rats hepatocytes
test concentrations: 5 dose level were tested: 10, 5, 2.5, 1.25 and 0.625 mM with and without S9 metabolic activation
Test principle
Hepatocytes were isolated from livers of rats. The primary hepatocyte cultures were exposed to the test article in the presence of 3H thymidine which is incorporated into the DNA, if DNA damage is occuring. DNA repair systems then stimulated UDS and increased the incorporation of thymidine

which was measured by grain counting after autoradiography of hepatocytes.
The following results are presented:
The average NNG and standard deviation
The percent of cells in repair and standard deviation (>=5) The average cytoplasmic and nuclear grain count
The number of cells in S-phase
Statistics
Results were judged without statistical analysis. Evaluation Criteria
Results are judged positive when:
At any dose tested, group, mean NNG value greater than 0 NNG and 20 % or more of cells responding (NNG value >=5)
An increase is seen in both NNG and the percentage of the cells in repair
A dose related increase is seen in both NNG and the percentage of the cells in repair Any induction of UDS can be reproduced in an independent experiment.
Validity Criteria
The assay is considered valid if:
Negative control slides have a group mean NNG value within the historical range.
The positive control have group mean NNG values of less than 5 NNG counts with 50% or more cells having NNG counts of 5 or more and statistically significant relative to the solvent control.
Results and discussion
Analytical determinations: None
Mutation assays: Negative control gave a group mean NNG value of less than zero with a percentage of cells in repair comparable with historical control data. In positive controls, group mean net nuclear grain count (NNG) values as well as percentage of cells in repair obtained were within the range of historical control. The sensitivity of the cell type used to a DNA damaging agent requiring metabolism for its action, 2-acetamidofluorene was demonstrated. Thus, the validity criteria of the test were fulfilled.
The findings of both experiments are summarised in the tables below.
Table B.6.8-7: UDS data of the first experiment
Concentration (mM)
Net Nuclear Grain Count (NNG)
Net Nuclear Grain
Count of cells in repair (NNG >5)
% cells in repair (NNG >5)
Mean
+/- sd
Mean
+/- sd
Mean
+/- sd
Solvent control
-2.38
5.20
5.63
0.51
4.21

1.53
0.625
-3.81
5.29
6.61
1.56
3.89
1.53
1.25
-3.61
5.23
6.99
0.56
4.02
1.53
2.5
-3.93
5.24
6.83
0.91
5.18
1.53
5
-3.35
5.10
6.28
1.66
3.45
1.00
10
-2.04
4.82
6.91
0.74
5.43
1.53
Positive control:
2-acetamidofluorene
6.25 µM
30.81
18.22
31.55
4.84
96.61
2.00

Table B.6.8-8: UDS data of the second experiment (amended by RMS because of errors)

Concentration (mM)
Net Nuclear Grain Count NNG
Net Nuclear Grain
Count of cells in repair NNG >5
% cells in repair NNG >5
Mean
+/- sd
Mean
+/- sd
Mean
+/- sd
Solvent control
-4.62
5.81
5.37
0.21
3.74
2.08
0.625
-4.77
5.46
6.90
0.60
1.78
0.00
1.25
-4.04
5.35
6.87
1.00
4.49
1.53
2.5
-3.81
5.82
7.07
1.61
6.18
2.08
5
-3.47
5.83
7.83
0.29
7.73
1.73

10
-5.58
6.06
6.97
1.29
3.76
1.53
Positive control:
2-acetamidofluorene
6.25 µM
17.57
11.18
17.73
3.29
94.94
9.45
Over the two experiments, group mean net nuclear grain count (NNG) values at the dose range tested from 10 to 0.625 mM were less than zero (-2.04 to –3.81 vs –2.38 in solvent control in the first UDS assay and –5.58 to –4.77 vs –4.62 in solvent control in the second UDS assay), that is to say below the threshold valure of 0 NNG for a positive response.
Furthermore, no significant increase in the percentage of cells in repair at any dose of AMPA tested when compared with the respective controls (5.43 to 3.89% vs. 4.21% in solvent control in the first assay and 3.76 to 1.78 % vs. 3.74% in solvent control in the second assay). In addition, in cells in repair, group mean net nuclear grain count (NNG≥5) values were comparable with the solvent controls (6.91 at 10 mM to 6.61 at 0.625 mM vs. 5.63 in control in the first assay and 6.97 ta 10 mM to 6.90 at 0.625 mM vs. 5.37 in control in the second assay).
Conclusion by the Notifiers
Under the conditions of this experiment, AMPA did not reveal any genotoxicity activity in the Unscheduled DNA synthesis assay.
RMS comments:
The study is considered acceptable. There was no evidence of a direct interaction of AMPA with the DNA of primary rat hepatocytes.
In contrast to what was mentioned in the GTF dossier, the test assay was performed in the “Institut Pasteur de Lille” in France and not in Japan.
B.6.8.2 Mechanistic studies on certain aspects of the toxicity of glyphosate
B.6.8.2.1 Studies on mechanism of salivary gland findings
Introduction by RMS:
A few studies have been submitted to investigate the mechanism behind the salivary gland findings that were obtained in quite many subchronic, long-term and reproduction studies in rats (see sections B.6.3, B.6.5 and B.6.6) and occasionally also in mice (, 1992, TOX9551954). The first experiment to elucidate the mode of action was performed by (1992, TOX9551954) as part of their comprehensive investigations within
the U.S. NTP and is described in the original DAR (1998, ASB2010-10302) on glyphosate in detail. The study was considered acceptable upon re-evaluation. In order to provide a most comprehensive evaluation of salivary gland changes, this part from the old DAR is copied here, followed by a summary Table B.6.8-9 and the detailed description of two more recent studies by (1996, ASB2012-

11537/ASB2012-11520) and (2010, ASB2012-11519),
commented by the RMS.
Copy from the previous DAR: Possible explanation for salivary gland alterations
“Because of morphologic similarities to salivary gland changes observed with the adrenergic agonist isoproterenol, a subacute study was designed to test the hypothesis that the salivary gland effects of glyphosate were also mediated through an adrenergic mechanism (
, 1992, TOX9551954). Groups of four male F344/N rats received glyphosate at a dietary level of 50000 ppm or were fed an untreated control diet. In three of the groups, the adrenergic agonist isoproterenol and/or the antagonist propranolol were administered by continuous subcutaneous infusion by osmotic minipumps. The study design was as follows: Group 1: control diet, only vehicle (water + 0.1% ascorbate) administered by minipump; Group 2: glyphosate diet, only vehicle administered by minipump;
Group 3: glyphosate diet, propranolol (1.2 mg/kg bw/d) administered by minipump; Group 4: control diet, isoproterenol (1.0 mg/kg bw/d) administered by minipump; Group 5: control diet, isoproterenol and propranolol administered by minipump.
After 14 days of treatment, the left parotid and submandibular/sublingual salivary glands were removed, weighed separately and processed for electron microscopy. The right parotid and submandibular/sublingual salivary glands were removed, sectioned and stained for histological evaluation.
Both isoproterenol and glyphosate induced significant enlargement of the salivary glands, glyphosate having much greater effect than isoproterenol. The parotid was most affected. Propranolol inhibited the effect of both substances on salivary gland weight but not completely in the case of glyphosate. Microscopically, similar changes were induced by glyphosate and isoproterenol consisting of cytoplasmic basophilic change, fine vacuolation and swelling of acinar cells resulting in a relative reduction in the number of ducts present. Glyphosate-treated animals were most severely affected. Propranolol, however, clearly protected the rats from the more severe lesions. Likewise, modest protection of histological effects caused by isoproterenol was seen. Cytoplasmic alteration of the submandibular gland
was more subtle and histologically detectable only in glyphosate-treated animals. However, electron microscopy elucidated an effect of isoproterenol on this gland, too. It could not be determined if the serous or mucous glandular acini were selectively affected by glyphosate. No changes were seen in the sublingual glands examined from any group demonstrating target specifity of glyphosate- and isoproterenol-associated lesions to those salivary glands which are mainly innervated by adrenergic fibers.
The authors assume that effects of glyphosate on salivary glands were due to an adrenergic mechanism. The biological significance of this finding is unkown (, 1992, TOX9551954).”
Table B.6.8-9: Summary of new mechanistic studies on salivary gland effects
Reference; study identification;
owner
Type of study; species, strain
Application route (dose)
Test substance
Purity [%]
Results

Studies not reviewed in the 2001 evaluation
2010; ASB2012- 11519;
Monsanto
8-week oral toxicity; Sprague-Dawley Rat, ♂
Citric acid: Oral gavage (791-1316
mg/kg bw) or via diet (14000 ppm); Trisodium citrate dehydrate: diet
(21400 ppm)
Citric acid, Trisodium citrate dihydrate
99.3
Higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands
1996;
ASB2012-
11520 and
2012-
115337;
Syngenta
4-week oral
toxicity; Sprague-Dawley (CD)/Fischer 344/Alpk:APSD (AP), Rat, ♂
Diet (20000
ppm)
Glypho-
sate acid, batch P24
95.6
Marked strain differences in
the severity of effect in the parotid salivary glands; most pronounced effect occurred in the F344 strain: diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells; similar but slighter an apparently reversible effects occurred in the AP and CD strains involving small foci
of cells only
Effects of citric acid (2010)
Reference: IIA, 5.10/01
Report: (2010)
An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats
Data owner: Monsanto/GTF Study No.: WIL-50361 Date: 2010-01-08
Unpublished ASB2012-11519
Guidelines: Guideline does not exist for this kind of study but data from the study report is similar to OECD 408.
Deviations:
not applicable
GLP:
yes
Acceptability:
See RMS comment
Dates of experimental work: 2009-02-24 to 2009-05-15
Materials and methods
Test materials:

Identification:
Anhydrous Citric Acid
Description:
White powder
Lot/Batch #:
XR3050
Purity:
99.9 %
Stability of test compound:
Stable at room temperature until 2010-01-06.
Identification:
Trisodium Citrate Dihydrate (TCD)
Description:
White crystalline solid
Lot/Batch #:
1387609
Purity:
99.3 %
Stability of test compound:
Stable at room temperature until 2011-03-01.
Vehicle:
Gavage: deionised water, Diet: plain diet
Test animals:
Species:
Rats
Strain:
Sprague-Dawley (CD)
Source:
Age:
approx. 6 weeks upon beginning of treatment
Sex:
Males
Weight at dosing:
177 - 227 g
Acclimation period:
14 days
Diet/Food:
Certified Rodent LabDiet #5002 (PMI Nutrition International, LLC), ad libitum
Water:
tap water, ad libitum
Housing:
Upon arrival, animals were housed three per cage for approximately 3 days. Thereafter, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 50 ± 20 %

Air changes: at least 10/hour 12 hours light/dark cycle
Animal assignment and treatment:
In a 8 week gavage and feeding study, groups of 10 Sprague Dawley rats received the respective vehicles or test substances for 56 consecutive days via oral gavage (Groups 1 and 3) or in the diet (Groups 2, 4 and 5; see Table B.6.8-10). A low pH diet containing 14000 ppm of citric acid in basal diet was offered continuously to Group 4. A high pH diet containing 21400 ppm of trisodium citrate dihydrate in basal diet (at an equivalent citrate ion concentration to Group 4) was offered continuously to Group 5. A concurrent control group (Group 2) received the basal diet on a comparable regimen. Citric acid in the vehicle, deionised water, was administered orally by gavage at a dose level of 791-1316 mg/kg/day to Group 3.
Concentrations of the Group 3 formulations were calculated and adjusted weekly, based on the average food consumption and body weights of the Group 4 animals from the previous week of dosing in order to maintain approximately equivalent citric acid dose levels to Group 4. A concurrent gavage control group (Group 1) received the vehicle on a comparable regimen.
Table B.6.8-10: Study group assignment

Group Number	Test Substance application	Dose Level (mg/kg bw/day or ppm)	Dose Volume (mL/kg)	Number of animals
1	Gavage Vehicle	0	10	10
2	Basal Diet	0	na	10
3	Gavage Citric Acid (low pH)	791-1316	10	10
4	Diet Citric Acid (low pH)	14,000	na	10
5	Diet Trisodium Citrate (high pH)	21,400	na	

10
na - not applicable
Observations
All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly.
Body weight
Individual body weights were recorded weekly.
Food consumption and compound intake Food consumption was recorded weekly.
Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: parotid salivary glands, mandibular salivary glands and sublingual salivary glands. The mandibular and sublingual salivary glands were weighed together as one organ since they were fused and could not be adequately separated for weighing.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum and femur (incl. joint)), brain (cerebrum at two levels; cerebellum with medulla/pons), caecum, colon, duodenum, epididymides, eyes with optic nerves, gross lesions, harderian glands, heart, ileum, jejunum, kidneys, lacrimal gland (exorbital), liver, lungs (incl. bronchi), mammary gland, lymph nodes (mandibular, mesenteric and axillary), nasal cavity, oesophagus, pancreas, Peyer's patches, pituitary,
prostrate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea and urinary bladder.
Microscopic examination was performed on the parotid salivary glands and gross lesions from all animals at the scheduled necropsy.
Statistics
All statistical tests were performed using the WIL Toxicology Data Management System (WTDMS™). Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1 % and 5 %, comparing each test substance-treated group to its respective control group.
Body weight, body weight change, food consumption, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA identified statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare each of the test substance-treated groups to the respective control group (Group 1 to Group 3 and Group 2 to Groups 4 and 5). Group 1 was also compared to Group 2. Statistical analysis of the severity of histological changes was conducted. Individual animals were assigned severity scores based on parotid salivary gland changes (0=without histological change, 1=minimal change, 2=mild change, and 3=moderate change). The severity scores were then compared statistically using the Mann-Whitney U-test by comparing Group 1 to Group 3 and Group 2 to Groups 4 and 5.
Results and discussion
Mortality:
No deaths occurred during the study.
Clinical observations:
All clinical findings in the test substance-treated groups were noted with similar incidence in the control groups, were limited to single animals, and/or were common findings for laboratory rats of this age and strain.

Body weight:
There were no statistically significant differences when the respective control and test substance-treated groups were compared.
Food consumption:
Food consumption was unaffected by citric acid or trisodium citrate dihydrate administration. A statistically significant decrease in food consumption of the gavage citric acid group (Group 3, Week 7/8) was probably due to biological variability and not considered related to test substance administration.
Organ weights:
Test substance-related effects on organ weights consisted of statistically significant higher absolute and relative parotid salivary gland weights in the low pH diet group (14,000 ppm citric acid) when compared to the dietary control group; the magnitude of change was > 40% (Table B.6.8-11). Higher absolute and relative parotid salivary gland weights were also observed in the low pH gavage group (791-1316 mg/kg bw/day citric acid) and in the high pH diet group (21,400 ppm TCD) when compared to their respective control groups. However, the parotid salivary gland weight differences in the low pH gavage and high pH diet groups were not statistically significant and were of much lesser magnitude of change.
There were no other statistically significant test substance-related effects on the fused mandibular/ sublingual or parotid salivary gland weights when the control groups and test substance-treated groups were compared.
Table B.6.8-11: Organ weight differences of salivary glands
Gavage Administration
Dietary Administration
aqueous control
791-1316 mg/kg bw/day citric acid
basal diet control
low pH diet, 14000 ppm citric acid
high pH diet, 21400 ppm trisodium citrate
dihydrate
Mean Absolute
Mandibular /
0.7625
0.7873
0.7682
0.8872
0.7869
Sublingual Fused
± 0.05446
± 0.08397
± 0.08670
± 0.16548
± 0.07028
Glands Weight (g)
Mean Relative
Mandibular /
0.179

0.180
0.173
0.199
0.183
Sublingual Fused
± 0.0105
± 0.0178
± 0.0221
± 0.0339
± 0.0201
Glands Weight (g)
Mean Absolute
Parotid Gland Weight (g)
0.3500
± 0.12450
0.4082
± 0.11990
0.2758
± 0.08514
0.3905*
± 0.10920
0.3502
± 0.08986
Mean Relative
Parotid Gland Weight (g)
0.083
± 0.0299
0.095
0.0304
0.062
± 0.0194
0.088*
± 0.0236
0.082
0.0220
* - significantly different from relevant control group ($p < 0.05$) using Dunnett's test
Necropsy:
All macroscopic findings noted were considered spontaneous and/or incidental in nature and unrelated to test substance administration.
Histopathology:
Test substance-related histological effects consisted of a higher severity of cytoplasmic alterations in the parotid salivary glands of the citric acid and trisodium citrate dihydrate- treated groups when compared to their respective control groups (Table B.6.8-12). The severity of cytoplasmic alteration was increased in all dose groups; however, the cytoplasmic alteration was clearly most severe in the low pH diet group (Group 4; 14000 ppm citric acid). Cytoplasmic alteration in the parotid salivary glands was characterized by the presence of hypertrophied acinar cells with basophilic granular

cytoplasm. The severity grades ranged from minimal to moderate, displayed by increasing numbers of affected acinar cells and more pronounced hypertrophy of acinar cells with increasing severity grade.

Cytotoxicity and hyperplasia were not observed and consequently, the observed changes were considered to be adaptive responses rather than adverse effects. There were no other test substance-related histological changes.

Table B.6.8-12: Toxicologically relevant histological changes

Gavage Administration
Dietary Administration
aqueous control
791-1316 mg/kg bw/day citric acid
basal diet control
low pH diet, 14,000 ppm citric acid
high pH diet, 21,400 ppm
trisodium citrate dihydrate
Parotid salivary
glands a
9
10
10
10
10
Incidence (%)
100
100
70
100
90
Minimal
8
6
5
0
4
Mild
1
3
2
6
5
Moderate
0
1
0
4
0

Average
severity b
1.1
1.5
0.9
2.4**
1.4
a - number of tissues examined from each group
b - 1= minimal, 2= mild and 3= moderate; animals without a histological change were assigned a severity score of 0
** - significantly different from relevant control group ($p < 0.01$) using the Mann-Whitney U-Test
Conclusion by the Notifiers
Citric acid administered orally via gavage or diet and trisodium citrate dihydrate administered via the diet to Sprague Dawley rats for 56 days resulted in higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands at all dose levels (791-1316 mg/kg bw/day gavage citric acid, 14000 ppm diet citric acid, and 21400 ppm diet trisodium citrate dihydrate). This effects were noted as most severe in the low pH dietary test group. In the absence of cytotoxicity and hyperplasia the noted effects are considered an adaptive response rather than an adverse effect and are consistent with the hypothesis that low pH diets result in adaptive cellular responses within the salivary glands.
RMS comments:
The study is considered supplementary because the test substance to be evaluated, i.e., glyphosate, was not included itself. However, it is suitable to demonstrate that organ weight and histological effects on salivary glands resembling very much those which were observed in some studies following glyphosate administration may be in fact due to low pH conditions in the oral cavity. Such conditions may occur when an acidic diet is administered as it was the case in feeding studies with glyphosate. It is agreed that the resulting salivary gland effects would be then rather adaptive than toxic. However, in principle, organ weight and histological changes may become adverse even if they are adaptive by nature. Furthermore, it cannot be excluded that other mechanisms might have also contributed to the observed findings in the glyphosate studies.
Comparison of sensitivity of different rat strains to salivary gland lesions (Allen, 1996)
Reference: IIA, 5.10/02
Report: (1996)
Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat.
Data owner: Syngenta Study No.: CTL/P/5160 Date: 1996-08-19
Unpublished
ASB2012-11520/11537
Guidelines: Guideline does not exist for this kind of study.
Deviations: not applicable
GLP: yes
Acceptability: See RMS comment
Dates of experimental work: 1996-01-15 to 1996-05-14
Materials and methods
Test materials:
Identification:
Glyphosate acid

Description:
White solid
Lot/Batch #:
P24
Purity:
95.6 %
Stability of test compound:
No data given in the report.
Vehicle and/
or positive control:
Plain diet
Test animals:
Species:
Rats
Strain 1:
Alpk:APfSD
Source:
Weight at dosing:
175.0 – 176.1 g
Strain 2:
Sprague-Dawley
Source:
Weight at dosing:
179.6 – 181.5 g
Strain 3:
Fischer 344
Source:
Weight at dosing:
107.4 – 108.9 g
Age:
approx. 28-30 days (on delivery)
Sex:
Males
Acclimation period:
11-13 days
Diet/Food:
CT1, ad libitum
Water:
Tap water, ad libitum
Housing:
Animals were housed by strain and four per cage.
Environmental conditions:
Temperature: 21 ± 3 °C
Humidity: 50 ± 20 %
Air changes: at least 15/hour 12 hours light/dark cycle
Animal assignment and treatment:

In a 28 days feeding study groups of 24 male Alpk:APfSD (Wistar-derived; AP), Sprague- Dawley (Charles River CD; CD) and Fischer 344 (F344) rats received 0 or 20000 ppm glyphosate acid. Eight animals from each group were killed on Day 29 and the remaining animals were retained without treatment for a further 4 (8 rats/group) or 13 weeks (8 rats/group).
Two test diet batches were prepared prior to start of treatment by mixing 1255 g test substance to 58.745 kg diet and blending. Samples of both preparations were analysed to verify the achieved concentration.
Clinical observations
Clinical examinations were performed daily. A detailed physical examination was performed prior to administration and weekly thereafter.
Body weight
Individual body weights were recorded on start of administration and weekly thereafter.
Food consumption
Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage.
Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination of the salivary glands. Thereafter the salivary glands were removed, weighed (left and right separately) and examined by light microscopy.
Statistics
All data were evaluated using analysis of variance and/or covariance by the GLM procedure in SAS (1989). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least- squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based in the error mean square in the analysis.
Results and discussion
Analysis of dose formulations
The mean achieved concentration of glyphosate acid in both batches of diet was within 2% of the target concentration.
Mortality
There were no treatment-related deaths. One treated AP rat was killed in Week 7 following accidental damage to its snout.
Clinical observations
There were no treatment-related findings in any of the groups noted during the study period.
Body weight
AP rats: During the administration period significant reductions in group mean bodyweight compared to control were seen. At the end of the administration period the difference was approximately 7 %. The reduction in bodyweight was maintained during the 4-week recovery period (approximately 7 % at the end of Week 9) but no differences in body weight were apparent by the end of the 13-week recovery period.
CD rats: Group mean bodyweights for treated animals were significantly reduced during the administration period in comparison to controls. The reduction in body weight was approximately 7 % (after adjusting for initial body weight) at the end of the administration period. However, body weights quickly recovered and were 5 % higher than controls (after adjusting for initial body weight)

by the end of the 13-week recovery period.
F344 rats: No treatment related effects were observed. Food consumption
AP rats: Overall, food consumption in the treated group tended to be slightly lower than the control during the administration period although this did not achieve statistical significance. No effects were seen at the end of the recovery period.
CD rats: Group mean food consumption for treated animals was generally lower than controls during the administration period although this did not always attain statistical significance. Food consumption for the recovery animals returned to control levels by Week 8.
F344 rats: There was no evidence of any treatment related effects. Organ weights
There was no evidence of any effects of glyphosate acid on the salivary gland weight at any time point in CD rats. On the contrary salivary gland weights were increased in the treated AP and F344 rats at the end of the administration period in comparison to control. While no effects were noted in the four or 13-week recovery AP animals, in F344 rats the salivary gland weights were still increased at these time points, although there was clear evidence of recovery.
Table B.6.8-13: Mean salivary gland weights at necropsy
Organ
AP
CD
F344
0
20000
0
20000
0
20000
Terminal weight (g)
Left salivary gland
0.652
0.740*
0.715
0.695
0.461
0.666**
Right salivary gland
0.523
0.659*
0.623
0.626
0.422
0.577*
Weight after 4 week recovery
Left salivary gland
0.748
0.703
0.844
0.742

0.488
0.555
Right salivary gland
0.639
0.623
0.701
0.637
0.428
0.505*
Weight after 13 week recovery
Left salivary gland
0.750
0.760
0.790
0.819
0.623
0.612
Right salivary gland
0.669
0.681
0.668
0.705
0.495
0.528

No macroscopic abnormalities were seen in salivary glands in any rat, either at the end of the administration period or after the four or 13-week recovery periods.

Treatment-related histological findings were confined to the parotid salivary gland and comprised alteration in the staining of the cytoplasm of the acinar cells. The affected cells appeared strongly basophilic and enlarged (recorded as basophilia of parotid acinar cells).

At the end of the four-week administration period this change was most prominent in F344 rats. All rats showed marked cytoplasmic basophilia that was diffuse, involving the whole of the parotid gland. However, no evidence of cell degeneration or necrosis was seen. Most of the control F344 rats also showed a minor degree of basophilia involving occasional acinar cells only.

The other two strains, AP and CD, both showed the same effect in the parotid gland after four weeks treatment but at a much reduced severity compared to the F344. In addition the distribution was different in that only small focal groups of acinar cells were affected in the AP and CD rats in contrast to the diffuse involvement seen in the F344. The effect was weakest in the CD rat.

The incidence data at the end of the administration period indicate that the background change varies in control rats in the three strains. None was seen in the AP controls, there was a single CD control rat with a minimal focal change, whereas 7 out of 8 F344 controls showed minor changes.

After four weeks recovery in the F344 strain the severity of the parotid basophilia was reduced to minimal or slight and affected small foci of acinar cells only. No changes were seen in the CD rats and only a single AP rat showed a minimal change. As an AP control rat showed changes at this time point this is considered not to be related to treatment.

After 13 weeks recovery no treatment related changes were seen in the AP and CD strains. Slightly more of the F344 rats showed minor focal changes compared to the corresponding control group but

this may reflect variations in the background spontaneous change rather than a residual effect of treatment.

Table B.6.8-14: Histopathological findings in salivary glands

Finding
AP
CD
F344
0
20000
0
20000
0
20000
Termination*
Atrophy (marked)
0 / 8
0 / 8
1/8
0 / 8
0 / 8
0 / 8
Interstitial fibrosis
(marked)
0 / 8
0 / 8
1/8
0 / 8
0 / 8
0 / 8
Basophilia of parotid
acinar cells
0 / 8
8 / 8
1/8
7 / 8
7 / 8
8 / 8
4 week recovery*
Mononuclear cell
infiltration (minimal)
0 / 8
1 / 8
0 / 8
0 / 8
1 / 8
0 / 8

Basophilia of parotid
acinar cells
1 / 8
1 / 8
0 / 8
0 / 8
0 / 8
6 / 8
Mucous metaplasia of
parotid (slight)
0 / 8
1 / 8
0 / 8
0 / 8
0 / 8
0 / 8
13 week recovery*
Mononuclear cell
infiltration (minimal)
0 / 8
0 / 8
0 / 8
0 / 8
1 / 8
1 / 8
Atrophy (minimal)
0 / 8
0 / 8
0 / 8
0 / 8
1 / 8
0 / 8
Basophilia of parotid acinar cells
1 / 8
1 / 8
1 / 8
1 / 8
1 / 8
5 / 8
* number of animals affected / total number of animals examined
Conclusion by the Notifiers
Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slighter effects occurred in the AP and CD) strains involving small foci of cells only.

Complete recovery of effects was apparent in AP and CD strains following the 4-week recovery period and significant recovery had occurred in the F344 strain. It is not clear whether the slightly higher incidence of minor focal changes in the salivary glands of the F344 strain after 13-week recovery was a residual effect of treatment or represented the random variation in the background incidence in this strain.
RMS comments:
The study is considered acceptable. Clear strain differences became obvious and might, to some extent, explain why salivary gland changes were observed in some but not all rat studies. However, it is still surprising that, e.g., histological lesions were seen in a long-term study in AP rats by Milburn (1996, TOX2000-1998) but not by Brammer (2001, ASB2012- 11488) in the same laboratory although nearly identical dose levels had been employed. (However, the test material was of slightly lower purity in the Milburn study.)
It cannot be excluded that similar salivary gland effects as in rats would also occur in humans if exposure to glyphosate was high enough. F344 rats were rarely used for toxicological testing of glyphosate. Thus, it cannot be argued that a rat strain of particular sensitivity was employed in studies that were used for risk assessment. Furthermore, there is no proof that particularly sensitive F344 rats, with regard to salivary gland effects, were a less suitable model for man than other rat strains.

Table B.6.8-15: Overview on newly submitted mechanistic studies for effects other than on salivary glands (provided by the Notifiers)

Reference; Study identification; Owner	Type of study Species, Strain	Application Route (Dose)	Test substance Purity [%]	Results
1996; ASB2012- 12054; Nufarm	Screening study for pharmaco- logical effects, Sprague-Dawley Rat, ♂ + ♀ for in vivo investi- gations, isolated organs from rats and guinea pigs for ex vivo experiments	Gavage (5000 mg/kg bw, single dose), for in vivo experiments; guinea pig ileum exposed in an isolated organ bath; for examination of neuromuscular activity follo- wing injection into previously killed animals	Glypho- sate technical 95.3	No haematological, electrocardiopgraphic or behavioural/functional changes after oral administration; contractile response similar to that seen with known parasympatho- mimetic agents in isolated guinea pig ileum; no neuromuscular blocking activity on innervated rat gastrognemius muscle
2012; ASB2012- 11521; Monsanto	Mice, B5C3F1 ♀			

Diet 0, 500,
1500, 5000
ppm (0, 150,
449,
1448 mg/kg bw/day)
Glypho-
sate
95.11
No suppression of the
humoral component of the immune system. No test- substance-related effects
Pharmacological activity (1996)
Reference: IIA, 5.10/03
Report: (1996)
Glyphosate Technical: Pharmacology Screening Study in the Rat
Data owner: Nufarm Study No.: 434/021 Date: 1996-06-28
Unpublished ASB2012-12054
Guidelines: JMAFF, 59 Nohsan No. 4200 (1985)
Deviations:
not applicable
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 1996-02-06 to 1996-04-04
Materials and methods
Test materials:
Identification:
Glyphosate Technical
Description:
White powder
Lot/Batch #:
H95D161A
Purity:
95.3 %
Stability of test compound:
No data given in the report.
in-vivo
1% carboxymethyl cellulose
Vehicle:
ex-vivo (guinea pig)
distilled water, krebs physiological buffer solution,
ex-vivo (guinea pig)
physiological saline
Test animals:
in-vivo
Species:

Rats
Strain:
Sprague-Dawley (CD)
Source:
Age:
no data
Sex:
Males and females
Weight at animal receipt (corrected by RMS):
176 - 200 g
Acclimation period:
At least 6 days
Diet/Food:
SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
By sex in groups of five in polypropylene cages with stainless steel grid floors.
Environmental conditions:
Temperature: 19 – 25 °C
Humidity: 40 – 75 %
Air changes: at least 15/hour 12 hours light/dark cycle
ex-vivo
Species:
Guinea pig
Strain:
Dunkin Hartley
Source:
David Hall Ltd., Staffordshire, UK
Age:
no data
Sex:
Males
Weight at animal receipt:
250 - 300 g
Acclimation period:
no data
Diet/Food:
Guinea Pig FDI Diet (Special Diets Services Ltd., Witham Essex, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
By sex in groups of up to three in polypropylene cages with solid floors and sawdust bedding.
Environmental conditions:
Temperature: 17 – 23 °C
Humidity: 30 – 70 %

Air changes: at least 15/hour 12 hours light/dark cycle
ex vivo (amended by RMS)
Species:
Rats
Strain:
Sprague-Dawley (CD)
Source:
Charles River UK Ltd., Margate, Kent, UK
Age:
no data
Sex:
Males and females
Weight at animal receipt :
110 - 125 g
Acclimation period:
no data
Diet/Food:
SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), ad libitum
Water:
Tap water, ad libitum
Housing:
By sex in groups of five in polypropylene cages with stainless steel grid floors.
Environmental conditions:
Temperature: 19 – 25 °C
Humidity: 30 – 70 %
Air changes: at least 15/hour 12 hours light/dark cycle
Animal assignment and treatment of in vivo studies:
Three groups of five male and five female rats each received glyphosate technical at a dose level of 5000 mg/kg bw by oral gavage. The control group was similar sized receiving vehicle only. The dosing volume was 10 mL/kg bw. Approximately one hour after dosing control and treated animals were examined for either haematological changes, electrocardiographic changes or behavioural/functional changes.
Blood parameters
Blood samples were taken from all animals via a tail vain. The following parameters were evaluated: Haemoglobin (Hb), total erythrocyte count (RBC), haematocrit (Hct), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (WBC), platelet count (PLT) and clotting (Prothrombin) time (CT).
Cardiovascular system
After animals were anaesthetised, cardiac activity was assessed using an electrocardiograph. A limb lead was attached to each limb and connected to the electrocardiogram. The equipment was set to lead II measurement at a sensitivity of either 10 mm/mvolt or 5 mm/mvolt and a chart speed of 25 mm/second. The following parameters were evaluated: Heart rate, P-R interval, QRS interval, Q-T interval, P-amplitude, R-amplitude, T-amplitude.
Nervous system
Animals were placed individually in a purpose built arena and assessed for behaviour and response to various stimuli using a modified Irwin Screen. The following parameters were evaluated: Salivation,

hypo/hyperthermia, skin colour, respiration, lacrymation, palpebral closure, pilo-erection, exophthalmia, gait, twitches, tremors, convulsions, abnormal behaviour, tail elevation, transfer arousal, urination, defaecation, vocalisation, finger approach, touch escape, tail & toe pinch, grasp response, auditory startle response, pupil response to light, palpebral reflex.

Animal assignment and treatment of ex vivo studies: Guinea pig - Isolated ileum

Sections of ileum were dissected from previously untreated guinea pigs killed by cervical dislocation. and were transferred to a purpose built isolated organ bath containing Krebs buffer solution with a test substance concentration of 12 mg/mL (maximum solubility). The isolated ileum was connected to the lever arm of an isotonic transducer by a cotton ligature. The transducer was connected to a chart recorder. Contractions of the isolated ileum could then be recorded. Standard solutions of acetylcholine, a known agonist, were prepared and added to the volume of buffer solution used to bathe the isolated ileum. A maximum volume of 2 mL was used for all experiments to ensure the integrity of the tissue in the medium. The contraction response of isolated ileum was recorded for each concentration of acetylcholine to produce a standard curve. Between additions of each new concentration of acetylcholine, the buffer in the organ bath was flushed out and replaced by fresh buffer. The test material, dissolved in buffer, was added and its response compared with standards. Following initial results an antagonist (atropine) to the effects of acetylcholine was added together with the agonist. The results were then compared with the effects of an antagonist and the test material.

The following parameters were evaluated: Response to acetylcholine (agonist), test material, atropine (antagonist).

Rat - Gastrocnemius muscle

Previously untreated rats were killed by cervical dislocation. The abdomen was immediately dissected open and the dorsal aorta exposed. A butterfly needle was inserted into the dorsal aorta, near to the bifurcation in a posterior direction.

A volume of 0.3 mL of lithium heparin at a concentration of 10 mg/mL in sterile saline was injected into the dorsal aorta followed by 0.5 mL of sterile saline.

The experiment itself was performed on separate animals with doses of either the test material dissolved in sterile saline at a concentration of 12 mg/mL (maximum solubility) or of tubocurarine (positive control) that were injected into the dorsal.

The gastrocnemius muscle of the hind limb was exposed with the sciatic nerve intact. The gastrocnemius muscle was detached from the ankle joint and this area was ligated with cotton which was then attached to the lever arm of a transducer. The limb was held in place by a series of pins. An electrical stimulus of 12 volts was applied to the sciatic nerve and the muscle response was recorded. This action was repeated at approximately twelve second intervals until sufficient responses had been recorded.

The following parameters were evaluated: Response to injection of sterile saline, tubocurarine and test material.

Results and discussion

Blood parameters

There were no biologically significant differences, among the parameters measured, between treated and control animals.

Cardiovascular system

There were no biologically significant differences, among the parameters measured, between treated and control animals.

Nervous system

There were no biologically significant differences, among the parameters measured, between treated and control animals.
Guinea pig isolated ileum
The addition of acetylcholine to the medium containing the isolated guinea pig ileum resulted in contraction of the tissue in a concentration related response. Incubation with atropine sulphate immediately prior to addition of acetylcholine diminished or abolished the contraction response in a concentration related manner.
The addition of glyphosate technical at the maximum solubility in buffer also resulted in contraction of the ileum. The force of contraction was increased by an increasing volume of the test material in solution. Incubation with atropine sulphate prior to addition of glyphosate technical also resulted in the abolition of contractile response.
Rat- Gastrocnemius muscle
Injection of tubocurarine at a concentration of 25 mg/mL resulted in a significant diminution of the contractile response of the rat gastrocnemius muscle when the sciatic nerve was stimulated. There was no effect on muscle contraction when either glyphosate technical (12 mg/mL) or physiological saline was injected. The difference in force of response seen with glyphosate technical and physiological saline can be attributed to individual animal variation.
Conclusion by the Notifiers
At a maximum dose level of 5000 mg glyphosate technical/kg bw there were no effects seen from the in vivo screens performed. When administered to the isolated guinea pig ileum glyphosate technical caused a contractile response similar to that seen with known parasympathomimetic agents. Evaluation of innervated muscle response using showed that glyphosate technical, when administered at the maximum solubility concentration in physiological saline, did not cause any neuromuscular blocking activity.
RMS comments:
The study is considered acceptable although it will not contribute that much to risk assessment of glyphosate. The conclusion is agreed with. A parasympathicomimetic activity of glyphosate, at least at a high dose level, was shown. This seems to be a bit contradictory to the adrenergic mechanism postulated by Chan and Mahler (1992, TOX9551954) for salivary gland effects but it must be taken into account that different tissues were investigated.
Immunotoxicity in mice (2012)
Reference: IIA, 5.10/04
Report: (2012)
Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice
Project No.: WI-10-460 (Study No.: WIL-50393) Data owner: Monsanto
Date: 2012-03-21
not published ASB2012-11521
Guidelines: US-EPA OPPTS 870.7800 (1998)
Deviations: None
GLP: Yes
Acceptability: See RMS comment
Dates of experimental work: 2010-10-05 - 2010-11-17
Materials and methods
Test material:
Identification:
Glyphosate

Description:
White powder
Lot/Batch #:
GLP-0807-19475-T
Purity:
95.11 % (dried)
Stability of test compound:
Expiry date: 2011-06-10
Vehicle and/
or positive control:
Basal diet
Cyclophosphamid monohydrate
Test animals:
Species:
Mouse
Strain:
B6C3F1/Crl
Source:
Age:
Approx. 37 days (on arrival)
Sex:
Female
Weight at dosing:
16.5 – 20.0 g
Acclimation period:
14 days
Diet/Food:
Certified Rodent LabDiet® # 5002 (meal) (PMI Nutrition International, LCC.), ad libitum
Water:
Tap water, ad libitum
Housing:
Individually in stainless steel, wire-mesh cages suspended above cage-board.
Environmental conditions:
Temperature: 22 ± 3 °C
Humidity: 50 ± 20 % Air changes: 10/hour 12 hours light/dark cycle
Animal assignment and treatment:
In a 28-day oral immunotoxicity study groups of 10 female B6C3F1/Crl mice received daily dietary doses of 0, 500, 1500 and 5000 ppm glyphosate (equivalent to 0, 150, 449 and
1448 mg/kg bw/day).
A further group of 10 females were used as positive immunosuppressive control group. These mice received basal diet for 28 days and were treated with an intraperitoneal (IP) injection of 50 mg/kg bw/day once daily for four consecutive days (study days 24-27).
Test diets were prepared weekly and stored at room temperature. For the negative and positive control groups an appropriate amount of basal diet was weighed into a plastic storage bag. For the test substance groups 500 g of basal diet was weighed (pre-mixture). An appropriate amount of glyphosate was weighted into a mortar, mixed with a small amount of the pre- mixture

and ground until uniform. This admixture was transferred to a Hobart mixer and mixed with the remainder of the pre-mixture basal diet for five minutes. The resultant mixture was then transferred to a V-blender with a sufficient amount of basal diet to achieve the correct diet concentration and mixed for an additional 10 minutes using an intensifier bar during the first and last three minutes of mixing to ensure a homogeneous mixture. The test diets were prepared from the lowest to highest concentration. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 450 and 5500 ppm. Analyses for achieved concentrations on the test diets were done during study weeks 0 and 3.

Mortality

Each animal was checked for mortality or signs of morbidity twice a day during the treatment period, including weekends and public holidays.

Clinical observations

A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at once a week during the study period, beginning one week prior to randomisation, and on the day of scheduled necropsy.

Body weight

Individual body weights were recorded twice weekly, beginning approximately one week prior to randomization, at the time of animal selection for randomization, on study day 0, and just prior to the scheduled necropsy. Mean body weights and mean body weight changes were calculated for the corresponding intervals.

Food consumption and test substance intake

The quantity of food consumed was recorded for each animal weekly, beginning approximately one week prior to randomization, and just prior to the scheduled necropsy. Food intake was calculated as g/animal/day for the corresponding body weight intervals. The mean amounts of glyphosate consumed (mg/kg/day) per dose group were calculated from the mean food consumed (g/kg of body weight/day) and the appropriate target concentration of glyphosate in the food (mg/kg of diet).

Serum collection for possible IgM antibody analysis

For determination of the possible extent of the suppression of IgM antibody production blood samples were collected from all animals at scheduled necropsy and processed to serum. Following euthanasia by carbon dioxide inhalation, approximately 0.75 mL of blood was collected from the inferior vena cava of each mouse into a tube containing no anticoagulant and allowed to clot. Serum was obtained and aliquots of approximately 150 µL (including any remainder serum) were transferred to cryovials and stored frozen (approximately -70 °C).

Sacrifice and pathology

A complete necropsy was conducted on all animals at scheduled termination or on animals that died or were sacrificed during the study period. Any macroscopic findings were recorded. The following organ weights were determined from all animals surviving to scheduled termination: spleen and thymus.

Tissue samples were taken from the spleen and thymus. Spleen samples were placed in EBSS/HEPES buffer. Thymus samples were preserved in 10% neutral-buffered formalin.

Spleen processing for immunotoxicological evaluation

For the determination of the number of specific IgM antibody-forming cells directed towards sRBC an AFC assay, as a modification of the Jerne plaque assay (Jerne et al., 1963, 1974) was conducted. Spleens were collected from all animals at the scheduled necropsy (study day 28) immediately following blood collection. Individual spleens were placed into individual tared tubes containing EBSS with 15 mM HEPES, supplemented with gentamicin as a bacteriostat, and maintained on ice. Each

tube was then weighed to provide a “wet” weight for each spleen. Spleen samples from Groups 1-4 animals were randomized and coded for antibody-forming cell (AFC) analysis. Spleen samples from Group 5 were labelled as positive control samples for analysis. The spleen samples were placed on crushed ice until procession for AFC analysis.
The spleen samples were processed into single-cell suspensions. The cell suspensions were then centrifuged and resuspended in EBSS with HEPES. Spleen cell counts were performed using a Model Z1™ Coulter Counter®. Viability of splenocytes was determined using propidium iodide and the Coulter® EPICS® XL-MCL™ Flow Cytometer

Statistics

Body weight, body weight change, and food consumption data were subjected to a parametric one way ANOVA to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test substance treated groups to the control group.
The positive control data were evaluated using the Student’s t-Test and compared to the basal diet control group.
Organ weight (wet spleen and thymus), final body weight, and AFC data obtained were first tested for homogeneity of variances using the Bartlett’s Chi Square test. Homogeneous data were evaluated using a parametric one-way ANOVA. When significant differences occurred, the treatment groups were compared to the basal diet control group using Dunnett’s test. Non-homogeneous data were evaluated using a non-parametric ANOVA. When significant differences occurred, the treatment groups were compared to the basal diet control group using the Gehan-Wilcoxon test when appropriate. The Jonckheere’s test was used to test for dose-related trends across the basal diet control and test substance treated groups. The
positive control data were evaluated using the Student’s t Test and compared to the basal diet control group. The criteria for accepting the results of the positive control group included a statistically significant ($p\leq0.05$) decrease in the response when compared to the response of the basal diet control group.
The AFC data were expressed as Specific Activity, IgM antibody forming cells per million spleen cells (AFC/106 spleen cells), and as IgM Total Spleen Activity (AFC/spleen).

Results and discussion

Diet analysis

The achieved concentrations of glyphosate in the dietary preparation were in the range of 85.6 - 97.5% of nominal, and therefore within the acceptable range of 85 – 115 %. The diet formulations were homogeneous and stable for 10 days when stored at room temperature with the following exception. During homogeneity/concentration acceptability testing, the 450 ppm diet formulation was 83.1% of target. The 5500 ppm diet formulation was within acceptable range (90.8 %) but was considered low, therefore, calibration standards were prepared as matrix-based samples and a cross-validation was conducted. The diet formulations were reanalyzed using matrix-based calibration standards and met the testing facilities SOP acceptance criteria for homogeneity and concentration acceptability. Based on these results, the protocol-specified doses of test substance were offered to the animals. The test substance was not detected in the basal diet that was offered to the basal diet control (Group 1) and positive control (Group 5) groups.

Mortality

There were no mortalities observed during the study period.

Clinical observations

There were no test substance-related clinical findings.

Body weight
There were no test substance related
Food consumption
There were no test substance-related effects on food consumption noted.
Gross pathology
There were no test substance-related macroscopic effects.
Treatment with the positive control CPS produced a small thymus in three of the 10 animals. These changes were consistent with the known effects of CPS in female B6C3F1 mice.
Organ weights
There were no test substance-related effects on terminal body weights or on spleen or thymus weights (absolute or relative to final body weight) when the test substance-treated groups were compared to the basal diet control group.
Treatment with the positive control CPS produced statistically significantly lower spleen and thymus weights (absolute and relative to final body weight) when compared to the basal diet control group. These changes were consistent with the known effects of CPS in female B6C3F1 mice.
The results of final body and organ weight determinations are presented in the Table B.6.8-16 below.
Table B.6.8-16: Final body weight and organ weight data
Dose group
Body weight
Spleen
Thymus
weight
% body weight
weight
% body weight
(g)#
(mg) #
(%)#
(mg) #
(%)#
1 (negative control)*
20.9 ± 0.3
85.3 ± 3.5
0.41 ± 0.02
44.3 ± 3.5
0.21 ± 0.02
2 (low)
20.6 ± 0.2
82.3 ± 4.6
0.40 ± 0.02
41.5 ± 1.9
0.20 ± 0.01
3 (mid)
21.6 ± 0.3
91.6 ± 6.5
0.42 ± 0.03

45.9 ± 2.7
0.21 ± 0.01
4 (high)
21.3 ± 0.2
86.0 ± 3.6
0.40 ± 0.02
42.0 ± 2.6
0.20 ± 0.01
5 (positive control)
21.5 ± 0.3
50.2 ± 3.2**
0.23 ± 0.02**
13.3 ± 0.8**
0.06 ± 0.01**
Values presented the mean ± SD derived from the number of animals evaluated per dose group
** Statistically significant from negative control at p ≤ 0.01
AFC assay
There were no test substance-related effects on spleen cell numbers, and in the functional evaluation of the IgM antibody-forming cell (AFC) response, treatment with glyphosate did not result in a statistically significant suppression of the humoral immune response when evaluated as either Specific Activity (AFC/106 spleen cells) or Total Spleen Activity (AFC/spleen). There were no statistically significant differences nor any dose-related trends noted when the basal diet control and test substance-treated groups were compared.
Statistically significantly lower spleen cell numbers, mean specific activity, and mean total spleen activity values were noted in the positive control (CPS treated) group when compared to the basal diet control group. These effects were consistent with the known immunosuppressant effects of CPS and validated the appropriateness of the AFC assay.
The results of the AFC assay are summarised in Table B.6.8-17 below.
Table B.6.8-17: Results of AFC assay
Dose group
Spleen cells
IgM AFC / 106
spleen cells
IgM AFC/spleen
(x 107)#
#
(x 103)#
1 (negative control)*
11.29 ± 0.65
1160 ± 131
127 ± 11
2 (low)
11.45 ± 0.64
1273 ± 123
144 ± 16
3 (mid)

13.45 ± 1.24
1368 ± 163
190 ± 37
4 (high)
12.51 ± 0.66
1514 ± 204
195 ± 32
5 (positive control)
5.18 ± 0.53**
0 ± 0**
0 ± 0**
Values presented the mean ± SD derived from the number of animals evaluated per dose group
** Statistically significant from negative control at $p \leq 0.01$
Conclusion by the Notifiers
Repeated dietary administration of glyphosate to females B6C3F1 mice did not suppress the humoral component of the immune system. The no-observed-effect level (NOEL) for suppression of the humoral immune response in female B6C3F1 mice offered glyphosate in the diet for 28 days was considered to be 5000 ppm (equivalent to 1448 mg/kg bw/day), the highest dietary concentration.
RMS comments:
The study is considered acceptable. For the parameters included, there was no evidence of immunotoxicity of glyphosate in mice up to a very high dose level.
Studies on mechanisms of toxicity and additive effects (copied from 1998 DAR, ASB2010-10302)
(1992, TOX9552421): Ammonium salt of glyphosate (MON-8750): General pharmacological study. Institute of Environmental Toxicology, Tokyo, Japan; Study no. IET 90-0149/ET-92-15). Dates of experimental work: 04 February 1992 - 02 March 1992. GLP: yes (self-certification of the laboratory). This report was submitted as part of the joint dossier of Monsanto and Cheminova.
The potential effects of MON 8750 (purity: 94.78%) on the nervous, respiratory and circulatory systems were assessed following single i.p. injection to male and female ICR mice at doses up to 5000 mg/kg bw and single i.v. injection to urethane-anesthetized and non- anesthetized male rabbits at doses up to 500 mg/kg bw. At the top dose levels, all mice died within 0.5 hours and all anesthetized rabbits within a few minutes after injection. Non- anesthetized rabbits survived i.v. application of 500 mg/kg bw although animals showed some neurological signs. In rabbits which died, heart rate was decreased and ECG changes have been noted. At the next lower dose levels (1250 mg/kg bw in mice or 125 mg/kg bw and 31.3 mg/kg bw in rabbits, respectively), transient symptoms like a decrease in blood pressure, reduced activity and neuromuscular signs were observed but cleared to normal values or behaviour within some hours at the latest. Respiratory rate was increased in surviving rabbits but decreased in anesthetized rabbits which died. It was concluded that an impact on cardiorespiratory functions is involved in acute toxicity. The lethal dose appears to be decreased under anesthesia.
(1987, TOX9551964): Synergism and potentiation in rats of glyphosate (tech.) of Excel Industries Ltd., Bombay. Study identification and dates of experimental work not given. GLP: no. This report was submitted independently by the notifiers Barclay and Luxan.
Glyphosate was administered to groups of 10 male Wistar rats as a single oral dose of 5000 mg/kg bw at a constant dose volume of 10 ml/kg in corn oil. Simultaneously, the animals received the compound 2,4-D sodium salt at dose levels of 376, 473, 596, 750, 944 or 1189 mg/kg bw. The second compound used for a potentiation experiment was dalapon at doses of 2500 and 5000 mg/kg bw.

After simultaneous dosing, the rats were observed for 14 days for toxic symptoms and mortality. According to the study authors, no potentiation has been observed with glyphosate and dalapon. However, two out of ten rats died after application of 5000 mg/kg bw glyphosate and 5000 mg/kg bw dalapon. When both compound were administered alone, no mortalities occurred.
The simultaneous administration of glyphosate and 2,4-D sodium salt caused a markedly higher mortality in all dose groups. In the groups receiving glyphosate and doses of 596 mg 2,4-D sodium salt/kg bw and above, all animals died. When the latter compound was administered alone, 100% mortality has been reached only at 1189 mg/kg bw. Hence, at least an additive acutely toxic effect of glyphosate and 2,4-D can be assumed.
1987, TOX9552430): Irritating effect of glyphosate, surfactant and Roundup on stomach and small intestine in dogs. Dep. of clinical medicine, University of Tsukuba, Japan. The study was submitted as part of the joint dossier of Monsanto and Cheminova.
The IPA salt of glyphosate, Roundup herbicide (41% IPA) and the surfactant MON 0818 (15 % of which is contained in Roundup) and 0.25 N hydrochloric acid solution (control) were directly administered on the gastric and small intestinal mucosa of fasted male beagle dogs. The specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues. Direct application of Roundup®
herbicide, and the surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup formulation than with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.
Further Studies on mechanisms of toxicity and additive effects (submitted after 2000)
Song, H.-Y. et al. (2012, ASB2013-10531): In vitro cytotoxic effect of glyphosate mixture containing surfactants.
The authors investigated wheter glyphosate influences the cellular toxicity of the surfactants polyoxyethylene tallow amine (TN-20) and polyoxyethylene lauryl amine ether (LN-10) on the mouse fibroblast like cells, alveolar epithelial cells and a heart cell line. The cytotoxicity of TN-20 qne LN-10 (0.4-100 µM), in the presence or absence of glyphosate was determined by assessing membrane integrity. TN-20 toxicity was significantly lower in the presence of 50 µM glyphosate for the fibroblast-like cell, for the alveolar cells and for the heart cell line compared to that of TN-20 alone. The cellular toxicity of LN-10 towards the fibroblast-like cells was found to be increased in the presence of 50 µM glyphosate. The authors conclude that these results suggest that the mixture toxicity may be a factor in glyphosate-surfactant toxicity in patients with acute glyphosate herbicide intoxication.
B.6.8.3 Studies in farm animals
Table B.6.8-18: Summary of studies in farm animals
Reference; Study identification; Owner
Type of study Species, Sex
Application route (Dose)
Test material
Purity [%]
Results
1987; TOX9552422;
Monsanto
Acute oral toxicity, Goat
♀

Gavage (0,
1980, 3090,
4620, and 10
000 mg/kg bw)
Glyphosate
98.7
LD50 =
3530 mg/kg bw
1987; TOX9552423;
Monsanto
Acute oral toxicity, Goat
♀
Gavage (0,
1400, 4290,
5360, 6700,
Glyphosate isopropyl-
amine salt
62.5
(46.2%
glyphosate)
LD50 =
5700 mg/kg bw
[CI 95%, 3.73–8.71]
Reference; Study
identification; Owner
Type of study Species, Sex
Application route (Dose)
Test material
Purity [%]
Results
and 10 000 and mg/kg
bw)
1987;
Subacute oral
Gavage (540,
Glyphosate
62.4
MLD =
TOX9552424 ;
toxicity (7 d),
830, 1290, and
isopropyl-
(46.2%
1290 mg/kg bw/d;
Monsanto
Cow

2000 mg/kg
amine salt
glyphosate)
LOAEL =
♀
bw/d;
830 mg/kg bw/d;
1000 mg/kg
NOAEL =
bw/d in pre-
540 mg/kg bw/d
test)
1987;
Subacute oral
Gavage (400,
Glyphosate
41.1
MLD =
ASB2010-8131;
toxicity (7 d),
500, 630, and
isopropyl-
(30.5%
790 mg/kg bw/d;
Monsanto
Cow
790 mg/kg
amine as
glyphosate)
LOAEL =
♀
bw/d;
Roundup ®
500 mg/kg bw/d
1000 mg/kg
formulation
NOAEL =
bw/d in pre-
MON2139
400 mg/kg bw/d
test)
MLD: minimum lethal dose
B.6.8.3.1 Acute toxicity in goats Study with glyphosate acid
Reference:
IIA, 5.10/05
Report:

(1987) The acute toxicology of glyphosate in female
goats,
Data owner: Monsanto Report No.: VT-80-450 Date: 1987-03-23

TOX9552422
Guidelines:
Non-guideline study
Deviations:
Not applicable.
GLP:
No
Acceptability:
See RMS comments
Remark: The goats used in this study were a priory not tested for pregnancies (in fact, at least three of the goats employed for the study were pregnant) and, thus, females of different hormonal and physiological status were used. Further, weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous.
Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.
Dates of experimental work: 1980-12-22 to 1981-05-05
Material and methods
Test material: Glyphosate Description: white crystalline solid Lot/Batch #: XHJ-64, NBP1494248
Purity: 98.7 % (N-phosphonomethyl glycine)
Stability of test compound: Stable for > 1 year (protocol date: 1980-08-28) Vehicle and/or positive control: Water
Test animals: Species: Goat, Strain:not applicable (Spanish goats) Source:
Age: 8 months–4 years Sex: female
Weight at dosing: 26.4 ± 7.2 kg Acclimation period: 30 days
Diet/Food: During the initial acclimatization (outdoor): bermudagrass hay and commercial goat meal containing not less than 16 % crude protein [Purina ® Goat Chow ® (Coarse) (Ralston Purina Company, Gonzales, TX, USA)]. After the initial acclimatisation (indoor) the goats were fed bermudagrass only.
Water: not specified
Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study
Environmental conditions: not stated
Study design and methods
Dose 1980, 3090, 4620, and 10 000 mg/kg bw 4 control groups of 5 goats each
Application route: Oral, gavage Application volume: 500 mL/goat Fasting time: not stated
Group size: 5
Post-treatment observation period: 14 days
Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology
In life dates: not stated
Animal assignment and treatment:

Groups of female Spanish goats received the test item, glyphosate, at dose levels of 10 000, 4620, 3090, and 1980 mg/kg bw by oral gavage in a sequential manner. Initially, the test item was given to a group receiving 10 000 mg/kg/bw and subsequent dosages were selected based on the observed responses. Control animals were treated with tap water. All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and
14. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Results and discussion

Mortality

All animals treated with 10 000 and 4620 mg/kg bw glyphosate died. 1/5 animals treated with 3090 mg/kg bw also died.

The results are summarised in the following table.

Table B.6.8-19: Mortality, survival time and animals used

Dose (mg/kg bw)	Toxicological result	Onset of death after	Mortality (%)	Dead animals	Animals with toxic signs	N
10 000						5
						5
						5
		3.9–19.2 h	100			
4620						5
						5
						5
		2.4–32.2 h	100			
3090						1
						5
						5
		71.7 h	20			
1980						0
						4
						5
						0

0
0
0
20
0
N = Total number of animals in group
The median lethal dose (LD50) was calculated to be LD50, goat, oral = 3530 mg/kg bw.
Clinical observations
Clinical signs of toxicity included colic, diarrhea, depression, and ataxia. Most of these signs were observed at all dosage levels except the 1980 mg/kg bw level at which only diarrhea was observed.
In detail: Clinical signs of goats that died included cecession of feeding activity, abdominal distress, depression, ataxia, mild diarrhea, and, shortly prior to death, recumbency. Toxic tubular nephrosis was the only consistent histopathological lesion observed in goats that died. Clinical signs of surviving goats included decreased feed consumption, diarrhea, and body weight loss.
Clinical signs of goats treated with the minimum dose included decreased feed consumption and diarrhea.
All clinical signs were absent at the end of the experiment (14–15 d).
One control goat gave birth to a single live kid approximately one hour before the time of treatment.
Another control goat gave birth to one normal and one small and very weak kid on day 4 of the experiment.
One goat of the 4.62 g/kg bw-treated animals gave birth to two full-term kids during the night before dosing.
Table B.6.8-20: Prominent clinical observations in female Spanish goats given glyphosate
Dose (mg/kg bw)
N
Observations (Number of goats affected)
10 000
5
CFA (5), apparent colic (2), depressed demeanor (2), slight ataxia (3), recumbency (4), death (5)
4620
5
CFA (5), apparent colic (2), depressed demeanor (2), ataxia (2), labored breathing (2), recumbency (4), diarrhea (1), death (5)
3090
5
fatally poisoned goat (1): CFA, diarrhea, apparent colic, subdued demeanor, thirst, ataxia, recumbency, nystagmus, death
survivors (4): decreased food consumption (4), apparent colic (2), diarrhea (4)
1980
5
Decreased food consumption (4), diarrhea (3)
CFA: cecession of feeding activity
Body weight
Most animals, which died while on study, exhibited losses in weight immediately prior to death.
Of the animals that survived until terminal sacrifice, those in the 3090 mg/kg bw group had

body weights less than their respective control group. Goats in the 1980 mg/kg bw group had mean body weights similar to their respective control group.

Food consumption was not precisely measured, but cessation of feeding activity was observed in all treatment groups.

Table B.6.8-21: Survival time, body weights and carcass weights (control groups not pooled)

Dose (mg/kg bw)
Mean
survival time (h)
Mean body weight (kg)
Mean carcass weight
N
Initial
Day 7
Day 14
N
Weight (kg)
Day
10 000
13.7
5
24.68
–
–
2
26.82
0
Control
S
5
24.36
23.22
23.68
–
–
–
4620
16.46
5
29.46
–
–
5
28.78
0–1
Control
S

5
27.76
26.81
26.63
–
–
–
3090
71.7*
5**
25.81
21.04
22.79
1
30.62
3
Control
S
5
28.58
29.89
28.12
–
–
–
1980
S
5
24.72
22.77
23.54
–
–
–
Control
S
5
25.95
24.95
25.08
–
–
–
–: not applicable
S: all animals survived
* One animal died at day 3 the others survived

** N = 4 for day 7 and 14.

Necropsy

Pulmonary edema to some degree was noted in several animals and was judged to be a terminal event.

The results of the post-mortem observations are summarised in the following table.

Table B.6.8-22: Summary of major gross pathologic diagnoses (animals affected/total)

Dose (mg/kg bw)	Died/ sacrificed	Body as a whole	Respiratory	Cardiovas cular	Gastro- intestinal	Urogenital
10 000	5/0	NGPD	Pulmonary edema (4/5) Pneumonia (1/5)	NGPD	NGPD	NGPD
4620	5/0	Serous atrophy of fat (1/5)	Pulmonary edema (4/5)	NGPD	Hepatic atrophy (1/5) Macerated fetus (1/5)	
3090	1/4	Serous atrophy of fat (1/5)	Pneumonia (1/5)	Pericarditis (1/5)	Fatty liver (1/5) Rumen haemorrhage (1/5)	Pallor, kidneys (4/5) Renal hypertrophy (1/5) Endometritis (2/5) Fetal death (1/5)
1980	0/5	NGPD	Pulmonary edema (3/5) Pneumonia (1/5)	NGPD	Fatty liver (1/5)	Chronic hepatitis (1/5)

Renal atrophy (1/5)
Controls
0/4*
Minimal fat stores (1/4)
Pulmonary edema (2/4)
NGPD
NGPD
Pallor kidney (2/4) Cystitis (1/4)
Metritis (1/4) Endometritis (1/4)
NGPD: No gross pathologic diagnosis
* only one randomly selected goat from each of the 4 control groups subjected to post mortem examination
Histopathology
Histological examination was performed only on heart, liver, kidney, spleen and other tissues with grossly visible lesions from animals given glyphosate at 4620 and 3090 mg/kg bw and of 4 sacrificed control animals.
The most consistent finding in fatally poisoned animals was mild to severe tubular nephrosis. 3090 mg/kg bw-treated animals that survived lack such lesions.
4/5 fatally poisoned goats had mild fatty change in the liver.
Clinical biochemistry and haematology
Elevation of blood urea nitrogen concentration, serum creatinine concentration, and numbers of circulating segmental neutrophils were the most consistent laboratory findings in goats given glyphosate. These changes were observed at all dosage levels used in this study.
Clinical laboratory findings were almost universally within or near control limits for surviving glyphosate-treated goats at the end of the experiment (day 14).
The results are summarized in the following tables.
Table B.6.8-23: Biochemical and haematological measurements of goats that died (min–max values)
Measurement (units)
Reference value
Control1
Death in
less than 4 h (1 goat)2
Death in 15–20 h
(3 goats)3
Death in 32–72 h
(2 goats)4
Death in 72 h
(1 goat)5
BUN (mg/dL)
10.0–20.0
2.3–21.3
12.7
16.2–25.0++
16.7–35.2++
55.5++
SC (mg/dL)

1.0–1.8
0.7–1.7
1.5
2.7–3.3++
4.0–6.0++
14.1++
GLU (mg/dL)
50.0–75.0
43–80
407++
48–57
35–38
268++
Na (mg/dL)
327–356
311–354
303++
333–335
313–321
327
K (mg/dL)
13.6–26.1
12.7–21.2
44.8++
21.8–31.5
15.2–20.5
12.9
Ca (mg/dL)
8.9–11.7
8.0–15.7
18.9++
15.2–18.6++
8.7–10.3
6.3++
P (mg/dL)
2.9–7.3
3.5–11.3
16.0++
5.4–9.5
8.8–17.0++
21.6++
Mg (mg/dL)
2.8–3.6
1.30–3.20
4.01++
2.87–3.61++

1.98–3.07
2.38
SGOT (IU/L)
167–513
31–151
78
49–126
60–85
129
LDH (IU/L)
123–392
108–385
295
172–543++
268–288
694++
SACH (IU/L)

319–478
426
272–475
266–287
324
ALKP (IU/L)
93–387
21–902
124
108–430
59–255
350
CPK (IU/L)
20–42
44–878
213
124–184
59–173
3285++
GGT (IU/L)

12–42

23–26
20–21
39
WBC5
4.0–15.0

5.5–24.8
14.5
22.5–28.0++
13.5–27.0++
57.3++
SEGS5
1.6–7.5
1.4–10.4
7.3
10.8–18.2++
4.9–14.9++
38.4++
BANDS5

0–1.4
2.8++
6.4–7.6++
4.1–10.0++
11.5++
LYMPHS5
1.8–9.0
2.4–15.4
3.8
3.1–7.0
1.4–4.5
5.7
EOS5
0.1–1.5
0–1.2
0.1
0
0
0
MONO5
0.1–0.9
0–0.9
0.6
0.2–0.5
0.1–0.8
1.7
BASO5
0–0.2
0–0.5
0
0–0.3
0

0
PCV(%)
19–40
23–42
26.0
31.0–40.0
27.0–28.0
39.0
HGB (g/dL)
8–16
8.1–16.2
8.6
9.4–12.3
8.2–11.0
15.4
RBC6
7–21
13.3–29.5
13.6
19.0–24.0
14.0–15.9
21.4
MCV (fL)
15–39
13.0–21.0
19.0
14.0–17.0
16.0–18.0
18.0
MCH (pg)
5.3–8.4
4.4–7.8
6.3
4.7–5.1
5.9–6.9
7.2
MCHC (g/dL)
32–40
26.9–40.8
33.1
30.3–30.8
30.4–39.3
39.5
TSP (g/dL)
6.4–7.0
5.0–8.2

8.9++
5.7–6.3
5.6–6.1
6.4
1: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
2: Goat sampled at death 3.8 h after receiving 10.0 g/kg
3:Goats sampled 11 h after receiving 10.0 g/kg 4:Goats sampled 24 h after receiving 4.62 or 3.09 g/kg
5:Goat sampled at death 72 h after receiving 3.09 g/kg
++: One or more values outside of control limits
Table B.6.8-24: Biochemical and haematological measurements of surviving goats (min–max values)
Measurement (units)
Reference value
Control1
Sign of intoxication observed (4 goats)2
No signs of intoxication observed (5 goats)3
days 1 and 3
days 7 and
14
days 1 and 3
days 7 and
14
BUN (mg/dL)
10.0–20.0
2.3–21.3
15.1–45.7++
3.1–26.4++
5.2–54.5++
7.0–31.3
SC (mg/dL)
1.0–1.8
0.7–1.7
1.8–5.3++
0.8–1.6
0.8–5.0++
0.8–1.3
GLU (mg/dL)
50.0–75.0
43–80
36–87
51–87
55–100
59–98
Na (mg/dL)
327–356
311–354

317–331
301–332
317–347++
319–350++
K (mg/dL)
13.6–26.1
12.7–21.2
6.7–18.1
9.4–17.0
12.3–18.3
11.9–18.2
Ca (mg/dL)
8.9–11.7
8.0–15.7
6.2–10.3++
7.9–10.2
6.1–10.1
8.5–11.4
P (mg/dL)
2.9–7.3
3.5–11.3
4.4–22.4++
4.1–8.0
3.7–9.0
5.3–8.7
Mg (mg/dL)
2.8–3.6
1.30–3.20
1.53–2.48
1.53–2.38
1.98–3.22
1.29–2.38
SGOT (IU/L)
167–513
31–151
44–69
47–229++
40–91
46–106
LDH (IU/L)
123–392
108–385
100–233
127–415++
145–276
144–226

SACH (IU/L)

319–478
231–447
221–390
293–440
291–445
ALKP (IU/L)
93–387
21–902
23–273
27–205
41–143
40–211
CPK (IU/L)
20–42
44–878
83–187
73–245
84–264
81–158
GGT (IU/L)

12–42
23–34
22–36
21–31
19–34
WBC5
4.0–15.0
5.5–24.8
17.5–41.5++
8.0–19-5
11.0–22.4
8.0–17.5
SEGS5
1.6–7.5
1.4–10.4
11.5–30.7++
2-4–14-8++
4.1–17.5++
2.0–7.2
BANDS5

0–1.4
0.2–7.5++

0.1–0.8
0–0.7
0–1.4
LYMPHS5
1.8–9.0
2.4–15.4
2.4–5.4
2.9–12.2
4.0–6.9
5.0–10.2
EOS5
0.1–1.5
0–1.2
0–0.2
0–0.3
0–0.3
0–0.7
MONO5
0.1–0.9
0–0.9
0–1.0
0.1–0.5
0.1–0.2
0.1–0.4
BASO5
0–0.2
0–0.5
0–0.2
0–0.3
0–0.3
0–0.3
PCV(%)
19–40
23–42
30.0–37.0
24.0–38.0
30.0–39.0
28.0–39.0
HGB (g/dL)
8–16
8.1–16.2
11.0–13.0
8.9–12.8
8.3–12.6
9.3–12.2
RBC6

7–21
13.3–29.5
17.4–22.4
13.3–20.3
15.0–24.5
16.6–23.6
MCV (fL)
15–39
13.0–21.0
14.0–18.0
15.0–20.0
13.0–19.0
14.0–19.0
MCH (pg)
5.3–8.4
4.4–7.8
5.6–6.9
5.6–7.1
4.8–6.6
4.4–6.8
MCHC (g/dL)
32–40
26.9–40.8
34.2–40.0
29.1–39.3
31.1–35.3
26.9–34.9
TSP (g/dL)
6.4–7.0
5.0–8.2
6.5–7.5
5.5–7.5
6.0–7.3
6.0–7.4
1: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
2: Goats given 3.9 g/kg
3:Goats given 1.98 g/kg
++: One or more values outside of control limits
Conclusion (Notifier)
The median lethal dose (LD50) was calculated to be LD50, goat, oral = 3530 mg/kg bw.
RMS comments: The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is good and it is suitable to provide additional information about acute oral toxicity in a ruminant species. The extent of investigations exceeded that one in acute toxicity studies in laboratory

rodents. However, pathology was confined to four of a total of 20 control animals. In addition, assessment was made a bit difficult by the fact that sometimes data obtained in the four control groups were pooled and sometimes reported separately. The conclusion of the notifier is agreed with. Acute oral toxicity of glyphosate in goats was low although the goat appeared more sensitive than the rat, both with regard to dose levels causing mortality and severity of effects.
Study with glyphosate isopropylamine salt
Reference: IIA, 5.10/06
Report: (1987) The acute toxicology of the isopropylamine salt of glyphosate in female goats
Data owner: Monsanto Report No.: VT-80-451 Date: 1987-04-16 TOX9552423
Guidelines: Non-guideline study
Deviations: Not applicable
GLP: Yes (Not checked by RMS. Not compulsory when the study was performed.)
Acceptability: See RMS comments below.
Remark: The goats used in this study were a priory not tested for pregnancies (at least two goats, one each in the low dose and the control groups, had been pregnant because they aborted) and, thus, females of different hormonal and physiological status were used.
Further, weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous.
Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.
Dates of experimental work: 1980-12-22 to 1981-05-08
Material and methods
Test material: MON 0139 (isopropylamine salt of glyphosate/ N-phosphonomethyl glycine isopropylamine)
Description: amber liquid Lot/Batch #: LURT 08020
Purity: 62.5 % (N-phosphonomethyl glycine isopropylamine)
46.2 % glyphosate/N-phosphonomethyl glycine
Stability of test compound: Stable for > 1 year (protocol date: 1980-08-28) Vehicle and/or positive control: water
Test animals:
Species: Goat
Strain: not applicable
(Spanish goats, the term is used to distinguish range meat goats from Angoras and dairy breeds. Most of the same origin as the Mexican Criollo but they may show traces of Nubian and Toggenburg blood.)
Source:
Age: 8 months–4 years Sex: Female
Weight at dosing: 27.1 ± 7.0 kg Acclimation period: 30 days
Diet/Food: During the initial acclimatisation (outdoor): bermudagrass hay and commercial goat meal containing not less than 16 % crude protein (Purina ® Goat Chow ® (Coarse)
(Ralston Purina Company, Gonzales, TX, USA). After the initial acclimatization (indoor) the goats were fed bermudagrass only.
Water: Fresh water
Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study
Environmental conditions: not stated

Study design and methods Animal assignment and treatment
Dose 1400, 4290, 5360, 6700, and 10 000 and mg/kg bw Application route: Oral, gavage
Application volume: 500 mL/goat Fasting time: not stated
Group size: 5
Four control groups of five animals each included Post-treatment observation period: 14 days
Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology
In life dates: not stated
Animal assignment and treatment:
Groups of females Spanish goats received the test item, MON 0139, at dose levels of 10 000, 6700, 5360, 4290, and 1400 mg/kg bw by oral gavage in a sequential manner. Initially, the test item was given at 10 000 mg/kg/bw and subsequent dosages were selected based on the observed responses. Control animals were treated with tap water. All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.
Results and discussion
Mortality
Treatment with 10 000, 6700, 5360, 4290, and 14000 mg/kg bw MON 0139 resulted in 5/5, 3/5, 2/5, 2/5, and 0/5 deaths, respectively. In the four control groups, there were also no deaths.
The results are summarised in the following table.
Table B.6.8-25: Mortality, survival time and animals used.
Dose (mg/kg bw)
Toxicological result
Onset of death after
Mortality (%)
Dead
animals
Animals with
toxic signs
N
10 000

5
5
5
15.6–42.0 h
100
0
0
0
5
0
6700

3
5
5
37.0–64.0 h
60
0
0
0
5
0
5360
2
5
5
47.5–49.0 h
40
0
0
0
5
0
4290
2
5
5
53.2–85.1 h
40
0
0
0
5
0
1400
0
2
5
--
0
0
0
0
5
0
N: Number of animals
The median lethal dose (LD50) was calculated to be LD50, goat, oral = 5700 mg/kg bw [CI 95 %, 3.73–8.71].

Clinical observations

Clinical signs of toxicity included colic, diarrhea, taxia and weakness. These signs of toxicity were observed at most dosage levels above 1400 mg/kg bw. Additional symptoms suggestive of central nervous system involvement were observed at dosages of 4290 mg/kg bw and above, including tremors, convulsions, and unusual behaviour.

In detail:

Clinical signs of goats that died included decreased food consumption, abdominal distress, ataxia and, shortly prior to death, recumbency.

One goat that died and one surviving goat each displayed an unusual “collapsing syndrome” of apparent neurological origin approximately 2 days after receiving MON 0139 while other goats displayed various other neurological signs.

One surviving goat developed extensive ulceration of the tongue and oral mucosa. These lesions healed completely by the end of the 14-day observation period.

Table B.6.8-26: Prominent clinical observations in female Spanish goats given glyphosate MON 0139

Dose (mg/kg bw)	N	Observations (Number of goats affected)
10 000	5	non-survivors (5): DFC (5); apparent colic (5); ataxia, shaking and jerking movements (2); depression (2); head bobbing (2); nystagmus (1); recumbency (4); diarrhea (1); paddling (2); mild convulsions (2)
6700	5	non-survivors (3): DFC (3); apparent colic (2); diarrhea (1); bloating (3); ataxia (3); “saw-horse” stance (2); tremor (1); recumbency (3); nystagmus (2); jerky movements (2); blinking (1); terminal clonic-tonic activity (3) survivors (2): DFC (2); bloating (1); abdominal distention (1); tremor (2); “collapsing syndrome” (1); diarrhea (2). (Abortion seen in one control goat)
5360	5	non-survivors (2): DFC (2); diarrha (1); ataxia (2); “saw-horse” stance (1); salivation (1); recumbency (1); tremor (1); “star-gazing” trance (1) survivors (3): DFC (3); diarrhea (3)
4290	5	non-survivors (2): DFC (2); ataxia (1); “collapsing syndrome (1); “chewing convulsions” (1); recumbency (2); salivation (1); terminal opisthotonus-like convulsions (1) survivors (3): DFC (3); diarrhea (3); apparent colic, salivation, lethargy, ulceration of oral mucosa (1)
1400	5	Minimal or no ill effects seen; reduced urination; abdominal distention (2) (Abortion seen in one control animal)

DFC: Decreased food consumption

Body weight

No statistically significant differences in body weight gain were observed between the groups treated with MON 0139 and their respective control groups.
Food consumption, although not precisely measured, was greatly reduced following treatment with MON 0139 at dosages of 4290 mg/kg bw and above. For most groups, feeding activity gradually increased, reaching normal levels by the end of the study.
Necropsy
No lesions considered to be treatment-related were noted at gross necropsy.
Table B.6.8-27: Summary of major gross pathologic diagnoses (animals affected/total)
Dose
(mg/kg bw)
Died/ sacrifiecd
Body as a whole
Respiratory
Cardiovas cular
Gastro- intestinal
Urogenital
10 000
5/0
NGPD
pulmonary
edema (2/5)
NGPD
NGPD
NGPD
6700
3/2
NGPD
pulmonary
edema (1/5)
NGPD
bloat (2/5)
pale kidneys (1/5)
renal hypertrophy (1/5)
5360
2/3
minimal
fat stores (1/5)
pulmonary edema (3/5)
NGPD
fatty liver (4/5)
pale kidneys (2/5)
4290
2/3
NGPD
pulmonary edema (3/5)
NGPD

fatty liver (1/5)
gallbladder edema (1/5)
pale kidneys (2/5) renal hypertrophy (1/5)
1400
0/5
minimal fat stores
(1/5)
pneumonia (1/5) pulmonary
edema (1/5)
pericarditis (1/5)
fatty liver (1/5)
metritis (1/5) pregnancy (1/5)
Control
0/4*
minimal fat stores (1/4)
pulmonary edema (2/4)
NGPD
NGPD
pale kidneys (2/4) mild cystitis (1/4)
metritis (1/4) endometritis (1/4)
NGPD: No gross pathologic diagnosis
*: 1 goats of each of the four control groups was subjected to post mortem investigation
Histopathology
Mild to severe tubular nephrosis was the only consistent histopathological lesion observed in goats that died. However, this tubular nephrosis was not observed in goats that survived until the end if the experiment.
Tubular nephrosis is considered to be diagnostically significant for goats that died a few days after an appropriate level of exposure.
These lesions may have contributed to the observed elevations in blood urea nitrogen (BUN) and serum creatine (SC).
Ischemic hippocampal neurons were observed in the brain of one goat that had displayed the so-called “collapsing syndrome”.
Clinical biochemistry and hematology
Slight to moderate elevations in blood urea nitrogen (BUN) and serum creatine concentrations (SC) were observed in all animals that died during the study. These findings may be related to the histopathologic kidney lesions observed in these animals.
Slight elevations in serum glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) activity were also observed in terminal animals immediately prior to death.
No other diagnostically or toxicologically significant changes were observed.
None of the biochemical parameters measured appeared to be involved in, or indicate the cause of, the unusual neurological manifestations seen in some goats receiving MON 0139.
The results are summarized in the following tables.
Table B.6.8-28: Biochemical and haematological measurements of goats that died (min–max values)
Measurement (units)
Reference value
Control1

Surviving < 49 h
Surviving > 50, < 90 h
Sampled 12–
14 h before
death (2 goats)2
Sampled < 4 h before
death (5
goats) 3
Sampled at 24 h post-
treatment (4
goats) 4
Sampled 6–
16 h before death
(3goats) 5
BUN (mg/dL)
10.0–20.0
2.3–21.3
26.7–44.1
31.5–62.4
13.8–22.0
44.2–111.4
SC (mg/dL)
1.0–1.8
0.6–1.7
4.5–6.1
3.3–5.8
1.4–2.9
5.1–6.7
GLU (mg/dL)
50.0–75.0
37–80
120–164
17–65
74–173
57–196
Na (mg/dL)
327–356
311–349
347–376
327–396
331–343
327–345
K (mg/dL)
13.6–26.1
13.4–21.2
16.2–16.5

10.9–23.2
10.3–16.9
9.0–11.0
Ca (mg/dL)
8.9–11.7
8.0–13.4
8.7–11.5
8.5–9.8
8.6–11.3
7.2–8.6
P (mg/dL)
2.9–7.3
4.1–11.9
2.9–16.0
3.5–10.0
3.9–6.4
2.9–5.9
Mg (mg/dL)
2.8–3.6
1.3–3.4
3.0–3.9
2.3–4.1
2.1–3.5
3.0–4.0
SGOT (U/L)
167–513
31–151
99–100
128–459
58–107
105–145
LDH (U/L)
123–392
145–385
303–310
394–880
220–298
266–394
SACH (U/L)

240–496
405–461
388–536
344–459
409–471
ALKP (U/L)

93–387
31–902
69–283
76–282
97–422
173–476
CPK (U/L)
20–42
44–878
144–185
181–2631
110–149
183–1145
GGT (U/L)

12–60
19–26
23–38
30–35
30–38
WBC5
4.0–15.0
5.5–17.5
12.0–14.0
3.7–21.5
9.0–23.0
10.0–18.0
SEGS5
1.6–7.5
1.4–10.4
3.1–7.0
1.3–4.8
4.1–16.6
1.8–6.9
BANDS5

0–1.4
3.6–5.9
1.3–12.1
0–4.8
1.4–9.7
LYMPHS5
1.8–9.0
2.4–9.2
2.6–2.8
1.0–6.5

1.4–4.9
1.7–4.4
EOS5
0.1–1.5
0–1.4
0–0
0–0
0–0.1
0–0
MONO5
0.1–0.9
0–0.9
0.4–0.6
0–0.9
0.1–0.4
0.1–0.5
BASO5
0–0.2
0–0.8
0–0
0–0
0–0.1
0–0
PCV(%)
19–40
23–47
26–49
33–45
34–45
31–42
HGB (g/dL)
8–16
7.8–16.7
9.6–15.6
10.6–15.6
10.8–14.4
10.6–14.8
RBC6
7–21
12.8–24.2
15.6–23.8
16.0–26.9
19.0–24.5
16.7–22.9
MCV (fL)
15–39

15–21
17.0–18.0
15.0–20.0
15.0–19.0
15.0–18.0
MCH (pg)
5.3–8.4
4.6–7.8
6.2–6.6
4.8–7.3
5.5–6.5
5.4–6.5
MCHC (g/dL)
32–40
28–41
31.8–36.9
31–35
32–37
32–35
TSP (g/dL)
6.4–7.0
5.0–8.2
6.7–8.6
5.8–7.3
7.2–7.7
6.2–7.7
1: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
2: Goats treated with 10 000 and 5360 mg/kg bw
3: Goats treated with 10 000 (n = 3), 6700 (n = 1), and 5360 (n = 1) mg/kg bw
4:Goats treated with 6700 (n = 2) and 4290 (n = 2) mg/kg bw
5:Goats treated with 6700 (n = 2) and 4290 (n = 1) mg/kg bw
Table B.6.8-29: Biochemical and haematological measurements of surviving goats (min–max values)
Measurement (units)
Reference value
Control1
Definitive signs of intoxication observed (8
goats) 2
Minimal or no signs of intoxication onserved (5
goats) 3
days 1 and 3
days 7 and
14
days 1 and 3
days 7 and
14

BUN (mg/dL)
10.0–20.0
2.3–21.3
7.6–28.7
3.7–135.6
3.9–13.6
6.0–19.1
SC (mg/dL)
1.0–1.8
0.6–1.7
1.0–1.9
0.8–9.7
0.8–1.4
0.9–1.4
GLU (mg/dL)
50.0–75.0
37–80
60–139
50–90
40–59
29–58
Na (mg/dL)
327–356
311–349
309–337
319–337
323–337
317–337
K (mg/dL)
13.6–26.1
13.4–21.2
12.2–17.6
8.0–19.5
13.9–19.5
15.0–19.4
Ca (mg/dL)
8.9–11.7
8.0–13.4
8.9–11.1
8.7–10.7
9.3–10.1
9.2–10.0
P (mg/dL)
2.9–7.3
4.1–11.9
3.5–10.2

3.8–9.7
4.5–9.3
4.9–8.8
Mg (mg/dL)
2.8–3.6
1.3–3.4
1.8–2.4
1.3–2.4
1.9–2.3
1.9–2.1
SGOT (U/L)
167–513
31–151
34–77
3.7–169
53–81
50–83
LDH (U/L)
123–392
145–385
118–232
141–3.17
170–242
151–238
SACH (U/L)

240–496
293–528
209–450
273–386
255–407
ALKP (U/L)
93–387
31–902
58–594
52–542
31–323
30–307
CPK (U/L)
20–42
44–878
58–124
44–180
54–154
44–152
GGT (U/L)

12–60
23–43
21–42
15–40
11–30
WBC5
4.0–15.0
5.5–17.5
5.7–19.5
8.0–35.5
7.5–12.5
7.5–20.0
SEGS5
1.6–7.5
1.4–10.4
2.0–14.0
2.0–9.7
2.9–7.2
3.4–16.4
BANDS5

0–1.4
0–1.0
0–1.8
0–0.3
0–0.6
LYMPHS5
1.8–9.0
2.4–9.2
2.0–6.7
3.1–8.4
2.9–6.4
2.0–6.3
EOS5
0.1–1.5
0–1.4
0–0.1
0–0.7
0–0.7
0–0.4
MONO5
0.1–0.9
0–0.9
0–0.8
0–0.7

0–0.2
0.1–3.9
BASO5
0–0.2
0–0.8
0–0.3
0–0.6
0–0.5
0–0.4
PCV(%)
19–40
23–47
29–39
27–38
24–32
24–34
HGB (g/dL)
8–16
7.8–16.7
10.4–12.9
9.4–12.4
11.3–12.2
9.8–11.3
RBC6
7–21
12.8–24.2
15.6–23.2
15.7–21.9
18.0–19.0
15.7–17.9
MCV (fL)
15–39
15–21
15.0–19.0
13.0–19.0
14.0–21.0
15.0–210
MCH (pg)
5.3–8.4
4.6–7.8
5.1–7.5
5.3–7.0
5.0–6.9
5.2–7.2
MCHC (g/dL)
32–40

28–41
32–38
31–37
33–40
34–41
TSP (g/dL)
6.4–7.0
5.0–8.2
5.9–8.7
5.6–7.7
6.1–7.2
5.9–7.1
1: Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
2: Goats treated with 6700 (n = 2), 5360 (n = 3) and 4290 (n = 3) mg/kg bw
3:Goats treated with14000 (n = 5) mg/kg bw
Conclusion
The median lethal dose (LD50) was calculated to be LD50, goat, oral = 5700 mg/kg bw [CI 95 %, 3.73–8.71].
RMS comments: The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is good and it is suitable to provide additional information about acute oral toxicity in a ruminant species. The extent of investigations exceeded that one in acute toxicity studies in laboratory rodents. However, pathology was confined to four of a total of 20 control animals. In addition, assessment was made a bit difficult by the fact that sometimes data obtained in the four control groups were pooled and sometimes reported separately.
The conclusion of the notifier is agreed with. Acute oral toxicity of the isopropylamine salt of glyphosate in goats was low and even lower than with the acid. This finding may confirm that the salt formulations of glyphosate tend to be less toxic than the acid.
B.6.8.3.2 Subacute toxicity in cows Subacute study with glyphosate isopropylamine salt
Reference: IIA, 5.10/07
Report: (1987) The subacute toxicity of the isopropylamine salt of glyphosate (MON 0139) in female cattle
Data owner: Monsanto Report No.: VT-82-003 Date: 1987-04-16 TOX9552424
Guidelines: None guideline study
Deviations: Not applicable.
GLP: Yes (Not checked by RMS. Not compulsory when the study was performed.)
Acceptability: See RMS comments below.
Dates of experimental work: 1982-02-02 to 1982-05-05
Material and methods
Test material: MON 0139 (isopropylamine salt of glyphosate/ N-phosphonomethyl glycine isopropylamine)
Description: amber liquid Lot/Batch #: LBRT 08023
Purity: 62.4%, (46.2% glyphosate only)
Stability of test compound: Stable for > 1 year (protocol date: 1980-01-18) Vehicle and/or positive

control: Water
Test animals:
Species: Cow
Strain: not applicable (Brahman cross) Source:
Age: not specified
Sex: Female (heifer/nulliparous) Weight at dosing: 215.6 ± 23.1 kg Acclimation period: at least 30 days
Diet/Food: During the initial acclimatization (outdoor): bermudagrass hay and commercial giat meal containing not less than 13 % crude protein (Purina ® Commercial Creep Chow ® (Ralston Purina Company, Gonzales, TX, USA). After the initial acclimatization (indoor) the heifers were fed bermudagrass only.
Water: Fresh water
Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study
Environmental conditions: not stated
Study design and methods Animal assignment and treatment
Dose (based on a preliminary trial with 1000 mg/kg bw) 540, 830, 1290, and 2000 mg/kg bw
Application route: Oral, gavage
Application volume: approx. 500 mL/animal Fasting time: not stated
Group size: 3/group or 2/group 4 control groups of 2 cows each
Post-treatment observation period: 14 days
Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology
In life dates: not stated
Animal assignment and treatment:
Brahman-cross heifers were treated in a sequential manner with MON 0139 by stomach tube in 7 consecutive daily doses of 540, 830, 1000 (pre-test, n = 2), 1290, and 2000 mg/kg bw (n = 3/group). Initially, the test item was given at 2000 mg/kg/bw/day and subsequent dosages were selected based on the observed responses. Control animals were treated with tap water. All animals were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Body weights were determined prior to dosing and for surviving animals on post- dosing days 7 and 14. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.
Results and discussion
Mortality
In the preliminary study neither heifer exposed to 1000 mg/kg bw died.
All animals treated with 2000 mg/kg bw MON 0139 died. 1/3 animals treated with 1290 mg/kg bw also died.
The results are summarised in the following table.
Table B.6.8-30: Mortality, survival time and animals used.
Dose (mg/kg bw)
Toxicological result
Onset of death after
Mortality (%)

Dead animals
Animals with toxic signs
N
2000
3
3
3
6.2–7.5
100
1290
1
3
3
7.5
33.3
1000*
0
2
2
--
0
830
0
3
3
--
0
540
0
0
3
--
0
0
0
0
8**
0
N: Number of animals
*: Preliminary test
**: All four control groups compiled
The minimum lethal dose (MLD) was 1290 mg/kg bw. The minimum toxic dose (MTD) was 830 mg/kg bw.
Clinical observations
In the preliminary study, treatment with 1000 mg/kg bw MON 0139 decreased food intake and induced diarrhea by the second day of treatment which continued throughout the seven day

treatment period. The signs ceased until the end of the study.
Treatment with 2000, 1290, and 830 mg/kg bw induced diarrhea.
Animals exposed to 2000 mg/kg bw MON 0139 additionally showed nervous system effects including head tremors, convulsions, ataxia, and possible visual impairment and sternal recumbancy.
Table B.6.8-31: Prominent clinical observations in female cows given MON 0139 (numbers of animals affected)
Dose (mg/kg bw)
N
Observations (Number of goats affected)
2000
3

diarrhea (3), decreased feed intake (3), nasal discharge (3), foamy salivation (1), head tremors (3), belligerency (1), whole-body tremors (3), ataxoa (3), head pressing (1), kicking at imaginary objects (1), apparent visual impairment (1), convulsions (1), falling (1), depression (1), recumbency (3), increased respiratory effort (2), death (3)
1290
3
diarrhea (3), decreased feed intake (3), depression (3), weakness (2), death (1)
1000
2
diarrhea (2); decreased feed intake (2)
830
3
diarrhea (3), decreased feed intake (3)
540
3
no signs of toxicosis (3)
CFA: cecession of feeding activity
Body weight and food consumption:
Treatment decreased body weight and food intake, as follows. However, the food intake was not precisely measured.
In the preliminary study, treatment with 1000 mg/kg bw MON 0139 decreased food intake.
Treatment with 2000 and 1290 mg/kg bw decreased food intake.
Treatment with 1290 mg/kg bw induced severe weight loss and depression for the first two weeks. While food consumption and fecal consistency returned to normal during the third week after treatment, the animals remained thin and weakened.
The results are summarized in the following table.
Table B.6.8-32: Mean percent body weight changes (from initial body weight) in cows (n) treated with MON 0139
Dose (mg/kg bw/day)
Study day
MON 0139-treated
Controls
2000
6

−1.2 (3)
−1.9 (2)
1290
6
−6.5a (3)
−2.1 (2)
14
−12.0b (2)
−1.6 (2)
21
−9.6 (2)
−3.4 (2)
830
6
−5.2a (3)
−1.5 (2)
14
−4.6 (3)
−0.4 (2)
21
−2.4a (3)
−5.1 (2)
540
6
0.3 (3)
−0.7 (2)
14
1.0 (3)
2.7 (2)
21
0.4 (3)
1.5 (2)
a:P < 0.083
b:P < 0.121
Necropsy
Few significant gross lesions were noted in fatally poisoned heifers other than dehydration, loss of weight and signs indicative of gastrointestinal irritation.
Kidney and liver to brain weight ratios were elevated at 2000 and 1290 mg/kg bw/day.
Table B.6.8-33: Mean kidney and liver weight/brain weight ratios
Dose
N
Kidney/brain
Liver/brain
2000
3
1.86a

7.57 a
0*
2
1.25
6.45
1290
3
1.49 a
5.96
0*
2
0.96
4.81
830
3
1.14 a
5.75
0*
2
1.27
6.08
540
3
1.18
6.14
0*
2
1.19
5.92
*: Concurrent control for preceding MON 0139-treated group
a: P < 0.83
Major gross pathologic findings are summarized in the following table.
Table B.6.8-34: Major gross pathologic findings (number)
Dose
Skin
Cardiovascular
Hemic & lymphatic
Hemic & lymphatic
Digestive
Urogenital
Dermatitis
Aspiration (1)
Epicardial
Petechiae,
Errosions, abomasal
HKBR (3)

(3)
Nodules (1)
petechiae (3)
capsule of
mucos a(1)
2000
(n = 3)
Pulmonary edema (2)
spleen (1)
Congestion intestine (3)
Diarrhea (3)
HLBR (3)
Distension, gall bladder (1)
Congestion liver (1)
0*
(n = 2)
Dermatitis
(2)
Infarct, rt
kidney (1)
1290**
Dermatitis (2)
Petechiae, pleura (1)
Epicardial petechiae (1)
Scars, capsule of liver (1) Errosions, abomasal
HKBR (2)
(n = 3)
Hemocyst,
mucos a(1)
heart valve (1)
Congestion intestine (1)
0*
Dermatitis
(2)
Petechiae,
trachea mucosa
Petechiae, duodenum,
congestion, peyer's
Petechiae,
urinary
(n = 2)
and pleura (1)
patches (1)
bladder (1)
Erosion, esophagus (1)
830

(n = 3)
Dermatitis
(1)
Congestion intestine (1)
Scars, liver capsule (1)
0*
(n = 2)
Dermatitis (1)
Cogestion, spleen (1)
Petechiae, abomasal mucosa
Congestion intestine (2)
Congestio n, vulvar
mucosa (1)
Subpleural
Erosions, esophagus (1)
HKBR (1)
focus (1)
HLBR (1)
540
Red foci,
Congestion intestine (1)
(n = 3)
subpleural (1)
Congestion peyer's
patches (1)
mottling, liver (1)
Dermatitis
Lymphoid
Lipoma (1)
0*
(n = 2)
(1)
hyperplasi
a pharyngeal
Parasitism (1)
nodes (1)
*: Concurrent control for preceding MON 0139-treated group
**: 1/3 bilateral hyphema NGL: no gross lesions
HLBR: high liver weight to brain weight ratio relative to controls HKBR: high kidney weight to brain weight ratio relative to controls
No findings for nervous, endocrine, muscular skeletal organs or systems, or the body as a whole
Histopathology
Microscopic examination of kidney from the dead heifer treated with 1290 mg/kg bw/day revealed marked renal tubular vacuolization.
Histologic examination of the abmomasum revealed multifocal superficial mucosal erosions, which appeared to be of recent development based on the minimal extent of the associated cellular

reaction.
Segmental congestion of the ileum was characterized microscopically by focal necrosis and inflammation of the mucosa overlying Peyer's patches.
The two surviving heifers treated with 1290 mg/kg bw/day each had mild renal tubular vacuolization.
No treatment-related microscopic lesions were observed at dosages of below 1290 mg/kg bw/day or in the 6 of eight control animals that had been subjected to histopathology.
Clinical biochemistry and hematology
Treatment with 1290 and 2000 mg/kg bw/day MON 0139 increased haematocrit, haemoglobin, red blood cells and increased serum levels of total protein, urea nitrogen (BUN), creatinine and serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), and creatome phosphokinase (CPK) activities.
The hematologic alterations were considered to be due to hemo-concentration secondary to fluid shifts resulting from diarrhea.
Elevations in CPK, SGOT, and LDH activities were attributed to muscle damage resulting from convulsions and/or prolonged sternal recumbancy.
Slight elevations of BUN and creatinine may have been due to decreased renal perfusion produced by dehydration secondary to diarrhea. However, the presence of histopathological kidney lesions at 1290 mg/kg bw/day and changes in serum electrolyte levels at several dosages suggest that these changes may have been partly due to some renal impairment.
An increase in the number of neutrophils and a decrease in the number of lymphocytes observed at 1290 and 2000 mg/kg bw/day probably represented a response to stress-induced release of corticosteroids.
Remark: Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.
The results are summarized in the following tables.
Table B.6.8-35: Days after treatment on which values for clinical laboratory measurements performed on cows treated with MON 0139 were significantly different from concurrent median control values
Measurement
Dose (mg/kg/bw/day)
a
2000
1290
830
540
Days after treatment
BUN
6
2, 6, 7, 8, 14

8–
CREAT
6
7, 8

8–

GLU

7

Na
6
6−, 14−

K
6−
6−, 8−, 14−
6−
14−
Ca
6−

8−

P
6
7, 21−
2

Mg
6
7, 8
2

Measurement
Dose (mg/kg/bw/day)
a
2000
1290
830
540
Days after treatment
SGOT
6§
2, 6, 7, 8, 14, 21

LDH
6§
6, 7

SACH

14–
21–

ALKP

7

CKN
6
2, 6, 7, 8

GGT

8,14 ,20
WBC

SEGSA
6

BANDSA
6

LYMPHSA
6–
6–

EOSA
2&, 6&
6&,8&

14−, 21−
MONOSA

BASOA

PCV
6
6§, 7, 8

HBG
6
6, 7, 8

RBC
6§
6, 7, 8

MCV

MCH

MCHC

6, 21

STP
6
6, 7, 8

---

a: represents data from 3 animals on days 2 and 6 and 2 animals on days 8, 14, and 21. Entries for day 7 represent one animal, and the value is outside the range of study-wide control values.
−: Medians significantly lower than the concurrent median control value, entries without a symbol represent medians significantly greater than the concurrent median control values
§: noteworthy non-significant increase
&:noteworthy non-significant decrease
Conclusion

The minimum lethal dose (MLD) was MLD, cow, oral = 1290 mg/kg bw. The minimum toxic dose (MTD) was MTD, cow, oral = 830 mg/kg bw.
RMS comments: The study is considered acceptable despite the low number of animals per dose group. However, studies of this type are usually not required in Europe for active ingredients in plant protection products and no guideline exists. The quality of the study is good and it is suitable to provide additional information about subacute oral toxicity in a ruminant species. The conclusion of the notifier is agreed with but the "MTD" should be rather considered the LOAEL to make it more comparable to routine toxicological studies and to avoid misunderstandings because "MTD" is often read as "maximum tolerated dose". The low dose of 540 mg/kg bw/day may be considered the NOAEL in this study. Subacute oral toxicity of the isopropylamine salt of glyphosate in young cattle (heifers) was low but signs and findings were more pronounced than in subacute studies (of longer duration) with glyphosate acid in rats and dogs.
Subacute study with a plant protection product
Reference: IIA, 5.10/08
Report: (1987) The subacute toxicity of Roundup ® herbicide (MON-2139) in female cattle
Data owner: Monsanto Report No.: VT-82-002 Date: 1987-03-23 ASB2010-8131
Guidelines: Non-guideline study
Deviations: Not applicable.
GLP: Yes (Not checked by RMS. Not compulsory when the study was performed.)
Acceptability: See RMS comments below.
Remark (Notifier): The animals were of different hormonal status (observed pregnancy) and partly infested with parasites. This heterogenicity of the study population combined with the small number of animals tested renders in particular the relevance of the clinical biochemistry and hematology findings questionable.
(Comment by RMS: It must be clarified that, according to the original study report, only in one control animal some evidence of parasitic infestation was obtained, based on eosinophilic perivascular infiltrates in the liver. In contrast, many animals on study were suffering from trichophytosis, i.e., a mycotic dermatitis.)
Dates of experimental work: 1982-02-02 to 1982-05-05
Material and methods
Test material: MON 2139 (Roundup ® formulation, N-phosphonomethyl glycine isopropylamine)
Description: amber liquid
Lot/Batch #: LBRP 01002
Purity: 41.1%, (N-phosphonomethyl glycine isopropylamine, 30.5% glyphosate only) Stability of test compound: Stable for > 1 year (protocol date: 1980-01-18)
Vehicle and/or positive control: Water Test animals: Species: Cow
Strain: not applicable (Brahman cross) Source:
Age: not specified

Sex: Female (heifer/nulliparous) Weight at dosing: 211.6 ± 21.5 kg Acclimation period: at least 30 days
Diet/Food: During the initial acclimatization (outdoor): bermudagrass hay and commercial giat meal containing not less than 13% crude protein (Purina ® Commercial Creep Chow ® (Ralston Purina Company, Gonzales, TX, USA). After the initial acclimatization (indoor) the heifers were fed bermudagrass only.
Water: Fresh water
Housing: Outdoor covered pens during acclimation and in indoor pens 11–15 days prior to treatment until the end of the study
Environmental conditions: not stated
Study design and methods Animal assignment and treatment
Dose (based on a preliminary trial with 1000 mg/kg bw) 400, 500, 630, and 790 mg/kg bw
4 control groups included
Application route: oral, rumen intubation Application volume: approx. 500 mL/animal Fasting time: not stated
Group size: 3/group or 2/group
Post-treatment observation period: 14 days
Observations: Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology
In life dates: not stated
Animal assignment and treatment:
Brahman-cross heifers were treated in a sequential manner with MON 2139 by intubation in 7 consecutive daily doses of 400, 500, 630,790 (n = 3/group), and 1000 mg/kg bw (n = 2/group, preliminary test). Initially, the test item was given at 1000 mg/kg/bw/day and subsequent dosages were selected based on the observed responses. Control animals (n = 2/group) were treated with fresh water. All animals were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment.
Body weights were determined prior to dosing and for surviving animals on post-dosing days 6, 14, and 21. Blood samples were collected on 3 separate days during pre-treatment and after dosing on days 2, 6, 8, 14, and 21. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.
Results and discussion
Mortality
In the preliminary study both heifers exposed to 1000 mg/kg bw died. 1/3 animals treated with 790 mg/kg bw MON 2139 died.
The results are summarized in the following table.
Table B.6.8-36: Mortality, survival time and animals used.
Dose (mg/kg bw)
Toxicological result
Onset of death after
Mortality (%)
Dead animals
Animals with toxic signs
N
1000*

2
2
2
3.3–6-3 days
100
790
1
3
3
8.8 days
33.3
630
0
3
3

0
500
0
2
3

0
400
0
0
3

0
0
0
0
8**

0
N: Number of animals
*: Preliminary test
**: Control groups compiled
The minimum lethal dose (MLD) was MLD, cow, oral = 790 mg/kg bw.
The minimum toxic dose (MTD) was MTD, cow, oral = 500 mg/kg bw. The no effect level (NOEL) was NOEL, cow, oral = 400 mg/kg bw.
Clinical observations
In the preliminary study, treatment with 1000 mg/kg bw MON 2139 induced severe watery diarrhea, cessation of feed intake, and, prior to death, labored respiratory movements.
Treatment with 790 mg/kg bw decreased food intake and induced diarrhea. The two surviving animals had normal stool by day 16 and were eating normally by day 20.

Treatment with 630 mg/kg bw decreased food intake and induced diarrhea.
Treatment with 500 mg/kg bw induced only diarrhea while treatment with 400 mg/kg bw had no effect.
Table B.6.8-37: Prominent clinical observations in female cows given MON 2139 (numbers of animals affected)
Dose (mg/kg bw)
N
Observations (Number of goats affected)
1000*
2
decreased feed intake (2), diarrhea (2), depression (1), expulsion of rumen ingest through the nose (1), labored breathing (2), death (2)
790
3
decreased feed intake (3), diarrhea (2), labored breathing (1), death (1)
630
3
decreased feed intake (3), diarrhea (2), soft feces (1)
500
3
soft feces (2), no signs of toxicosis (1)
400
3
no signs of toxicosis (3)
*: Preliminary test
Body weight and food consumption
Treatment 630 and 400 mg/kg bw MON-2139 decreased body weight on day 14 and treatment with 500 mg/kg bw increased body weight on day 6 compared to controls. However, statistical significance was not reached.
The results are summarised in the following table.
Table B.6.8-38: Mean percent body weight changes (from initial body weight) in cows (n) treated with MON 2139
Dose (mg/kg bw/day)
Study day
MON 2139-treated
Controls
790
6
−1.3 (3)
−2.1 (2)
14
−7.6 (2)
−1.6 (2)
21
−9.0 (2)
−3.4 (2)

630
6
1.4 (3)
−0.7 (2)
14
−0.5a (3)
2.7 (2)
21
− 0.5 (3)
1.5 (2)
500
6
5.3b (3)
−1.9 (2)
14
−0.2 (3)
−0.8 (1)
21
1.9 (3)
1.2 (1)
400
6
−1.2 (3)
−1.4 (2)
14
−2.5a (3)
−0.4 (2)
21
−0.3 (3)
5.1 (2)
a:approaches being significantly less than control mean (0.05 < P < 0.10)
b:approaches being significantly greater than control mean (0.05 < P < 0.1)
Necropsy
No consistent gross post-mortem lesions were observed in any of the MON 2139- treated or control animals.
The only treatment-related finding was aspiration pneumonia in the 790 mg/kg bw-treated animal that died. One of the animals in the preliminary study also exhibited gross lung lesions consistent with broncho-pneumonia.
Mean kidney and liver weights are presented in the following table.
Table B.6.8-39: Mean kidney and liver weight/brain weight ratios after treatment with MON 2139
Dose (mg/kg bw)
N
Kidney/brain
Liver/brain
790
3

1.44a
5.80a
0*
2
0.96
4.81
630
3
1.28
5.91
0*
2
1.19
5.92
500
3
1.35a
5.88
0*
2
1.25
6.45
400
3
1.31
5.53
0*
2
1.27
6.08
*: Concurrent control for preceding MON 2139-treated group
a: P < 0.83
Major gross pathologic findings are summarized in the following table.
Table B.6.8-40: Major gross pathologic findings (number)

Body as a whole
Skin
Respiratory
Cardiovascular
Hemic & Lymphatic
Digestive
Urogenital
790 mg/kg bw (n = 3)
Autolysis
Dermatitis
Congestion,

Petechiae,
Congestions,
Abomastitis,
Congestion,
(1/3)
(3/3)
thracheal
epicardial (1/3)
bronchial
congestion
kidneys
Weight
mucosa,
Pallor, heart (1/3)
lymph nodes
colon (1/3)
(1/3)
loss (1/3)
broncho-
(1/3)
Discoloration,
Fetus (1/3)
pneumonia
rumen mucosa
HKBR (2/3)
(1/3)
(1/3)
Hyperkeratosis,
tongue (1/3)
erosions
esophagus
(1/3)
darkening,
rumen (1/3)
petechiae,
abomasum 1/3)
Concurrent control for 790 mg/kg bw (n = 2)
Dermatitis
Petechia,
Erosion,
(2/2)
trachea &
esophagus
pleura (1/2)
(1/2)
Petechia,

tuodenum (1/2)
congestions,
Peyer’s patches
(1/2)
Body as a whole
Skin
Respiratory
Cardiovascular
Hemic & Lymphatic
Digestive
Urogenital
630mg/kg bw (n = 3)
Dermatitis
Lymphoid
Focal fibrosis,
HKBR (1/3)
(1/3)
hyperplasia,
liver (1/2)
pharyngeal
Parasitism
nodes (1/3)
(2/3)
Slight
congestion,
spleen (1/3)
Concurrent control for 630mg/kg bw (n = 2)
Dermatitis
Lymphoid
Parasitism
(1/2)
hyperplasia,
(1/2)
pharyngeal
Lipoma (1/2)
nodes (mild)
(1/2)
500 mg/kg bw (n = 3)
Weight
Dermatitis
Petechiae,
Congestion
Erosion,
HKBR (3/3)
loss (3/3)
(3/3)

tracheal
(1/3)
esophagus
mucosa (1/3)
(1/3)
Petechiae, gall
bladder (1/3)
Concurrent control for 500 mg/kg bw (n = 2)
Weight loss (2/2)
Dermatitis (2/2)
Infarct, right kidney (1/2)
400 mg/kg bw (n = 3)a
Dermatitis
Adhesions,
Congestion,
HKBR (2/3)
(1/3)
pleura (1/2)
ileum (1/3)
Parasitism
(1/3)
Concurrent control for 500 mg/kg bw (n = 2)
Dermatitis
Congestion
Congestion,
(1/2)
ileum & cecum
vulvar
(1/2)
mucosa
Congestion,
(1/3)
illeal mucosa
(1/2)
Petechia,
abomasal
mucosa (1/2)
a: 1/3 animals showed melanosis in the adrenal capsule and 1/3 animals showed hydrocephalus NGL: no gross lesions
HKBR: high kidney weight to brain weight ratio relative to controls No findings for special senses or the skeletal muscle.
Histopathology
Microscopic tissue examination confirmed the presence of aspiration pneumonia in the 790 mg/kg bw-treated animal that died. One of the 790 mg/kg bw-treated animals was found to be carrying a live, apparently normal fetus when sacrificed at study termination.
There were no other treatment-related microscopic findings.

Clinical biochemistry and hematology

Treatment with 790 mg/kg bw/day MON 2139 increased serum urea nitrogen (BUN) and electrolytes, serum enzyme activities (SECT, GGT), hemoglobin concentration, and red blood cell counts. Significant changes in group median and individual clinical laboratory values at lower dose levels were not considered to be treatment-related since no dose-response relationship was evident. The results are summarized in the following tables.

Table B.6.8-41: Days after treatment on which values for clinical laboratory measurements performed on cows treated with MON 2139 were significantly different from concurrent median control values

Measurement	Dose (mg/kg/bw/day)			
	790	630	500	400
Days after treatment				
BUN	8, 14	---	---	21a
CREAT	---	---	---	2a, 6a, 8a, 14a
GLU	21a	---	2a, 8a	---
Na	14a	---	---	---
K	14a , 21a	---	---	---
Ca	---	---	---	---

P
14a , 21a
6a

Mg

SGOT
14 , 21

LDH
14a

8a, 14a

SACH

ALKP
8a, 14a , 21a

2a, 6a, 8a

CKN

GGT
21

WBC

SEGSA

BANDSA
8

6, 14
LYMPHSA

EOSA
14a

MONOSA

BASOA

PCV

HBG
6, 8

RBC
6, 8

MCV

MCH

MCHC
6, 14
2

STP

8a, 14a
---
a: medians significantly lower than the concurrent median control value; entries without this symbol represent medians significantly greater than the concurrent median control values.
Conclusion
The minimum lethal dose (MLD) was MLD, cow, oral = 790 mg/kg bw. The minimum toxic dose (MTD) was MTD, cow, oral = 500 mg/kg bw. The no effect level (NOEL) was NOEL, cow, oral = 400 mg/kg bw.
RMS comments: The study is considered supplementary because of the uncertainty with regard to parallel parasitic infestation and dermal mycosis that might have weakened the animals. Nontheless, the study is suitable to provide additional information about subacute oral toxicity of a Roundup formulation in a ruminant species and for comparison with effects of the isopropylamine salt. The conclusion of the notifier is agreed with but the “MTD” should be rather considered the LOAEL to make it more comparable to routine toxicological studies and to avoid misunderstandings because “MTD” is often read as “maximum tolerated
dose”. The low dose of 400 mg/kg bw/day was the NOAEL in this study. Toxic signs and mortality occurred at dose levels that were lower than in the similar study with the isopropylamine salt of glyphosate suggesting a higher toxicity of MON 2139. This finding is in line with information from other sources pointing to a higher toxicity of certain formulations as compared to glyphosate and its salts.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.11)
For extensive discussion on reference values please see Volume 1.
RMS comment (August 2013):
Despite comments by GTF (please refer to commenting table, July 2013) the justification for deriving the ADI and AOEL based on developmental studies in rabbits as given under 2.6.12 in Vol.1 of the DRAR is still considered convincing and was not changed.
B.6.11 Acute toxicity including irritancy and skin sensitisation of preparations (Annex IIIA 7.1)
B.6.11.1 Summary
Table B.6.11-1: Information on MON 52276*

Product name and code
MON 52276
Formulation type
Soluble concentrate (SL)
Active substance (incl. content)
Glyphosate, 360 g/L as its isopropylamine salt, 486 g/L; Remark: This formulation does not contain any
Function
Herbicide
Product already evaluated as the ‘representative formulation’ during the first Annex I inclusion
Yes
* Information on the detailed composition of MON 52276 can be found in the confidential part of this dossier, Doc. J (Vol.4).
MON 52276, the lead formulation of the Glyphosate Task Force submission, was one of the representative formulations supporting the 2001 Annex I inclusion of glyphosate. This formulation is still registered in Europe and its composition has not changed.
Justified proposals for classification and labelling
In accordance with Directives 67/548/EEC and 1999/45/EC and according to the criteria giv- en in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 the following classification and labelling with regard to toxicological data is proposed for the preparation:
Table B.6.11-2: Justified proposals for classification and labelling
C&L according to Directives 67/548/EEC and 1999/45/EC
Hazard symbols:
None
Indications of danger:
None
Risk phrases:
None
Safety phrases:
None
Additional labelling phrases:
To avoid risks to man and the environment, comply with the instructions for use.
C&L according to Regulation (EC) No 1272/2008
Hazard classes, categories:
None
Signal word:
None
Hazard statements:
None
Additional labelling phrases:
To avoid risks to man and the environment, comply with the instructions for use. [EUH401]
'15.8 percent of the mixture consist of ingredients of unknown inhalation toxicity.'
Table B.6.11-3: Summary of risk assessment for operators, workers, bystanders and residents for MON 52276
Result

PPE / Risk mitigation measures
Operators
Acceptable
German model
- Avoid any unnecessary contact with the product. Misuse can lead to health damage.
- Keep out of the reach of children.
- Wear protective gloves when handling the undiluted product.
UK POEM *
- Avoid any unnecessary contact with the product. Misuse can lead to health damage.
- Keep out of the reach of children.
- Wear protective gloves when handling the undiluted product.
- Wear protective gloves when handling/applying the product ready for application.
- Wear an impermeable coverall when applying/handling the product ready for application.
Workers
Acceptable
- Treated areas/crops may not be entered until the spray coating has dried.
Bystanders
Acceptable
None
Residents
Acceptable
None
* only tractor-mounted applications are acceptable
The risk assessment according to the German model has shown that the estimated exposure towards glyphosate in MON 52276 does not exceed the systemic AOEL for operators, workers, bystanders and residents, if prescribed PPE is worn by operators in the case of applications using knapsack sprayers. No specific PPE is necessary for operators or for workers.
The risk assessment according to the UK-POEM has shown that the estimated exposure towards glyphosate in MON 52276 will not exceed the systemic AOEL for operators applying MON 52276 in field crops using tractor-mounted equipment provided that prescribed PPE is worn (gloves during mixing/loading and application). Operator exposure will be below the systemic AOEL only, if prescribed PPE is worn.
On the other hand, as far as hand-held applications under high crops are concerned, no safe use could be demonstrated using the UK-POEM for exposure estimation irrespective of whether or not PPE is used (gloves mixing/loading and application as well as impermeable coverall during application).
B.6.11.2 Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for MON 52276
The conclusions of the 2001 EU evaluation of MON 52276 (acute toxicity profile) are summarised in Table B.6.11-4. All data are still relevant to this submission. However a new dermal sensitisation study was subsequently conducted under GLP conditions, following the revised OECD 406 test guideline (modified Buehler; 9 applications). The new dermal sensitisation study confirms both the results of the previously submitted non-GLP study and the 2001 EU evaluation for this end point.
Table B.6.11-4: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for MON 52276
Annex point (2001 EU

Monograph Annex Point)
Type of test, model system (Guideline)
Result
Acceptability
Classification (acc. to the criteria in Dir. 67/548/EEC)
Classification (acc. to the criteria in Reg. 1272/2008)
Reference
IIIA 7.1.1 (B.5.11.1.1)
LD50 oral, rat (OECD 401)
> 5000 mg/kg bw
Yes
None
None
1991
TOX9552438
IIIA 7.1.2 (B.5.11.1.2)
LD50 dermal, rat (OECD 402)
> 5000 mg/kg bw
Yes
None
None
1991
TOX9552439
IIIA 7.1.3 (B.5.11.1.3)
LC50 inhalation, rat
Not submitted, not necessary. Justification presented in Vol. 3, B.6.11.5)
IIIA 7.1.4 (B.5.11.1.4)
Skin irritation, rabbit (OECD 404)
Non-irritant
Yes
None
None
1991
TOX9552440
IIIA 7.1.5 (B.5.11.1.5)
Eye irritation, rabbit (OECD 405)
Non-irritant
Yes
None
None
1992
TOX9552441
IIIA 7.1.6/01 (B.5.11.1.6)

Skin sensitisation, guinea pig (OECD 406,
Buehler (3 applications)
Non- sensitising
No
None
None
1992
TOX9552442
IIIA 7.1.6/02
Skin sensitisation, guinea pig (OECD 406,
Buehler (9 applications)
Non- sensitising
Yes
None
None
2001
TOX2005- 1135
Supplementary studies for combinations of plant protection products
No data – not required
Summaries of previously reviewed studies (Sections 7.1.1 – 7.1.5) and the new dermal sensitisation study (IIIA 7.1.6/02) are presented below.
B.6.11.3 Acute oral toxicity
Reference: OECD IIIA 7.1.1
Report: 1991
Acute Oral Toxicity Study in Rats., BD-91-261,
TOX9552438
Guidelines: US EPA FIFRA guideline 81-1 (1984)
OECD 401 (1987),
EEC directive 84/449/EEC method B.1 (1984)
Deviations: No
GLP: Yes
Acceptability: Yes
Materials and methods
Test material (Lot/Batch No.)
MON 52276 (LLN-9105-3135-F)
Species
Rat, Sprangue-Dawley [CD® - Crl : CD® (SD)BR]
No. of animals (group size)
5 males and 5 females
Dose
5000 mg/kg bw
Exposure
Once by gavage
Vehicle/Dilution
None
Post exposure observation period

14 days
Remarks
None
Results and discussions
Table B.6.11-5: Results of acute oral toxicity study in rats of MON 52276
Dose (mg/kg bw)
Toxicological results 1)
Duration of signs
Time of death
LD50 (mg/kg bw) (14 days)
Males
5000
0/5/5
1 day
--
> 5000
Females
5000
0/5/5
1 day
--
> 5000
1) Number of animals which died/number of animals with clinical signs/number of animals used
Table B.6.11-6: Summary of findings of acute oral toxicity study in rats of MON 52276
Mortality:
There were no mortalities during the study.
Clinical signs:
Faecal staining and / or soft stool was noted in all animals after dosing on day 1. A few animals also showed oral and / or nasal discharge, as well as hypoactivity.
Body weight:
Body weight gain was unaffected by the administration of the test substance.
Macroscopic examination:
The gross necropsy conducted at termination of the study revealed no observable abnormalities.
Conclusion
Under the experimental conditions, the oral LD50 of MON 52276 is higher than 5000 mg/kg bw in rats. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.
B.6.11.4 Acute percutaneous toxicity Reference: OECD IIIA 7.1.2
Report: 1991
Acute Dermal Toxicity Study in Rats., BD-91-262,
TOX9552439
Guidelines: US EPA FIFRA guideline 81-2 (1984),
OECD 402 (1987),
EEC directive 84/449/EEC method B.3 (1984)
Deviations: No

GLP: Yes
Acceptability: Yes
Materials and methods
Test material (Lot/Batch No.)
MON 52276 (LLN-9105-3135-F)
Species
Rat, Sprangue-Dawley [CD® - Crl : CD® (SD)BR]
No. of animals (group size)
5 males and 5 females
Dose(s)
5000 mg/kg bw
Exposure
24 hours (dermal, semi-occlusive)
Vehicle/Dilution
None
Post exposure observation period
14 days
Remarks
None
Results and discussions
Table B.6.11-7: Results of acute dermal toxicity study in rats of MON 52276
Dose (mg/kg bw)
Toxicological results 1)
Duration of signs
Time of death
LD50 (mg/kg bw) (14 days)
Males
5000
0/0/5
--
--
> 5000
Females
5000
0/0/5
--
--
> 5000
1) Number of animals which died/number of animals with clinical signs/number of animals used
Table B.6.11-8: Summary of findings of acute dermal toxicity study in rats of MON 52276
Mortality:
There were no mortalities during the study.
Clinical signs:
There were no dermal effects observed in any of the animals throughout the study period.
Two animals showed red ocular discharge and one additional animal had red-stained urine at day 1.
Body weight:

Body weight gain was unaffected by the administration of the test substance.
Macroscopic examination:
The gross necropsy conducted at termination of the study revealed no observable abnormalities.
Conclusion
Under the experimental conditions, the dermal LD50 of MON 52276 is higher than 5000 mg/kg bw in rats. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.
B.6.11.5 Acute inhalation toxicity
An acute inhalation toxicity study on MON 52276 has not been performed, because the criteria listed in Annex II (7.3.1) of Commission Regulation (EU) 545/2011 are not met (see below):
MON 52276 is not / does not
a) a gas or liquified gas,
The pure active substance, glyphosate acid, is in the form of colourless crystals at ambient temperature, with a melting point of 189.5 °C. The preparation MON 52276 is a soluble liquid (SL) formulation
b) a smoke generating formulation or fumigant,
c) used with fogging/misting equipment,
d) a vapour releasing preparation,
The preparation is not a vapour releasing preparation. It is a soluble liquid (water- based), which is mixed with water for application by hydraulic sprayers
e) an aerosol,
f) a powder or a granule containing a significant proportion of particles of diameter < 50 µm (> 1 % on a weight basis), MON 52276 is a soluble liquid, not a powder
g) to be applied from aircraft in cases where inhalation exposure is relevant,
h) contain an active substance with a vapour pressure > 1x10-2 Pa and is not to be used in enclosed spaces such as warehouses or glasshouses,
The active ingredient, glyphosate acid, is essentially non-volatile. Its vapour pressure is well below 1 x 10-2 Pa., the threshold for consideration as a volatile substance:
Vapour pressure: 1.31 × 10-5 Pa (25 °C) Henry's Law Constant: 2.1 × 10-7 Pa × m3 × mol-1
Based on volatility, the calculated vapour density of glyphosate is less than 1 mg × m-3 at 25 °C (equivalent to less than 6 x 10-9 moles × m3).
In MON 52276, the active ingredient is formulated as the isopropylamine salt of glyphosate. The salt is less volatile than the acid:
Vapour pressure: 2.1 x 10-6 Pa (25 °C) Henry's Law Constant: 4.6 x 10-10 Pa × m3 × mol-1
The calculated vapour density of the isopropylamine salt of glyphosate is less than 0.2 mg × m-3 at 25 °C (equivalent to less than 1 x 10-9 moles × m3).
i) to be applied in a manner which generates a significant proportion (greater than 1 % on a weight basis) of particles or droplets of diameter <50 µm unless the applicant can justify an alternative approach under Directive 1999/45/EC or Regulation (EC) No 1272/2008, where applicable.
The product is recommended for spraying through hydraulic nozzles. Label recommendations propose that the nozzles used to atomise the spray mixture should produce a "medium" to "medium/coarse" spray quality as defined by the International (BCPC) spray classification system. Such nozzles produce a size range of droplets suitable to optimise their deposition on target weeds while reducing the proportion of droplets susceptible to drift.
Droplet spectra were measured for MON 52276 using standard nozzles typical for the type used on

field sprayers in a supplementary study (1999; ASB2012-12069). The Spraying Systems 11003 nozzle used in the study is classified as producing a “fine/medium” spray and, therefore, represents a worst case in terms of the proportion of small droplets produced. The results for MON 52276 are comparable to those from studies on other formulations of glyphosate. The droplet size data are measured and reported as % volume, however, the specific gravity of a spray solution is close to 1.00.

Volume median diameter 246 µm Number median diameter 55 µm

% total spray volume < 50 µm

0.71 %

< 10 µm

0.00 %

Less than 1 % (w/w) of the droplets have a smaller diameter than 50 µm, the threshold specified in Commission Regulation 545/2011 as a trigger to perform a mandatory acute inhalation toxicity study. Larger nozzles, such as Spraying Systems 11004, or “low drift nozzles”, produce fewer small droplets which would even represent a lower risk than those produced by standard nozzles.

B.6.11.6 Skin irritation

Reference: OECD IIIA 7.1.4

Report: 1991

Primary Dermal Irritation Study in Rabbits., BD-91-263,

TOX9552440

Guidelines: OECD 404 (1992);

Commission Directive 92/69/EEC method B.4 (1992), US EPA FIFRA guideline 81-5 (1984)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Test material (Lot/Batch No.)

MON 52276 (LLN-9105-3135-F)

Species

Rabbit, New Zealand White

No. of animals (group size)

4 males and 2 females

Initial test using one animal

No

Exposure

0.5 mL (4 hours, semi-occlusive)

Vehicle/Dilution

None

Post exposure observation period

3 days

Remarks

The test substance was applied on two sites of each animal (right and left).

Results and discussions

Table B.6.11-9: Skin irritation of MON 52276

Animal No.

Scores after treatment

1)
Mean scores (24-72 h)
Reversible [day]
0.5 h
24 h
48 h
72 h
1 (male)
Right
Left
Erythema Oedema
Erythema Oedema
0
0
0
0
0.0
0
1
0
0
0
0
0
0
0.0
0.0
1
0
0
0
0
0.0
2 (male)
Right
Left
Erythema Oedema Erythema
Oedema
1
0
0
0
0.0
0
2
0

0
0
0
0
0
0.0
0.0
1
0
0
0
0
0.0
3 (female)
Right
Left
Erythema Oedema
Erythema Oedema
2
1
1
0
0.67
0
1
0
0
1
0
0
1
0
0
0
0 2)
0.0
0.67
0.0
3
4 (male)
Right
Left
Erythema Oedema
Erythema Oedema
1
0

0
0
0.0
0
1
0
0
0
0
0
0
0.0
0.0
1
0
0
0
0
0.0
5 (male)
Right
Left
Erythema Oedema
Erythema Oedema
1
0
0
0
0.0
0
1
0
0
0
0
0
0
0.0
0.0
1
0
0
0
0
0.0
6 (female)

Right
Left
Erythema Oedema Erythema
Oedema
1
0
0
0
0.0
0
1
0
0
0
0
0
0
0.0
0.0
1
0
0
0
0
0.0
1) scores in the range of 0 to 4
2) desquamation reported
Clinical signs::No mortality occurred. No clinical signs were reported.
Conclusion
Under the experimental conditions, MON 52276 is not a skin irritant. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.
B.6.11.7 Eye irritation
Reference: OECD IIIA 7.1.5
Report: 1992
Primary Eye Irritation Study in Rabbits, BD-91-60,
TOX9552441
Guidelines: OECD 405 (1987);
EC Directive 92/69/EEC method B.5 (1992), US EPA FIFRA guideline 81-4 (1984)
Deviations: No
GLP: Yes
Acceptability: Yes
Materials and methods
Test material (Lot/Batch No.)
MON 52276 (LLN-9102-2794-F)
Species

Rabbit, New Zealand White
No. of animals (group size)
3 males and 3 females
Initial test using one animal
No
Exposure
0.1 mL (single instillation in conjunctival sac)
Irrigation (time point)
No
Vehicle/Dilution
None
Post exposure observation period
7 days
Remarks
None
Results and discussions
Table B.6.11-10: Eye irritation of MON 52276
Animal No.
Scores after treatment
1)
Mean scores (24-72 h)
Reversible [day]
1 h
24 h
48 h
72 h
1 (female)
Corneal opacity
0
0
0
0
0.0
0
Iritis
0
0
0
0
0.0
0
Redness conjunctivae
1
1
1
0

0.67
3
Chemosis conjunctivae
1
0
0
0
0.0
1
2 (male)
Corneal opacity
0
0
0
0
0.0
0
Iritis
0
0
0
0
0.0
0
Redness conjunctivae
1
1
1
0
0.67
3
Chemosis conjunctivae
1
0
0
0
0.0
1
3 (female)
Corneal opacity
0
0
0
0
0.0
0

Iritis
0
0
0
0
0.0
0
Redness conjunctivae
2
0
0
0
0.0
1
Chemosis conjunctivae
1
0
0
0
0.0
1
4 (male)
Corneal opacity
0
0
0
0
0.0
0
Iritis
0.5
0
0
0
0.0
1
Redness conjunctivae
2
2
2
1
1.67
7
Chemosis conjunctivae
1
0

0
0
0.0
1
5 female)
Corneal opacity
0
0
0
0
0.0
0
Iritis
0
0
0
0
0.0
0
Redness conjunctivae
1
2
2
1
1.67
7
Chemosis conjunctivae
1
0
0
0
0.0
0
6 (male)
Corneal opacity
0
0
0
0
0.0
0
Iritis
0
0
0
0

0.0
0
Redness conjunctivae
1
2
2
2
2.0
7
Chemosis conjunctivae
1
0
0
0
0.0
1
1) scores in the range of 0 to 4 for cornea opacity and chemosis, 0 to 3 for redness of conjunctivae (1 is not considered positive by the applicants) and 0 to 2 (including 0.5 which is not considered positiv) for iritis
Clinical signs: No mortality occurred. No clinical signs of systemic toxicity were reported.
Conclusion
Under the experimental conditions, MON 52276 is not an eye irritant. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.
In addition to the toxicological study on rabbits an open publication was submitted by the applicants (Acquavella, J.F. et al., 1999; TOX2002-699) dealing with human ocular effects from self-reported exposure towards Roundup® herbicides. According to this publication no serious, long-lasting eye injury occurred during a period of 5 years among callers to a single regional poison center.
B.6.11.8 Skin sensitisation
For a summary of the non-GLP compliant study on skin sensitisation (IIIA 7.1.6/01, 1992 ; TOX9552442) which was evaluated during the first Annex 1 inclusion
procedure of glyphosate and which was not submitted for this renewal it is referred to the DAR.
Reference: OECD IIIA 7.1.6/02
Report: , 2001
Skin Sensitisation Test in Guinea Pigs (Modified Buehler test: 9 Applications),
CI-2001-153, TOX2005-1135
Guidelines: OECD 406 (1992);
Commission Directive 96/54/EC B.6 (1996)
Deviations: No
GLP: Yes
Acceptability: Yes
Remark: The LLNA, or, if not possible, the M&K-test is clearly pre- ferred to the Buehler-test according to the current state of knowledge and the expected data requirements for plant protection products for authorisation in the EU. According to REACH, the LLNA is the first choice method, too, and a justification for the use of a different test shall be provided. Test Method Guideline B.6 by the European Commission (Reg. (EC) No. 440/2008) or even by its previous ver- sion 96/54 also

recommends the preferential use of an adjuvant-test (e.g. M&K-test) instead of the Buehler-test without adjuvant unless a justification is given for using the Buehler-method. However, no justification is available.
But, since the provided Buehler-test is valid this is to be accepted against the background of animal welfare.
Materials and methods
Test material (Lot/Batch No.)
MON 52276 (A1C1204104)
Species
Guinea pig, Hartley Crl: (HA) BR
No. of animals (group size)
Test substance group: 10 male and 10 female guinea pigs
Vehicle control goup: 5 male and 5 femaleguinea pigs
Range finding:
Yes
Exposure (concentration(s), no. of applications)
Topical induction: Undiluted (9 x) Challenge: Undiluted
Vehicle
Purified water
Pretreatment prior to topical application
No
Reliability check
Mercaptobenzothiazole (topical induction: 1st to 4th ind. 20 % w/w, 5th and 6th ind. 10 % w/w, 7th and 8th ind. 5 % w/w and 9th ind. 2.5 % w/w and challenge: 20 %)
Remarks
None
Results and discussions
Table B.6.11-11: Summary of skin responses after challenge exposure towards MON 52276
24 hours
48 hours
After challenge
MON 52276
0/20
0/20
Control Group (Vehicle)
0/10
0/10
Positive control
2/10
7/10
1) Number of animals with positive dermal response (scores of 1-3)/number of animals in dose group
Clinical signs::No deaths occurred. No signs of systemic toxicity were reported.
Conclusion
Under the experimental conditions, MON 52276 is not a skin sensitiser. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations as well as according to Regulation (EC) No. 1272/2008.

B.6.11.9 Supplementary studies for combinations of plant protection products

Combined application of plant protection products is not intended. Therefore, no such studies were performed.

B.6.12 Dermal absorption (Annex IIIA 7.6)

Introduction into this chapter by RMS

In the 2001 EU glyphosate evaluation, dermal absorption of glyphosate was considered to be less than 3% (DAR 1998, ASB2010-10302). This estimate was based on in vivo data in Rhesus monkeys, as well as on in vitro data in human skin, using the original glyphosate formulation Roundup (1983, TOX9552417; 1991, TOX9552418). However, these studies do not comply to current standards and should not be taken into consideration anymore even though the 3% assumption was supported by a new study with the active ingredient (2012, ASB2012-11459).

For exposure calculation and risk assessment, it is necessary to determine or estimate dermal absorption of an active ingredient from the formulation under evaluation since co-formulants may have a crucial impact on the absorption rate. Such studies are available for a small number of formulations containing glyphosate. For the representative formulation MON 52276, dermal absorption was assessed in vitro on human skin (isolated epidermis). Valid studies of this type are usually accepted in the EU as ”stand alone-information” with their results (after appropriate rounding) being directly and without further adjustment used for exposure calculation and risk assessment (OECD, 2011, ASB2013-2; EFSA, 2012, ASB2012-
6959). Thus, the absence of in vivo data is not considered a data gap. This in vitro study (Ward, 2010, ASB2012-5383) and their results are reported in detail below.

Subsequently, a study is adressed in which dermal absorption of the active ingredient glyphosate itself (i.e., not contained in a commercial formulation) was investigated in vitro on rabbit skin (Hadfield, 2012, ASB2012-11459). Even though rabbit skin is a rather unusual model for studies of this type, the study is reported because it may give an idea of the low dermal absorption of glyphosate that is not formulated to generate a specific plant protection product.

Four more in vitro dermal absorption studies were submitted as part of the GTF dossier (Davies, 2003, ASB2012-11518; Ward, 2010, ASB2012-11515; Ward, 2010, ASB2012-11516;
Hadfield, 2012, ASB2012-11517) that all confirmed a low dermal absorption of glyphosate. However, since these studies were performed with products other than the representative formulation, there is no need to take them into consideration for the current re-evaluation of glyphosate. Accordingly, they were not reviewed by the RMS and have been excluded from Volume 3. If necessary, they should be evaluated in future on national or zonal level.

B.6.12.1 Dermal absorption of glyphosate from MON 52276 in vitro (Human epidermis)

Reference:

IIIA, 7.6.2/01

Report:

Ward, R.J. 2010 360 g/L Glyphosate SL Formulation (MON 522276) – In vitro absorption of Glyphosate through human epidermis

Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK Study No.: JV2084, Report No.: JV2084-REG,

Date: 2010-02-19, Unpublished ASB2012-5383

Guidelines:

OECD 428 with regard to the OECD guideline, however, some
deficiencies are mentioned in the RMS comment below

Deviations:

None
GLP:
Yes
Acceptability:
See RMS comment
Dates of experimental work: 2009-06-09 - 2009-08-26
The objective of this study was to evaluate the potential dermal absorption of glyphosate from a 360 g/L SL formulation concentrate, as well as from two representative in-use dilutions prepared as 1:12.5 (v/v) and 1:150 (v/v) aqueous dilutions.
14C-glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The doses were applied to human epidermal membranes at a rate of 10 µL/cm2 and left unoccluded for an exposure period of 24 hours. The absorption process was followed
by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout the exposure period. The distribution of glyphosate within the test system and a 24-hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).
Material and methods
Test materials:
Non radio-labelled test substance:
Identification:
Isopropyamine salt of glyphosate techn. material (glyphosate-IPA)
Description:
Clear, water white to amber viscous liquid (solution in water)
Lot/Batch #:
A8B60170S0
Chemical purity/a.i. content:
Glyphosate-IPA: 63.81 % Glyphosate acid: 47.28 %
Stability of test compound:
Expiry date: 2012-01-25
Analytical reference standard:
Identification:
Glyphosate acid
Description:
White solid
Lot/Batch #:
GLP-0810-19515-A
Chemical purity:
99.8 %
Stability of test compound:
Expiry date: 2011-01-31
Radio-labelled test substance
Identification:
14C-glyphosate (as glyphosate acid)
Lot/Batch #:
53463-3-23
Chemical purity:
99.8 %

Radiochemical purity:
97.8 % (confirmed by analysis)
Specific activity:
47 mCi/mmol; 1739 MBq/mmol; 277.9 µCi/mg; 10.28 MBq/mg
Stability of test compound:
Not reported
Blank formulation
Identification:
Proprietary surfactant blend (MON 8153)
Concentration of a.s.:
0 %
Description:
Not reported
Lot/Batch #:
Not reported
Stability of test compound:
Not reported
Formulated test substance
Identification:
MON 52276
The formulation concentrate used was not supplied as complete formulation, but had to be prepared from the ingredients a) and c) described above, to allow the
incorporation of the radiolabel.
The test substance concentration in the prepared formulation was confirmed by analysis.
Test skin source:
Species:
Human
Source:
Tissue bank (not further specified)
Study design and methods
Preparation of skin samples: Human skin samples (details regarding the donors not given) were immersed in water at 60 °C for 40-45 seconds and the epidermis was teased away from the dermis. Each membrane was given an identifying number and stored frozen, at approximately -20 ºC, on aluminium foil until required for use.
Test substance preparation
Three test substance concentrations representing the formulation concentrate and two field dilutions were prepared at target concentrations of 360 g/L, 29.6 g/L and 2.51 g/L. The nominal radioactivity contained in the dose preparations was 3.3 MBq.
Radioactive stock solution of 14C-glyphosate
Dry 14C-glyphosate was solubilised in 2 mL of water and mixed thoroughly.
High dose (formulation concentrate, 360 g/L)
A pre-mix was prepared by mixing 3900 mg glyphosate-IPA technical material with an appropriate amount of proprietary surfactant blend. 78 µL (m 78 mg) of the radioactive stock solution was mixed with 482 mg of the pre-mix. Water was added to give a total weight of 585 mg. The solution was mixed well. Assuming a density of 1.17 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 369 g glyphosate/L. According to the raw data, only four cells with epidermal

samples from three different donors were used
Intermediate dose, (1:12.5 (v/v) aqueous dilution, 29.6 g/L)
A pre-mix was prepared by mixing 305.92 mg glyphosate-IPA technical material with an appropriate amount of proprietary surfactant blend. 78 µL (r 78 mg) of the radioactive stock solution was mixed with 38.01 mg of the pre-mix. Water was added to give a total weight of 500 mg. The solution was mixed well. Assuming a density of 1 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 29.6 g glyphosate/L. According to the raw data, six cells with epidermal samples from three different donors were used.
Low dose (1:150 (v/v) aqueous dilution, 2.51 g/L)
A pre-mix was prepared by mixing 76.90 mg glyphosate-IPA technical material with an appropriate amount of proprietary surfactant blend. 78 µL (A 78 mg) of the radioactive stock solution was mixed with 2.64 mg of the pre-mix. Water was added to give a total weight of 500 mg. The solution was mixed well. Assuming a density of 1 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 2.51 g glyphosate/L. According to the raw data, agin, only four cells but with epidermal samples from four different donors were used.
Analyses of dose preparations
The radioactivity content of the stock solution was determined by liquid scintillation counting (LSC) analyses of sub-samples of solvent dilutions. The radiochemical purity of the radiolabelled test substance was determined by high performance liquid chromatography (HPLC).
The radioactivity content and homogeneity of the dose preparations were checked by LSC analyses. The radiochemical purity and stability was measured by HPLC analyses.
Preparation of diffusion cells
The skin membranes were placed in static glass diffusion cells providing an exposure area of 2.54 cm2 of skin. The cells had a receptor chamber volume of approximately 4.5 mL.
An integrity test was performed by measuring the electrical resistance across the skin membranes. Membranes with a resistance of ≥ 10 kA were considered having a normal integrity and used for the absorption study.
Physiological saline was chosen as receptor fluid to ensure that the test substance (taking into account its physico-chemical properties) could freely partition into this compartment. However, it was not reported if solubility of the test substance was tested. The skin surface temperature was maintained at 32 ± 1 °C using a water bath.
Test substance application and sampling
Prior to dosing a pre-treatment sample of 500 µL was taken from each diffusion cell, and replaced by an equal amount of fresh receptor fluid.
Each dose formulation was applied to the skin membrane at the rate of 10 µL/cm2 exposed skin area (25.4 µL dose), corresponding to target concentration of 3693 µg/cm2, 296 µg/cm2 and 25.1 µg/cm2 for the high, intermediate and low dose level, respectively. The applications were left un-occluded for 24 hours.
Receptor fluid samples (500 µL) were taken by an auto-sampler at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. After each sampling the removed amount of receptor fluid was replaced by an equal amount of fresh receptor fluid.
Terminal procedures
After the last sampling 24 hours after application, the remaining receptor fluid was discarded. The receptor chamber was rinsed with receptor fluid that was also discarded.
The donor chamber was carefully removed and the underside wiped with a single natural sponge, pre-wetted with 3 %Teepol L® in water, which was added to the wash sponges. The donor chamber

was washed with deionised water and a sample was taken for LSC analysis.
The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The skin surface was washed with further sponges pre-wetted with water. All the sponges were combined and digested in Soluene 350® and made up to a recorded volume. A sample was taken for analysis.
The surface of the skin was allowed to dry naturally
Each skin membrane was tape stripped using 3M Scotch 'Magic' tape to a maximum of five strips. The tape strips were soaked individually in 30 % v/v methanol in water to extract any test material. The extracts were sequentially numbered and analysed by LSC. In some cases, it was not possible to take the full five tape strips as the epidermis began to tear, therefore tape stripping was discontinued. The last tape strip for these diffusion cells was digested with the remaining epidermis, so as not to underestimate residues in the remaining epidermis compartment. The remaining epidermis was carefully removed from the receptor chamber and digested in Soluene 350® and the whole digest analysed by LSC.
Analysis of samples
Liquid samples of the receptor fluid, washing solutions, digested wash sponges, tape strip extracts and digested epidermis by LSC using a Packard 3100 TR LSC counter and Goldstar as scintillation fluid. Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of glyphosate in the receptor solution in terms of µg/cm2. The amounts absorbed, rates of absorption (µg/cm2/h) and 'percentage of dose absorbed' were calculated. Membranes with absorption profiles that indicate membrane damage during the course of the experiment have been excluded from calculations. The results of the mass balance and distribution determinations are expressed in terms of amount absorbed and 'percentage of applied dose The absorbed dose is considered the glyphosate detected in the receptor fluid, while the potentially biologically available proportion of the dose is regarded as the sum of absorbed dose and the amount recovered from the epidermis after tape stripping. The test material removed from the surface of the epidermis by the washing procedure, as well as the glyphosate recovered from the epidermis at the end of the exposure is considered unabsorbed.
Results and discussion
Analysis of unformulated 14C-glyphosate
HPLC analysis of the unformulated sample of 14C-glyphosate confirmed a radiochemical purity of 97.8 %.
Analyses of dose preparations
The achieved concentration of glyphosate in the dose preparations was calculated to be 369.3, 29.6 and 2.52 g glyphosate /L in the formulation concentrate, 1/12.5 v/v dilution and 1/150 v/v dilution, respectively.
LCS analyses confirmed the dose solutions to be homogeneous.
Dermal absorption of glyphosate
The determined distribution of radioactivity for the different dose groups are summarised in Table B.6.12-1 below.
Table B.6.12-1: Summary of results for dermal absorption of 14C-glyphosate from representative SL formulation MON 52278
Dose preparation
High (concentrate)

Intermediate (1:12.5 v/v dilution)
Low
(1:150 v/v dilution)
Nominal concentration [g/L]
360
29.6
2.51
Actual concentration [g/L]
369.3
29.6
2.52
Applied dose [µL/cm2]
10
10
10
Applied dose [µg/cm2]
3693
296
25.2
Number of cells accessed
4*
6
4*
Distribution of radioactivity (mean values)
µg/cm2
% of
applied dose
µg/cm2
% of
applied dose
µg/cm2
% of
applied dose
Surface compartment
Stratum corneum (5 tape strips)
2.39
0.065
0.386
0.130
0.081
0.320
Stratum corneum (first 2 tape
strips)
1.57
0.043
0.283

0.096
0.065
0.256
Skin wash
3656
99.0
288
97.4
24.8
98.4
Donor chamber
83.4
2.26
6.67
2.26
<LOQ
0.008
Receptor compartment
Receptor fluid (0-24 h)
0.322
0.009
0.086
0.029
0.023
0.092
Total absorbed
0.322
0.009
0.086
0.029
0.023
0.092
Remaining epidermis (after 5 tape strips)
2.02
0.055
0.310
0.105
0.047
0.185
Remaining epidermis (after 2 tape strips)
2.84
0.077
0.413
0.140

0.063
0.250
Total potentially absorbable**
(after 5 tape strips)
2.343
0.063
0.396
0.134
0.070
0.276
Total potentially absorbable** (after 2 tape strips)
3.162
0.086***
0.499
0.169
0.086
0.342***
Total recovery
3744
101
296
100
25.0
99.0
Absorption rates [µg/cm2/h]
0.014
0.003
0.001
(0-24h)
* Some cells for these applications were excluded from calculations as the analytical data indicated that the epidermal membrane may have been damaged during application.
** Total potentially absorbable = total absorbed + remaining epidermis
*** Dermal absorption values used for exposure assessment
Dose preparation
High (concentrate)
Intermediate (1:12.5 v/v dilution)
Low
(1:150 v/v dilution)
Nominal concentration [g/L]
360
29.6
2.51
Actual concentration [g/L]
369.3
29.6
2.52

Applied dose [µL/cm2]
10
10
10
Applied dose [µg/cm2]
3693
296
25.2
Number of cells accessed
4*
6
4*
Distribution of radioactivity (mean values with standard deviation)
Surface compartment
µg/cm2 % of applied dose, based on the mean
µg/cm2 % of applied dose, based on the mean
µg/cm2 % of applied dose, based on the mean
Stratum corneum (total) 2.39 ± 0.71
0.065 0.386 ±
0.371
0.130 0.081±
0.082
0.320
Stratum corneum - first tape strip (to be excluded)
Stratum corneum - second tape strip (to be excluded)
1.12 ± 0.81
0.45 ± 0.26
0.030 0.182 ±
0.209
0.012 0.101 ±
0.106
0.096 0.048 ±
0.051
0.034 0.016 ±
0.019
0.192
0.064
Skin wash 3656 ±
181
99.0 288 ±
5.54
97.4 24.8 ±
0.496
98.4
Donor chamber 83.4 ± 167
2.26 6.67 ±

6.12
2.26 <LOQ n.a.
Receptor compartment
Receptor fluid (0-24 h) 0.322 0.009 0.086 0.029 0.023 0.092
Receptor fluid (0-12 h) 0.190 0.005 0.045 0.015 0.017 0.067
Total absorbed 0.322 ± 0.318
0.009 0.086 ±
0.038
0.029 0.023 ±
0.017
0.092
Remaining epidermis (after tape stripping)
2.02 ± 0.82
0.055 0.310 ±
0.143
0.105 0.047 ±
0.041
0.185
Remaining epidermis including Stratum corneum (after exclusion of 2 tape strips)
Total potentially absorbable** (after exclusion of the two upper tapes strips)
2.84 0.077 0.413 0.140 0.063 0.250
3.162 0.086 0.499 0.169 0.086 0.342
Total recovery 3744 ± 104
101 296 ±
4.45
100 25.0 ±
0.54
99.0
Absorption rates [µg/cm2/h] (0-24h)
0.014 ± 0.07 0.003 ± 0.001 0.001 ± <0.001
* Some cells for these applications were excluded from calculations as the analytical data indicated that the epidermal membrane may have been damaged during application.
** Total potentially absorbable = total absorbed + remaining epidermis (including lower layers of the Stratum corneum), SD values not provided in the original study
n.a. not applicable
The overall total recovery for the three dose levels was good, with mean values of 99 – 101 % of the applied dose.
Glyphosate absorption from the 360 g/L concentrate formulation was essentially constant over the entire 24 hour exposure period (mean rate = 0.014 µg/cm2/h). By the end of the exposure period, the mean total amount of absorbed glyphosate was 0.322 µg/cm2 (0.009 % of applied dose).
From the intermediate and low-dose aqueous dilutions of the formulation, absorption was fastest during the early period of absorption, with 0.010 µg/cm2/h, (0-1h) and 0.004 µg/cm2/h, (0-2h), respectively. The rates after this early period until the end of the exposure at 24h were 0.003 µg/cm2/h and 0.001 µg/cm2/h for the intermediate and low dose dilutions, respectively.
At the end of the exposure period, the mean total amounts of absorbed glyphosate were 0.086 and 0.023 µg/cm2 (0.029 % and 0.092 % of applied dose), respectively.

For the formulation concentrate and both aqueous dilutions, the vast majority of the applied glyphosate was removed from the surface of the epidermis during the washing procedure at the end of the 24-hour exposure period (mean 97.4-99.0 %). The mean total amount of glyphosate recovered from the epidermis was 0.120 %, 0.235 % and 0.505 % of the applied dose for the concentrate, intermediate and low dose dilution, respectively.
The amount of potentially biologically available glyphosate (absorbed + epidermis after tape striping) for the concentrate, intermediate and low dose dilutions were 0.064 %, 0.134 % and 0.277 % respectively for 5 tape strips and more conservatively 0.086 %, 0.169 % and 0.342 % respectively for only 2 tape strips.
Conclusions by the Notifier
The results of this in vitro dermal absorption study indicate that the absorption of glyphosate through human skin is very limited and very slow. The vast majority of glyphosate was removed from the skin by the washing procedures.
The total absorbed amounts after 24 hour exposure were 0.009 %, 0.029 % and 0.092 % of the applied dose for the formulation concentrate, the 1:12,5 (v/v) and 1:150 (v/v) dilution, respectively.
The corresponding total potentially absorbable amounts, represented by the mean absorbed dose together with the amounts in the remaining skin were 0.063 %, 0.134 % and 0.276 %, respectively for 5 tape strips and more conservatively 0.086 %, 0.169 % and 0.342 % respectively for only 2 tape strips.
Thus, the results predict that the dermal absorption of glyphosate from potential exposure to this 360 g/L glyphosate / L SL formulation would be less than 1 %, irrespective of whether two or five tape strips were considered to contain non-biologically available glyphosate.
Table B.6.12-2: Summary of glyphosate dermal absorption from MON 52276
Study
% of applied dose*
Reference
Concentrate
Spray dilutions
SL formulation
360 g/L
29.6 g/L
2.51 g/L
In vitro (human skin)
0.086
0.169
0.342
IIIA 7.6.2
Ward, 2010a
* The absorption values correspond to total amounts potentially absorbable through human skin (i.e. amounts of radioactivity recovered in the receptor fluid and remaining skin after tape stripping with two strips).
RMS comments
The study is considered acceptable. The mass balance was very good and any adjustment for test material losses not needed. However, a few deficiencies were noted:
• The intended number of cells (membranes) was six for each application. However, in the low and high dose experiments, 2 membranes had to be excluded because analytical data indicated that

epidermal membranes might have been damaged during treatment.
• Detailed information on the skin samples (sex and age of donors, body site from which the samples were taken) was not provided.
• The number of cells (samples) even at the mid dose level and of skin donors in the high and mid dose experiements was lower than required according to current EFSA guidance (EFSA, 2012, ASB2012-6959). This cannot be considered a deviation that would put the quality and acceptability of the study into question because it was
commissioned and performed before the guidance document was prepared and came into force but the current guidance must be taken into account for interpretation of the results.
Very low dermal absorption of glyphosate from the representative formulation has been shown. It must be emphasised that the use of isolated epidermis (instead of dermatomed skin) and an exposure period of 24 hours will most probably result in overestimation of the dermal absorption rate.
The approach taken by the applicant to calculate the dermal absorption is agreed with. In line with current EU practice and also with the new European Guidance document on dermal absorption (EFSA, 2012, ASB2012-6959) the first two (upper) tape strips were excluded. The amount retained in the lower (three) tape strips, in contrast, should be considerd potentially absorbable because raw data have shown that 75 % penetration (expressed as the percentage of radioactivity in the receptor fluid) was not achieved for any of the three applied concentrations after one half of the study duration, i.e., after 12 hours (see Table B.6.12-1). The achieved mean values were rounded according to new rules (EFSA, 2012, ASB2012- 6959) giving dermal absorption rates of 0.1, 0.2, and 0.3 % for the high, mid and low concentration that should be used for exposure calculation and risk assessment purposes. However, uncertainties with regard to the origin of skin samples and their donors, their actual number in the different experiments and also the occasionally rather high standard deviations should be taken into account. Thus, for exposure calculation and risk assessment purposes, it is proposed to use a (conservative) estimate of 1% dermal absorption for the formulation concentrate as well as for both dilutions.
The RMS is aware that the new European Guidance document has come into force long after conduct of the study and also after submission of the glyphosate dossier. Thus, in a strict sense, it might not be applicable. However, it was used here to support the approach taken with regard to inclusion/exclusion of tape strips and for rounding in the hope that it might be helpful for a harmonised evaluation of this study.
B.6.12.2 Dermal absorption of glyphosate active substance in vitro (abraded rabbit skin)
Reference:
IIA, 5.3.7/02
Report:
Hadfield, N. 2012a Glyphosate acid - In Vitro Absorption through Abraded Rabbit Skin using [14C]-glyphosate
Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK Study No.: JV2182, Report No.: JV2182-REG
Date: 2012-04-18, Unpublished, ASB2012-11459
Guidelines:
OECD 428
Deviations:
None
GLP:

yes
Acceptability:
See RMS comment
Dates of experimental work: 2011-12-12 to 2011-12-22
The purpose of this study was to determine the in vitro percutaneous absorption of glyphosate acid through abraded rabbit skin following a 6-hour exposure period and subsequent 18 hour monitoring period. This study was designed to assess the potential dermal penetration of test material through rabbit skin and will be of use in estimating the systemic dose achieved in a previous in vivo rabbit dermal toxicity study (see IIA 5.3.7, Johnson, 1982, TOX9552366). Therefore, the application rate and exposure conditions used in this study were calculated to be equivalent to 5000 mg/kg bw/day as applied to rabbits in the in vivo dermal study (IIA 5.3.7).
14C-glyphosate was incorporated into a wet cake preparation prior to application. The preparations were applied as a paste to abraded rabbit skin membranes at a rate of
79.8 mg/cm2 (corresponding to 48.3 mg glyphosate acid/cm2) and left unoccluded for an exposure period of 6 hours, after which the skin surface was washed. The absorption process was followed by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout a total time-period of 24 hours. The distribution of glyphosate within the test system and a 24-hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).
Materials and methods
Test materials:
Non radio-labelled test substance:
Identification:
MON 77973 (glyphosate acid)
Description:
White wet cake
Lot/Batch #:
GLP-1103-21149-T
Chemical purity/a.i. content:
85.14 % as glyphosate acid (purity: 95.93 %)
Stability of test compound:
Expiry date: 2012-03-09
Analytical reference standard:
Identification:
Glyphosate acid
Lot/Batch #:
GLP-0810-1915-A
Chemical purity:
Not reported
Radio-labelled test substance
Identification:
14C-glyphosate (as glyphosate acid) [phosphonomethylene-14C]-glyphosate
Lot/Batch #:
4675JJN002-1
Radiochemical purity:
96.7 % (confirmed by analysis)
Specific activity:

48 mCi/mmol; 1776 MBq/mmol; 2523µCi/mL; 9.35 MBq/mL
Test skin source:
Species:
Rabbit
Strain:
New Zealand White Albino
Source:
Harlan
Age:
At least 12 weeks
Type:
Complete pelt
Study design and methods:
Preparation of skin samples
Skin pelts from New Zealand White albino rabbits at least 12 weeks old were obtained from Harlan. The skin samples were transported on cold blocks and were stored on arrival at -20°C, the day after sacrifice. The skin samples arrived clipped and excised and were examined for scars and blemishes. Any extraneous subcutaneous tissue was removed after defrosting and the pelts clipped further if necessary. The pelts were given an identifying number and individually stored frozen, at approximately -20°C, on aluminium foil until required for use.
Test substance preparation
The doses were prepared, to mimic as closely as possible a 5000 mg/kg dose from a previous rabbit in vivo study (IIA 5.3.7, 1982, TOX9552366). The dose equivalency was calculated on a dose per unit area of skin basis using an average in vivo rabbit weight of
2.78 kg. The doses were prepared as close to the time of application as was practicable.
Radioactive stock solution of 14C-glyphosate
The radiolabelled 14C-glyphosate was supplied as a solution in water.
Trial preparation of the radiolabelled glyphosate acid
Glyphosate acid trial preparation was prepared using the method described below, with the exception that different volumes or smaller amounts of radioactivity or unlabelled material were used, where applicable. Three individual vials were prepared as part of the trial preparation, to assess dosing methodology. The paste like composition of the dose preparation was investigated to ensure that it visually provided good skin contact during application to the membranes.
Preparation of radiolabelled glyphosate acid
Firstly 8008 mg of non-labelled glyphosate wet cake was added to a vial, followed by 4162 µL of radiolabelled glyphosate stock solution, providing a nominal 3.85 mg of glyphosate (40 MBq) radioactivity. 5 mL of water was then added and the preparation mixed thoroughly. The preparation was then freeze dried to remove the water added and the water present in the wet cake. When dry, the glyphosate wet cake preparation was then weighed to confirm the removal of all the water. Approximately 521 mg of the dried wet cake preparation was then added to 8 individual vials together with approximately 300 µL of saline to each vial to create a paste. A final weight of each vial was recorded and the preparation was thoroughly mixed with a spatula into a paste before dosing.
Preparation of non-labelled glyphosate acid
To demonstrate that the dose preparations have a close contact during the application procedure, an additional dose preparation without radiolabel was prepared according to the procedure described above.

Analyses of dose preparations
The radioactivity content of the stock solution was determined by liquid scintillation counting (LSC) analyses of sub-samples of solvent dilutions. The radiochemical purity of the radiolabelled stock solution of the test substance was determined by thin layer chromatography (TLC) using unlabelled test substance as reference standard.
The radioactivity content and homogeneity of the dose preparations were checked by LSC analyses. The radiochemical purity and stability was measured by TLC analyses.
Preparation of diffusion cells
The skin membranes were placed in static glass diffusion cells providing an exposure area of 2.54 cm2 of skin. The cells had a receptor volume of approximately 4.5 mL.
An integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. Non-abraded membranes with a resistance of 1.5 - 5 kA were considered having a normal integrity and used for the skin abrasion. Rabbit skin was abraded using a blunt spatula drawn over the skin area approximately six to eight times, in the form of a grid, in order to mimic 'Draize' abrasion as conducted in the in vivo study (IIA 5.3.7, 1982, TOX9552366). After the abrasion a further integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. For abraded skin samples membranes with ER values in the range of 0.7 – 1.0 kf were selected for the study.
Cells were selected such that the application rate was represented by eight intact skin samples from five different animals. Physiological saline was chosen as receptor fluid. The skin surface temperature was maintained at 32 ± 1 °C using a water bath.
Test substance application and sampling
Prior to dosing a pre-treatment sample of 500 µL was taken from each diffusion cell, and replaced by an equal amount of fresh receptor fluid.
Each dose formulation was applied to the abraded skin membrane as a dried glyphosate acid wet cake paste and spread over the skin surface using a spatula. The weight of each individual preparation and spatula were recorded before and after dosing to allow the applied dose to be calculated.
Each dose was applied at the nominal rate of 79.8 mL/cm2 exposed skin area (202.8 mg/cell), corresponding to 48.3 mg glyphoate/cm2. The applications were left un-occluded for 24 hours.
Receptor fluid samples (500 µL) were taken by an auto-sampler at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. After each sampling the removed amount of receptor fluid was replaced by an equal amount of fresh receptor fluid.
After the 6-hour sampling, the skin samples were washed by gently swabbing the application site application site with at least three natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. Two further sponges, pre- wetted with water, were used to further swab the surface.
Terminal procedures
After the last sampling, 24 hours after application the remaining receptor fluid was discarded. The receptor chamber was rinsed with receptor fluid that was also discarded.
The donor chamber was carefully removed and the underside wiped with a single natural sponge, pre-wetted with 3 %Teepol L® in water, which was added to the wash sponges. The donor chamber was washed with deionised water and a sample was taken for LSC analysis.
The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be

complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The skin surface was washed with further sponges pre-wetted with water. All the sponges were combined and digested in Soluene 350® and made up to a recorded volume. A sample was taken for analysis.
Due to the fragility of the abraded skin samples tape stripping could not be performed. Instead a heat separation technique was used to separate the epidermis from the dermis.
The skin was carefully removed from the receptor chamber and the flange area cut away and digested in Soluene 350® and aliquots taken for analysis by LSC.
The remaining skin disc was placed dermis side down, on cling film. A second piece of cling film was then used to cover the epidermis side. A 200g weight was placed in a water bath at 65ºC for an hour prior to use. The weight was placed onto the epidermal surface with moderate pressure for approximately 90 seconds. The epidermis was peeled away from the dermis using forceps. The dermis was digested in Soluene 350® and aliquots taken for analysis by LSC. The epidermis was digested in Soluene 350® and the whole sample analysed by LSC.
Analysis of samples
The radiochemical purity and stability of the 14C-glyphosate preparations was determined by TLC using silica gel plates and methanol : water : acetic acid (6 : 3 : 0.5, v/v/v). Radioactivity on the TLC plates were measured using a Packard Instant Imager (SOP E003). Unlabelled material was visualised under UV light at 254 nm.
For visualising the test material on the TLC plates a 2 % ninhydrine solution in acetone was used.
In addition, for analyses of dose preparations K2 cellulose plates and a revised solvent system (methanol : water : acetic acid (8 : 1.5 : 0.5, v/v/v) was used.
Liquid samples of the receptor fluid, washing solutions, digested wash sponges, and digested dermis and epidermis were measured by LSC using a Packard 3100 TR LSC counter and Goldstar as scintillation fluid.
Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of glyphosate in the receptor solution in terms of µg/cm2. The amounts absorbed, rates of absorption (µg/cm2/h) and 'percentage of dose absorbed' were calculated. Membranes with absorption profiles that indicate membrane damage during the course of the experiment have been excluded from calculations. The results of the mass balance and distribution determinations are expressed in terms of amount absorbed and 'percentage of applied dose The absorbed dose is considered the glyphosate detected in the receptor fluid, while the potentially biologically available proportion of the dose is regarded as the sum of absorbed dose and the amount recovered from the dermis. The test material removed from the surface of the epidermis by the washing procedure, as well as the glyphosate recovered from the epidermis at the end of the exposure is considered unabsorbed.
Results and discussion
Analyses of the 14C-glyphosate stock solution
TLC analysis of the 14C-glyphosate stock solution confirmed a radiochemical purity of greater than 95 %.
LSC analysis revealed a radioactivity content of 72.1 MBq, equivalent to a concentration of 0.924 mg/mL. The stock solution was homogeneous with a 1.31 % deviation between the replicates.
Analyses of dose preparations
LSC analyses confirmed the mean application rate to be 48.3 mg glyphosate/cm2.
The dose preparations had low variability between the replicates analysed (1.66 %-6.26 %) and, considering the physical nature of the preparation, the dose preparations were considered to have

acceptable homogeneity.
Membrane integrity check
Based on the ER measurements eight cells with abraded skin samples were selected for the absorption study.
Dermal absorption of glyphosate
Absorption profiles were assessed from eight abraded skin samples. Since one skin sample showed an atypical absorption profile, this was excluded from the calculation of means and SD.
The determined distribution of radioactivity are summarised in Table B.6.12-3 below.
Table B.6.12-3: Summary of results for dermal absorption of 14C-glyphosate (rabbit skin)
Dose preparation
Applied dose “wet cake” [mg/cm2]
79.8
Applied dose glyphosate [mg/cm2]
48.3
Number of cells assessed
7
Distribution of radioactivity (mean values)
µg/cm2
SD
% of applied dose
SD
Surface compartment
Dermis (after heat separation)
118
19.4
0.243
0.040
Skin wash at 6 hours
42802
3008
87.9
6.30
Skin wash at 24 hours
1159
1224
2.38
2.51
Donor chamber
59.2
56.9
0.121
0.117
Receptor compartment
Receptor fluid (0-24 h)
1177
244

2.42
0.503
Total absorbed*
1177
--
2.42
--
Epidermis (after heat separation)
20.1
9.97
0.041
0.020
Flange area
132
68.6
0.270
0.141
Total potentially absorbable**
1295
--
2.663
--
Total recovery
45468
2096
93.3
4.46
Absorption rates [µg/cm2/h]
(0-24h)
53.1
10.2
--
--
SD Standard deviation
* Amount in receptor fluid.
** Total potentially absorbable = total absorbed + remaining dermis (after heat separation)
The total recovery of the individual cells was in the range of 87.3 % to 98.2 %, with an overall mean recovery of 93.3 % of applied dose.
The majority of the applied glyphosate acid (mean 87.9 %) was washed off the skin at 6 hours, with a further 2.38 % washed off at 24 hours. A small proportion (0.041 %) of the dose applied was recovered from the epidermis, with 0.243 % remaining in the dermis.
The mean rate of absorption of glyphosate acid between 0-1 hours was 47.0 µg/cm2/h, which increased to 166 µg/cm2/h between 1-4 hours. The mean absorption rate subsequently slowed to 72.3 µg/cm²/h between 4-10 hours and declined further to 13.3 µg/cm²/h for the remainder of the absorption period (10-24 hours). The overall absorption rate (0-24 hours) was
53.1 µg/cm2/h.

The mean amount of glyphosate acid that penetrated abraded rabbit skin into the receptor fluid over the entire 24-hour experimental period was 1177 µg/cm2, corresponding to 2.42 % of the applied dose.
Considering that the amount found in the remaining dermis after 24 h is potentially available and could further penetrate through the skin, the total amount of glyphosate potentially available was 2.66 % of the applied dose
Conclusions by the Notifier
The results of this in vitro study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow. The vast majority of glyphosate will be washed off during normal washing procedures. The mean total amount absorbed after 24 hours was 2.42 %. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 %.
RMS comments
The study is considered supplementary despite its good quality. Using abraded rabbit skin as an in vitro model, it could be shown that dermal absorption of glyphosate active ingredient was less than 3 %, confirming to some extent a previous assumption (DAR 1998, ASB20110- 10302) that was based on data obtained in Rhesus monkeys in vivo (1991, TOX9552418). However, even though the information provided by this study is interesting from a purely scientific point of view, its regulatory value will be quite limited. Dermal absorption of an active ingredient must be always determined or estimated for a certain formulation under evaluation. Exposure to glyphosate in a ”wet cake” in this study does neither reflect real exposure conditions of operators, bystanders or workers to liquid concentrates or spray dilutions nor a possible impact of co-formulants.
B.6.12.3 Publications on dermal absorption of glyphosate
Wester et al. (2005, ASB2012-12050) compared dermal absorption through human skin in vitro after exposure either to a 1 % solution (not further specified) or to cotton sheets that were treated with the same solution on the same day or up to 2 days before to reflect the abundance of glyphosate residues infabric that may occur due to applications of this active ingredient in cotton. The authors could show a lower absorption of glyphosate across the skin from the treated cotton as compared to the liquid solution itself but found also remarkable residues on and in the skin when the cotton sheets had been treated on the same day. Adding of water to the cotton sheets resulted in an increase in absorption rate.