B.6.1.3 Published information

Toxicokinetics and metabolism of glyphosate were seldom subject to investigations of industry-indpendent researchers and, thus, experimental data in open literature is scarce. The following paragraphs were transferred from the original DAR (1998, ASB2010-10302) and slightly amended for purposes of the RAR:

(1991, TOX9551791) reported an absorption rate from the gastrointestina tract of 35 - 40 % of the total dose following the single oral administration of 10 mg of a mixture of 12C- and 14C-glyphosate per kg bw to male Sprague-Dawley rats. Urine and faeces were considered equally important routes of elimination. 7 days after application, total body burden was approximately 1 % of the administered dose and was primarily associated with the bone. Two hours following a single dose of the mixed test material, traces of a minor metabolite (<0.1 % of the dose applied) were detected beside the predominating parent in the colon tissue. This compound was also found in the gastrointestinal tract content of one rat at 28 hours post dosing and was considered likely to be aminomethyl phosphonic acid (AMPA) although the retention time for this metabolite was not identical to that for AMPA. The authors reported AMPA to be a product of metabolic activity of intestinal microbes.

(1992, TOX9551954) investigated (with contributions of
who are mentioned above) the elimination and tissue distribution of 14C-glyphosate in male F344/N rats following oral and intravenous administration. After single low (5.6 mg/kg bw) or high (56 mg/kg bw) oral doses, more than 90 % of the applied radioactivity was eliminated within 72 hours. During the first 24 hours, approximately 50 % had been excreted in the faeces and nearly 30 % via the urine. It was assumed that the urinary radioactivity represented the amount of glyphosate absorbed. The peak blood levels occurred at 1 (low dose) or 2 (high dose) hours after dosing. Following an i.v. dose of 5.6 mg/kg bw, 90 % of the radioactivity was excreted in urine within the first 6 hours already. Glyphosate did not accumulate in the body. In a further group of rats receiving 5.6 mg/kg bw by oral gavage, only 1 % of the dose remained in the tissues after 24 hour. It is also stated that pretreatment with Roundup via drinking water did not change the elimination pattern of glyphosate.

In a more recent paper that was included in the GTF dossier submitted for current evaluation, (2009, ASB2012-11542) reported some parts of toxicokinetics of glyphosate
(obtained from SIGMA CHEMICALS) in rats after single intravenous (i.v.) administration of 100 mg/kg bw or a single oral dose of 400 mg/kg bw. The focus was on plasma characteristics and distribution to the different compartments: “Serial blood samples were obtained after i.v. and oral administration. Plasma concentrations of glyphosate and its metabolite amiomethyl phosphonic acid (AMPA) were determined by HPLC method. After i.v. and oral administration, plasma concentration-time curves were best described by a two-compartment open model. For glyphosate, the elimination half-lives (T1/2/) from plasma were 9.99 h after
i.v. and 14.38 h after oral administration. The total plasma clearance was not influenced by dose concentration or route and reached a value of 0.995 l h-1kg-1. After i.v. administration, the apparent volume of distribution in the second compartment (V2) and volume of distribution at steady state (Vss) were 2.39 and 2.99 l kg-1, respectively, suggesting a considerable diffusion of the herbicide into tissues. After oral administration, glyphosate was partially and slowly absorbed with a Tmax of 5.16 h. The oral bioavailability of glyphosate was found to be 23.21 %. Glyphosate was converted to AMPA. The metabolite AMPA represented 6.49 % of the parent drug plasma concentrations. The maximum plasma concentrations of glyphosate and AMPA were 4.62 and 0.416 µml-1, respectively. The
maximum plasma concentration of AMPA was achieved at 2.42 h. For AMPA, the elimination half-life

(T1/2/) was 15.08 h after oral administration of glyphosate parent compound (quoted from original article)."

The RMS is not aware of any further scientific publications dealing with toxicokinetics and metabolism of glyphosate in laboratory animals or man. However, interesting additional information on urinary excretion of glyphosate in humans was provided that, however, did not alter the conclusions that were drawn from the many studies described above. In the original draft, this data was reported here but, for the revised version, the information was substantially amended (because more data had become available in the meantime) and transferred to section B.6.9.3 where a new sub-section on human biomonitoring was created.

More recently, in 2013, a biomonitoring study was performed on behalf oft he NGO "Friends of the earth" and its German partner organisation BUND by Hoppe (Medical Laboratory Bremen, Haferwende 12, D-28357 Bremen, Germany) and submitted to the RMS (ASB2013- 8037). To our knowledge, this data has not been published in a scientific journal so far but is available in the internet. 182 urine samples from 18 European (EU and non-EU) countries (6 – 12 per country but mostly 10) were examined for glyphosate and AMPA by means of a modern analytical method (transformation of both compounds to two different derivatives followed by GC-MS/MS). This method appears very selective but it is not known whether it has been sufficiently validated so far. The LOQ for both, glyphosate and AMPA, was 0.15 µg/L. Creatinine was also measured as an internal proof for validity of the urine measure- ments. The measured values themselves are considered reliable by the RMS. The results suggest that there is a certain exposure of European population to glyphosate, mainly by dietary intake. This is not surprising since glyphosate is a widely used active substance worldwide. Residues in food and feed may occur and are allowed if below the MRLs. Systemically available glyphosate (i.e., the rather low percentage that is absorbed from the GIT) is excreted via the urine, virtually unchanged. Apparently, there is also some exposure to AMPA although its origin is less clear. However, due to the limited number of involved participants and the ab- sence of any information about them (such as age, gender, body weight, social background, origin from urban or rural environments, nutrition habits) and the way how they were recruit- ed, the study was only explorative and cannot be regarded as representative. The mean dietary exposure level cannot be estimated on this basis, neither for a single country nor for Europe it its whole. Moreover, no conclusion can be drawn to which extent the apparent differences in urinary levels of glyphosate in samples obtained in the different countries might reflect the actual use of glyphosate. (It was reported, e.g., that 8 out of 10 samples from Austria and 10 out of 12 from Switzerland were below the LOQ in contrast to only 3 of 10 from the UK or even 1 of 10 from Malta.)

In any case, none of the measured concentrations was of health concern since the exposure that may be calculated on this basis is far below the ADI. For an adult with 70 kg body weight, the newly proposed ADI of 0.5 mg/kg bw (see B.6.10 and Volume 1), would mean that the total daily intake of glyphosate might be as high as 35 mg. If 20 % is assumed to be orally absorbable (see above), up to 7 mg might be eliminated via the urine. Since the average urine volume is 1.5 – 2 L, theoretical urine concentrations of glyphosate in the magnitude of 3.5 to 4.7 mg/L would result. Even the maximum values measured by Hoppe (2013, ASB2013-8037) are less than 0.1 % of these expected concentrations proving a very low sys- temic dose that was received by the participants, presumably via the dietary route. With re- gard to AMPA, it must be emphasised that the glyphosate ADI also covers this metabolite and that the assessment does not change even when the low AMPA concentrations and those of glyphosate would be summed up.

It is interesting to note that the mean value of ca 0.2 µg/L was by five times lower than the geometric

mean of glyphosate concentrations that were measured in a study in U.S. farmers on the third day following application, i.e., with mainly dermal and inhalative exposure to be assumed. However, the maximum value on that post-application day 3 was 68 µg/L and on the day of glyphosate spraying even 233 µg/L (Acquavella et al., 2004, ASB2012-11528). This comparison suggests that exposure of operators will normally exceed that of consumers and that, if operators are not on risk, dietary exposure should not be a matter of concern.

B.6.1.4 Data obtained with formulations

Not relevant for this section dealing with toxicokinetic behaviour and metabolism of the active substance. For dermal absorption, see B.6.12.

Published information

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):

20 F344/N rats 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. Ten rats/sex and group were used for evaluation of haematological and clinical chemistry parameters. All rats survived until the end of the study and there were no clinical signs of toxicity apart from diarrhea at the top dose level in both sexes. Body weight gain was markedly reduced in high dose males and slightly decreased in high dose females. There were some minor alterations in haematological and clinical chemistry parameters at least at the upper dose levels. Morphologic changes at necropsy were confined to parotid and submandibular (submaxillary) salivary glands in both sexes. This "cytoplasmatic alteration" consisted of basophilic change and hypertrophy of acinar cells. The parotid gland was more affected. Here, the normal granular, eosinophilic staining cytoplasm of the acinar epithelial cells was replaced by basophilic and finely vacuolated cytoplasm. A NOEL could not be established since these lesions were observed already at the lowest dose level but not in the control groups. The degree of change showed a clear dose response. The outcome of this study shows that glyphosate is of low toxicity when administered orally over a period of 3 months to rats since the animals tolerated daily doses as high as 50000 ppm (more than 3000 mg/kg bw/day) without mortality or clinical signs of overt toxicity and without pathological changes other than the rather equivocal salivary gland findings.

B.6.4.8 Published data (released since 2000)

B.6.4.8.1 Introduction

An earlier review of the toxicity of glyphosate and the original Roundup™ formulation concluded that neither glyphosate nor the formulation pose a risk for the production of heritable/somatic mutations in humans (Williams et al., 2000, ASB2012-12053). This review of subsequent glyphosate genotoxicity publications includes analysis of study methodology and incorporation of all the findings into a weight of evidence for genotoxicity. Two publications provided limited additional support for the conclusion that glyphosate and glyphosate based formulations (GBFs) are not active in the gene mutation assay category. The weight of evidence from in vitro and in vivo mammalian chromosome effects studies supports the earlier conclusion that glyphosate and GBFs are predominantly negative for this end point category. Exceptions are mostly for unusual test systems but there are also some unexplained discordant positive results in mammalian systems. Several reports of positive results for the SCE and comet DNA damage endpoints have been published for glyphosate and GBFs. The data suggest that these DNA damage effects are likely due to cytotoxic effects rather than DNA reactivity. This weight of evidence review concludes that there is

no significant in vivo genotoxicity and mutagenicity potential of glyphosate or GBFs that would be expected under normal exposure scenarios.

B.6.4.8.2 General review and analysis considerations

The published studies for review consideration were identified by literature searches for published reports containing references to glyphosate or glyphosate based formulations (GBFs) that also contained searchable terms which indicated that genotoxicity studies were performed. Literature search utilised Chemical Abstracts (provided by Chemical Abstracts Service, a division of the American Chemical Society) and Web of Knowledge (Thompson Reuters), using the following modules: Web of ScienceSM, BIOSIS Previews®, MEDLINE®, and CAB Abstracts® (CABI) abstracting services. Search criteria were as follows (glyphosate acid and the various salts): glyphosat* OR glifosat* OR glyfosat* OR 1071-83-6 OR 38641- 94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR
40465-66-5 OR 69254-40-6 OR (aminomethyl w phosphonic*) OR 1066-51-9. Each identified publication was evaluated to verify that it contained original results of one or more genotoxicity studies on glyphosate or GBFs. Emphasis was placed on publications in peer- reviewed journals and abstracts or other sources with incomplete information were not considered. Reviews without original data were not considered for evaluation; however, these reviews were examined to determine if there were any cited publications that had not been detected in the literature searches. Each relevant publication was examined using several criteria to characterize the scientific quality of the reported genetic toxicology studies. Useful, objective criteria for this purpose were international guidelines for genetic toxicology studies developed by expert groups. These include principles for conducting studies, reporting results and analyzing and interpreting data. Some of the principles of the guidelines are generally applicable to categories of studies or all studies while others are specific for a particular type of test system and end point. Some of the specific types of studies encountered in the review do not yet have international guidelines; however, some of the guideline elements should be generically applicable to these studies. The guidelines for genetic toxicology tests developed for the Organisation for Economic Cooperation and Development (OECD) are a pre-eminent source of internationally agreed and expert guidelines. Other regulatory international and national regulatory genetic toxicology testing guidance are usually concordant with the OECD guidelines. Table B.6.4-28 presents some key OECD guideline criteria that were found to be relevant to analysis of the studies considered in this review.

Comparison of the published studies to the criteria in guidelines used for regulatory purposes does not represent an absolute judgment standard but it does serve to provide one means of characterization of the various published studies. Some of the criteria are rarely met in scientific publications. For example, data for individual cultures and individual animals are not commonly included in publications in scientific journals. These data are presumably collected but are usually summarised as means with a measure of variance for the treatment and control groups. This is not considered to be a significant omission in a scientific publication. However, other guideline features are more essential in demonstrating scientific quality standards and should be considered as having greater weight in evaluating a study. For example, there are consistent recommendations that assays involving visual scoring (e.g. chromosome aberration, micronucleus and sister chromatid exchange) should use slides that are independently coded so that scoring is performed without knowledge of the treatment or control group being scored. This guidance is good scientific practice and studies that do not include a description of coding or “blind” scoring in the methodology would appear to have a deficiency either in the methodology or the description of the methodology used. Other examples of guideline features that have clear experimental scientific value are the use of concurrent negative

and positive controls and concurrent measurement and reporting of toxicity endpoints in main experiments, especially in in vitro mammalian cell assays.

Test materials, as described in the publications, were reviewed by industry experts to identify any publicly available and useful information on composition for the reported formulations to assist in interpreting the relevance of findings to glyphosate and/or formulation components. It should be noted that a common problem encountered in the published literature is the use of the terms "glyphosate", "glyphosate salt" or "Roundup" to indicate what may be any GBF that contains additional components such as surfactants. Published results from studies with different formulations have sometimes been incorrectly or inappropriately attributed to the active ingredient. The original Roundup formulation (MON 2139), containing 41 % isopropyl amine glyphosate salt and 15.4 % MON 0818 (a polyethoxylated based surfactant blend), is no longer sold in many markets. However, other glyphosate based formulations are sold under the Roundup brand name with varying glyphosate forms, concentrations and surfactant systems. Clear identification of the test material is very important in toxicology studies because toxicity of formulations can be dramatically different than the active ingredient. The fact that test materials identified as Roundup formulations may actually have different compositions should be considered when comparing results of different studies. A major consideration, especially for DNA damage endpoints and for in vitro mammalian cell assays, is an assessment of whether observed effects might be due to toxicity or extreme culture conditions rather than indicating DNA-reactive mediated processes. Relevant considerations include control of medium pH and osmolality for in vitro mammalian cell studies and whether effects are observed only at cytotoxic doses or in association with severe toxicity to the test system. Other important generic considerations in evaluating experimental results of each published study are evidence of experimental reproducibility and whether a biologically plausible dose response has been demonstrated.

Table B.6.4-28: Genetic Toxicology Test Guideline Criteria

Area	Guidance	Reference
All studies	Test material purity and stability should be reported	OECD 471 (1997) OECD 473 (1997)
	Concurrent negative and positive controls should be included with each assay	
Assays with visual scoring	All slides should be independently coded before analysis (i.e. scored without knowledge of the treatment or control group)	OECD 473 (1997) OECD 479 (1986)
In vitro mammalian cell assays	Assay should be usually be conducted in the presence and absence of an appropriate exogenous metabolic activation system	OECD 473 (1997)
	Cytotoxicity should be determined in the main experiment	

At least three analyzable concentrations should be used
Maximum dose determined by toxicity or 5 µg/ml, 5 mg/ml or
10 mM for soluble non-toxic test materials
Individual culture data should be provided
In vivo mammalian assays
Five analyzable animals per group. Single sex may be used if there are no substantial difference in toxicity between sexes
OECD 475 (1997)
OECD 474 (1997)
Limit dose for non-toxic substances of 2000 mg/kg for treatments up to 14 days and 1000 mg/kg for treatments longer
than 14 days
In vitro chromosome aberration
Treatment for 3-6 hours in one experiment and harvest at 1.5 cell cycles. If negative a second experiment with continuous
treatment for 1.5 cell cycles
OECD 473 (1997)
Scoring of at least 200 metaphases ideally divided between duplicate cultures
In vitro sister
chromatid exchange
Treatment for 1-2 hours up to two cell cycles with harvest after
two cell cycles in the presence of bromodeoxyuridine
OECD 479 (1986)
Scoring of 25 metaphases per culture (50 per treatment group)
In vitro micronucleus
Most active agents detected by treatment for 3-6 hours with harvest at 1.5-2 cell cycles after treatment. An extended treatment for 1.5-2 cycles in the absence of metabolic activation
is also used
OECD 487 (2010)
Scoring of at least 2000 binucleated cells or cells for micronuclei
for each treatment or control group
In vivo bone marrow chromosome
aberration
Single treatment with first harvest at 1.5 cell cycles after treatment and second harvest 24 hour later or single harvest 1.5
cycles after last treatment for multiple daily treatments
OECD 475 (1997)
Three dose levels usually recommended except when limit dose
produces no toxicity
Concurrent measures of animal toxicity and toxicity to target
cells
At least 100 cells analyzed per animal
Individual animal data should be reported
In vivo erythrocyte
micronucleus
Three dose levels for first sampling time

OECD 474 (1997)
Treatment once with at least 2 harvests usually at 24 and 48 h after treatment or one harvest 18-24 h after final treatment if two
or more daily treatments are used
Scoring of 2000 immature erythrocytes per animal or 2000
mature erythrocytes for treatments of 4 weeks or longer
Table B.6.4-29 presents a summary of genotoxicity test results for glyphosate and GBFs published subsequent to Williams et al. (2000, ASB2012-12053). Test results are organised by the major genotoxicity assay categories of gene mutation, chromosome effects and DNA damage and other end points. Major features presented for each publication are the assay endpoint, the test system, the test material, the maximum dose tested and comments relevant to the reported conduct and results of the assay. For brevity, earlier reviewed individual publications of genotoxicity study results are referred to by citation of (Williams et al., 2000, ASB2012-12053) rather than the original references reviewed in (Williams et al., 2000, ASB2012-12053).
Table B.6.4-29: Genetic toxicology studies of glyphosate and glyphosate formulations published on or after 2000
End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference
In Vitro Gene Mutation
End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference
Point
Ames strains
Perzocyd 10
2 µg/plate
Negative
TA1535 not
Chrusciels
mutation
SL
(toxic)
used
ka et al.,
formulation

2000,
(ASB2013-
9830)
Wing spot test
Drosophila
glyphosate
10 mM in
Negative/
Negative or
Kaya et al.,
(96%)
larval stage
inconclusive
c
inconclusive in crosses not
2000, (ASB2013-
sensitive to
9832)
recombination
events
In Vitro Chromosome Effects—Mammalian Systems
Cytokinesis
Bovine
Glyphosate
560 µM
Positive?
PH, MA, SC,
Piesova,
block
lymphocytes
formulation
48 h –S9
TO
2004
micronucleus
(62%
(ASB2012-
glyphosate
12001)
Monsanto
source)
Cytokinesis
Bovine
Glyphosate
560 µM
Positive?

PH, SC, TO
Piesova,
block
lymphocytes
formulation
48 h –S9
Negative
2005
micronucleus
(62%
2 h –S9
Negative
(ASB2012-
glyphosate
2 h +S9
12000)
Monsanto
source)
Chromosome
Mouse spleen
herbazed
50 µM?
Positive
Concentrations
Amer et
aberration
cells
formulation
used not clear.
al., 2006
PH, MA, SC,
(ASB2012-
TO, RE
11539)
Chromosome
Bovine
Glyphosate
1.12 mM
Negative
Chromosome 1
Holeckova,
aberration
lymphocytes
formulation
(toxic)
FISH analysis.

2006
(62%
(24 h)
PH, MA, PC,
(ASB2012-
glyphosate)
SC, TO, RE
11847)
Monsanto
source
Chromosome
Bovine
Glyphosate
1.12 mM
Negative
PH, MA, SC,
Sivikova
aberration
lymphocytes
formulation
(toxic)
RE
and
(62%
(24 h)
Dianovsky,
glyphosate)
2006
Monsanto
(ASB2012-
source
12029)
Chromosome
Human
Glyphosate
6 mM (not
Negative
MA, IC, RE
Manas et
aberration
lymphocytes
(96%)
toxic)
al., (2009
ASB2012-
11892)

Cytokinesis
Human
Glyphosate
580 µg/mL
Negative
SC, RE
Mladinic et
block
lymphocytes
(technical,
(toxic)
(-S9)
al., 2009
micronucleus
96%)
(est. 3.43
Positive
(ASB2012-
mM)
(+S9)
11906)
Cytokinesis
Human
Glyphosate
580 µg/mL
Negative
SC, RE
Mladinic et
block
lymphocytes
(technical,
(toxic)
(-S9)
al., 2009
micronucleus
96%)
(est. 3.43
Positive
(ASB2012-
mM)
(+S9)
11907)
In Vitro Chromosome Effects— Non Mammalian Systems
Chromosome aberration
Onion root tip meristem
Roundup formulation (Bulgaria)

1% active ingredient (estimated
Negative
TO, IC, RE
Dimitrov et al., 2006 (SB2012-
End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference
4.4-5.9
mM)
11607)
Micronucleus
Onion root
Roundup
1% active
Negative
TO, RE
Dimitrov et
tip meristem
formulation
ingredient
al., 2006
(Bulgaria)
(estimated
(SB2012-
4.4-5.9
11607)
mM)
In Vivo Chromosome Effects—Mammalian Systems
Bone marrow erythrocyte micronucleus
Mouse
Glyphosate
300 mg/kg i.p.
Perzocyd 10 SL
formulatio n
Negative Negative
DL, TO, SC, IM, RE
DL, TO, SC, IM, RE
2000, (ASB2013- 9830)
Bone marrow
Mouse
Roundup 69

2 x 200
Negative
TO, SC, IE, RE
erythrocyte
formulation
mg/kg i.p.
micronucleus
2000
(ASB2013-
11477)
Bone marrow
Mouse
Roundup™
2 x 200
Negative
TO, SC, IE, RE
,
erythrocyte
formulation
mg/kg i.p.
2002
micronucleus
(Monsanto)
(SB2012-
11834)
Bone marrow
Rabbit
Roundup™
750 ppm in
Positive?
DL, PC, TO,
Chromosome
formulation
drinking
SC, IC
aberration
water
2005
(ASB2012-
11841)
Bone marrow Chromosome aberration
Mouse
Herbazed formulation (84%
glyphosate)
50 mg/kg
i.p. (1,3, 5 days)

Negative
TO, SC, RE
2006 (ASB2012- 11539)
100 mg/kg
oral (1,7,
14, and 21 days)
Positive
Spermatocyte Chromosome aberration
Mouse
Herbazed formulation (84%
glyphosate)
50 mg/kg
i.p. (1,3, 5 days)
Negative
TO, SC, RE
2006
(ASB2012- 11539)
100 mg/kg
oral (1,7,
14, and 21 days)
Positive
Bone marrow
Mouse
Roundup
1080 mg/kg
Negative
DL, TO, IC,
Chromosome
formulation
p.o. (1/2
RE
2006
aberration
(Bulgaria)
LD50)
(ASB2012-
11607)
Bone marrow
Mouse
Analytical
2 x 200
Positive
Erythrocytes
erythrocyte
glyphosate
mg/kg i.p.

scored?
2009
micronucleus
(96%)
TO, SC, IC, RE
(ASB2012-
11892)
Bone marrow
Mouse
Roundup™
50 mg/kg
Positive
DL, SC, IC, RE
End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference
Chromosome
formulation
i.p.
, 2009
aberration
(Monsanto)
(ASB2012-
12005)
In Vivo Chromosome Effects—Non-Mammalian Systems
Erythrocyte
Oreochromis
Roundup 69
170 mg/kg
Negative?c
TO, RE
micronucleus
niloticus
i.p.
(Tilapia)
(maximum
tolerated)
2000
(ASB2013-
11477)
Wing spot test

Drosophila
Glyphosate (96%)
10 mM in larval stage
Positive/inco nclusiveb
2000
(ASB2013-
9832)
Erythrocyte
Tilapia
Roundup™
170 mg/kg
Positive
TO, RE
micronucleus
formulation
(abdominal
2002
(Monsanto)
injection)
(ASB2012-
11834)
Erythrocyte
Crasseus
Roundup
15 ppm
Positive
TO, IE, RE
micronucleus
auratus
formulation
glyphosate
(goldfish)
in water (2,
2007
4 and 6
(ASB2012-
days)
11587)
Prochilodus
Roundup™
10 mg/l (6,
Negative
DL, TO, SC,
lineatus
formulation
12 and 24

RE
2008
(tropical fish)
(75% of 96 h
h) in water
(ASB2012-
LC50)
11586)
Erythrocyte
Caiman eggs
Roundup®
1750
Positive
RE
micronucleus
Full II
ug/egg
2009
formulation
(ASB2012-
12002)
Erythrocyte
Caiman eggs
Roundup®
Sprayed 2x
Positive
DL, TO, RE
micronucleus
Full II
with 100
2009
formulation
litres of
(ASB2012-
3%/ha 30
12002)
days apart
Micronucleus
Fish (Guppy)
Roundup®
5.65 µg/l
Positive
(and alkaline
Transorb
SCGE)
2013

(ASB2014-
7617)
In Vitro DNA Damage Mammalian Systems
Alkaline
GM38 human
Glyphosate
6.5 mM
Positive
MA, PH, TO,
Monroy et
SCGE
fibroblasts
(technical
SC, RE
al., 2005
and
grade)
(ASB2012-
HT1090
11910)
human
fibrosarcoma
Sister
mouse spleen
herbazed
50 µM?
Positive
Concentrations
Amer et
chromatid
cells
formulation
used not clear
al., 2006
exchange
MA, PH, TO,
(ASB2012-
SC, RE
11539)
Sister chromatid exchange
bovine lymphocytes
Glyphosate formulation (62%
1.12 mM (toxic)
Positive
PH, SC, RE
Sivikova and Dianovsky,

End point
Test System
Test Material
Maximum
Dose
Result
Commenta
Reference
glyphosate, Monsanto)
2006 (ASB2012-
12029)
Alkaline single cell gel electrophoresi s (SCGE,
comet)
Hep-2 cells
Glyphosate (analytical, 96%)
7.5 mM (limited by toxicity)
Positive
MA, PH, RE
Manas et al., 2009 (ASB2012- 11892)
Alkaline SCGE
Human lymphocytes
Glyphosate (technical, 96%)
580 µg/ml (toxic) (est. 3.43
mM)
Positive (- S9)
Positive (+S9)
Mladinic et al., 2009 (ASB2012-
11906)
SCGE
Human lymphocytes (compared with Tilapia erythrocytes and Tradescantia
nuclei)
Glyphosate (96%)
700 µM
Positive (according to authors)
Inconsitent and not clear dose dependent
Alvarez- Moya et al., 2014 (ASB2014- 6902)
SCGE
Human buccal epithelial cells
Glyphosate (95%) and Roundup
Ultra Max
200 mg/l
Positive
Higher activity of formulation than pure a. s.
Koller et al., 2012 (ASB2014-
7618)
In Vitro DNA Damage Non-Mammalian Systems

SOS
E. coli
Roundup BIO
formulation
2.5
ug/sample
Positive
Raipulis et al. 2009 (ASB2012- 12008)
Alkaline SCGE
Tradescantia flowers and nuclei
Glyphosate(technical, 96%)
700 µM
Positive
PH, SC
Alvarez- Moya et al., 2011 (ASB2012-
11538)
In Vivo DNA Damage Mammalian Systems
Spermatocytes and bone marrow
Mouse
herbazed formulation (84%
glyphosate)
200 mg/kg p.o.
Positive
TO, SC, RE
Amer et al., 2006
(ASB2012- 11539)
SCGE
blood cells, liver cells,
Mouse
Glyphosate (96%) and AMPA
400 mg/kg bw/day Glyphosate or 100 mg/kg
bw/day AMPA
Glyphosate and AMPA positive
Manas et al., 2013 (ASB2014- 6909)
In Vivo DNA Damage Non-Mammalian Systems
Erythrocyte alkaline SCGE
Crasseus auratus (goldfish)
Roundup formulation
15 ppm glyphosate in water (2,
4 and 6 days)
Positive
TO, RE
2007 (ASB2012- 11587)
Erythrocyte and gill cell alkaline
SCGE
Prochilodus lineatus (tropical fish)

Roundup™ formulation (75% of 96 h
LC50)
10 mg/l (6,
12 and 24
h) in water
Positive
DL, TO, RE
2008 (ASB2012- 11586)
Erythrocyte
Caiman
Roundup®
1750
Positive
RE
End point
Test System
Test Material
Maximum Dose
Result
Commenta
Reference
alkaline SCGE
eggs/hatchlin gs
Full II
formulation
µg/egg
., 2009 (ASB2012-
12002)
Erythrocyte alkaline SCGE
European eel
Roundup formulation
166 µg/liter
Positive
DL, SC, RE
2010 (ASB2012- 11836)
Erythrocyte alkaline SCGE
Caiman eggs/hatchlin gs
Roundup® Full
II
formulation
Sprayed 2x with 100 l of 3%/ha 30 days
apart
Positive
DL, RE
2009 (ASB2012- 12002)
SCGE

blood cells
European eel
Roundup Ultra and
Glyphosate and
POAE
116 µg/l
35.7 µg/l
18.6 µg/l
positive
No increased effect of glyphosate in combination with POAE
Guilherme et al., 2012 (ASB2014- 7619)
SCGE
Fish (Prochilidus)
Roundup Transorb and Glyphosate
5 mg/l
2.4 mg/l
positive
Inconsistent and not clearly dose dependent
Moreno et al., 2014 (ASB2014- 7522)
a MA, Mammalian metabolic activation system not used and short exposure not used; PH, no indication of pH or osmolality control;
DL, less than three dose levels used; PC, no concurrent positive control;
TO, no concurrent measurement of toxicity reported or toxicity not observed for highest dose level;
SC, independent coding of slides for scoring not indicated for visually scored slides;
IC, less than 200 cells scored per treatment or less than 100 metaphases scored per animal for chromosome aberrations.;
IE, less than 2000 erythrocytes scored per animal;
RE, results not reported separately for replicate cultures or individual animals;.
b Positive for small wing spots only in one cross. Negative or inconclusive for all spot categories for three other crosses.
c Statistically significant increase in micronucleated PCE frequency only at mid dose level but overall result judged negative.
B.6.4.8.3 Structure Activity Analysis
Glyphosate was evaluated using Derek for Windows (Llhasa Ltd., Leeds, UK, Version 11.0.0, October 24, 2009). No structural alerts were identified for chromosome damage, genotoxicity, mutagenicity or carcinogenicity. This small molecule consists of the amino acid, glycine, joined with a phosphonomethyl group. These moieties are not known to be genotoxic; therefore, the lack of structure activity alerts for glyphosate is expected.
B.6.4.8.4 Gene Mutation
As reviewed by Williams et al., (2000, ASB2012-12053), most gene mutation studies for glyphosate and GBFs were negative. Gene mutation assays included numerous Ames/Salmonella and E. coli WP2 bacterial reversion assays, Drosophila sex-linked recessive lethal assays and a CHO/HGPRT in vitro mammalian cell assay. Of fifteen gene mutation assays reported, there were only two positive observations. A reported positive Ames/Salmonella result for Roundup formulation was not replicated in numerous other studies. There was one report of a positive result for a GBF in the Drosophila sex-linked recessive lethal assay but this was contradicted by a negative result for the

same GBF in this assay reported by another laboratory. Further, the positive study had some features that hampered interpretation, including the lack of concurrent negative controls (Williams et al., 2000).

Subsequent to the Williams et al. (2000, ASB2012-12053) review only two gene mutation studies have been reported (Table B.6.4-29). One negative Ames/Salmonella assay result was reported for a GBF of undefined composition, Percozyd 10 SL (Chruscielska et al., 2000, ASB2013-9820). Although this result is consistent with a large number of negative Ames/Salmonella results for glyphosate and GBFs, the reported study results have significant limitations. One of the recommended test strains, TA1535, was not used and results were only presented as "-" without presentation of revertant/plate data. A positive result for glyphosate was reported in the Drosophila wing spot assay which can indicate both gene mutation and mitotic recombination endpoints (Kaya et al., 2000, ASB2013-9832). Small increases in small wing spot frequencies were observed in one of four crosses of larvae treated with up to 10 mM glyphosate. The lack of a positive response in the balancer-heterozygous cross offspring, which are insensitive to mitotic recombination events, suggests that there is no evidence for effects on gene mutation endpoint events such as intragenic mutations or deletions in this publication.

These gene-mutation publications add very limited data to the weight of evidence conclusion that glyphosate and GBFs do not pose significant risk for gene mutation.

B.6.4.8.5 Chromosome effects

Assays to detect chromosome effects such as structural chromosome aberrations and micronucleus incidence constitute a second major genotoxicity end point category. A large number of publications with chromosome effects endpoints have been reported since the Williams et al. (2000, ASB2012-12053) review. These are described in Table B.6.4-29 and are separated into various test system categories which include in vitro cultured mammalian cell assays, in vitro tests in non-mammalian systems, in vivo mammalian assays and in vivo assays in non-mammalian systems. A Drosophila wing spot test (discussed previously) is also included in this category because results are relevant to somatic recombination.

B.6.4.8.5.1 In vitro chromosome effects

Two human and one bovine in vitro peripheral lymphocyte chromosome aberration studies of glyphosate were considered in the earlier review (Williams et al., 2000, ASB2012-12053). One human lymphocyte in vitro study had negative results for glyphosate tested up to approximately 2-3 mM (calculated from reported mg/ml) in the absence and presence of an exogenous mammalian activation system. The other two studies with human and bovine lymphocytes and no metabolic activation system reported positive results at concentrations more than two orders of magnitude lower. The earlier review noted several other unusual features about the positive result studies including an unusual exposure protocol and discordant positive results for another chemical found negative in other laboratories.

As indicated in Table B.6.4-29 both positive and negative results have been reported for glyphosate and GBFs in the nine in vitro chromosome effects assays published after the Williams et al. (2000, ASB2012-12053) review. It is noteworthy that many of these studies have various deficiencies in conduct or reporting compared to internationally accepted guidelines for conduct of in vitro chromosome aberration or micronucleus studies (see Table B.6.4-28). Perhaps the most significant deficiency was that coding and scoring of slides without knowledge of the treatment or control group was not indicated in seven of nine publications. This could be a deficiency in conducting the studies or perhaps a deficiency in describing methodology in the publications. Other common deficiencies included failure to indicate control of exposure medium pH, no use of exogenous metabolic

activation and no reporting of concurrent measures of toxicity.

Results for glyphosate active ingredient

Three publications reported testing of technical glyphosate for micronucleus or chromosome aberration endpoints in cultured human lymphocytes (Manas et al., 2009, ASB2012-11892; Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012-11907). Negative results for the micronucleus or chromosome aberration end points were observed in the absence of exogenous metabolic activation (S9) in all three publications. The maximum exposure concentration in the absence of S9 was in the range of 3-6 mM in these studies.

Two publications by one author reported cytokinesis block micronucleus results for cultured bovine lymphocytes treated with what was reported as 62 % by weight isopropyl amine salt of glyphosate from a Monsanto Belgium source (Piesova, 2004, ASB2012-12001; Piesova, 2005, ASB2012-12000). This test material appears to be a manufacturing batch of the isopropylamine salt of glyphosate in water without surfactants, which is not sold as a GBF. In one publication no statistically significant increases in binucleated cell micronucleus frequency were observed with 24 hours of treatment (Piesova, 2004, ASB2012-12001). For

48 hours of treatment a statistically significant increase in micronucleus frequency was observed in one donor at 280 µM but not at 560 µM and in a second donor at 560 µM but not

280 µM. The second publication reported negative results for the cytokinesis block micronucleus assay in bovine lymphocytes incubated with glyphosate formulation up to 560 µM for two hours in the absence and presence of a mammalian metabolic activation system (Piesova, 2005, ASB2012-12000). This publication also reported positive results for 48 hours of treatment without S9. Curiously, in this second publication the same inconsistent dose response pattern was observed in which a statistically significant increase in micronucleus frequency was observed in one donor at 280 µM but not at 560 µM and in a second donor at 560 µM but not 280 µM. The lack of a consistent dose response pattern between donors suggests that the results with 48 hours of treatment are questionably positive. Two other publications found negative results for the chromosome aberration endpoint in cultured bovine lymphocytes treated with what appears to be the same test material of 62 %

by weight isopropylamine salt of glyphosate from a Monsanto Belgium source, (Holeckova, 2006, ASB2012-11847; Sivikova and Dianovsky, 2006, ASB2012-12029). Both the studies used a maximum concentration of 1.12 mM which was reported to cause a decrease in mitotic inhibition of >50 %. These two studies have several limitations including that an exogenous mammalian metabolic activation system was not used for chromosome aberration and scoring was not reported to be on coded slides. In addition, Holeckova (2006, ASB2012-11847) only examined effects detectable by staining of chromosome 1 and did not report positive control results (Holeckova, 2006, ASB2012-11847). Despite these limitations and the variable donor results, the results from these two studies are generally consistent with a lack of chromosome aberration effects of the isopropylamine salt of glyphosate on in vitro cultured mammalian cells in several experiments using high, toxic dose levels and exposures of 2-24 hours in the absence of S9.

One laboratory reported increases in cytokinesis-blocked micronucleus frequency in cultured human lymphocytes exposed to glyphosate for 4 hours in the presence of an exogenous human liver metabolic activation system (S9) in two publications (Mladinic et al., 2009a; Mladinic et al., 2009b). In both publications a statistically significant increase in micronuclei was observed with S9 at the highest dose level of glyphosate tested (580 µg/mL, ≈ 3.4 mM). Increased proportions of centromere- and DAPI-positive micronuclei were observed for the high dose with S9 suggesting that the induced micronuclei were derived from chromosomes rather than chromosome fragments. Statistically

significant increases in the frequency of nuclear abnormalities (buds and bridges) and DNA strand breakage were also observed at the highest dose tested in both publications. In parallel experiments cytotoxic effects such as early apoptosis, late apoptosis and necrosis were observed and these effects were uniquely or preferentially observed in the presence of S9 and at the highest dose level tested (Mladinic et al., 2009, ASB2012-11906). Also, the negative control level of such end points as necrosis and alkaline SCGE tail moment was significantly increased in the presence of S9 (Mladinic et al., 2009, ASB2012-11906). It should be noted that glyphosate is mostly excreted unmetabolised in vivo in mammals with only very small levels of aminomethylphosphonic acid (AMPA) or an AMPA-related structure observed (, 2009, ASB2012-11542; 1991, TOX9551791). These observations suggest that the observations of S9
mediated effects by Mladinic et al. are not likely to be due to in vivo relevant metabolites. The preponderance of in vitro genotoxicity studies conducted with exogenous mammalian metabolic activation systems has been negative, including a previously reviewed chromosome aberration study in human lymphocytes conducted up to a similar dose level (Williams et al., 2000, ASB2012-12053) and a bovine lymphocyte cytokinesis block micronucleus study (Piesova, 2005, ASB2012-12000). Overall these results suggest the possibility of a weak aneugenic rather than clastogenic (chromosome breaking) effect occurring in the presence of S9 at high dose levels of glyphosate. The pattern of activity as well as the failure to observe activity in several other in vitro genotoxicity assays conducted with S9 suggests that the activity observed in the Mladinic et al. studies does not have a significant weight of evidence for in vitro genotoxicity and is not likely to be relevant to in vivo genotoxicity.

The recently published results for mammalian in vitro chromosome aberration and micronucleus assays demonstrate a weight of evidence that technical glyphosate and glyphosate salt concentrates are negative for these end points in cultured mammalian cells in the absence of an exogenous mammalian metabolic activation system. Five publications from four laboratories report negative in vitro mammalian cell chromosome or micronucleus results in the absence of exogenous activation while three publications from two laboratories report positive results. These results reinforce the Williams et al. (2000, ASB2012-12053) conclusion that positive chromosome aberration results reported for glyphosate in cultured human lymphocytes in the absence of an exogenous metabolic activation system are aberrant.

Recent reports of positive chromosome aberration and micronucleus results for glyphosate in the presence of an exogenous mammalian activation system in cultured human lymphocytes in one laboratory (Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012- 11907) have no substantial reproducibility verification from other laboratories in the recent in vitro chromosome effects studies considered in this review because most of the studies performed by other laboratories (Table B.6.4-29) did not employ an exogenous mammalian activation system. These results are discordant with one previously reviewed result demonstrating a negative result for glyphosate in cultured human lymphocytes with mammalian metabolic activation using the chromosome aberration endpoint (Williams et al 2000, ASB2012-12053) and a negative result in the presence of S9 for the micronucleus endpoint in bovine lymphocytes (Piesova, 2005, ASB2012-12000). The numerous consistent negative results for glyphosate and GBFs in gene mutation studies which employed exogenous mammalian metabolic activation and careful examination of the data suggests that the positive results indicate a possible threshold aneugenic effect associated with cytotoxicity rather than a DNA-reactive mechanism resulting in chromosome breakage. Thus, the weight evidence for the in vitro chromosome effect assays indicates a lack of DNA-reactive clastogenic chromosome effects.

Results for GBFs

Amer et al. (2006, ASB2012-11539) reported positive in vitro chromosome aberration effects in mouse spleen cells for a formulation described as herbazed, which was reported to contain 84 % glyphosate and 16 % solvent, an unusually high glyphosate concentration for a formulation. The test material is not further characterised, lacking description of the glyphosate salt form and inert ingredients. The glyphosate concentrations used in the study are not clear because there are different descriptions of the concentration units (M or M glyphosate/ml medium) in the publication. Thus, the maximum concentration might have been 5 x 10-5 M (50 µM) or 5 x 10-5 M glyphosate/mL medium (50 mM). The former concentration, which was reported as toxic, would indicate effects at concentrations well below those typically found toxic for GBFs in cultured mammalian cells. The latter level of 50 mM would be well in excess of the limit level of 10 mM recommended in OECD guidelines (OECD473, 1997). In addition to a question about the concentration used there are several other limitations to the reported study including no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Given these limitations, the uncertainty about the concentrations used and the nature of the test material, these results should not be considered to have significant relevance or reliability with respect to glyphosate or GBFs.

In addition to in vitro mammalian cell studies there is also a report of negative results for the chromosome aberration and micronucleus endpoints in onion root tips incubated with a Roundup formulation (Dimitrov et al., 2006, ASB2012-11607). The maximum exposure concentration (stated as 1 % active ingredient) is estimated to be on the order of 4-6 mM. This study did not employ an exogenous mammalian metabolic activation system; however, it does provide evidence for a lack of chromosome effects for glyphosate and a GBF in a non- mammalian in vitro system. The result agrees with earlier reported negative onion root tip chromosome aberration results for glyphosate but is discordant with earlier reported positive results for a Roundup GBF in this system (Williams et al., 2000, ASB2012-12053).

B.6.4.8.5.2 In vivo Chromosome Effects—Mammalian Systems

The Williams et al. (2000, ASB2012-12053) glyphosate toxicity review presented results from in vivo mammalian chromosome effect assays. Results from several mouse bone marrow erythrocyte micronucleus studies of glyphosate and GBFs (e.g. Roundup, Rodeo and Direct) were negative for micronucleus induction. These included studies from different laboratories mostly following modern guidelines. The intraperitoneal (i.p.) route was used for most of the negative studies and maximum doses for many of the studies were toxic or appropriately close to LD50 values. In addition to i.p. studies a 13 week mouse feeding study was also negative for the micronucleus endpoint with an estimated maximum daily glyphosate dose of over 11,000 mg/kg/day. There was one published report of a weak positive mouse bone marrow micronucleus response observed for glyphosate and Roundup GBF. This study, which employed a smaller number of animals per group than other negative studies, was clearly aberrant from the numerous other negative studies not only in micronucleated cell frequency finding but also the finding of altered polychromatic erythrocyte to normochromatic erythrocyte (PCE/NCE) ratios. The overall weight of evidence from the earlier reviewed studies was that glyphosate and GBFs were negative in the mouse bone marrow erythrocyte micronucleus assay. The earlier review also noted a negative mouse dominant lethal result for glyphosate administered by gavage at a maximum dose level of 2000 mg/kg.

As indicated in Table B.6.4-29, there are numerous subsequent publications of in vivo mammalian chromosome effects assays. With one exception, all of the in vivo mammalian studies were conducted in the mouse using either the bone marrow chromosome aberration or micronucleus

endpoints. It should be noted that there are some fairly consistent limitations in the reported conduct of these studies compared to OECD guidelines. In most studies concurrent indications of toxicity (other than effects on the bone marrow) are not reported, coding of slides for scoring is not reported, individual animal data are not reported and fewer than recommended cells or metaphases per animal were scored. Other limitations encountered include use of only a single or two dose levels rather than three dose levels.

Results for glyphosate active ingredient

Two publications reported results for glyphosate in the mouse bone marrow erythrocyte micronucleus assay. Negative results were reported in one study which used a dose of 300 mg/kg of glyphosate administered once i.p. with sacrifices at 24, 48 and 74 hours after dosing (2000, ASB2013-9820). This study had some limitations including the use of only one dose level, no reporting of toxicity other than PCE/NCE ratio, no reported coding of slides for scoring and scoring of 1000 PCE's per animal (scoring of 2000 PCE's per animal is recommended by OECD guidelines). A second publication reported positive results for glyphosate administered at 50, 100 and 200 mg/kg via i.p. injections repeated at 24 hours apart with sacrifice 24 hours after the second dose (., 2009, ASB2012-11892). A statistically significant increase in micronucleated erythrocytes was observed in the high dose group. This study had limitations comparable to the negative study. A more significant potential difficulty with this second publication is that "erythrocytes" rather than polychromatic erythrocytes were indicated as scored for micronuclei. This does not appear to be a case of using "erythrocytes" to mean polychromatic erythrocytes because the term "polychromatic erythrocytes" is used elsewhere in the publication describing measurements of PCE/NCE ratios. Scoring of total erythrocytes instead of immature polychromatic erythrocytes for micronuclei would be inappropriate in an assay with the stated treatment and harvest times because of the transient nature of micronucleated PCE's in bone marrow (OECD474, 1997).

There is no definitive explanation for the discrepancy between the two publications. Although one study used a single dose with multiple harvest times and the second used two doses and a single harvest time, both are acceptable protocols and would not be expected to lead to such discordant results (OECD474, 1997). The negative result reported for the 13 week feeding study in the earlier review (Williams et al., 2000, ASB2012-12053) confirms that positive results are not simply due to repeat dosing. The reported negative result (., 2000, ASB2013-9820) seems to be in accord with a majority of earlier reviewed mouse bone marrow micronucleus studies of glyphosate using similar doses and the

i.p. or feeding routes (Williams et al., 2000, ASB2012-12053). Also, the apparent scoring of micronuclei in erythrocytes rather than just polychromatic erythrocytes raises a significant methodological question for the reported positive study.

Results for GBFs

There are several publications reporting in vivo mammalian bone marrow chromosome aberration and micronucleus endpoint results for Roundup GBFs. Three publications report negative results for Roundup branded GBF in mouse chromosome aberration or micronucleus assays. Negative results were reported for two different Roundup branded GBFs administered at 2 x 200 mg/kg i.p. in mouse bone marrow erythrocyte micronucleus assays (

2000, ASB2013-11477; 2002, ASB2012-11834). The

second study did not report coding of slides for scoring. Another publication reported negative results in mouse bone marrow studies for both the chromosome aberration and erythrocyte micronucleus endpoints (2006, ASB2012-11607) using a dose of 1080 mg/kg administered orally (p.o.). In contrast, one publication reported positive results for Roundup GBF in mouse bone marrow

for the chromosome aberration and erythrocyte micronucleus endpoints using a single maximum dose of 50 mg/kg i.p. (2009, ASB2012-12005). Both the positive results and the magnitude of the increases in the chromosome aberration and micronucleus endpoint reported in this study are remarkably discordant with other reported results for Roundup and other GBFs in mouse bone marrow chromosome aberration and erythrocyte studies in a number of laboratories and publications (Table B.6.4-29 and Williams et al., 2000, ASB2012-12053). The reasons for this discordance are not clear. One unusual feature of the positive study is that the Roundup GBF was administered in dimethylsulfoxide. This is an unusual vehicle to use in in vivo genotoxicity studies, particularly for glyphosate which is water soluble and especially so in a formulated product. A published toxicity study found that use of a dimethylsulfoxide/olive oil vehicle by the i.p. route produced dramatically enhanced toxicity of glyphosate formulation or the formulation without glyphosate compared to saline vehicle and that the enhanced toxicity observed with this vehicle was not observed when the oral route was used (., 2008, ASB2012-11845). These observations suggest that use of DMSO as a vehicle for administration of formulation components by the i.p. route might produce unusual toxic effects that are not relevant to normally encountered exposures. Regardless of the reasons for the discordant positive results it is clear that a large preponderance of evidence indicates that GBFs are typically negative in mouse bone marrow chromosome aberration and erythrocyte assays.

One publication reported positive results for bone marrow chromosome aberration in rabbits administered Roundup GBF in drinking water at 750 ppm for 60 days (, 2005, ASB2012-11841). This study is relatively unique in terms of species and route of administration. The results do not report water intake in the test and control groups. Given the potential for water palatability issues with a formulated product, this is a significant shortcoming, as any effects noted may be attributable to dehydration. This study had further limitations including the use of only a single dose level and not coding slides for scoring.

Examination of the chromosome aberration scoring results showed that large increases for the treated group were observed for gaps and “centromeric attenuation” which were included in the summation and evaluation of structural chromosome aberration effects. Ordinarily gaps are scored but are not recommended for inclusion in total aberration frequency and centromeric attenuation is not included in ordinary structural aberrations (OECD475, 1997). These unusual scoring and interpretive features raise significant questions about using this study to make conclusions about clastogenicity of the GBF tested.

Two other publications report in vivo mammalian chromosome aberration or micronucleus results for GBFs. An uncharacterisd GBF, Percozyd 10L, was reported to be negative in a mouse bone marrow erythrocyte micronucleus assay (2000, ASB2013- 8929 and ASB2013-8931). The maximum dose level tested, 90 mg/kg i.p., was reported to be 70 of the i.p LD50 as determined experimentally by the authors. This study had several limitations including use of less than three dose levels and no reported coding of slides for scoring. Positive results were reported for another uncharacterized GBF, herbazed, in mouse bone marrow and spermatocyte chromosome aberration studies (2006, ASB2012- 11539). No statistically significant increases in aberrant cells were observed in bone marrow cells for i.p. treatment of 50 mg/kg for 1, 3 or 5 days or in spermatocytes for 1 or 3 days treatment. Statistically significant increases in frequency of spermatocytes with aberrations were reported for 5 days of treatment with 50 mg/kg (i.p.). Oral treatment of 50 mg/kg and 100 mg/kg were reported to produce increases in aberrant cell frequency in bone marrow cells after extended treatments (14 and 21 days) but not after shorter 1 and 7 day treatments. Similarly, significant increases in aberrant cell frequencies of spermatocytes were reported at 14 and 21 days of 50 mg/kg

oral treatment (negative for 1 and 7 days treatment) and at 7, 14 and 21 days of 100 mg/kg treatment (negative for 1 day treatment). Although not a genotoxic endpoint per se, it should be noted that statistically significant increases in frequency of sperm with abnormal morphology were also observed in mice treated with 100 and 200 mg/kg p.o. for 5 days. The positive results for the uncharacterized herbazed GBF were only observed after extended oral treatments (bone marrow and spermatocytes) and extended i.p. treatments (spermatocytes). The fact that positive results were not observed in an erythrocyte micronucleus test of mice treated with glyphosate up to 50,000 ppm in feed for 13 weeks (Williams et al., 2000, ASB2012-12053) provides direct evidence that extended glyphosate treatment by the oral route does not induce detectable chromosome effects. This treatment was longer and up to much higher glyphosate exposures than those used for the . (2006, ASB2012-11539) studies. Thus, it appears likely that these effects were due to some component(s) of the specific herbazed GBF tested rather than glyphosate.

In vivo mammalian assays for chromosome effects are an important category for characterisng genotoxicity that complements the gene mutation category. While some positive results have been reported the preponderance of evidence and published results are negative for glyphosate and GBFs.

B.6.4.8.5.3 In vivo Chromosome Effects—Non-Mammalian Systems

The Williams et al. (2000, ASB2012-12053) review reported a few in vivo plant assays for chromosome effects in non-mammalian systems. These included negative results for glyphosate and positive results for Roundup GBFs for chromosome aberrations in an onion root tip assay and negative results for glyphosate with the micronucleus end point in a Vicia faba root tip assay. Subsequent to the earlier review a number of publications reported results for erythrocyte micronucleus assays conducted on GBFs in several non-mammalian fish and reptile species with discordant results. One publication reported apparently negative results for the erythrocyte micronucleus test in Oreochromis niloticus (Nile tilapia) administered a test material described as Roundup 69 GBF, at an upper dose of 170 mg/kg i.p. (
2000, ASB2013-11477). Although there was an increase in micronucleated erythrocyte frequency at the mid-dose level this was not observed at the high dose level and considerable variability in frequencies in different groups was noted. Negative results were also reported in another fish species (Prochilodus lineatus) exposed to 10 mg/liter Roundup branded GBF for 6, 24 and 96 hours (2008, ASB2012- 11586). This concentration was reported to be 96 % of a 96 hour LC50. Positive results were reported for the erythrocyte micronucleus assay conducted in the fish Tilapia rendalii exposed to 170 mg/kg i.p. of another Roundup GBF (2002, ASB2012-11834). Examination of the micronucleus frequencies in this publication indicated that the negative control micronucleus frequency was considerably lower than the frequencies for all but one of 21 treatment groups for 7 different test materials. This suggests an unusually low control frequency and at least one treatment group was statistically significantly elevated for each of the 7 test materials, including many instances where the statistically significant increases were not consistent with a biologically plausible dose response. The possibility that the apparently significant increases were due to a low negative control value should be considered for this publication. Another publication reported positive erythrocyte micronucleus results in goldfish (Carassius auratus) exposed to 5 to 15 ppm of a Roundup GBF for 2 to 6 days (, 2007, ASB2012-11587). The reasons for the discordant results are not clear for these fish erythrocyte micronucleus assays of Roundup GBFs. Although different species and GBF's were used in the different studies there were pairs of studies with positive and negative results that used similar treatment conditions (170 mg/kg i.p. or 10-15 mg/litre in water).

Results for an unusual test system of exposed caiman eggs are reported by 2009, ASB2012-12002. Eggs were topically exposed in a laboratory setting to Roundup Full II GBF, and erythrocyte

micronucleus formation was measured in hatchlings (., 2009, ASB2012-12002). The GBF tested was reported to contain the potassium salt of glyphosate and alkoxylated alkylamine derivatives as surfactants. Statistically significant increases in micronucleated erythrocytes were observed in hatchlings from eggs treated with 500-1750 µg/egg. This system is quite unusual in the species tested and even more so in using an egg application with measurement of effects in hatchlings. Although there is some experience with a hen's egg erythrocyte micronucleus assay using in ovo exposure the erythrocytes are evaluated in embryos with only a few days between treatment and the erythrocyte micronucleus end point. In the reported caiman egg assay there was presumably a single topical exposure followed by an egg incubation period of about 10 weeks before hatching. Biological plausibility raises questions whether genotoxic events in ovo can produce elevated micronucleated erythrocyte frequencies detectable after 10 weeks, given the number of cell divisions occurring in development of a hatchling.

One published study reported a weak positive result in a Drosophila wing spot assay (Kaya et al., 2000, ASB2013-9832). Statistically significant positive increases were only in one of four crosses for small twin spots and not for the two other wing spot categories (large wing spots and twin wing spots). As discussed above, only negative or inconclusive results were observed for crosses that were not subject to mitotic recombination effects. If the result was actually treatment related it only would indicate an increase in recombination events and not in somatic mutations.

The above in vivo chromosome effect assays in non-mammalian systems give discordant results for reasons that aren't precisely defined. Typically these results would be given lower weight than mammalian systems in being predictive of mammalian effects, especially since
there is little or practically no assay experience with these systems in comparison with in vivo mammalian chromosome effects assays, such as the rat or mouse bone marrow chromosome aberration or erythrocyte micronucleus assays.

B.6.4.8.6 DNA damage and other end points

A number of studies of glyphosate and GBFs have been published since 2000 which used various DNA damage end points in a variety of in vitro and in vivo systems. The DNA damage category includes end points such as sister chromatid exchange and DNA repair response in bacteria, but the most common DNA damage end point encountered was the alkaline single cell gel electrophoresis end point (alkaline SCGE) also commonly referred to as the "comet" assay. The alkaline SCGE end point has been applied to both in vitro and in vivo test systems.

In addition to DNA damage there are a few reports of other types of studies which can be associated with genotoxic effects even though the end points are not specific indicators of genotoxicity per se. These include sperm morphology and carcinogenicity studies.

In vitro DNA Damage Studies

Some positive results for glyphosate or GBFs in the SCE end point were reported in cultured human and bovine lymphocytes in the earlier review (Williams et al., 2000, ASB2012- 12053). These results tended to be weak, inconsistent and with limited evidence for dose response. A number of limitations were observed for the studies such as the failure to control pH and abnormally low control values. Additional in vitro DNA damage end point results described in the earlier review included negative results for glyphosate in the B. subtilis rec- assay and in the primary hepatocyte rat hepatocyte unscheduled DNA synthesis assay.

There are two subsequent publications using in vitro cultured mammalian cells and the SCE endpoint. Positive SCE results were reported for the uncharacterised herbazed GBF in mouse spleen cells (Amer et al., 2006, ASB2012-11539). The dose response pattern for SCE response in this study was similar to the response for chromosome aberrations in this publication. Limitations of this study are in common

to those described above for the chromosome aberration end point portion of the study; no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Positive SCE results were also reported for cultured bovine lymphocytes treated with up to 1.12 mM glyphosate for 24 and 48 hours without exogenous mammalian metabolic activation (Sivikova and Dianovsky, 2006, ASB2012-12029). The highest dose of 1.12 mM significantly delayed cell cycle progression with 48 hour treatment. These same concentrations for 24 h exposures did not induce statistically significant increases in chromosome aberrations which provides a clear example of a differential response of the SCE endpoint (Sivikova and Dianovsky, 2006, ASB2012-12029). This is an important consideration in these publications, as chromosome effects are considered more relevant to genotoxicity than DNA damage.

Positive results for glyphosate are reported for the alkaline SCGE end point in three publications. Positive SCGE results were observed for two mammalian cell lines exposed to glyphosate for 4 hours at concentrations of 4.5-6.5 mM (GM39 cells) and 4.75-6.5 mM (HT1080 cells) (Monroy et al., 2005, ASB2012-11910). These concentrations are close to the upper limit dose of 10 mM generally recommended for in vitro mammalian cell assays and control of medium pH is not indicated. Characterisation of nuclear damage was done by visual scoring without coding of slides being indicated. Positive alkaline SCGE results were also reported in Hep-2 cells exposed for 4 hours to 3.5-7.5 mM glyphosate (Manas et al.,

2009, ASB2012-11892). Higher concentrations of glyphosate were reported to result in viability of <80 % as determined by dye exclusion. As noted for the preceding publication, the concentrations employed were reasonably close to a limit dose of 10 mM and control of medium pH was not reported. This publication reported negative results for the chromosome aberration endpoint in cultured human lymphocytes exposed to up to 6 mM glyphosate for 48 hours and it should be noted that in this case an appropriate control of medium pH was reported for this human lymphocyte experiment. Positive alkaline SCGE results have also been reported for cultured human lymphocytes exposed to glyphosate at concentrations up to 580 µg/ml (estimated 3.4 mM) for 4 hours (Mladinic et al., 2009, ASB2012-11906). Effects were observed both in the presence and absence of S9 with statistically significant increases in tail intensity at 3.5, 92.8 and 580 µg/ml without S9 and at 580 µg/ml with S9. A modification of the alkaline SCGE assay employing human 8-hydroxyguanine DNA-glycosylase (hOGG1) to detect oxidative damage only indicated statistically significant effects on tail length for treatment with 580 µg/ml with S9. Increases in nuclear abnormalities (nuclear buds and/or nucleoplasmic bridges) were also observed at 580 µg/mL with and without S9 and in micronucleus frequency at 580 µg/ml with S9. Measurements of total antioxidant capacity and thiobarbituric acid reactive substances showed statistically significant increases at 580 µg/ml in the presence or absence of S9. Interpretation of the significance of metabolic activation effects is complicated by the observation that several of the end points (alkaline SCGE tail intensity and nuclear abnormalities) tended to show increases in the presence of S9 in negative controls or at the very lowest concentrations of glyphosate. A reasonable summation of the results in this publication is that alkaline SCGE effects and other effects such as nuclear abnormalities, early apoptosis, necrosis and oxidative damage were consistently observed at 580 µg/mL.

In addition to mammalian cell studies there are publications reporting positive alkaline SCGE effects for glyphosate in Tradescantia flowers and nuclei exposed to up to 700 µM glyphosate (Alvarez-Moya et al., 2011, ASB2012-11538) and in the E. coli SOS chromotest for DNA damage conducted on a Roundup BIO GBF (Raipulis et al., 2009, ASB2012-12008). Observations of DNA damage in plants exposed to glyphosate are of questionable significance because of the herbicidal nature of

glyphosate and the SOS chromotest provides only indirect evidence of DNA damage in a bacterial system.

Overall there appear to be a number of studies in which glyphosate or GBFs have been reported to produce positive responses in DNA damage endpoints of SCE or alkaline SCGE in vitro in mammalian cells. Most of these have occurred with exposures to mM concentrations of glyphosate. Although this dose level range is lower than the limit dose of 10 mM recommended for several in vitro mammalian cell culture assays (OECD473, 1997; OECD476, 1997; OECD487, 2010), an even lower limit dose of 1 mM was recently recommended for human pharmaceuticals, particularly because of concerns about relevance of positive in vitro findings observed at higher dose levels. In addition, many of the studies have limitations such as not indicating control of medium pH and not coding slides for visual scoring. Concerns over the possibility of effects induced by toxicity have led to several suggestions for experimental and interpretive criteria to distinguish between genotoxic DNA-reactive mechanisms for induction of alkaline SCGE effects and cytotoxic or apoptotic mechanisms. One recommendation for the in vitro alkaline SCGE assay is to limit toxicity to no more than a 30 % reduction in viability compared to controls. Importantly, dye exclusion measurements of cell membrane integrity, such as those reported in some of the above publications may significantly underestimate cytotoxicity that could lead to alkaline SCGE effects. Other

recommendations include conducting experiments to measure DNA double strand breaks to determine if apoptotic process might be responsible for alkaline SCGE effects. Measurement of apoptotic and necrotic incidence were only performed in one publication (Mladinic et al., 2009, ASB2012-11906) and these measurements indicated both apoptotic and necrotic processes occurring in parallel with observations of alkaline SCGE effects. These direct observations as well as the reported dose responses, consistently suggest that biological effects and cytotoxicity accompany the observations of DNA damage in vitro in mammalian cells and therefore confirm the likelihood that the observed effects are secondary to cytotoxicity and are thresholded.

In vivo DNA damage studies

In the earlier review positive results for DNA strand breakage were reported for mice treated by the i.p. route with glyphosate and GBFs and for the alkaline SCGE endpoint in tadpoles of the frog Rana catesbiana exposed to a GBF (Williams et al., 2000, ASB2012-12053).

. (2006, ASB2012-11539) reporten an increase in SCE frequency in bone marrow cells of mice treated with uncharacterised herbazed GBF. Statistically significant positive effects were only observed at the highest dose level tested (200 mg/kg administered p.o.).

Several recent publications report alkaline SCGE results for GBFs in aquatic species. Three publications reported positive alkaline SCGE results in aquatic vertebrates exposed to Roundup GBFs in water. These publications have a common feature that alkaline SCGE results were reported as visually scored damage category incidence rather than instrumental measurements of properties such as the tail length or tail intensity. In one publication increases in nuclei exhibiting alkaline SCGE visual damage effects were observed in erythrocytes and gill cells of the tropical fish Prochilodus lineatus exposed to 10 mg/litre of a Roundup GBF in water (2008, ASB2012-11586). Results were variable with cell type and incubation; statistically significant positive responses were observed for erythrocytes at 6 hours and 96 hours, but not 24 hours or for branchial cells from the gills at 6 hours and 24 hours. Measurement of erythrocyte micronucleus frequency and nuclear abnormalities did not show statistically significant increases in these endpoints. The concentration used was reported to be 75 % of the 96 hour LC50, but trypan blue dye measurements apparently indicated >80 % viability of cells used in the alkaline SCGE assays. A second publication reported positive alkaline SCGE results in erythrocytes of the goldfish, Carasseus auratus, exposed to 5, 10 and 15 ppm of a

Roundup GBF for 2, 4 or 6 days (
2007, ASB2012-11587). Similar effects were observed for other end points (micronucleus and nuclear abnormalities). In general, effects increased with concentration and time. This publication did not report toxicity measurements or, more specifically, measurements of cell viability in the population studied. Positive results were also reported in erythrocytes of the European eel, Anguilla anguilla, exposed to 58 and 116 µg/liter of a Roundup GBF in water for 1 or 3 days ., 2010, ASB2012-11836). Increases in nuclear abnormalities were also observed in erythrocytes from animals exposed for 3 days. Measurement of toxicity was not reported for the animals or erythrocytes; however, several endpoints relevant to antioxidant responses and oxidant effects were made in whole blood samples. No statistically significant effects were observed for catalase, glutathione transferase, glutathione peroxidase, glutathione reductase or reduced glutathione content. A large statistically significant increase for thiobarbituric acid reactive substances (TBARS, a measure of lipid peroxidation) was observed for the 115 µg/litre concentration group at 1 day. Statistically significant TBARS increases were not observed at 3 days, but, the 3-day negative control value appeared to be several fold higher than the 1-day value.

Significance of DNA damage end point results

DNA damage end points such as SCE or alkaline SCGE are generally regarded as supplementary to the gene mutation and chromosome effects end point categories. DNA damage endpoints do not directly measure effects on heritable mutations or events closely associated with chromosome mutations. In vitro DNA damage endpoints such as the SCE or alkaline SCGE can be induced by cytotoxicity and cell death processes rather than from DNA-reactive mechanisms.

The observation of effects of sodium dodecyl sulfate is also interesting because it suggests responses to surfactants which are typically components of GBFs. As a more specific example, polyoxyethylenealkalylmine (POEA), a surfactant component of some GBFs has been shown to elicit cytotoxic effects such as perturbation of the mitochondrial membrane and disruption of mitochondrial membrane potential in cultured mammalian cells (, 2007, ASB2009-9030). Surfactant effects provide a plausible mechanism for observations of GBFs inducing DNA damage responses. Such responses would be expected to be associated with cytotoxicity-inducing exposures and exhibit a threshold.

B.6.4.8.7 Human and environmental studies

A number of human and environmental studies have been published in or after 2000 where some exposures to GBFs in the studied populations were postulated. These publications are summarised in Table B.6.4-30.

Table B.6.4-30: Studies of Human and Environmental Populations with Reported or Assumed Glyphosate Exposure

Exposed Population
End point
Exposures
Result
Reference
Human Studies
Open field and fruit
Bulky DNA adducts
glyphosate
No effects attributed

., 2007
farmers
formulation use
to glyphosate
(ASB2012-11543)
reported in only 1
formulation
of 29 fruit farmers
exposure
Humans in areas
Lymphocyte
Aerial or manual
Increase in CB MN
where glyphosate
cytokinesis block
spraying of
but no clear
2009
formulation is
micronucleus (CB
glyphosate
relationship to
(ASB2012-11570)
applied
MN)
formulation for
assumed or reported
illicit crop control
exposures
and sugar cane
maturation
Floriculturists
Lymphocyte CB
Glyphosate
Increase in CB MN
MN
formulation use
but not statistically
2004
reported in 21/51
significant
(ASB2012-11572)
workers with
average of 106.5 kg
applied
Floriculturists

Lymphocyte CB
Glyphosate
Statistically
MN
formulation use
significant increase
2002
reported in 57/107
in CB MN
(ASB2012-11573)
workers. Numerous
other pesticides
reported as used by
a similar number or
more of workers
Exposed Population
End point
Exposures
Result
Reference
Agricultural workers
Buccal cell
Glyphosate
Statistically
micronucleus
formulation use
significant increase
2009
reported along with
in MN
(ASB2012-11570)
numerous other
pesticides
Fruit growers
Lymphocyte
Glyphosate use
No effects
Alkaline SCGE;
reported in 2/19 1
attributable to
2003
Ames test on urine
day before captan
glyphosate
(ASB2012-11878)
spraying and 1/19

formulation
on the day of
exposure
captan spraying
Agricultural workers
Lymphocyte CB
Glyphosate
No statistically
2003
MN; buccal cell
formulation use
significant increases
(ASB2012-11991)
micronucleus
reported in 16% of
in CB MN or buccal
one of four
cell micronucleus
populations studied
frequencies
(Hungary)
Individuals on or
Lymphocyte
Glyphosate
Statistically
near glyphosate
alkaline SCGE
formulation
significant increases
2007
spraying
aerially sprayed
in damaged cells
(ASB2012-11992)
within 3 km
Greenhouse Farmers
Lymphocyte SCE
Glyphosate
Statistically
formulation use
significant increases
2001
reported in 99/102
in SCE
(ASB2012-12025)
workers; numerous

other pesticides
used
Farmers
Lymphocyte CB
Glyphosate
Statistically
2006
MN
formulation use
significant increase
(ASB2012-12045)
reported in 3/11
in micronucleus
farmers
frequency but not in
frequency of
binucleated cells
with micronuclei
Environmental Studies
Meadow voles living
Blood cell alkaline
Glyphosate
Some effects judged
on golf cousres
SCGE; erythrocyte
formulation use
possibly related to
2004
micronucleus
reported along with
Daconil® fungicide
(ASB2012-11871)
numerous other
pesticides
Fish from dams
Erythrocyte
Glyphosate
Higher MN
2011
(various species)
micronucleus
formulation use
frequencies than
(ASB2012-12017)
reported in adjacent
normal or expected

lands along with
but no negative
other pesticides
concurrent controls
used
Many of the human studies either found no effects attributable to GBFs or the reported GBF usage by the studied population was too low to be associated with observed population effects (., 2007, ASB2012-11543; ., 2004, ASB2012-11572; ., 2003, ASB2012-11878; ., 2003, ASB2012-11991; ., 2006, ASB2012-
12045).
In some studies, incidence of GBF use by the population studied was significant but high incidence of use of other pesticides was also reported (2002, ASB2012- 11573; 2001, ASB2012-12025). Even though positive effects were observed in these populations, ascribing these effects to any particular environmental exposure is not
scientifically justifiable and such results certainly cannot be considered as definitive evidence for GBF-induced human genotoxic effects.
Two published studies focused on populations believed to be exposed to GBFs by their presence at or near aerial or manual spraying operations. One publication reported induction of alkaline SCGE effects in blood lymphocytes of populations living within 3 km of areas sprayed with glyphosate formulation for illicit crop eradication 2007, ASB2012-11992). The populations studied were relatively small (24 exposed individuals and 21 non-exposed individuals). The sprayed material was reported to be Roundup Ultra, a GBF containing 43.9 % glyphosate, polyethoxylated surfactant and a proprietary component, Cosmoflux 411F. Specific methods for collection, storage, and transport of blood samples are not described for either the exposed population or control group. The publication also does not indicate that slides were coded for scoring which consisted of visual classification into damage categories and measurement of DNA migration (tail length). There were fairly large differences in ages and sex distribution of the exposed and control populations but these did not appear to be statistically significant. The study reported increases in damaged cell categories and statistically significant increases in DNA migration (tail length) in the presumably exposed population. Interpretation of the results of this study should consider numerous reported signs of toxicity in the exposed population and the reported application rate of 24.3 litres/ha which was stated to be 20 times the maximum recommended application rate. Some of the reported human health effects described by
(2007, ASB2012-11992) appear to be consistent with severe exposures noted in clinical reports of acute poisoning incidents with GBFs and other pesticide formulations (often self- administered) rather than typical bystander exposures. Given the considerably favorable general toxicology profile of glyphosate as reported by the WHO/FAO Joint Meeting on Pesticide Residues (WHO/FAO, 2004, ASB2008-6266) and in Williams et al. (2000, ASB2012-12053), factors related to either high surfactant exposure, unusual GBF components in this formulation or other undocumented variables appear to be confounding factors in this study. It appears that the reported alkaline SCGE effects could well have been secondary to the ailments reported in this study population.
A second publication reported results for a blood lymphocyte cytokinesis-block micronucleus study of individuals in areas treated with glyphosate formulation by aerial spraying or manual application (2009, ASB2012-11570). Although the title of the publication contains the term “agricultural workers”, most of the populations studied do not appear to be agricultural workers who are involved in application of GBFs. The human lymphocyte culture and scoring methodology employed in the .

(2009, ASB2012-11570) study appear to be generally consistent with commonly used and recommended practices for this assay. However, there is a significant question as to how long the blood samples used in the study were stored prior to initiating cultures and this may have affected the micronucleus numbers observed in the different sets of samples and populations. Also, the populations in the aerially sprayed regions had a second sampling a few days after the first sampling and this second sampling was not performed in the control populations. The publication reported a small increase in the frequency of binucleated cells with micronuclei and micronuclei per cell in samples collected from people living in three regions after spraying of GBFs compared with control values of samples collected just before spraying. However, the pattern of the increases did not correlate either with the application rate or with self-reported exposure. The largest post-spraying increase in binuclated cell micronucleus frequency was reported for a population with a much lower glyphosate active ingredient application rate and only 1 of 25 people in this region reported contact with sprayed glyphosate formulation. Increases in binucleated cell micronucleus frequency did not have a statistically significant relationship with self-reported exposure for two other populations. Some interpretative statements in
(2009, ASB2012-11570) suggest a small transient genotoxic effect of glyphosate formulation spraying on frequencies of binucleated cells with micronuclei, but other statements indicate that causality of the observed effects could not be determined using reasonable criteria and that lack of exposure data precluded conclusions. This study has a combination of uncontrolled or inadequately characterized variables, such as uncharacterisd exposure to ”genotoxic pesticides”, that would appear to preclude using the data to support any conclusion that exposure to GBFs affects binucleated micronucleus frequencies. Actually, the available data, while certainly limited in nature, support a conclusion that the observed effects do not appear to be attritubable to glyphosate formulation exposure. This conclusion is reinforced by (2004, ASB2012-11528), where biomonitoring of agricultural workers applying GBFs reports systemic exposures orders of magnitude below in vivo model chromosome aberration and micronucleus study doses, the majority of which were negative for glyphosate and GBFs.

There are two publications related to environmental monitoring for genotoxic endpoints. One study using blood cell alkaline SCGE and micronucleus endpoints was conducted on samples from meadow voles living on or near golf courses where pesticides had been applied (2004, ASB2012-11871). Results were significantly inconsistent between two seasons. Although some suggestions of effects were reported, glyphosate was only one of a number of applied pesticides and the effects observed were considered as possibly attributable to exposure to Daconil® fungicide. A second publication reported results for the erythrocyte micronucleus assay applied to fish collected from several dams in Brazil (, 2011, ASB2012-12017). Glyphosate formulation was one of a number of pesticides reported to be used in the area of the dams. No efforts appear to have been made to measure glyphosate or other pesticide concentrations in any of the ten dams from which fish were sampled. This study reported what were considered to be high levels of micronucleated cell frequency but there were no concurrent negative controls. In the absence of these controls the results cannot be interpretted as indicating any effect of pesticide exposure.

Although there have been a fairly large number of human genotoxicity studies reported where there was some exposure to GBFs, the large majority of these studies do not allow any conclusions about possible effects of glyphosate or GBFs because the exposure incidence was low or because there were reported exposures to a large number of pesticides. One report found an increase in alkaline SCGE effects in humans living in or near areas where a GBF was sprayed but that study had a number of methodology reporting and conduct deficiencies and the reported effects could well have been

due to toxicity reported in the study population. A second study found some increases in cytokinesis-block micronucleus frequency in humans possibly exposed to GBFs but the effects were not concordant with application rates or self- reported exposures and thus do not constitute reliable indications of effects for this endpoint in humans exposed to GBFs. Neither of the two environmental monitoring studies in meadow voles or fish provide any reliable evidence of exposures to glyphosate or GBFs or adverse effects resulting from potential exposures to glyphosate or GBFs.

After submission of the first draft of this RAR for public comment the following additional studies have been included.

Koureas et al. (2014, ASB2014-9724) performed a study aimed at estimating the oxidative damage to DNA in different subpopulations in Thessaly region (Greece) and investing its correlation with exposure to pesticides and other potential risk factors. The study produced findings that support the hypothesis that pesticide exposure is involved in the induction of oxidative damage to DNA. A correlation was found in this study between exposure to formulations containing neonicotinoids or glufosinate ammonium and oxidative damage to DNA. However, no significant correlation was reported for glyphosate.

Gentile et al. (2012, ASB2014-9482) submitted results of the micronucleus assay as a biomarker of genotoxicity in the occupational exposure to agrochemicals in rural workers in Argentina. The authors found significant differences in the frequency of micronuclei between occupationally exposed (20 individuals) and unexposed (10 individuals) workers. However, no conclusion on genotoxicity of glyphosate or other specific pesticides is possible on basis of this study.

Da Silva et al. (2014, ASB2014-9358) performed a genotoxic assessment in tobacco farmers at different crop times. The study sought to determine genotoxic effects in farmers occupationally exposed to agrochemicals and nicotine. A significant increase of micronucleated cells in the off-season group was observed. However, no conclusion on genotoxicity of glyphosate or other specific pesticides is possible on basis of this study.

Benedetti et al. (2013, ASB2014-9279) studied genetic damage in soybeans workers exposed to pesticides. The evaluation was performed with the comet and buccal micronucleus assays. The results of both tests revealed DNA damage in soybean workers. No special pesticide can be identified as cause of the observed effects.

B.6.4.8.8 DNA-Reactivity and carcinogenesis

As noted in the earlier review, 32P-postlabelling DNA adduct studies in mice did not indicate formation of adducts from glyphosate and questionable evidence of adducts from Roundup GBF administered as a high 600 mg/kg i.p. dose in an unusual dimethylsulfoxide/olive oil vehicle (Peluso et al., 1998, TOX1999-318; Williams et al., 2000, ASB2012-12053). Another earlier reviewed study reported DNA strand breakage in liver and kidneys of mice injected i.p with glyphosate and Roundup GBF. This study also reported an increase in 8- hydroxydeoxyguanosine (8-OHdG) residues in liver DNA from mice injected with glyphosate but not GBF. Increased 8-OHdG was found in kidney DNA from mice injected with GBF but not glyphosate (Bolognesi et al., 1997, Z59299; Williams et al., 2000, ASB2012-12053). No new direct studies of DNA reactivity of glyphosate or GBFs were encountered in publications since 2000. One publication did report on studies in mice to further investigate toxic effects and 8-OHdG levels associated with the routes, vehicles and dose levels employed in earlier 32P-postlabelling and DNA strand breakage and 8-OHdG studies (Heydens et al., 2008, ASB2012-11845). This publication reported that high i.p. dose levels of GBF induced significant liver and kidney toxicity that were not observed with oral administration. Statistically significant increases in 8-OHdG were not observed in this study under the same conditions as employed by the earlier study. The dimethylsulfoxide/olive oil vehicle dramatically enhanced toxicity of GBF administered by the i.p.

route and the toxicity was also observed for formulation components without glyphosate. These results indicated that the effects reported in the earlier studies were associated with high liver and kidney toxicity that was primarily due to the non-glyphosate components of the formulation and which were produced by the i.p. route of exposure to very high dose levels. The enhancement of toxicity by the unusual dimethylsulfoxide/olive oil dosing vehicle further calls into question whether the 32P-postlabelling finding represented effects associated with unusual toxicity rather than being indicative of adducts formed from glyphosate or glyphosate formulation components.
Carcinogenicity is not a direct endpoint for genotoxicity but it is one of the possible consequences of genotoxicity and, conversely, lack of carcinogenicity in well-conducted experimental studies provides some evidence that a significant genotoxic mode of action is not operating in vivo. The earlier review of glyphosate concluded that it was not carcinogenic
in mouse or rat chronic studies and notes that glyphosate was not considered carcinogenic by numerous regulatory agencies and scientific organisations (Williams et al., 2000, ASB2012- 12053).
B.6.4.8.9 AMPA and POEA
In addition to glyphosate and GBFs, the earlier review included information on the toxicity and genotoxicity of the major environmental breakdown product of glyphosate, aminomethylphosphonic acid (AMPA), and what was at that time a common GBF surfactant mixture of polyethoxylated long chain alkylamines synthesized from animal-derived fatty acids (polyethoxylated tallow amine, ethoxylate, POEA). Today a wide variety of surfactant systems are employed by different companies for different regions and end uses.
In the earlier review, summarised genotoxicity results for AMPA included negative results in the Ames/Salmonella bacterial reversion assay, an in vitro unscheduled DNA synthesis assay in primary hepatocytes and a mouse bone marrow erythrocyte micronucleus assay (Williams et al., 2000, ASB2012-12053). One publication of AMPA genotoxicity results was observed subsequent to 2000. In this publication analytical grade AMPA was reported to have positive effects in several assays including an alkaline SCGE endpoint in cultured mammalian Hep-2 cells, a chromosome aberration endpoint in cultured human lymphocytes and in a mouse bone marrow erythrocyte micronucleus assay (Manas et al., 2009, ASB2012-11891). Experimental limitations in the conduct of the alkaline SCGE assay included no inclusion of mammalian metabolic activation and no reported control of medium pH even though relatively high concentrations of AMPA acid (2.5-10 mM for 4 hours) were used. Although nucleoid images were analyzed with software rather than visual analysis the methodology doesn't indicate that slides were coded and there may have been a visual judgment component in selection of images for analysis. The positive results were statistically significant increases in tail length,
% DNA in tail and tail moment at 4.5 to 7.5 mM AMPA. The human lymphocyte chromosome aberration assay also did not employ an exogenous mammalian metabolic activation system but control of medium pH and blind scoring of slides were reported for this assay. A small increase in chromosome aberrations per 100 metaphases was observed in cells exposed to 1.8 but not 0.9 mM AMPA for 48 hours. The increase was marginally significant ($p<0.05$) and no statistically significant increases were observed for any specific chromosome aberration category. Although number of cells with aberrations are commonly used to describe results from in vitro chromosome aberration assays (OECD473, 1997) these data were not presented. Given the marginal significance, these omissions are a significant limitation in interpreting the results. Positive results were also reported for a mouse micronucleus bone marrow assay in mice administered 2 x 100 mg/kg or 2 x 200 mg/kg i.p at 24 hour intervals. The methodology description did not indicate that slides were coded for analysis in this assay. Results were reported as a statistically significant increase from a negative control value of

3.8/1000 micronucleated erythrocytes to 10.0 and 10.4/1000 micronucleated erythrocytes in the 2 x 100 and 2 x 200 mg/kg dose groups, respectively. These data do not indicate a reasonable dose response and a third dose level was not employed as recommended for this assay (OECD474, 1997). The publication indicates micronucleus scoring results for “erythrocytes” and not polychromatic or immature erythrocytes as would be appropriate for the acute dose protocol employed. Although this might be an inadvertent error in methodology description the term polychromatic erythrocytes was used in the methods section and PCE was used in the results table to describe scoring of PCE/NCE ratio.

The reported positive effects for AMPA in the in vitro studies are not concordant with in vitro results for other endpoints or the lack of genotoxic structural alerts in the structurally similar parent molecule moieties from DEREK in silico analysis. The alkaline SCGE effect could be due to cytotoxicity, especially considering the relatively high dose levels employed (close to the 10 mM upper limit dose) and the lack of indication of pH control. Although limited cytotoxicity (>80 % viability) was reported using the trypan blue exclusion method this endpoint may grossly underestimate cytotoxic effects observed with other end points.

The in vitro chromosome aberration assay positive result was of low magnitude and was of particularly questionable significance, considering the lack of statistical significance for any individual chromosome aberration category and that the results for number or percent of cells with chromosome aberrations were not reported.

There is a clear discordance in results for AMPA in the mouse bone marrow micronucleus assay. In the earlier review negative results were reported for AMPA in a mouse bone marrow micronucleus assay conducted with dose levels up to 1000 mg/kg i.p. (Williams et al., 2000, ASB2012-12053) The maximum dose level was much higher than those used by Manas et al. (2009, ASB2012-11891) Although Manas et al. used a protocol with two doses separated by 24 hours and a single harvest time, this protocol would not be expected to give different results than a single dose with multiple harvest times, particularly when the maximum single dose was much higher (OECD474, 1997). PCE/NCE ratio data from the Manas et al. (2009, ASB2012-11891) study do not indicate that there were detectable bone marrow toxic effects observed under the conditions of their study. It appears possible that Manas et al. may have inappropriately scored erythrocytes for micronuclei instead of polychromatic erythrocytes, but if this is the case lower sensitivity rather than higher sensitivity would be expected. These limitations suggest the possibility that the aberrant result might be that of Manas et al. (2009, ASB2012-11891) but further studies might be necessary to resolve the discordance.

The earlier review reported negative results for POEA in an Ames/Salmonella assay (Williams et al., 2000, ASB2012-12053). No other genotoxicity results were reported for POEA individually but numerous genotoxicity results were presented, as described earlier, for GBFs containing POEA. Examination of subsequent literature for this review did not produce any new publications reporting genotoxicity results for POEA as an individual test material (i.e. not as a glyphosate formulation). However, there were some publications confirming that POEA can be a significant contributor to toxicity of GBFs and that it exhibits biological effects consistent with surfactant properties. As noted earlier, experiments with a POEA- containing formulation without glyphosate administered i.p. in DMSO/olive oil vehicle to mice produced the same severe liver and kidney toxicity as a GBF indicating that the toxicity primarily resulted from the formulation components rather than glyphosate (Heydens et al., 2008, ASB2012-11845). Similarly, dose-response curves were superimposed for an in vitro system evaluating a GBF and the same formulation without glyphosate present (Levine et al., 2007, ASB2009-9030). Effects on mammalian cells consistent with membrane disruption and

consequent cytotoxicity were observed for POEA (Benachour and Seralini, 2009, ASB2012- 11561).

B.6.4.8.10 Genotoxicity of glyphosate mixtures and photoactivation

physico-chemical environment. The mixture made with the four pesticides exhibited the most potent cytogenetic toxicity, which was 20-fold higher than those of the most active compound AMPA, and 100-fold increased after light-irradation.

B.6.4.8.11 Genotoxicity Weight of Evidence

The earlier review applied a weight of evidence analysis to the available genotoxicity data. Various weighted components included assay system validation, test system species, relevance of the endpoint to heritable mutation, reproducibility and consistency of effects and dose-response and relationship of effects to toxicity (Williams et al., 2000, ASB2012-12053). The conclusion of this analysis was that glyphosate and Roundup GBFs were not mutagenic or genotoxic as a consequence of direct chemical reaction with DNA. This was supported by a strong preponderance of results indicating no effects in in vivo mammalian assays for chromosome effects and consistently negative results in gene mutation assays. Although some DNA damage responses were noted, these were judged likely to be secondary to toxicity rather than DNA reactivity.

Since this earlier review, a large number of genotoxicity studies have been conducted with glyphosate and GBFs. For gene mutation, one of the two primary endpoint categories with direct relevance to heritable mutation, one subsequent publication contains a summary of results from a bacterial gene mutation endpoint assay (Ames/Salmonella bacterial reversion assay). Although there were very significant limitations to the information published, the negative result is consistent with the majority of negative results reported for glyphosate and GBFs in Ames/Salmonella bacterial reversion assays. Another publication reported results for a Drosophila wing spot assay of glyphosate. Results were negative or inconclusive in this assay for crosses that would have detected gene mutation as loss of heterozygosity. The new results provide some support to reinforce the earlier conclusion that glyphosate and GBFs are not active for the gene mutation endpoint category.

The second primary endpoint category with direct relevance to heritable mutation is chromosome effects. The earlier review noted mixed results for two in vitro chromosome effects assays in mammalian cells but concluded that the most reliable result was the negative assay. A number of in vitro mammalian cell chromosome aberration or micronucleus assay results have been subsequently published using bovine or human lymphocytes. These assays suffer from some technical limitations in conduct or reporting of methodology that frequently included failure to indicate control of medium for pH and failure to indicate coding of slides for visual scoring. Both positive and negative results are reported in these assays. A large preponderance of results in the absence of an exogenous mammalian metabolic activation system were negative up to high (mM) dose levels that were toxic or close to toxic levels observed in parallel experiments. The exceptions were a weak and inconsistent response reported in two publications from the same laboratory and a positive response for the uncharacterized formulation, herbazed. In addition to these findings in mammalian cells negative results were also reported for Roundup GBF in an onion root tip assay conducted without exogenous mammalian metabolic activation. Thus, the preponderance of evidence from assays not employing an exogenous mammalian metabolic activation system indicates that glyphosate and GBFs are not structural chromosome breakage inducers (clastogenic) in in vitro mammalian chromosome aberration or micronucleus assays.

Two publications from one laboratory reported an increase in micronucleus frequencies for glyphosate in in vitro cultured mammalian cells in the presence of an exogenous S9 metabolic activation system (Mladinic et al., 2009, ASB2012-11906; Mladinic et al., 2009, ASB2012- 11907). An enrichment for centomeric-containing micronuclei suggested that the increased

micronuclei observed in these studies were derived from aneugenic processes, probably mediated through toxicity, rather than chromosome breakage. Thus, these two reports of weak micronucleus responses in the presence of exogenous mammalian metabolic activation appear to result from toxicity-associated aneugenic rather than clastogenic mechanisms. A number of other gene mutation and in vitro chromosome effect genotoxicity studies are negative with exogenous metabolic activation which supports the conclusion that the weight of evidence does not indicate a DNA-reactive clastogenic activity in in vitro assays using mammalian cells.

All except one of a number of in vivo mouse bone marrow chromosome aberration or micronucleus assays of glyphosate and GBFs were reported as negative in the earlier review. In the updated review both positive and negative results were reported for glyphosate and GBFs in these types of assays. Many of these studies had limitations or deficiencies compared to international guidelines with the most common and significant being no indication of slide coding for visual scoring. Four publications from three laboratories reported negative results in mouse bone marrow erythrocyte micronucleus assays of glyphosate and GBFs which are consistent with the earlier reviewed studies. These studies used high, peri-lethal dose levels administered by the i.p. or oral routes.

Two publications from two laboratories reported positive results for glyphosate and GBFs in the mouse bone marrow erythrocyte micronucleus assay. One positive result for glyphosate was encountered using dose levels and routes that were similar to those employed in the negative glyphosate studies in the same assay system. The publication reporting this result indicates that erythrocytes rather than polychromatic erythrocytes were scored which would be inappropriate for the treatment protocol but it is possible that this is a misreporting of what types of cells were actually scored. Although there is no definitive explanation for the discordance, the preponderance of mouse bone marrow erythrocyte micronucleus studies of glyphosate are clearly negative. The reported positive result for Roundup GBF is discordant with a number of negative results for Roundup or other GBFs conducted at higher dose levels. The most unique feature of this study was the use of dimethylsulfoxide as a vehicle. The preponderance of mouse bone marrow erythrocyte micronucleus studies for Roundup and other GBF studies is negative.

Positive results were reported in an unusual test system (rabbit) and route (drinking water), but water intake was not reported and effects may therefore be attributable to dehydration. Furthermore, most of the effects were on endpoints not usually considered as indicators of clastogenicity and structural chromosome aberration. One laboratory reported positive results for chromosome aberration effects in bone marrow and spermatocytes after extended dosing. However, the herbazed formulation test material was not characterised.

While more discordant results in the important in vivo mammalian chromosome effect assay category have been reported in publications subsequent to the earlier 2000 review the preponderance of evidence continues to indicate that glyphosate and GBFs are not active in this category of end point.

Several in vivo erythrocyte micronucleus assay results for GBFs in non-mammalian systems (fish and caiman eggs) have been published since the earlier review. These test systems have relatively little experience and are largely unvalidated in comparison to the mouse bone marrow erythrocyte micronucleus assay. Two publications report negative results and two publications report positive results in different fish species and there is no definitive explanation for the discordance. Both the positive and negative studies employed maximum dose levels that were toxic or close to toxic dose levels. One possible explanation for the discordance is that the positive effects were associated with toxicity that only occurred beyond an exposure threshold and over a fairly narrow dose range. Positive results in hatchlings derived from caiman eggs exposed to Roundup formulation are given

relatively
little weight because of extremely limited experience with this assay system and because of significant questions about how DNA damage effects induced in embryos can persist and be evident in cells of hatchlings after several weeks and numerous cell divisions. The reported weak and inconsistent response in one of four crosses for somatic recombination in the Drosophila wing spot assay is also accorded relatively low weight. These non-mammalian test systems are generally considered of lower weight for predicting mammalian effects than mammalian test systems. Also, the environmental significance of effects for GBFs should consider the relationship between concentrations or exposures producing effects and likely environmental concentrations or exposures. This is particularly important if the effects are produced by threshold mediated toxic processes.
There have been a significant number of publications since the earlier review of results for assays in the DNA damage category with some SCE and a large number of alkaline SCGE endpoint publications. In general, the DNA damage end point category is considered supplementary to the gene mutation and chromosome effect categories because this endpoint category does not directly measure heritable events or effects closely associated with heritable events. Regulatory genotoxicity testing recommendations and requirements focus on gene mutation and chromosome effect end points for initial core testing, particularly for in vitro testing. This consideration is underscored by the observation of some cases of compounds where positive effects are observed in these assays that are not observed for gene mutation or chromosome effect assays. Also, there are numerous examples of responses in these endpoints that do not appear to result from mechanisms of direct or metabolite DNA-reactivity. The unique response consideration is reinforced in this data set by observations of responses in DNA damage endpoints but not in chromosome effect end points.
Many DNA damage endpoint assays of glyphosate or GBFs have produced positive results at high, toxic or peri-toxic dose levels for the SCE and alkaline SCGE endpoints in a variety of test systems including cultured mammalian cells, several aquatic species and caiman eggs. The only new report of positive in vivo mammalian DNA damage effects are for an uncharacterised formulation, herbazed. There are several examples of negative results for a chromosome aberration or micronucleus endpoint and positive results for the alkaline SCGE or SCE endpoint in the same publication (Cavalcante et al., 2008, ASB2012-11586; Manas et al., 2009, ASB2012-11892; Mladinic et al., 2009, ASB2012-11906; Sivikova and Dianovsky, 2006, ASB2012-12029). These examples confirm the impression that the DNA damage endpoints are not necessarily predictive of heritable mutation effects and are also consistent with the DNA damage endpoints reflecting toxic effect mechanisms. While the number of reported positive responses in these endpoints does suggest that effects in these endpoints can be induced by glyphosate or GBFs, comparison with results for gene mutation and chromosome effects endpoints, examination of the dose response and association of the effects with toxic endpoints indicates that these effects are likely secondary to toxicity and are threshold mediated. Surfactants in GBFs increase toxicity compared to the active ingredient of glyphosate salts and are shown to induce effects such as membrane damage and oxidant stress which are likely capable of inducing DNA damage effects at cytotoxic doses. These factors as well as other considerations presented in Section 6.3 indicate that these DNA damage effects have negligible significance to prediction of hazard or risk at lower and more relevant exposure levels.
Most of the human studies do not provide interpretable or relevant information regarding whether there are in vivo human genotoxic effects of GBFs because the reported incidence of glyphosate formulation exposure in the population was low or because there were reported exposures to a relatively large number of pesticides. Two studies with focus on glyphosate exposure through presence in or near areas of glyphosate formulation spraying found increases in the DNA damage

alkaline SCGE end point. In one study clinical signs of toxicity
were reported in the population and spraying concentrations were reported to be many times the recommended application rate. Given the nature of the end point a reasonable interpretation is that effects might well be due to the overt toxicity that was reported in the publication. This would be a threshold mediated, non-DNA reactive mechanism and is consistent with experimental system results showing alkaline SCGE effects in animals exposed to high levels of formulation components. The low weight of evidence for significant genotoxic hazard indicated by this particular endpoint in human monitoring is reinforced by findings that exercise induces alkaline SCGE effects in humans. The other study found increases in binucleated micronucleated cell frequency in population in spraying areas but the increases were not consistent with spraying levels or self-reported exposure. These latter observations are not consistent with the study presenting clear evidence of GBF effects on this endpoint. In sum, the available human data do not provide any clear indications that exposed humans are substantially different in response than mammalian animal models or that exposure to GBFs produces DNA-reactive genotoxicity.

Carcinogenicity is an adverse effect that is a possible consequence of genotoxic and mutagenic activity. Conversely, lack of carcinogenicity in properly conducted animal models is supportive for lack of significant in vitro mammalian genotoxicity. The updated review provides one new study of glyphosate formulation which is negative for either initiator or complete carcinogenesis activity which provides additional evidence to reinforce the conclusion from earlier mammalian carcinogenicity assays that glyphosate and GBFs are non- carcinogenic. These findings support the conclusion that glyphosate and GBFs do not have in vivo mammalian genotoxicity or mutagenicity.

In addition to considering the results relevant to genotoxicity hazard assessment, an important additional perspective on risk can be provided by comparing levels used in experimental studies with expected human and environmental exposure levels. A study of farmers indicated a maximum estimated systemic glyphosate dose of 0.004 mg/kg for application without protective equipment and a geometric mean of 0.0001 mg/kg (Acquavella et al., 2004, ASB2012-11528). When compared with in vivo mammalian test systems that utilize glyphosate exposures on the order of 50-300 mg/kg, the margins of exposure between the test systems and farmers is 12,500-75,000 for the maximum farmer systemic exposure and 0.5-3 million for the geometric mean farmer systemic exposure. These margins are quite substantial, especially considering that many of the in vivo genotoxicity studies are negative. Assuming reasonable proportionality between exposure to glyphosate and GBF ingredients, similar large margins of exposure would exist for GBF components. The margins of exposure compared to in vitro mammalian cell exposures are estimated to be even larger. Assuming uniform distribution, the systemic concentration of glyphosate from the Aquavella et al. (2004, ASB2012-11528) farmer biomonitoring study would be on the order of 24nM for the maximum and 0.59 nM for the geometric mean exposure. A typical maximum in vitro mammalian exposure of 1-5 mM represents a margin of exposure of 42,000-211,000 for the maximum farmer exposure and 1.7-8.4 million for the geometric mean farmer systemic exposures, respectively.

Overall, the weight of evidence of the studies considered in the earlier review as well as the studies considered in this review indicates that glyphosate and GBFs are not genotoxic in the two general endpoint categories most directly relevant to heritable mutagenesis, gene mutation and chromosome effects. This conclusion results from a preponderance of evidence; however, there are reports of positive discordant results in both end point categories. The new studies considered in this review provide some evidence for DNA damage effects induced by high, toxic exposures, particularly for the alkaline SCGE end point and for GBFs containing surfactant. Several considerations, including the lack of response in other endpoint

categories, suggest that these effects result from toxic and not DNA-reactive mechanisms and that they do not indicate in vivo genotoxic potential under normal exposure levels.
Regulatory and authoritative reviews of glyphosate supporting registrations and registrations in all regions of the world over the last 40 years have consistently determined that glyphosate is nongenotoxic (Commission, 2002, ASB2009-4191; WHO/FAO, 2004, ASB2008-6266). Scientific publications contrary to these regulatory reviews should be evaluated using a weight of evidence approach with consideration for reliability of the assay used and data quality presented.
Abbreviations
AMPA, aminomethylphosphonic acid ; CB MN, cytokinesis block micronucleus; GBF, glyphosate based formulation; i.p., intraperitoneal ; NCE, normochromatic erythrocyte; OECD, Organization for Economic Cooperation and Development; PCE, polychromatic erythrocyte; POEA, polyethoxylated tallow amine, ethoxylate; SCE, sister chromatid exchange; SCGE, single cell gel electrophoresis (comet).
Author(s)
Year
Study title
Alvarez-Moya, C., Silva, M.R.,
Arambula, A.R.V., Sandoval, A.I., Vasquez, H.C.,
Gonzales Montes, R.M.
2011
Evaluation of genetic damage induced by glyphosate isopropylamine salt using Tradescantia bioassays Genetics and Molecular Biology
Volume: 34
Number: 1
Pages: 127-130 ASB2012-11538
Abstract*
Glyphosate is noted for being non-toxic in fishes, birds and mammals (including humans). Nevertheless, the degree of genotoxicity is seriously controversial. In this work, various concentrations of a glyphosate isopropylamine salt were tested using two methods of genotoxicity assaying, viz., the pink mutation assay with Tradescantia (4430) and the comet assay with nuclei from staminal cells of the same plant. Staminal nuclei were studied in two different forms, namely nuclei from exposed plants, and nuclei exposed directly. Using the pink mutation assay, isopropylamine induced a total or partial loss of color in staminal cells, a fundamental criterion utilised in this test. Consequently, its use is not recommended when studying genotoxicity with agents that produce pallid staminal cells. The comet assay system detected statistically significant ($p < 0.01$) genotoxic activity by isopropylamine, when compared to the negative control in both the nuclei of treated plants and directly treated nuclei, but only the treated nuclei showed a dose-dependent increase. Average migration in the nuclei of treated plants increased, when compared to that in treated nuclei. This was probably due, either to the permanence of isopropylamine in inflorescences, or to the presence of secondary metabolites. In conclusion, isopropylamine possesses strong genotoxic activity, but its detection can vary depending on the test systems used.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:

Exposure conditions of plants (immersion) not representative for glyphosate. Inappropriate test model
as herbicides are toxic to plants. Presentation of results not sufficient for assessment. Reporting deficiencies (e.g. positive controls).
Relevance of study:
Not relevant (Due to reliability, and exposure
conditions of plants and inappropriate test model).
Klimisch code:
3
Author(s)
Year
Study title
Bolognesi, C. Bonatti, S. Degan, P. Gallerani, E. Peluso, M. Rabboni, R. Roggieri, P.
Abbondandolo, A.
1997
Genotoxic activity of glyphosate and its technical formulation roundup
Journal of Agricultural and Food Chemistry Volume: 45
Pages: 1957-1962 Z59299
Abstract*
Glyphosate (N-phosphonomethylglycine) is an effective herbicide acting on the synthesis of aromatic amino acids in plants. The genotoxic potential of this herbicide has been studied: the results available in the open literature reveal a weak activity of the technical formulation. In this study, the formulated commercial product, Roundup, and its active agent, glyphosate, were tested in the same battery of assays for the induction of DNA damage and chromosomal effects in vivo and in vitro. Swiss CD1 mice were treated intraperitoneally with test substances, and the DNA damage was evaluated by alkaline elution technique and 8- hydroxydeoxyguanosine (8-OHdG) quantification in liver and kidney. The chromosomal damage of the two pesticide preparations was also evaluated in vivo in bone marrow of mice as micronuclei frequency and in vitro in human lymphocyte culture as SCE frequency. A DNA-damaging activity as DNA single-strand breaks and 8-OHdG and a significant increase in chromosomal alterations were observed with both substances in vivo and in vitro. A weak increment of the genotoxic activity was evident using the technical formulation.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Basic data given, however, the study is performed with methodological and reporting deficiencies (only data without metabolic activation generated in in vitro tests, no positive controls included in in vitro SCE and in vivo
experiments, in some experiments only two test substance concentrations tested).
Relevance of study:
Not relevant (Due to methodological and reporting deficiencies data considered to be supplemental information; i.p. exposure route is not relevant for human exposure)
Klimisch code:
3
Author(s)

Year
Study title
Bolognesi, C., Perrone, E., Landini, E.
2002
Micronucleus monitoring of a floriculturist population from western Liguria, Italy
Mutagnesis Volume: 17
Number: 5
Pages: 391-397 ASB2012-11573
Abstract*
A biomonitoring study was carried out to investigate whether exposure to complex pesticide mixtures in ornamental crop production represents a potential genotoxic risk. Exposed and control subjects were selected in western Liguria (Italy). The area was chosen for its intensive use of pesticides. The main crops produced were roses, mimosas, carnations and chrysanthemums, as ornamental non-edible plants, and tomato, lettuce and basil, as edible ones. The levels of micronuclei (MN) were analysed in peripheral blood lymphocytes of 107 floriculturists (92 men and 15 women) and 61 control subjects (42 men and 19 women). A statistically significant increase in binucleated cells with micronuclei (BNMN) was detected in floriculturists with respect to the control population (4.41 +/- 2.14 MN/1000 cells versus
3.04 +/- 2.14, $P < 0.001$). The mean number of BNMN varied as a function of sex and age. Smoking habit had no effect on MN frequency. A positive correlation between years of farming and MN frequency in peripheral blood lymphocytes was observed ($r = 0.30$, $P = 0.02$). The conditions of exposure were also associated with an increase in cytogenetic damage, with a 28 % higher MN frequency in greenhouse workers compared with subjects working only outdoors in fields. Workers not using protective measures during high exposure activities showed an increase in MN frequency. Our findings suggest a potential genotoxic risk due to pesticide exposure.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable for glyphosate
Comment:
MN-test comparable to OECD guidelines, but not equal. Exposures to multiple pesticides with no information on exposure concentrations to individual pesticides make result unreliable for glyphosate.
Relevance of study:
Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenicity possible. Not relevant for
glyphosate).
Klimisch code:
3
Author(s)
Year
Study title
Bolognesi, C.,
Landini, E., Perrone, E., Roggieri, P.
2004
Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by

fluorescence in situ hybridization (FISH) with an all-chromosome centrometric probe
Mutation Research Volume: 557
Number: 2
Pages: 109-117 ASB2012-11572
Abstract*
Flower production in greenhouses associated with a heavy use of pesticides is very wide- spread in the western part of the Ligurian region (Italy). The formation of micronuclei in peripheral blood lymphocytes is a valuable cytogenetic biomarker in human populations occupationally exposed to genotoxic compounds. In the present study we investigated the micronucleus frequency in peripheral blood lymphocytes of 52 floriculturists and 24 control subjects by use of the cytokinesis-block methodology associated with fluorescence in situ hybridization with a pan-centromeric probe that allowed to distinguish centromere-positive (C+) and centromere-negative (C−) micronuclei. The comparison between floriculturists and controls did not reveal any statistically significant difference in micronucleus frequency, although an increase was observed with increasing pesticide use, number of genotoxic pesticides used and duration of exposure. An increase in C+ as well as in C− micronuclei and in the percentage of C+ micronuclei with respect to the total number of micronuclei was detected in floriculturists, suggesting a higher contribution of C+ micronuclei in the total number scored. The percentage C+ micronuclei was not related to the duration of exposure or to the number of genotoxic pesticides used, but a higher percentage (66.52 % versus 63.78 %) was observed in a subgroup of subjects using benzimidazolic compounds, compared with the floriculturist population exposed to a complex pesticide mixture not including benzimidazolics. These results suggest a potential human hazard associated with the exposure to this class of aneuploidy-inducing carcinogens.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable for glyphosate
Comment:
Well-documented study. MN-test comparable to OECD guidelines, but not equal. No information on exposure concentrations to individual pesticides
Relevance of study:
Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenic non-statistically significant differences possible. No statistically relevant findings
reported for glyphosate alone).
Klimisch code:
2
Author(s)
Year
Study title
Cavas, T., Könen S.
2007
Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (Carassius auratus) exposed to a glyphosate formulation using the micronucleus test and the comet assay
Mutagenesis 22
263-268

ASB2012-11587
Abstract*
Glyphosate is a widely used broad-spectrum weed control agent. In the present study, an in vivo study on the genotoxic effects of a technical herbicide (Roundup®) containing isopropylamine salt of glyphosate was carried out on freshwater goldfish Carassius auratus. The fish were exposed to three doses of glyphosate formulation (5, 10 and 15 ppm). Cyclophosphamide at a single dose of 5 mg/L was used as positive control. Analysis of micronuclei, nuclear abnormalities and DNA damage were performed on peripheral erythrocytes sampled at intervals of 48, 96 and 144 h post treatment. Our results revealed significant dose-dependent increases in the frequencies of micronuclei, nuclear abnormalities as well as DNA strand breaks. Our findings also confirmed that the alkaline comet assay and nuclear deformations in addition to micronucleus test on fish erythrocytes in vivo are useful tools in determining the potential genotoxicity of commercial herbicides.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not Reliable
Comment:
Methodological and reporting deficiencies (e.g. test substance source, no concurrent measurement of toxicity reported, less than 2000 erythrocytes scored per animal and results not reported separately for replicates).
Relevance of study:
Relevant with restrictions (Due to reliability. Discussion confuses glyphosate with glyphosate formulated products.)
Klimisch code:
3
Author(s)
Year
Study title
Guilherme, S.
Gaivao, I. Santos, M.A.
Pacheco, M.
2010
European eel (Anguilla Anguilla) genotoxic and pro-oxidant responses following short-term exposure to Roundup® - a glyphosate-based herbicide.
Mutagenesis Volume: 25
Number: 5
Pages: 523-530 ASB2012-11836
Abstract*
The glyphosate-based herbicide, Roundup®, is among the most used pesticides worldwide. Due to its extensive use, it has been widely detected in aquatic ecosystems representing a potential threat to non-target organisms, including fish. Despite the negative impact of this commercial formulation in fish, as described in literature, the scarcity of studies assessing its genotoxicity and underlying mechanisms is evident. Therefore, as a novel approach, this study evaluated the genotoxic potential of Roundup® to blood cells of the European eel (Anguilla anguilla) following short-term (1 and 3 days) exposure to environmentally realistic concentrations (58 and 116 mg/L), addressing also the possible association with oxidative stress. Thus, comet and erythrocytic nuclear abnormalities (ENAs) assays

were adopted, as genotoxic end points, reflecting different types of genetic damage. The prooxidant state was assessed through enzymatic (catalase, glutathione-S-transferase, glutathione peroxidase and glutathione reductase) and non-enzymatic (total glutathione content) antioxidants, as well as by lipid peroxidation (LPO) measurements. The Roundup® potential to induce DNA strand breaks for both concentrations was demonstrated by the comet assay. The induction of chromosome breakage and/or segregational abnormalities was also demonstrated through the ENA assay, though only after 3- day exposure to both tested concentrations. In addition, the two genotoxic indicators were positively correlated. Antioxidant defences were unresponsive to Roundup®. LPO levels increased only for the high concentration after the first day of exposure, indicating that oxidative stress caused by this agrochemical in blood was not severe. Overall results suggested that both DNA damaging effects induced by Roundup® are not directly related with an increased pro-oxidant state. Moreover, it was demonstrated that environmentally relevant concentrations of Roundup® can pose a health risk for fish populations.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
No positive controls were included, which significantly detracts from the utility of a non-validated, non- standard test method. Less than the standard of a minimum of three dose levels used, independent coding of slides for scoring and results not reported separately
for replicates.
Relevance of study:
Not relevant (Non-standard test system, no positive
controls to verify test method/study validity.)
Klimisch code:
3
Author(s)
Year
Study title
Kale, P.G.
Petty, B.T. Jr. Walker, S. Ford, J.B. Dehkordi, N. Tarasia, S. Tasie, B.O. Kale, R.
Sohni, Y.R.
1995
Mutagenicity Testing of Nine Herbicides and Pesticides Currently Used in Agriculture.
Environmental and Molecular Mutagenesis Volume: 25
Pages: 148-153
Z73986, ASB2012-11860
Abstract*
Nine herbicides and pesticides were tested for their mutagenicity using the Drosophila sex- linked recessive lethal mutation assay. These are Ambush, Treflan, Blazer, Roundup, 2,4-D Amine, Crossbow, Galecron, Pramitol, and Pondmaster. All of these are in wide use at present. Unlike adult feeding and injection assays, the larvae were allowed to grow in medium with the test chemical, thereby providing long and chronic exposure to the sensitive and dividing diploid cells, i.e., mitotically active spermatogonia and sensitive spermatocytes. All chemicals induced significant numbers of mutations in at least one of the cell types tested. Some of these compounds were found

to be negative in earlier studies. An explanation for the difference in results is provided. It is probable that different germ cell stages and treatment regimens are suitable for different types of chemicals. Larval treatment may still be valuable and can complement adult treatment in environmental mutagen testing.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Comparable to 1984 OECD guideline, but with several deficiencies (no positive controls reported and thus study validity not verifiable; wild type male treatment age different than recommended, purity of test substances not reported, tested formulation other
ingredients such as surfactants not reported.)
Relevance of study:
Not relevant for glyposate (Glyphosate not tested;
formulation tested)
Klimisch code:
3
Author(s)
Year
Study title
Manas, F. Peralta,
L. Raviolo, J. Garcia Ovando, H. Weyers, A. Ugnia,
L. Gonzalez Cid,
M. Larripa, I. Gorla, N.
2009
Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests.
Ecotoxicology and Environmental Safety Volume: 72
Pages: 834-837 ASB2012-11891
Abstract*
Formulations containing glyphosate are the most widely used herbicides in the world. AMPA is the major environmental breakdown product of glyphosate. The purpose of this study is to evaluate the in vitro genotoxicity of AMPA using the Comet assay in Hep-2 cells after 4h of incubation and the chromosome aberration (CA) test in human lymphocytes after 48 h of exposition. Potential in vivo genotoxicity was evaluated through the micronucleus test in mice. In the Comet assay, the level of DNA damage in exposed cells at 2.5-7.5 mM showed a significant increase compared with the control group. In human lymphocytes we found statistically significant clastogenic effect AMPA at 1.8 mM compared with the control group. In vivo, the micronucleus test rendered significant statistical increases at 200-400 mg/kg. AMPA was genotoxic in the three performed tests. Very scarce data are available about AMPA potential genotoxicity.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:

Reporting deficiencies (purity of AMPA not specified, several parameters in the MNT not reported, only 2 dose levels used in both CA and MNT). Exposure route used in the MNT is not relevant for human exposure;
methodological deficiencies (see guideline deviations).
Relevance of study:
Not relevant (Due to reliability)
Klimisch code:
3
Author(s)
Year
Study title
Manas, F. Peralta,
L. Raviolo, J. Garcia Ovando, H. Weyers, A. Ugnia,
L. Gonzalez Cid,
M. Larripa, I. Gorla, N.
2009
Genotoxicity of glyphosate assessed by the comet assay and cytogenic tests
Environmental Toxicology and Pharmacology Volume: 28
Pages: 37-41 ASB2012-11892
Abstract*
It was evaluated the genotoxicity of glyphosate which up to now has heterogeneous results. The comet assay was performed in Hep-2 cells. The level of DNA damage in the control group (5.42±1.83 arbitrary units) for tail moment (TM) measurements has shown a significant increase ($p < 0.01$) with glyphosate at a range concentration from 3.00 to 7.50mM. In the chromosome aberrations (CA) test in human lymphocytes the herbicide (0.20–6.00mM) showed no significant effects in comparison with the control group. In vivo, the micronucleus test (MNT)was evaluated in mice at three doses rendering statistical significant increases at 400 mg/kg (13.0±3.08 micronucleated erythrocytes/1000 cells, $p < 0.01$). In the present study glyphosate was genotoxic in the comet assay in Hep-2 cells and in the MNT test at 400 mg/kg in mice. Thiobarbituric acid reactive substances (TBARs) levels, superoxide dismutase (SOD) and catalase (CAT) activities were quantified in their organs. The results showed an increase in these enzyme activities.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Guideline deviations and reporting deficiencies. Several parameters in the MNT not reported. Blind scoring reported for the CA but not MNT. Exposure route used in the MNT is not relevant for human exposure. (see guideline deviations). No indication of pH or osmolality control for the comet assay. Results not reported separately for replicates.
Relevance of study:
Not relevant (Due to guideline deviations and reporting
deficiencies).
Klimisch code:
3
Author(s)

Year
Study title
Mladinic, M. Berend, S. Vrdoljak, A.L. Kopjar, N. Radic, B. Zeljezic, D.
2009
Evaluation of Genome Damage and Its Relation to Oxidative Stress Induced by Glyphosate in Human Lymphocytes in Vitro
Environmental and Molecular Mutagenesis Volume: 50
Number: 9
Pages: 800-807 ASB2012-11906
Abstract*
In the present study we evaluated the genotoxic and oxidative potential of glyphosate on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure. Testing was done with and without metabolic activation (S9). Ferric-reducing ability of plasma (FRAP), thiobarbituric acid reactive substances (TBARS) and the hOGG1 modified comet assay were used to measure glyphosate's oxidative potential and its impact on DNA. Genotoxicity was evaluated by alkaline comet and analysis of micronuclei and other nuclear instabilities applying centromere probes. The alkaline comet assay showed significantly increased tail length (20.39 m) and intensity (2.19 %) for 580 mg/mL, and increased tail intensity (1.88 %) at 92.8 eg/mL, compared to control values of 18.15 /m for tail length and 1.14 % for tail intensity. With S9, tail length was significantly increased for all concentrations tested: 3.5, 92.8, and 580 mg/mL. Using the hOGG1 comet assay, a significant increase in tail intensity was observed at 2.91 gg/mL with S9 and 580 /g/mL without S9. Without S9, the frequency of micronuclei, nuclear buds and nucleoplasmic bridges slightly increased at concentrations 3.5 /g/mL and higher. The presence of S9 significantly elevated the frequency of nuclear instabilities only for 580 /g/mL. FRAP values slightly increased only at 580 /g/mL regardless of metabolic activation, while TBARS values increased significantly. Since for any of the assays applied, no clear dose-dependent effect was observed, it indicates that glyphosate in concentrations relevant to human exposure do not pose significant health risk.
* Quoted from article
Klimisch evaluation
Reliability of study:
Reliable with restrictions
Comment:
Non-GLP, non-guideline in vitro study, meeting scientific principles
Relevance of study:
Relevant with restrictions (Assessment of Genotoxicity in vitro at concentrations relevant to human exposure levels; authors state that no clear dose-dependent effect was observed, and results indicate that glyphosate in concentrations relevant to human exposure do not pose significant health risk.
Klimisch code:
2
Author(s)
Year
Study title
Mladinic, M.,
Perkovic, P., Zeljezic, D.
2009b

Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay Toxicology Letters
Volume: 189
Number: 2
Pages: 130-137 ASB2012-11907
Abstract*
Possible clastogenic and aneugenic effects of pesticides on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure were evaluated with and without the use of metabolic activation (S9). To get a better insight into the content of micronuclei (MN) and other chromatin instabilities, lymphocyte preparations were hybridized using pancentromeric DNA probes. Frequency of the MN, nuclear buds (NB) and nucleoplasmic bridges (NPB) in cultures treated with glyphosate slightly increased from
3.5 μg/mL onward. Presence of S9 significantly elevated cytome assay parameters only at 580 μg/mL. No concentration-related increase of centromere (C+) and DAPI signals (DAPI+) was observed for glyphosate treatment. Terbuthylazine treatment showed a dose dependent increase in the number of MN without S9 significant at 0.0008 μg/mL and higher. At concentration lower than 1/16 LD50 occurrence of C + MN was significantly elevated regardless of S9, but not dose related, and in the presence of S9 only NBs containing centromere signals were observed. Carbofuran treatment showed concentration dependent increase in the number of MN. The frequency of C + MN was significant from 0.008 μg/mL onward regardless of S9. Results suggest that lower concentrations of glyphosate have no hazardous effects on DNA, while terbuthylazine and carbofuran revealed a predominant aneugenic potential.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-GLP, non-guideline study in vitro. Positive and negative control results almost indistinguishable for MN assay without metabolic activation. Negative control NB and NBP results not reported.
Relevance of study:
Not relevant (Proposed mechanism of genotoxicity (in vitro) is not relevant to human exposure levels. Authors express confidence that estimated maximum human exposure levels correspond to acceptable safety levels based on evaluated in vitro endpoints, and that their
findings need to be verified in vivo.)
Klimisch code:
3

Author(s)
Year
Study title
Paz-Y-Mino, C. Sanchez, M. E. Arevalo, M. Munoz, M. J. Witte, T.
De-La-Carrera, G. O.
Leone, P. E.
2007
Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate.
Genetics and Molecular Biology Volume: 30

Number: 2
Pages: 456-460 ASB2012-11992
Abstract*
We analyzed the consequences of aerial spraying with glyphosate added to a surfactant solution in the northern part of Ecuador. A total of 24 exposed and 21 unexposed control individuals were investigated using the comet assay. The results showed a higher degree of DNA damage in the exposed group (comet length = 35.5 mu m) compared to the control group (comet length = 25.94 mu m). These results suggest that in the formulation used during aerial spraying glyphosate had a genotoxic effect on the exposed individuals.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Documentation of Comet assay insufficient for assessment.
Relevance of study:
Not relevant (Glyphosate formulation was applied at much higher dose rates than recommended for the intended uses in the EU. In addition, the herbicide was combined with the adjuvant (Cosmoflux 411F) that can increase the biological action of the herbicide. This
adjuvant will not be used in the EU.)
Klimisch code:
3
Author(s)
Year
Study title
Peluso, M. Munnia, A. Bolognesi,
C. Parodi, S.
1998
32P-postlabeling detection of DNA adducts in mice treated with
the herbicide Roundup.
Environmental and Molecular Mutagenesis Volume: 31
Number: 4
Pages: 55-59 TOX1999-318
Abstract*
Roundup is a postemergence herbicide acting on the synthesis of amino acids and other important endogenous chemicals in plants. Roundup is commonly used in agriculture, forestry, and nurseries for the control or destruction of most herbaceous plants. The present study shows that Roundup is able to induce a dose-dependent formation of DNA adducts in the kidneys and liver of mice. The levels of Roundup-related DNA adducts observed in mouse kidneys and liver at the highest dose of herbicide tested (600 mg/kg) were 3.0 +/- 0.1 (SE) and 1.7 +/- 0.1 (SE) adducts/10(8) nucleotides, respectively. The Roundup DNA adducts were not related to the active ingredient, the isopropylammonium salt of glyphosate, but to another, unknown component of the herbicide mixture. Additional experiments are needed to identify the chemical specie(s) of Roundup mixture involved in DNA adduct formation. Findings of this study may help to protect agricultural workers from health hazards and provide a basis for risk assessment.
* Quoted from article

Klimisch evaluation
Reliability of study:
Not Reliable
Comment:
A non-guideline study with confounding results based on testing a surfactant containing formulation. Reporting deficiencies (statistical methods). Toxic surfactant effects subsequently verified in Heydens et al. (2008, ASB2012-11845) reporting the same study type with a glyphosate formulated product and an appropriate control; formulation blank without
glyphosate.
Relevance of study:
Not relevant (i.p. administration of high doses of a surfactant containing formulation a relevant exposure scanario for human risk assessments. In addition, the DNA adducts observed were not related to the active ingredient (isopropylammonium salt of glyphosate), but to another, unknown component of the herbicide
mixture.)
Klimisch code:
3
Author(s)
Year
Study title
Poletta, G.L. Larriera, A. Kleinsorge, E.
Mudry, M.D.
2009
Genotoxicity of the herbicide formulation Roundup® (glyphosate) in broad-snouted caiman (Caiman latirostris) evidenced by the Comet assay and Micronucleus test Mutation Research
Volume: 672
Number: 2
Pages: 95-102 ASB2012-12002
Abstract*
The genotoxicity of pesticides is an issue of worldwide concern. The present study was undertaken to evaluate the genotoxic potential of a widely used herbicide formulation, Roundup® (glyphosate), in erythrocytes of broad-snouted caiman (Caiman latirostris) after in ovo exposure. Caiman embryos were exposed at early embryonic stage to different sub-lethal concentrations of Roundup® (50, 100, 200, 300, 400, 500, 750, 1000, 1250 and 1750)g/egg). At time of hatching, blood samples were obtained from each animal and two short-term tests, the Comet assay and the Micronucleus (MN) test, were performed on erythrocytes to assess DNA damage. A significant increase in DNA damage was observed at a concentration of 500 ag/egg or higher, compared to untreated control animals ($p<0.05$). Results from both the Comet assay and the MN test revealed a concentration-dependent effect. This study demonstrated adverse effects of Roundup® on DNA of C. latirostris and confirmed that the Comet assay and the MN test applied on caiman erythrocytes are useful tools in determining potential genotoxicity of pesticides. The identification of sentinel species as well as sensitive biomarkers among the natural biota is imperative to thoroughly evaluate genetic damage, which has significant consequences for short- and long-term survival of the natural species.
* Quoted from article
Klimisch evaluation
Reliability of study:

Not reliable
Comment:
Non-GLP studies in a unique test model. Micronucleus assay followed guideline, Comet assay similar to guideline.
Test methods have been modified to be applied caiman species. Methodological deficiencies: housing and feeding conditions of parents not specified; sex not distinguished, stability and homogeneity assessment of test substance preparations not reported. Results not reported separately for replicate individual animals.
Relevance of study:
Not relevant. Highly artificial in ovo exposure scenario not relevant to real world environmental exposures.
Caiman eggs are covered and not exposed to the surface. Any glyphosate in a potential herbicide overspray would sorb to sediment and organic matter. and not transport to the egg surface.
Klimisch code:
3
Author(s)
Year
Study title
Rodrigues, H.G. Penha-Silva, N.
Ferreira Pereira de Araujo, M. Nishijo, H.
Aversi-Ferreira, T.A.
2011
Effects of Roundup® Pesticide on the Stability of Human Erythrocyte Membranes and Micronuclei Frequency in Bone Marrow Cells of Swiss Mice
The Open Biology Journal, Volume: 4 Pages: 54-59
ASB2012-12010
Abstract*
Pesticides can affect the health of living organisms through different mechanisms such as membrane denaturation. The evaluation of the deleterious effects of chemical agents on biological membranes can be performed through the analysis of the stability of erythrocytes against a concentration gradient of certain chemical agent in physiologic saline solution. This work analyzed the effect of the herbicide Roundup® on the membrane of human erythrocytes in blood samples collected with EDTA or heparin as anticoagulant agent. The results were analyzed through spectrophotometry at 540 nm and light microscopy. There was an agreement between spectrophometric and morphologic analyses. At the concentration limit recommended for agricultural purposes, Roundup® promoted 100 % of hemolysis. The D50Roundup® values obtained for human blood samples collected with EDTA were not significantly different from those obtained for samples collected with heparin. However, the lysis curves presented lower absorbance values at 540 nm in the presence of blood collected with EDTA in relation to that collected with heparin, probably due to haemoglobin precipitation with EDTA. This work also analyzed the effects of three different Roundup® doses (0.148, 0.754 and 1.28 mg/kg) on the micronuclei frequency in bone marrow cells of Swiss mice in relation to a positive control of cyclophosphamide (250 mg/kg). The two highest Roundup® doses showed the same genotoxicity level as the positive control.
* Quoted from article
Klimisch evaluation
1Reliability of study:

Not reliable. Determination of the stability of human erythrocytes: Results are not surprising because surfactants are known to compromise cell membrane integrity. Doses not reflective of physiological concentrations of either glyphosate or surfactant.
Micronucleus test in vivo: Irrelevant route of exposure for surfactant containing formulated products. Results confounded by presence of surfactant toxicity; refer to Heydens et al. (2008, ASB2012-11845)
Comment:
Non-guideline, non-GLP studies
Determination of the stability of human erythrocytes Results attributable to surfactant induced cytotoxicity Micronucleus test in vivo
Major reporting deficiencies (no information on number of cells evaluated, only graphical documentation of results, no information on absolute MN frequencies).
Relevance of study:
Not relevant (Test material containing surfactant is not
appropriately evaluated in either model).
Klimisch code:
3
Author(s)
Year
Study title
Vigfusson, N.V. Vyse, E.R.
1980
The effect of the pesticides Dexon, Captan and Roundup on sister chromatid exchanges in human lymphocytes in vitro. Mutation Research
Volume: 79
Pages: 53-57
TOX9700576, ASB2012-12044
Abstract*
Three pesticides at varying concentration were tested for the induction of SCE [sister chromatid exchanges] in human lymphocytes in vitro. The fungicide, Dexon, sodium (4-(dimethylamino)phenyl)diazene sulfonate, caused the greatest increase in SCE frequency and the response was dose related. The herbicide, Roundup, isopropylamide salt of N-(phosphonomethyl)glycine, had the least effect on SCE requiring the use of much higher concentrations to produce an effect. Limited results were obtained with the fungicide Captan, cis-N-((trichloromethyl)thio)-4-cyclo-hexene-1, 2-dicarboximide, because of toxic levels of the fungicide or solvent used.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Test material was a formulated product containing surfactant. Authors acknowledge cytotoxicity was a confounding factor for data interpretation; since the time of this study, around 1980, surfactant effects on in vitro test systems have been well documented. Only very minor changes in SCE were reported, with a limited data set of two donors and a lack of dose- response. Statistical analysis was not feasible with this very limited data set.

Relevance of study:
Not relevant (Limited data set, internally consistent
findings, no statistics conducted and no dose-response)
Klimisch code:
3

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.

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.

B.6.5.3 Published data on carcinogenicity (released since 2000) Epidemiology studies

A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the specificity of their conclusions regarding pesticides in general, classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, few draw tenable associations with any specific cancer outcome. Publications suggesting glyphosate is associated with any cancer outcome are discussed below.

An essential consideration in both, risk assessment and interpreting the relevance of toxicology data is exposure assessment. An inherent low level of confidence exists for epidemiological studies where tenuous links to exposure exist. Suggested associations between health outcomes and any possible causative agent are merely speculation if exposures are not identifiable. Pivotal to the understanding of glyphosate exposure are data published by Acquavella et al. (2004, ASB2012-11528; 2005, ASB2012-11530), which quantified human systemic glyphosate exposure levels in farmer applicators and their families. The geometric mean systemic dose for farmers applying glyphosate, some of whom applied glyphosate to areas up to 400 acres, was 0.0001 mg/kg/day, approximately 0.03 % of the EU glyphosate acceptable operator exosure Level (AOEL) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). The highest systemic dose, skewed well above the geometric mean, was 0.004 mg/kg/day, which is 1.95 % EU glyphosate AOEL according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191) and 1.3 % of the current EU glyphosate attapcable daily intake (ADI) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). Even lower systemic doses were determined for spouses and children, 0.00004 mg/kg and 0.0008 mg/kg, respectively. Multiple carcinogenicity studies have since been conducted by numerous glyphosate registrants demonstrating NOAELs of at least ten-fold higher than the highest dose tested in the study driving the current EU ADI calculation.

The largest epidemiological study of pesticide exposure and health outcomes in the United States is the Agricultural Health Study (AHS), which included glyphosate. Dozens of publications have resulted from data generated in this study of approximately 57,000 enrolled farmer applicators. Blair et al. (2009, ASB2012-11566) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was not reported to be associated with leukemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos et al. (2005, ASB2012-11605) reported AHS data evaluating glyphosate use and multiple cancer endpoints; no association was noted for glyphosate with all cancers, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic

cancers, non-Hodgkin's lymphoma (NHL) and leukemia. In an earlier publication based on another data set, however, De Roos et al., (2003, ASB2012-11606) reported an association between NHL and glyphosate use. McDuffie et al. (2001, ASB2011-364) reported a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. Blair et al. (2009, ASB2012-11566) did not report an association between glyphosate use and NHL in the AHS data, but a "possible association" between glyphosate use and multiple myeloma was mentioned. The AHS publication reporting this refers to a "suggested association" between glyphosate use and multiple myeloma (De Roos et al., 2005, ASB2012- 11605), yet it did not demonstrate significant increase in relative risk for multiple myeloma. Both De Roos papers will be discussed in more detail below. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009, ASB2012-11623) specifically references De Roos (2005 ASB2012-11605) as providing no observed incidents of cancers of any type being associated with glyphosate.

Lee et al. (2005, ASB2012-11882) reported a glyphosate association with gliomas, with the odds ratio differing between self-respondents (OR = 0.4) and proxy respondents (OR = 3.1). The authors expressed concern that higher positive associations observed for proxy respondents with glyphosate and several other pesticides, and suggested perhaps more accurate reporting of proxies for cases, and underreporting by proxies for controls; proxy respondents were spouses in 62 % of cases versus 45 % of controls, lending to lower reported incidents in the control group.

Monge et al (2007, ASB2012-11909) investigated associations between parental pesticide exposures and childhood Leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with "other pesticides", including paraquat, chlorothalanil and "others". No association was noted for paternal exposures, but elevated leukaemias were associated with maternal exposures to "other pesticides" during pregnancy. Similarly, glyphosate is captured under "other pesticides" being associated with NHL by Fritschi et al. (2005, ASB2012-11624) and therefore should not be interpreted as an association with glyphosate.

Some further epidemiologic studies are focused on an association between pesticide exposure and Non-Hodgkin`s Lymphoma (NHL). Hardell and Eriksson (1999, ASB2012-11838) investigated in a case-control study the incidence of NHL in relation to pesticide exposure in Sweden. 404 cases and 741 controls have been included. The authors discussed an increased risk for NHL especially for phenoxyacetic acids. Glyphosate was included in the uni-variate and multi-variate analyses. However, only 7 of 1145 subjects in the study gave exposure histories to this agent. The authors reported a moderately elevated odds ratio (OR) of 2.3 for Glyphosate. This OR was not statistically significant oand was based on only 4 "exposed" cases and 3 "exposed" controls. The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43 % of the pesticide use information, and the dificulty in the controlling for potential confounding factors given the small number of exposed subjects.

A further study was submitted by Hardell et al. (2002, ASB2012-11839). This study pools data from the above mentioned publication by Hardell and Eriksson (1999, ASB2012-11838) with data from a previously submitted publication from Nordström, Hardell at al. (1998, TOX1999-687).

The authors found increased risks in an uni-variate analysis for subjects exposed to herbicides, insecticides, fungicides and impregnating agents. Among herbicides, significant associations were found for glyphosate and MCPA. However, in multi-variate analyses the only significantly increased risk was for a heterogenous category of other herbicides than above, not for glyphosate. No information is given about exposure duration, exposure concentration, as well as medical history,

lifestyle factors (e.g. smoker, use of prescribed drugs etc.). In all, the above mentioned limitations of the publication from Hardell and Eriksson (1999, ASB2012-11838) are also the limitions of the publication from Hardell et al. (2002, ASB2012-11839).

Fritschi et al. (2005, ASB2012-11624) submitted a case-control study with 694 cases of NHL and 694 controls in Australia. Substantial exposure to any pesticide was associated with an increase of NHL. However, no association between NHL and glyphosate can be made on basis of this study. No information was given about exposure duration, used glyphosate products, exposure duration and application rates. Therefore, the documentation is considered to be insufficient for assessment.

Eriksson et al. (2008, ASB2012-11614) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02. Results and reliability of the study are discussed below.

Alavanja et al. (2013, ASB2014-9174) reviewed studies on cancer burden among pesticide applicators and others due to pesticide exposure. In this article the epidemiological, molecular biology, and toxicological evidence emerging from recent literature assessing the link between specific pesticides and several cancers including prostate cancer, non-Hodgkin lymphoma, leukemia, multiple myeloma, an breast cancer were integrated. Glyphosate was reported to be the most commonly used in conventional pesticide active ingedient worldwide. The only association between the use of glyphosate and cancer burden described in this review was the result of Eriksson et al. (2008, ASB2012-11614) which was described above.

The following epidemiology publications report a lack of association between glyphosate and specific cancer types.

• Alavanja et al. (2003, ASB2012-11535) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.

• Multigner et al, (2008, ASB2012-11917) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong et al. (2009, ASB2012-11922).

• The lack of association between glyphosate use and prostate cancer was also supported recently in an epidemiology study of Farmers in British Columbia, Canada by Band et al. (2011, ASB2012-11555).

• Lee et al. (2004, ASB2012-11883) reported a lack of association between glyphosate use and stomach and esophageal adenocarcinomas.

• Carreon et al. (2005, ASB2012-11585) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.

• Engel et al. (2005, ASB2012-11613) reported AHS data on breast cancer incidence among farmers' wives, with no association between breast cancer and glyphosate.

• Flower et al (2004, ASB2012-11620) reported AHS data on parental use of specific pesticidesa and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.

• Andreotti et al. (2009, ASB2012-11544) reported AHS data where glyphosate was not associated with pancreatic cancer.

• Landgren et al. (2009, ASB2012-11875) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.

• Karunanayake et al. (2011, ASB2012-11865) reported a lack of association between glyphosate and Hodgkin's lymphoma.

• Pahwa et al. (2011, ASB2012-11987) reported a lack of association between glyphosate and

multiple myeloma.
• Schinasi and Leon (2014, ASB2014-4819) published the results of epidemiologic research on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides. Phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL. However, no association between NHL an glyphosate was reported.
• Kachuri et al. (2013, ASB2014-8030) investigated the association between lifetime use of multiple pesticides and multiple myeloma in Canadian men. Excess risks of multiple myeloma were observed among men reported using at least one carbamate pesticide, one phenoxy herbicide and ≥ organochlorines. However, no excess risk was observed for glyphosate.
• Cocco et al. (2014, ASB2014-7523) investigated the role of occupational exposure to agrochemicals in the aetiology of lymphoma overall, B cell lymphoma and its most prevalent subtypes. No increased CLL risk in relation to glyphosate was evidenced.
• Alavanja and Bonner (2012, ASB2014-9173) reviewed studies on occupational pesticide exposure and cancer risk. Twenty one pesticides identified subsequent to the last IARC review showed significant exposure-response associations in studies of specific cancers. No significant association was observed for glyphosate.
• El-Zaemy and Heyworth (2012, ASB2014-9473) reported a case control study on the association between pesticide spray drift from agricultural pesticide application areas and breast cancer in Western Australia. The findings support the hypothesis that woman who ever noticed spray drift or who first noticed spray drift at a younger age had increased risk of breast cancer. However, it was not possible to examine whether the observed associations are the result of a particular class of pesticides.
• Pahwa et al. (2011, ASB2014-9625) investigated the putative associaton of specific pesticides with soft-tissue sarcoma (STS). A Canadian population-based case-control study conducted in six provinces was used on this analysis. The incidence of STS was associated with insecticides aldrin and diazinon after adjustment for other independent predictors. However, no statistically significant association between STS and exposure to glyphosate or other herbicides was observed.
• Koutros et al. (2011, ASB2014-9594) studied associations between pesticide and prostate cancer. No statistically significant positive association between pesticides and prostate cancer were observed. There was suggestive evidence on an increased risk (OR>1.0) with an increasing number of days of use of petroleum oil/petroleum distillate used as herbicide, terbufos, fonofos, phorate and methyl bromide. However, no increased risk (OR>1.0) was observed for glyphosate.
In summarizing AHS publications, Weichenthal et al. (2010, ASB2012-12048) noted that increased rates in the following cancers were not associated with glyphosate use; overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, esophagus cancer and thyroid cancer.
Mink et al. (2012, ASB2014-9617) submitted a comprehensive review of epidemiologic studies of glyphosate and cancer. To examine potential cancer risks in humans they reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. They also reviewed relevant methodological and biomonitoring studies of glyphosate. The review found non consistent pattern of positive assciations indicating a causal relationship between total cancer (in adults or in children) or any site- specific cancer and exposure to glyphosate.
Animal studies
Just recently (i.e., after submission of the GTF dossier), a two-year study in rats was published (Séralini et al., 2012, ASB2012-15514). Its main objective was to show a possible impact of long-term

feeding of genetically modified (and glyphosate treated) maize to rats but three of the test groups were administered a commercially available formulation (Roundup
GT Plus, apparently authorised at least in Belgium) containing 450 g glyphosate/L at different concentrations ranging from 0.1 ppb (50 ng glpyphosate/L) to 0.5 % (2.25 g glyphosate/L) in drinking water. In these groups, the authors reported alterations in some clinical chemistry (blood and urine) parameters and hormone levels and histopathological lesions concerning the liver and the gastrointestinal tract but also a higher incidence of mammary tumours in females resulting in a shorter lifespan. This study was heavily discussed in the scientific community as well as in the general public where it gained remarkable attention due to massive promotion although it was clearly flawed by many serious deficiencies. A major point of concern was the small group size of only 10 males and 10 females per dose, i.e., the test design was that one of a subchronic study. Such a small number of animals is not appropriate for a long-term study because age-related changes cannot be adequaetely taken into account. Following the receipt of contributions from many MS authorities, a comprehensive critical assessment was published by EFSA (2012, ASB2012-15513, EFSA Journal, 2012, 10 (11), 2986). The
conclusion was that ”the currently available evidence does not impact on the ongoing re- evaluation of glyphosate…”. This opinion on the Séralini study is agreed with and supported by the RMS.
In reaction to this publication a large number of letters was send to the editor: Barale-Thomas (2012, ASB2013-10998), Berry (2012, ASB2013-10988), Grunewald (2012, ASB2013-
11001), Hammond et al. (2012, ASB2013-10995), Heinemann (2012, ASB2013-10987), Langridge (2012, ASB2013-10986), Ollivier (2012, ASB2013-11000), Panchin (2013, ASB2013-10937), Pilu (2012, ASB2013-10992), Schorsch (2013, ASB2013-10996), Tester (2012, ASB2013-10994), Tien & Huy (2012, ASB2013-10984), Trewavas (2012, ASB2013- 10989), Tribe (2012, ASB2013-10997), Wager (2012, ASB2013-10993), de Souza (2012, ASB2013-10999).
Chruszielska et al. (2000, ASB2013-9829) published a combined long term toxicity and carcinogenicity study in rats. The active substance glyphosate was used in the study and the study was performed on basis of OECD guideline 453. The number of animals per dose group and sex (85 animals) was even higher than required in guideline 453. Therefore, the study is considered to be relevant. No carcinogenic effects have been registered in the study.
George et al., (2010, ASB2012-11829) used a 2-stage cancer model in mice to evaluate a glyphosate formulation for tumor promotion. A known tumor promoter, 12-o-tetradecanoyl- phorbol-13-acetate (TPA) was used as a positive control and for comparison with glyphosate effects after exposure to a tumor initiator, 7, 12-dimethylbenz[a]anthracene. Proteomics were later applied to extrapolate a basis for glyphosate formulation tumor promotion. The results are considered by the authors to indicate a tumor promoting potential of glyphosate. However, the formulation Roundup was used in the study and not the active substance glyphosate. Furthermore, the up- and down-regulation of protein expression is not sufficient to prove a carcinogenic effect.
Mechanistic studies
the authors emphasize that glyphosate was not associated with prostate cancer risk in the main effect studies (Agricultural Health Study AHS).
Barry et al. (2011, ASB2014-9247) evaluated interactions between 39 pesticides and 394 tag single-nucleotide polymorphisms (SNPs) for 31 BER genes among 776 prostate cancer cases and 1444 male controls in a nested case-control study of Agricultural Healt Study (AHS) pesticide applicators. The authors used likelihood ratio tests from logistic regression models to determine p-values for interactions between three-level pesticide variables and SNP (assuming a dominant model) and the false discovery rate multiple comparison adjustment approach. The authors observed notable

interactions between several pesticides and BER gene variants with respect to prostate cancer. However, only fonofos x NEIL3 rs 1983132 showed an interaction fitting an expected biological pattern that remained significant after adjustment for multiple comparisons. No significant association was observed for glyphosate.

The following studies are described more detailed:

Author(s)

Year

Study title

Hardell, L. Eriksson, M.

1999

A Case-Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides.

Cancer, Volume: 85, Number: 6, Pages: 1353-1360 ASB2012-11838

Abstract*

Background. The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL.

Methods. A population-based case–control study in northern and middle Sweden encompassing 442 cases and twice as many controls was performed. Exposure data were ascertained by comprehensive questionnaires, and the questionnaires were supplemented by telephone interviews. In total, 404 cases and 741 controls answered the questionnaire. Uni- variate and multi-variate analyses were performed with the SAS statistical data program.

Results. Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0 –2.5) and fungicides (OR, 3.7; 95% CI, 1.1–13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9 –2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0–6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL.

ConclusionS. Exposure to herbicides in total, including phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Study prone to selection and recall bias. No evidence of relevant glyphosate exposures. Medical history was assessed, but not reported.

Relevance of study:

Not relevant (Exposure to multiple chemicals and though glyphosate exposure data were convincing (7/1145 subjects) and statistically non-significant positive associations reported.)

Klimisch code:

3
Additional comments:
Hardell and Eriksson (1999, ASB2012-11838) conducted a case control study to look for associations between reported pesticide use and non-Hodgkin's lymphoma (NHL). The study included 404 NHL cases and 741 controls. The measure of association in this study was the odds ratio (OR), a statistic that estimates of the ratio of disease rates (in this case NHL rates) for exposed and unexposed populations.
The authors reported statistically significant associations for NHL with: reported use of any herbicide (OR = 1.6), reported use of any fungicide (OR = 3.7), and reported use of 4-chloro- 2-methylphenoxyacetic acid (OR = 2.7). The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43 % of the pesticide use information, and the difficulty in controlling for potential confounding factors, given the small number of exposed subjects.
The authors also reported a moderately elevated OR of 2.3 for glyphosate. This OR was not statistically significant and was based on only four "exposed" cases and three "exposed" controls. This study has several important limitations: no exposure assessment, dependence on next-of- kin's recollections of study subjects' pesticide use for approximately 43 % of study subjects, potential recall bias, and the very small number of subjects who reported using specific herbicides. The latter leads to findings that are statistically imprecise. Due to the potential for bias and the statistical imprecision, the results of this study are not convincing.
Author(s)
Year
Study title
Hardell, L. Eriksson, M. Nordstrom, M.
2002
Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies.
Leukemia & Lymphoma Volume: 43
Number: 5
Pages: 1043-1049
ASB2012-11839
Abstract*
Increased risk for non-Hodgkin's lymphoma (NHL) following exposure to certain pesticides has previously been reported. To further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL a pooled analysis was performed on two case-control studies, one on NHL and another on hairy cell leukemia (HCL), a rare subtype of NHL. The studies were population based with cases identified from cancer registry and controls from population registry. Data assessment was ascertained by questionnaires supplemented over the telephone by specially trained interviewers. The pooled analysis of NHL and HCL was based on 515 cases and 1141 controls. Increased risks in uni-variate analysis were found for subjects exposed to herbicides (OR 1.75, CI 95% 1.26-2.42), insecticides (OR 1.43, CI 95%
1.08-1.87), fungicides (OR 3.11, CI 95% 1.56-6.27) and impregnating agents (OR 1.48, CI 95% 1.11-1.96). Among herbicides, significant associations were found for glyphosate (OR 3.04, CI 95% 1.08-8.52) and 4-chloro-2-methyl phenoxyacetic acid (MCPA) (OR 2.62, CI 95% 1.40-4.88). For several categories of pesticides the highest risk was found for exposure during the latest decades before

diagnosis. However, in multi-variate analyses the only significantly increased risk was for a heterogeneous category of other herbicides than above.
• Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
This publication combines the results of two previous studies by the authors on HNL (Hardell and Eriksson, 1999, ASB2012-11838) and HCL (Nordström,et al., 1998, TOX1999-687). No information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc). Study documentation is insufficient for assessment.
Relevance of study:
Not relevant (Due to reliability of data set drawn from Hardell and Eriksson, 1999, ASB2012-11838)
Klimisch code:
3
Additional comments:
This study pools data from the previously reviewed publication by Hardell and Eriksson (1999, ASB2012-11838) with data from Nordström et al. (1998, TOX1999-687). Therefore the discussion of limitations of Hardell and Eriksson (1999, ASB2012-11838) also applies to Hardell et al. (2002, ASB2012-11839) (see above).
Author(s)
Year
Study title
Fritschi, L. Benke, G. Hughes, A. M. Kricker, A. Turner, J. Vajdic, C. M. Grulich, A. Milliken, S. Kaldor, J. Armstrong, B.
K.
2005
Occupational exposure to pesticides and risk of non-Hodgkin's lymphoma
American Journal of Epidemiology Volume: 162, Pages: 849-857 ASB2012-11624
Abstract*
Pesticide exposure may be a risk factor for non-Hodgkin's lymphoma, but it is not certain which types of pesticides are involved. A population-based case-control study was undertaken in 2000-2001 using detailed methods of assessing occupational pesticide exposure. Cases with incident non-Hodgkin's lymphoma in two Australian states (n = 694) and controls (n = 694) were chosen from Australian electoral rolls. Logistic regression was used to estimate the risks of non-Hodgkin's lymphoma associated with exposure to subgroups of pesticides after adjustment for age, sex, ethnic origin, and residence. Approximately 10 % of cases and controls had incurred pesticide exposure. Substantial exposure to any pesticide was associated with a trebling of the risk of non-Hodgkin's lymphoma (odds ratio = 3.09, 95 % confidence interval: 1.42, 6.70). Subjects with substantial exposure to organochlorines, organophosphates, and "other pesticides" (all other pesticides excluding herbicides) and herbicides other than phenoxy herbicides had similarly increased risks, although the increase was statistically significant only for "other pesticides." None of the exposure metrics (probability, level, frequency, duration, or years of exposure) were associated with non- Hodgkin's lymphoma. Analyses of the major World Health Organization subtypes of non- Hodgkin's lymphoma suggested a

stronger effect for follicular lymphoma. These increases in risk of non-Hodgkin's lymphoma with substantial occupational pesticide exposure are consistent with previous work.
Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
No information about exposure duration, used glyphosate products, exposure duration and application
rates. Documentation is insufficient for assessment.
Relevance of study:
Not relevant (Multiple pesticide exposures. No definitive association between NHL and glyphosate can
be made.)
Klimisch code:
3
Additional comments:
No information about exposure duration, used glyphosate products, exposure duration and application rates. Only multiple pesticide exposures are reported. No association between NHL and glyphosate can be made on basis of this study.
Author(s)
Year
Study title
De Roos, A. J. Zahm, S. H. Cantor, K. P. Weisenburger, D. D.
Holmes, F. F. Burmeister, L. F. Blair, A.
2003
Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occupational and Environmental Medicine Volume: 60, Number: 9, Pages: -E11
ASB2012-11606
Abstract*
Background: An increased rate of non-Hodgkin's lymphoma (NHL) has been repeatedly observed among farmers, but identification of specific exposures that explain this observation has proven difficult.
Methods: During the 1980s, the National Cancer Institute conducted three case-control studies of NHL in the midwestern United States. These pooled data were used to examine pesticide exposures in farming as risk factors for NHL in men. The large sample size (n = 3417) allowed analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides in the model, and adjusting the estimates based on a prespecified variance to make them more stable.
Results: Reported use of several individual pesticides was associated with increased NHL incidence, including organophosphate insecticides coumaphos, diazinon, and fonofos, insecticides chlordane, dieldrin, and copper acetoarsenite, and herbicides atrazine, glyphosate, and sodium chlorate. A subanalysis of these "potentially carcinogenic" pesticides suggested a positive trend of risk with exposure to increasing numbers.
Conclusion: Consideration of multiple exposures is important in accurately estimating specific effects and in evaluating realistic exposure scenarios.
Quoted from article

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc were reported. Specific lymphomas are not identified (NHL captures all types of lymphoma other than Hodgkin's lymphoma). Documentation is insufficient to associate exposures with specific NHL
diseases.
Relevance of study:
Not relevant (No report of identifying various types of lymphoma under the NHL umbrella; no definite association between specific NHL diseases and
glyphosate can be made)
Klimisch code:
3
Additional comments:
No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc) were reported. Specific lymphomas are not identified. The reported hierarchical regression did not find a statistically significant odds ratio for ever use of glyphosate and NHL.
Author(s)
Year
Study title
De Roos, A.J. Blair, A. Rusiecki, J.A. Hoppin, J.A. Svec, M. Dosemeci, M. Sandler, D.P.
Alavanja, M.C.
2005
Cancer Incidence among Glyphosate-Exposed Pesticide Applicators in the Agricultural Health Study
Environmental Health Perspectives
Volume: 113, Number: 1, Pages: 49-54 ASB2012-11605
Abstract*
Glyphosate is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. Although there has been little consistent evidence of genotoxicity or carcinogenicity from in vitro and animal studies, a few epidemiologic reports have indicated potential health effects of glyphosate. We evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrolment (1993–1997). Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which > 97% were men. In this analysis, glyphosate exposure was defined as a) ever personally mixed or applied products containing glyphosate; b) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and c) intensity-weighted cumulative exposure days (years of use
× days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between glyphosate and incidence of all cancers combined and 12 relatively common cancer subtypes. Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes we studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the AHS. Given the widespread

use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects, including less common cancers.
* Quoted from article
Klimisch evaluation
Reliability of study:
Reliable without restrictions
Comment:
Well documented publication. Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.
Relevance of study:
Relevant (Evaluation focussed on glyphosate, although other pesticides were also considered in the data
evaluation)
Klimisch code:
2
Additional comments:
Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.
Response 1 – summary from Letter to the Editor by Farmer et al. (2005, ASB2012- 11616)
Authors provided an incomplete genotoxicity review which was inconsistent with opinions of regulatory agencies and experts around the world, that glyphosate is not genotoxic. An extensive toxicology review of glyphosate was cited by the authors, mentioning a lack of carcinogenicity with glyphosate exposures, yet neglected to cite the extensive genotoxicity review in the same publication by Williams et al. (2000, ASB2012-12053)
Biological plausibility of a cancer effect should be considered in the light of exposure. Acquavella et al (2004, ASB2012-11528) reported the maximum systemic dose to resulting from application of glyphosate to areas as large as 400 acres was 0.004 mg/kg, and the geometric mean systemic dose was 0.0001 mg/kg in farmers. If these glyphosate applications and exposures continued daily over the course of a lifetime, the systemic dose would be at least 250,000-fold lower than the cancer no-effect level in rodents.
The authors were requested to further evaluate their models for confounding and selection bias in the multiple myeloma analysis.
Response 2 – summary from Lash (2007, ASB2012-11877)
Table 2 of De Roos et al. (2005, ASB2012-11605) noted 32 cases of multiple myeloma associated with “ever-use” of glyphosate and when compared with “never-use” (adjusted for age only) yielded a rate ratio of 1.1 (95 % CI 0.5-2.4). However, when the data set was adjusted for age, demographic and lifestyle factors and other pesticide use, the rate ratio increased to 2.6 (95 % CI 0.7-9.4).
The adjusted estimate merits careful inspection and can only be undertaken with access to the primary data, not made available by the authors.
Bias analysis was conducted, accounting for confounding and exposure misclassification. Adjustment for confounders in De Roos et al. (2005, ASB2012-11605), which resulted in limiting the data set by 25 % because of missing data on the adjustment variables, likely introduced selection bias and produced the a rate ratio of 2.6 that was substantially biased.
Author(s)
Year

Study title
Eriksson, M. Hardell, L. Carlberg, M.
Akerman, M.
2008
Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis
International Journal of Cancer Volume: 123, Pages: 1657-1663 ASB2012-11614
Abstract*
We report a population based case-control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18-74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91 %) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18-2.51. Regarding phenoxyacetic acids highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27-6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10-3.71 and with
>10 years latency period OR 2.26, 95% CI 1.16-4.40. Insecticides overall gave OR 1.28, 95% CI 0.96-1.72 and impregnating agents OR 1.57, 95% CI 1.07-2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between
exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.
Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Multiple avenues for bias were introduced in study design, execution and data processing. No information about exposure duration, used glyphosate products and application rates. Other factors (i.e. smoking habits, medication etc.) were assessed but not included in the
evaluation.
Relevance of study:
Relevant with reservation
Klimisch code:
3
Additional comments:
The authors (Eriksson et al. 2008, ASB2012-11614) conducted a population-based case- control study of exposure to a variety of pesticides and non-Hodgkin lymphoma (NHL), including separate analyses of histopathological categories of NHL. Study subjects were males and females, ages 18-74, living in Sweden between December 1, 1999 and April 30, 2002. The final study group included 910 cases and 1016 controls. Exposure, ascertained via an interviewer-administered questionnaire, focused on pesticide and other chemical agents, and included a total work history (although a job-exposure matrix was not used). For pesticide exposure, information on number of years, number of days per year, and approximate length of exposure per day was also obtained. A minimum of one full day of exposure was required for categorization as “exposed.”
The authors reported a statistically significant positive association between “herbicide exposure” and NHL (OR = 1.72; 95% CI: 1.18-2.51). Glyphosate exposure was reported by 29 cases and 18 controls,

and the corresponding odds ratio (OR) was 2.02 (95% CI:
1.10-3.71). The ORs for glyphosate exposure of <10 days and >10 days were 1.69 (95% CI:
0.70-4.07) and 2.36 (1.04-5.37), respectively. The ORs for glyphosate were 1.11 (95% CI:
0.24-5.08) and 2.26 (95% CI: 1.16-4.40) for "latency" periods of 1-10 years and >10 years, respectively. In analyses of glyphosate and type of NHL, statistically significant positive associations were observed for small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) (OR = 3.35; 95% CI: 1.42-7.89) and for "unspecified NHL" (OR = 5.63; 95% CI:
1.44-22.0). Odds ratios for the other types (total B-cell lymphomas, grade I-III follicular lymphoma, diffuse large B-cell lymphoma, other specified B-cell lymphoma, unspecified B- cell lymphoma, and T-cell lymphomas) were above 1.0, but were not statistically significant (i.e., the 95% confidence intervals were relatively wide and included the null value of 1.0).
The authors concluded, "Glyphosate was associated with a statistically significant increased OR for lymphoma in our study, and the result was strengthened by a tendency to dose- response effect…" (p. 1662). The authors suggested that their findings are consistent with results of a previous case-control study (Hardell and Eriksson 1999, ASB2012-11838) and pooled analysis (Hardell et al. 2002, ASB2012-11839) that they conducted. In the case- control study, an OR of 2.3 (95% CI: 0.4-13.0), based on 4 exposed cases and 3 exposed controls, was reported for glyphosate and NHL. In the pooled analysis of two case-control studies, which included data from Hardell and Eriksson (1999, ASB2012-11838), an OR of
3.04 (95% CI: 1.08- 8.52) was reported, based on 8 exposed cases and 8 exposed controls. The authors also cited three studies (De Roos et al. 2003, ASB2012-11606; McDuffie et al. 2001; ASB2011-364, De Roos et al. 2005, ASB2012-11605) by other groups as being consistent with their results in that they "also associate glyphosate with different B-cell malignancies such as lymphomas and myelomas." It should be noted, however, that the relative risk (RR) reported by De Roos et al. (2005, ASB2012-11605) for the highest versus lowest category of cumulative exposure days of glyphosate and NHL in the prospective Agricultural Health Study was 0.9.

Interpretation Issues

Identification of Cases and Potential Referral Bias. It is noteworthy that the cases in the current analysis were identified from some of the same hospitals as the authors' prior publication; thus, referral bias may have been an issue. In particular, the researchers approached the patients after diagnosis if the physicians deemed it appropriate. Therefore, if the physicians were concerned that their patient's NHL was associated with agricultural exposures, they may have suggested participation in the study.
Participation Rates and Potential Selection Bias. The authors report a participation rate of 91% and 92% for cases and controls, respectively; however, these figures are based on completed questionnaires out of those who had previously said they would participate in the study. The number of eligible patients (i.e., prior to physician approval to "approach") was not reported, so the computation of an exact participation rate is difficult. Based on information provided in the paper, participation among cases is estimated to be about 80%. Nonparticipation is a concern for several reasons. First, in a case-control study, an odds ratio will be an accurate representation of the exposure-disease association when the cases are representative of all cases and the controls are representative of the exposure experience of the population that gave rise to the cases. If the final study sample is not representative of this "target population" then measures of effect (e.g., the odds ratio) may not be valid. In addition, one must be concerned about selection bias. Selection bias occurs in a case-control study when the exposure distribution for cases and controls differ for those

who participate in the study compared to those who are eligible but do not participate in the study. It is not possible to determine whether there is selection bias without information about nonparticipants.

Strengths and Limitations of Using Living Cases Only versus All Cases (Living + Dead).

The authors noted that 88 potential cases died before they could be interviewed and were therefore excluded from the study. It is also stated in the Discussion that restricting the study to living cases and controls was an "advantage" of the study, as interviewing cases and controls directly compared to interviewing next-of-kin was preferable. While it is generally true that this would be an advantage, the following statement by the authors, therefore, is not accurate, "The study covered all new cases of NHL during a specified time" (p. 1660). The study did not include all new cases; it included only those cases who survived until the time of the interview. Thus, while there may have been an advantage to restricting the study to living cases, there was a trade-off in that the study population did not represent all cases, specifically those cases with more aggressive disease. This disadvantage was not discussed by the authors, nor was the potential bias that could have resulted from excluding many eligible cases.

Exposure Measurement and Information Bias. Exposure was ascertained via a questionnaire oriented towards pesticide and other chemical agents. In addition, interviewers collected information by telephone if "important" data were lacking, incomplete, or unclear. It is unknown what is meant by "important," and the proportion of cases and controls who received phone calls was not reported. Thus, information bias may be a concern. Even though interviewers were blinded to case and/or control status, they may have been able to determine this information during the course of the interview. Furthermore, recall bias may be an issue because exposure information was based on participant response and cases and controls may recall and/or report past pesticide exposures differently. No exposure validation techniques were implemented, nor did an industrial hygienist (or any other type of personnel trained in assessing occupational exposures) independently validate/estimate the frequency and/or intensity of exposure. The authors assumed that "some misclassification regarding quantity of exposure has probably occurred, but such misclassification would most probably be nondependent of case/control status, and therefore only weaken any true risks" (p. 1660). They do not provide any explanation as to why they believe that exposure misclassification would be "most probably" nondifferential. If NHL cases believe that pesticides may be related to their disease, then it is certainly possible that they may recall and/or report pesticide exposure differently than NHL-free controls, which could result in odds ratios that are inflated as a result of bias.

Interpretation of "dose-response" analyses. The referent group in the statistical analyses consisted of participants who were unexposed to all pesticides. The dose-response analyses were based on a dichotomy of the median number of days exposed to a particular agent. It is difficult to analyze "dose-response" when only two exposure categories are considered. Furthermore, the dose-response analyses were based on median values of exposure but heterogeneity of cut-points is evident across agents. For example, glyphosate was analyzed as < 10 days and > 10 days, whereas, "other" herbicides were analyzed as < 32 days and > 32 days. Although analytical cut-points were data driven, interpretation across the wide variety of exposures is complicated by the variability in exposure cut-points. In addition, even though the OR for the higher category of exposure days was greater than the OR for the lower category, the two 95% confidence intervals were wide and overlapped considerably (0.70- 4.07 and 1.04-5.37). Thus, it is not clear whether the two point estimates reported (1.69 and 2.36) are significantly different from each other. Finally, this result cited in the "dose-response" analyses may have been

confounded by exposure to other herbicides. In Table II (Eriksson et al. 2008, ASB2012- 11614), the authors observed elevated associations for other herbicides, including MCPA, 2,4,5-T and/or 2,4-D. The correlation between exposure to glyphosate and other herbicides was not provided nor were analyses of glyphosate-exposed individuals after accounting for the collinear relation between this agent and other agents. The odds ratio for "ever" exposure to glyphosate was attenuated after additional adjustment for other pesticides (Table VII, Eriksson et al. 2008, ASB2012-11614), but multi-variate -adjusted estimates for the "dose- response" odds ratios were not reported.

Unusual Pattern of Positive Associations. The authors conducted multiple comparisons, and one would expect a certain proportion of their findings to be statistically significant (whether in the positive or inverse direction) simply as a result of chance. It is somewhat surprising, therefore, that the vast majority of the ORs presented in this manuscript are greater than 1.0, regardless of the statistical significance. The authors do note that for some of the analyses (e.g., latency), only chemicals for which ORs were greater than 1.5 and for which there were at least 10 exposed cases, or for which there was a statistically significant OR were evaluated. On the other hand, dose-response was evaluated based on the number of exposed subjects and not on the strength or significance of the findings. The authors do not address this directly, but do state in their Discussion, "…several pesticides are chemically related and may exert their effects on humans through a similar mechanism of action, which may explain the wide range
of pesticides that have been related to NHL over time in different countries and with different exposure conditions" (p. 1661). On the other hand, this pattern of positive findings could be a result of bias, including recall bias (or other information bias), selection bias, uncontrolled confounding, or a combination of these and other factors.

Interpretation of Eriksson et al. (2008, ASB2012-11614) in Context of Other Studies. Despite the statement by the authors that, "Recent findings from other groups also associate glyphosate with different B-cell malignancies such as lymphomas and myeloma" (p. 1662), most multi-variate analyses of glyphosate and NHL do not report statistically significant associations (De Roos et al. 2005, ASB2012-11605; De Roos et al. 2003; ASB2012-11606, Hardell and Eriksson 1999, ASB2012-11838; Hardell et al. 2002; ASB2012-11839, Lee et al. 2004; ASB2012-11883, McDuffie et al. 2001; ASB2011-364, Nordström et al. 1998, TOX1999-687) (Tables B.6.5-62 and B.6.5-63). It is notable that Hardell et al. (2002, ASB2012-11839) reported a significant positive association between glyphosate association and NHL, but the multi-variate -adjusted odds ratio was attenuated and not statistically significant. Similar findings were reported by Eriksson et al. (2008, ASB2012-11614). Specifically, the association reported by the authors in the abstract (OR = 2.02; 95% CI: 1.10- 3.71) was adjusted for age, sex and year of diagnosis or enrollment. When other pesticides were added to that model (i.e., agents with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subjects), the adjusted odds ratio was 1.51 (95% CI: 0.77-2.94). Thus, the authors' final statement, "Furthermore, our earlier indication of an association between glyphosate and NHL has been considerably strengthened" is questionable. Their previous findings showed a non-significant association after multi-variate adjustment (OR = 1.85; 95% CI: 0.55-6.20). The 2008 study similarly reported a statistically non-significant association between glyphosate and NHL after multi-variate adjustment (OR = 1.51; 95% CI: 0.77-2.94). The results reported for analyses of duration of exposure and latency of exposure did not adjust for other pesticides, and one would expect that those ORs would also be attenuated.

Summary of Findings: Cohort and Case-Control Studies of Exposure to Glyphosate and Non-Hodgkin Lymphoma

Table B.6.5-62: Cohort Studies

Author Year
Description
No. of Exposed Cases
Type of Relative Risk Estimate
Relative Risk Estimate
95%
Confidence Limits
Variables Included in Statistical Model
De Roos et al.
2005 (ASB2012
-11605)
57-2,678 vs.
1-20
Cumulative Exposure Daysa
17
RR
0.9
0.5-1.6
Age at enrollment, education, pack-years of cigarette smoking, alcohol consumption in the past year, family history of cancer in first-degree
relatives, and state of residence
337.2-18,241
22
RR
0.8
0.5-1.4
Also adjusted for other
vs. 0.1-79.5
pesticides
Intensity-
Weighted
Exposure
Daysb
a Years of use x days per year; categorized by tertiles
b Years of use x days/year x estimated intensity level; categorized by tertiles
Table B.6.5-63: Case Control Studies
Author Year
Exposure Evaluated
Subgroup Description
No. of Expose d Cases
No. of Expose d Control
s
OR
95% CI
Variables Included in Statistical Model
De Roos et al.

2003 (ASB201 2-11606)
Ever exposure to specific pesticide; men only (all 47 pesticides were regressed simultaneously)
Glyphosate (Logistic Regression)
Glyphosate (Hierarchical Regression)
36
36
61
61
2.1
1.6
1.1-4.0
0.9-2.8
Age, study site and other pesticides
Second-level model incorporated what was known about each true effect parameter prior to seeing the study data
Hardell and Eriksson 1999 (ASB201 2-11838)
Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)
Glyphosate (conditional logistic regression; uni-variate analysis)
Glyphosate (conditional logistic regression;
multi-variate analysis)
4
4
3
3
2.3
5.8
0.4-13
0.6-54
Age and country (matching factors)
Multi-variate variables not listed by authors
Hardell et al.
2002 (ASB201 2-11839)
Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)
Glyphosate (conditional logistic regression; uni-variate analysis)
Glyphosate (conditional logistic regression;
multi-variate analysis)
8
8
8
8
3.04
1.85
1.08-8.52
0.55-6.20

Age and county (matching factors); study, study area (county), and vital status
Multi-variate variables not listed by authors
Lee et al. 2004 (ASB201 2-11883)
Exposure to individual pesticides
Glyphosate use, Non- asthmatics
Glyphosate
use, Asthmatics
53
6
91
12
1.4
1.2
0.98-2.1
0.4-3.3
Age, state, vital status
McDuff- ie et al. 2001 (ASB201 1-364)
Exposure to individual active chemicals
Glyphosate (Round-Up)
Glyphosate (Round-Up)
51
NR
133
NR
1.26
1.20
0.87-1.80
0.83-1.74
Strata for age and province of residence
Plus statistically significant
medical variables
Author Year
Exposure Evaluated
Subgroup Description
No. of Expose d Cases
No. of Expose d Control
s
OR
95% CI
Variables Included in Statistical Model
Nordst- röm et al.
1998 (TOX199 9-687)
Exposure to specific herbicides, insecticides, and fungicides
Glyphosate
4
5

3.1
0.8-12
Age and country (matching factors)
Eriksson et al.
2008 (ASB201 2-11614)
Exposure to specific herbicides regardless if they also had been exposed to phenoxyacetic acids or not
Glyphosate
29
29
18
18
2.02
1.51
1.10-3.71
0.77-2.94
Age, sex, and year of diagnosis or enrollment
Age, sex, and year of diagnosis or enrollment and pesticides with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subject
Exposure to herbicide stratified by median number of days among exposed controls
Glyphosate ≤ 10 days
Glyphosate
>10 days
12
19
9
9
1.69
2.36
0.70-4.07
1.04-5.37
Age, sex, and year of diagnosis or enrollment
Exposure to Glyphosate:
NR
NR
1.87
0.998-
Age, sex, and specific herbicides
B-Cell
3.51
year of diagnosis according to

lymphomas
or enrollment
different
lymphoma entities
Lymphocytic
NR
NR
3.35
1.42-7.89
lymphoma/B-
CLL
Follicular
NR
NR
1.89
0.62-5.79
grade I-III
Diffuse large
NR
NR
1.22
0.44-3.35
B-cell
Lymphoma
Other
NR
NR
1.63
0.53-4.96
specified
B-cell
lymphoma
Unspecified
NR
NR
1.47
0.33-6.61
B-cell
Lymphoma
T-cell
NR
NR
2.29
0.51-10.4
lymphomas
Unspecified

NR
NR
5.63
1.44-22.0
NHL
Author(s)
Year
Study title
George, J. Prasad, S. Mahmood, Z.
Shukla, Y.
2010
Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach
Journal of Proteomics Volume: 73, Pages: 951-964 ASB2012-11829
Abstract*
Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12- dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF-
1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu–Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth- inhibition, anti-oxidant responses, etc. The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu–Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.
* Quoted from article
Klimisch evaluation
Reliability of study:
Reliable with restrictions
Comment:
Non-guideline mechanistic study. Scientifically acceptable study with deficiencies (controls with glyphosate alone, and co-formulants were not included)
Relevance of study:
Relevant with restrictions (Glyphosate formulation not
glyphosate alone was tested.)
Klimisch code:
2
Additional comments:
The authors use glyphosate as a synonym for what is really a glyphosate based formulated product. Doses in this study are not representative of human exposures to glyphosate or glyphosate based

formulations. Mice in the tumor promoting group VIII received topical applications of concentrated glyphosate formulated product three times per week for over thirty weeks without washing after an initial treatment with the potent tumor initiator DMBA. Glyphosate had been shown to have very low dermal absorption, even in formulated products, and since is non-volatile, would likely accumulate on mouse skin. Surfactants are typically irritating and non-volatile. Given the irritation potential of the unwashed exposed mouse skin over the course of thirty or more weeks, tumor promotion may be a physical response to substantial localized dermal irritation. Epidemiological studies reported above note no association with glyphosate and either skin or lip cancers.

Label directions outline appropriate personal protective equipment such as gloves and long sleeves. Furthermore, any dermal exposure of concentrated product to human skin would prove irritating and prompt handlers to wash off soon after dermal exposure.

Human in vitro dermal absorption studies reported for a range of glyphosate based formulations containing different surfactant systems all demonstrate extremely low dermal absorption of glyphosate active ingredient for concentrated products, of less than 0.2 %. Test material recovery in each of the four reported dermal absorption studies was very good, close to 100 %. Most of the glyphosate was removed during skin surface washing at either eight or twenty four hours of in vitro human skin exposure. This also suggests significant potential for accumulation of glyphosate on the surface of the mice skin in George et al. (2010, ASB2012- 11829).

The up-regulation / down-regulation of protein expression reported after a single dermal dose of a glyphosate formulated product (proteomics experiment, group II), while interesting, does not demonstrate any toxicological endpoint. Rather, perturbations may well represent normal homeostatic fluctuations and be a natural response to insult.

Author(s)

Year

Study title

Seralini, G.-E. Clair, E. Mesnage, R. Gress, S. Defarge, N. Malatesta, M. Hennequin, D. Spiroux de Vendomois, J.

2012

Long term toxicity of a Roundup herbicide and a Roundup- tolerant genetically modified maize. Food and Chemical Toxicology 50, 4221-4231

ASB2012-15514

Abstract*

The health effects of a Roundup-tolerant genetically modified maize (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water), were studied 2 years in rats. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls, the pituitary was the second most disabled organ; the sex hormonal balance was modified by GMO and Roundup treatments. In treated males, liver congestions and necrosis were 2.5–5.5 times higher. This pathology was confirmed by optic and transmission electron microscopy. Marked and severe kidney nephropathies were also generally 1.3–2.3 greater. Males presented 4 times more large palpable tumors than controls which occurred up to 600 days earlier. Biochemistry data confirmed very significant kidney chronic deficiencies; for all treatments and both sexes, 76% of the altered parameters were kidney related. These results can be explained by the non linear endocrine-disrupting effects of Roundup, but also by the overexpression of the transgene in the GMO and its metabolic consequences.

* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
The study was performed to investigate the long term toxicity and carcinogenicity. However the study design does not agree with the OECD guidelines on long term toxicity and carcinogenicity.
Relevance of study:
Relevant with restrictions (Glyphosate formulation not
glyphosate alone was tested.)
Klimisch code:
3
Comments:
Seralini et al. (2012, ASB2012-15514) submitted a report of long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. The health effects have been studied 2 years in rats. Six groups of rats were fed with 11, 22 and 22 % of genetically modified NK603 maize either treated or not with Roundup. Three further groups of rats were fed with control diet and had access to water supplemented with 50 ng/L, 400 mg/L and
2.25 g/L of the commercial product Roundup (GT Plus, 450 g/L of glyphosate). The pure active substance glyphosate was not tested in this study.
The study is not considered reliable because of several important limitations. According to the authors the studies have been performed to investigate the long term toxicity and carcinogenicity. However, the number of animals per dose and sex was only 10 and also the further study design does not agree with the OECD guidelines on long term toxicity and carcinogenicity. The spontaneous incidence of mammary tumors in the used Sprague Dawley rats is much higher than in most other rat strains. Therefore, a higher number of animals would be necessary for the differentiation between treatment related carcinogenicity and accidental aberrations. Also for the assessment of mortality and further described toxic effects a higher number of animals would be needed.
The presented results in the publication are incomplete and therefore, an evaluation of the presented results was complicated.
The study was extensively discussed and criticized in the public. In an additional paper Seralini et al. (2013, ASB2013-10985) gave some answers to the critics. The authors admit that the study “should not be considered as a final point in knowing the toxicological effects of NK603 and R (oundup)” and that the study has limits.
Jany (2012, ASB2014-9580) submitted a critical review of the study by Seralini et al. (2012). The authors conclude that the scientific value of this publication would be limited and non conclusions are possible concerning maize NK603 with and without Roundup treatment.
Ollivier (2012, ASB2013-11000) proposes to use the Chi-square test to compare mortality rates in the study of Seralini et al. (2012). In result of this test there would be no statistical significance.
In a further paper Seralini et al. (2014, ASB2014-9632) discuss criticisms which have been published in reaction on the study by Seralini et al. (2012, ASB2012-15514).
John (2014, ASB2014-9584) reacts in a letter on the decision of the publisher to retract the article of Seralini et al. (2012). John concludes that there would be no grounds for retraction. Wallace-Hayes (2014, ASB2014-9559), the editor-in-chief of Food and Chemical Toxicology, gives answers on questions on the retraction of the paper of Seralini et al. (2012). He concludes once more that “a careful and time-consuming analysis found that the data were inconclusive, and therefore the

conclusion described in the article were unreliable. Accordingly, the article was retracted."
Folta (2014, ASB2014-9478) writes in a letter to the editor that he would see this work of Seralini (2012) as a manipulation of the scientific process to achieve activist gains. He stands behind the journal`s decision to retract the work.
Rosanoff (2014, ASB2014-9397) proposes in a letter concerning the Seralini (2012) study that the raw data should be published.
Roberfroid (2014, ASB2014-9393) writes in a letter concerning the Seralini (2012) study that he is ashamed about the decision to retract this paper.
In a further letter Roberfroid (2014, ASB2014-9392) writes that in his understanding the study of Seralini (2012) remains an important scientific (not a regularory) observation that can not be ignored.
Pilu (2012, ASB2014-9387) writes in a letter to the editor on the Seralini (2012) study that mycotoxins in maize could have influenced the results of the study. Therefore, he asks for further information on the mycotoxin content in the maize used in the Seralini study.
Author(s)
Year
Study title
Chruscielska, K.
Brzezinski, J. Kita, K. Kalhorn, D. Kita, I. Graffstein, B. Korzeniowski,
P.
2000
Glyphosate Evaluation of chronic activity and possible far- reaching effects. Part 1. Studies on chronic toxicity Pestycydy 2000, (3-4), 11-20
ASB2013-9829
Abstract*:
The combined test of chronic toxicity and carcinogenicity of glyphosate was performed on Wistar-RIZ rats. The herbicide was administered in water at concentrations: 0, 300, 900, 2700 m/L. The examination of the peripheral blood parameters and the smears of bone marrow did not reveal harmful effect of the herbicide on haematopoietic system of rats. The biochemical parameters determined on blood and urine only in some cases showed significant deviations in comparison with the control group, but in any examined indices dose-effect-time occurred what could manifest the toxic influence of glyphosate. In pathomorphological studies on the organs no correlation was stated between the number of observed tumours and the concentrations of the herbicide. It indicates lack of pathogenic influence of glyphosate on neoplastic pathogenesis.
* Quoted from article
Klimisch evaluation
Reliability of study:
Reliable with restrictions
Comment:
The published details of the study are limited. However, according to the authors the study was performed on basis of OECD guideline No. 453
Relevance of study:
Relevant
Klimisch code:
2
Comments:
The active substance glyphosate was used in the study and the study was performed on basis of

OECD guideline 453. The number of animals per dose group and sex (85 animals) was even higher than required in guideline 453. Therefore, the study is considered to be relevant. No carcinogenic effects have been registered in the study.

B.6.6.12 Published data (released since 2000)

A large number of studies on developmental and reproductive toxicity (DART) was published since 2000. These studies are reported and discussed below. Furthermore, also studies on endocrine disruption (ED) have been included in this chapter because they are mainly related to developmental and reproductive toxicity.

Published studies on developmental toxicity, reproductive toxicity and an endocrine disrupting potential of glyphosate and glyphosate based formulations include in vitro studies, in vivo studies and epidemiological studies. Many studies since 2000 are specifically discussed in a comprehensive glyphosate DART review publication by Williams et al. (2012, ASB2012-12052). Further discussions of significant papers follow.

In addition, glyphosate was included on the US EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds to Tier 1 Screening. The US EPA published the criteria for inclusion on List 1 was strictly based on exposure potential, not hazard, specifically stating in the Federal Register (2009, ASB2012-12041);

"This list should not be construed as a list of known or likely endocrine disruptors".

A consortium of glyphosate registrants in North America, the Joint Glyphosate Task Force, LLC (JGTF), coordinated the conduct of the glyphosate battery of Tier 1 screening assays under the EDSP and submitted these assays to the US EPA. The US EPA will evaluate the full battery of Tier 1 screening assays together using a weight of evidence approach, for glyphosate's potential to interact with the estrogen, androgen and thyroid endocrine pathways. The following below were submitted by the JGTF to the US EPA in early 2012 and are reviewed. However, the Agency has announced they will not release their Data Evaluation Records (DERs) for individual EDSP studies until a weight of evidence review has been completed for List 1 compounds.

In Vitro EDSP Glyphosate Studies submitted to the US EPA

- Androgen Receptor Binding (Rat Prostate Cytosol); OCSPP 890.1150
- Aromatase (Human Recombinant); OCSPP 890.1200
- Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC); OCSPP 890.1250
- Estrogen Receptor Transcriptional Activation (Human cell Line, HeLa-9903); OCSPP 890.1300; OECD 455
- Published OECD Validation of the Steroidogenesis Assay (Hecker et al., 2010, ASB2012-11840)

In Vivo EDSP Glyphosate Studies submitted to the US EPA

- Amphibian Metamorphosis (Frog) OCSPP 890.1100; OECD 231
- In Vivo Hershberger Assay (Rat); OCSPP 890.1600; OECD 441
- Female Pubertal Assay; OCSPP 890.1450; OECD None
- Male Pubertal Assay; OCSPP 890.1500
- Uterotrophic Assay (Rat); OCSPP 890.1600; OECD 440
- Fish Short-Term Reproduction Assay; OCSPP 890.1350; OECD 229

The glyphosate Tier 1 screening assay study reports are owned by the JGTF. The European Glyphosate Task Force (GTF) is negotiating to procure access rights to the battery of glyphosate EDSP Tier 1 screening study reports. Results of the Hershberger and Uterotrophic in vivo rat studies, now in the public domain, as are the published results of the OECD validation of the Steroidogenesis assay, in which glyphosate clearly had no impact on steroidogenesis, are discussed below.

Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA

Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia 2012, ASB2012-12016) for the Hershberger and
Uterotrophic assays. No effects were noted for any potential for glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.
Bailey et al. (2013, ASB2013-3464) summarized the first results of the male and female Pubertal assay of this programm. Based on these results, glyphosate does not exhibit endocrine disruption in Male and Female Pubertal assays.
Levine et al. (2012, ASB2014-9609) published a short summary of the results of tests with glyphosate in the EPA`s Endocrine Disruptor Screening Program (EDSP). They conclude that from the weight of evidence provided by the Tier 1 assays, performed at independent labs, under the EDSP along with the higher Tier regulatory safety studies, with a high level of confidence glyphosate would not be an endocrine disruptor.
In Vitro Glyphosate DART/ED Publications
Many in vitro research publications have characterised pesticide formulations, including glyphosate based formulations, as toxic and endocrine disrupting products. Researchers and editorial boards did in some cases not consider the fact that surfactants (which are often components of formulated pesticide products), by their physico-chemical nature, are not suitable test substances using in vitro cell models. Surfactants compromise the integrity of cellular membranes, including mitochondrial membranes, and thus confound endpoint measurements considered as representative of specific toxicological modes of action or pathways.
A laboratory at the University of Caen, France, has multiple recent publications of in vitro research with glyphosate and glyphosate based formulations (Richard et al., 2005, ASB2009- 9024; Benachour et al., 2007, ASB2009-9018; Benachour and Seralini, 2009, ASB2012- 11561; Gasnier et al., 2009, ASB2012-11629; Gasnier et al., 2010, ASB2012-11628; Gasnier et al., 2011, ASB2012-11630; Clair et al., 2012, ASB2012-11592; Mesnage et al., 2012, ASB2012-11900), with proposed extrapolations to an array of in vivo effects including potent endocrine disruption, aromatase inhibition, estrogen synthesis, placental toxicity, foetotoxicity, embryotoxicity and bioaccumulation. These publications are in some cases replicates of earlier studies, using different cell lines or primary cell cultures and in some cases the same data are reported again in a subsequent publication. Firstly, the in vitro synergism claims are conjecture, because no control groups of surfactant without glyphosate were tested. Secondly, the extrapolations to in vivo effects are unjustifiable based on both the unsuitability of surfactants in such test systems and the supraphysiological cytotoxic concentrations at which in vitro effects are reported. Again often overlooked by in vitro researchers and editorial boards, Levine et al. (2007, ASB2009-9030) presented convincing data demonstrating a lack of in vitro synergism for glyphosate with other formulation ingredients. Regarding Seralini's repeated claims of glyphosate induced aromatase inhibition in mircosomes (Richard et al., 2005; TOX2005-1743, Benachour et al., 2007, ASB2009- 9018; Gasnier et al., 2009, ASB2012-11629), the data are confounded and thus uninterpretable where surfactants are introduced to such in vitro systems. This is noted in the US EPA Aromatase Inhibition Test Guideline, OECD 890.1200, in which notes, "Microsomes can be denatured by detergents [surfactants]. Therefore, it is important to ensure that all glassware and other equipment used for microsome preparations be free of detergent residue."
Another in vitro publication claiming a specific developmental toxicity pathway has gained significant public attention. Paganelli et al. (2010, ASB2012-11986) conducted three in vitro
assays, (i) frog embryos exposed to glyphosate formulation; (ii) frog embryos directly injected without injection blank negative controls; and (iii) fertilised chicken embryos exposed directly to a

glyphosate formulation through a hole cut in the egg shell. Key issues surrounding this research include irrelevant routes of exposure as well as excessively high and environmentally unrealistic doses.

Thongprakaisang et al., (2013, ASB2013-11991) submitted a study on the effects of pure glyphosate on estrogen receptors mediated transcriptional activity and their expressions. The following cell lines have been used: a hormone-dependent breast cancer, T47D, a stably EREC-luc construct tranfected hormone-dependent breast cancer T47D-KBluc and a hormone-independent human breast cancer, MDA-MB231. Glyphosate (purity ≥ 98 %) was tested in concentrations from 10-12 to 10-6 M. Glyphosate exerted proliferative effects on human hormone-dependent cell lines but not in hormone-independent cell lines. Furthermore, an additive estrogenic effect between glyphosate and genistein, a phytoestrogen, was reported. The authors conclude that these in vitro results need further investigation in an animal study. It must be emphasised that no increase in mammary tumours was reported in any of the numerous long-term studies in rats or mice (see Vol. 3, B.6.5 and Vol. 1, B.2.6).

Cavalli et al. (2013, ASB2014-7495) studied the effects of the formulation Roundup Original in rat testis and Sertoli cells in vitro. The authors propose that Roundup toxicity, implicated in Ca2+ overload, cell signalling misregulation, stress response of the endoplasmatic reticulum, and/or depleted antioxidant defenses, could contribute to Sertoli cell disruption in spermatogenesis that could have an impact on male fertility.

In Vivo Glyphosate DART/ED Publications

Relatively few in vivo publications on glyphosate DART and ED exist in comparison with the list of in vitro publications. Some lack appropriate interpretation of basic toxicology; e.g. Daruich et al. (2001, ASB2012-11601). Beuret et al. (2005, ASB2012-11564) investigated the effects of 1 % Glyphosate oral exposure (a trade product from Argentina described as “Herbicygon” was used which is a commercial herbicide formulation) on lipoperoxidation and antioxidant enzyme systems in pregnant rats and in fetuses. Lipoperoxidation was higher in both maternal and fetal livers in the glyphosate treated groups. Catalase and Superoxide dismutase activity were not altered. Both studies are reviewed in Williams et al. (2012, ASB2012-12052).

Dallegrave et al. (2003, ASB2012-11600; 2007, ASB2012-2721) published results of two non-guidelines rat developmental toxicity studies, in which a glyphosate based formulation containing POEA was evaluated. However, reporting deficiencies and inconsistencies pose difficulties in data interpretation. These studies are dicussed in detail in the Appendix on
(please refer to B.6.13).

Romano et al. (2010, ASB2012-12012) evaluated a glyphosate based formulation in a male pubertal-like assay in Wistar rats, reporting decreased preputial separation, reduced seminiferous epithelial height, increased luminal diameter of seminiferous tubules, and increased relative testicular and adrenal weights. Given the gravity of the reported findings in this publication, a review was undertaken by Kelce et al. (2010, ASB2012-11867). Most recently, Romano et al. (2012, ASB2012-12011) reported additional findings in male rats after supposed in utero and post natal exposures which include “behavioral changes and histological and endocrine problems in reproductive parameters and these changes are reflected by a hypersecretion of androgens and increased gonadal activity, sperm production
and libido”. As in their first publication, Romano et al. (2012, ASB2012-12011) base their hypothesis on selectively discussed literature implicating glyphosate as an endocrine disruptor, predominantly with citations to research from the Seralini laboratory.

Kimmel et al. (2013, ASB2013-3462) analyzed the information from 7 unpublished developmental

studies in rabbits and 6 developmental toxicity studies in rats to determine if glyphosate poses a risk for cardiovascular malformations. In summary, assessment of the reviewed data fails to support a potential risk for increased cardiovascular defects as a result of glyphosate exposure during pregnancy.

Chruscielska et al. (2000, ASB2013-9831) submitted a teratogenicity study in Wistar outbred rats. The used test guideline was not indicated. Doses of 0-750-1500-3000 mg/kg bw/day have been administered from day 7-14 of pregnancy to 20 females per dose group. No embryotoxic and no teratogenic effects have been administered.

Omran and Salama (2013, ASB2014-7614) report that the exposition of snails to atrazine or glyphosate resultet in signs of endocrine disruption and cellular toxicity. However, in this study only the formulation “Herfosate” was used and no pure active substance glyphosate.

Razi et al. (2012, ASB2014-9390) consider that glyphosate (125 mg/kg bw/d oral administered for 10, 20, 30 & 40 days) effects testicular tissue and sperm parameters in male Wistar rats. Clear effects were already seen after 10 days administration and thereafter, however accompanied by significant clinical symptoms (decreased movement, staggering gait, occasional trembling, diarrhea) and reduced body weight gain of 20 %. These findings are in contrast to those in rat studies submitted for EU evaluation. For comparison, the current EU evaluation of glyphosate proposes an overall subchronic (90-d) NOAEL of 414 mg/kg bw/d (rats) and for reproductive toxicity of 351 mg/kg bw/d, albeit generated from feeding studies. Similarily, after oral administration in female rats an NOAEL of 300 mg/kg bw/d for maternal and developmental effects was established, toxic effects were observed at much higher dose levels, only. The high toxicity described in the present publication is hardly to explain, because the publication does not give any information whether technical material or a glyphosated based formulation was tested. To conclude, the results of the publication does not affect the current assessment of glyphosate.

Cassault-Meyer et al. (2014, ASB2014-5615) investigated the effects of a glyphosate-based herbicide (Roundup Grand Travaux Plus) after an 8-day exposure of adult rats. Endocrine (aromatase, estrogen and androgen receptors, Gperl in testicular and sperm mRNAs) and testicular functions (organ weight, sperm parameters and expression of the blood-testis barrier markers) were monitored at day 68, 87, and 122 after treatment, spermiogenesis and spermatogenesis. A significant and differential expression of aromatase in testis and a diminution of mRNA expression of nuclear markers in spermatozoa were observed. The authors conclude that results suggest changes in androgen/estrogen balance and in sperm nuclear quality.

POEA DART Studies

Polyethoxylated alkylamine (POEA) surfactants are a class of non-ionic surfactant, containing a tertiary amine, an aliphatic group of variable carbon chain length and two separate sets of ethoxy (EO) chains of variable length. A dietary exposure assessment of POEAs was submitted by Bleeke et al. (2010, ASB2010-6123). This exposure assessment report also refers to the US EPA Alky Amine Polyalkoxylates Human Health Risk Assessment, which includes POEAs (http://www.regulations.gov/search/Regs/home.html#document Detail?R=09000064809b983b).

Williams et al. (2012, ASB2012-12052) recently evaluated and detailed the results of DART studies with two different POEA surfactants.

Furthermore, a detailed comparison of the toxicity of glyphosate was
submitted in the appendix “Toxicological evaluation of the surfactant (CAS no. ” which is attached to this report.

Epidemiology Glyphosate DART/ED Publications

Several epidemiology studies in which glyphosate exposure was considered have evaluated the

following range of reproductive outcomes; miscarriage, fecundity, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, attention-deficit disorder / attention-deficit hyperactive disorder (ADD/ADHD). In most instances, glyphosate and reproductive outcomes lack a statistically significant positive association, as described in a recent review of glyphosate non-cancer endpoint publications (Mink et al., 2011, ASB2012-11904). In evaluating ADD/ADHD, a positive association with glyphosate use was reported by Garry et al. (2002, ASB2012-11626), but cases were reported by parents with no clinical confirmation and the reported incidence rate of approximately 1 % for the study population was well below the general population incidence rate of approximately 7 %. Regarding in utero exposures, McQueen et al. (2012, ASB2012-11898) report very low measured dietary exposures, from 0.005 % to 2 % of the current glyphosate ADI in Europe. Given the low perfusion rate of glyphosate across the placenta (Mose et al., 2008, ASB2012-11914), human in utero exposures would be very limited.

Campana et al. (2010, ASB2013-10559) estimated the frequency of 27 birth defects in 7 geographical regions of Argentina. A sample of 21,844 newborn with birth defects was selected, ascertained from 855,220 births, between 1994 and 2007, in 59 hospitals. The study results suggested that frequencies of 14 of the 27 examined birth defects were higher in one or more regions. This study was discussed in some publications in relation to the use of glyphosate pesticides. However, Campana et al. (2010, ASB2013-10559) commented on secular trends, altitude above sea level, folic acid fortification and ethnic factors and further variables. It was not indicated that any of these variables was associated with an increased occurrence of any type of birth defects.

Two studies of residential proximity to agriculture-related pesticide applications (California) by Carmichael et al. (2013, ASB2014-9307) and Yang et al. (2013, ASB2014-9644) examined whether early gestational exposure to pesticides were associated with an increased risk of hypospadia, neural tube defects or orofacial clefts in offspring. In both studies formulated glyphosate was mentioned only as one out of five chemicals to which controls were most frequently exposed. The authors of both studies concluded the few positive findings on chemicals, but other than glyphosate, should be interpreted with caution and need to be repeated in other populations.

training to enable improvement of equipment and efficiency of application to minimize exposure risks.

Further reviews on DART

Antoniou et al. (2012, ASB2012-15927) submitted a review article on “Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence”. According to the authors published studies “have raised concern regarding the potential for glyphosate and its commercial formulations to cause birth defects and other reproductive problems”. The “draft assessment report revealed that … industry tests contained clear evidence of glyphosate-mediated teratogenicity and reproductive toxicity”. The EU adopted “an acceptable daily intake (ADI) for glyphosate that is unreliable and could potentially result in exposures that cause harm to humans.” The authors suggest that a “new risk assessment should be conducted with full public transparency by scientists who are independent of industry.”

Lopez et al. (2012, ASB2013-10534) submitted a review article on “Pesticides used in South American GMO-Based Agriculture: a review on their effects on humans and animal models”. The authors discuss the results of genetic studies in agricultural regions in the province of Cordoba, Argentina, biomarkers in agricultural regions in the province of Santa Fe, Argentina and congenital malformations and genotoxicity in populations exposed to pesticides in Paraguay. According to the authors, human health in these areas was damaged by pesticides. However, a relation to glyphosate or another substance or pesticide was not evidenced. Nevertheless, based on the results of Paganelli

et al. (2010, ASB2012-11986), it was concluded that glyphosate-based herbicides) would be linked to an increased activity of the retinoic acid signaling pathways and this might explain the higher incidence of embryonic malformations and spontaneous abortions observed in populations exposed to pesticides.

Basrur (2006, ASB2014-7492) submitted a review on disrupted sex differentiation and feminization of men. In this review the studies of Arbuckle and associates are cited which report a relation between pesticide exposure (including glyphosate) and reproductive risk.

Vandenberg et al. (2012, ASB2014-9635) submitted a review on low dose effects an nonmonotonic dose responses of hormones and endocrine disrupting chemicals. The authors reviewed two major concepts on EDC studies: low dose and nonmonotonicity. They conclude that nonmonotonic responses and low-dose effects would be remarkably common in studies of natural hormones and EDSs. Whether low doses of EDCs influence certain human disorders would be no longer conjecture, because epidemiologic studies would show that environmental exposures to ECDs would be associated with human diseases and disabilities. The authors demand that fundamental changes in chemical testing and safety determination woud be needed to protect human health.

In a direct response on the article of Vandenberg et al. (2012, ASB2014-9635) a discussion paper was submitted by Rhomberg and Goodman (2012, ASB2014-9391). These authors conclude that Vandenberg et al. (2012, ASB2014-9635) presented examples as anecdotes without attempting to review all available pertinent data, selectively citing studies without evaluating most of them or examining whether their putative examples are consistent and coherent with other relevant information. Many of their examples have been questioned by many scientists. Overall, Vandenberg et al. (2012, ASB2014-9635) put forth many asserted illustrations of their two conclusions without providing sufficient evidence to make the case for either and while overlooking evidence that suggest the contrary.

Lamb et al. (2014, ASB2014-9605) submitted a review with critical comments on the WHO- UNEP state of the science of endocrine disrupting chemicals – 2012. The authors conclude
that the 2012 report does not provide a balanced perspective, nor does it accurately reflect the state fo the science on endocrine disruption.

Borgert et al. (2013, ASB2014-9292) reviewed literature on thresholds of endocrine activity. The brief review highlights how the fundamental principles governing hormonal effects – affinity, potency, and mass action – dictate the existence of thresholds and why these principles also define the potential that exogenous chemicals might have to interfere with normal endocrine functioning.

The review by Sengupta and Banerjee, (2013, ASB2014-9730) is related to impacts of pesticides on male fertility. With respect to glyphosate the authors only cited in vitro data published by Richard et al. (2005, ASB2009-9025), and these have been already reported and evaluated in the present renewal assessment report (please refer to ‘In vitro Glyphosate DART/ED Publications’).

‘Epidemiology DART/ED Publication’.

Publication’.

present renewal assessment report (please refer to ‘Further reviews on DART’).

The English abstract of a Chinese publication by Zhang et al. (2013, ASB2014-9643) give notice of a summary on reproductive and developmental toxicity studies on glyphosate and the related mechanisms on humans and animals to provide suggestions for further research.

Comparison of the active substance glyphosate and glyphosate containing formulations concerning DART and ED

For the active substance glyphosate a very comprehensive data package of guideline conform studies on developmental and reproductive toxicity is available. This data package was prepared over the

last decades and updated within the last years.
In these submitted studies it was demonstrated that glyphosate is not a teratogenic substance. NOEL values for developmental toxicity and reproductive toxicity can be derived from the results of these studies. There are no relevant indications of an endocrine disrupting activity of the active substance glyphosate. Additionally, also in the further guideline conform toxicological studies (e.g. the subchronic and chronic toxicity studies) no indications of an endocrine disrupting activity of glyphosate (e.g. organ weight and histology of sexual organs, behaviour etc.) have been observed. Therefore, on basis of this comprehensive and high quality data package the active substance glyphosate is not considered to be an endocrine disruptor or a teratogenic substance.
Additionally to the studies which have been performed according to validated EU- and OECD guidelines a large number of studies has been published on DART and ED. Most of these studies use glyphosate containing preparations instead of the pure active substance glyphosate. However, some studies directly compare the toxicity of the active substance glyphosate and glyphosate conaining preparations. Furthermore, studies have been performed on the toxicity of surfactants which are used in preparations together with glyphosate, especially The results of these surfactant studies can be compared with the results of the above mentioned guideline conform studies on glyphosate.
In result of these comparisons it can clearly be concluded that the toxicity of preparations and the toxicity of surfactants like / polyethoxylated alkylamine is significantly higher than the toxicity of the active substance glyphosate.
A detailed comparison of the toxicity of tallowamin and glyphosate was submitted in the appendix “Toxicological evaluation of the e surfactant (CAS no. 61791-26- 2)” which is attached to this report. In this evaluation is clearly demonstrated that there is a significantly higher toxicity of the surfactant tallowamin with regard to all of the following endpoints investigated:
- acute oral toxicity
- acute dermal toxicity
- skin irritation
- eye irritation
- skin sensitization
- short term toxicity, rat
- short term toxicity, dog
- reproduction toxicity study, parental toxicity
- reproduction toxicity study, reproductive toxicity
- reproduction toxicity study, offspring toxicity
- developmental toxicity, rat, fetal effects

Walsh et al. (2000, ASB2012-12046) published research claiming that a glyphosate based formulation, but not glyphosate alone, adversely affected the steroidogenesis pathway by inhibiting progesterone production resulting in downstream reduction in mitochondrial levels of StAR protein. Subsequent research by Levine et al. (2007, ASB2009-9030) demonstrated no synergism between glyphosate and the surfactant since the cytotoxic effects were completely independent of glyphosate. Identical dose-response curves were noted for formulated product with and without the glyphosate active ingredient.
Further research addressing the steroidogenesis pathway confirmed glyphosate lacked endocrine disruption potential specific to this pathway. Quassinti et al. (2009, ASB2012- 12007) evaluated effects on gonadal steroidogenesis in frog testis and ovaries on glyphosate and another active substance, noting that glyphosate unequivocally demonstrated no effect. Forgacs et al. (2012,

ASB2012-11621) also tested glyphosate alone and demonstrated no effect on testosterone levels in BLTK1 murine leydig cells in vitro. Furthermore, the OECD multi-laboratory validation of the Steroidogenesis Assay used for Tier 1 screening of the US EPA EDSP, evaluated glyphosate and concluded no impact on steroidogenesis (Hecker et al., 2011, ASB2012-11840). Consequently, the US EPA considered reference to the OECD validation report sufficient for meeting the glyphosate Steroidogenesis Assay Test Order in the EDSP Tier 1 screening of glyphosate.

Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia, 2012, ASB2012-12016) for the Hershberger and

Uterotrophic assays. No effects were noted for any potential for the active substance glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.

Richard et al. (2005, TOX2005-1743) studied effects of glyphosate and roundup on human placental cells and aromatase. Summarising their results they stated that "roundup is always more toxic than its active ingredient."

In a further study from the same institute Benachour et al. (2007, ASB2009-9018) studied time- and dose-dependent effects of roundup on human embryonic and placental cells. They summarized that "in all instances, roundup … is more efficient than its active ingredient, glyphosate…". And in a further publication by Benachour and Seralini (2009, ASB2012- 11561) it was stated "this work clearly confirms that the adjuvants in roundup formulations are not inert." In a response to this publication by the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532) it was answered that surfactant effects … are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis. In the most recent publication from the same institute, Mesnage et al. (in press, ASB2012-13917) the potential active principle for toxicity on human cells for 9 glyphosate-based formulations was studied. The authors summarized that "ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity".

In a comprehensive analysis of the available literature in developmenat and reproductive outcomes in humans and animals after glyphosate exposure, Williams et al. (2012, ASB2012- 12052) summarized: "An evaluation of this database found no consistent effects of glyphosate exposure on reproductive health or the developing offspring. Furthermore, no plausible mechanism of action for such effects were elucidated. Although toxicity was observed in studies that used glyphosate-based formulations, the data strongly suggest that such effects were due to surfactants present in the formulations and not the direct result of glyphosate exposure."

In vitro DART/ED publications

Author(s)

Year

Study title

Walsh, L.P. McCormick, C. Martin, C. Stocco, D.M.

2000

Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression.

Environmental Health Perspectives Volume: 108

Number: 8

Pages: 769-776 ASB2012-12046

Abstract*

Recent reports demonstrate that many currently used pesticides have the capacity to disrupt

reproductive function in animals. Although this reproductive dysfunction is typically characterized by alterations in serum steroid hormone levels, disruptions in spermatogenesis, and loss of fertility, the mechanisms involved in pesticide-induced infertility remain unclear. Because testicular Leydig cells play a crucial role in male reproductive function by producing testosterone, we used the mouse MA-10 Leydig tumor cell line to study the molecular events involved in pesticide-induced alterations in steroid hormone biosynthesis. We previously showed that the organochlorine insecticide lindane and the organophosphate insecticide

Dimethoate directly inhibit steroidogenesis in Leydig cells by disrupting expression of the steroidogenic acute regulatory (StAR) protein. StAR protein mediates the rate-limiting and acutely regulated step in steroidogenesis, the transfer of cholesterol from the outer to the inner mitochondrial membrane where the cytochrome P450 side chain cleavage (P450scc) enzyme initiates the synthesis of all steroid hormones. In the present study, we screened eight currently used pesticide formulations for their ability to inhibit steroidogenesis, concentrating on their effects on StAR expression in MA-10 cells. In addition, we determined the effects of these compounds on the levels and activities of the P450scc enzyme (which converts cholesterol to pregnenolone) and the 3 a-hydroxysteroid dehydrogenase (3 -HSD) enzyme (which converts pregnenolone to progesterone). Of the pesticides screened, only the pesticide Roundup inhibited dibutyryl [(Bu)2]cAMP-stimulated progesterone production in MA-10 cells without causing cellular toxicity. Roundup inhibited steroidogenesis by disrupting StAR protein expression, further demonstrating the susceptibility of StAR to environmental pollutants.

*Quoted from article

Klimisch evaluation

Reliability of study:

Reliable with restrictions

Comment:

Non-standard test systems, but publication meets basic scientific principles. However, surfactant blend in

Roundup confounds results.

Relevance of study:

Relevant with restrictions: Different effects of glyphosate alone and glyphosate formulations were observed. No conclusion can be drawn that the

observed effects are result of glyphosate exposure.

Klimisch code:

2

Additional comments:

Glyphosate did not affect steroidogenesis in the test system.

Roundup formulation data was confounded by mitochondrial membrane damage, attributable to the surfactant in the tested formulation.

Roundup results were comprehensively addressed in Levine et al. (2007, ASB2009-9030): Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have “indistinguishable” dose response curves for reductions in progesterone production in hCG stimulated MA-10 Leydig cells. Therefore the effect on progesterone levels shown by Walsh (2000, ASB2012-12046) were independent of glyphosate and attributable to the surfactant component of the formulation.

Comparable rates of progesterone inhibition for several different surfactants suggest a common mode of action for surfactants.

Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have almost identical concentration-dependent decreases in MTT activity in MA-10 cells, suggesting the surfactant alone was responsible for the observed cytotoxicity and effect on mitochondrial function.
The JC-1 assay demonstrated the decreased progesterone production in MA-10 Leydig cells was accompanied by loss of mitochondrial membrane potential. These results confirm StAR protein function and steroidogenesis require intact mitochondrial membrane potential.
StAR protein expression were not affected by treatments, indicating that perturbed mitochondrial membrane, not StAR protein inhibition, was responsible for the effects noted by Walsh et al. (2000, ASB2012-12046).
Author(s)
Year
Study title
Paganelli, A. Gnazzo, V. Acosta H. Lopez, S.L. Carrasco, A.E.
2010
Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signalling
Chemical Research in Toxicology Volume: 23
Pages: 1586-1595 ASB2010-11410
Abstract*
The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosatebased herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate in development. Xenopus laevis embryos were incubated with 1/5000 dilutions of a commercial GBH. The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior-posterior (A-P) axis. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed, rather than a surfactant or other component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in Xenopus embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of otx2 expression and with the disruption of cephalic neural crest development. The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:

Non-guideline study that is not sufficiently described for assessment. Inadequate positive and negative control experiments.
Relevance of study:
Not relevant: Irrelevant routes of exposure and inappropriately high doses. Test system not adequate for human risk assessment.
Klimisch code:
3
Additional comments:
Response 1 – summarized from Williams et al. (2012, ASB2012-12052)
No pH adjustment for doses and thus effects may be in response to the acidic nature of glyphosate technical acid.
Inappropriate and irrelevant routes of exposure.
Data requires further substantiation before consideration in risk assessment.
Response 2 – Saltmiras et al. (2011, ASB2012-12015) letter to the Editor
Multiple high quality toxicological studies and expert review panels consistently agree glyphosate is not a teratogen or reproductive toxicant.
The authors' justification for this research is flawed, providing no valid basis, other than an opinion, of an increase in the rate of birth defects in Argentina.
Direct injection of frog embryos and through chicken shells do not reflect real world exposure scenarios to either environmental species or humans.
Doses were excessively high and irrelevant for risk assessment purposes. Frog embryos were also bathed in glyphosate formulation at doses 9-15 times greater than the acute LC50 same species of frog. Calculating equivalent oral doses based on pharmacokinetics studies, such doses are 150000000 times greater than worst case human exposure monitoring data.
"…. the results from this research cannot be used in isolation to reach the conclusions expressed in the publication. Instead, the type of data in this research paper must be interpreted relative to all other available data on the specific materials under study and with balanced consideration for higher tier apical studies."
Response 3 – Mulet (2011, ASB2012-11916) letter to the Editor
Notes the premise for this research is falsely based on an incorrectly cited local pediatric bulletin from Paraguay.
"…. this article refers to a study in a single hospital in Paraguay showing a correlation between pesticide use (not herbicides as mentioned by Paganelli et al., ASB2010-11410) and birth malformations. In the cited study (Benitez et al., ASB2012-11563), the authors state that the results are preliminary and must be confirmed. Is important to remark that the Benitez et al. study does not include any mention to glyphosate, so does not account for what the authors are stating in the introduction…..This journal is also wrongly cited in the discussion referring to increased malformations due to herbicides, which is not the result of the study."
Response 4 – comments from BVL (2010, ASB2012-11579)
Highly artificial experimental conditions.
Inappropriate models to replace validated mammalian reproductive and developmental toxicity testing methods for use in human health risk assessment.
Inappropriate routes of exposure.
Lack of corroborative evidence in humans.
"In spite of long-lasting use of glyphosate-based herbicides worldwide, no evidence of teratogenicity in humans has been obtained so far."

Response 5– comments from European Commission Standing Committee on the Food Chain and Animal Health (2011, ASB2012-11615)

The EU commission supports the German Authorities position, “that that there is a comprehensive and reliable toxicological database for glyphosate and the effects observed have not been revealed in mammalian studies, nor evidenced epidemiologically in humans.” “…. the Commission does not consider there is currently a solid basis to ban or impose specific restrictions on the use of glyphosate in the EU.”

Response 6– Palma, G. (2010, ASB2012-11989) letter to the Editor

The author of the letter claims that the study by Paganelli et al., 2010 (ASB2010-11410), described effects of glyphosate only at unrealistic high concentrations or via unrealistic routes of exposure. The data are thought to be inconsistent with the literature, and therefore not suitable or relevant for the risk assessment for humans and wildlife. Furthermore the author asserts that findings do not support the extrapolation to human health as stated in the publication.

Author(s)

Year

Study title

Richard, S. Moslemi, S. Sipahutar, H. Benachour, N. Seralini, G.E.

2005

Differential effects of glyphosate and roundup on human placental cells and aromatase.

Environmental Health Perspectives Volume: 113

Pages: 716-720 TOX2005-1743

Abstract*

Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems, but its mechanism of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Study design is insufficient for risk assessment of real exposure concentrations. Methodological deficiencies (no controls were included). Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems. EPA Test Guideline OCSPP 890.1200 specifically notes that microsomes are denatured by detergents (i.e. surfactants) and that all

glassware should be
thoroughly rinsed.
Relevance of study:
Not relevant: Excessive doses exceed typical in vitro
limit doses. In vitro test system is inappropriate with surfactants.
Klimisch code:
3
Additional comments:
Response 1 – summarized from Williams et al. (2012, ASB2012-12052)
Glyphosate at non-cytotoxic concentrations in this test system was demonstrated to have no effects on aromatase activity.
Likewise, did not affect mRNA levels after 18 hours treatment at ≤ 0.1% glyphosate. Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.
The in vitro test system is non-validated Physiologically irrelevant concentrations tested.
Testing surfactant-like substances in such systems is now recognized to be not valid.
Response 2 – summarized from the French Ministry of Agriculture and Fish, Committee for Study of Toxicity (2005, ASB2009-9025)
Major methodological gaps.
JEG3 cells, a choriocarcinoma human cell line (average of 70 chromosomes vs 46 in normal human cells).
Concentrations of Roundup used in the various experiments considered to be extremely high. In consideration of limiting factors (oral absorption, 30 %; skin absorption, 0.3 %; rapid elimination kinetics), such levels would involve considerable human exposure, or several dozen liters of Roundup diluted at 2 %.
concentrations of Roundup that trigger an effect on aromatase (0.5 % - 2 %) are at least 1000 times more effective than those of known aromatase inhibitors, such as azole derivatives Study design does not make it possible to show the influence of the adjuvants, nor synergism of adjuvants and glyphosate.
Multiple non-specific effects of surfactant agents on a broad range of cellular targets not discussed.
No comparison with comparable surfactant agents intended for household use. Multiple instances of bias in its arguments and its interpretation of the data.
The authors over-interpret their results in the area of potential health consequences for humans (unsuitable references, non-sustained in vitro-in vivo extrapolation, etc.).
Author(s)
Year
Study title
Benachour, N. Sipahutar, H. Moslerni, S. Gasnier, C. Travert, C.
Seralini, G. E.
2007
Time- and dose-dependent effects of roundup on human embryonic and placental cells.
Archives of Environmental Contamination and Toxicology Volume: 53
Pages: 126-133 ASB2009-9018
Abstract*
Roundup® is the major herbicide used worldwide, in particular on genetically modified plants that have been designed to tolerate it. We have tested the toxicity and endocrine disruption potential of Roundup (Bioforce®) on human embryonic 293 and placental-derived JEG3 cells, but also on normal

human placenta and equine testis. The cell lines have proven to be suitable to estimate hormonal activity and toxicity of pollutants. The median lethal dose (LD50) of Roundup with embryonic cells is 0.3 % within 1 h in serum-free medium, and it decreases to reach 0.06 % (containing among other compounds 1.27 mM glyphosate) after 72
h in the presence of serum. In these conditions, the embryonic cells appear to be 2-4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1 -2 %, i.e., with 21-42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup. We demonstrated that serum-free cultures, even on a short-term basis (1 h), reveal the xenobiotic impacts that are visible 1-2 days later in serum. We also document at lower non-overtly toxic doses, from 0.01 % (with 210 µM glyphosate) in 24 h, that Roundup is an aromatase disruptor. The direct inhibition is temperature-dependent and is confirmed in different tissues and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). Furthermore, glyphosate acts directly as a partial inactivator on microsomal aromatase, independently of its acidity, and in a dose-dependent manner. The cytotoxic, and potentially endocrine-disrupting effects of Roundup are thus amplified with time. Taken together, these data suggest that Roundup exposure may affect human reproduction and fetal development in case of contamination. Chemical mixtures in formulations appear to be underestimated regarding their toxic or hormonal impact.
Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Study report has several reporting deficiencies in the methods section (e.g. test conditions for the pH- and temperature dependent assay not reported). There is no information on the suitability of the used HEK 293 cell line for assessment of hormonal activity. Exceedingly high doses above the limit dose for this study type.
Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:
Not relevant: Excessive doses exceed typical in vitro
limit doses. In vitro test system is inappropriate with surfactants.
Klimisch code:
3
Additional comments:
Glyphosate at and above relevant concentrations for this test system was demonstrated to have no effects on aromatase activity.
Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.
Comparable research to Richard et al (2005, TOX2005-1743), but with an additional cell line, HEK 293, derived from aborted human embryo kidneys, transformed by inserting adenovirus DNA.
Excessively high doses tested, not environmentally relevant for human health or environmental risk assessment.
Aromatase production within the steroidogenesis pathway. Therefore, aromatase inhibition would be detected in the steroidogenesis assay. The OECD multi-laboratory validation of the steroidogenesis assay evaluated glyphosate, demonstrating no impact on the steroidogenesis pathway (Hecker et al., 2011, ASB2012-11840).

Response – summarized from Williams et al. (2012, ASB2012-12052)
pH of test system not adjusted to physiologically appropriate levels; Negative controls were not pH adjusted to appropriate levels.
Confounding surfactant effects due to cell membrane damage render data generated with formulated products in this test system null.
Author(s)
Year
Study title
Benachour, N. Seralini, G. E.
2009
Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells.
Chemical Research in toxicology Volume: 22, Pages: 97-105 ASB2012-11561
Abstract*
We have evaluated the toxicity of four glyphosate (G)-based herbicides in Roundup formulations, from 10(5) times dilutions, on three different human cell types. This dilution level is far below agricultural recommendations and corresponds to low levels of residues in food or feed. The formulations have been compared to G alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA. HUVEC primary neonate umbilical cord vein cells have been tested with 293 embryonic kidney and JEG3 placental cell lines. All R formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage. They also induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells. G provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants. AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. This should be discussed when analyzing the in vivo toxic actions of R. This work clearly confirms that the adjuvants in Roundup formulations are not inert. Moreover, the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from formulation-treated crops.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption
potential of surfactants are well known for in vitro test systems. No positive controls were included.
Relevance of study:
Not relevant (Excessive doses exceed typical in vitro
limit doses. In vitro test system is inappropriate with

surfactants)
Klimisch code:
3
Additional comments:
Response – summarized from the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532)
Cell lines used present characteristics which may be at the source of a significant bias in the interpretation of the results.
Experiments were conducted with 24 hours exposure in a medium without serum, which could lead to disturbance of the physiological state of the cells.
The glyphosate used in the study is glyphosate acid, whereas in the preparations tested it is in the form of an isopropylamine salt. No precise information is given about the pH of test concentrations except the highest dose.
No mention of any positive evidence for the apoptosis test.
Cytoxicity and induction of apoptosis may due to pH and/or variations in osmotic pressure on cell survival at the high doses tested.
Surfactant (tensoactive) effects and increased osmolality are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis.
Conclusions are based on unvalidated, non-representative cell models (in particular tumour or transformed cell lines) directly exposed to extremely high product concentrations in culture conditions which do not observe normal cell physiological conditions.
No new information is presented on mechanism of action of glyphosate and preparations containing glyphosate.
The authors over-interpret their results with regard to potential health consequences for humans, based in particular on an unsupported in vitro–in vivo extrapolation
The cytotoxic effects of glyphosate, its metabolite AMPA, the tensioactive POAE and other glyphosate-based preparations proposed by Benachour and Seralini do not add any pertinent new facts which call into question the conclusions of the European assessment of glyphosate or those of the national assessment of the preparations.
Author(s)
Year
Study title
Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M. C., Seralini, G. E
2009
Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines.
Toxicology
Volume: 262, Number: 3, Pages: 184-191 ASB2012-11629
Abstract*
Glyphosate-based herbicides are the most widely used across the world; they are commercialised in different formulations. Their residues are frequent pollutants in the environment. In addition, these herbicides are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. Up to 400 ppm of their residues are accepted in some feed. We exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity, to four different formulations and to glyphosate, which is usually tested alone in chronic in vivo regulatory studies. We measured cytotoxicity with three assays (Alamar Blue, MTT, ToxiLight), plus genotoxicity (comet assay), anti-estrogenic (on ERa, ER,) and anti-androgenic effects (on AR) using gene reporter tests. We also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters

were disrupted at sub-agricultural doses with all formulations within 24h. These effects were more dependent on the formulation than on the glyphosate concentration. First, we observed a human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. A real cell impact of glyphosate-based herbicides residues in food, feed or in the environment has thus to be considered, and their classifications as carcinogens/mutagens/reprotoxics is discussed.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Due to reporting deficiencies (e.g. correlation between concentration used in toxicity tests and concentrations used in comet assay) assessment of results difficult.
Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test
systems.
Relevance of study:
Not relevant: Excessive doses exceed typical in vitro
limit doses. In vitro test system is inappropriate with surfactants.
Klimisch code:
3
Additional comments:
Response 1 – summarized from Williams et al. (2012, ASB2012-12052)
Glyphosate demonstrated no significant anti-estrogenic potential
Glyphosate demonstrated some anti-androgenic potential at lower concentrations, but not as doses increased and therefore results are considered unrelated to treatment
Four glyphosate based formulations demonstrated both estrogenic and androgenic activity. Results are confounded due to surfactants within the formulated products tested, which affect cell membrane integrity and produces false findings.
Response 2 – summarized from BfR Review (2009, ASB2012-11565)
Numerous methodological flaws are noted. Test substance(s) not characterized
Source of materials for cell culture not provided. Dosing concentrations not well described
Serum free media only appropriate for short term (3-4 hour) in vitro exposures. pH control of dilutions not clear.
Osmolality of test solutions not reported.
Electrophoresis parameters insufficiently or inaccurately reported. Numerous reporting deficiencies are noted.
Influence of serum-free cell culturing on endpoints can not be determined
Incomplete data reporting, in cluding β-galactosirase activity, cototoxicity for select assays. Positive control data not reported.
Confusion between maximum residue levels verses systemic concentrations in humans.
Author(s)
Year

Study title

Clair, E., Mesnage, R., Travert, C., Seralini, G.E.

2012

A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels.

Toxicology in Vitro

Volume: 26, Number: 2, Pages: 269-279 ASB2012-1628

Abstract*

The major herbicide used worldwide, Roundup, is a glyphosate-based pesticide with adjuvants. Glyphosate, its active ingredient in plants and its main metabolite (AMPA) are among the first contaminants of surface waters. Roundup is being used increasingly in particular on genetically modified plants grown for food and feed that contain its residues. Here we tested glyphosate and its formulation on mature rat fresh testicular cells from 1 to 10000 ppm, thus from the range in some human urine and in environment to agricultural levels. We show that from 1 to 48 h of Roundup exposure Leydig cells are damaged. Within 24–48 h this formulation is also toxic on the other cells, mainly by necrosis, by contrast to glyphosate alone which is essentially toxic on Sertoli cells. Later, it also induces apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures. At lower non toxic concentrations of Roundup and glyphosate (1 ppm), the main endocrine disruption is a testosterone decrease by 35%. The pesticide has thus an endocrine impact at very low environmental doses, but only a high contamination appears to provoke an acute rat testicular toxicity. This does not anticipate the chronic toxicity which is insufficiently tested and only with glyphosate in regulatory tests.

* Quoted from article

Klimisch evaluation

Reliability of study:

Not reliable

Comment:

Non-guideline in vitro test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified).

Relevance of study:

Not relevant (Due to reliability. In addition, in vitro data do not reflect real in vivo exposure situations, and therefore not relevant for human risk assessment purposes.)

Klimisch code:

3

Additional comments:

In vitro test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified). The concentrations used in these experiments are not relevant to human exposures to glyphosate and the experimental system used is not relevant to whole animal outcomes. Importantly, the alleged impacts on endocrine function have not been observed in animal studies of glyphosate or other components of glyphosate formulations at relevant concentrations. Authors state that the lowest concentration of glyphosate tested was 50 ppm, several orders of magnitude higher than an anticipated human intake (based on pharmacokinetics described in Anadon et al., 2009, ASB2012-11542) following worst case dietary exposure at the ADI.

Author(s)

Year
Study title
Hokanson, R. Fudge, R. Chowdhary, R. Busbee, D.
2007
Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate.
Human & Experimental Toxicology Volume: 26, Pages: 747-752,
ASB2012-11846
Abstract*
Gene expression is altered in mammalian cells (MCF-7 cells), by exposure to a variety of chemicals that mimic steroid hormones or interact with endocrine receptors or their co- factors. Among those populations chronically exposed to these endocrine disruptive chemicals are persons, and their families, who are employed in agriculture or horticulture, or who use agricultural/horticultural chemicals. Among the chemicals most commonly used, both commercially and in the home, is the herbicide glyphosate. Although glyphosate is commonly considered to be relatively non-toxic, we utilized in vitro DNA microarray analysis of this chemical to evaluate its capacity to alter the expression of a variety of genes in human cells. We selected a group of genes, determined by DNA microarray analysis to be dysregulated, and used quantitative real-time PCR to corroborate their altered states of expression. We discussed the reported function of those genes, with emphasis on altered physiological states that are capable of initiating adverse health effects that might be anticipated if gene expression were significantly altered in either adults or embryos exposed in utero.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Not acceptable in vitro methods for test mixtures containing surfactant. Well documented study publication, but surfactants are inappropriate test
substance in cell lines.
Relevance of study:
Not relevant Temporal altered gene expression is not a biomarker for toxicity, but rather, may be within the range of normal biological responses of homeostasis. In vitro cytotoxicity of surfactants, however, is a significant confounder in data interpretation. Data do
not reflect real in vivo exposure situations, and therefore not relevant for human risk assessment purposes.
Klimisch code:
3
Additional comments:
In vitro cytotoxicity of surfactants is a significant confounder in data interpretation. Relevance of altered gene expression in a cell line derived from a breast cancer should not be extrapolated to reflect human health endpoints. Altered gene expression should not be confused with adverse health outcomes. Rather altered gene expression may equally be considered a biological response within the range of normal homeostasis.
In vivo DART/ED publications
Author(s)
Year

Study title
Yousef, M.I.,
Salem, M.H.,
Ibrahim, H.Z.,
Helmi, S.,
Seehy, M.A., Bertheussen, K.
1995
Toxic Effects of Carbofuran and Glyphosate on Semen Characteristics in Rabbits.
Journal of Environmental Science and Health. Part B. Volume: 30, Number: 4, Pages: 513-534
ASB2012-12058
Abstract*
The present study was undertaken to investigate the effect of chronic treatment with two sublethal doses of Carbofuran (carbamate insecticide) and Glyphosate (organophosphorus herbicide) on body weight and semen characteristics in mature male New Zealand white rabbits. Pesticide treatment resulted in a decline in body weight, libido, ejaculate volume, sperm concentration, semen initial fructose and semen osmolality. This was accompanied with increases in the abnormal and dead sperm and semen methylene blue reduction time. The hazardous effect of these pesticides on semen quality continued during the recovery period, and was dose-dependent. These effects on sperm quality may be due to the direct cytotoxic effects of these pesticides on spermatogenesis and/or indirectly via hypothalami-pituitary- testis axis which control the reproductive efficiency.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-GLP, non-guideline study with major reporting deficiencies. Dose-levels poorly defined as 1/10 and 1/100 LD50. Purity of the test substances, source of animals, environmental conditions, mortality and clinical signs not reported. No testis and epididymis weights were determined or reported and no histopathological examination conducted. In addition, stability and homogeneity assessment of test substance preparations were not done or not reported. Rabbits
have low body weights at study start, suggesting impaired health status.
Relevance of study:
Not relevant (Due to low confidence in study conduct
and the inadequacy of reporting.)
Klimisch code:
3
Additional comments:
Response – summarized from Williams et al. (2000, ASB2012-12053)
Numerous serious deficiencies in the design, conduct, and reporting of this study which make the results uninterpretable.
Only four rabbits per treatment group were used, and therefore statistics are questionable.
Rabbits appeared to be small for their age; at study start (32 weeks) tested animals had 16- 25 % lower body weight than historical weights for commercially bred animals of the same age and strain. Low body weights as study start suggest compromised health status of the animals at initiation.
Dose levels were not quantified.
Purity of glyphosate and composition of the glyphosate formulation were not reported. Inadequate

description of test material administration.
Improper semen collection technique reported.
Report is unclear whether control animal sham handling was undertaken, a critical factor in stress related outcomes in this species.
Food consumption of test and control groups not adequately reported.
Variability not adequately reported for endpoint measurements in test and control groups, preventing statistical analysis to support the author's conclusions.
Dose-responses not observed, despite the wide dose spread.
Sperm concentrations of all groups within normal ranges for this strain of rabbit. No meaningful conclusions can be drawn from this publication.
Author(s)
Year
Study title
Daruich, J. Zirulnik, F. Gimenez, M. S.
2001
Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses
Environmental Research Volume: 85
Pages: 226-231 ASB2012-11601
Abstract*
To prevent health risk from environmental chemicals, particularly for progeny, we have studied the effects of the herbicide glyphosate on several enzymes of pregnant rats. Glyphosate is an organo-phosphorated nonselective agrochemical widely used in many countries including Argentina and acts after the sprout in a systemic way. We have studied three cytosolic enzymes: isocitrate dehydrogenase-NADP dependent, glucose-6-phosphate dehydrogenase, and malic dehydrogenase in liver, heart, and brain of pregnant Wistar rats. The treatment was administered during the 21 days of pregnancy, with 1 week as an acclimation period. The results suggest that maternal exposure to agrochemicals during pregnancy induces a variety of functional abnormalities in the specific activity of the enzymes in the studied organs of the pregnant rats and their fetuses.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Basic data given, however, the study is performed with methodological and reporting deficiencies (unknown exposure levels, only cytosolic enzymes measured,
inappropriate controls, lack of consistent dose-response data).
Relevance of study:
Not relevant (Due to reliability. In addition, study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate).
Klimisch code:
3
Additional comments:
The study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate. Test substance administration is poorly described, but rough calculations on approximate surfactant intake show excessively high and unrealistic exposures when compared to DART systemic parental and reproductive/developmental NOAEL values for POEA formulation surfactants.

Response summarized from Williams et al. (2012, ASB2012-12052)
Test substance and doses not adequately described. Inappropriate control groups.
Results suggest that the effect of treatment on body and organ weights may be due to reduced food and water intakes.
A consistent effect of treatment was not observed and dose-response relationships were generally lacking
The information gathered may be misleading because the enzymes monitored are found in both the cytosol and mitochondria.
Food restriction affects the activity of many enzymes, including those examined in this study.
Author(s)
Year
Study title
Romano, R.M. Romano, M.A. Bernardi, M.M. Furtado, P.V. Oliveira, C.A.
2010
Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology.
Archives of Toxicology Volume: 84, Pages: 309-317 ASB2012-12012
Abstract*
Glyphosate is a herbicide widely used to kill weeds both in agricultural and non-agricultural landscapes. Its reproductive toxicity is related to the inhibition of a StAR protein and an aromatase enzyme, which causes an in vitro reduction in testosterone and estradiol synthesis. Studies in vivo about this herbicide effects in prepubertal Wistar rats reproductive development were not performed at this moment. Evaluations included the progression of puberty, body development, the hormonal production of testosterone, estradiol and corticosterone, and the morphology of the testis. Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in semineferous tubules' morphology, decreased significantly the epithelium height ($P < 0.001$; control = 85.8 ± 2.8 µm; 5 mg/kg = 71.9 ± 5.3 µm; 50 mg/kg =
69.1 ± 1.7 µm; 250 mg/kg = 65.2 ± 1.3 µm) and increased the luminal diameter ($P < 0.01$; control = 94.0 ± 5.7 µm; 5 mg/kg = 116.6 ± 6.6 µm; 50 mg/kg = 114.3 ± 3.1 µm; 250 mg kg =
130.3 ± 4.8 µm); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups ($P < 0.001$; control = 154.5 ± 12.9 ng/dL; 5 mg/kg = 108.6 ± 19.6 ng/dL; 50 mg/dL = 84.5 ± 12.2 ng/dL; 250
mg/kg = 76.9 ± 14.2 ng/dL). These results suggest that commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disturbances in the reproductive development of rats when the exposure was performed during the puberty period.
Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Study with methodological and reporting deficiencies or conflicting findings (e.g., increased relative testicular
weights, but decreased testosterone measurements.
Relevance of study:

Relevant study type for investigating male reproductive endpoints, but questionable relevance of this specific study based on low reliability of data and interpretation. Not relevant for glyphosate (test material was a
formulated product, not glyphosate).
Klimisch code:
3
Additional comments:
Test material was a formulated product, not glyphosate. The authors failed to measure many of the key parameters in the validated pubertal male assay protocol and hence generated data that were internally inconsistent or incomplete.
Author(s)
Year
Study title
Romano, M.A. Romano, R.M. Santos, L.D. Wisniewski, P. Campos, D.A. de Souza, P.B. Viau, P. Bernardi, M.M. Nunes, M.T.
de Oliviera, C.A.
2012
Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression
Archives of Toxicology
Volume: 86, Number: 4, Pages: 663-673 ASB2012-12011
Abstract*
Sexual differentiation in the brain takes place from late gestation to the early postnatal days. This is dependent on the conversion of circulating testosterone into estradiol by the enzyme aromatase. The glyphosate was shown to alter aromatase activity and decrease serum testosterone concentrations. Thus, the aim of this study was to investigate the effect of gestational maternal glyphosate exposure (50 mg/kg, NOAEL for reproductive toxicity) on the reproductive development of male offspring. Sixty-day-old male rat offspring were evaluated for sexual behavior and partner preference; serum testosterone concentrations, estradiol, FSH and LH; the mRNA and protein content of LH and FSH; sperm production and the morphology of the seminiferous epithelium; and the weight of the testes, epididymis and seminal vesicles. The growth, the weight and age at puberty of the animals were also recorded to evaluate the effect of the treatment. The most important findings were increases in sexual partner preference scores and the latency time to the first mount; testosterone and estradiol serum concentrations; the mRNA expression and protein content in the pituitary gland and the
serum concentration of LH; sperm production and reserves; and the height of the germinal epithelium of seminiferous tubules. We also observed an early onset of puberty but no effect on the body growth in these animals. These results suggest that maternal exposure to glyphosate disturbed the masculinization process and promoted behavioral changes and histological and endocrine problems in reproductive parameters. These changes associated with the hypersecretion of androgens increased gonadal activity and sperm production.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline, non-GLP study meeting scientific principles. Unusual and short dosing regiment

commencing towards the end of pregnancy (GD18, rather than GD6 as per OECD Test Guidelines 414) through post natal day 5. In vivo study with reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). A
number of atypical endpoints evaluated.
Relevance of study:
Not relevant (due to questionable dosing regimen and
atypical array of endpoints measured).
Klimisch code:
3
Additional comments:
Study with some reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). Dosing was limited to dams, starting on gestation day 18, well after organogenisis, through post natal day 5. No controls for litter effects appear to be reported, confounding interpretation of results. With the very short window of maternal exposure, biological plausibility of any test substance related effects in the mature offspring is questionable. However, the normal variability of some unusual or atypical endpoint measurements, such as "sexual partner preference" along with mRNA and protein expression, is not known. Of particular concern, however, are differences in critical endpoints for control animals reported in Romano et al. (2010, ASB2012-12012) compared to Romano et al. (2012, ASB2012- 12011); these include increased day of preputional sepratarion (PPS) of control male rate (37 days in 2010; 47 days in 2012), body weight at day of PPS (146 grams in 2010; 245 grams in 2012), serum testosterone concentrations (155 ng/dL in 2010; 63 ng/dL in 2012), estradiol concentrations (32 pg/mL in 2010; 1.4 pg/mL in 2012), subular diameter (266 µm in 2010;
479 µm in 2012), epithelial height (86 µm in 2010; 92 µmin 2012) and luminal height (94 µm in 2010; 257µm in 2012). Therefore, results are difficult to interpret, particularly for relevance to human health risk assessment.
system in offspring whose dame were treated with glyphosate". Romano and Romano (2012, ASB2014-9396) rebutted these comments and conclusions.
Epidemiology DART/ED Publications
Author(s)
Year
Study title
Arbuckle, T. E. Lin, Z.
Mery, L. S.
2001
An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population
Environmental Health Perspectives Volume: 109
Pages: 851-857 ASB2012-11545
Abstract*
The toxicity of pesticides on human reproduction is largely unknown—particularly how mixtures of pesticide products might affect fetal toxicity. The Ontario Farm Family Health Study collected data by questionnaire on the identity and timing of pesticide use on the farm, lifestyle factors, and a

complete reproductive history from the farm operator and eligible couples living on the farm. A total of 2,110 women provided information on 3,936 pregnancies, including 395 spontaneous abortions. To explore critical windows of exposure and target sites for toxicity, we examined exposures separately for preconception (3 months before and up to month of conception) and postconception (first trimester) windows and for early (< 12 weeks) and late (12–19 weeks) spontaneous abortions. We observed moderate increases in risk of early abortions for preconception exposures to phenoxy acetic acid herbicides [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1–2.1], triazines (OR = 1.4; 95% CI, 1.0–2.0), and any herbicide (OR = 1.4; 95% CI, 1.1–1.9). For late abortions, preconception exposure to glyphosate (OR = 1.7; 95% CI, 1.0–2.9), thiocarbamates (OR = 1.8; 95% CI, 1.1–3.0), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0–2.4) was associated with elevated risks. Postconception exposures were generally associated with late spontaneous abortions. Older maternal age (> 34 years of age) was the strongest risk factor for spontaneous abortions, and we observed several interactions between pesticides in the older age group using Classification and Regression Tree analysis. This study shows that timing of exposure and restricting analyses to more homogeneous endpoints are important in characterizing the reproductive toxicity of pesticides.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide.
Relevance of study:
Not relevant (Study design is not suitable for assessment
of glyphosate exposure).
Klimisch code:
3
Additional comments:
Pre-conception glyphosate exposure odds ratio for spontaneous abortion is considered of borderline significance (OR = 1.4). Post-conception glyphosate exposure was not associated
with spontantous abortin (OR = 1.1). Authors note multiple limitations of the study relating to exposure, likely misclassification of pesticides and correct assignment of exposure window to pre- or/and post-conception
OFFHS information gathering methodology has high potential recall bias. Blair and Zahm (1993, ASB2012-11567) report 60 % accuracy when comparing self reported pesticide usage with purchasing records.
OFFHS relied exclusively on maternal self-reports of adverse pregnancy outcomes, not all of which were confirmed via medical or other records.
Three highly relevant confounding factors were not considered in the OFFHS questionnaire: history of previous spontaneous abortion(s), maternal age and smoking.
Response summarized from Williams et al. (2012, ASB2012-12052)
395 spontaneous abortions were reported out of 3936 pregnancies; rate of spontaneous aborting in Arbuckle et al. (2001, ASB2012-11545) was 10 %.
The baseline rate of spontaneous abortions in the general populations is much higher, ranging from 12 % to 25 %.
Recall bias is reflected in the recall of spontaneous abortion over the previous 5 years (64 % of all

spontaneous abortions reported) being much higher than the recall of those greater than 10 years prior to the survey (34% of all spontaneous abortions reported).
Substantial exposure misclassification may have occurred (pre- versus post-conception) due to likely author extrapolation of exposure data.
Strong confounding variables are not apparent in previous data analyses published by the authors of the OFFHS, and therefore odds ratios are crude.
Published results fail to demonstrate a significant association of glyphosate exposure spontaneous abortion risk and therefore must be considered cautiously.
Author(s)
Year
Study title
Savitz, D.A. Arbuckle, T.
Kaczor, D. Curtis, K.M.
1997
Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology
Volume: 146, Number: 12, Pages: 1025-1036 ASB2012-12022
Abstract*
Potential health effects of agricultural pesticide use include reproductive outcomes. For the Ontario Farm Family Health Study, the authors sampled Ontario farms from the 1986 Canadian Census of Agriculture, identified farm couples, and obtained questionnaire data concerning farm activities, reproductive health experience, and chemical applications. Male farm activities in the period from 3 months before conception through the month of conception were evaluated in relation to miscarriage, preterm delivery, and small-for- gestational-age births. Among the 1,898 couples with complete data (64 % response), 3,984 eligible pregnancies were identified. Miscarriage was not associated with chemical activities overall but was increased in combination with reported use of thiocarbamates, carbaryl, and unclassified pesticides on the farm. Preterm delivery was also not strongly associated with farm chemical activities overall, except for mixing or applying yard herbicides (odds ratio = 2.1, 95 % confidence interval 1.0-4.4), Combinations of activities with a variety of chemicals (atrazine, glyphosate, organophosphates, 4-[2,4-dichlorophenoxy] butyric acid, and insecticides) generated odds ratios of two or greater. No associations were found between farm chemicals and small-for-gestational-age births or altered sex ratio. Based on these data, despite limitations in exposure assessment, the authors encourage continued evaluation of male exposures, particularly in relation to miscarriage and preterm delivery.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not Reliable
Comment:
No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide. Due to study design and evaluation methods, study results are not reliable.
Relevance of study:
Not Relevant (Study design is not suitable for
assessment of glyphosate exposure).
Klimisch code:
3

Additional comments:
Glyphosate is one of many pesticides mentioned in three epidemiological reports that examine possible links between on-farm pesticide use and reproductive outcomes. All three reports - Savitz et al. (1997, ASB2012-12022), Curtis et al. (1999, cited in ASB2012-11545) and Arbuckle et al. (2001, ASB2012-11545) - use data from the Ontario Farm Family Health Study (OFFHS) (Arbuckle 1994, cited in ASB2012-11545). Savitz et al. (1997, ASB2012- 12022) investigated associations between reported pesticide use by males and pregnancy outcomes, specifically: miscarriage, pre-term delivery and small-for-gestational-age birth. Curtis et al. (1999, cited in ASB2012-11545) studied whether reported pesticide use by males or females was associated with delayed pregnancy, while Arbuckle et al. (2001, ASB2012- 11545) looked for associations between reported pesticide use and spontaneous abortion.
In the study by Savitz et al. (1997, ASB2012-12022), a number of specific pesticides had weak statistical associations with miscarriages and pre-term deliveries, but pesticides tended not to be associated with small for gestational age births. There were no statistically significant findings for glyphosate. In the study by Curtis et al.(1999, cited in ASB2012- 11545), for farms on which glyphosate was used, there was no significant association for women being engaged in pesticide activities. For men, glyphosate use was associated with a slight, but statistically significant, decrease in time to pregnancy. The authors dismissed this finding, which was contrary to their hypothesis that pesticide exposure delayed pregnancy, as probably due to uncontrolled factors or chance. Arbuckle et al. (2001, ASB2012-11545) found that reported preconception use of phenoxyacetic acids, triazines, glyphosate, and thiocarbamates were weakly, but statistically significantly, associated with spontaneous abortions. Post conception reported use was not associated with increased risk. The authors characterized the associations between pesticides and spontaneous abortions as "hypothesis generating" pending confirmation from other epidemiologic studies.
These studies are not convincing evidence of a relationship between glyphosate exposure and adverse pregnancy outcomes for a number of reasons:
There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. For example, according to a study by the National Cancer Institute, self-reports of pesticide usage were found to be only 60 percent accurate when compared with purchasing records (Blair & Zahm 1993, ASB2012- 11567). Further increasing the potential for inaccuracy is the fact that study subjects were only asked about pesticide use for the 5 years before the OFFS survey. These responses were
assumed to be applicable to the entire farming careers of study subjects, an assumption inconsistent with changes in agricultural practice. Lastly, basing exposure estimation on questionnaire responses has the potential to be influenced by what epidemiologists call "recall bias." This refers to the likelihood that families that experienced an adverse reproductive outcome are more likely to remember use of certain pesticides than families that had only normal births.
The most widely used pesticides, like atrazine, glyphosate, and 2,4-D, are most easily recalled and most likely to be over-reported.
The OFFHS study relied exclusively on maternal self-reports of adverse pregnancy outcomes with no medical or other validation. Generally, scientists place less confidence in reports of health outcomes that are not validated with medical records.
A confounding factor is a cause of a disease that is correlated with another exposure being studied. Failure to control confounding factors, especially those that are strong causes of a disease, can create spurious associations between benign exposures and diseases. In the Arbuckle study, there were at

least three important potential confounding factors that were not controlled: history of previous spontaneous abortion, maternal age, and smoking. Even a weak correlation between these factors and use (or recall of use) of pesticides would produce spurious associations. In addition, in all three studies, the authors did not control the putative effect of one pesticide for the putative effects of other pesticides. So, for example, since farmers tend to use 4 or more pesticides each year, a disease that is associated with one pesticide will likely be associated with all, since their use patterns are correlated. In the absence of an analysis that controls for multiple pesticides, the best that can be said is that the findings for any individual pesticide might be due to its correlation with another pesticide.

In summary, three publications based on data collected in the OFFHS found associations between several pesticides and various adverse reproductive outcomes. There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. Glyphosate was not significantly associated with adverse reproductive outcomes in two of these studies (Savitz et al. 1997, ASB2012-12022, Curtis et al. 1999, cited in ASB2012-11545). Glyphosate and other pesticides were weakly associated with spontaneous abortion in the study by Arbuckle (2001, ASB2012-11545). However, the author did not control for important personal confounding factors or for multiple exposures and no actual exposure data was used, casting doubt on the validity of the findings in this study.

Biomonitoring data for glyphosate, collected as part of the Farm Family Exposure Study (FFES), provide assurance that human health effects related to glyphosate exposure are very unlikely. In the FFES, researchers from the University of Minnesota collected 5 days of urine samples from 48 farm families before, during, and after a glyphosate application (Mandel et al., 2005, ASB2012-11893, accepted for publication). Only 60% of farmers showed detectable exposure to glyphosate, with a 1 part per billion limit of detection, and the maximum estimated absorbed dose was 0.004 mg/kg (Acquavella et al., 2004, ASB2012- 11528). For farmers who apply glyphosate 10 times per year for 40 years, this maximum dose is more than 30,000-fold less than the EPA reference dose1 of 2 mg/kg/day. For spouses, only 4% showed detectable exposures and the maximum systemic dose was 0.00004 mg/kg/day. Since glyphosate is not a reproductive toxic in high dose animal studies and since actual exposures on farms are so low, it is very unlikely that glyphosate would cause adverse reproductive outcomes for farmers or their spouses.

Author(s)

Year

Study title

Garry, V. F. Harkins, M. E. Erickson, L. L.
Long-Simpson, L. K. Holland, S. E. Burroughs, B. L.

2002

Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA.

Environmental Health Perspectives Volume: 110

Pages: 441-449 ASB2012-11626

Abstract*

We previously demonstrated that the frequency of birth defects among children of residents of the Red River Valley (RRV), Minnesota, USA, was significantly higher than in other major agricultural regions of the state during the years 1989-1991, with children born to male pesticide applicators having the highest risk. The present, smaller cross-sectional study of 695 families and 1,532 children,

conducted during 1997-1998, provides a more detailed examination of reproductive health outcomes in farm families ascertained from parent- reported birth defects. In the present study, in the first year of life, the birth defect rate was
31.3 births per 1,000, with 83% of the total reported birth defects confirmed by medical records. Inclusion of children identified with birth or developmental disorders within the first 3 years of life and later led to a rate of 47.0 per 1,000 (72 children from 1,532 live births). Conceptions in spring resulted in significantly more children with birth defects than found in any other season (7.6 vs. 3.7%). Twelve families had more than one child with a birth defect (n = 28 children). Forty-two percent of the children from families with recurrent birth defects were conceived in spring, a significantly higher rate than that for any other season. Three families in the kinships defined contributed a first-degree relative other than a sibling with the same or similar birth defect, consistent with a Mendelian inheritance pattern. The remaining nine families did not follow a Mendelian inheritance pattern. The sex ratio of children with birth defects born to applicator families shows a male predominance (1.75 to 1) across specific pesticide class use and exposure categories exclusive of fungicides. In the fungicide exposure category, normal female births significantly exceed male births (1.25 to 1). Similarly, the proportion of male to female children with birth defects is significantly lower (0.57 to 1; p = 0.02). Adverse neurologic and neurobehavioral developmental effects clustered among the children born to applicators of the fumigant phosphine (odds ratio [OR] = 2.48; confidence interval [CI], 1.2-5. 1). Use of the herbicide glyphosate yielded an OR of 3.6 (CI, 1.3-9.6) in the neurobehavioral category. Finally, these studies point out that a) herbicides applied in the spring may be a factor in the birth defects observed and b) fungicides can be a significant factor in the determination of sex of the children of the families of the RRV. Thus, two distinct classes of pesticides seem to have adverse effects on different reproductive outcomes. Biologically based confirmatory studies are needed.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Epidemiological study with some methodological / reporting deficiencies (selection of study subjects, no information about exposure duration, exposure concentration, pesticide use frequency).
Relevance of study:
Not relevant because of methodological deficiencies.
Klimisch code:
3
Additional comments:
Response 1 – summary from Mink et al. (2011) (ASB2012-11904)
Publication reports on different classes of pesticides and several birth defects and developmental outcomes.
Paternal use of glyphosate was associated with parent-reported ADD/ADHD in children (OR = 3.6). Six out of 14 children with parent reported ADD/ADHD also reported exposure to glyphosate. Diagnoses of ADD/AHDH were not all confirmed. However, overall rate for the sample population (14/1532) was well below ADD/ADHD rates for the general population (7%).
Variables in statistical model analyses were not reported.
Response 2 – summary from Williams et al. (2012, ASB2012-12052)
Health data obtained via parent reporting for 695 families via written questionnaire and confirmed

where possible.
Pesticide use information obtained initially via telephone then followed up by written questionnaire.
Reproductive health outcomes for births occurring between 1968 and 1998 were obtained for 1532 live births. Over half the births occurred prior to 1978, approximately 20 years after study initiation.
All pesticide use classes (herbicide only; herbicide and insecticide; herbicide, insecticide and fungicide; herbicide, insecticide and fumigant) were associated with birth defects.
Authors state neurobehavioral disorder would not be considered based lack consistent diagnoses. However, a detailed analysis was conducted for ADD/ADHD.
43% (6/14) parent reported children with ADD/ADHD were associated with glyphosate formulation use.
14 cases of ADD/ADHD reported out of 1532 live births, which is substantially lower that the diagnosed incidence of 7% for the general population.
No conclusions regarding glyphosate exposure and ADD/ADHD outcome can be drawn. No other glyphosate specific data were reported.
Author(s)
Year
Study title
Garry, V.F.,
Holland, S.E., Erickson, L.L., Burroughs, B.L.
2003
Male Reproductive Hormones and Thyroid Function in Pesticide Applicators in the Red River Valley of Minnesota Journal of Toxicology and Environmental Health, Part A Volume: 66, Number: 11, Pages: 965-986
ASB2012-11627
Abstract*
In the present effort, 144 pesticide applicators and 49 urban control subjects who reported no chronic disease were studied. Applicators provided records of the season's pesticides used by product, volumes, dates, and methods of application. Blood specimens for examination of hormone levels were obtained in summer and fall. In the herbicide-only applicator group, significant increases in testosterone levels in fall compared to summer and also elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the fall were noted. With respect to fungicide use, in an earlier cross-sectional epidemiologic study, data demonstrated that historic fungicide use was associated with a significant alteration of the sex ratio of children borne to applicators. As before, among current study subjects it was noted
that historic fungicide use was associated with increased numbers of girls being born. Lower mean total testosterone concentrations by quartile were also correlated with increased numbers of live-born female infants. A downward summer to fall seasonal shift in thyroid- stimulating hormone (TSH) concentrations occurred among applicators but not among controls. Farmers who had aerial application of fungicides to their land in the current season showed a significant shift in TSH values (from 1.75 to 1.11 mU/L). Subclinical hypothyroidism was noted in 5/144 applicators (TSH values >4.5 mU/L), but not in urban control subjects. Based on current and past studies, it was concluded that, in addition to pesticide exposure, individual susceptibility and perhaps economic factors may play a supporting role in the reported results.
* Quoted from article
Klimisch evaluation
Reliability of study:

Not reliable
Comment:
Epidemiological study with some methodological / reporting deficiencies (e.g. selection of control subjects/samples, no details of exposure).
Documentation is insufficient for assessment.
Relevance of study:
Not relevant for glyphosate (due to reliability; in addition, no direct assessment of glyphosate exposure
was made).
Klimisch code:
3
Additional comments:
The publication brings little information on endpoints attributable to glyphosate.
Given the subjects were pesticide applicators, little can be drawn from the findings other than perhaps certain endpoints which may be associated with this specific occupation exposed to multiple chemical substances.
Of the 136 participants volunteering blood samples, only one individual (subject D) was noted with one abnormally high thyroid hormone levels associated with glyphosate use; thyroid stimulating hormone (FSH) was about double the normal range in the fall and thyroid stimulating hormone (TSH) higher than normal in the summer.
Another individual (subject E) had abnormally high TSH levels associated with multiple pesticide usage of 12 different active ingredients.
Author(s)
Year
Study title
Bell, E.M.
Hertz-Picciotto, I. Beaumont, J.J.
2001
A Case-Control Study of Pesticides and Fetal Death Due to Congenital Anomalies
Epidemiology
Volume: 12, Number: 2, Pages: 148-156 ASB2012-11559
Abstract*
We examined the association between late fetal death due to congenital anomalies (73 cases, 611 controls) and maternal residential proximity to pesticide applications in ten California counties. A statewide database of all applications of restricted pesticides was linked to maternal address to determine daily exposure status. We examined five pesticide chemical classes. The odds ratios from logistic regression models, adjusted for maternal age and
county, showed a consistent pattern with respect to timing of exposure; the largest risks for fetal death due to congenital anomalies were from pesticide exposure during the 3rd– 8th weeks of pregnancy. For exposure either in the square mile of the maternal residence or in one of the adjacent 8 square miles, odds ratios ranged from 1.4 (95 % confidence interval = 0.8 – 2.4) for phosphates, carbamates, and endocrine disruptors to 2.2 (95 % confidence interval =
1.3 – 3.9) for halogenated hydrocarbons. Similar odds ratios were observed when a more restrictive definition of nonexposure (not exposed to any of the five pesticide classes during the 3rd– 8th weeks of pregnancy) was used. The odds ratios for all pesticide classes increased when exposure occurred within the same square mile of maternal residence.

* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Epidemoliogical study with methodological deficiencies (e.g. glyphosate was included in the pesticide class of phosphates, thiophosphates, phosphonates; no differentiation between single and multiple exposures).
Relevance of study:
Not relevant (No glyphosate-specific results.)
Klimisch code:
3
Additional comments:
Response – summary from Williams et al. (2012, ASB2012-12052)
Classes of pesticides were evaluated in this study, with glyphosate included as one of 47 active ingredients in the broad category of "phosphates/thiophosphates/phosphonates".
Of the 47 active ingredients, many were organophosphate insecticide with known mammalian modes of action. The glyphosate mode of action is on the EPSPS enzyme in plants, which is not present in the animal kingdom.
Given the very low volatility of glyphosate and the low potential for inhalation exposures to aerosol sprays up to two miles away from the subjects, systemic doses to glyphosate would be considered negligible.
Mose et al., (2008, ASB2012-11914) demonstrated a low perfusion rate of glyphosate across the placenta. Coupled with the known low dermal and gastrointestinal absorption of glyphosate and the rapid elimination of systemic doses of glyphosate in the urine, human in utero exposures would be extremely limited.
The reported congenital anomalies associated with fetal death in Bell et al. (2001, ASB2012- 11559) can in no way be linked to glyphosate exposure.
Author(s)
Year
Study title
Aris, A. Leblanc, S.
2011
Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada.
Reproductive toxicology Volume: 31, Pages: 528-533 ASB2012-11547
Abstract*
Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3- methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without

pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Exact levels of PAGMF, glyphosate or AMPA in the diets were not determined. It is not clear if the measured concentrations could have been resulted from other exposure routes.
Relevance of study:
Relevant with restrictions (Provides real life actual exposure concentrations in humans. Data are limited due to the absence of any information on applied
pesticides, application rates, etc.).
Klimisch code:
3
Author(s)
Year
Study title
Benítez-Leite,
S. Macchi, ML and Acosta, M.
2009
Malformaciones congénitas asociadas a agrotóxicos. Arch Pediatr Urug
Volume: 80, Number: 3, Pages: 237-247 ASB2012-11563
Abstract*
Introduction: exposure to pesticides is a known risk for human health. This paper describes the relationship between parental exposure and congenital malformations in the newborn. Objective: to study the association between exposure to pesticides and congenital malformations in neonates born in the Regional Hospital of Encarnación, in the Department of Itapúa, Paraguay. Materials and methods: a prospective case-controlled study carried out from March 2006 to February 2007. Cases included all newborns with congenital malformations, and controls were all healthy children of the same sex born immediately thereafter. Births outside the hospital were not counted. Exposure was considered to be any contact with agricultural chemicals, in addition to other known risk factors for congenital defects. Results: a total of 52 cases and 87 controls were analyzed. The average number of births each month was 216. The significantly associated risk factors were: living near treated fields (OR 2,46, CI95% 1,09-5,57, p<0,02), dwelling located less than 1 km (OR 2,66, CI95% 1,19-5,97, p<0,008), storage of pesticides in the home (OR 15,35, CI95% 1,96-701,63), p<0,003), direct or accidental contact with pesticides (OR 3,19, CI95% 0,97-11,4, p<0,04), and family history of malformation (OR 6,81, CI95% 1,94-30,56, p<0,001). Other known risk
factors for malformations did not show statistical significance. Conclusion: the results show an association between exposure to pesticides and congenital malformations. Further studies are required to confirm these findings.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:

Study design of epidemiological study for developmental toxicity insufficient for assessment, as well as methological and reporting deficiencies (no assessment to which pesticides / active substances the mothers were exposed, use frequency not specified, selection of control group after study period is questionable, no information on exposure situation of mother for this control group assessed, etc.).
Relevance of study:
Not relevant (The exposure to several pesticides was assessed in general, but no pesticide or active substance,
including glyphosate, was specified or assessed).
Klimisch code:
3

B.6.7.1 Published data on neurotoxicity
Several publications over the last decade have evaluated glyphosate with respect to neurotoxicity endpoints. Three papers report a total of two human cases of Parkinson's disease. The first case followed acute exposure to a glyphosate formulation while spraying a garden (Barbosa et al., 2001, ASB2012-11557; da Costa et al, 2003, ASB2012-11598). The second case reported chronic exposures to a factory worker in China, where a variety of pesticides including glyphosate were produced (Wang et al, 2011, ASB2012-12047). Several questions arise in attempting to link glyphosate exposures with each case of Parkinson's disease. Firstly, significant systemic exposures to glyphosate in each instance are questionable, given the poor dermal absorption and low volatility of the compound. Secondly, if glyphosate was a causative agent of this fairly common disease, a significant number of cases associated with either acute and/or chronic exposures would be evident. Glyphosate formulations are sometimes readily accessible for suicide attempts, which are usually unsuccessful, as less than 10% of glyphosate self administered ingestions result in death. No reports of Parkinson's disease in survivors following very acute ingestions of glyphosate products have been documented. Glyphosate has been manufactured and widely used in agriculture and consumer markets for approximately 40 years, so a single case of a pesticide factory worker developing Parkinson's disease, while unfortunate, does not constitute cause and effect; there is no evidence of a higher frequency of Parkinson's disease in glyphosate production workers.
Multiple long term animal studies with glyphosate have failed to demonstrate any evidence of neurotoxicity, and certainly have not shown evidence of Parkinson's-like abnormalities. While some studies have suggested statistical associations with general pesticide exposure or general insecticide or herbicide exposure (Engel et al., 2001, ASB2012-11612), there is no evidence suggesting a specific association between glyphosate and Parkinson's disease. In the largest study to date of US Farmers (Agricultural Health Study), no increased risk of Parkinson's disease was found in association with reported glyphosate use (Kamel et al., 2007, ASB2012-11862). Human non-cancer epidemiologic outcomes related to glyphosate have recently been reviewed (Mink et al. 2011, ASB2012-11904), and there is no convincing
evidence for an increased incidence of Parkinson's disease or other neurological disorders in individuals reporting glyphosate exposure.
Several publications open with the premise that pesticide exposures are linked with Parkinson's disease, and then proceed to report a priori research linking glyphosate with a measurable endpoint. This endpoint is then extrapolated to link with Parkinson's disease in humans. Despite the lack of compelling human associations between glyphosate exposure and Parkinson's disease, such research continues to be published. Astiz et al., (2009, ASB2012-11549), Negga et al. (2011, ASB2012-11923) and Gui et al. (2012, ASB2012-

11835) all conducted glyphosate research in the above mentioned manner, all in very different test systems. Negga et al. (2011, ASB2012-11923) notes neurodegeneration in Caenorhabditis elegans worms following exposure to glyphosate (trimesium form, which has a different toxicology profile than glyphosate) uses concentrations equal to the LD25, LD50 and LD75, or actual concentrations of glyphosate of 3 to 10 percent, i.e.- the high concentration is approximately 10-fold higher than concentrations applied directly in the field. The relevance of such high-dose exposures to the trimesium salt in this experimental model to human Parkinson's disease is highly questionable and irrelevant to the Annex 1 renewal of glyphosate technical acid. Astiz et al. (2009, ASB2012-11549) and Gui et al. (2012, ASB2012-11835) both affirm their test models (in rats and in PC-12 cells respectively) for evaluating neurodegenerative disorders, then directly link their research results to Parkinson's disease in humans; these two studies are addressed below.

Cole et al. (2004, ASB2012-11594) evaluated 15 different pesticides for neurotoxic end points in C. elegans with analytical grade active ingredients, noting reduced cholinesterase for pesticides with this mode of action, but not glyphosate. Interestingly, the authors report a low pH effect resulting in reduced cholinesterase activity in the high dose of glyphosate and a plant growth promoter. Glyphosate formulations contain salt forms of glyphosate, not the technical acid and thus do not have a low pH. Additionally, human incidents of self induced glyphosate poisonings do not report the common symptoms of acute acetylcholinesterase inhibition; salivation, lacrimation, urination and defecation (SLUD).

After preparation of the original DAR in 2013, the following publications became available:

Cattani et al. (2014, ASB2014-3919) studied neurotoxic effects of the formulation Roundup in the hippocampus on immature rats following acute (30 min) and chronic (pregnancy and lactation) exposure. Results showed that acute exposure to Roundup increased CA2+ influx leading to oxidative stress and neural cell death. Taken together, the results demonstrate that Roundup might lead to excessive extracellular glutamate levels and to glutamate excitotoxicity and oxidative stress in rat hippocampus.

is ascribed to a disturbance of gut microflora rather than to a direct effect on the neuronal system. Thus, there assumptions are based on certain in vitro results (e.g., Shehata et al., 2013, ASB2012-16301) that are discussed in depth in section B.6.8.3.3. The authors propose dietary and lifestyle changes to prevent autism.

Narayan et al. (2013, ASB2014-9620) reviewed literature on Parkinson`s disease. The authors conclude that houshold use of organophosphorus pesticides is associated with increased risk of developing Parkinson`s disease. Glyphosate is considered by these authors to be an organophosphorus pesticide.

McConnell et al. (2012, ASB2014-9615) tested multi-well microelectrode arrays for neurotoxicity screening. In result of these tests glyphosate was considered negative concerning neurotoxic effects.

LeFew et al. (2013, ASB2014-9608) evaluated microelectrode array data using Bayesian modeling as an approach to screening and prioritization for neurotoxicity testing. Glyphosate was identified to be negative in these neurotoxicity tests.

Further studies are reported more detailed:

Author(s)
Year
Study title
Barbosa, E.R.
Leiros da Costa M.D. Bacheschi, L.A. Scaff M.
2001

Parkinsonism After Glycine-Derivate Exposure Movement Disorders
Volume: 16, Number: 3, Pages: 565-568 ASB2012-11557
Abstract*
This 54-year-old man accidentally sprayed himself with the chemical agent glyphosate, a herbicide derived from the amino acid glycine. He developed disseminated skin lesions 6 hours after the accident. One month later, he developed a symmetrical parkinsonian syndrome. Two years after the initial exposure to glyphosate, magnetic resonance imaging revealed hyperintense signal in the globus pallidus and substantia nigra, bilaterally, on T2- weighted images. Levodopa/benserazide 500/125 mg daily provided satisfactory clinical outcome.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not assignable
Comment:
Medical case report, single incident
Relevance of study:
Relevant with restrictions (Data are limited due to the absence of any information on purity and application concentrations of glyphosate formulation, as well as co- formulations.)
Klimisch code:
4
Author(s)
Year
Study title
Wang, G., Xiao-Ning, F., Yu-Yan, T., Qi,
Ch., Shen-Di, CH.
2011
Parkinsonism after chronic occupational exposure to glyphosate.
Parkinsonism and related disorders ASB2012-12047
Abstract*
Here we report a patient with parkinsonism following chronic occupational exposure to glyphosate. A previously healthy 44-year-old woman presented with rigidity, slowness and resting tremor in all four limbs with no impairment of short-term memory, after sustaining long term chemical exposure to glyphosate for 3 years as a worker in a chemical factory. The chemical plant produced a range of herbicides including: glyphosate, gibberilins, and dimethyl hydrogen phosphite; however, the patient worked exclusively in the glyphosate production division. She only wore basic protection such as gloves or a face mask for 50 h each week in the plant where glyphosate vapor was generated. She frequently felt weak. … Physical examination revealed a parkinsonian syndrome. …
We cannot exclude the coincidence of idiopathic PD with exposure to glyphosate on our patient. …
* Quoted from article
Klimisch evaluation
Reliability of study:
Not assignable
Comment:
Medical case report, single incident
Relevance of study:
Relevant with restrictions

Klimisch code:
4
Author(s)
Year
Study title
Astiz, M. de Alaniz, M.J.
Marra, C.A.
2009
Effect of pesticides on cell survival in liver and brain rat tissues
Ecotoxicology and Environmental Safety Volume: 72, Pages: 2025-2032, ASB2012-11549
Abstract*
Pesticides are the main environmental factor associated with the etiology of human neurodegenerative disorders such as Parkinson's disease. Our laboratory has previously demonstrated that the treatment of rats with low doses of dimethoate, zineb or glyphosate alone or in combination induces oxidative stress (OS) in liver and brain. The aim of the present work was to investigate if the pesticide-induced OS was able to affect brain and liver cell survival. The treatment of Wistar rats with the pesticides (i.p. 1/250 LD50, three times a week for 5 weeks) caused loss of mitochondrial transmembrane potential and cardiolipin content, especially in substantia nigra (SN), with a concomitant increase of fatty acid peroxidation. The activation of calpain apoptotic cascade (instead of the caspase-dependent pathway) would be responsible for the DNA fragmentation pattern observed.
Thus, these results may contribute to understand the effect(s) of chronic and simultaneous exposure to pesticides on cell survival.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Unsuitable test system (i.p exposure route is not relevant for human exposure). No information on purities of test substances used. Small group size (4
males/dose group), reporting deficiencies
Relevance of study:
Not relevant (intraperitoneal injection is a non-relevant
route of exposure for humans)
Klimisch code:
3
Additional comments
This non-guideline study utilized very small group numbers (4 rats/group) and therefore is not sufficiently robust to appropriately identify changes attributable to the test material administration. The test materials are not well described, without indication of whether a glyphosate salt form or acid was used and purity was not reported.
The publication focuses on the post necropsy data analysis and reporting. Data on animal husbandry, clinical observations, feed and water intake, weekly body weight were not reported, but the authors note there were no adverse observations.
No statistically significant effects were noted for liver endpoints, yet the liver is in close proximity to test material administration via intraperitoneal injection.

Statistically significant effects were noted for brain tissue endpoints in the substantia nigra and cerebral cortex. However, there is a lack of biological plausibility for brain exposures to glyphosate, given the necessity to pass the blood-brain barrier and the known rapid elimination kinetics of this polar molecule via urine.

Author(s)
Year
Study title
Gui, Y.X.,
Fan, X.N.,
Wang, H.M.,
Wang, G.,
Chen, S.D.
2012
Glyphosate induced cell death through apoptotic and autophagic mechanisms.
Neurotoxicology and teratology
Volume: not specified (accepted manuscript) Pages: not specified
ASB2012-11835

Abstract*
Herbicides have been recognized as the main environmental factor associated with human neurodegenerative disorders such as Parkinson's disease (PD). Previous studies indicated that the exposure to glyphosate, a widely used herbicide, is possibly linked to Parkinsonism, however the underlying mechanism remains unclear. We investigated the neurotoxic effects of glyphosate in differentiated PC12 cells and discovered that it inhibited viability of differentiated PC12 cells in dose- and time-dependent manners. Furthermore, the results showed that glyphosate induced cell death via autophagy pathways in addition to activating apoptotic pathways. Interestingly, deactivation of Beclin-1 gene attenuated both apoptosis and autophagy in glyphosate treated differentiated PC12 cells, suggesting that Beclin-1 gene is involved in the crosstalk between the two mechanisms.
* Quoted from article

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Documentation insufficient for assessment (not clearly stated dose levels and duration of exposure, as well as treatment conditions for all tests. In addition, tested doses were much higher than real in vivo concentrations).
Relevance of study:
Not relevant (Due to reliability)
Klimisch code:
3
Additional comments:
In this paper, the authors apply glyphosate to adrenal cancer cells in culture at concentrations sufficient to cause cell death. Two major interacting pathways leading to cell death (autolysis and apoptosis) are evaluated, and the results are hardly surprising - the cells do indeed die via known mechanisms leading to cell death. The authors use these observations, and the fact that Parkinson's disease involves the death of certain nerve cells in the brain, to try and create a link between glyphosate and Parkinson's disease. There are, however, many problems with this extrapolation.

The cells used are not the neurons involved in Parkinson's, but rather a cell line derived from an adrenal gland cancer (pheochromocytoma), and the doses used are very high- the high dose killed nearly 50 % of cells in 72 hours, and the low dose was ¼ this level. The high dose equates to approximately 1/10 the concentration applied directly in the field, and is far higher than any internal glyphosate concentration that could ever occur following glyphosate use. A sufficiently high dose of every substance will kill cells - but this does not mean that every substance causes Parkinson's disease.
Unprotected cells in culture are highly susceptible to changes in pH and other non-specific effects, and it is not clear that the researchers assessed or accounted for these possible effects. This being said, the concentrations of glyphosate used (40 mM) are known to kill other cell types in culture (Heu et al., 2012, ASB2012-11844) via induction of apoptosis. Thus, no particular specificity or neuronally-specific susceptibility exists for the cell line tested. While 40 mM glyphosate is toxic to cells in culture, the LD50 in rodents is over 5000 mg/kg and C. elegans will have a 25 % survival following exposure to a 10 % solution of glyphosate. In- vitro results do not appear to reflect in vivo events.
Anadon et al. (2009, ASB2012-11542) dosed rates with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved glyphosate peak modeled plasma concentrations of approximately 5 ug/mL (5 ppm). Assuming linear kinetics, the current maximum allowable EU daily intake (0.3 mg/kg/day) would give an approximated blood concentration of 0.17 ppm (170 ppb). This is conservative, as McQueen et al (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4 % of the current European acceptable daily intake.
The lowest glyphosate concentration used in this experiment is 5mM (830 ppm), or 5000 times higher than the estimated blood concentration following the current EU maximum allowable daily exposure. It is also 166 times higher than the concentrations Anadon et al. (2009, ASB2012-11542) achieved using doses of 400 mg/kg glyphosate. In short, the concentrations used in this work are massively higher than any concentration is blood (let alone brain tissue) that can be achieved following normal human exposures.

B.6.8.3.3 Published data
Urinary concentrations of glyphosate in cattle and other species
Krüger et al. (2013, ASB2013-11599) reported the abundance of glyphosate in the urine of a total of 240 cows from Danmark. From each of eight dairy farms, the same number of 30 cows (15 fresh calving, 15 high yielding cows, i.e., at the top level of milk production) was selected. All these 240 cows excreted glyphosate in the urine, however, at very different concentrations. Urine samples were diluted 1:20 with distilled water and tested for glyphosate by means of an ELISA kit (Abraxis, USA). The limit of detection (LOD) or a limit of quantification (LOQ) were, unfortunately, not mentioned. However, it is stated in the paper that validation of test results had been done by a comparison with GC-MS which is considered a more suitable method. It was mentioned that the correlation coefficient between the two methods was 0.96 and, thus, sufficiently high but this validation data was not shown in this paper. However, for further interpretation of the results, it is assumed that the method was in fact valid and that the measured values were reliable.
It is worth mentioning that the cows (breed not given) were 4 to 7 years old and had an average body weight between 550 – 600 kg and that the total number of cows in the farms ranged from 140 to 400 animals. The average daily milk yield in the different farms ranged from 8.6 to 11.2 kg, for i.e., not very high was representative for countries with modern and efficient agriculture.

Mean urinary glyphosate concentrations differed very much among the eight farms, ranging from 10 ng/mL µg/L up to 103.3 ng/mL µg/L. It is a reasonable assumption that urinary excretion of glyphoate was due to dietary exposure and, thus, detection of glyphosate in the urine of cattle ist not surprising. Residues of glyphosate may occur in feedstuffs for ruminants and, so far the maximum residue limits (MRLs) are not exceeded, are allowed by law and of no concern. (Therefore, the word "contamination" as used by the authors is not correct and somehow misleading.) The reason for the remarkable differences between the farms is unknown but most probably the diet was different, including a high variability in glyphosate residues. It is one of the main deficiencies of the publication that no details on feeding regime have been given and that the diets were not analysed for glyphosate content.

It is interesting to note that the mean urinary concentrations in cows exceeded the mean value in human urines of 0.2 µg/L as found by Hoppe (2013, ASB2013-8037, see B.6.9.3) by at least 50 (up to more than 500) to more than 500 times suggesting higher residues of glyphosate in cattle rations than in human diet. This big difference is also confirmed by comparison to further data on glyphosate findings in human urine samples (see B.6.9.3)

The maximum mean value of ca 103 µg/L can be used to calculate a systemic dose to which the cows in that farm had been exposed to since glyphosate does not accumulate but is rapidly excreted. The study authors have estimated a maximal glyphosate excretion via urine of
3.1 mg/day. If an oral absorption rate of only 20 % is assumed instead of 30 % as used by the study authors (for justification, see B.6.1), the maximum daily oral intake should have been in the magnitude of 15 – 16 mg. This dose might be compared either to the ADI (even though
but it is not clear if a reference dose established for humans is per se also applicable to farm animals) and in particular to ruminants. or to Thus, the caclulated intake was compared to the NOAELs in the subacute studies in cattle mentioned above.

If the first approach is taken, the use of the proposed ADI of 0.5 mg/kg body weight for glyphosate (see Vol. 1, B.6.12) would result in a tolerable intake of 225 mg for a cow of 550 kg body weight. This amount is by about 14 – 15 times higher than the expected maximal exposure of the Danish cows on study or, in other words, the systemic dose in the cow with highest urinary excretion of glyphosate would account for not more than about 7 % of the ADI.

Using the second approach, Thus, the maximum expected systemic dose was better compared to the NOAELs in subacute studies with either the isopropylamine salt of glyphosate (540 mg/kg bw/day according to Rowe et al., 1987, TOX9552424) or a Roundup formulation (400 mg/kg bw/day according to Rowe et al., 1987, ASB2010-8131). For a cow of 550 kg, the latter NOAEL would correspond to a daily intake of 220 g. In the study description (see above), a glyphosate content of 30.5% is mentioned. Thus, a daily glyphosate intake of 67 g can be calculated. If this amount of 67000 mg is compared to the expected maximum systemic exposure of 15 or 16 mg per day in the study in Danish cows, a margin of safety of about 1:4200 would result. Based on these considerations, an impairment of animal health in these Danish cows is very unlikely.

In contrast, the authors reported increased activities of the enzymes creatine kinase (CK), glutamate oxaloacetate transaminase (GOT, synonymous to ASAT), and glutamate dehydrogenase (GLDH) in blood serum but also changes in cholesterol levels and an increase in blood urea concentrations. when compared to (mostly not precisely mentioned) reference values of unknown origin. For this comparison, they used reference values of unknown origin
relationship with the
detection of glyphosate in urine d interpreting the alterations in laboratory
parameters as indicative for liver damage or nephrotoxicity.

Leaving aside the serious methodical deficiencies of the study (e.g., the absence of a control group with no glyphosate residues in the urine), there is no evidence that the altered clinical chemistry parameters in cows were indicative of any health deterioration. There could be many different reasons of such alterations and, taking into account the very low glyphosate concentrations (see above), exposure to glyphosate is not a likely one. Moreover, the statistical correlation between glyphosate excretion in urine and changed clinical chemistry parameters as claimed by the autors was in fact rather poor. (Thus, for the comparison of glyphosate with creatine kinase, an R value of 0.135 was considered in the article as indicative of "positive correlation" which in fact is not the case. In contrast, higher R values of up to 0.809 were obtained when two different clinical chemistry parameters such as zinc and cobalt concentrations were measured and may indicate some correlation between them but apparently not with glyphosate excretion. Unfortunately, due to the way of reporting, the reader may be misleaded here to assume correlation with glyphosate.)
Since, in parallel, the authors claimed very low serum levels of several trace elements such as cobalt or manganese, a possible chelating mode of action of glyphosate was suspected. However, these considerations appear purely speculative, in particular against the background of the very low exposure. Even if glyphosate would have chelating properties, the ingested and absorbed amount is not expected to bind trace elements to such an extent that clinical signs might be expected to occur even though such an effect was suspected by scientists from the Aarhus university uin Danmark (Sørensen et al., 2014, ASB2014-5761).
Again, statistical correlation was rather weak. Indeed, the almost complete absence of the two elements in cattle from all farms rather points to either an analytical problem or to a deficiency in the diet.
Thus, to conclude, the urinary levels in Danish cows might well reflect the abundance of glyphosate residues in their feed. The systemic exposure that may be calculated is very low and no health concern is anticipated. Final conclusion on the clinical chemistry findings is not possible but, on one hand, it seems not proven that they were adverse and, on the other, it is very unlikely that they might be related to glyphosate.
Furthermore, glyphosate was found in the urine of fattening rabbits (n = 77) in the magnitude of about 60 µg/L (mean value, standard deviation showing values ouf up to ca 120 µg/L). Less glyphosate (mean of 20-30 µg/L) was determined in the urine of hares (n = 193). Nothing is known on the origin of these samples and no conclusions can be drawn.
(In human samples that were analysed by the same group, the mean concentration was nearly 2 µg/L with a maximum in the magnitude of 5 µg/L. For details, see section B.6.9.3.) Information on glyphosate residues in the organs of slaughtered cows was also given in this paper and is reported in chapter B.7.
Possible impact on the microflora in ruminant's GIT
A number of papers has been published recently in which a possible causal link of glyphosate exposure and subsequent Clostridium botulinum (C. botulinum) overgrowth with a new disease in cattle is suggested. The scientific background of this assumption is the herbicidal mode of action of glyphosate. In plants, the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP synthase) is inhibited resulting in a lack of formation of aromatic amino acids by the shikimate pathway that is common in the plant kingdom but not does not occur in
animals. However, this pathway is operative in most bacteria and yeast and many protozoan species. Thus, an impact of glyphosate on microflora, e.g., in the intestines, is at least conceivable. In line with that, concern on this issue was expressed by scientists from the Aarhus University in Denmark (Sørensen et al., 2014, ASB2014-5761).

Rodloff and Krüger (2012, ASB2013-13311) hypothesised that an emerging new disease in cattle but also symptoms in a small number of farmers might be caused by Clostridium botulinum. This animal disease of so far unknown etiology and pathogenesis was reported to have occurred from the late 1990ies onwards in cattle mainly from some parts of Germany but, according to the authors, cases had been observed also in France, the Netherlands, and the
U.K. even though references were not given. Clinical signs in cattle are predominately seen in the perinatal period and comprise indigestion with alternating constipation and diarrhea, apathy, ataxia, paralysis, retracted abdomen, breathing difficulties, a decrease in milk yield, and death. In some farmers taking care of affected herds, symptoms such as dizziness, weakness, fatigue, blurred vision, nausea, and difficulties to speak, to swallow and to breathe have been occasionally reported.
The authors suggested a causal link to Clostridium botulinum because these signs and symptoms appear similar to what is known from the rarely occurring cases of botulism in animals and man that are not food-borne (i.e., not caused by acute intoxication with the bacterial toxin). By means of an ELISA (no details given), they have detected Clostridium botulinum neurotoxin (BoNT) in fecal samples (in total in 16 out of 33) obtained from cattle on six German farms where the suspicious clinical signs or even fatalities had been noted but not in a total of 10 samples from two farms without any evidence of this disease. Toxin types were different (A, B, C, D, and E, in various combinations). Health state of the donor animals was not reported. In addition, neurotoxin of different types was found in various organs such as the rumen and the liver in 15 sick cows after slaughter. However, it is not known how many cows were examined in total post mortem and by which method and if they belonged to the group of which fecal samples had been taken before.
In man, 16 out of 77 fecal samples were positive for BoNT that were taken from humans who were reported to have been in close contact with diseased cows. It is not clear if one or more samples were obtained from the same person and, thus, the total number of humans under investigation is not known. Again, health status of the involved people was not reported. With regard to neurotoxin types, mainly type E had been found.
Unfortunately, these numbers do not completely match with a table in the paper in which 12 BoNT-positive human fecal samples in a total number of 33 and 29 BoNT-positive bovine fecal samples out of 118 under examination were mentioned to be obtained from 7 “selected” German cattle farms.
Conclusion by RMS:
This paper does not contribute anything to risk assessment of glyphosate and, in fact, this herbicidal active ingredient was not mentioned therein. However, it is important for understanding of subsequent publications and, therefore, is referred to in this RAR.
In itself, this paper is adequate to suggest a scientific hypothesis, based on some data that might require further research. However, no causal relationship between a new disease in cattle and C. botulinum has been established. C. botulinum neurotoxin could not be quantified. Qualitative detection of the neurotoxin might be also a random co-incidence. The publication is flawed by many reporting deficiencies. In particular, the method by which the neurotoxin was detected and its different types were distinguished, is not described.
In a similar paper, Krüger et al. (2012, ASB2013-13312) reported the abundance of (different types) of the micro-organism C. botulinum itself in 44 out of 196 bovine fecal samples (22.5%) and in 17 out of 77 human fecal samples (22%) but also in silages (9 / 21 = 47%), concentrate feed specimens (4 / 14 = 28.6%), and in all 7 tested house dust specimens from a total of 41 dairy farms in the German Federal State of Schleswig-Holstein. This finding was based on the immunological detection of antigens of C. botulinum by an ELISA technique using polyclonal antibodies. All the cattle had been reported to have shown clinical signs as described above. In addition, in four of the involved humans,

symptoms were claimed to have occurred but without further specification or medical confirmation.
Conclusion by RMS:
Again, this paper, in principle, is not relevant for risk assessment of glyphosate but it is mentioned therein that ingested spores might germinate in the intestinal tract if protective indigenous bacterial flora is lacking (as observed in cases of infant botulism) or altered and this might be linked to the glyphosate hypothesis explained below. Therefore, it is mentioned here despite its many reporting deficiencies. The findings rather point to ubiquitious occurrence of C. botulinum but are not suitable to prove a causal relationship of its abundance to clinical signs or symptoms.
By the same group, it was published that different bacteria such as Enterococcus faecalis, Enterococcus faecium or Bacillus badius were able to inhibit growth of C. botulinum and/or the production of its neurotoxins in vitro whereas other bacterial species did not exhibit such an effect (Shehata et al., 2012, ASB2013-8529). Subsequently, Krüger et al. (2013, ASB2013-8527) reported that glyphosate (analytical grade) and the herbicide Roundup UltraMax® containing 450 glyphosate/mL was able to suppress this antagonizing of effect of Enterococcus species on C. botulinum in vitro. What they actually observed was a growth inhibition of both C. botulinum (and a reduction in neurotoxin type B production) and Enterococcus faecalis by glyphosate and Roundup herbicide but at different concentrations. While growth of E. faecalis was completely inhibited by 0.1 mg glyphosate or Roundup/mL, the same effect on C. botulinum was seen only at a concentration of 1 mg Roundup/mL or of 10 mg glyphosate/mL.
Conclusion by RMS:
This data suggests a different susceptibility of E. faecalis and C. botulinum to cytotoxic effects of glyphosate and a glyphosate-based herbicide in vitro. With regard to C. botulinum, the Roundup UltraMax formulation was more toxic than the active ingredient confirming similar evidence from various fields of toxicological testing. No conclusions can be drawn if similar effects might occur in vivo because the situation in, e.g., the GITs of ruminants or monogastric animals is different with hundreds or thousands of mirobial species co-existing. In any case, even the lowest tested concentration of 0.1 mg/mL appears extremely high if a (maximum) systemic dose of 15 or 16 mg per cow is assumed to result from feeding the animals with a ration containing glyphosate residues (based on 2013, ASB2013-11599, see above). Thus, the possible impact of glyphosate (herbicides) on bacteria due to inhibition of the enzyme EPSP was somehow confirmed in vitro but there is no health concern and no impact on realistic risk assessment.
A different toxicity of Roundup UltraMax® to various microbial species was also observed by Shehata et al. (2013, ASB2012-16301) who measured the effect of different concentrations on 23 bacterial species and strains mostly of chicken origin and also on sporulated Eimeria tenella (i.e., a protozoon in poulty) oocytes in vitro. It is not clear whether the mentioned
concentratios ranging from 0.075 mg to up to 5 (bacteria) or 1.2 (Eimeria tenella) mg per mL are related to the herbicide formulation or had been adjusted to the active ingredient. In general, the authours found lower minimum inhibitory concentrations (0.075 – 0.6 mg/mL) for beneficial bacteria whereas, in contrast, some pathogenic germs such as Clostridium perfringens or several Salmonella species appeared much less sensitive with growth inhition seen only at the highest tested concentration of 5 mg/mL. With regard to Eimeria tenella, the threshold for an effects was around 0.3 mg/mL with a clear effect to be seen at 0.6 mg/mL.
Conclusion by RMS:
Different cytototoxicity of a glyphosate-based herbicide to micro-organisms was confirmed once more and might be due to either the active ingredient or, e.g., a surfactant. (It is not known whether a surfactant was contained.) However, antibiotic activity of the herbicide (expressed in the minimum

inhibitory concentrations) was lower than that of known antibiotics that are used in veterinary medicine. Even the lowest effect concentrations in this study were by far higher than the expected glyphosate concentrations in poultry feed (GTF, 2013; ASB2013-11007) and, thus, must be considered unrealistically high. Furthermore, in vitro exposure of selected indivdual species and strains to a herbicide might be not a good model for complex interactions in GIT of poultry when residues are ingested.

To conclude, a link of glyphosate residues in ruminants diet to a new disease in cattle has not been established and is not likely. Furthermore, there is no convincing proof that clinical signs in cattle (of which the occurrence cannot be doubted) were indeed caused by C. botulinum or its toxins. Meanwhile, a comprehensive case-control study on a possible causal relationship between C. botulinum and that chronic disease in cattle has been conducted in Germany. Preliminary results suggest that this was not the case even though further investigations have been considered necessary. In addition, use of glyphosate on the included farms had no impact on the occurrence of clincial signs (Seyboldt and Hoedemaker, 2014, ASB2014- 10736).

However, because of the growing public concern about this cattle disease especially in Germany and because an effect on micro-organisms due to EPSP inhibition cannot be excluded, the German Federal Institute for Risk Assessment (BfR) has commisioned a study with a glyphosate-based herbicide (containing a surfactant) in an artifical rumen system (RUSITEC) to investigate wheter (1) quantitative composition of ruminal microflora might be compromised and (2) there is evidence of C. botulinum overgrowth. Unfortunately, results of this project were not available when this RAR was finalised (October, 2013) but it

is hoped that they will be published in 2014 have not been published so far. However, an internal research report of the Veterinary High School in Hannover (Riede et al., 2013; ASB2013-14684) has been submitted to the Federal Institute for Risk Assessment and is reported here in brief. Two different experiments were performed. In the first one, the effects of a glyphosate-based herbicide (Plantaclean® XL; 360 g/l glyphosate, containing a

surfactant) on rumen fermentative parameters were studied. Total glyphosate

doses per day were 0.26 or 2.31 mg per fermentation vessel. No major changes in rumen parameters were detected except slight decreases in NH3-N concentrations and increases in isovalerate production in response to the high dosage. There was an increase in (beneficial)

Bifidobacterium spp. but, in general, the microbial communities were not affected. In the second trial, no effects of the herbicide on the growth of C. sporogenes was found that had been artificially added to serve as a surrogate for C. botulinum.

Conclusion by RMS:

This publication cannot be considered to describe a reliable scientific study. First, the analytical data obtained from the piglets appear questionable since no information was given whether the ELISA had been modified for these investigations or validated for analysing tissue samples. No LOD or LOQ was mentioned. For the changing glyphosate content in the diet as claimed by the farmer, there is no confirmation in the publication.

The second main weakness of the study is that only malformed piglets had been investigated for glyphosate concentrations in their organs. Thus, there was no control group to (possibly) prove the hypothesis of a potential correlation.

For the following considerations, such a correlation is unlikely:

• In a multitude of developmental studies and multi-generation studies in rats, no evidence of teratogenicity was obtained. Even in rabbits which proved more vulnerable, developmental effects were confined to exaggerated dose levels causing also clear maternal toxicity (see sction B.6.6). It is

very unlikely that pigs, receiving much lower amounts of glyphosate by ingestion of residues in the diet, should be that much more sensitive and, if so, it is hardly conceivable that such effects would not have become apparent before and also in other countries and on other farms.
• Many different malformations were reported. However, most chemical teratogens produce a specific teratogenic effect or a certain pattern of findings. Moreover, teratogenic effects usually follow a dose response. In this case, the glyphosate concentrations in the organs and tissues were so variable that such a dose response may be excluded.
• Malformations in piglets are quiet frequent and have often a genetic background. Infectious diseases may also play a role. There is no indication in the paper that a differential diagnosis has been considered.
Samsel and Seneff, 2013 (ASB2013-8535) reviewed toxicological literature on glyphosate and concluded that glyphosate inhibits cytochrome P450 enzymes. The authors believe that this activity would result in nearly all diseases as inflammatory diseases, obesity, depression, ADHD, autism, Alzheimer`s disease, Parkinson`s disease, ALS, multiple sclerosis, cancer, cachxia, infertility, developmental diseases, gastrointestinal disorders, heart disease, diabetes. Antoniou et al. (2011, ASB2011-7202) reviewed toxicological literature on Roundup and glyphosate. The authors conclude that Roundup and glyphosate would cause endocrine disruption, damage to DNA, reproductive developmental toxicity, neurotoxicity and cancer as well as birth defects. Many of these effects would be found at very low doses, comparable to levels of pesticide residues found in food and the environment. Mostafalou and Abdollahi (2013, ASB2014-9618) published a review on the relation between pesticides and elevated ranges of a broad range of different diseases. According to the authors pesticides cause diseases as different types of cancers, diabetes, neurodegenerative disorders like Parkinson, Alzheimer and amyotrophic lateral sclerosis, birth defects, and reproductive disorders, resipiratory problems, cardiovascular diseases, chronic nephropaties, autoimmune diseases, chronic fatique syndrome and aging. Mesnage and Seralini (2014, ASB2014-9616) submitted a review on pesticide toxicity and genetically modified organisms (GMOs) which are used in agriculture. The authors propose to pay more attention on the mixtures of pesticides with further substances and to test relevant combinations of pesticides at levels which occur in genetically modified plants. NABU (2011, ASB2012-8016) reviewed some ecological and toxicological literature on glyphosate and formulations. The active substance, metabolites and further substances in the formulations are considered toxic especially for aquatic organisms. They would disturb human cells and the development of vertebrates. In result of resistance of wild plants the amount of glyphosate products is expected to grow in the future. PANAP (2009, ASB2012-8017) reviewed literature on toxicity, environmental effects and environmental fate of glyphosate. The authors conclude that independent scientific studies and poisonings in Latin America are beginning to reveal that use of glyphosate would not be safe.
Antoniou et al. (2010, ASB2012-803) reviewed toxicological and ecological literature on glyphosate and genetically modified Soya. The authors conclude that the toxic activity of glyphosate is increased by the combination with further substances in the formulations. Toxicity was already observed at concentrations which occur in agriculture and environment. The authors conclude that there would be a relation between glyphosate and increased malformations. Furthermore, epidemiologicas studies would demonstrate a relation between glyphosate use and carcinogenicity and genotoxicity. Brändli and Reinacher (2012, ASB2012-804) submitted a short survey on use and health effects of glyphosate. The authors conclude that the use of glyphosate for siccation would be a scandal and would be considered to be bodily injury by negligence.
Greenpeace (2011, ASB2012-810) reviewed literature on ecological and health effects of glyphosate.

The authors conclude that the submitted evidence in this report demonstrates that glyphosate-based products can have advers impacts on human and animal health, and that a review of their safety for human and animal health is urgently needed. The authors demand that no genetically modified glyphosate-tolerant crops should be authorised. They would be linked to unsustainable farming practices that damage the basic natural resources food production is based upon, and their cultivation should be banned.

Altenburger et al. (2012, ASB2014-9176) submitted a review that provides an overview on experimental studies from the past decade that address diagnostic and/or mechanistic questions regarding the combined effects of chemical mixtures using toxicogenomic techniques. By joining established mixture effect models with toxicokinetic and –dynamic thinking the authors suggest a conceptual framework that may help to overcome the current limitation of providing mainly anecdotal evidence on mixture effects.

Furthermore, some studies have been published in which the authors investigate the activity of glyphosate and/or glyphosate formulations on selected biochemical or morphological structures. However, based on the provided information the impact of these results for the in vivo situation of the whole organism of animals or humans with autoregulation and feedback mechanisms is questionable. The dose dependency of the described effects and their importance for real life situations is often not sufficiently discussed by the authors. Many authors conclude that further studies would be necessary.

In some cases the authors compare the toxicity of glyphosate with the toxicity of glyphosate formulations or of surfactants. A frequent result in these cases is a higher toxicity of the formulations or the surfactants. In some further cases only glyphosate formulations have been used in the studies. Some authors use these results for a conclusion concerning the toxicity of the active substance glyphosate but do not consider the activity of surfactants or further substances in the formulation. study the commercial formulation Roundup was used. Therefore, the results can not be attributed to the substance glyphosate only. Hedberg et Wallin, 2010 (ASB2014-7494) studied the effects of glyphosate, Roundup and further substances on intracellular transport in Xenopus laevis. The chemicals inhibited retrograde transport of melanosomes in the range of 0.5 – 5 mM. Cellular morphology and localization of microtubules and actin filamentes were affected. The effects are pH-dependent. El-Shenawy, 2009 (ASB2012-11611) compared the cytotoxicity of Roundup and the active substance glyphosate. Male rats were i. p. treated with Roundup or glyphosate. The results characterize Roundup as a stronger antioxidant than the active substance glyphosate itself. Caglar and Kolankaya, 2008 (ASB2012-11580) treated rats with formulation Roundup orally during 5 and 13 weeks and studied hepatotoxicity. The authors concluded that high doses of Roundup can be a potential riks for human health. Modesto and Martinez (2010, ASB2012-811) studied effects of Roundup Transorb on fish. They observed haematologic alterations and effects on antioxidant defenses and on acetylcholinesterase activity.

Zhao et al. (2013, ASB2014-9645) investigated the effect of different doses of glyphosate on apoptosis and expression of androgen-binding protein and vimentin mRNA in mouse Sertoli cells. The authors conclude that glyphosate can cause cellular damages, inhibit cell proliferation, induce cell apoptosis, and decrease expression of ABP and vimentin mRNAs in mouse Sertoli cells in vitro. Xia et al. (2013, ASB2014-9642) studied the induction of vitellogenin gene expression in the fish medaka exposed to glyphosate and potential molecular mechanism. While glyphosate markedly up-regulated VTG transcription levels in both female and male fish, the upward trend was inhibited at the high glyphosate concentrations. Wunnapak et al. (2014, ASB2014-9638) used Roundup to induce nephrotoxicity in rats. A panel of kidney injury biomarkers was evaluated in terms of suitability to

detect acute kidney injury and dysfunction. Martini et al. (2012, ASB2014-9613) used 3Z3-L1 fibroblasts to investigate the effect of a commercial formulation of glyphosate on proliferation, survival and differentiation. According to the results, a glyphosate-based herbicide inhibits proliferation and differentiation in this mammalian cell line and induces apoptosis suggesting GF-mediated cellular damage.
Kilinc et al. (2013, ASB2014-9588) studied the influence of pesticide exposure on carbonic anhydrase II from sheep stomach. The authors conclude that both glyphosate isopropylamine and dichlorvos inhibited CA-II isoenzym in a noncompetetive manner.
Jasper et al. (2012, ASB2014-9583) evaluated the toxicity of hepatic, haematological, and oxidative effects of glyphosate Roundup on male and female albino Swiss mice. The results of this study indicate that glyphosate-Roundup can promote haematological and hepatic alterations, even at subacute exposure, which could be related to the induction of reactive oxygen species.
Chaufan et al. (2014, ASB2014-9314) studied the effects on oxidative formulation in HepG2 cells. The authors conclude that the results confirm that G formulations have adjuvants working together with the active ingredient and causing tolxic effects that are not seen with acid glyphosate.
Gencer et al. (2012, ASB2014-9481) studied in vitro effects of Imazethapyr, 2,4-D, glyphosate and propanocarb on human erythrocyte carbonic anhydrase activity. Imazethapyr was the most effective inhibitor for CA-H isoencyme. The lowest inhibition was caused by glyphosate.
Campo et al. (2009, ASB2014-9281) evaluated the toxicity of ten pesticides used in the municipality of Popayan., Colombia, using bioassay with Bacillus subtilis. Glyphosate was slightly toxic in this test.
Kwiatkowska et al. (2014, ASB2014-9603) published a study that was undertaken to evaluate toxic potential of glyphosate, ist metabolites AMPA, methylphosphonic acid and its impurities N-(phosphonomethyl)iminodiacetic acid (PMIDA), N-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine. The authors evaluated the effect of those compounds on haemolysis, haemoglobin oxidation, reactive oxygen species (ROS) formation and changes in morphology of human eythrocytes. Glyphosate, its metabolites and impurities induced a little haemolysis and haemoglobin oxidation. All changes were very low, even after 24 h incubation. Most of the investigated compounds induced reactive oxygen species formation from 0.25 mM, except the N-methylglyphosate wich caused an increase in ROS formation from 0,5 mM. Moreover, the investigated xenobiotics did no change the size and shape (except bis-(phosphonomethyl)amine) of the human erythrocytes. Changes in human erythrocytes were observed only when high concentrations of the compounds were applied. Some investigated metabolites and impurities caused a slight stronger damage to human erythrocytes than glyphosate.
to the present calculation of the ADI. However, this calculation would never replace the direct study of the commercial formulation with its adjuvants in regulatory tests. Coalova et al. (2014, ASB2014-7615) studied the influence of spray adjuvant on the toxicity effects of a glyphosate formulation in Hep-2-cell line. They determined the median lethal concentration of Atanor (glyphosate formulation), Impacto (spray adjuvant) and a mixture of both agrochemicals. The substances and mixtures induced dose- and time-dependent cytotoxicity. The toxicity of a mixture of Atanor and Impacto was additive in Hep2-cells. The authors conclude that the addition of adjuvant to glyphosate formulation would increase the toxicity of the mixture in cell culture. Kwiatkowska et al. (2014, ASB2014-8085) investigated the effect of glyphosate, its metabolites and impurities on acetylcholinesterase (AchE) activity (in vitro) in human erythrocytes. The authors conclude that the compounds studied (used in concentrations that are usually determined in the environment) do not disturb function of human erythrocyte acetylcholinesterase.
Hoare (2014, ASB2014-9157) submitted a QSAR assessment on the toxicological properties of

glyphosate and its impurities. To assess the toxicological properties of glyphosate and five impurities (AMPA, IBMPA, MAMPA, NMG and IDA) present in the technical grade of material, the QSAR models ACD labs, DEREK NEXUS, TOXTREE, EPA T.E.S.T. VEGA and OECD Toolbox were employed. None of the structures analysed triggered any alerts on DEREK NEXUS for carcinogenicity, chromosome damage, genotoxicity or mutagenicity. Eye and skin irritation were not anticipated in any of the QSAR models evaluated. No alert for skin sensitisation was triggered in DEREK NEXUS for any compound. Equivocal alerts for nephrotoxicity and plausible alerts for hepatotoxicity were triggered by DEREK NEXUS for glyphosate, NMG, MAMPA and AMPA.

A further study was published which describes cases of intoxication in dogs and cats:

Bates and Edwards (2013, ASB2014-9249) inform in a letter about cases of intoxication of dogs and cats by glyphosate in UK, registered by the Veterinary Poisons Information Service. Vomiting, diarrheoea and lethargy were the most common signs in dogs. Vomiting, anorexia and lethargy were the most common signs in cats.

Two further studies have been submitted which investigated ecotoxicological effects of glyphosate:

Relyea (2005, ASB2012-204) examined the impact of four globally common pesticides including glyphosate on the biodiversity of aquatic communities containing algae and animals.

In a further study Relya (2012, ASB2012-2791) created wetland communities including water plants and animals and exposed thes communities to Roundup. The author reports different effects on nontarget species.

Further studies are presented in detail below:

Author(s)

Year

Study title

Robert Bellé, Ronan Le Bouffant, Julia Morales, Bertrand Cosson, Patrick Cormier et Odile Mulner-Lorillon

2007

L'embryon d'oursin, le point de surveillance de l'ADN endommagé de la division cellulaire et les mécanismes à l'origine de la cancérisation.

Journal de la Société de Biologie,

Volume : 201, Number: 3, Pages: 317-327 ASB2012-11560

Abstract*

Sea urchin embryo, DNA-damaged cell cycle checkpoint and the mechanisms initiating cancer development (translation from original article)

Cell division is an essential process for heredity, maintenance and evolution of the whole living kingdom. Sea urchin early development represents an excellent experimental model for the analysis of cell cycle checkpoint mechanisms since embryonic cells contain a functional DNA-damage checkpoint and since the whole sea urchin genome is sequenced. The DNA- damaged checkpoint is responsible for an arrest in the cell cycle when DNA is damaged or incorrectly replicated, for activation of the DNA repair mechanism, and for commitment to cell death by apoptosis in the case of failure to repair. New insights in cancer biology lead to two fundamental concepts about the very first origin of cancerogenesis. Cancers result from dysfunction of DNA-damaged checkpoints and cancers appear as a result of normal stem cell (NCS) transformation into a cancer stem cell (CSC). The second aspect suggests a new definition of "cancer", since CSC can be detected well before any clinical evidence. Since early development starts from the zygote, which is a primary stem cell, sea urchin early development allows analysis of the early steps of the cancerization process. Although sea urchins do not develop cancers, the model is alternative and complementary to stem cells which

are not easy to isolate, do not divide in a short time and do not divide synchronously. In the field of toxicology and incidence on human health, the sea urchin experimental model allows assessment of cancer risk from single or combined molecules long before any epidemiologic evidence is available. Sea urchin embryos were used to test the worldwide used pesticide Roundup that contains glyphosate as the active herbicide agent; it was shown to activate the DNA-damage checkpoint of the first cell cycle of development. The model therefore allows considerable increase in risk evaluation of new products in the field of cancer and offers a tool for the discovery of molecular markers for early diagnostic in cancer biology. Prevention and early diagnosis are two decisive elements of human cancer therapy.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not assignable
Comment:
Documentation insufficient for evaluation.
The publication overview provides information on the general application of the sea urchin embryo model for the prediction of “cancerogenicity”. Only a short reference to another study with a glyphosate-containing herbicide is given. Details of the glyphosate product are not provided. Common surfactants have previously shown the same effects in this model. This model is not appropriate for testing materials containing surfactants
because surfactant induced cytotoxicity via membrane disruption is well documented using in vitro systems.
Relevance of study:
Not relevant (Prevention of cell cycle transition was determined
for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants.)
Klimisch code:
4
Author(s)
Year
Study title
Marc J.,
Mulner-Lorillon, O., Boulben, S, Hureau, D.
Durand, G., Belle, R.
2002
Pesticide Roundup Provokes Cell Division Dysfunction at the level of CDK1/Cyclin B Activation..
Chem. Res. Toxicol. 2002, 15, 326-331 ASB2013-9838
Abstract*
To assess human health risk from environmental chemicals,we have studied the effect on cell cycle regulation of the widely used glyphosate-conaining pesicide Roundup. As a model system we have used sea urchin embryonic first divisions following fertilization., which are appropriate for the study of universal cell cycle regulation without interference with transcription. We show that 0.8 % Roundup (containing 8 mM glyphosate) induces a delay in the kinetic of the first cell cleavage of sea urchin embryos. The delay is dependent on the concentration of Roundup. The delay in the cell cycle could be induced using increasing glyphosate concentrations (1-10 mM) in the presence of a subthreshold concentration of Roundup 0.2 %, while glyphosate alone was ineffective, thus

indicating synergy between glyphosate and Roundup formulation products. The effect of Roundup was not lethal and involved a delay in entry into M-phase of the cell cycle, we analysed CDK1/cyclin B activation during the first division of early development. Roundup delayed the activation of CDK1/cyclin B in vivo. Roundup inhibited also the global protein synthetic rate without preventing the accumulation of cyclin B. In summary, Roundup affects cell cycle regulation by delaying activation of the CDK1/cyclin B complex, by synergic effect of glyphosate and formulation products. Considering the universality among species of the CDK1/cyclin B regulator, our results question the safety of glyphosate and Roundup on human health.
* Quoted from article
Author(s)
Year
Study title
Marc J.,
Mulner-Lorillon, O., Durand, G.,
Belle, R.
2003
Embryonic cell cycle for risk assessment of pesticides at the molecular level.
Environmental Chemistry Letters Volume: 1, Number: 1, Pages: 8-12 ASB2009-9013
Abstract*
Cell cycle mechanisms are highly conserved from unicellular eukaryotes to complex metazoans including humans. Abnormalities in the regulation of the cell cycle result in death or diseases such as cancer. Early development of sea urchin has proved to be a powerful model for cell division studies and offers the opportunity to study synchronous cell divisions in the absence of transcriptional control. We have analyzed pesticide induced dysfunctions in the first cell division following fertilization in sea urchin embryos, using Roundup, a widely used pesticide formulation containing isopropylamine glyphosate as the active substance. The pesticide induced cell cycle dysfunction by preventing the in vivo activation of the universal
cell cycle regulator CDK1/cyclin B. We further show that synthesis of the regulator protein, cyclin B, as well as its association to the catalytic protein, CDK1, were not affected by the pesticide. Therefore, our results suggest that the pollutant impedes the processing of the CDK1/cyclin B complex, which is required in its physiological activation. Our studies demonstrate the relevance of sea urchin embryonic cells as a sensitive model to assess pesticide toxicity at the level of the universal cell cycle checkpoints.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Mechanistic study. Outcome with little additional information compared to the authors' previously published work. Non-standard, non-guideline.
Commonly used surfactants have previously shown the same effects in this model.
Relevance of study:
Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using in vitro
systems.)

Klimisch code:
3
Author(s)
Year
Study title
Marc, J.
Mulner-Lorillon, O. Belle, R.
2004
Glyphosate-based pesticides affect cell cycle regulation Biology of the Cell
Volume: 96, Pages: 245-249, ASB2009-9014
Abstract*
Cell-cycle dys-regulation is a hallmark of tumor cells and human cancers. Failure in the cell- cycle checkpoints leads to genomic instability and subsequent development of cancers from the initial affected cell. A worldwide used product Roundup 3plus, based on glyphosate as the active herbicide, was suggested to be of human health concern since it induced cell cycle dysfunction as judged from analysis of the first cell division of sea urchin embryos, a recognized model for cell cycle studies. Several glyphosate-based pesticides from different manufacturers were assayed in comparison with Roundup 3plus for their ability to interfere with the cell cycle regulation. All the tested products, Amega, Cargly, Cosmic, and Roundup Biovert induced cell cycle dysfunction. The threshold concentration for induction of cell cycle dysfunction was evaluated for each product and suggests high risk by inhalation for people in the vicinity of the pesticide handling sprayed at 500 to 4000 times higher dose than the cell- cycle adverse concentration.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not Reliable
Comment:
Non-standard, non-guideline study. Commonly used
surfactants have previously shown the same effects in this model.
Relevance of study:
Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using in vitro
systems.
Klimisch code:
3
Author(s)
Year
Study title
Marc, J. Belle, R. Morales, J. Cormier, P.
Mulner-Lorillon, O.
2004
Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition.
Toxicological Sciences, Volume: 82, Pages: 436-442 ASB2012-11894
Abstract*

A glyphosate containing pesticide impedes at 10 mM glyphosate the G2/M transition as judged from analysis of the first cell cycle of sea urchin development. We show that formulated glyphosate prevented dephosphorylation of Tyr 15 of the cell cycle regulator CDK1/cyclin B in vivo, the end point target of the G2/M cell cycle checkpoint. Formulated glyphosate had no direct effect on the dual specific cdc25 phosphatase activity responsible for Tyr 15 dephosphorylation. At a concentration that efficiently impeded the cell cycle, formulated glyphosate inhibited the synthesis of DNA occurring in S phase of the cell cycle. The extent of the inhibition of DNA synthesis by formulated glyphosate was correlated with the effect on the cell cycle. We conclude that formulated glyphosate's effect on the cell cycle is exerted at the level of the DNA-response checkpoint of S phase. The resulting inhibition of CDK1 cyclin B Tyr 15 dephosphorylation leads to prevention of the G2/M transition and cell cycle progression.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-standard, non-guideline study. Commonly used surfactants have previously shown the same effects in this model.
Relevance of study:
Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well
documented using in vitro systems.
Klimisch code:
3
Additional comments:
Comments of the notifier are submitted on the web site of Monsanto (2006, ASB2013-5455): http://www.monsanto.com/products/Documents/glyphosate-background-materials/Response_ISIS_apr_06.pdf
The following two recent publications, by Heu et al. (2012, ASB2012-11843 and ASB2012- 11844) are commented on collectively after the second summary/Klimisch rating, below.
Author(s)
Year
Study title
Heu, C.,
Berquand, A., Elie-Caille, C., Nicod, L.
2012
Glyphosate-induced stiffening of HaCat keratinocytes, a Peak Force Tapping study on living cells.
Journal of Structural Biology Volume: 178, Number: 1, Pages: 1-7 ASB2012-11843
Abstract*
The skin is the first physiological barrier, with a complex constitution, that provides defensive functions against multiple physical and chemical aggressions. Glyphosate is an extensively used herbicide that has been shown to increase the risk of cancer. Moreover there is increasing evidence suggesting that the mechanical phenotype plays an important role in malignant transformation. Atomic force microscopy (AFM) has emerged within the last decade as a powerful tool for providing a nanometer-scale resolution imaging of biological samples. Peak Force Tapping (PFT) is a newly

released AFM-based investigation technique allowing extraction of chemical and mechanical properties from a wide range of samples at a relatively high speed and a high resolution. The present work uses the PFT technology to investigate HaCaT keratinocytes, a human epidermal cell line, and offers an original approach to study chemically-induced changes in the cellular mechanical properties under near- physiological conditions. These experiments indicate glyphosate induces cell membrane stiffening, and the appearance of cytoskeleton structures at a subcellular level, for low cytotoxic concentrations whereas cells exposed to IC50 (inhibitory concentration 50 %) treatment exhibit control-like mechanical behavior despite obvious membrane damages. Quercetin, a well-known antioxidant, reverses the glyphosate-induced mechanical phenotype.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline in vitro tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)
Relevance of study:
Not relevant (in vitro data on the effects on an immortalised epidetmal cell-line does consider low exposure potential due to stratum cornea protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not in formulated glyphosate based
products)
Klimisch code:
3
Author(s)
Year
Study title
Heu, C.,
Elie-Caille, C., Mougey, V., Launay, S., Nicod, L.
2012
A step further towards glyphosate-induced epidermal cell death: Involvement of mitochondrial and oxidative mechanisms. Environmental Toxicology and Pharmacology Volume: 34, Number: 2, Pages: 144-153
ASB2012-11844
Abstract*
A deregulation of programmed cell death mechanisms in human epidermis leads to skin pathologies. We previously showed that glyphosate, an extensively used herbicide, provoked cytotoxic effects on cultured human keratinocytes, affecting their antioxidant capacities and impairing morphological and functional cell characteristics. The aim of the present study, carried out on the human epidermal cell line HaCaT, was to examine the part of apoptosis plays in the cytotoxic effects of glyphosate and the intracellular mechanisms involved in the apoptotic events. We have conducted different incubation periods to reveal the specific events in glyphosate-induced cell death. We observed an increase in the number of early apoptotic cells at a low cytotoxicity level (15%), and then, a decrease, in favour of late apoptotic and necrotic cell rates for more severe cytotoxicity conditions. At the same time, we showed that the glyphosate-induced mitochondrial membrane potential disruption could be a cause of apoptosis in keratinocyte cultures.
* Quoted from article

Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline in vitro tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)
Relevance of study:
Not relevant (in vitro data on the effects on an immortalized epidermal cell-line does consider low exposure potential due to stratum cornea protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not contained in formulated
glyphosate based products)
Klimisch code:
3
Additional comments:
Glyphosate technical acid evaluated was not reported to be pH adjusted and therefore does not reflect real world exposures to the more neutral pH formulations, which contain glyphosate salts, not glyphosate acid
The pH range of test concentrations (850-1150 mg/L) is very acidic, approximately 1.7-2- 2 pH units. Keeping in mind the pH scale is logarithmic, these values are substantially lower than those of viable skin and in vitro cell cultures.
Exposure potential to live human epidermal skin cells in the field is likely to be considerably lower than the authors have considered. The epidermis is protected by the stratum cornea. Human in vitro dermal absorption studies for a range glyphosate formulated products are presented in the chapter on dermal absorption, showing a very low dermal absorption of glyphosate; nearly all of the glyphosate is washed off the skin surface after 24 hour exposures (88% to >99 % before stratum cornea removal). Therefore, the studies of Heu et al., while
representative of glyphosate spray concentrations, are approximately two or more orders of magnitude higher of those which may result for 8-24 hour dermal exposures.
Author(s)
Year
Study title
Axelrad, J.C. Howard, C.V. McLean, W.G.
2003
The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon
Toxicology, Volume: 185, Pages: 67-78 ASB2012-11553
Abstract*
Speculation about potential neurotoxicity due to chronic exposure to low doses of organophosphate (OP) pesticides is not yet supported by experimental evidence. The objective of this work was to use a cell culture model of chronic OP exposure to determine if such exposure can alter the sensitivity of nerve cells to subsequent acute exposure to OPs or other compounds. NB2a neuroblastoma cells were grown in the presence of 25 μM diazinon for 8 weeks. The OP was then withdrawn and the cells were induced to differentiate in the presence of various other pesticides or herbicides, including OPs and OP-containing formulations. The resulting outgrowth of neurite-like structures was measured by light microscopy and quantitative image analysis and the IC50 for each OP or formulation was calculated. The IC50 values in diazinon-pre-exposed cells were compared with the equivalent values in cells not pre-exposed to diazinon. The IC50 for inhibition of neurite outgrowth by acute application

of diazinon, pyrethrum, glyphosate or a commercial formulation of glyphosate was decreased by between 20 and 90 % after pre-treatment with diazinon. In contrast, the IC50 for pirimiphos methyl was unaffected and those for phosmet or chlorpyrifos were increased by between 1.5- and 3-fold. Treatment of cells with chlorpyrifos or with a second glyphosate-containing formulation led to the formation of abnormal neurite-like structures in diazinon-pre-exposed cells. The data support the view that chronic exposure to an OP may reduce the threshold for toxicity of some, but by no means all, environmental agents.
* Quoted from article
Klimisch Evaluation
Reliability of study:
Not reliable
Comment:
Incorrect characterisation of glyphosate as an organophosphate pesticide. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactant are well known for in vitro test systems. Exposure route not relevant for human risk assessment. Rationale for chosen test substance concentration not given.
Relevance of study:
Not relevant (in vitro data, do not reflect real in vivo
exposure situations. Pre-exposure to diazinon is not relevant for this submission).
Klimisch code:
3
Author(s)
Year
Study title
Benedetti, A. L.
2004
The effects of sub-chronic exposure of Wistar rats to the
Vituri, C.D. Trentin, A.G. Domingues, M.A.C.
Alvarez-Silva, M.
herbicide Glyphosate-Biocarb®
Toxicology Letters, Volume: 153, Pages: 227-232 ASB2012-11562
Abstract*
The object of this study was to analyze the hepatic effects of the herbicide Glyphosate- Biocarbo (as commercialized in Brazil) in Wistar rats. Animals were treated orally with water or 4.87, 48.7, or 487 mg/kg of glyphosate each 2 days, during 75 days. Sub-chronic treatment of animals starting from the lowest dose of glyphosate induced the leakage of hepatic intracellular enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggesting irreversible damage in hepatocytes. We observed the increase of Kupffer cells in hepatic sinusoid of glyphosate-treated animals. This was followed by large deposition of reticulin fibers, composed mainly of collagen type III. We may conclude that Glyphosate- Biocarbo may induce hepatic histological changes as well as AST and ALT leaking from liver to serum in experimental models.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comments:

Study report meets basic scientific principles. Study design and documentation is insufficient for assessment.
Relevance of study:
Not relevant because study design not sufficient for assessment of toxicity of the active substance Glyphosate. Toxicity is attributable to high oral dosing of surfactant component. There are several reporting
deficiencies.
Klimisch code:
3
Author(s)
Year
Study title
Mesnage, R. Clair, E. Gress, S. Then, C. Szekacs, A.
Seralini, G.E.
2012
Cytotoxicity on human cells of Cry1Ab and Cry 1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide.
Journal of Applied Toxicology
doi: 10.1002/jat.2712. [Epub ahead of print] ASB2012-11900
Abstract*
The study of combined effects of pesticides represents a challenge for toxicology. In the case of the new growing generation of genetically modified (GM) plants with stacked traits, glyphosate-based herbicides (like Roundup) residues are present in the Roundup-tolerant edible plants (especially corns) and mixed with modified Bt insecticidal toxins that are produced by the GM plants themselves. The potential side effects of these combined pesticides on human cells are investigated in this work. Here we have tested for the very first time Cry1Ab and Cry1Ac Bt toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293, as well as their combined actions with Roundup, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release
by membrane alterations and caspase 3/7 inductions. Cry1Ab caused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. The Roundup tested alone from 1 to 20 000ppm is necrotic and apoptotic from 50ppm, far below agricultural dilutions (50% lethal concentration 57.5ppm). The only measured significant combined effect was that Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by Roundup; this could delay the activation of apoptosis. There was the same tendency for the other markers. In these results, we argue that modified Bt toxins are not inert on non-target human cells, and that they can present combined side-effects with other residues of pesticides specific to GM plants.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-guideline, non-GLP in vitro tests meeting scientific principles. Deficiencies: No positive controls were specified, test conditions not described (referenced to a description elsewhere). Exceedingly high doses and an inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.

Relevance of study:
Relevant with restrictions (Due to reliability. The
assessed combinatory effects are of limited relevance)
Klimisch code:
3
Additional comments:
Direct exposure to cells in culture bypasses normal processes limiting absorption and cellular exposure and avoids normal metabolism, excretion, serum protein binding, and other factors that would protect cells in the intact organism.
Anadon et al. (2009, ASB2012-11542) dosed rates with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved peak modeled plasma concentrations of glyphosate of approximately 5 ug/mL (5mg/L or 5 ppm). Assuming linear kinetics, the maximum allowable US daily intake (2 mg/kg/day) would give an approximated blood concentration of 0.025 ppm (25 ppb). McQueen et al. (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4 % of the acceptable daily intake.
The “Roundup” LC50 concentration used (57.5 ppm) is more than 2000-fold higher than the anticipated concentration (based on Anadon et al., 2009, ASB2012-11542) following maximum allowable intake.
The co-application of Cry protein with the glyphosate-surfactant reduces the apparent degree of cellular injury (as measured by induction of caspase levels). This occurs even at concentrations of Cry1Ab which the authors report to cause cellular injury and membrane disruption. This is worth noting for several reasons:
First, it brings into question the toxicity observations with Cry1Ab, as the argument that membrane disruption and impaired mitochondrial function should be protective seems to be highly untenable, especially in view of the studies (Levine et al, 2007, ASB2009-9030) demonstrating the mitochondrial membrane activity of surfactants.
Second, it should take off the table any implications of a “synergistic effect” of Cry proteins and glyphosate-surfactant herbicides. (The direction is, if anything, antagonistic, but the entire system is fundamentally irrelevant.)
Third, this probably is demonstrating the artificiality of the system itself. As noted above, this is a protein-free medium. Protein protects cells in culture by multiple mechanisms- binding to toxic materials, binding to potential receptor sites, or other non-specific surface-stabilisation effects. It appears from Mesnage’s own data that simple addition of protein to their system, even at low concentrations (and even if that protein is a Cry protein) protects from toxicity.
Author(s)
Year
Study title
Clair E., Linn, L., Travert, C., Amiel, C., Seralini, G.E
2012
Effects of Roundup® and Glyphosate on Three Food Microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus
Current Microbiology, Volume: 64, Number: 5, Pages: 486-
491
ASB2012-11592
Abstract*

Use of many pesticide products poses the problem of their effects on environment and health. Amongst them, the effects of glyphosate with its adjuvants and its by-products are regularly discussed. The aim of the present study was to shed light on the real impact on biodiversity and ecosystems of Roundup®, a major herbicide used worldwide, and the glyphosate it contains, by the study of their effects on growth and viability of microbial models, namely, on three food microorganisms (Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus) widely used as starters in traditional and industrial dairy technologies. The presented results evidence that Roundup® has an inhibitory effect on microbial growth and a microbicide effect at lower concentrations than those recommended in agriculture. Interestingly, glyphosate at these levels has no significant effect on the three studied microorganisms. Our work is consistent with previous studies which demonstrated that the toxic effect of glyphosate was amplified by its formulation adjuvants on different human cells and other eukaryotic models. Moreover, these results should be considered in the understanding of the loss of microbiodiversity and microbial concentration observed in raw milk for many years.
* Quoted from article
Klimisch evaluation
Reliability of study:
Not reliable
Comment:
Non-validated, non-guideline test with methodological and reporting deficiencies (e.g. dose concentrations in media not specified, no positive controls or controls that show the validity of the test system /and concentration range tested). Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:
Not relevant (Due to reliability)
Klimisch code:
3
Additional Comments
Glyphosate at 1 % had no effect on lactobacilli but did impair Geotrichum, which is unsurprising as glyphosate at herbicidal concentrations will impact metabolism of many fungi, which (like plants) use the shikimate pathway for aromatic amino acid production.
Surfactants are known to be bacteriostatic, with (for example) quaternary ammonium compounds typically being active in the 30-150 ppm range.
Clair et al. demonstrate that surfactants are bacteriostatic for 3 microorganisms at concentration ranges well within the range of concentrations generally found to be useful for sanitation purposes. However, surfactant solutions are routinely used to sanitize food processing equipment at concentrations at or above those tested by Clair et al. (2012, ASB2012-11592).
B.6.9 Medical data and information (Annex IIA 5.9)
B.6.9.1 Report on medical surveillance on manufacturing plant personnel
Industrial hygiene air monitoring data for glyphosate with workers at the Monsanto Luling, Louisiana manufacturing facility are available for the years 1981-1998 and have been submitted as part of the GTF dossier (no particular reference available). No such data are available from a Monsanto European manufacturing facility. Based on the measured low exposures to glyphosate in the manufacturing setting (well below the ADI) and low toxicological concern, glyphosate specific medical monitoring was not considered necessary by Monsanto. The following data are air

concentration measurements which are conservatively applied as 100 % bioavailable to calculations of mean and maximum daily exposures.

Table B.6.9-1: Particulate exposures from glyphosate technical acid operations involving wetcake, e.g., supersack or container filling operations. Values are time weighted averages

Glyphosate Technical Dust (mg/m3)	Mean Daily Exposure* (mg/kg/day)	Maximum Daily Exposure* (mg/kg/day)	Sample Type	# Samples	Range	Mean	SD
All				179	0.0003-0.2594	0.00647	0.0218
	0.00108	0.04323					
Personal				176	0.0003-0.2549	0.00655	0.022
	0.00109	0.04248					
Area				3	0.0008-0.024	0.00153	0.0008
	1	0.00026	0.00400				
Operator				158	0.0008-0.2594	0.00727	0.0235
	0.00121	0.00393					
Maintenance				16	0.0005-0.0053		

0.00206
0.0014
4
0.00034
0.00088
Lab
2
0.0003-0.0004
0.00035
N/A
0.00006
0.00007
* based on breathing 10 m3 air/shift and 60 kg worker
Table B.6.9-2: Glyphosate isopropylamine salt liquid formulation bottling, drumming and tote filling operations. Values are time weighted averages
Glyphosate IPA Salt- Liquid Formulations (mg/m3)
Mean Daily Exposure**
(mg/kg/day)
Maximum Daily Exposure**
(mg/kg/day)
Sample Type
Samples
Range
Mean
SD
All
72
0.0001-0.47
0.085
0.105
0.01050
0.05804
Personal
58
0.0001-0.47
0.0251
0.106
0.00310
0.05804
Area
14
0.004-0.28
0.0932
0.105
0.01151
0.03458

Operator
54
0.0001-0.47
0.0966
0.11
0.01193
0.05804
Maintenance
4
0.0041-0.0088
0.00792
0.00187
0.00098
0.00099
** based on breathing 10 m3 air/shift and 60 kg worker and divided by 1.3496 to convert IPA salt to technical acid
Improvements in manufacturing facility containment and ventilation systems over recent years further reduce the likelihood of operator exposures within glyphosate manufacturing facilities.

B.6.9.2 Reports on clinical cases and poisoning incidents
Glyphosate is worldwide used extensively as herbicide. According to this extensive use a large number of poisoning incidents happened and was published. An extensive review of clinical cases was submitted by 2004, ASB2012-11576. Another review of cases was published by ., 2008, ASB2012-11879.
(2009, ASB2013-11831) briefly mentioned a total of 60 reports by physicians from Germany on cases of poisoning with glyphosate herbicides since 1990. Without further specification, in the vast majority of 52 cases only slight health impairment was reported. In four cases, health disturbances were considered “moderate” whereas the only one actually life-threatening case was the result of ingestion of 200 mL of a herbicide containing glyphosate and a surfactant with suicidal intent. In the three remaining cases, no symptoms were reported or their severity could not be evaluated.
More than 650 cases of intoxication/irritation ascribed to ingestion of/contact to glyphosate- based herbicides are mentioned in an overview on poisoning incidents from Brazil that was just recently kindly provided to the RMS by the Brazilian National Health Surveillance Agency (Paumgartten/ANVISA, 2012, ASB2013-13413). This data was collected by up to 10 Brazilian poison centers between 2010 and 2012. It is not clear if it is representative for the whole huge country in which the agricultural conditions in general and also those of pesticide use are extremely different. In addition, there is even a higher number of poison information centers in Brazil that have not provided data and, thus, the number of incidents might underestimate the real incidence. On the first glance, the exposure routes, ingested amounts, circumstances (accident, suicidal attempt?), clinical signs and medical treatment are similar to what is known from Germany and from the literature. The much higher total number of cases (as compared to Germany) seems to reflect the applied amount of glyphosate and its formulations that is by orders of magnitude higher in Brazil. Unfortunately, severity of symptoms is not graded and vey often the final outcome was not reported. Another problem is that the product to which exposure was claimed is not specified. Frequently, “Roundup” is mentioned but different formulations are marketed under this name. In many other cases, the incident is ascribed to”glyphosate”. However, this is not credible because there is no

simple access to the active ingredient and a certain product must have been applied. Furthermore, it is not clear whether a causal relationship had been actually confirmed. Thus, this potentially most interesting data require further thorough analysis before it may be used for evaluation of health risks.

Jayasumana et al. (2014, ASB2014-3085) published a hypothesis on an association between glyphosate exposition, hard water and nephrotoxic metals in an epidemic of chronic kidney disease in Sri Lanka. The authors conclude that although glyphostate alone does not cause an epidemic of chronic kidney disease, it seems to have acquired the ability to destroy the renal tissues when forms complexes with localized geo environmental factor (hardness) and nephrotoxic metals. This conclusion is mainly based on a metal chelating property of glyphosate and the extensive use of glyphosate in Sri Lanka, and the occurance of hard water in the concerned areas. However, the hypothesis was not experimentally evidenced up to now. Sirinathsinghji (2014, ASB2014-10742) report that Sri Lanka is set to partially ban glyphosate-based herbicide use following a study linking it to a fatal chronic kidney disease epidemic affecting the country. This decision is also based on the above described publication of Jayasumana et al. (2014, ASB2014-3085)

Zouaoui et al. (2012, ASB2014-9734) reported 13 cases of acute intoxication with glyphosate (mostly suicidal). The most common symptoms were oropharyngeal ulceration, nausea and vomiting. The main altered biological parameters were high lactate and acidosis. We also noted respiratory distress, cardiac arrhythmia, hypercaleamia, impaired renal function, hepatic toxicity and altered consciousness. In fatalities, the common symptoms were cardiovascular shock, cardiorespiratory arrest, haemodynamic disturbance, intravascular disseminated coagulation and multiple organ failure. Concentrations of glyphosate and AMPA have been determined in blood and urine.

Sribanditmongkol et al. (2012, ASB2014-9731) report a case of a woman who died after ingestion of approximately 500 ml Roundup formulation. Toxic effects of the pesticide were caused by the abilitiy to erode tissues including mucous membranes and linings of the gastrointestinal and respiratory tracts. A mild degree of pulmonary congestion and edema was observed in both lungs.

Lee et al. (2012, ASB2014-9607) report the case of a 60-year orld patient who intentionally ingested 450 ml Roundup. He experienced cardiac arrest but was successfully resuscitated and treated with continuous venovenous haemodiafiltration.

Hour at al. (2012, ASB2014-9571) describe the case of a 66 year old man with a history of alcohol abuse who ingested 500 ml of rum and 350 ml of Roundup. He was hypotensive, diaphoretic and hypoxic. After veno-venous hemodiafiltration his condition improved within 24 hours.

Hinojosa et al. (2011, ASB2014-9566) submitted a retrospective study to identify substances involved in poisonings at Lariboisiere hospital. 315 patients were included with 891 announced substances. Only 1 case of glyphosate was identified.

Garlich et al. (2014, ASB2014-9480) report the case of a 62-year old man who drank a bottle of 41% glyphosae formulation. He was bradycardic and obtunded with respiratory depression. The patient underwent haemodialysis 16 h post ingestion after which he demonstrated improvement in clinical status.

A case of an inhalative intoxication by glyphosate is reported by BfR (2007, ASB2014-9290). A 59 years old farmer sprayed a glyphosate formulation without protective equipment over approximately 3 hours. He suffered from laboured breathing, cough and fever. A biopsy showed alveolitis and bronchiolitis.

Beswick and Millo (2011, ASB2014-9283) describe a fatal poisoning with a glyphosate surfactant herbicide. A 29-year old man was admitted following deliberate ingestion of approximatly 300 ml of Roundup Ace. He developed severe and persistent lactic acidosis, hyperkalaemia, hypotension,

torrential watery diarrhoea and abdominal distension in the first 24 hours. The clinical course was complicated by cardiac arrhythmia and an episode of cardiac arrest. On day three following poisoning, the patient died.

Malhotra et al. (2010, ASB2012-11890) report a case of a 71-year old male who attempted suicide with a glyphosate formulation and developed a prolonged but reversible encephalopathy suggestive of acute CNS toxicity. He was in cardiogenic shock with severe metabolic acidosis. Neurologic investigations were performed to exclude structural pathology. CT brain scan was normal. An EEG reading on day 8 demonstrated generalised slow wave acitivity with triphasic sharp and slow wave complex consistent with an encephalopathy although non convulsive seizures could not be excluded.

B.6.9.3 Observations on exposure of the general population and epidemiological studies

Two studies on concentrations of glyphosate in human urine samples (Acquavella et al., 2004, ASB2012-11528) and Hoppe (2013, ASB2013-8037) are available and reported in detail in chapter B.6.1 under B.6.1.3 (Published information).

Human biomonitoring based on urine measurements

Systemic exposure of humans following application of glyphosate in the field or presumed dietary intake may be roughly calculated on the basis of urinary concentrations even though it is sometimes not easy to distinguish between the routes of exposure (occupational vs. dietary) and their respective contributions to the total intake. In the following, the available data is reported separately for Europe and the U.S. because agricultural conditions are different. In particular, the wide-spread plantation of glyphosate-resistant crops in the Americas must be taken into account because a generally higher exposure level of the population can be assumed there. For comprehensive evaluation and comparison of the resulting exposure or “systemic dose” estimates with the proposed reference values, see Vol. 1 (2.6.11). In parallel to preparation of this RAR, this data and its evaluation have been separately published by the German Federal Insitute for Risk Assessment in a scientific journal (Niemann et al., ASB2014-11029)

For better comparability of the results, the detection/quantification limits in urine as well as the measured and calculated values are always given as µg/L here although, in the original studies, sometimes the unit “ppb” had been used instead (1 µg/L = 1 ppb).

• Data from the United States

glyphosate, with a limit of detection (LOD) of 1 µg/L whereas a separate limit of quantification (LOQ) was not mentioned.

Sixty percent of the farmers had detectable levels of glyphosate in their urines on the day of application with a remarkable difference between the origin of the samples since glyphosate was detected in the urine of 87% of South Carolina farmers as compared to only 36% positive findings from Minnesota. The geometric mean of the concentrations for the whole group of farmers was 3.2 µg/L on the day of application (Minnesota: 1.4 µg/L, South Carolina: 7.9
µg/L). It seems that the explanation for this gap might be a different use that was made of personal protective equipment. Farmers who did not wear rubber gloves had higher urinary concentrations than found in the other men (nearly 10 µg/L as compared to 2 µg/L) and, in fact, use of rubber gloves was much more common in Minnesota.

In all participants, a decline over the next days was observed.

The maximum value was 233 µg/L. On post-application day 3, the urinary concentration had fallen to 68 µg/L. Based on this highest so far measured value, a” systemic dose” of
0.004 mg/kg bw was calculated by the study authors. For this purpose, they had taken into consideration the individually measured excretion for days 0 through 3 and assumed a daily urine volume of 2 L. Some corrections for incomplete excretion and pharmacokinetic recovery were made.

If, however, only the extraordinarily high concentration on the day of application itself is considered, the systemically available amount of glyphosate would be at least 466 µg (rounded for 500 µg in case that not all of it had been excreted in urine the same day) giving, for a 60 kg weighing person, a systemic dose of 0.0083 mg/kg bw.
Among spouses, only very few (4 %) had detectable levels in their urine on the day of application but not later. The maximum value was 3 µg/L. In children, 12 % (all from South Carolina) had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 µg/L. (It is remarkable that this teenage boy's father was the same man displaying the highest urine concentration among all applicators.) All but one of the children with detectable concentrations had helped their fathers or were, at least, present during herbicide mixing, loading, or application.
This study is considered of good quality and reliable although Mage (2006, ASB2012-11888) claimed some methodological deficiencies with regard to urine collection and absent correction for prior glyphosate exposure. This field investigation is considered important because it was the first one to give an idea about urinary excretion of glyphosate in humans following proven occupational exposure. Of course, it is not known to which extent dietary exposure may have contributed to the measured values but it is presumed to have been low. At least the extremely high values of up to 233 µg/L that have been occasionally measured were most certainly due to direct application of glyphosate-based herbicides, most likely without the use of adequate protective equipment.
• Data from Europe
urine in one of the children cannot be explained with certainty but might be either due to dietary intake (although it would be surprising then that the mother and siblings had none in their urines) or to track-in of traces of the herbicide by the father resulting in residues, e.g., in house dust or yard dirt to which children might be exposed to via the oral, dermal or inhalativ routes, respectively.
In this study, urine was also analysed for aminomethylphosphonic acid (AMPA), i.e., the most important plant and soil metabolite of glyphosate. AMPA is normally found at very low levels in conventional plants, however, several genetically engineered varieties of glyphosate- tolerant plants degrade glyphosate very quickly giving higher amounts of this metabolite (see chapter B.7). In mammals, AMPA is formed only in traces, most likely due to the activity of intestinal bacteria (see section B.6.1). Thus, it is not surprising that Mesnage et al. (2012, ASB2014-3846) could not detect AMPA in any sample since the amount of glyphosate received was relatively small and the main exposure route, at least for the father, was certainly dermal and/or inhalative but not by ingestion.
A Europe-wide biomonitoring study (Hoppe, 2013, ASB2013-8037) was recently performed on behalf oft he NGO "Friends of the Earth" and its German partner organisation "Bund für Umwelt- und Naturschutz Deutschland" (BUND) and submitted on request to the RMS. This data has not been published in a scientific journal so far but is available in the Internet. 182 frozen urine samples from 18 European (EU and non-EU) countries (6 – 12 per country but mostly 10) were examined for glyphosate and AMPA by means of a modern and selective analytical method, i.e., transformation of both compounds to two different derivatives followed by GC-MS/MS. The LOQ for both, glyphosate and AMPA, was 0.15 µg/L. As in the previous studies by Acquavella et al. (2004, ASB2012-11528) and Curwin et al. (2007, ASB2012-11597), creatinine was also measured as an internal proof for the validity of the urine measurements.
For glyphosate, nearly 44% (80 samples) and, for AMPA, more than one third (65) of the participants had urine concentrations above the LOQ. Maximum values of 1.82, 1.64 or 1.55 µg/L for glyphosate were found in samples obtained from Latvia, the UK, and Malta, respectively, but the mean value of 0.21 µg/L was much lower. (For calculation of the mean, the

study author had included the samples with values below the LOQ and assumed a concentration of 0.075 µg/L, i.e., half the LOQ, for them. Thus, in reality, the actual mean value might be either a bit higher or lower.) For AMPA, the maximum values of 2.63, 1.26, and 0.89 µg/L were measured in samples from Croatia, Belgium, and Malta with a mean urinary concentration of 0.18 µg/L for all involved people. It was surprising that in more than 30 cases the AMPA concentrations were higher than those of glyphosate, sometimes by 10 times or more. In a few samples, AMPA values were rather high with glyphosate concentrations below the LOQ.

Apart from this data, the author also mentioned a "reference value" of 0.8 µg/L for glyphosate in urine, based on analytical investigations in a total of 90 people from a not further described "urban collective" from the region of the German city of Bremen (where its laboratory is situated). This figure was the 95th percentile of the individual values and was established in 2012 in preparation of the main study. For AMPA, a "reference value of 0.5 µg/L was given. The measured values themselves are considered reliable by the RMS. The results confirmed the previous assumption that there is in fact evidenc of a certain exposure of European

population to glyphosate, most likely by dietary intake. This is not surprising since glyphosate is a widely used active substance worldwide. Residues in food and feed may occur (see

On behalf of the German Federal Environmental Agency, frozen urine samples that had been taken for other purposes in 1996 and 2012 in the city of Greifswald in the north-eastern part of Germany and its surrounding region were analysed in retrospect for glyphosate residues. This so far unpublished data was kindly submitted to the German Federal Insitute for Risk Assessment (Markard, 2014; ASB2014-2057) to support ongoing evaluation of glyphosate. In each of the two sampling years, urine analysis for glyphosate and AMPA was performed in samples from ten male and ten female students (age 20 – 29 years at the time of sampling). The LOQ of the test method (i.e., gas chromatography) of 0.15 µg/L was exceeded for glyphosate in 22 of the totally 40 samples. The maximum value was 0.65 µg/L. There was a tendency towards an increase in glyphosate concentrations in urine in the 2012 samples compared to those from 1996, possibly reflecting a more frequent use of glyphosate in agriculture resulting in a higher dietary intake. The LOQ was exceeded more frequently and individual values tended to be higher.

Again, there were indications that AMPA concentrations in the urine may be higher than those of glyphosate. 10 out of 40 results were above the LOQ of 0.15 µg/L with a maximum value 1.31 µg/L. However, in contrast to glyphosate, the AMPA concentrations appeared to decrease between 1996 and 2012 suggesting that there is poor correlation between glyphosate and AMPA residues and that other routes or sources for exposure to AMPA than by (plant) metabolism of glyphosate should be considered. In addition, the stability of glyphosate in deep-frozen urine over more than 16 years was not investigated, maybe resulting in a shift of the glyphosate/AMPA ratio.

Human biomonitoring based on breast milk

In nursing mothers and lactating animals, milk is a common route of elimination of xenobiotics but its actual relevance depends on the substances and the chemical classes they belong to. For substances that have very long half-lives and accumulate in the body, like many organochlorine compounds, systematic surveillance of excretion via breast milk may provide useful information on the exposure level of the general population and its long-term trends as well as on the intake by infants. For instance, the decrease in exposure to pesticides such as DDT or lindane after their ban but also excretion of chemicals such als dioxin-like and non dioxin-like polychlorinated biphenyls was followed by this method (Fürst, 2006, ASB2014-8168; UBA, 2008, ASB2014-8167; BfR, 2011, ASB2014-8171; Verdugo-Raab,

2013, ASB2014-8173). For glyphosate, because of its physico-chemical properties, accumulation in

the body is not likely. The substance is not lipophilic and, thus, a deposit in body fat that might be released during nursing/lactation cannot arise. In line with these more theoretical considerations, the numerous kinetic studies (see section B.6.1) have clearly shown rapid and quantitative elimination from the body, no potential for accumulation and no affinity to fatty tissues. Investigations in lactating cows and goats following oral administration revealed very limited excretion via the milk accounting for not more than 0.1% of the administered total dose (see chapter B.7). There is no reason to suspect that this might be different in nursing mothers.

Measuring of pesticides and their residues in breast milk might be also useful if offspring toxicity in reproduction studies was due to exposure of the pups via the milk. There are examples for that (in humans mainly related to medical drugs) but, with glyphosate, evidence of such effects was completely lacking, despite the large number of multi-generation studies (see section B.6.6.1). Thus, in principle, there is no need to investigate breast milk for glyphosate and, accordingly, no data was available when the first draft of this report was prepared.

Recently, Honeycutt and Rowlands (2014, ASB2014-6793) published data on glyphosate findings in breast milk that gained considerable public attention but neither are the measured values reliable nor the conclusions of the authors agreed with. Ten samples were obtained from ten women who live in different U.S. Federal States. It is clear that this low number is in no way representative and that the findings can be considered at best expolarative. In three out of the 10 samples (all three from different states), the detection limit of 75 µg/L was exceeded with individual values of 76, 99, and 166 µg glyphosate/L. Detection of a chemical in milk does not necessarily mean that the substance must have accumulated before. It may be simply widely distributed throughout the body and excreted, among other routes, also by the milk. However, in this case, the values appear extremely high, in particular if compared to the urinary concentrations mentioned above, taking into account that urine is the main excretion route for ingested and systemically absorbed glyphosate (see section B.6.1). Based on the Internet publication itself and the sample number given therein, the group of nursing mothers was different from the people of whom urine samples had been analysed (see above). Thus, it is not possible to compare excretion of glyphosate via urine and breast milk for the same woman. Taking into account the physico-chemical properties of glyphosate and its well known pattern of distribution and elimination (based on animal studies), it is simply not conceivable that breast milk concentrations might be higher than those in urine. In contrary, urinary concentrations are expected to be much higher. However, the maximum ever measured urine concentration of 233 µg/L (Acquavella et al., 2004, ASB2012-11528) was due to immediately preceeding direct application of a herbicide by a farmer. Based on these considerations, the values reported for breast milk should be seriously doubted.

It was noted that apparently the same ELISA, without further modification, has been used for analysis of urine, “household water”, and breast milk for glyphosate. It is well known that validation of an assay for different matrices is inevitable but was not reported to have been performed in this case. The LOD of 75 µg/L in milk is by ten times higher than in urine (see above) pointing to large differences. Thus, the obvious deficiencies in the analytical method make the results not reliable. Even if the measured values were trusted in, they would not allow to give a rough estimate of the women’s exposure since it is not known to which extent of ingested or otherwise absorbed glyphosate is excreted by this route although ruminant data suggest that it should be a very minor one. For phtalates (Fromme et al., 2011, ASB2014-8169) and, more recently, for certain organochlochlorine and perfluorinated substances (Raab et al., 2013, ASB2014-8170), exposure of exclusively breast-fed infants was calculated on the basis of (much more reliable) concentrations measured in breast milk and then compared to the respective reference values. If the same is tried

for glyphosate, despites the doubts about the validity of the results, the highest measured value of 166 µg/L in breast milk could be taken as a provisional point of departure. Assuming a daily amount of breast milk of 700-900 mL that is produced (and consumed) to feed a baby in the first six month after giving birth, a total excretion of up to 150 µg glyphosate would result. For an exclusively breast-fed infant of 10 kg, the resulting exposure of 15 µg/kg bw would be by about 33 times lower than the (proposed) ADI of 500
µg/kg bw (0.5 mg/kg bw) and the margin would become bigger if the infant grows. Thus, even a glyphosate concentration in this unreliably high magnitude in breast milk would not be of health or developmental concern.

Epidemiology

A number of human studies on genotoxicity have been published since 2000 in which exposures of the studied populations to glyphosate-based formulations were postulated. These publications are presented and discussed in chapter B.6.4 (Genotoxicity), in the “Published data” section under B.6.4.8.7 (Human and environmental studies).

Likewise, several epidemiology studies on a possible relationsship of exposure to glyphosate (and further pesticides) and cancer have been published since 2000 that are presented and discussed in chapter B.6.5 under “Published data” (B.6.5.3 Published data).

With regard to reproductive outcome in humans, a number of epidemiological studies in which glyphosate exposure was considered are presented and discussed in chapter B.6.6 (Reproductive toxicity).

Corsini et al. (2012, ASB2014-9352) submitted a comprehensive review on pesticide induced immunotoxicity in humans. The authors conclude that the available studies on the effects of pesticides on human immune system have several limitations including poor indications on exposure levels, multiple chemical exposures, heterogeneity of the apprach and diffuculty in giving a prognostic significance to the slight changes often observed. Further studies would be necessary.

Sugeng et al. (2013, ASB2014-9733) performed a hazard ranking of agricultural pesticides for cancer, endocrine disruption and reproductive/developmental toxicity in Yuma County, Arizona. Glyphosate was not considered relevant concerning carcinogenicity and reproductive toxicity in result of this ranking. Concerning endocrine disruption the authors concluded a low hazard.

Lesmes-Fabian et al. (2012, ASB2014-9726) reported results of dermal exposure assessment of pesticide use by sprayers in potato farms in the Colombian highlands. The authors conclude that the results would suggest that to reduce the health risk, three aspects have to be considered: avoiding to modification of nozzles, using adequate work clothing made of thick fabrics and cleaning properly the tank sprayer before the application activity.

Perry et al. (2014, ASB2014-9626) report the epidemiology of pesticide exposures reported to poison centres in the UK over a 9-year period. The authors conclude that the data from this survaillance study indicate that poison centre resources can usefully monitor pesticide exposures resulting in health care contact in the UK. The NPIS may usefully be one competent to the UK`s response to European legislation requiring survailance of complications resulting from pesticide use.

Labite and Cummins (2012, ASB2014-9604) submitted a quantitative approach for ranking human health risks from pesticides in Irish gorundwater. According to human health based risk glyphosate was ranked by this method at number 37 of 40 pesticide substances.

Horiuchi et al. (2007, ASB2014-9570) describe 394 cases of dermatitis in Saku district in Japan. Three of these 394 cases have been related to glyphosate.

Goldner at al. (2013, ASB2014-9492) evaluted the assciation between thryoid disease and use of insecticides, herbicides and fumigants/fungicides in male application in the Agricultural Health Study

(AHS). The authors conclude that there is a association between hypothyroidism and specific herbicides (especially 2,4-D, 2,4,5-T and 1,4,5-TP) and insecticides in male applicators. There was no significantly increased association observed for glyphosate.
Roberts et al. (2012, ASB2014-9394) submitted a review on pesticide exposure in children. The authors conclude that childrens exposures to pesticides should be limited as much as possible. According to the authors there would also be numerous reports in the medical literature of adverse events after human exposure. Patients would have presented with signs and symptoms consistent with an aspiration pneumonia-like syndrome.
In response on this article of Roberts et al. a letter was published by Goldstein (2012, ASB2014-9493) from Monsanto company. In this letter the author is correcting some inaccuracies regarding glyphosate in the Roberts review.
Chien et al. (2012, ASB2014-9326) submitted a retrospective cohort study on risk and prognostic factors of inpatient mortality associated with unintentional insecticide and herbicide poisonings. 3968 inpatients recruited at hospitalization between 199 and 2008 in Taiwan have been considered in this study. The authors conclude that overall survival for herbicide impatients was significantly worse than for insecticide poisoning patients. Further information on the specific type of pesticide was not available.
A study on the epidemiology of glyphosate-surfactant herbicide poisoning in Taiwan, 1986- 2007 was submitted by Chen et al. (2009, ASB2014-9318). A total of 2186 patients were eligible for analysis. Most of the exposures were related to oral ingestion and attempted suicide. The authors conclude that age, ingested amount, delayed presentation and reason for exposure were likely to be determinants of the severety of GlySH exposure. Because shock is the major cause of death and usually develops early after GlySH exposure, prompt fluid replacement therapy seems critical in the initial management of such exposure.
Carroll et al. (2012, ASB2014-9308) studied diurnal variation in probability of death following self-poisoning in Sri Lanka. No evidence of diurnal variation in the outcome was observed for glyphosate.
B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests
The summary in this section is based on well over 30 years of experience with numerous formulations of glyphosate in a wide range of situations. The extensive use of glyphosate has encouraged clinical assessment of various interventions and has resulted in reporting of alleged associations of symptoms with exposures to glyphosate products. The clinical toxicology of glyphosate and of glyphosate-surfactant formulations have been the subject of an extensive review (2004, ASB2012-11576), and a review of cases with assessment of clinical prognostic factors was more recently published (. 2008, ASB2012-11879).
Animals do not have the shikimic acid pathway; and no direct target-mediated mode of action in mammalian systems has been clearly identified to date (2004, ASB2012- 11576). Glyphosate does not inhibit the cholinesterases, and has no cholinergic effect. While incidental exposure in glyphosate-surfactant herbicide mixtures is common, review of available case reports (AAPCC 2003-2011) indicates that the vast majority of reported non- suicidal exposures involve skin and/or eye irritation or irritation of the respiratory tract by inhalation of spray mist, and that systemic symptoms are rare following non-suicidal exposures to glyphosate products. Based upon human experience and animal data, even those systemic symptoms reported following incidental exposure appear unlikely to be causally related to exposure (., 2002, ASB2012-11831).
The following clinical effects are divided into those expected following minor and significant exposures for each category based upon expected severity of systemic symptoms. The factors which determine if the exposure was minor or significant include:

• Route of exposure.
Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures for purposes of the symptom descriptions below. Ingestions more than 50 mL (one mouthful if amount unknown) of a product with >10 % glyphosate concentration may be significant.
• Concentration of the product.
Glyphosate concentrations of less than 10 % rarely if ever produce significant toxicity. Most serious illness has historically resulted from ingestion of the 41 % (glyphosate IPA) concentrate. In the absence of extensive clinical experience for the 11-40 % concentration range, any ingestion of greater than 50 mL of a glyphosate preparation having a greater than 10 % concentration of glyphosate salts should be considered potentially significant for purposes of the symptom descriptions below.
• Intent of the exposure.
Accidental ingestion rarely involves large quantities of concentrated formulations. Intentional ingestion cases may not present with a reliable history and may require observation if the amount ingested cannot be reliably determined.
Route and organ system specific syptoms of exposure:
Dermal
Minor exposures:
Contact with skin may produce a dermatitis similar to that of detergents (Bradberry et al., 2004, ASB2012-11576)
It is expected that the severity of injury following skin exposure will be significantly decreased with a less concentrated product and with a reduced duration of contact.
Phototoxic reactions (sunlight or ultraviolet (UV) light induced skin reactions) have been reported. These symptoms are believed to be due to an antimicrobial additive (benzisothiazolone) which is present in selected residential use (i.e. non- agricultural) products containing 10 % glyphosate or less (2004, ASB2012-11576).
Significant absorption through the skin does not occur (see also B.6.12). Significant exposures:
Skin exposures are not expected to cause systemic effects or serious cutaneous effects when the results of animals studies and the low dermal absorption are taken into account. There are no reports that would suggest the contrary. Symptoms as noted in the minor exposure may occur.
Ocular
Minor exposures:
A review of ocular exposures to US glyphosate-surfactant formulations (1513 exposures over a 5-year period), showed no permanent eye injury (1999, TOX2002-699).
Human eye exposures have generally resulted in temporary conjunctival irritation, clearing after irrigation or in 1-2 days and permanent eye damage is said to be “most unlikely” (2004, ASB2012-11576).
It is expected that the severity of injury following eye exposure will be significantly decreased with a less concentrated product or with a reduced contact time.
Significant exposures:
Eye exposures are not expected to cause systemic effects or serious ocular injury (1999; TOX2002-699, 2004, ASB2012-11576).
Systemic exposure – ingestion or inhalation
• Neurologic
Minor exposures:

There is no clinical or experimental evidence that glyphosate or glyphosate-surfactant formulations cause neurological symptoms or injury after exposure by any route.
Significant exposures:
There have been no reports of primary convulsions after ingestion.
One author reports most patients present with a clear sensorium unless another substance, such as alcohol, has been co-ingested or severe hypoxemia has occurred (1989, TOX9552426); however "moderate disorders of consciousness" have been reported within 48 hours of suicidal ingestions of the concentrate (, 1987, Z35531;
, 1988, Z35532). This has occurred in patients with significant systemic illness and is not believed to be the result of reduced organ perfusion (., 2004, ASB2012-11576) or perhaps other factors such as metabolic disturbance but the possibility of a direct toxicological effect cannot be excluded (., 2004, ASB2012-11576).
There are two isolated case report of Parkinson's disease developing in individuals with a history of glyphosate product exposure (2001, ASB2012-11557;
2011, ASB2012-12047). These publications are reported in detail with Klimisch rating in chapter B.6.7 (Neurotoxicity) under B.6.7.2 (Published data), because they are discussed there in context with other studies on neurotoxicity and on Parkinson`s disease.
• Gastrointestinal:
Minor exposures:
Minor exposures are likely to be asymptomatic, but the patient may experience an unpleasant taste, tingling, mild self-limited nausea and vomiting.
Self-limited diarrhoea may also occur which is thought to be due to the surfactant.
Significant exposures:
A burning sensation in the mouth and throat, salivation, oral erythema, sore throat, dysphonia, dysphagia, epigastric pain, nausea, spontaneous vomiting, abdominal pain and diarrhoea are common and may last up to a week.
Serum amylase may be elevated; isoenzyme analysis done in a few cases identified a salivary gland origin (1989, TOX9552426).
In severe cases with large ingested doses, hematemesis, GI bleeding, melena and hematochezia may occur. Paralytic ileus has been reported as a rare event.
Endoscopy has noted erosions of the pharynx and larynx, esophagitis and gastritis with mucosal oedema, erosions and haemorrhage. However, transmural injury and perforation have not been noted (1999, ASB2012-11510).
In fatal cases, autopsy notes mucosal or transmural oedema and necrosis throughout the small bowel with erosion and haemorrhage; in the large bowel, mucosal oedema and focal haemorrhage was noted (, 1989, TOX9552426).
Clinical, autopsy and experimental evidence (1987, TOX9552430) indicate a potential for gastrointestinal damage from glyphosate components of glyphosate formulations, but the frequency of severe injury appears to be low.
Chien et al. (2013, ASB2014-9321) studied the spectrum of corrosive esophageal injury after intentional paraquat or glyphosate surfactant herbicide ingestion. They performed an observational study on 47 patients with paraquat or glyphosat ingestion. The authors conclude that paraquat and glyphosate are mild caustic agents that produce esophagea injuries of grad 1, 2a and 2b only. The data suggest a potential relationship between the degree of esophageal injury and systemic complications.
• Cardiovascular: Minor exposures:

Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Cardiovascular effects are not expected to result from such minor exposures and no reports are available.
Significant exposures:
Hypotension is common after ingestions of a mouthful or more of the concentrated product (not the diluted forms) and usually responds to IV fluids and pressor amines. Shock as manifested by oliguria, anuria and hypotension which was unresponsive to fluids and pressors, ultimately resulting in death, has been reported. (1989, TOX9552426, 2004, ASB2012-11576). Transient hypertension may be noted.
• Upper respiratory: Minor exposures:
Dermal, eye and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Significant upper respiratory effects are not expected from minor exposures, but minor irritation or discomfort may occur (., 2004, ASB2012-11576).
Significant exposures:
Significant systemic exposures are not anticipated to occur via the inhalational route. However, if occurring, they would most probably also affect the lower respiratory tract (see below).
• Lower respiratory: Minor exposures:
Because of the non-volatile nature of glyphosate and the surfactant, exposures to vapour is not possible. The spray equipment that is commonly used will produce particles that are non- respirable.
Significant exposures:
Tachypnea, dyspnea, cough and bronchospasm including cyanosis have been seen in severe ingestions (more than a mouthful of concentrated product). These effects are indiactive of systemic toxicity.
Aspiration pneumonia, pulmonary oedema and respiratory failure have been seen although the exact role of aspiration has not been fully investigated.
An isolated case report suggests the development of acute pneumonitis in a worker following his performing maintenance on non-operating spray equipment used to apply a glyphosate- surfactant formulation (1998, ASB2012-11513). However, actual exposure and its extent could not be really substantiated in this case. Accordingly, the occurrence of pneumonitis in this individual is more likely to be coincidental by nature although a (different) occupational origin seems plausible (1999, ASB2012-11511).
There is also a case report from Germany in which a glyphosate-surfactant product (tallowamine or “POEA” based) was applied by knapsack spayer in a 0.5ha forestry application at the registered application rate at 25° C for approximately 3 hours without
respiratory protection (Burger et al., 2009, ASB2013-11831). About 7 hours after application he developed chest pain with rapidly increasing severe respiratory distress and fever up to approximately 38° C. On hospital admission, radiographic changes of lungs could be demonstrated. To further assess possible causes, bronchoscopy and closed lung biopsy was performed. Histopathology revealed “toxic inflammation of the lungs” that was significantly different from bacterial infection. After 7-days of drug treatment, changes in lung reversed but six months after the incident, the patient still experienced moderate respiratory complaints on exertion. In the X-ray examination, there were still detectable lung changes although some improvement had been noted. In addition, in the same reference, 20 cases of inhalative exposure among a total of 60 reports on confirmed or presumed poisoning incidents with glyphosate herbicides from Germany (since 1990) were mentioned with breathing difficulties occurring in 50 % of the affected people. No more details

on clinical courses or outcomes were given but it was emphasised by the authors as ”striking” that the involved products nearly always contained

• Renal:

Minor exposures:

Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Renal effects are not expected to result from such minor exposures and no reports are available.

Significant exposures:

Hypotension and hypovolemic shock may result in oliguria and anuria, following severe ingestions (2004, ASB2012-11576). Abrupt rises in BUN and serum creatinine may be seen.

• Metabolic: Minor exposures:

Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Metabolic effects are not expected following minor exposures and no reports are available.

Significant exposures:

Mild fever may be noted even in the absence of infection (2004, ASB2012- 11576)

Metabolic acidosis is often seen in a severely poisoned patient 2004, ASB2012-11576) and this acidosis may fail to respond to bicarbonate therapy. Although the exact nature was not elucidated, a lactic acidosis was suspected.

• Hematologic: Minor exposures:

Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Haematological effects are not expected from minor exposures and no reports are available.

Significant exposures:

Leukocytosis without evidence of bacterial infection has been noted in peripheral blood after ingestion of the concentrate (, 2004, ASB2012-11576).

Hemoconcentration has been seen as a result of intravascular volume depletion and might indicate severe capillary fluid leakage (1989, TOX9552426).

No primary toxic effects on bone marrow or formed elements have been reported to date.

• Hepatic: Minor exposures:

Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Hepatic effects are not expected from such minor exposures and no reports are available.

Significant exposures:

No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin are reported (1989, TOX9552426; 2004, ASB2012-11576).

• Clinical chemistry (electrolytes): Minor exposures:

Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance. However, such signs are not expected following a minor exposure.

Significant exposures:

Electrolytes (Na, K, Cl and Ca) in the absence of renal failure generally remain normal. Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance.

POTASSIUM SALTS: While potentially toxic ingestions of all glyphosate products may result in fluid and electrolyte disturbances, particular attention to potassium may be important following ingestion of the potassium salt products. Close monitoring of serum potassium levels and/or electro-cardiographic monitoring (for peaked T-waves or rhythm disturbances) is recommended following

significant ingestion of potassium salt products, particularly for high risk individuals. Individuals with the following may be at elevated risk following acute potassium exposure: known hyperkalemia, renal failure / renal dysfunction, use of potassium sparing diuretics, hypoaldosteronism, co-ingestion of other K+ containing materials, underlying heart disease, use of digoxin, digitoxin, oabain, or exposure to other cardiac glycosides. The quantity of potassium ingested from a glyphosate potassium salt product can be estimated from the weight percent of glyphosate potassium as:
Percent K+ salt x 5.3 = mEq potassium per 100 cc of product
Several case reports indicate that after ingestions of large amounts of glyphosate-potassium salt concentrate solutions, clinically significant hyperkalemia may occur. (2001, ASB2012-11556) reported an intoxication in a 65 year old female who ingested a glyphosate- potassium salt (350 mL Roundup Maxload missing from container, in addition to 250 mL of another glyphosate formulation which was not a potassium salt but amount actually ingested unclear) in a suicidal attempt. On admission, serum potassium level was 9.3mEq/L (typical normal value < 5) with electrocardiographic changes consistent with hyperkalemia. The patient did have a concomitant acidosis (pH 7.272) which may account for some portion of the elevation in potassium (acidosis displaces intracellular potassium). The patient responded to medical management and survived.
(2012, ASB2012-11863) reported the case of a 69 year old female who ingested approximately 500 mL of the same product. On arrival in the hospital, the patient had
hyperkalemia (10.7 mEq/L), pulseless ventricular tachycardia, and a severe metabolic acidosis (pH 7.005, will elevate potassium.) The patient required aggressive cardiopulmonary resuscitation and hemodialysis but did recover.
According to the GTF dossier (no particular reference given), Monsanto is aware of one additional (unpublished) case of a similar ingestion with dramatically elevated potassium level in which the patient was moribund when medical care was instituted. The patient could not be resuscitated. Because serum potassium levels rise rapidly following death (due to redistribution of intracellular potassium), it is not possible to know how much of the observed hyperkalemia was the result of the ingestion versus profound acidosis and post mortem redistribution (which is partially due to acidosis).
It should be noted that the issue of hyperkalemia is limited to cases involving the suicidal ingestion of glyphosate-potassium concentrates. Potassium is a normal component of the human diet, and potassium intake attributable to occupational glyphosate-surfactant herbicide exposure will be negligible compared to typical dietary intake. While the concentrate formulations may contain up to approximately 250 mEq of potassium per 100 mL, product diluted for use (1 % glyphosate concentration) will contain about 6 mEq potassium per 100 mL. By way of reference, a medium size banana contains about 10 mEq (425 mg) of potassium.
Finally, it should be noted that the apparently very large (>150 mL) ingestions of glyphosate-surfactant concentrates observed in these cases are well within the range isopropylamine salt products reported to produce fatalities, and that elevations in potassium concentrations are reported (probably due to acidosis) following ingestions of glyphosate IPA salt products. While the cases do suggest that potassium salt products likely contribute to the risk of hyperkalemia, it is not clear at this time if the use of postassium salts will increase the overall clinical severity and/or mortality associated with glyphosate concentrate product ingestions.
Specific diagnostic testing and prognostic considerations
Serum or other body fluid measurements of glyphosate will be generally not available in a time frame that would be useful for acute clinical diagnosis. As the management of symptoms associated with glyphosate-surfactant product ingestion is symptom-driven in any event, the lack of rapidly available

knowledge on concentrations of glyphosate will generally not impair clinical care. Levels may be more helpful in addressing forensic issues.
Attention should be paid to electrolyte concentrations in individuals with significant ingestion exposures, particularly to glyphosate-potassium concentrate solutions.
Respiratory distress requiring intubation, pulmonary oedema, shock (systolic BP <90 mmHg), altered consciousness, abnormal chest X-ray, ingestion of over 200 cc concentrate (41 %), or renal failure necessitating dialysis have been associated with a higher risk of poor clinical outcomes including mortality (2008, ASB2012-11879). A prognostic index based upon these factors was developed but its use is not expected to contibute significantly to improved medical care. As symptom onset may be delayed, early use of such prognostic indicators may in fact result in under-estimation of clinical severity of a case.
B.6.9.5 First aid measures
The following, quite general measures have been proposed by notifiers but were not evaluated by RMS toxicologists because this is beyond the scope of this RAR :
Skin exposure:
Remove all contaminated clothing and flood the skin surface with water. Wash the exposed skin twice with soap and water.
A close examination of the skin may be required if pain or irritation exist after decontamination.
All clothing that are contaminated should be laundered before they are worn again
Eye exposure:
Remove contact lens from the affected eye(s) if appropriate.
Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.
A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably with an eye specialist.
Ingestion exposure:
Dilute preparations (Glyphosate <10 %): An ingestion of a dilute preparation of glyphosate (<10 %) probably does not require treatment other than dilution with milk or water, and symptomatic care. Further gastrointestinal decontamination is not needed, even if spontaneous emesis has not occurred.
Concentrated (> 10 %) preparations: Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 mL/kg in a child or 250 mL in an adult.
Inhalation exposure:
No pulmonary treatment is necessary for occasional, accidental breathing of mist.
Severe, acute pulmonary injury has not been reported following inhalation exposure. Individuals with respiratory distress from any cause should be relocated (if medically stable) to fresh air and receive supplemental oxygen if available.
In the event of respiratory failure or lack of respiration, administer artificial respiration (or if pulse not detectable, cardiopulmonary resuscitation).
B.6.9.6 Therapeutic regimes
The following therapeutic regimes have been proposed by notifiers but were not evaluated by RMS toxicologists because this is beyond the scope of this RAR :
“The registrants believe that the following represent general best practices for medical management of serious ingestions of glyphosate-surfactant products.

Establish respiration and assure adequacy of ventilation. Eye exposure:
Remove contact lens from the affected eye(s) if appropriate.
Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.
A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably by an eye specialist.
Ingestion exposure:
Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 mL/kg in a child or 250 mL in an adult.
patient disposition:
Concentrated preparations (Glyphosate 41 % or greater):
Any person ingesting greater than a large mouthful (50 mL in an adult, 0.5 mL/kg in a child) of a 41 % or greater glyphosate concentrate product should be admitted to a hospital and observed for 24 hours.
Any adult ingesting greater than 100 mL of a 41 % or greater glyphosate concentrate product (>1.4 mL/kg in a child) should be admitted to the intensive care unit.
Any suicide attempt by person ingesting a concentrated product should be evaluated for psychological status and should be admitted if necessary for observation with suicide precautions.
Concentrated preparations (Glyphosate 10 %-40 %):
An ingestion of concentrated glyphosate (10 % - 40 %) will usually result in spontaneous emesis. There is limited experience with glyphosate formulations in this concentration range. In view of this limited information, the registrants currently recommend managing these ingestions in a manner similar to the management of the 41 % concentrate.
Prevention of absorption (This lists various methods for “Prevention of Absorption". These should NOT be construed as being in order of preference. Consult with Poison Center or medical personnel to determine the need for and preferred method for decontamination. In many instances, no intervention is required.)
Gastric aspiration: If no significant spontaneous vomiting has occurred gastric aspiration may be considered. If performed soon after ingestion, gastric emptying by aspirating liquid gastric content with a lavage or standard NG tube may possibly remove some of the ingested glyphosate. The intent is to remove unabsorbed liquid by aspiration not to use lavage fluid. As absorption of liquids is likely to be relatively rapid, gastric aspiration after 1 to 2 hours is unlikely to be effective.
Emesis: Emesis is controversial at this time. Glyphosate/surfactant products are irritants. The registrants do not recommend the routine use of syrup of ipecac for glyphosate / surfactant ingestions because of the risk of exacerbating the irritant effects on the GI tract.
Activated charcoal: There are no data to support or refute the use of activated charcoal in glyphosate/surfactant product ingestions. Low molecular weight, amphoteric compounds and detergents do not always bind well to activated charcoal. In the event of a mixed ingestion, activated charcoal may be advisable.
Assessment of gastro-intestinal injury
Injury to the upper gastrointestinal tract may occur following ingestion of glyphosate concentrates. A study of upper gastrointestinal endoscopy following glyphosate–surfactant ingestions suggested that Zarger grade 2 lesions (erosions) were associated with longer hospital stay and with a higher incidence of serious complications (Chang 1999, ASB2012- 11510). However, no major esophageal or gastrointestinal injury was observed, and strictures have not been reported following uncomplicated

glyphosate-surfactant ingestion.
Because no serious gastrointestinal injury is reported, and because the need for hospitalisation and/or treatment of complications can be determined without endoscopic evaluation, the registrants recommend that endoscopy be reserved for patients with co-ingestions suggesting a need for endoscopy or for patients with signs and symptoms suggestive of more serious injury (serious oral burns, inability to handle secretions, clinical obstruction) regardless of clinical history.
Monitor blood pressure:
Monitor the patient closely for signs of hemodynamic instability. The insertion of a Swan- Ganz catheter may be warranted.
Hypotension:
If the patient is hypotensive, administer IV fluid boluses and place in Trendelenburg position. If the patient is unresponsive to these measures, administer a vasopressor (dopamine, epinephrine, norepinephrine, phenylephrine, isoproterinol, etc.) if needed.
Monitor blood gases and obtain chest radiograph:
Consider the use of repeat blood gases and a peripheral pulse oximeter to monitor hypoxemia. Observe closely for sign of acidosis.
Pulmonary oedema:
Closely monitor arterial blood gases. If PO2 cannot be maintained above 50 mm Hg with inspiration of 60 % oxygen by face mask or mechanical ventilation, then positive end expiratory pressure (PEEP) or continuous positive airway pressure (CPAP) may be needed. Avoid a positive fluid balance by careful administration of crystalloid solutions. Monitor fluid status through a central venous line or Swan Ganz catheter as needed.
Acidosis:
Correction of acidosis should be guided by blood gases, electrolytes and clinical judgment. Attention should be directed to volume status and correction of poor perfusion in mild cases. Sodium bicarbonate may be used to correct the acidosis in severe cases.
Hyperkalemia (from ingestion of Potassium salt formulations):
For moderate hyperkalemia (K+ of 6.0-7.0 mEq/L), administer sodium polystyrene sulfonate with sorbitol. For more severe hyperkalemia (K+ > 7 mEq/L) or serious complications of hyperkalemia, correct metabolic or respiratory acidosis if present to allow potassium to enter the intracellular space. Additional management may include a glucose/insulin drip, intravenous sodium bicarbonate or calcium, and dialysis to remove excess potassium.
Monitor renal function closely:
Assure adequate urine output. Catheterise severely ill patients. Hemodialysis may be needed in the event of renal failure or electrolyte disturbances.
Enhanced elimination:
Forced diuresis: Glyphosate is excreted very well by the kidneys. Adequate urine flow will ensure the rapid elimination of glyphosate. Although elimination may perhaps be enhanced by forced diuresis, there is no clinical evidence that this is necessary, and fluid overload may precipitate pulmonary oedema.
Hemodialysis: Hemodialysis may be useful to correct fluid, electrolyte and metabolic disturbances in the patient with renal failure. The institution of hemodialysis solely to enhance
the removal of glyphosate or other product components is not of proven benefit. Nevertheless, it is reasonable to consider the initiation of hemodialysis in the significantly ill patient who fails to respond to routine supportive management.
Serious exposure via inhalation is not expected:

Inhalation exposures are not expected due to the aerodynamics of droplet size from sprayers and because the product is not volatile. Monitor the patient for signs of respiratory compromise. Create an artificial airway if necessary. Check adequacy of tidal volume. Monitor the patient for respiratory distress; if a cough or dyspnea develop, evaluate the patient for respiratory irritation, bronchitis and/or pneumonia, but these are not expected.
Serious exposure via skin is not expected:
Significant skin exposures are not expected; however, the patient should be treated empirically if a dermal exposure is suspected. Remove all contaminated clothing and flood the skin surface with water. Wash the exposed skin twice with soap and water. A close examination of the skin may be required if pain or irritation exist after decontamination. All contaminated clothing should be laundered before wearing.
Laboratory:
Monitor electrolytes, especially if the patient is experiencing vomiting and diarrhea.15 Patients ingesting concentrated products based on the potassium salt of glyphosate may ingest large amounts of potassium (see calculations above). Observe serum potassium and/or electrocardiogram carefully. Patients experiencing pulmonary symptoms or having chest radiograph changes should have arterial blood gas monitoring. A peripheral pulse oximeter and a Swan Ganz catheter may be needed."
Gil et al. (2013, ASB2014-9488) examined the potential therapeutic effects of intravenous lipid emulsion (ILE) on the patients with acute glyphosate intoxication. The authors conclude that ILE administration was associated with lower incidence of hypotension and arrhythmia in patiens with acute glyphosate intoxication. ILE administration seems to be an effective treatment modality in patients who ingested glyphosate.
Subchronic in vivo studies have been performed with formulation Bushfire in Wistar rats (Tizhe et al., 2013 (ASB2014-6963; Tizhe et al., 2013 (ASB2014-6965) and Tizhe et al., 2013 (ASB2014-6964). The authors concluded that toxicity of glyphosate would be amelioreted by zinc supplementation. However, the conclusions are considered not clearly evidenced because of the use of a formulation instead of the active substance, low animal numbers, no clear dose dependency of effects and further limitations.
Astiz at al., 2013 (ASB2014-7493)studied the protective effect of lipoic acid (LA) against the intoxication by mixtures of pesticides including glyphosate. A mixture of different pesticides including glyphosate was i. p. injected to male rats. The results suggest that LA administration would be a promising therapeutic strategy for coping with disorders suspected to be caused by oxidative stress generators such as pesticides.
Astiz et al. (2012, ASB2014-9201) investigated the protective effects of lipoic acid as antioxidant in the case of oxidative stress caused by glyphosate or other pesticides. The authors conclude that lipoic acid displayed a protective role against pesticide-induced damage, suggesting that LA administration is a promising therapeutic strategy.
B.6.9.7 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion
Dermal exposure:
Skin irritation following exposure to glyphosate-only or glyphosate-surfactant materials is generally limited to topical irritation which will resolve within 3 days to 1 week following exposure. If exposure is aggravated by occluded conditions or physical abrasion, more severe skin injury with open skin injury may rarely result and may take longer to fully resolve.
Eye exposure:
Irritant symptoms generally resolve within 3-7 days of exposure. Most irritation is minor, but

exposure to concentrate or the occurrence of a foreign body or of abrasions (from rubbing the eye) may result in corneal abrasion requiring topical antimicrobial therapy, often given in conjunction with topical corticosteroids and temporary eye patching to provide symptomatic relief. As noted above, a large study of (U.S.) ocular exposures to glyphosate-surfactant products demonstrated no long term eye injury.

Inhalation exposure:

Glyphosate-surfactant products generally do not contain readily volatile ingredients and thus inhalation exposure is limited to inhalation of agricultural droplets, which will deposit primarily in the upper airway. Resulting irritant symptoms will generally resolve within hours to a few days following exposure.

Ingestion:

Following minor or incidental ingestions, or ingestion of fully diluted formulations, gastrointestinal upset with nausea, vomiting, and diarrhoea may occur. Nausea and vomiting usually resolve within a few hours of ingestion. Diarrhoea may last for several days but is generally not severe. Following a major ingestion, the onset of systemic symptoms may be delayed by several hours. Fatalities due to cardiovascular failure are generally delayed by 12 – 36 hours. For serious but non-fatal cases, primary clinical injury generally is manifest within 72 hours but secondary complications such as infection or respiratory distress syndrome may supervene. The majority of serious but surviving cases will be fully recovered within 7-10 days of ingestion. Individuals with complicated hospital courses may require a more extended and highly variable time to recover.

B.6.9.8 Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment

The outcome of eye, dermal, and inhalational exposures, which are not expected to result in serious injury in any event, will not be significantly altered by delays in medical management. Similarly, minor oral exposures are symptomatically managed and unlikely to result in severe gastrointestinal symptoms. Medical management with intravenous fluids may provide some symptomatic relief in the event of dehydration, but recovery is anticipated in any event.

For serious ingestions having major electrolyte disturbances or life threatening alterations of cardiovascular performance, medical intervention may be life saving. Fortunately, as noted above, the onset of serious symptoms following ingestion is generally delayed by at least several hours, allowing for medical transport in all but the most remote or extreme circumstances.

B.6.13.2 Available toxicological data for each formulant

Please refer to the material safety data sheets provided in Document J of this dossier. There is no toxicological classification of co-formulants which has to be considered for classification and labelling of MON 52276 according to these MSDS.

Remarks on surfactants included into glyphosate-containing plant protection products

All glyphosate-containing plant protection products contain surfactants or - if not present as an integral component – are to be mixed with surfactants as a compulsory additive to produce the ready-to-use dilution. As has already been discussed during the first Annex I inclusion procedure for glyphosate it became apparent that glyphosate-containing products were more toxic than glyphosate alone. This phenomenon was attributed to the presence of particular surfactants predominantly, namely the POE-

Some MS may wish to allow for this in the context of the national risk assessment for POE- containing glyphosate formulations. Therefore, a toxicological evaluation for

POE- (including reference values) is provided in a separate paragraph within Vol. 3 (B.6.13.3) of this

RAR.
MON 52276 which is the representative formulation here does not contain any POE- Instead, a different type of surfactant, i.e. a quarternary ammonium compound,
is used for MON 52276.
Since studies on MON 52276 concerning acute toxicity, skin and eye irritation as well as skin sensitisation were performed with the original preparation of MON 52276 the results for these toxicological short-term endpoints also reflect possible effects provoked by the surfactant. No further studies are needed according to the data requirements for plant protection products. Therefore, no toxicological long-term studies were submitted using the formulated product or the surfactant alone. Moreover, up to now no reference values have been considered necessary for the surfactant used, thus, no respective risk assessment was required.
According to the material safety data sheet for the surfactant provided by the applicants this co-formulant was not mutagenic in an Ames-test. No further information on toxicological long-term endpoints was given in this material safety data sheet.
In addition, MON 52276 has been authorised within the EU for many years. There are no medical data which have been collected by occupational physicians or poisoning emergency centres describing long-term adverse health effects for operators provoked by this plant protection product until today.