Glyphosate &
its IPA-, K-, NH4- and DMA salts

Representative formulation:

Product MON 52276
(360 g/L glyphosate acid)

Herbicide

Application for Renewal of Approval (AIR 2) according to Commission Regulation (EC) N° 1141/2010

ANNEX II -III
TIER III summary
Overall assessment and conclusion

Document N:

Overall assessment and conclusion

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1. The substance, its properties, uses, proposed classification and labelling

1.1 Identity of the active substance and preparations containing it

All relevant information and data concerning the identity of glyphosate acid and the salts of glyphosate (glyphosate IPA-salt, glyphosate NH_4-salt, glyphosate K-salt and glyphosate DMA-salt) and of the formulated product, MON52276, have been provided in Section 1 of the Annex II and Annex III dossier except in the case of confidential information which is included in Document J.

1.2 Physical and chemical properties

Glyphosate acid is a 'glycine' herbicide. Each member of the glyphosate taskforce (GTF) has included one or more specifications covering different source(s) of glyphosate acid as manufactured in Document J.

Glyphosate acid is a white odourless crystalline solid comprised of one basic amino function and three ionizable acidic sites. The purified substance has a relative density of 1.704 $g.cm^{-3}$ and melts at 189.5 °C. The free acid dissociates readily (pKa: 2.34, 5.73, 10.2) resulting in a moderate water solubility of 10.5 g/l (20°C). Its solubility in water increases substantially when converted to monobasic salts by isopropylamine, KOH, NH_4OH and dimethylamonium. Glyphosate is generally formulated as water soluble formulations based on these more soluble monobasic salts. The n-octanol/water partition coefficient (log P_{OW} = -3.2 at 25°C) indicates no potential for bioaccumulation. Its vapour pressure amounts to 1.31 x 10^{-5} Pa at 25°C. Henry's law constant is 2.1 x 10^{-7} $pa.m^3.mol^{-1}$. Glyphosate is stable to hydrolytic degradations in sterile water in most environmentally relevant pH ranges. The pure active substance does not absorb light significantly at wavelengths longer than 230 nm indicating no sensitivity to direct photolysis. Photochemical oxidative degradation in air is expected to occur fast in 1.6 hours. Its (auto)flammability and oxidizing properties are not critical. Glyphosate acid is not explosive.

MON 52276 is an aqueous based soluble concentrate of the isopropylamine (IPA) salt of glyphosate containing 360 g/L glyphosate acid. It is not flammable, explosive or oxidizing. It is slightly acidic and moderately viscous. Stability testing shows that the product has a shelf-life in excess of two years and is not affected by short periods of low temperature exposure.

1.3 Details of uses and further information.

MON 52276 is a systemic contact herbicide for use in agriculture, horticulture, forestry, viticulture, amenity, weed control of non-cultivated areas, home and garden uses and aquatic weed control. The active ingredient, glyphosate, binds to and blocks the activity of its target enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) and enzyme of the aromatic acid biosynthetic pathway. The inhibition of the enzyme prevents the plant from synthesizing the essential aromatic amino acids needed for protein biosynthesis.

The joint representative GAP for the re-registration of glyphosate includes only a selection of these uses including (1) pre-planting applications to all crops, (2) post-planting pre-emergence of all crops, (3) pre-harvest in cereals, peas, beans, oil seed rape, flax musteard and line seed, (4) direct spray applications under foliage, inter-row and around the base of the trunk in orchards and

vines and (5) spot applications (spray and knapsack) under foliage, inter-row and around the base of the trunk in orchards and vines.

The maximum cumulative application rate is 4.32 kg/ha. The maximum application rate per treatment is 2.16 kg/ha except for spot applications in orchards and vines where the maximum application rate is 2.88 kg/ha. Mutliple applications pre-emergence in all crops and in orchards& vines are possible provided the maximum annual rate is not exceeded. Typical spray dilutions are 100-400 L/ha but undiluted or low volume applications especially in orchards and vines are also included in the joint GAP.

1.4 Classification and labelling

1.4.1 Classification and labelling of the a.s.

GLYPHOSATE ACID

Physical and chemical properties:	No classification
Toxicological data:	Eye damage 1 (hazard statement: H318)
Environmental fate and behaviour data:	Aquatic Chronic 2 (Hazard statement: H411)

Label:

Symbol: GHS05, GHS09

Indication of Danger: 'Danger'

Hazard Statement: H 318 (Causes serious eye effects), H411 (Toxic to aquatic life with long lasting effects).

On the label, the Hazard Statements (H-phrases: H318 & H 411) as well as the following Precautionary statements (P-phrases) need to be mentioned: P305, P351, P338, P310, P273, P391 & P501.

According to Part 4 of CLP, following wording shall be included on the label of Plant Protection Products: EUH 401 "To avoid risks to human health and environment, comply with the instructions for use'

GLYPHOSATE SALTS

(with the exception of those specified elsewhere in Annex IV of Regulation 1272/2008)

Physical and chemical properties:	No classification
Toxicological data:	No classification
Environmental fate and behaviour data:	Aquatic chronic 2 (Hazard statement: H411)

Label:

Symbol: GHS09

Indication of Danger: None

Hazard Statement: H411 (Toxic to aquatic life with long lasting effects)

On the label, the Hazard Statement (H-phrase H411) as well as the following Precautionary statements (P-phrases) need to be mentioned: P273, P391 & P501

According to Part 4 of CLP, following wording shall be included on the label of Plant Protection Products: EUH 401 "To avoid risks to human health and environment, comply with the instructions for use'.

1.4.2 Classification and labelling of the PPP

Physical and chemical properties:	No classification.
Toxicological data:	No classification
Environmental fate and behaviour data:	No classification
Environmental effects:	No classification

Label:

Symbol:	None
Indication of danger:	None
Risk phrases:	None

Safety phrases: No precautionary statements (P-phrases) need to be mentioned.

According to Part 4 of CLP, following wording shall be included on the label of Plant Protection Products: EUH 401 "To avoid risks to human health and environment, comply with the instructions for use'

2. Methods of analysis

2.1 Methods for analysis of the active substance as manufactured

Members of the Glyphosate Task Force have submitted methods of analysis of glyphosate as manufactured in the confidential section of this dossier (DOC J).

The requirements for specificity, linearity, accuracy and repeatability of the HPLC-VIS methods proposed for analysis of the two toxicologically relevant impurities (formaldehyde and N-Nitroso glyphosate) in glyphosate as manufactured, have been fulfilled.

2.2 Methods for formulation analysis

For the determination of the active ingredient in the representative plant protection product, an HPLC method with UV detection is proposed. The method has been shown to have satisfactory specificity, linearity, accuracy and repeatability. CIPAC method 284/SL/(M)/- is suitable for the determination of glyphosate in SL formulations.

The requirements for specificity, linearity, accuracy and repeatability of the HPLC-VIS methods proposed for analysis of two joint significant impurities (formaldehyde and N-Nitroso glyphosate) in glyphosate containing formulations, have been fulfilled.

2.3 Methods for residue analysis

2.3.1 Multi-residue methods for residue analysis

Multi-residue method S19 Deutsche Forschungsgemeinschaft and Multi-residue method No. 5, and the Dutch "Analytical methods for residues in pesticides" were found to be not suitable for the analysis of glyphosate residues.

2.3.2 Methods for residue analysis of plants and plant products

The residue of concern was defined on the basis of several plant metabolism study as glyphosate and aminomethylphosphonic acid (AMPA). Several analytical methods for the determination of glyphosate and AMPA in representative plant matrices have been available over time in line with the improvement of analytical techniques.
The most commonly used analytical method (Method DFG 405) for glyphosate and AMPA residues in crops involves a clean up through Chelex 100 resin (Fe^{+3} form), followed by anion exchange chromatography and analysis with HPLC coupled to a post column reaction system to produce a fluorescent derivative which is quantitated with a fluorescence detector (HLPC FLD). Determination involves post-column hypochlorite oxidation and reaction of the amine product with ortho-phtalaldehyde and mercapto-ethanol to produce the fluorescent derivative. The methods have shown to provide satisfactory recoveries for both glyphosate and AMPA yielding limits of quantification of 0.05 mg/kg. The method DFG 405 was validated for analysis of residues of glyphosate and AMPA in barley (grain and straw), maize (green plant and corn), oil seed rape, sugar beet (roots) and citrus at an LOQ of 0.05 mg/kg. The method proved to be highly specific. Good linearity was obtained for glyphosate and AMPA in a concentration range from 0.016 to 2.5 µg/mL for each analyte. Correlation coefficients (r^2) for glyphosate of 0.9996 and 1.000 for AMPA were obtained. The method is accurate (mean recoveries within the 70-110% range). The standard deviations of their recoveries determined following spiking at the limit of quantification provided evidence that the method has satisfactory repeatability. The inter-laboratory validation study conducted demonstrated satisfactory reproducibility.

The second method is based on gas chromatography (GC) coupled with the mass spectrometric (MS) mass selective detection (GC-MSD) after derivatization with trifluoroacetic anhydride and heptafluorobutanol (method RAM 328/01). This method has been successfully validated by interlaboratory studies and is thus suitable for enforcement. The method was validated for analysis of residues of glyphosate and AMPA in flax seed, coffee, cabbage, melon, oat grain and rye straw at levels of 0.05 mg/kg (LOQ), 5 mg/kg and 5 mg/kg. Additionally the method was validated for high residue levels of glyphosate and AMPA in oranges (0.5 mg/kg) and sunflower seeds (20 mg/kg). The method proved to be highly specific (one monitoring ion and two qualifier ions). Good linearity was obtained for glyphosate and AMPA in a concentration range from 0.0003 to 0.02 µg/mL for each analyte. The correlation coefficients (r^2) for glyphosate for the three target ions (m/z 612, 611 and 584) ranged from 0.9977 to 0.9998. For AMPA the correlation coefficients (r^2) for the three target ions (m/z 446, 372 and 502) ranged from 0.9972 to 0.9998. The method is accurate (mean recoveries within the 70-110% range). The standard

deviations of the recoveries determined following spiking at the limit of quantification provided evidence that the method has satisfactory repeatability. The inter-laboratory validation study conducted demonstrated satisfactory reproducibility.

In a more recently developed method glyphosate and AMPA residues are determined directly without derivatization by liquid chromatography with tandem mass spectrometer (LC-MS/MS) in negative multiple reaction monitoring (MRM) mode, monitoring two ions (glyphosate: quantifier: 168→68, qualifier: 168→79; AMPA: quantifier: 110→63, qualifier: 110→79). Glyphosate and AMP A are isolated from crop matrices by high speed blender extraction using 0.1 % formic acid in water and methylene chloride. Following centrifugation, an aliquot of the aqueous phase extract is mixed with isotopically enriched glyphosate and AMPA internal standards then passed through solid phase extraction media for final cleanup. The analytes are analyzed by LC-MS/MS and quantitated using internal standards. The method has been validated for the analysis of the raw agricultural commodities of corn, soybeans, canola, cotton, sugar beets, alfalfa, citrus, cotton oil, potato tuber, carrot roots, onion bulbs, cucumber fruit, cabbage heads, cauliflower heads, lettuce leaves, leek plants and tomato fruit. The limit of quantification (LOQ) is 0.05 mg/kg for both analytes for all crops. The method proved to be highly specific (one monitoring ion and one qualifier ion). Good linearity was obtained for glyphosate and AMPA in a concentration range from 1.25 to 250 ng/mL for each analyte. The correlation coefficients (r^2) of ≥ 9983 for glyphosate and ≥ 0.9991 for AMPA were obtained for standards. Mean recoveries obtained at each level of fortification and overall for each matrix were in the range 70-110% in the method validation for both glyphosate and AMPA. The accuracy of the method is within the limits specified by current EU guidance. Coefficients of variation (relative standard deviation) of recoveries obtained at each level of fortification and overall for each matrix were less than 20% in the method validation for both glyphosate and AMPA. The repeatability of the method is within the limits specified by current EU guidance.

2.3.3 Methods for residue analysis of food of animal origin

Adequate analytical methods for glyphosate and AMPA (including confirmatory methods and interlaboratory validation studies) are available for representative animal matrices. The methods are based on GC-MSD) in the select ion monitoring (SIM) mode after derivatization with trifluoroacetic anhydride and heptafluorobutanol (method RAM 308/01 and RR 94-018B). The methods have shown to provide satisfactory recoveries for both glyphosate and AMPA yielding limits of quantification of 0.1 mg/kg.

Method RAM 308/01 (identical to method RR 94-018B with the exception of the substitution solvent for the crude matrix extract) was validated for analysis of residues of glyphosate and AMPA in bovine kidney, liver and fat at an LOQ of 0.1 mg/kg and in bovine milk and poultry eggs at an LOQ of 0.01 mg/kg. A limit of quantification of 0.03 mg/kg for muscle has been set in the method. The method proved to be highly specific (one monitoring ion and two qualifier ions). Good linearity was obtained in egg (r^2 = 0.9795 and 0.9784 for glyphosate and AMPA respectively) and kidney (r^2 = 0.9946 and 0.9981 for glyphosate and AMPA respectively). In addition good linearity was obtained in kidney, liver and fat with correlation coefficients of 0.9963, 0.9953 and 0.9953 for glyphosate and correlation coefficients of 0.9940, 0.9970 and 0.9971 for AMPA, respectively. Independent validation of the method established linearity for glyphosate and AMPA in milk, liver and egg using matrix-matched standards.

The methods are accurate (mean recoveries within the 70-110% range). The standard deviations of the recoveries determined following spiking at the limit of quantification provided evidence that the method has satisfactory repeatability. The inter-laboratory validation study conducted demonstrated satisfactory reproducibility.

2.3.4 Methods for residue analysis of soil

One primary and two confirmatory methods were provided for the analysis of glyphosate and AMPA residues in soil. The primary and one confirmatory method are based on GC-MSD in the select ion monitoring (SIM) mode after derivatization. In one method (Alferness, 1994), glyphosate and AMPA residues are extracted from soil samples and the aqueous extracts are derivatized with a mixture of trifluoroacetic anhydride and heptafluorobutanol to produce the heptafluorobutyl esters of the acid functional groups and the trifluoroacetyl derivatives of the amines. In the second method (Schneider, 2001) the soil extracts are derivatized with trifluoroacetic acid, trifluoroacetic anhydride and trifluoroethanol to form the volatile ester derivatives. The glyphosate and AMPA derivatives are analyzed by capillary GC and the derivatives are quantified by mass-selective detection using single ion monitoring. The second confirmatory method (Method DFG 405). is based on HPLC-FLD with post-column hypochlorite oxidation and reaction of the amine product with ortho-phtalaldehyde and mercapto-ethanol similar to the corresponding crop method (Method DFG 405).

The limits of quantification of all the tree methods were 0.05 mg/kg of glyphosate and AMPA in soil. All three methods of analysis have been validated for analysis of glyphosate and AMPA residues in soil (recovery, linearity, specificity, limit of quantification and repeatability).

2.3.5 Methods for residue analysis of water

A highly specific method was provided for the analysis glyphosate and AMPA residues in groundwater, surface water and drinking water. The method is based LC-MS/MS and selective ion monitoring quantitation after derivatization with FMOC-Cl, using isotopically enriched internal standards. The method has a limit of quantification of 0.03µg/L for both glyphosate and AMPAin drinking water, surface water and ground water. The linearity of the detector response was in the working range of 0.2 ng/mL to 10 ng/mL. The correlation coefficients of the calibration curves were ≥0.9992. Mean recovery values for glyphosate and AMPA for all fortification levels (LOQ and 10x LOQ) in all matrices were in the range of 70 to 110%. Method validation results from an independent laboratory were well within guideline requirements, demonstrating the reproducibility of the method and confirming that this method is suitable for use in support of post-registration data requirements for glyphosate in the EU.

2.3.6 Methods for residue analysis of air

On the basis of the vapour pressure and Henry's Law constant for glyphosate, it is clear that glyphosate is unlikely to be be found in air. In addition glyphosate is not applied as an aerosol or mist. Consequently, exposure of operators, workers and bystanders by the inhalation route will be minimal. It is therefore contended that an analytical method for air is not required.

However one primary and two confirmatory methods have been presented for the analysis of glyphosate in air. The primary method is based on GC-MSD after derivatization with trifluoroacetic acid anhydride and trifluoroethanol. The method for the determination of

glyphosate in air was successfully validated at an LOQ of 5µg/m³ also meeting requirements for specificity, linearity, accuracy and repeatability.

The confirmatory methods are based on HPLC-FLD with post-column hypochlorite oxidation and reaction of the amine product with ortho-phtalaldehyde and mercapto-ethanol (LOQ = 8µg/m^3) and GC-ECD (LOQ = 7.2µg/m^3).

3. Impact on human and animal health

3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities in the active substance or to their transformation products

3.1.1. Absorption, distribution, metabolism and excretion

Absorption, distribution and elimination

The 2001 EU evaluation of glyphosate concluded that following oral administration, glyphosate is rapidly absorbed from the gastrointestinal tract but only to a limited extent of approximately 30 - 40 %. Data on the extent of biliary excretion in bile-cannulated rats, that was not available for the 2001 EU glyphosate evaluation, confirms the systemically available glyphosate is excreted exclusively in the urine [redacted] 1996). Elimination of ingested glyphosate via faeces and systemic glyphosate via urine is rapid and is nearly complete within 48 hours. The pulmonary route of elimination is negligible (< 0.2%, [redacted] 1995 and [redacted] 1996). Faeces contain unabsorbed glyphosate.

Distribution into the organs and tissues after an oral dose is rapid but limited with generally low residues found in organs and tissues at termination. After a period of 3 to 7 days following oral administration, total body burden accounted for less than 1% of the applied radioactivity. There is no evidence of a potential for accumulation in animals based on residue analysis in organs and tissues after 72h -168h. Elimination from bone is slower than from other tissues. However, the amount of radiolabel in bone after 168h after a single oral dose was relatively low at 0.02 – 0.03% of the applied dose ([redacted] 1995). The highest residues were measured in bone, followed by kidney and liver. This pattern of absorption, distribution and elimination was not significantly changed either by single high doses administered or by repeated administration of low doses. The sex of the test animals did not affect the results. The pattern of distribution of radioactivity in whole-body autoradiograms showed the greatest intensity of radioactivity to be in bone and gastrointestinal tract at up to 24 hours after dosing which was reduced to negligible amounts within 48 hours ([redacted] 1996). Peak plasma levels were observed within 4 – 6 h and elimination from blood and plasma was rapid with no evidence of accumulation in blood cells. A biphasic pattern of elimination of radiolabel in plasma has been suggested from the plasma radiolabel in a range of studies and terminal half lives have been estimated at 8 – 10h. Radiolabel in plasma was negligible after 24h and not detected at 168h.

Metabolism

Metabolism of glyphosate is very limited. Most of the parent glyphosate is eliminated unchanged and a small amount, just under 0.5% of the applied dose is eliminated as aminomethylphosphonic acid (AMPA). While AMPA is known to be the major metabolite of glyphosate in plants, metabolism in mammals has been shown to be very limited.

3.1.2 Acute toxicity

The 2001 EU evaluation of glyphosate concluded that glyphosate acid and its salts exhibit a low acute toxicity in laboratory animals by the oral and dermal route with LD_{50} values greater than 2000 mg/kg bw in previously conducted studies. These results were confirmed in other and new studies recently performed since the last review. Given all LD_{50} values exceed the highest dose tested and differences between EU and GHS classification criteria, the acute oral and dermal toxicity endpoints should be amended to greater than 5000 mg/kg bw/day.

Glyphosate acid is of low acute inhalation toxicity with LC_{50} values above the limit test dose of 5 mg/L air per 4 hours obtained for the acid and the isopropylammonium salt (IPA), and above the maximum attainable concentration for the ammonium salt.

Regarding primary irritation, glyphosate acid and the salts were found to be non-irritant to intact skin and only slightly irritant to abraded skin. Studies conducted since the last EU review confirm these findings. No classification is required.

Glyphosate acid was found to be strongly irritating to rabbit eyes requiring classification; previously as R41 – **'Risk of serious damage to eyes'** and now **'Irreversible effects on the eye/serious damage to eyes (Category 1)'** under GHS. Recently performed studies on the eye irritating potential of glyphosate acid supported the previous findings and classification. There was markedly less eye irritation observed with the salts which are used in glyphosate based formulations. Glyphosate salts in formulations are of a more neutral pH than glyphosate acid which is not the form used in commercial products. Thus, the glyphosate salts should be classified separately for eye irritation.

Glyphosate acid has been tested for skin sensitisation in guinea pigs by the stringent Magnusson-Kligman test method and the Buehler test method, and in mice in the local lymph node assay. In all study types glyphosate acid (and IPA salt) was unequivocally negative for skin sensitisation potential.

Table 0-1: Summary of acute toxicity of glyphosate acid and its salts

Type of study	Species	Glyphosate Acid	Glyphosate IPA salt	Glyphosate ammonium salt
Oral route	Rat	LD_{50} > 5000 mg/kg	LD_{50} > 5000 mg/kg	LD_{50} = 4613 mg/kg
	Mouse	LD_{50} > 5000 mg/kg	LD_{50} = 3669 mg/kg	-
Dermal route	Rat	LD_{50} > 5000 mg/kg	LD_{50} > 5000 mg/kg	LD_{50} > 5000 mg/kg
Inhalation route	Rat	LC_{50} > 5 mg/L	LC_{50} > 5 mg/L	LC_{50} > 1.9 mg/L#
Primary skin irritation	Rabbit	Non/mild irritant	Non/slight irritant	Non irritant
Eye irritation	Rabbit	Severe irritant/corrosive	Slight irritant	Slight irritant

Skin sensitisation	Guinea pig (M&K/Buehler)	Not sensitising	Not sensitising	-
	Mouse (LLNA)	Not sensitising	-	-

- Highest attainable concentration.

The representative formulation, MON52276, was found to be of low acute oral and dermal toxicity in the rat. It is a slight irritant to rabbit skin and is a mild irritant to the rabbit eye. It is not a skin sensitiser in the guinea pig Buehler test, thus indicating that it poses no risk to man under normal handling conditions.

Table 0-2: Summary of acute toxicity of the representative formulation MON 52276

Type of study	Species	MON52276
Oral route	Rat	LD_{50} > 5000 mg/kg
Dermal route	Rat	LD_{50} > 5000 mg/kg
Inhalation route	Rat	#
Primary skin irritation	Rabbit	Slight irritant
Eye irritation	Rabbit	Mild irritant
Skin sensitisation	Guinea pig (9 induction Buehler)	Not sensitising

- An acute inhalation toxicity study has not been performed with MON 52276, because the criteria listed in Annex II (7.3.1) of Commission Regulation (EU) 545/2011 are not met. See IIIA Section 7.1.3.

3.1.3 Genotoxicity

In the 2001 EU evaluation glyphosate was examined for mutagenicity and clastogenicity in a wide range of test systems covering all relevant endpoints *in vitro*. Additional studies have been conducted on glyphosate since the last EU review. All additional studies were negative and are considered confirmatory data. Glyphosate has clearly been proved to have no genotoxicity potential a wide range of regulatory studies *in vitro*.

Table 0-3: Summary of *in vitro* genotoxicity testing with glyphosate acid

Type of study (reference)	Test organism/test system	Dose range tested and metabolic activation	Results
***In vitro* gene mutation tests in bacteria**			
Bacterial reverse mutation assay (Ames test)#	*S. typhimurium* TA 98, 100, 102 1535, 1537, 1538 *E.coli* WP2 *uvrA* pKM 101, WP2 pKM 101, WP2 hcr	1 – 5000 µg/plate +/- S9	negative
***In vitro* tests for gene mutation in mammalian cells**			

Type of study (reference)	Test organism/test system	Dose range tested and metabolic activation	Results
Mouse lymphoma test (Jensen, 1991)	Mouse lymphoma cells (L5178Y)	- S9: 0.61 – 5.0 mg/L + S9: 0.52 – 4.2 mg/L	negative
HGPRT assay (Li, 1983)	Chinese hamster ovary (CHO) cells	- S9: 5 – 22.5 mg/L + S9: 5 – 252 mg/L	negative
Mouse lymphoma test (Clay, 1996)	Mouse lymphoma cells (L5178Y $TK^{+/-}$)	+/- S9: 296 – 1000 µg/mL	negative
***In vitro* tests for clastogenicty in mammalian cells**			
Cytogenicity (Van de Waart, 1995)	Peripheral human lymphocytes (-S9: 24, 48 h exposure; +S9: 3 h, harvest after 24 or 48 h)	- S9: 33 – 333 µg/mL + S9: 237 – 562 µg/mL	negative
Cytogenicity (Kyomu, 1995)	CHL cells	- S9: 62.5 – 1000 µg/mL + S9: 255 – 2000 µg/mL	negative
Cytogenicity (Wright, 1996)	CHL cells	+/- S9: 0 - 1250 µg/mL	negative
Cytogenicity (Fox, 1998)	Human lymphocytes	- S9: 100 – 1250 µg/mL + S9: 100 – 1250 µg/mL	negative
***In vitro* tests for DNA damage and repair in mammalian cells**			
UDS assay (Rossberger, 1994)	Primary rat (Sprague-Dawley) hepatocytes	0.13 – 111.69 mM	negative
UDS assay (Williams, 1983)	Primary rat (F344) hepatocytes	Up to 125 µg/mL	negative
***In vitro* tests for DNA damage and repair in bacteria**			
Rec assay (Mie, 1995)	*B. subtilis* stains H17 and M45	+/- S9: 7.5 – 240 µg/disc	negative

- Many bacterial reverse mutation assays have been performed, all strains and overall concentration range tested are presented here. All studies were negative for evidence of mutagenic potential.

During the 2001 EU glyphosate evaluation, a number of *in vivo* cytogenicity studies and bone marrow micronucleus tests in rats and mice were evaluated. The last review concluded that glyphosate is not clastogenic *in vivo.* Since the last review the ability of glyphosate to cause chromosomal aberrations has been further investigated in the *in vivo* micronucleus test [redacted] 2009b, [redacted], 2007, [redacted] 2008, [redacted], 1999, [redacted] 2006, [redacted], 1996 & [redacted] 2008). All the new studies demonstrating glyphosate was negative for clastogenic potential *in vivo*.

Table 0-4: Summary of *in vivo* genotoxicty testing with glyphosate

Type of study (reference)	Test organism/test system	Dose levels Sampling	Results
Cytogenicity in bone marrow [redacted] 1994)	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day sampling 24 h after second dose	Negative for clastogenicity; mitotic index ↓ at 5000 mg/kg bw
Micronucleus test in bone marrow ([redacted] 1993c)	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day sampling 24 h after second dose	♂: negative ♀:equivocal
Micronucleus test in bone marrow [redacted] 2009b)	CD rat, single oral application	0, 500, 1000, 2000 mg/kg bw/day sampling after 24 and 48 h	♂: negative ♀: negative
Micronucleus test in bone marrow [redacted] 2007)	Swiss albino mice ♂, daily oral applications for 2 successive days	0, 8, 15, 30 mg/kg bw/day sampling 24 h after second dose	negative
Micronucleus test in bone marrow [redacted], 2008)	Swiss albino mice ♂+♀, daily i.p. applications for 2 successive days	0, 15.62, 31.25, 62.5 mg/kg bw/day sampling 24 h after second dose	negative
Micronucleus test in bone marrow [redacted], 1991)	NMRI mice, single oral application	0 – 5000 mg/kg bw (98.6%) sampling after 24, 48,72 h	negative
Cytogenicity in bone marrow [redacted] 1983)	Sprague-dawley rats, single i.p. injection	0 – 1000 mg/kg bw (98.7%) sampling after 6, 12,24 h	negative
Micronucleus test ([redacted], 1999)	Swiss albino mice, ♂ + ♀, two i.p. injections (24 h interval)	0, 187.5, 375, 562.5 mg/kg bw Sampling 24 h after 2nd application	negative
Micronucleus test in bone marrow ([redacted], 2006)	CD-1 mice ♂; single i.p. dose	0, 150, 300, 600 mg/kg bw sampling after 24 and 48 h	negative
Micronucleus test in bone marrow ([redacted] 1996)	CD-1 mice, 5 ♂+ 5 ♀/dose / sampling point; single oral dose	0, 5000 mg/kg bw sampling after 24 and 48 h	negative
Micronucleus test in bone marrow ([redacted] 2008)	NMRI mice 6 ♂/dose/sampling point; single oral dose	0, 2000 mg/kg sampling after 24 and 48 h, 500 & 1000 mg/kg bw sampling after 24 h only.	negative

In the previous 2001 EU glyphosate evaluation genotoxic effects on germ cells were examined in dominant lethal assays in rats and mice. In both species no genotoxcic effect of glyphosate on germinal tissues was found.

Table 0-5: Summary of *in vivo* germ cell genotoxicty testing with glyphosate acid

Type of study (reference)	Test organism/test system	Dose levels	Results
Dominant lethal assay ([redacted] 1992)	Wistar rats, single oral dose, 10 successive one-week mating periods (1:1 sex ratio)	0, 200, 1000, 5000 mg/kg bw/day	negative
Dominant lethal assay [redacted] 1980)	CD-1 mice, single oral dose; each treated male mated with a total of 16 females over a period of 8 weeks	0, 200, 800, 2000 mg/kg bw/day	negative

Glyphosate has been tested in a wide array of *in vitro* and *in vivo* genotoxicity assays. **Overall, in the vast majority of studies performed, glyphosate proved clearly negative and it can be concluded from the weight of evidence presented that the active ingredient does not exhibit a genotoxic hazard to humans.**

3.1.4 Sub-chronic and chronic toxicity

Short-Term

Sub-acute and sub-chronic toxicity studies note a low oral toxicity of glyphosate and its salts in rats, mice and dogs.

3.1.4.1 Sub-chronic toxicity in rodents

In rats, the previous 2001 EU glyphosate evaluation concluded that the lowest NOEL was about 100 mg/kg bw/day in 90-day feeding studies in rats with the first effects occurring in the range 250-300 mg/kg bw/day, however in most studies higher NOAELs were established. Liver effects were observed indicated by clinical chemistry and organ weight changes in rats. Soft stools and diarrhoea, together with occasionally reduced bodyweight gain and food consumption, suggest irritation of the gastrointestinal tract at high dose levels. In some oral rat studies cellular alterations in salivary glands were observed upon histopathological examination. Overall, the mouse is less sensitive than the rat with only effects observed on body weight at very high dose levels.

Additional studies included in this submission that have not been previously reviewed also demonstrate that glyphosate is of low oral toxicity. The NOAELs ranged between 79-765 mg/kg bw/day and the lowest LOAEL observed in rats was 569 mg/kg bw/day ([redacted] 1995). Consistent with the previously reviewed studies effects were observed on clinical chemistry parameters (often non-specific markers of mild toxicity), bodyweight gain and food consumption at high dose levels. Additionally, caecum distension and an increase in caecum weight were observed in both a 13 week rat study ([redacted] 1995) and a 13 week mouse study ([redacted] 1995). This effect appeared to be dose related at very high dose levels in both species but was not associated with any corollary histopathological changes and is therefore of uncertain toxicological relevance. In contrast, in another 13 week dietary rat study ([redacted], 1996) mucosal atrophy of the caecum was observed at 730 mg/kg bw/day where there was no associated weight change. Another finding observed in male mice that had been previously reported was cystitis of the urinary bladder in animals dosed at 6295 mg/kg bw/d..

The salivary gland findings were driving the NOEL/NOAEL range of 250-300 mg/kg bw/day in the previous EU review. The glyphosate taskforce believes these salivary gland findings are a non-adverse adaptive response to treatment with a low pH diet. Two studies were conducted to

further investigate alterations of salivary glands, described as increased basophilic staining and enlargement of cytoplasm especially in the parotid salivary glands. The first study evaluated the potential effects of low pH diet on the parotid salivary glands. Citric acid was selected as an appropriate surrogate for glyphosate, having both a similar pH-dilution curve and low toxicity. Citric acid was given to male rats in diet (14000 ppm) and via gavage (791-1316 mg/kg bw/day). Trisodium citrate dihydrate (21400 ppm, an equivalent citrate ion concentration) was also given in a diet for eight weeks. Higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands at all dose levels were observed. These effects were noted as most severe in the low pH dietary test group. In the absence of cytotoxicity and hyperplasia the noted effects were considered as an adaptive response rather than an adverse effect and are consistent with the hypothesis that low pH diets result in adaptive cellular responses within the salivary glands ([redacted] 2010). The second study investigated the effect and it's reversibility in 3 different rat strains (Allen, 1996). Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effects in the parotid salivary gland. Salivary gland weights were increased after 4 weeks of treatment in the F344 and AP (Alpk:AP$_f$SD, Wistar-derived) strains. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but less pronounced effects occurred in the AP (Alpk:AP$_f$SD, Wistar-derived) and CD (Sprague-Dawley; Charles River) strains involving small foci of cells only. Complete recovery of both salivary gland weights and histopathological changes was apparent in AP and CD strains following the 4-week recovery period. The salivary gland weight increase reversed in the F344 strain however there was evidence that the basophilia of parotid acinar cells had not fully recovered in all the F344 strain animals after a 13 week recovery period.

Based on the weight of evidence across the studies presented by the glyphosate taskforce it is proposed that the changes observed in the salivary gland (basophilia of the parotid acinar cells) are a non-adverse adaptive response in the rat to treatment with a low pH diet for the following reasons:

- The effect is observed with another organic acid with a similar pH-dilution curve to glyphosate.
- The effect is only observed following treatment in the diet. The same effect has not been observed across an extensive database following other exposure routes. The ADME radiolabel studies indicate glyphosate does not accumulate in the salivary gland.
- The effect, seen primarily in the rat, is variable in severity and has not been observed consistently across sex, dose or strain.
- From a histopathological perspective across an extensive database, there is no accompanying evidence of cytotoxicity leading to necrosis or apoptosis, no evidence of inflammation or change in function and the cellular alterations do not progress with time to preneoplastic or neoplastic lesions (but in fact decrease in incidence and severity or disappear altogether with time).
- The effect is reversible upon cessation of treatment with glyphosate.

Overall the NOEL/NOAEL levels established in the 90-day dietary studies in rats varied between approximately 80 and 1600 mg/kg bw/day and because the salivary gland alterations are considered adaptive the lowest low observed effects of treatment were in the range of 550 mg/kg bw day [redacted] 1995). Over the range of available studies the appropriate NOAEL was 5000 ppm (equivalent to 414/447 mg/kg bw/day in male and female rats respectively). Mice appeared

to be less sensitive, with substantially higher NOAEL values, the lowest of which was 600 mg/kg bw/day.

Table 3.1-6: Summary of glyphosate acid subchronic toxicity studies in rodents

Type of study (reference)	Species	Dose levels	NOEL / NOAEL	Targets / Main effects
90-day, oral diet ([redacted] 1989)	Rat, Sprague-Dawley	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	1000 mg/kg bw/day: Clinical chemistry, cellular alterations in paratoid salivary glands, decreased urinary pH (♂ only)
13-week, oral diet ([redacted] 1993)	Rat, Sprague-Dawley	0, 2000, 6000, 20000 ppm (≅ 0, 125.2/156.3, 371.9/481.2, 1262/1686.5 mg/kg bw/day ♂/♀)	NOAEL = 6000 ppm (≅ 379.1/481.2 mg/kg bw/day ♂/♀)	1262.1/1686.5 mg/kg bw/day ♂/♀ (calculated values): Diarrhea, blood in urine, organ weight changes
90-day, oral diet (+ 2-week recovery) ([redacted] 1992)	Rat, Wistar	0, 200, 2000, 20000 ppm	2000 ppm (147/196 mg/kg bw/day (♂/♀))	1000 mg/kg bw/day: Clinical chemistry, reduced body weight gain
13-week, oral diet (+ 5-week recovery) ([redacted] 1989)	Rat, CD	0, 2000, 3000, 5000, 7500 ppm	NOEL: 7500 ppm (ca. 375 mg/kg bw/day)	No treatment-related effects
90-day, oral diet ([redacted] 1987)	Rat, Sprague-Dawley	0, 1000, 5000, 20000 ppm	NOEL: 20000 ppm (1267/1623 mg/kg bw/day (♂/♀))	No treatment-related effects
90-day, oral, diet ([redacted] 1996)	Rat, Alpk:APfSD	0. 1000, 5000, 20000 ppm	NOEL: 5000 ppm (414/447 mg/kg bw/day ♂/♀	1612/1821 mg/kg bw/day (♂/♀): Reduced body weight, food consumption and utilisation reduced in ♂ only, clinical chemistry changes (changes (↑ALP, ALT)
90-day, oral, diet ([redacted] 1996)	Rat, Sprague-Dawley	0, 1000, 10000, 50000 ppm (≅ 0, 79/90, 730/844, 3706/4188 mg/kg bw/day (♂/♀)	1000 ppm (79/90 mg/kg bw/day ♂/♀)	730/844 mg/kg bw/day (♂/♀): Clinical chemistry changes, mucosal atrophy of the caecum

Type of study (reference)	Species	Dose levels	NOEL / NOAEL	Targets / Main effects
13-week, oral, diet ([redacted] 1995)	Rat, Sprague-Dawley	0, 3000, 10000, 30000 ppm (≅ 168/195, 569/637, 1735 / 1892 mg/kg bw/day (♂/♀))	NOAEL: 3000 ppm (168/195 mg/kg bw/day ♂/♀)	10000 ppm (≅ 569/637 mg/kg bw/day (♂/♀)): caecum distention; caecum weight increased without histopathological findings 30000 ppm (≅ 1735 / 1892 mg/kg bw/day (♂/♀)): caecum distention; caecum weight increased without histopathological findings, reduced body weight and lower food efficiency; increased AP activity in ♀
13-week, oral, diet ([redacted] 1995)	Mouse, ICR	0, 5000, 10000, 50000 ppm (≅ 0, 600/765, 1221/1486, 6295/7435 mg/kg bw/day (♂/♀)	NOAEL: 5000 ppm (600/765 mg/kg bw/day ♂/♀)	10000 ppm (1221 / 1486 mg/kg bw/day ♂/♀): caecum distention ♀, increased absolute and relative caecum weight 50000 ppm 6295 / 7435 mg/kg bw/day ♂/♀):: reduced bodyweight and food consumption, decreased fodd efficiency ♀, haematological changes in ♀, blood chemistry changes, caecum distention and increased absolute and relative caecum weight in both sexes without histopathological changes in the caecum; cystitis of the urinary bladder in ♂
13-week, oral, diet ([redacted] 1991)	Mouse, CD-1	0, 200, 1000, 4500 mg/kg bw/day	NOEL: 4500 mg/kg bw/d	No treatment-related effects

↓ = decreased; ↑ = increased;

One study on sub-acute inhalation toxicity (14 days) in rodents ([redacted] 1985: non-GLP (pre-GLP) study) has been reviewed in the 2001 EU evaluation. The previous review concluded no treatment-related effects were observed and the NOEL was 3.8 mg/L. No further studies have been conducted.

The short-term percutaneous toxicity of glyphosate has been investigated in the rat and rabbit. In both Sprague-Dawley (SD) ([redacted] 1993) and Wistar derived ([redacted] 1996) rats no signs of systemic toxicity were noted following dosing for 21 days at 1000 mg/kg bw/day, the limit dose for this study type. Three studies were conducted in New Zealand White rabbits ([redacted] and doses ranged from 1000 mg/kg bw/day to 5000 mg/kg bw/day. No signs of treatment related systemic toxicity were noted in any study, the highest NOAEL being 5000 mg/kg bw/day. The NOAEL for short term percutaneous toxicity was 1000 mg/kg bw/day in the

rat and 5000 mg/kg bw/day in the rabbit as previously concluded in the 2001 EU glyphosate evaluation.

Table 3.1-7: Summary of glyphosate acid repeat dose percutaneous toxicity studies

Type of study (reference)	Species	Dose levels (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Targets / Main effects
21-day, dermal ([redacted] 1993)	Rat, Sprague-Dawley	0, 1000	1000	Weak dermal irritation at 1000 mg/kg bw/day; no systemic effects
21-day, dermal ([redacted] 1996)	Rat, Alpk: AP_fSD	0, 250, 500, 1000	1000	No treatment-related effects
21-day, dermal ([redacted] 1985)	Rabbit, NZW	0, 500, 1000, 2000	2000	No treatment-related effects
21-day, dermal ([redacted] 1982)	Rabbit, NZW	0, 100, 1000, 5000	5000	Dermal irritation at 5000 mg/kg bw/day; no systemic effects
In vitro dermal absroption (Hadfield, (2012)	Rabbit	Equivalent of 5000	133 systemic NOAEL in rabbits	2.42% absorbed 0.243% remaining in dermis 2.66% systemic = 133 mg/kg/day
28-day, dermal ([redacted] 1994a)	Rabbit, NZW	0, 500, 1000, 2000	2000	No effects

3.1.4.2 Sub-chronic toxicity in the dog

In oral sub-chronic toxicity studies in the dog previously evaluated in the 2001 EU glyphosate evaluation, only unspecific signs of toxicity (decrease in body weight gain and food consumption) were observed at high dose levels. In two dietary 3- and 12-month dog studies performed at the same laboratory, liver effects of equivocal toxicological significance were observed at low doses (8-29 mg/kg bw/day). However, the previous evaluation found that because these findings were not confirmed in more recent studies using much higher dose levels they were not considered to be compound-related. The previous review concluded the lowest relevant NOAEL was 300 mg/kg bw/day for glyphosate acid and the IPA salt.

This NOAEL is supported by four additional studies ([redacted] 2007, [redacted] 1999, [redacted] 1996 & [redacted] 1996). In the [redacted] (1999), [redacted] (1996) and [redacted] 1996) studies only minor effects on bodyweight and clinical chemistry parameters were observed at 1000 mg/kg bw/day. In the [redacted] (2007) study at 1000 mg/kg bw/day the test item administration induced marked clinical signs and mortality leading to the early termination of the group at week 11, however, the 300 mg/kg bw/day was the NOAEL. Overall the lowest NOAEL observed was 253 mg/kg bw/d and the highest was 1000 mg/kg bw/day. The lowest effect level was 1000 mg/kg bw/d in 90 day dog studies.

Oral one year toxicity in the dog was previously evaluated in the 2001 EU glyphosate review. [redacted] (1991) and [redacted] (1985) studies have been previously evaluated and

like the 90-day studies only non-specific signs of toxicity (slight effect body weight and an increase in clinical signs of soft, liquid stools) were observed at limit dose. The previous review concluded the lowest relevant NOAEL was 300 mg/kg bw/day for glyphosate acid. These previous conclusions were confirmed by 3 additional one year dog studies. Again non-specific effects of toxicity were observed at doses at, or close to limit dose. These effects were characterised as reduction in body weight gain, reduction in urinary pH and minor effects on clinical pathology parameters. The lowest dose level where treatment related effects were observed was 926 mg/kg bw/day in the [redacted] (1996) study.

Overall NOEL/NOAELs ranged from 182 - 1015 mg/kg bw/day in dogs. The lowest effect level observed in dogs was a slight reduction in body weight gain in male dogs at 30000 ppm (equivalent to 926 mg/kg bw/day). Over the range of available studies in dogs the appropriate NOAEL was 500 mg/kg bw/day.

Table 0-8: Summary of glyphosate acid toxicity studies in the dog that have not been previously reviewed in the 2001 EU glyphosate evaluation

Type of study Species (Reference)	Dose levels	NOEL/NOAEL	Targets/Main effects
13-week, oral capsule Dog, Beagle [redacted] 2007)	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	1000 mg/kg bw/day: Liquid/soft faeces, dehydration, thin appearance, vomiting, pallor, body weight gain ↓ ♂, body weight loss ♀, food consumption ↓, ALT ↑, AP ↓, protein ↓, albumin ↓, adipocytes in sternum ↑, Prostrate and uterine atrophy
90-day, oral diet, Dog, Beagle ([redacted] 1999)	0, 200, 2000, 10000 ppm (0, 5.3, 53.5, 252.6 mg/kg bw/day)	NOAEL: 10000 ppm (252.6 mg/kg bw/day)	No treatment-related effects
13-week, oral diet Dog, Beagle ([redacted] 1996)	0, 1600, 8000, 40000 ppm	NOAEL: 40000 ppm (1015/1014 mg/kg bw/day ♂/♀)	No treatment-related effects
13-week, oral capsule Dog, Beagle ([redacted] 1996)	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	1000 mg/kg bw/day: Decreased body weight, clinical chemistry changes

Type of study Species (Reference)	Dose levels	NOEL/NOAEL	Targets/Main effects
52-week, oral capsule Dog, Beagle ([redacted] 2008)	0, 30, 125, 500 mg/kg bw/day	NOAEL: 500 mg/kg bw/day	No treatment-related effects
12-month, oral diet Dog, Beagle ([redacted] 1997)	0, 1600, 8000, 50000 ppm	8000 ppm (182/184 mg/kg bw/day ♂/♀)	50000 ppm: (≅ 1203/1259 mg/kg bw/day (♂/♀)) loose stool, retarded body weight gain, reduced body weight at termination without stat. significance, urinary pH ↓, slight anemic changes in ♀, slight focal pneumonia / focal granulomatous pneumonia in the lung of all ♀ (extent of the lesion was very focal and slight in intensity), statistically, no significant differences, blood chemistry changes (Cl ↑, albumin ↓, P ↓ ♀)
1-year, oral, diet Dog, ([redacted] 1996)	0, 3000, 15000, 30000 ppm	NOELs ♀: 15000 ppm (447 mg/kg bw/day) ♂: 30000 ppm (906 mg/kg bw/day)	30000 ppm (926 mg/kg bw/d) ♀ only: Slight ↓ body weight gain.

↓ = decreased; ↑ = increased;

Long-Term & Carcinogenicity

The long-term toxicity and carcinogenic potential of glyphosate has been assessed in rats and mice. The 2001 EU glyphosate evaluation concluded that in long-term studies in rats and mice there was no evidence of carcinogenicity. It also concluded that in rats, there were no adverse effects on survival or clinical signs. A reduction in body weight gain, increases in alkaline phosphatase and liver weight changes, an increase in incidence of cataracts, inflammation of the gastric mucosa and histopathological changes in the salivary glands were observed sporadically across the studies previously reviewed. In the mouse the previous 2001 review concluded that non-neoplastic treatment related effects were limited to high dose males in the [redacted] (1983) study and comprised of a reduction in body weight gain, hepatocyte hypertrophy and bladder epithelial hyperplasia.

Five additional long term studies have been conducted in the rat and 3 in the mouse that have not been previously reviewed at the EU level. There was no evidence that glyphosate acid is carcinogenic in any of these studies that have not been previously submitted.

3.1.4.3 Long term toxicity and carcinogenicity in the rat

A 1-year toxicity study ([redacted] 1996) was performed in rats with dietary doses of 0, 2000, 8000 and 20000 ppm glyphosate acid. Based on body weight and salivary gland effects at 20000

ppm, the NOAEL for toxicity for glyphosate acid was 8000 ppm equivalent to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females.

In 2-year dietary rat study, by [redacted] (1997), rats received diets providing 0, 3000, 10000 or 30000 ppm glyphosate. The NOAEL for toxicity is 3000 ppm equivalent to 104 and 115 mg/kg bw/day for males and females, respectively, based on histopathological and clinical effects of the caecum together with follicular hyperkeratosis and/or folliculitis/follicular abscess in the mid and high dose groups.

In another combined chronic toxicity and carcinogenicity study ([redacted] 2001) which was performed with glyphosate technical in rats receiving diets providing 0, 2000, 6000 or 20000 ppm glyphosate acid, the NOAEL was set at 6000 ppm equivalent to 361 and 437 mg/kg bw/day for males and females, respectively. The LOAEL was 20000 ppm. It was based on liver and kidney effects, prostatitis, periodontal inflammation, urinary acidosis and haematuria, which may be attributed to the acidity of the test substance.

The 2 year dietary rat study conducted by [redacted] (1997) concluded that there were no adverse treatment related effects and the NOAEL was 1290/1740 mg/kg bw/day in males and females respectively.

The most recent rat dietary carcinogenicity study was conducted in 2009 by [redacted] again there were no adverse treatment related effects at the highest dose tested. The NOAEL for this study was 1230 mg/kg bw/day.

In the previous review salivary glands have been suggested as a possible target organ. Histological changes described as "cellular alteration" in the parotid and mandibular salivary glands and a higher organ weight of these glands were noted at 100 mg/kg bw/day and higher ([redacted] 1993). These findings determined the lowest NOAEL in the previous review from the long-term studies. The glyphosate taskforce has demonstrated that these changes are a non-adverse adaptive response to treatment with a low pH diet. When the salivary glands are viewed in perspective, as an adaptive change, the lowest effect level in the long-term rat studies is 354/393 mg/kg bw/day in males and females respectively [redacted] 1997). Overall the NOEL/NOAEL levels established in the long term studies in rats varied between approximately 31 and 1290(♂)/1720(♀) mg/kg bw/day but given the lowest low effect level the appropriate overall NOAEL for glyphosate in long-term studies in rats is 300 mg/kg bw/day.

3.1.4.4 Long term toxicity and carcinogenicity in the mouse

A combined toxicity and carcinogenicity study in mice [redacted] 2001) demonstrated a slightly higher mortality in the high dose group. Mortality was within the upper end of the historical control range. However, treatment with glyphosate might slightly have affected the mortality at the highest dose of 10000 ppm, and because a relationship to treatment was unclear a conservative NOAEL for toxicity at the mid dose of 1000 ppm (150.5 mg/kg bw/day for combined sexes) was set for this study. The number of malignant lymphoma, the most common tumour in the mouse, was slightly elevated in the high dose group compared to control, but this was considered as incidental background variation based on historical control data and was not considered to be related to treatment. However it should be noted that the high dose group received a daily achieved dose of 1460 mg/kg bw/day which is in excess of the limit dose recommended by most current international guidelines.

In the study by [redacted] 1997 the low effect level was 8000 ppm (equivalent to 787 mg/kg bw/day) in females only based on a reduction in body weight gain. At the top dose of 40000 ppm (equivalent to 4348/4116 mg/kg bw/day in males and females respectively) signs of toxicity

included loose stools, reduced body weight gain, food consumption and food utilisation, caecum distension and increased absolute and relative caecum weight (without corollary histopathological findings), increased incidence of anal prolapsed consistent with histopathological erosion/ulceration of the anus.

The most recent 80-Week dietary mouse study was conducted by [redacted] 2009. There were no adverse treatment related effects at the highest dose tested. The NOAEL for this study was 810/1081 mg/kg bw/day in males and females respectively.

Overall the lowest effect level observed in the long-term mouse studies was 787 mg/kg bw/day in females in the [redacted] (1997) study and the wide range of NOEL/NOAELs of 151 – 1081 mg/kg bw/day is an artefact of dose selection.

There was no evidence for a carcinogenic potential of glyphosate noted in any of the studies performed in rats and mice.

Table 0-9: Summary of glyphosate acid long-term toxicity studies in rodents

Type of study Species/Strain (Reference)	Dose levels (mg/kg bw/day)	NOAEL (NOAEL)* (mg/kg bw/day)	LOAEL (mg/kg bw/day) Targets / Main effects
1-year, oral diet Rat, Wistar Alpk: APfSD [redacted] 1996)	♂ 0, 141, 560, 1409 ♀ 0, 167, 671, 1664 (0, 2000, 8000, 20000 ppm)	560/671 ♂/♀ (1409/1664)	1409/1664: Salivary glands, body weight reduction
2-year, oral diet Rat, Wistar [redacted] 1996)	0, 7.4, 74, 741 ♂ 0, 6.3, 59.4, 595 ♀ 0, 8.6, 88.5, 886 (0, 100, 1000, 10000 ppm)	595/886 ♂/♀ 741 ♂+♀ (741 ♂+♀)	> 595/886: Only mild effects on clinical chemistry (liver enzymes) without histopathological changes
2-year, oral diet Rat, Sprague-Dawley [redacted] 1997)	♂ 0, 104, 354, 1127 ♀ 0, 115, 393, 1247 (0, 3000, 10000, 30000 ppm)	104/115 (1127/1247)	354/393: Caecum weight increased, distension of caecum, loose stool, follicular hyperkeratosis and/or folliculitis/ follicular abscess, reduced body weight
2-year, oral diet Rat, Sprague-Dawley [redacted] 1997)	♂ 0, 150, 780, 1290 ♀ 0, 210, 1060, 1740 (0, 3000, 15000, 30000 ppm)	1290/1740 (1290/1740)	> 1290/1740: Only mild toxic effects without histopathological changes
2-year, oral diet Rat, Wistar Alpk: APfSD [redacted] 2001)	♂ 0, 121, 361, 1214 ♀ 0, 145, 437, 1498 (0, 2000, 6000, 20000 ppm)	361/437 (1214/1498)	1214/1498: Kidney and liver findings. Increased survival due to bw reduction, reduced food consumption, prostatitis, periodontal inflammation

Type of study Species/Strain (Reference)	Dose levels (mg/kg bw/day)	NOAEL (NOAEL)* (mg/kg bw/day)	LOAEL (mg/kg bw/day) Targets / Main effects
2-year, oral diet Rat, Sprague-Dawley [redacted] 1993)	0, 10, 100, 300, 1000	300 (1000)	1000: Decreased body weights, decreased urinary pH, salivary glands (histopathology at terminal and interim kill, organ weight ↑ at interim kill); evidence of weak liver toxicity (clinical chemistry AP ↑, organ weight ↓)
26-month, oral diet Rat, Sprague-Dawley [redacted] 1981)	♂ 0, 3, 10, 31 ♀ 0, 3.4, 11, 34 (0, 30, 100, 300 ppm)	31/34	No treatment-related effects
2-year, oral diet Rat, Sprague-Dawley [redacted] 1990)	♂ 0, 89, 362, 940 ♀ 0, 113, 457, 1183 0, 2000, 8000, 20000 ppm)	362/457 (940/1183)	940/1183: Systemic effects: cataracts ♂, reduced body weight in ♀, increased liver weight. Local effects: inflammation of gastric mucosa
2-year, oral diet Rat, Wistar [redacted] 2009)	0, 95, 317, 1230	1230 (1230)	> 1230: No treatment-related effects
2-year, oral diet Mouse, CD-1 [redacted] 1993)	0, 100, 300, 1000	1000 (1000)	> 1000 Not clearly identified
2-year, oral diet Mouse, CD-1 [redacted] 1983)	♂ 0, 157, 814, 4841 ♀ 0, 190, 955, 5874	157/190 (4841/5874)	841/955 Decreased body weight, histological changes in liver and urinary bladder (epithelial hyperplasia)
18-month, oral diet Mouse, Swiss albino [redacted] 2001)	0, 15, 151, 1460 (0, 100, 1000, 10000 ppm)	151 (1460)	1460 Increased mortality

Type of study Species/Strain (Reference)	Dose levels (mg/kg bw/day)	NOAEL (NOAEL)* (mg/kg bw/day)	LOAEL (mg/kg bw/day) Targets / Main effects
18-month, oral diet Mouse, ICR [redacted] 1997)	0, 1600, 8000, 40000 ppm	8000 / 1600 ppm (= 838/153 mg/kg bw/day (♂/♀) (4348/4116 (♂/♀))	8000 ppm (≅ 787 mg/kg bw/day) (♀): retarded growth 40000 ppm: pale-coloured skin ♂, loose stool, retarded growth, reduced food consumption and food efficiency, caecum distension and increased absolute and relative caecum weight without histopathological findings increased incidence of anal prolapse, in consistent with histopathological erosion/ulcer of the anus
18-month, oral diet Mouse, CD-1 [redacted] 2009)	0, 500, 1500, 5000 ppm	810/1081 (♂/♀) 946 (♂+♀) (810/1081 (♂/♀)	No treatment-related effects

* NOAEL for carcinogenicity
↓ = decreased; ↑ = increased

3.1.4.5 Reproduction and Developmental Toxicity

Two Generation Reproductive Toxicity

The potential of glyphosate to cause toxic effects on **reproduction** (reproductive performance, fertility, development) was examined in several multi-generation studies in rats. In the previous 2001 EU glyphosate evaluation no specific reproductive toxicity potential was shown for the active substance. Minor effects on the offspring consisting of a reduced pup weight were seen only at high dose levels and were associated with signs of parental toxicity. Treatment-related effects in parent animals were similar to those seen in sub-chronic and chronic toxicity studies and occurred at comparable dose levels. Since the last review three new studies have been performed.

In the first additional study by [redacted] (1997) parental toxicity was evident at doses of 30000 ppm and consisted of reduced body weight, soft stool and distension of the caecum which was consistent with findings in the sub-chronic and chronic rats studies conducted at this laboratory. In this study, effects in offspring consisted mainly of reduced body weight and distension of the caecum and were observed at 30000 ppm only.

In the [redacted] (2000) study the only effect of treatment was a reduction in the bodyweight of the F1A pups in the 10000 ppm group (1063/1634 mg/kg bw/day in males and females respectively) with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the mating period. The fertility and reproductive performance of each generation of parental animals and the clinical condition and survival of their offspring were not adversely affected by treatment.

In the most modern study by [redacted] (2007) there were no treatment-related effects on reproductive performance, parents or offspring.

Overall the lowest effect level for parental toxicity was 668-771 & 752-841 mg/kg bw/day in males and females respectively based on slightly reduced body weight in F1 males and increased food and water consumption F1 females in the [redacted] (1992) study. The lowest effect level for

the offspring was 1063/1634 mg/kg bw/day in males and females respectively based on reduced body weight of first generation pups during lactation ([redacted] 2000).

There were no effects on reproduction (reproductive performance, fertility, parturition, lactation, sperm parameters and oestrus cycle) noted in any of the dose groups in any of the studies.

The relevant parental and offspring NOEL/NOAELs ranged from 197-1063 mg/kg bw/day for males and 226-1634 mg/kg bw/day for females. Given the lowest low effect level (668-771 & 752-841 mg/kg bw/day in males and females respectively) the appropriate overall NOAEL for glyphosate in two-generation studies in rats is 6000 ppm (equivalent to 417-458 and 485-530 mg/kg bw/day in males and females respectively).

Table 0-10: Summary of glyphosate acid two generation toxicity studies in the rat that have not been previously reviewed in the 2001 EU glyphosate evaluation

Type of study Species (Reference)	Dose levels	NOEL/NOAEL		Targets/Main effects
		Parental	Offspring / reproductive	
2-generation, diet, rat, Sprague-Dawley ([redacted] 2007)	0, 1500, 5000, 15000 ppm	15000 ppm (≅1063/1634 mg/kg bw/day ♂/♀)	15000 ppm (≅1063/1634 mg/kg bw/day ♂/♀)	No treatment-related effects on parents, offspring and reproduction
2-generation, diet, rat, Alpk:AP_fSD [redacted] 2000)	0, 1000, 3000, 10000 ppm	3000 ppm (≅322/459 mg/kg bw/day ♂/♀)	3000 ppm (≅322/459 mg/kg bw/day ♂/♀)	10000 ppm (≅1063/1634 mg/kg bw/day ♂/♀): **Parental:** body weight of F1 males ↓ during pre-mating. **Offspring:** reduced body weight of F1A pups during lactation. No effects on reproduction.
2-generation, diet, rat, Sprague-Dawley [redacted] 1997)	0, 1200, 6000, 30000 ppm	6000 ppm (≅417-458 and 485-530 mg/kg bw/day ♂/♀)	6000 ppm (≅417-458 and 485-530 mg/kg bw/day ♂/♀)	30000 ppm (≅2150-2411 & 2532-2760 mg/kg bw/day ♂/♀): **Parental:** loose stool, slight decrease in mean body weight in F1 ♂ at 2nd generation selection, caecum distension. **Offspring:** reduced body weight (F0 ♂ and F1 ♂ from week 1 to necropsy), caecum distension. No effects on reproduction.

Developmental Toxicity in the Rat

The previous 2001 EU glyphosate review concluded that in the rat the lowest relevant NOEL for both maternal and developmental effects was 300 mg/kg bw/day and the lowest effect level was 1000 mg/kg bw/day. The evaluation found there was no evidence of teratogenicity. Two additional teratogenicity studies have been performed in rats that have not been previously reviewed in the 2001 EU glyphosate evaluation. These studies are considered to be confirmatory data. Overall the lowest effect level for maternal and foetotoxicity was 1000 mg/kg bw/day and

the appropriate overall NOAEL was 500 mg/kg bw/day for both the dams and the foetuses based on the [redacted] 1996.

Table 0-11: Summary of glyphosate acid developmental toxicity studies in the rat that have not been previously reviewed in the 2001 EU glyphosate evaluation

Type of study Species (Reference)	Dose levels	NOEL/NOAEL		Targets/Main effects
		Maternal	Offspring / reproductive	
Developmental toxicity, rat, Alpk: APfSD [redacted] 1996)	0, 250, 500, 1000 mg/kg bw/day	1000 mg/kg bw/day	1000 mg/kg bw/day	**Maternal:** no effects **Developmental:** no effects
Developmental toxicity, rat [redacted] 1995)	0, 30, 300, 1000 mg/kg bw/day	300 mg/kg bw/day	1000 mg/kg bw/day	1000 mg/kg bw/day: **Maternal:** slightly loose stool **Developmental:** No effects

Developmental Toxicity in the Rabbit

The previous 2001 EU glyphosate review concluded that the NOEL for developmental effects was 350 mg/kg bw/day [redacted] 1980) and that effects on the foetuses were only observed in the presence of marked maternal toxicity. Overall the previous evaluation determined that glyphosate was not teratogenic in rabbits. Three additional studies have been included in this submission. The results from these studies are consistent with the data that has been previously reviewed, the pattern of maternal toxicity is consistent and effects on the foetuses were only observed in the presence of maternal toxicity.

In rabbits, glyphosate exposure via oral gavage led to clinical signs of toxicity in dams consistent with gastro-intestinal disturbances. Rabbits were more sensitive to oral gavage dosing than other species. Clinical signs observed included diarrhoea/soft faeces, reduced faecal output, reduced body weights, reduced food consumption and increased mortality. These effects are consistent with gastro-intestinal stasis (ileus) likely caused by the mucosal membrane irritation potential of glyphosate acid. Rabbits (caecotrophs) are particularly sensitive to disruption of the gastro-intestinal tract. Stress and other environmental factors such as pain can lead to the normal muscular contractions of the stomach and intestines being greatly diminished which in turn leads to disruption of the normal intestinal/caecum bacterial flora. It is likely that the mucosal membrane of the rabbit gastro-intestinal tract is irritated by bolus administration of glyphosate acid and that the associated stress or pain leads to gastro-intestinal stasis. The gross necropsy signs observed in maternal animals in the studies by [redacted] (1995), [redacted] (1996) and [redacted] (1996), such as hair like boluses in the stomach, fluid filled large intestines and gas distension in the lower gastrointestinal tract are indicative of gastro-intestinal stasis. The severity of this finding appears to be relevant to only hindgut fermenters as it is not seen in rats or dogs following administration of an oral bolus dose.

Further supporting evidence that these findings are related to gastro-intestinal disturbance has been generated by the Glyphosate Task Force. Dermal penetration through rabbit skin was determined [redacted] 2012) under the same conditions as exposure in a dermal toxicity study in rabbits [redacted] 1982). This study demonstrated that glyphosate acid systemic exposure following percutaneous administration in the [redacted] study (1982) was at least equivalent to the

systemic exposure following oral gavage administration in many of the rabbit developmental toxicity studies, where significant clinical signs (soft faeces, reduced faecal output, reduced body weights) and mortality were observed. In contrast, rabbits exposed to the same systemic dose by the percutaneous route demonstrated no comparable clinical signs and survived until study termination. It is concluded that the effects observed in rabbits after oral gavage are a local effect on the gastrointestinal system of the rabbit, not observed in other test species, and very unlikely relevant for humans.

Overall maternal toxicity was observed at dose levels of 150 mg/kg bw/day and above. The highest relevant NOAEL for maternal toxicity was 100 mg/kg bw/day.

Foetotoxicity/developmental toxicity occurred at doses that were above (or rarely at the same dose as) the dose that caused maternal toxicity. Most indications of developmental toxicity were reduced ossifications of skull, phalangeal and sternebral bones, which are typically seen in the litters of pregnant animals that do not eat well and lose weight during pregnancy. The importance of this observation should not be misconstrued to mean that maternal toxicity in those cases was the proximate agent that injured the fetus, but rather that if exposures to the causative agent are kept below the doses that cause maternal toxicity, the developing offspring are protected. The lowest observed effects on the foetuses occurred at 300 mg/kg bw/day and were characterized by delayed ossification and decreased foetal weights ([redacted] 1996). The relevant NOAEL for foetotoxicity is 250 mg/kg bw/day.

A report from an independent source [redacted] 2011) has claimed that congenital malformations, especially of the cardiovascular system, were caused by glyphosate exposure in this same series of studies. A variety of malformation was reported across the database of glyphosate studies; these included:

- Dilated aorta/narrow pulmonary artery
- Narrow aorta/dilated pulmonary artery
- Interventricular septal defect
- Cardiomegaly
- Single ventricle
- Retro-esophageal right subclavian artery
- Interrupted aorta
- Right subclavian artery arising from aortic arch
- "Seal-shaped" heart

If glyphosate does cause congenital heart defects, it would be anticipated that the prevalence of congenital heart defects would be increased and one would expect the malformation rate to increase with increasing dose until the pregnant does would become intoxicated or the fetuses would die. The malformations occurred at a low incidence across all dose groups; they did not exhibit a positive dose-response; and often clusters of the malformations occurred in the same fetuses.

The incidence of aorticopulmonary septum-related defects in the combined control groups was 1/879 (0.1%); in the combined glyphosate-treated groups the incidence was 12/2250 (0.5%). One half of the malformed fetuses was found in litters exposed to the highest doses (450 and 500 mg/kg/day), which also experienced severe maternal toxicity including maternal deaths, abortions, and weight loss. If these groups are not considered because of the potential confounding factor introduced by maternal health issues, the incidence of the defects is 6/2049 (0.3%). These data show that the overall incidence of aorticopulmonary septum-related defects in offspring from mothers exposed to glyphosate at doses below those that cause severe maternal toxicity is similar to that seen in non-exposed rabbits.

The other prominent cardiovascular malformation is dilated heart. All observations of this finding (among both control and treated groups) occurred in a study conducted in a single laboratory [redacted] 1993). This study has several weaknesses including a small number of litters available for examination due to low pregnancy rates and maternal deaths in the mid- and high-dose groups. None of the other six studies reported dilated hearts, although there was a single case of cardiomegaly reported in the mid dose group of 100 mg/kg/day in the [redacted] (1993) study (this was considered not to be related to treatment). Neither the criteria used to diagnose dilated heart nor measurements of the hearts were provided, so it is not possible to directly compare the dilated heart findings to the hearts of the more than 2800 fetuses in the other studies. It is possible that the observation of dilated hearts is due to overly stringent inspection compared to criteria used by other laboratories.

Taken together, overall data regarding potential cardiovascular malformations in the seven rabbit developmental toxicology studies do not support the contention that there is a clear compound related effect on the foetal heart.

Table 0-12: Summary of glyphosate acid developmental toxicity studies in the rabbit

Strain (Reference)	Dose level (mg/kg bw/day)	Maternal Mortality #	Diarrhoea/ loose faeces #	Reduced faecal output #	Body weight effect	Necropsy findings	Number of dams with live young or litters at Day 29 #
Japanese White [redacted] 1995)	300	1/18	4/17	0/17	Lower than control	Erosion in the stomach, hair bolus in stomach, watery contents in large intestine/caecum	15/18
NZW [redacted] 1996)	400	2/18	10/16	2/18	Initial loss and then statistically significant lower bodyweight gain than control	Fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, duodenum congested and colon, rectum and appendix gas distended.	16/18
	200	1/18*	0/16	2/18	Lower body weight gain than control	-	16/18

Strain (Reference)	Dose level (mg/kg bw/day)	Maternal Mortality #	Diarrhoea/ loose faeces #	Reduced faecal output #	Body weight effect	Necropsy findings	Number of dams with live young or litters at Day 29 #
NZW [redacted] 1996)	300	2/20	19/20	9/20	Reduction in maternal body weight gain	Hair-like substance in the stomach	17/20
	175	2/20	11/20	9/20	Reduction in maternal body weight gain	-	17/20
Dutch Belted [redacted] 1980)	350	10/16	16/16	not recorded	No effect	-	6/16
	175	2/16	slight increase in incidence	not recorded	No effect	-	11/16
NZW [redacted] 1991)	450	1/20	13/20	12/20	No effect	-	13/20
	150	0/16	5/16	11/16	No effect	-	15/16
NZW [redacted] 1993)	500	8/15	12/15	0/15	Statisticaly significant lower body weights than controls	-	6/15
NZW [redacted] 1989)	500	0/15	0/15	0/15	Statisticaly significant lower body weight gain	-	12/15

- x/y: number of animals affected/total number of animals in group
* - due to mal-dosing.

3.1.5 Neurotoxicity (acute, delayed, sub-chronic)

The previous 2001 glyphosate evaluation concluded that there was no evidence of neurotoxicity in acute, subchronic or chronic studies in rodents and dogs. To add weight to this conclusion an acute neurotoxicity study in rats was performed by [redacted] (1996a) has been included in this assessment that was not previously reviewed during the 2001 glyphosate evaluation. Administration of glyphosate acid produced clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) at approximately 6 hours after dosing on day 1 in 3/10 females, only, which received 2000 mg/kg. One of these females was subsequently found dead on day 2. Quantitative assessment of landing foot splay, sensory perception, muscle weakness and locomotor activity revealed no changes indicative of neurotoxic potential. Histopathological evaluation of the central and peripheral nervous system revealed no treatment-related changes in animals receiving 2000 mg/kg. The no-observed effect level (NOEL) for neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg.

In addition a sub-chronic neurotoxicity study was also performed by [redacted] (1996b). In this study administration of glyphosate acid produced no clinical signs of toxicity or effects on any of

the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. In addition, there were no treatment-related changes in brain weight, length or width. Comprehensive histopathological evaluation of the peripheral and central nervous system revealed no evidence of any changes which could be attributed to administration of glyphosate acid. The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm (equivalent to 1547/1631 mg/kg bw/day in males and females respectively).

There was no evidence that glyphosate has neurotoxic potential in specific neurotoxcity assessments or in any of the studies performed throughout the toxicology database.

Table 0-13: Summary of glyphosate acid neurotoxicity studies in rat that were not been previously reviewed in the 2001 EU glyphosate evaluation

Type of study Species (Reference)	Dose levels	NOEL/NOAEL	Targets/Main effects
Acute, oral gavage, Rat Alpk:APfSD [redacted] 1996a)	0, 500, 1000, 2000 mg/kg bw/day	Systemic toxicity: 1000 mg/kg bw/day Neurotoxicity: 2000 mg/kg bw/day	Systemic toxcity - 2000 mg/kg bw/day ♀ only: On day 1 subdued behaviour, decreased activity, hunched posture, sides pinched in, tip-toe gait and hypothermia transient signs consisitent with general systemic toxicity. Neurotoxicity: No treatment related effects.
13-week oral diet, Rat Alpk:APfSD [redacted] 1996b)	0, 2000, 8000, 20000 ppm	Systemic toxicity: 20000/8000 ppm (≅617.1/1630.6 mg/kg bw/day) in ♂/♀ respectively. Neurotoxicity: 20000 ppm (≅1546.5/1630.6 mg/kg bw/day in ♂/♀ respectively)	Systemic toxicity - 20000 ppm (≅1546.5 mg/kg bw/day) ♂ only: Reduced body weights and food utilisation Neurotoxicity: No treatment related effects.

3.2 Toxicological end point for assessment of risk following long-term dietary exposure (ADI)

In the previous evaluation a chronic study was considered the most appropriate to derive the ADI. Since the rat proved the most sensitive species upon long-term exposure, it was suggested to derive the ADI for glyphosate be based on the basis of the chronic toxicity data obtained in rats. The ADI was based on a NOAEL of 31/34 mg/kg bw/day (males/females) derived from a two year rat study. This was the highest dose tested in this study and animals at this dose showed no signs of significant toxicity. Since then further chronic toxicity studies have been performed that indicate the appropriate NOAEL in long-term toxicity studies in rats is appreciably higher.

The 2004 JMPR review of glyphosate established an ADI for glyphosate of 1.0 mg/kg bw/day on the basis of the NOAEL of 100 mg/kg bw/day for salivary gland alterations in a long-term study

of toxicity and carcinogenicity in rats and a safety factor of 100. At that time the JMPR review of glyphosate concluded that this treatment-related effect was of unknown toxicological significance. In addition, it has to be noted that in the previous EU glyphosate evaluation the NOAEL for some 2-year rat studies were lower than presented in this review based on salivary gland effects. However, the cellular alterations observed in the salivary glands are considered to be an adaptive response to the acidic diet from glyphosate technical acid and are of no adverse consequence (see Annex II, Document M, Section 3 Point 5.10, Part 1):

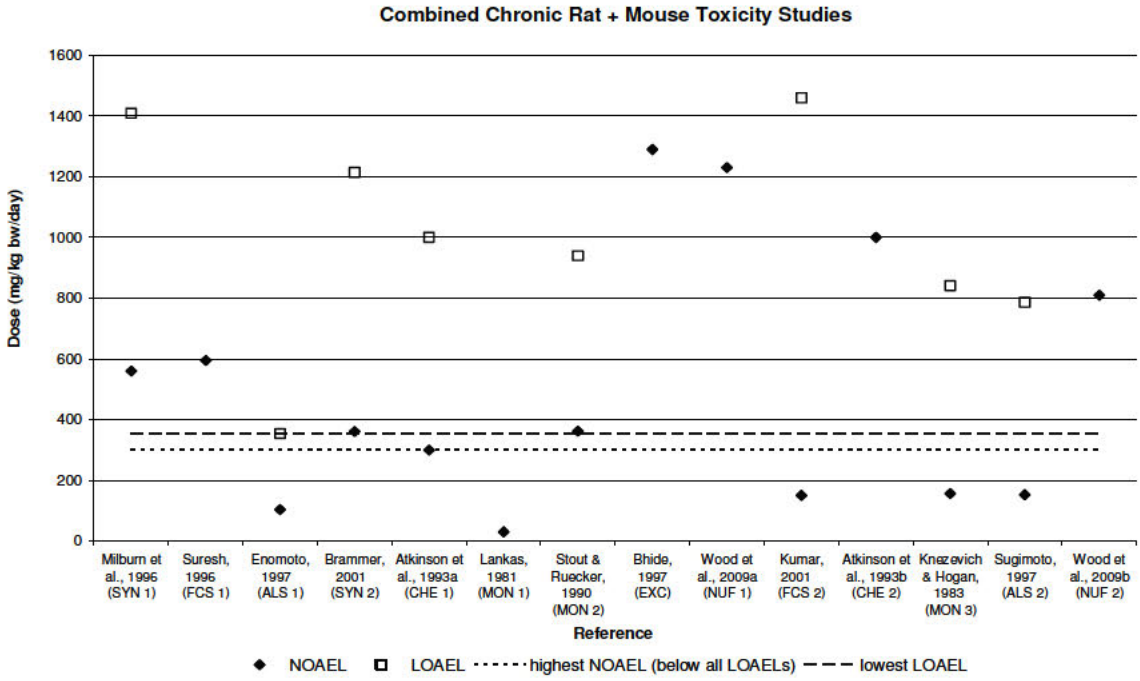
- The effect is observed with another organic acid with a similar pH-dilution curve to glyphosate.
- The effect is only observed following treatment in the diet. The same effect has not been observed across an extensive database following other exposure routes. The ADME radiolabel studies indicate glyphosate does not accumulate in the salivary gland.
- The effect, seen primarily in the rat, is variable in severity and has not been observed consistently across sex, dose or strain.
- From a histopathological perspective across an extensive database, there is no accompanying evidence of cytotoxicity leading to necrosis or apoptosis, no evidence of inflammation or change in function and the cellular alterations do not progress with time to preneoplastic or neoplastic lesions (but in fact decrease in incidence and severity or disappear all together with time).
- The effect is reversible upon cessation of treatment with glyphosate.

Based on sub-chronic toxicity studies performed in rats and mice, the mouse seems to be the least sensitive species. Due to the dose-spacing chosen in the long-term toxicity studies, the lowest LOAELs observed in mice were equal or greater than 841 mg/kg bw/day, with NOAELs of about 150 mg/kg bw/day and higher. The LOAELs observed in chronic rat studies were lower as compared to mice. Thus the rat is considered the most appropriate species for ADI derivation.

The highest relevant NOAEL observed in chronic toxicity studies is the **NOAEL of 300 mg/kg bw/day** from a 2-year rat study [redacted] 1993). This value is supported by two other studies in rats ([redacted] 2001; [redacted] 1990) with slightly higher NOAELs of 361 mg/kg bw/day and 362 mg/kg bw/day, respectively.

Applying a safety factor (SF) of 100 the **ADI** is considered to be **3 mg/kg bw/day** (i.e. 300 mg/kg bw/day/ 100 (SF)).

Figure 3.2-1: NOAELs and LOAELs observed in chronic rat and mouse studies with glyphosate



3.3 Toxicological end point for assessment of risk following acute dietary exposure – ArfD (Acute reference dose)

Due to the low acute toxicity profile of glyphosate the derivation of an ARfD for glyphosate is not necessary for the following reasons:

- Glyphosate is not acutely toxic; it did not produce mortality, overt clinical signs, changes in behaviour or relevant pathological lesions after a single dose up to 1000 mg/kg bw.
- No significant changes in clinical signs, behaviour, body weight or food consumption were observed in repeated-dose toxicity studies during the first few days with doses up to and above 500 mg/kg bw/day.

3.4 Toxicological end point for assessment of occupational and bystander risks – AOEL/MOE

In the previous 2001 EU glyphosate evaluation the AOEL based on maternal effects observed in rabbit developmental toxicity studies. The relevant NOAEL was 75 mg/kg bw/day. In addition to the multiple rabbit developmental toxicity studies reviewed in the initial Annex I inclusion of glyphosate, three more developmental toxicity studies in rabbits ([redacted] 1995, [redacted] 1996, and [redacted] 1996) have confirmed that adult rabbits are sensitive to oral gavage dosing with glyphosate.

In rabbits, glyphosate exposure via oral gavage led to clinical signs of toxicity consistent with gastro-intestinal disturbances. Rabbits were more sensitive to oral gavage dosing than other species. Clinical signs observed included diarrhoea/soft faeces, reduced faecal output, reduced body weights, reduced food consumption and increased mortality, all consistent with gastro-

intestinal stasis (ileus). Rabbits (caecotrophs) are particularly sensitive to disruption of the gastro-intestinal tract. Stress and other environmental factors such as pain can lead to the normal muscular contractions of the stomach and intestines being greatly diminished which in turn leads to disruption of the normal intestinal/caecum bacterial flora. It is likely that the mucosal membrane of the rabbit gastro-intestinal tract is irritated by bolus administration of glyphosate acid and that the associated stress or pain leads to gastro-intestinal stasis. The gross necropsy signs observed in maternal animals in the studies by [redacted] (1995), [redacted] (1996) and [redacted] 1996), such as hair like boluses in the stomach, fluid filled large intestines and gas distension in the lower gastrointestinal tract are indicative of gastro-intestinal stasis. The severity of this finding (gastro-intestinal stasis) appears to be relevant to hindgut fermenters only as both the rat and the dog are better adapted to tolerate the irritation potential of an oral bolus dose administration of glyphosate acid.

Further evidence, that these findings are related to gastro-intestinal disturbance comes from the Hadfield (2012) study that measured dermal absorption *in vitro* through rabbit skin. Based on the results of this study, 2.66% of the dermally applied dose in the [redacted] 21-day dermal toxicity study in the rabbit (1982), where there was no evidence of gastro-intestinal effects, was systemically available. Thus, the NOAEL for systemic effects after dermal application of 5000 mg/kg bw in the Johnson study was 133 mg/kg bw. Since lower systemic doses resulted in significant gastro-intestinal toxicity in dams from the rabbit developmental toxicity studies, such effects were likely attributable to a route specific toxicity rather than systemic toxicity.

In general the AOEL is derived from the highest dose at which no adverse effects are observed in relevant studies in the most sensitive species. Since operators are normally not exposed over long time periods to plant protection products, relevant studies for the AOEL derivation are sub-chronic (i.e. 90-day) or developmental toxicity studies.

The consistent delayed onset of symptoms, suggests that the effects may be due to repeated dosing of a low pH organic acid via oral gavage to the rabbit causing local irritation of the gastric mucosa. These effects are not representative of glyphosate-related systemic toxicity, but due to gastrointestinal tract (GIT) disturbances caused by a large bolus dose of acidic material.

After dietary administration (e.g. as in the sub-chronic toxicity studies) effects observed at the LOAELs consisted mainly on systemic effects (e.g. changes in clinical chemistry parameters, decrease of urinary pH). Some effects caused by GIT disturbances (e.g. diarrhoea) were also present. However, these effects occurred at higher dose levels as compared to effects observed after gavage dosing in the rabbit.

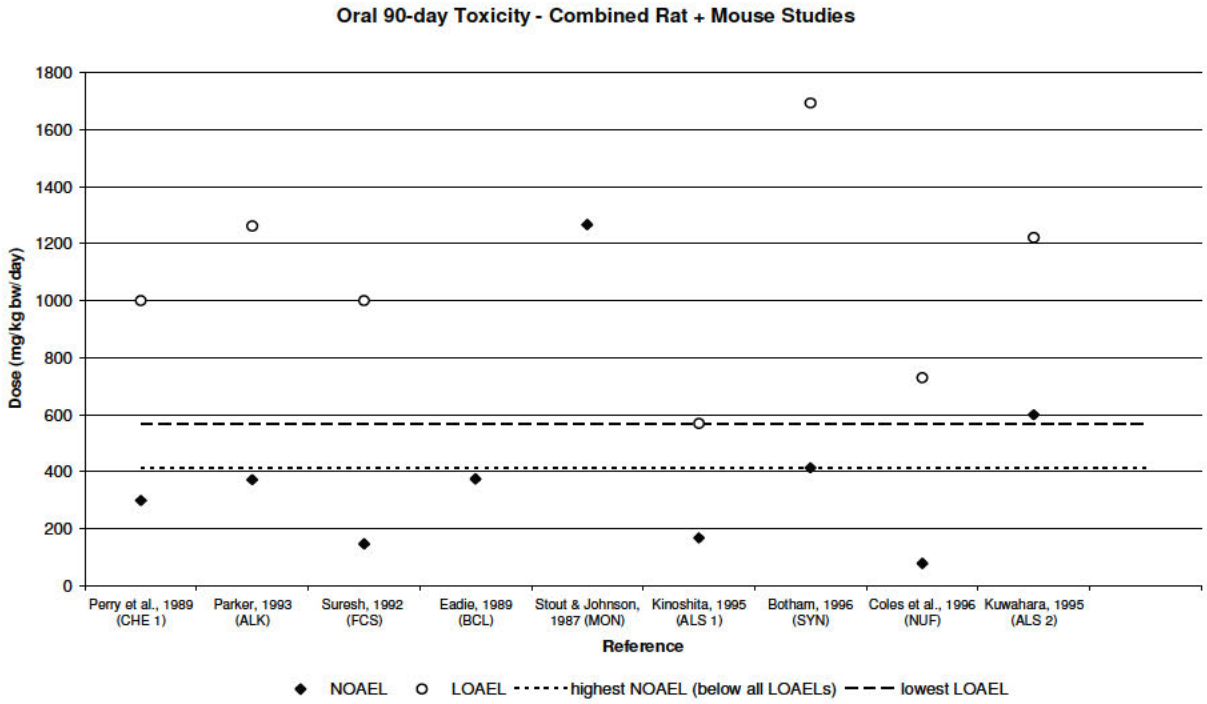
Regarding the facts outlined above the rabbit developmental toxicity studies are considered inappropriate for derivation of the AOEL, since the observed effects are not representative of systemic toxicity.

This is substantiated by the rationale given in the draft guidance document for AOEL derivation[1]. According to the draft guidance the dependency of the observed toxicity on the exposure route is essential for the determination of the most appropriate study for AOEL setting (see 3.14 of the guidance document).

Therefore, the sub-chronic toxicity studies performed in rodents by dietary administration of glyphosate are used for AOEL derivation, since the observed effect levels based on systemic toxicity.

[1] Working Document – Draft Guidance for the setting and application of acceptable operator exposure levels (AOELs); SANCO 7531 – rev. 10; European Commission, Health & Consumer Protection Directorate-General, 2006-07-07

Figure 3.4-1: NOAEL / LOAELs from 90-day toxicity studies in rodents



*The NOAEL of 4500 mg/kg bw/day derived from [redacted] 1991 (CHE 2) was not considered in this Figure.

As can be seen from the graph, the 90-day rat study conducted by [redacted] 1996 (SYN) provides the most sensitive endpoint. The observed **NOAEL was 414 mg/kg bw/day**.

With a safety factor of 100, as well as a correction for 30% oral absorption, the resulting AOEL **is 1.2 mg/kg bw/day**.

3.5 Drinking water limit

The drinking water limit can be derived using the ADI as starting point. Allowing 10 % of the ADI to be contributed by drinking water and assuming that an adult person of 60 kg bodyweight consumes 2 L water per day, the **MAC_{DW}** is calculated to be **9 mg/L** (i.e. MAC_{DW} = (ADI x 0.1 x 60 kg bw/2 L)).

3.6 Impact on human an animal health arsing from exposure to the active substance or to impurities contained in it

MON 52276 is formulated as a soluble liquid (SL) containing nominal 360 g glyphosate acid/L as the active substance. The product is used as herbicide for the control of annual, perennial and biennial weeds and will be applied up to 3 times per season. Applications are made pre- and post-planting, and pre-emergence or pre-harvest of the crops, as well as post-emergence of weeds. Spray treatments are performed using tractor-mounted ground-boom sprayers and knapsack sprayers. A summary of the representative uses for MON 52276 is presented below.

Table 06-1: Summary representative uses of MON 52276

Crop(s)	F	Application rate per treatment		Spray volume	Maximum in-use concentration	Number of treatments	Application technique
		[L product/ha]	[kg a.s./ha]	[L/ha]	[kg a.s./hL]	min - max	
All crops (pre-planting)	F	1 – 6*	0.36 – 2.16	100 - 400	2.16	1 – 2*	Tractor-mounted ground boom sprayer with hydraulic nozzles
All crops (post-planting/pre-emergence of crops)	F	1 - 3	0.36 – 1.08	100 - 400	1.08	1	
Cereals, oil seeds (both pre-harvest)	F	2 - 6	0.72 – 2.16	100 - 400	2.16	1	
Orchard crops, vines, incl. citrus & tree nuts (post emergence of weeds)	F	2 – 8*	0.72 – 2.88	100 - 400	2.88	1 – 3*	Knapsack sprayer
Orchard crops, vines, incl. citrus & tree nuts (post emergence of weeds; spot treatment)	F	2 – 8*	0.72 – 2.88	100 - 400	2.88	1 – 3*	

F = field use

3.6.1 Operators – estimates relevant for Europe

For the risk assessment operator exposure to the active substance glyphosate was evaluated using the German model and the UK-POEM for tractor-mounted applications, as well as the UK-POEM for hand-held applications to low-level targets. The exposure estimates were compared to the respective AOEL of 1.2 mg/kg bw/day. The results are summarised below.

Table 0-2: Operator exposure estimates for glyphosate from the use of MON 52276 – no PPE

	PPE Scenario*	Total systemic exposure** (mg/kg bw/day)	Total systemic exposure as % of AOEL*** (%)
Tractor-mounted spray application to low crops			
German model: Tractor-mounted ground boom sprayer • 20 ha/day • 6 L product/ha (≅ 2.16 kg a.s./ha) • 70 kg operator	None	0.0066	0.55
	None / with standard work wear	0.0034	0.28
UK-POEM: Tractor-mounted ground boom sprayer • 50 ha/day • 6 L product/ha (≅ 2.16 kg a.s./ha) • 100 L/ha • 60 kg operator	None	0.081	6.75
Knapsack applications to low-level targets – outdoors			
UK-POEM: knapsack sprayer • 1 ha/day • 8 L product/ha (≅ 2.88 kg a.s./ha) • 100 L/ha • 60 kg operator	None	0.226	18.8

* No PPE German Model: Operator wearing T-shirt and shorts.
No PPE / standard work wear German model: Operator wearing long work wear (coverall) but no PPE
No PPE UK POEM: Operator wearing long sleeved shirt, long trousers ("permeable") but no gloves

** Taking into account a dermal absorption of 0.09 % for the concentrated product and 0.34% for the spray solution

*** Compared to the proposed AOEL of 1.2 mg/kg bw/day

It is concluded that MON 52276 can be applied safely operators using tractor-mounted and hand-held application techniques without the use of PPE.

3.6.2 Operators – estimates relevant for North America

As this dossier is prepared for registration of MON 52276 in the EU, no operator exposure calculations relevant for North America were performed.

3.6.3 Bystanders

Bystander and resident exposure assessment was performed using the German guidance for evaluation of bystander and resident exposure of Martin *et al.* (2008)[2] . Estimations are presented for both, adults and children. The results are presented in the following.

Table 0-3: Bystander exposure estimates for glyphosate

	Bystander		Resident	
	Adult	**Child**	**Adult**	**Child**
Dermal exposure (mg/kg bw/day)	0.000036	0.000028	0.000004	0.000006
Inhalation exposure (mg/kg bw/day)	0.00001	0.000021	0.000276	0.000515
Oral exposure (mg/kg bw/day) – due to hand-to-mouth transfer	n.a.	n.a	n.a.	0.000039
Oral exposure (mg/kg bw/day) – due to mouthing	n.a	n.a	n.a.	0.000010
Total systemic bystander exposure (mg/kg bw/day)	0.000046	0.000049	0.00028	0.000568
Total systemic exposure as % of AOEL* (%)	< 0.01	< 0.01	0.02	0.05

* Compared to the AOEL of 1.2 mg/kg bw/day
n.a = not applicable

It is concluded that neither bystanders, nor residents are at risk due to the intended use of MON 52276.

3.6.4 Workers

For the intended uses of MON 52276 as a herbicide applied pre- and post-planting, and pre-emergence or pre-harvest there are no foreseen re-entry activities. The only reasonable re-entry scenario is inspection of the crops. However, for spray treatments pre- and post-planting, and pre-emergence of the crops, as well as post-emergence of weeds in orchards, crop inspection activities normally require no dermal contact to the foliage, but rather consist of a visual inspection.

As worst-case re-entry exposure during 2 hours of crop inspection activities following pre-harvest treatment of cereals and oilseeds were assessed. Exposure evaluations were done according to the German worker re-entry model (Krebs et al., 2000)[3] . The results are presented below.

[2] Martin et al., Guidance for exposure and Risk Evaluation for Bystanders and Residents exposed to Plant Protection Products during and after application, J. Verbr. Lebensm., Vol 3, No. 3, p. 272-281, August 2008.
[3] Krebs. et al.; 2000; Uniform Principles for Safeguarding the Health of Workers Re-entering Crop Growing Areas after Application of plant protection products (Nachrichtenbl. Deut. Pflanzenschutzdienstes, 52(1), p. 5-9, 2000

Table 0: Re-entry worker exposure estimates for glyphosate

Scenario	Unprotected professional worker during crop inspection after pre-harvest treatments in cereals or oilseeds*
Dermal exposure (mg/person/day)	21.6
Absorbed dose (mg/kg bw/day)	0.0012
Total systemic exposure as % of AOEL** (%)	0.1

* Worker wearing shoes, socks, long-sleeved shirt, and long trousers

** Compared to the AOEL of 1.2 mg/kg bw/day

It is concluded that workers are not at risk during re-entry activities in treated crops.

3.6.5 Consumers

Please refer to section Residues, Dietary Safety.

4. Residues

4.1 Definition of the residues relevant to MRLs

4.1.1 Definition of the residues in crops relevant to MRLs

Crop metabolism studies

The metabolism and distribution of ^{14}C-glyphosate in more than 20 varieties of conventional crops has been reviewed in the 2001 EU evaluation. These crop varieties represent the major crop groups required for plant metabolism testing. The routes of uptake considered in these studies included root uptake from soil and hydroponic solutions, applications to stems and trunks, and foliar applications of glyphosate to conventional crops. The majority of the plant-contained ^{14}C-radioactivity was released by aqueous extraction in almost all cases. Glyphosate was the major ^{14}C-component of the extract, and AMPA was the major ^{14}C-containing metabolite. Glyphosate was almost always present in higher amounts than AMPA, except in corn foliage following hydroponic application of ^{14}C-glyphosate, where glyphosate and AMPA were present at comparable levels. In addition to glyphosate and AMPA several minor metabolites that typically constituted less than 1% of the TRR were also occasionally detected. Several of these minor metabolites were identified, as N-methylaminomethylphosphonic acid (N-methyl-AMPA), methylphosphonic acid, and N-methyl-glyphosate. No significant metabolites other than AMPA were observed.

Additional studies in citrus, grapes, soybeans and wheat treated with glyphosate trimesium were also evaluated in the 2001 EU evaluation albeit in a parallel submission. These studies also considered different application methods (direct to soil, foliar overspray, pre-emergence and pre-harvest) and led to similar conclusions. When ^{14}C-glyphosate was applied directly to the crop, as the pre-harvest application in wheat or deliberate overspray in grapes, the majority of the residues remained as glyphosate. The only significant metabolite was AMPA. It was usually a minor component of the TRR, but in several of the soybean commodities, AMPA residues exceeded those of glyphosate. No other significant metabolites were identified in the glyphosate TMS metabolism studies.

While glyphosate-tolerant crop uses are not being included in the current dossier, the 2001 EU evaluation included also four metabolism studies in glyphosate-tolerant crops. Two of the studies were in crops (soybean and cotton) that included only CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) conferring glyphosate tolerance, and two of the studies were in crops (maize and oilseed rape) that included both CP4 EPSPS and GOX (glyphosate oxidoreductase), which metabolizes glyphosate to AMPA. The studies on metabolism of glyphosate in tolerant maize and oilseed rape plants demonstrated a rapid metabolism of glyphosate to AMPA caused by the presence of GOX. In contrast, cotton and soybean did not contain GOX and thus were similar to the non-tolerant plants, and metabolised glyphosate only slowly to AMPA.

Conclusion: The results of all the numerous plant uptake and metabolism studies demonstrate that glyphosate is slowly metabolised in plants to AMPA. With only a few exceptions (some soybean commodities and hydroponically-grown maize forage where AMPA levels were comparable to or greater than glyphosate levels), glyphosate is the major compound present in plant tissues. In all cases, AMPA accounts for less than 27 % of the radioactive residues, and typically is less than 10 %. With the exception of AMPA, no other metabolites of glyphosate are detected that account for greater than 5% of the total radioactive residues.

Environmental chemistry and fate relevant for residue uptake from soil

Chemical hydrolysis and photodecomposition do not contribute significantly to the degradation pathways of glyphosate in soil. However, glyphosate is extensively degraded in soil, under both aerobic and slightly anaerobic conditions, by indigenous soil micro-flora. The metabolite distribution resulting from the degradation of glyphosate in soil is similar under both aerobic and anaerobic conditions. Main metabolic pathway in soil is degradation to AMPA as the only significant degradation product of glyphosate, which is further metabolized to CO_2. Uptake from glyphosate and AMPA from soil through the roots is demonstrated to be extremely limited because of the tight binding of both compounds with soil particles. Soybeans, cotton, wheat, maize, barley, oats, rice, sorghum, potatoes, sugar beets, and pasture crops were treated with a pre-emergence application of glyphosate at application rates equivalent to 4.48 kg/ha.

For root uptake from the soil in apple trees, grapes, coffee plants, citrus, walnut, almond, and pecan trees, glyphosate was applied to the soil surface of pots containing the emerged crops, while shielding the foliage, at glyphosate application rates of between 2.24 kg/ha and 5.07 kg/ha.

In all cases, maximum uptake of radioactivity into plants grown in soil treated with ^{14}C-glyphosate was less than 1 % of the total applied radioactivity, demonstrating that very little of the applied glyphosate is taken up from the soil.

Confined crop rotation studies

A confined rotational crop study was reviewed in the 2001 EU evaluation of glyphosate (Nichols, 1990, RIP95-01201; McMullan et al, 1990, RIP95001202). The primary crop, soybeans, received a pre-plant application of 4.15 kg/ha of ^{14}C-glyphosate. Carrots, lettuce and barley were planted as rotational crops at 30, 119 and 365 days after application. Total ^{14}C-radioactivity expressed as glyphosate equivalents, was less than 0.2 mg/kg in all rotational crop samples and decreased with time. Release of ^{14}C-radioactivity upon aqueous extraction of rotational crop samples was less than 60 % of the radioactivity in the plants in all cases, and typically less than 40 %. The non-extractable ^{14}C-radioactivity in 30 day rotational barley grain and straw samples harvested 125 days after treatment was characterized as biopolymers of glucose. Aqueous extracts of the rotational crop tissues contained less than 0.02 mg/kg glyphosate in all cases.

The results of this study demonstrate that only very low levels of glyphosate or glyphosate metabolites are present in the soil and plant tissues of rotational crops planted after treatment of a primary crop with glyphosate. The only metabolite of glyphosate found was AMPA. The majority of glyphosate derived radioactivity in the soil and plant tissues has been attributed to natural products derived by incorporation of one carbon compounds such as CO_2 into natural metabolic pools. The distribution of radioactivity in rotational crops was found to be similar to the distribution found in plants exposed to $^{14}CO_2$. The results of this study show that glyphosate residues in emergency replant and rotational crops will be less than those found in the primary crop.

An additional confined rotational crop study was reviewed in the 2001 EU evaluation of glyphosate-trimesium (Spillner, 1993, RIP95-00018; Subba-Rao, 1994, RIP95-00019). ^{14}C-Glyphosate-trimesium (labelled in the glyphosate portion) was applied either as a single or as sequential applications, at a total rate equivalent to 3.9 – 6.6 kg/ha of glyphosate acid. Soybeans were planted as the primary crop. Lettuce, wheat and radishes were planted as the rotational crops, at 35 days, 125 and 370 days after the initial application. There was minimal uptake of residues in the samples. Glyphosate residue levels were <0.01 mg/kg in all samples, and the maximum AMPA residues were 0.03 mg/kg. All other extractable and unextractable radioactivity was associated with [^{14}C] incorporated or bound to natural products.

There is an additional rotational crop study not included in the glyphosate or glyphosate-trimesium 1999 monograph but submitted prior to ECCO review. The results are comparable to those included in the monographs. In this study crop rotation experiments were performed with [^{14}C] glyphosate on lettuce, radish and wheat - crops considered to be representative of leafy, root, and cereal crops, respectively. The active substance was applied to sandy loam soil at a single application rate of 6.5 kg/ha, exceeding the maximum annual application rate of 4.32 kg/ha. After the application, soil was aged 30, 120, and 365 days prior to planting. Soil samples were taken after application and after harvest of the mature crops for each plant back interval. Parent glyphosate residues above the LOQ were not found in any plant parts destined for human consumption. AMPA residues were found in the first and second planting of wheat. The residues in grains were 0.40 and 0.20 mg/kg, respectively. In the third planting no residues of AMPA were found in any wheat matrices.

No residues of parent glyphosate or AMPA were found in any of the mature radish and lettuce samples harvested from any of the planting intervals. This indicates that glyphosate and AMPA do not accumulate in rotational crops tested and that the majority of carbon which was initially

part of the glyphosate molecules applied to the soil that is taken up by these plants becomes incorporated into plant components or is converted into compounds other than glyphosate and AMPA.

Storage stability of residues in plant commodities

As reviewed during the 2001 EU glyphosate evaluation, the stability of spiked crop samples (exogenous fortifications) has been determined over a period of 0 to 31-32 months while the stability of endogenous (plant incorporated) residues has been determined over a period of 2 to 5 years in frozen storage (Mueth, 1991 RIP95-01332). Endogenous residues of both glyphosate and AMPA are proven to be stable in the seven crop commodities included in this study (corn grain, soy forage, sorghum stover, clover, tomatoes, alfalfa seed and potatoes) after 2-5 years in frozen storage. Although the exogenous AMPA residues show some decline over the course of this stability study, the decline is minimal. Coupled with the high stability of endogenous residues of AMPA, these results show that both glyphosate and AMPA are stable in different crop types (water, oil, protein, and starch containing and dry material) in frozen storage.

The stability of glyphosate and AMPA residues in representative raw agricultural commodities stored at -20 °C, including sorghum grain, soy bean, soy bean straw, and wheat grain, has been demonstrated (McKay, 1989, RIP95-00028) as reviewed in the 2001 EU Glyphosate Trimesium evaluation. Samples were removed for analysis at intervals up to 2 years after fortification. In addition, sorghum grain was also analysed at 4 years after fortification. Analysis showed that glyphosate and AMPA were stable in all samples taken. A further storage stability study (Lant, 1995, RIP9600003) on samples of wheat and oats processed products including grain, groats, glumes, flakes, bread, and flour confirms that incurred residues of glyphosate are stable over periods of up to 20 months.

Additional studies have been presented in the current submission in order to fulfil the current EU data requirements (supplementary or confirmatory data). In these studies, samples of soybean seed and straw, pasture grass, wheat, rye and barley grain and straw, maize (corn), sugar beet root & leaves and oranges were spiked with glyphosate and AMPA and stored at a temperature of -10°C to -20°C over a period of one year and up to 3.5 years. Glyphosate and AMPA were stable for at least 6 months in the soybean seeds, 12 months in pasture grass and at least 13 months in soybean straw. In wheat and rye grain and straw, glyphosate was stable for at least 3.5 years and AMPA was stable for at least 288 days in grain and at least 190 days in straw. Glyphosate and AMPA residues in barley (grain and straw), maize and sugar beet were stable for at least 18 months. In oranges, glyphosate and AMPA were stable for at least 2 years. Samples of beans, oilseed rape and linseed were spiked with glyphosate and stored at about -18 °C. The residues in all matrices were stable for about 18 months.

Together these studies provide new data on stability of glyphosate and AMPA in acidic crop commodities (oranges), and supplement the previous data on stability in oil seeds, cereals, root crops, forage and straw.

Proposed residue definition (food of plant origin)

The current residue definition for enforcement for glyphosate was established in the 2001 EU evaluation.

Plant metabolism studies demonstrated that glyphosate is the primary residue in crop commodities, AMPA is the major metabolite and in most cases the residues of AMPA are not significant.

Glyphosate is the primary residue in plant commodities and it was concluded that the residue definition for enforcement should be: **glyphosate**.

In 2009, under the framework of Article 10 of Regulation (EC) No 396/2005 the metabolism of glyphosate in genetically modified soya bean and maize containing the glyphosate-N-acetyl transferase (GAT) gene was assessed[4]. Submitted studies indicated that the metabolism of glyphosate in these transgenic crops proceeds in a different pathway, producing two additional metabolites, N-acetyl-glyphosate and N-acetyl-AMPA.

Several options for the definition of the residue for enforcement were proposed by EFSA, including maintaining the current definition. No change is currently proposed, so the definition of the residue for enforcement should be: **glyphosate**.

Taking into account the differences in metabolism in crops containing the GAT gene, the definition of the residue for risk assessment for plant products was recently amended to be: **the sum of glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA, calculated as glyphosate.**

4.1.2 Definition of the residues in food of animal origin relevant to MRLs

Poultry

Two different studies on laying hens were reviewed in the 2001 EU glyphosate evaluation to determine the absorption, distribution, metabolism and excretion in livestock. In one study (Boden, 1988, RIP95-01205; Feng and Patanella, 1988, RIP95-01206), animals were dosed with a 9:1 ratio of glyphosate and aminomethylphosphonic acid, AMPA, which is the primary plant metabolite of glyphosate. The hens were dosed at a level corresponding to a total dietary concentration of 120 and 400 mg/kg. For the other study (Powles, 1994, RIP95-01208), hens were dosed with glyphosate alone at a level corresponding to a total dietary concentration of 200 mg/kg. Glyphosate and AMPA were rapidly excreted mainly in the faeces and urine, primarily as unchanged parent compound, resulting in low residue levels in edible tissues and eggs. There was minimal metabolism of glyphosate to AMPA, as clearly demonstrated in the study conducted with glyphosate alone. Metabolites resulting from the degradation of glyphosate and AMPA in tissues were either insignificant or entirely absent.

[4] EFSA (European Food Safety Authority), 2009. Reasoned opinion on the modification of the residue definition of glyphosate in genetically modified maize grain and soybeans, and in products of animal origin. EFSA Journal 2009; 7(9):1310, 42 pp.

An additional study in hens was reviewed in the 2001 EU glyphosate trimesium evaluation (Bowler, 1994, RIP95-00020). The animals were dosed with ^{14}C-glyphosate in the form of the trimesium salt at a level equivalent to 62-64 mg/kg of glyphosate acid in the diet.Glyphosate-trimesium radiolabelled in the glyphosate portion was rapidly and nearly completely excreted by hens. The radioactive residues found in tissues and eggs consist mainly of glyphosate and the metabolite AMPA. In addition, a part of the radioactivity was incorporated into naturally occurring products.

Conclusion:

Results from all three sets of animal metabolism studies are consistent. Both glyphosate and AMPA were rapidly and extensively excreted after dosing in hens. Tissue levels were generally low, and AMPA was the only significant metabolite present. Other metabolites resulting from degradation of glyphosate and AMPA were either insignificant or absent.

Metabolism in Lactating ruminants

Two different studies on lactating goats were reviewed in the 2001 EU glyphosate evaluation to determine the absorption, distribution, metabolism and excretion in livestock. In one study (Boden, 1988, RIP95-01203; Feng and Patanella, 1988, RIP95-01204), animals were dosed with a 9:1 ratio of glyphosate and aminomethylphosphonic acid, AMPA, which is the primary plant metabolite of glyphosate. The goats were dosed at a level corresponding to a total dietary concentration of 120 mg/kg. For the other study (Powles, 1994, RIP95-01207), goats were dosed with glyphosate alone at a level corresponding to a total dietary concentration of 200 mg/kg.

Glyphosate and AMPA were rapidly excreted mainly in the faeces and urine, primarily as unchanged parent compound, resulting in low residue levels in edible tissues and milk. There was minimal metabolism of glyphosate to AMPA, as clearly demonstrated in the study conducted with glyphosate alone. Metabolites resulting from the degradation of glyphosate and AMPA in tissues were either insignificant or entirely absent.

An additional study in hens was reviewed in the 2001 EU glyphosate trimesium evaluation (Ericson, 1994, RIP95-00022). The animals were dosed with ^{14}C-glyphosate in the form of the trimesium salt at a level equivalent to 62-64 mg/kg of glyphosate acid in the diet. In goats, the glyphosate portion of glyphosate-trimesium is rapidly excreted mainly in the faeces. Tissue residues were generally low with the highest value reached in the kidneys. The radioactive residues found in tissues consisted mainly of glyphosate itself and the metabolite AMPA. The major radioactive residues in milk were natural products in the form of lactose, triglycerides and protein. Lactose and triglycerides constituted over 45 % TRR in milk, while material associated with post extraction milk solids comprised 20 % TRR, which is consistent with natural incorporation of radiocarbon into proteins. Residues of glyphosate did not accumulate in fat, tissues or milk.

Conclusion:

Results from all three sets of animal metabolism studies are consistent. Both glyphosate and AMPA were rapidly and extensively excreted after dosing in goats. Tissue levels were generally low, and AMPA was the only significant metabolite present. Other metabolites resulting from degradation of glyphosate and AMPA were either insignificant or absent.

Storage Stability of residues in animal commodities

The stability of exogenous residues of glyphosate and AMPA in animal commodities has been demonstrated (Manning, 1998, RIP95-01253). Samples of swine, cow, and chicken fat, muscle, liver and kidney along with cow milk and chicken eggs were fortified with a solution of glyphosate and AMPA and stored frozen at ≤-20 °C. Samples were stored for up to 13 to 32 months. The data, reviewed during the 2001 EU evaluation, indicate a slight decrease in the glyphosate and AMPA residues for most matrices over the course of the study. However, these results show that losses due to instability have a negligible effect on the results of the feeding studies on swine, dairy cow and laying hens.

In addition, as part of the 2001 EU glyphosate trimesium evaluation, storage stability of glyphosate and AMPA has been demonstrated in muscle, liver, kidney, eggs and milk for a minimum of 689 days (1.9 years). (Graham, 1987, RIP95-00024 and Graham, 1987, RIP95-00025).

Proposed residue definition (food of animal origin)

The current residue definition for enforcement for glyphosate was established in the 2001 EU evaluation.

Radiolabelled studies in lactating goats and laying hens following oral administrations of glyphosate and AMPA showed that metabolites resulting from the degradation of these compounds in edible tissues, milk and eggs were either insignificant or entirely absent.

Glyphosate is the primary residue in animal commodities and it was concluded that the definition of the residue for enforcement should be: **glyphosate**.

In 2009, under the framework of Article 10 of Regulation (EC) No 396/2005 the metabolism of glyphosate in genetically modified soya bean and maize containing the glyphosate-N-acetyl transferase (GAT) gene was assessed[5]. Submitted studies indicated that the metabolism of glyphosate in these transgenic crops proceeds in a different pathway, producing two additional metabolites, N-acetyl-glyphosate and N-acetyl-AMPA.

Several options for the definition of the residue for enforcement were proposed by EFSA, including maintaining the current definition. No change is currently proposed, so the definition of the residue for enforcement should be: **glyphosate**.

Taking into account the differences in metabolism in crops containing the GAT gene, the definition of the residue for risk assessment for animal products was recently amended to be: **the sum of glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA, calculated as glyphosate.**

[5] EFSA (European Food Safety Authority), 2009. Reasoned opinion on the modification of the residue definition of glyphosate in genetically modified maize grain and soybeans, and in products of animal origin. EFSA Journal 2009; 7(9):1310, 42 pp.

4.2 Residues relevant to consumer safety

Residues from applications in crops

Numerous supervised residue trials have been conducted to establish MRLs for glyphosate. In cases where residues resulting from different glyphosate formulations have been compared in side-by-side field trials, no differences were been found which allows to extrapolate residue data across formulations.

From all uses listed in the representative GAP, pre-harvest use results in the highest residue levels in food commodities. These in-crop, pre-harvest applications are currently approved in various European Union Member States for cereals (wheat, barley, oats, and rye), pulses (beans and peas), oil seed crops and forage grasses. Maximum glyphosate residues in grain and seed resulting from pre-harvest applications according to approved uses reached 20 mg/kg.

In-crop selective equipment or between-the-row applications of glyphosate may also result in detectable residues in crops. For example, an MRL of 1 mg/kg was set for maize that has received inter-row selective applications.

A major method of glyphosate application is as a pre-plant or pre-emergence treatment that does not result in significant residues. The latter is reconfirmed by a series of recent pre-emergence residue trials in representative crops of all major crop groups which are presented in the current submission. The new trials have been conducted in crop-relevant countries across Europe (Spain, Italy, France, UK, Bulgaria, Germany, Hungary and Poland) at nominal dose rates of 6.0 L/ha applied three days after seeding (carrots, bulb onions and sugar beet), three days before planting seedlings (tomatoes, cucumber, zucchini, cauliflower, head cabbage, lettuce and leek) or three days after planting (potato) but in all instances before crop emergence. Samples from all crops were taken at BBCH 49 or BBCH 89 (commercial maturity in each crop) and the relevant crop commodities were analyzed for glyphosate and AMPA with crop-validated analytical methods.

The glyphosate and AMPA residues for all trials of all crops were below the LOQ (<0.05 mg/kg), and therefore support the existing MRLs of 0.1 mg/kg for pre-plant/pre-emergence uses.

Residues from feeding animals treated commodities

Animal feeding studies using glyphosate and AMPA with lactating cows, poultry, and swine, have been reviewed during the 2001 EU glyphosate evaluation. For these studies, test groups of animals were fed a daily ration containing a nine to one mixture of glyphosate and AMPA at total combined daily dietary levels of 40, 120, and 400 mg/kg for 28 days. The dosing levels are assumed to represent, respectively, lx, 3x, and l0x the maximum expected residue levels of glyphosate and AMPA in the diet. Animals were sacrificed either following the last day of treatment or following a 28 day depuration period. Milk samples were taken in the cow study and eggs were collected in the poultry study at various time points during treatment and depuration. At sacrifice, residue levels were determined in fat, muscle, liver and kidney.

For all three species, glyphosate and AMPA residues were less than 0.05 mg/kg (undetectable) in all fat and muscle samples from all treatment levels following the 28-day dosing period, except muscles samples from swine and fat samples from chickens dosed at the highest level, which had residues of 0.06 to 0.07 mg/kg of glyphosate.

The highest glyphosate and AMPA residues were found in kidneys. At the end of the 28-day dosing period glyphosate residues in kidney of cow, swine and chicken dosed at the 10x level

were 3.0, 7.63, and 3.82 mg/kg, respectively. AMPA residue levels in the same tissues were 0.07, 0.29, and 0.96 mg/kg, respectively. Significantly lower levels of glyphosate and AMPA were found in liver tissues collected at the end of the 28-day dosing period. For the l0x dose level liver samples, glyphosate residues were 0.20, 0.60, and 0.61 mg/kg, respectively. AMPA residues in the same tissues were <0.05, 0.12, and 0.39 mg/kg, respectively.

Analysis of tissues following the 28 day depuration period demonstrate that glyphosate and AMPA are rapidly eliminated. Following a 28-day depuration period, AMPA residues were less than 0.05 mg/kg in all samples. Glyphosate residues in the 28-day depurated animal tissues were less than 0.05 mg/kg in all tissues except kidney samples at the 3x and l0x dose levels, which contained average glyphosate residues of 0.08 and 0.18 mg/kg, respectively. Glyphosate and AMPA residues were less than 0.025 mg/kg (undetectable) in all milk samples collected from cows dose at the l0x level. Glyphosate residues were undetected in all egg samples collected from hens dosed at the 1x level, and were up to 0.131 mg/kg in eggs of hens dosed at the 10x level. AMPA residues in the same samples were less than 0.025 mg/kg in all cases. All glyphosate residues in eggs collected after a 7-day depuration period were less than 0.025 mg/kg.

Additional animal feeding studies with glyphosate-trimesium in cattle and poultry were evaluated in the 2001 EU glyphosate trimesium evaluation. Laying hens were fed with glyphosate-trimesium at dose levels of 0.5, 5 and 50 mg glyphosate-trimesium/kg in feed (equivalent to 0.34, 3.4 and 34 mg/kg of glyphosate acid). The hens were dosed for 28 consecutive days. Certain hens were selected for an additional withdrawal period of 7 days in which no glyphosate-trimesium was administered. No treatment-related effects on feed consumption, body weight or egg production were evident throughout the study. Glyphosate-trimesium, when fed continuously at a level equivalent to 34 mg/kg of glyphosate acid to laying hens, produced low concentrations of residues in eggs and edible tissues. Residues of glyphosate in eggs ranged from <0.01 – 0.015 mg/kg. Residues of glyphosate in kidney were 0.31 mg/kg, and were not detected (<0.05 mg/kg) in liver, fat and muscle. Residues of AMPA were below the limit of determination in all tissues and eggs. All residues were below the limit of determination by 7 days after cessation of dosing.

Lactating dairy cattle were dosed daily for 28 days with five rates of glyphosate-trimesium technical, at rates equivalent to 0.5, 5, 50, 300 and 1000 mg/kg in the diet (equivalent to 0.34, 3.4, 34, 207 and 690 mg/kg of glyphosate acid in the diet). Two animals from each group were sacrificed after 28 days and the remainder were sacrificed after 7 days of withdrawal. Feed consumption, milk production and body weights of dairy cows were not affected by daily administration of glyphosate-trimesium at dose levels up to 300 mg/kg in the diet. At a dose level of 1000 mg/kg treatment related effects were observed including lethargy with reduced feed consumption, milk production and bodyweight.

Glyphosate-trimesium, when fed continuously for 28 days, at a level equivalent to 207 mg/kg of glyphosate acid to dairy cattle, produced low concentrations of residues in milk and edible tissues. One milk sample had glyphosate residues at 0.02 mg/kg, all others were below the limit of determination (<0.02 mg/kg). In kidney, glyphosate residues were 1.8 – 2.6 mg/kg and AMPA residues were 0.47 – 0.58 mg/kg immediately after dosing, and declined to 0.12 mg/kg and <0.05 mg/kg, respectively, 7 days after cessation of dosing. In fat, glyphosate residues were 0.06 mg/kg and AMPA was <0.05 mg/kg. Glyphosate and AMPA levels in liver and muscle were below the limit of determination in all samples.

Conclusion

Results in both sets of livestock feeding studies are consistent. Glyphosate and AMPA are rapidly excreted. The highest residues are in kidney, with lower residues in the liver. Residues in milk, eggs, tissue and fat were either not detected or were very low. Residues declined quickly after dosing was stopped.

Effects on residues from industrial processing

As part of the current submission a nature of the residue study was presented. This study evaluates the degradation of [^{14}C]glyphosate under hydrolytic conditions at high temperatures in sterile aqueous buffers at pH 4, 5 and 6 for periods of up to 60 minutes, simulating common processing practices as pasteurisation, baking, brewing, boiling and sterilisation (OECD 507). The experiments showed that glyphosate did not degrade at temperatures ranging from 90°C to sterilizing conditions (121°C) in any of the buffer systems tested, indicating that glyphosate should be stable in/on processed commodities during common processing practices.

In addition, processing studies in many crops were included in the 2001 EU glyphosate and glyphosate trimesium evaluations. Glyphosate concentrates primarily in processed fractions such as hulls and bran of cereals and citrus peel due to surface residues in meal after removal of oil fractions and in concentrated liquid fractions such as molasses. Glyphosate does not partition into oil, and is removed from highly processed fractions such as sugar.

Consumer risk assessment

Long-term consumer exposure to potential glyphosate residues was estimated according to the EFSA PRIMo model[6] for chronic risk assessment. The most recent chronic risk assessment for glyphosate was published by EFSA in January 2012 in support of the application to set an import tolerance for glyphosate in lentils[7]. In that assessment, EFSA used the MRL values for most crops, and added the median residue value of 1.47 mg/kg for lentils, based on data in the import tolerance petition.

Residue input values for several glyphosate-tolerant crops were conservatively calculated as the sum of the glyphosate MRL and a proposed AMPA MRL, expressed as glyphosate. These calculated residue input values were: rape seed (10.8 mg/kg), soybean (28.4 mg/kg) and maize (2.6 mg/kg). The AMPA MRLs were proposed in the 2000 Germany peer review[8] but were not included in the MRL legislation.

Using the above input values and the current established ADI of 0.3 mg/kg, the total calculated intake values accounted for up to 46.7% of the ADI (WHO Cluster B).

Based on toxicology data presented in this dossier, the proposed ADI for glyphosate has been increased to 3.0 mg/kg bw/day. A revised chronic risk assessment has been conducted using the

[6] Revision 2.0 of the EFSA model, downloaded Sep 2011. Reasoned Opinion on the Potential Chronic and Acute Risk to Consumers' Health Arising from Proposed Temporary EU MRLs According to Regulation (EC) No 396/2005 on Maximum Residue Levels of Pesticides in Food and Feed of Plant and Animal Origin, European Food Safety Authority, 15 March 2007

[7] European Food Safety Authority; Modification of the existing MRL for glyphosate in lentils. EFSA Journal 2012;10(1):2550. [25 pp.] doi:10.2903/j.efsa.2012.2550. Available online: www.efsa.europa.eu/efsajournal

[8] Germany, 2000. Complete list of end points (available on CIRCA in "Archive individual substances/glyphosate")

proposed ADI. The residue level present in each commodity is set at the established MRL. In addition, the proposed MRL of 10 mg/kg in lentils (see Document E-2) is also included in the assessment.

The TMDI calculation gives an unrealistic worst-case estimate of intake, because it assumes that 100% of crops with established and proposed uses will contain residues at the MRL. No account is taken of the potential reduction in residues during transport and storage or during commercial and domestic processing. In practice, the actual intake is likely to be much lower than the calculated values. For all population groups in all models the estimated TMDI is at or below 4.4% of the ADI.

The results indicate that there is no unacceptable chronic risk to human health from the consumption of commodities treated with glyphosate according to the uses considered.

A risk assessment for acute consumer exposure is not required since no acute reference dose (ARfD) has been set or proposed for glyphosate. No unacceptable acute risk to human health from the consumption of commodities treated with glyphosate according to the uses considered is indicated.

4.3 Residues relevant to worker safety

For the glyphosate uses in the representative GAPs there are no foreseen re-entry activities. The only reasonable re-entry scenario is inspection of the crops. However, for spray treatments pre- and post-planting, and pre-emergence of the crops, as well as post-emergence of weeds in orchards, crop inspection activities normally require no dermal contact to the foliage, but rather consist of a visual inspection. At all times these activities should only occur when the spray deposit has dried.

As worst-case re-entry exposure during 2 hours of crop inspection activities following pre-harvest treatment of cereals and oilseeds were assessed. Exposure evaluations were done according to the German worker re-entry model (Krebs *et al.*, 2000)[9]. Systemic dose obtained consumes less than 1% of the AOEL indicating low exposure and safe uses.

4.4 Proposed MRLs and compliance with existing MRLs

4.4.1 Compliance with existing MRLs

Glyphosate uses respecting the GAPs specified on the label recommendation will result in residue levels below the established MRLs.

4.4.2 Proposed MRLs

The MRLs as presented in the Commission Regulation 839/2008/EC. All MRLs for raw agricultural commodities still apply.

[9] Krebs. et al.; 2000; Uniform Principles for Safeguarding the Health of Workers Re-entering Crop Growing Areas after Application of plant protection products (Nachrichtenbl. Deut. Pflanzenschutzdienstes, 52(1), p. 5-9, 2000

These MRLs were determined from the results of supervised field trials conducted in Europe, with the exception of soybeans and tea (for which import tolerances are recommended). For soybeans, data for MRL determinations were derived from supervised field trials conducted in the United States. For tea, data for MRL determinations were derived from supervised field trials conducted Taiwan and Sri Lanka. In all cases, MRLs for raw agricultural commodities are based on currently approved, critical Good Agricultural Practices in the European Union.

For the estimation of the residues in animal products, the STMR of cereal grain and straw was used as proposed by the JMPR FAO panel, resulting in very low residue situation expected for all products of concern. Therefore, the MRLs for foodstuff of animal origin have been revised in Regulation 839/2008/EC.

No new MRLs are being proposed as part of this submission.

4.5 Proposed import tolerances

This submission proposes no new import tolerances.

4.6 Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs

None

5. Fate and behaviour in the environment

5.1 Definition of the residue relevant to the environment

Multiple studies from the glyphosate taskforce (GTF) members all independently show that chemical degradation, photodecomposition and volatilization are, at most, very minor pathways for the dissipation of glyphosate in the environment. However, studies have conclusively demonstrated that glyphosate is degraded in soil, under aerobic, anaerobic, and aerobic flooded (water sediment) conditions, by indigenous soil and sediment micro-flora. In all of the environmental fate studies conducted with ^{14}C-glyphosate, aminomethylphosphonic acid (AMPA) was identified as the most significant metabolite (>10% of the applied glyphosate).

In the aerobic studies AMPA was the only significant metabolite and its maximum concentration levels ranged from 14.7-50.1% of the applied glyphosate. In the anaerobic soil metabolism studies again AMPA was the only significant metabolite and its maximum concentration levels ranged from 0.5-44.2% of the applied glyphosate and in the soil photolysis studies AMPA, the only significant degradation product observed, ranged from 8.2-28.4% of the applied glyphosate.

Water sediment studies with glyphosate demonstrated that 6 to 48% of the applied glyphosate is mineralized to carbon dioxide during 100 or 120 days of incubations. The principal metabolite of glyphosate in water/sediment system was AMPA. The maximum amount of AMPA detected ranged from 2 to 16% (water phase) and up to 27% (total system) of the total glyphosate applied. Hydroxymethylphosphonic acid (HMPA) was detected only in the water phase of one of the studies with a maximum amount of about 10% of the dose after 61 days.

Water sediment studies on AMPA showed that from 8 to 40% of the applied AMPA is mineralized to carbon dioxide. In one study other minor transient metabolites of AMPA were also detected in the water/sediment system. The major metabolite of AMPA which was found mainly in sediments was identified as 1-oxo AMPA (8.8–22.9% of the applied AMPA in both sediment systems of the study respectively). Another transient degradation product was detected mainly in the water phases of both aquatic systems (1.8-9.8% of applied AMPA) which was postulated to be phosphonoformic acid, a logical and labile metabolic transformation product expected in the pathway of mineralization of AMPA to CO_2. However, neither 1-oxo-AMPA nor phosphonoformic acid were detected in any significant amounts from the four water/sediment studies conducted with glyphosate; confirming the significant transitory nature of these aquatic degaradtion products.

Thus, AMPA is the major soil and aquatic metabolite of glyphosate but is of no environmental significance as consistently demonstrated throughout the dossier (very low leaching potential, no herbicidal activity, lower ecotoxicological and toxicological effects compared to parent). HMPA is the second most significant aquatic metabolite of glyphosate but is of no aquatic-ecological significance as demonstrated in the dossier. On the basis of the maximum concentrations detected, the AMPA water/sediment transitory metabolites 1-oxo-AMPA and phosphonoformic acid, do not reach levels to qualify them as major aquatic metabolites of parent glyphosate.

It is therefore proposed that for the purpose of monitoring the residue relevant to the environment is defined as **glyphosate (parent compound). For the purpose of risk assessment in this dossier, all major metabolites have been considered in the residue definition:**

Residue in soil & groundwater: glyphosate and AMPA
Residue in surface water: glyphosate, AMPA and HMPA

5.2 Fate and behaviour in soil

Studies show that chemical hydrolysis and photodecomposition do not contribute significantly to the degradation pathways of glyphosate in soil. However, glyphosate is extensively degraded in soil, under both aerobic and slightly anaerobic conditions, by indigenous soil micro-flora. The metabolite distribution resulting from the degradation of glyphosate in soil is similar under both aerobic and anaerobic conditions. Main metabolic pathway in soil is degradation to AMPA as the only significant degradation product of glyphosate, which is further metabolized to CO_2.

The **aerobic metabolism** of glyphosate in soil was investigated under laboratory conditions in a series of 7 separate and independent soil metabolism studies covering a range of different soil textures and soil characteristics. The soil pH ranged from 5.9-7.1, the soil organic carbon levels ranged from 0.3% to 2.2%. The duration of the tests ranged from 31 to 364 days whereas glyphosate application rates across the tests ranged from 2.4-10 µg/g of soil. Only one major metabolite, AMPA, was consistently identified at levels ranging from 14.7-50.1% of the applied glyphosate. These studies demonstrated that the production of CO_2 was a significant route of degradation indicating that from 23% to 79.6% of the applied glyphosate is mineralized to carbon dioxide. The proposed metabolic pathway under aerobic soil conditions is therefore the direct microbial conversion from glyphosate into AMPA and further mineralization into carbon dioxide, although at a slower rate than glyphosate.

The **rate of degradation under aerobic conditions** was derived from 12 aerobic route and rate studies covering 15 different soil textures, a wide range in soil pH (5.2-8.3) and soil organic carbon (0.5-6.8%). The aerobic degradation half-lives of glyphosate from all studies were re-calculated in accordance with the kinetic approaches recommended in the latest guidance (FOCUS, 2006, 2011). The results of all degradation studies are consistent with the conclusions of the first EU review of glyphosate (2001) and have consistently demonstrated the ready degradation of glyphosate in soil over time. Glyphosate shows a clear non-linear degradation pattern in all soils. The DT_{50} values ranged from 1.0 days to 60.2 days with a median DT_{50} of 5.8 days across all soils. In most cases (12 out of 15 soil types examined) the DT_{50} values for glyphosate degradation were less than 10 days. For those studies where the pattern of decline of glyphosate is clearly established within the experimental period of the study (12 out of 15 soils), the calculated time for 90% degradation of glyphosate (DT_{90}) ranged from 7.2 to 159.4 days. However, due to the relatively slower rate of degradation of glyphosate in 3 soil types and the study duration of only 120 days, 10% of the initial glyphosate concentration was not reached within the experimental period in these three soils. This indicates that a reliable DT_{90} estimate cannot be derived in these three soils, since extrapolation of the bi-phasic non-linear model beyond the duration of the study generally results in unrealistically long DT_{90} values. The aerobic degradation rate of glyphosate at lower temperatures (10°C) was conducted in one study; establishing a laboratory aerobic degradation DT_{50} of 10 days and DT_{90} of 53 days at 10°C.

Degradation half-lives for the major soil metabolite, AMPA were calculated from the laboratory aerobic rate and route of degradation studies of glyphosate in accordance with FOCUS (2006, 2011) guidance. Separate aerobic soil degradation studies with AMPA as applied test substance are not available. The best-fit DT_{50} values of AMPA ranged from 39.0 days to 134.8 days, indicating AMPA also degrades in soil, although at a rate somewhat slower than parent glyphosate.

The **anaerobic metabolism** of glyphosate in soil was also investigated under laboratory conditions in a series of 5 separate and independent anaerobic soil metabolism studies in loamy sand and sandy loam textured soils. The soil pH ranged from 5.8-6.9, the soil organic carbon levels ranged from 1% to 1.8%. Glyphosate application rates across the tests ranged from 5–30 µg/g of soil. Four studies were conducted according to old SETAC anaerobic soil test guideline. Only one study was conducted according to requirements of the current OECD guideline 307 for anaerobic transformation.

In general the same metabolic pathway as in the aerobic studies was observed. AMPA was identified as the only significant degradation product of glyphosate under anaerobic conditions at maximum levels ranging from 0.5-44.2% of the applied glyphosate. The mineralization into carbon dioxide ranged from 0.87% to 45.42% at the end of the studies.

The most reliable estimate of the **rate of degradation under anaerobic conditions for glyphosate** was obtained from the study conducted under OECD test guidance (anaerobic conditions instead of anoxic conditions). In this study the route and rate of degradation of [^{14}C] glyphosate was investigated in a flooded sandy loam soil following an aerobic aging period equivalent to one half-life. The results of the new anaerobic laboratory degradation study show that glyphosate also degrades under anaerobic conditions although at a slower rate than under aerobic conditions. The anaerobic DT_{50}, calculated over a period of 120 days of anaerobic incubation was established as 142 and 205 days for the soil and total system (soil/water compartments), respectively. The metabolite distribution resulting from the degradation of

glyphosate in soil is similar under both aerobic and anaerobic conditions. Levels of AMPA, the most significant degradation product, increased to 30% of applied dose after 84 days and subsequently declined to 28% of the dose after 120 days of anaerobic incubation. Due to the relatively fast rate of formation of AMPA in the study and the short duration of the study (120 days) a clear pattern of decline of AMPA was not established in the study and no accurate DT_{50} value can be calculated for AMPA. However, from visual inspection of the AMPA data, it can be concluded that AMPA also degrades in soil under anaerobic conditions, although at a slower rate than under aerobic soil conditions. According to GAP information, for the glyphosate use in arable cropping systems fully anaerobic conditions are expected to be rare throughout the surface soil zone where glyphosate occurs. Where anaerobic conditions occur in the surface soil layer, aerobic conditions are normally re-established quickly resulting in rapid degradation of glyphosate as shown previously.

Three existing **soil photolysis** studies, according to US EPA Section 161-3 guidance, were presented covering sandy loam and silt loam soils. Soil pH ranged from 6.1-8.3 and soil organic carbon ranged from 0.3-1.4%. Two of the studies had a 12h light/dark-cycle. One study had a natural light/dark cycle. Observed AMPA levels ranged from 8.2% to 28.4%. However, the degradation profile of glyphosate was similar for both light exposed and dark control, indicating that degradation of glyphosate in these studies is most likely due to soil microbial degradation and not by photodegradation. The rate of degradation of glyphosate was faster however in light exposed samples of one out of the three studies. Although some differences were found among the studies, taken together, the results of these soil photolysis studies show that the degradation of glyphosate on soil surfaces to AMPA is a slow process and is, at most, a very minor pathway for the degradation of glyphosate in soil.

Field soil dissipation of glyphosate in areas representative of Central Europe (multiple field locations in Germany and Switzerland) and areas with climate and soil characteristics comparable with those in Southern Europe (USA/ Tennessee, California, Georgia) and Northern Europe (Canada) has been extensively evaluated. These studies not only represent a range in geographical and climatological conditions but also a wide variety in key soil characteristics such as texture, pH (4.7-8.5) or soil organic matter (0.4-6%). Application rates were in the range of 3-6 kg a.s./ha. The duration of the studies varied from 61-581 days. The results of all dissipation studies have consistently demonstrated that glyphosate dissipates in soil over time under field conditions. In trials where soil cores were sectioned into segments (*ca* 0-10, 10-20, and 20-30 cm) and were separately analysed for residual glyphosate and AMPA showed majority of residues at the top soil layer, suggesting that leaching is not a most likely route of dissipation in these dissipation studies. Consistent with the laboratory studies, glyphosate showed a clear non-linear degradation pattern in all soil dissipation studies. The DT_{50} values (persistence endpoints) for glyphosate dissipation were obtained from 18 out of 21 trials, with sufficient data to describe the decline of glyphosate and representative of the various soil types and climatic conditions in Europe and USA. They ranged from 2.3 to 40.9 days with the exception of only one site in Iowa, USA, for which the DT_{50} was 143.3 days. The corresponding DT_{90} values ranged from 22.6 to 706.6 days, but were typically less than one year (15 out of 18 trials) with a median DT_{90} value of 113 days across all 18 locations. Due to pronounced and continuous non-linear degradation of glyphosate to AMPA in soil, only nine out of 21 soil dissipation locations contain sufficient data to adequately describe the pattern of decline of AMPA in these studies. DT_{50} values of AMPA from these studies ranged from 48.5 to 514.9 days, but were typically less than one year (7 out of 9 trials).

The **adsorption/desorption characteristics of glyphosate** were investigated in four studies, representing 14 different soils and a wide range of soil characteristics. The pH ranged from 5.2-8.4; the soil organic carbon ranged from 0.29-3% and the cation exchange capacity ranged from 1.8-28.3 meq/100g. Three of the studies followed OECD 106 guidance, one study followed US EPA guidance (US EPA section 163-1).

The K_f / K_{foc} and 1/n values for glyphosate were all derived from Freundlich Isotherms. The Kf values ranged from 9.4 to 897 mL/g (arithmetic mean: 259 mL/g). The Koc values ranged from 1600 to 60000 mL/g (arithmetic mean: 16810 ml/g). The soil parameters such as the pH, % organic carbon, % clay, or cation-exchange capacities (CEC) showed minimal effect upon glyphosate adsorption to soils. **The results of all studies show that glyphosate has a high adsorption and therefore a low potential for leaching in soil.**

The **adsorption/desorption characteristics of AMPA** were also investigated in four studies, representing 17 different soils and a range of soil characteristics. The pH ranged from 4.6-8.4; the soil organic carbon ranged from 0.29-2.6% and the cation exchange capacity ranged from 1.8-32.8 meq/100g. All studies followed OECD 106 guidance.

The K_f and K_{foc} values for AMPA ranged from 10 to 509 mL/g (arithmetic mean: 112 mL/g) and 1119 to 45900 mL/g (arithmetic mean: 9749 mL/g), respectively. As for glyphosate, the major soil physicochemical parameters such as the OC, pH, % clay, or cation-exchange capacities (CEC) show minimal effect upon AMPA adsorption to soils. **The results of all studies show that AMPA has a high adsorption and therefore a low potential for leaching in soil.**

The low propensity of glyphosate and AMPA for **leaching** was confirmed in three column leaching studies with glyphosate and an aged column leaching study with glyphosate and AMPA. In addition predicted environmental concentrations in groundwater were calculated for glyphosate and its major soil metabolite AMPA for a range of uses in various crops in the EU following the latest modelling guidance by FOCUS. The exposure assessment was based on a representative use pattern derived from the representative GAP. Depending on the crop, two- or three-consecutive applications (respective intervals as defined in the representative GAP) at rates ranging from 720 to 2880 g glyphosate acid/ha were evaluated. In order to cover a wide range of uses, the representative FOCUS crop-scenarios were chosen so as to ensure that all FOCUS groundwater scenarios are considered for representative uses chosen for modelling. The assessment was performed using the leaching models FOCUS PEARL 4.4.4 and FOCUS PELMO 4.4.3.

The predicted environmental concentrations in groundwater (PEC_{gw}) of glyphosate and AMPA were calculated to be < 0.001 µg/L in all scenarios for both models. Therefore, it can be concluded that the use of glyphosate is not likely to pose an unacceptable risk to groundwater if the active substance is used in compliance with the label recommendations.

Although lysimeter studies were not conducted by the glyphosate taskforce companies, three lysimeter studies conducted close to the BBA test guideline from the public literature were presented in order to address the precautionary language regarding risk to groundwater in the inclusion directive 2001/99 EC, as completely as possible. An overview of the conditions of these studies is provided in the table below. Glyphosate was either not detected in the leachate (Stadlbauer et al., 2005; Grundmann et al., 2008) or the mean annual concentrations were significantly below 0.1 µg/L (Fomsgaard et al. 2003). A similar pattern was observed for AMPA.

Table 5.2-1: Lysimeter studies

Authors	Study design	Soil / Crop / Location	Application rate	Precipitation	Sampling
Stadlbauer et al., 2005	2 field plots, soil was saturated with water before application	Quaternary substrates of the "Mur" valley, Austria (spatially heterogeneous) Crops: maize monoculture or crop rotation Steiermark, Austria	3.872 L Roundup/ha in 242 L water (one application)[1)]	2002: average annual precipitation (dry spring and autumn; high rainfall in December) 2003: 68% of the mean annual precipitation were observed (650 mm)	Leaching (suction caps and leaching collectors in 0.8 and 1.05 m depths) and mixed soil samples (0-90 cm depth, taken up to three month after application) Duration: about 2 years
	2 lysimeters, soil was saturated with water before application		8.8 and 10.08 L of Roundup solution (2 applications, see above)		Leachate and monolith lysimeters (down to 3 m depth) Duration: about 2 years
Grundmann et al., 2008	2 lysimeters which allowed the trapping of gaseous ^{14}C losses using soil and plant chambers (surface: 1 m², depth: 2 m)	Sandy soil Crop: transgenic soybeans Germany	1 kg glyphosate / ha, (3 applications), ^{14}C labelled glyphosate was mixed with Roundup	Precipitation was not reported	Leachate and soil cores Duration: about 15 months
Fomsgaard et al., 2003	2 lysimeters of a low tillage field (surface: 0.5 m², depth 1.1 m)	Sandy loam soil, not ploughed in the last 20 – 30 years Crops: spring barley and winter wheat Denmark	0.8 kg glyphosate / ha, (one application), ^{14}C-labelled and unlabelled glyphosate (Roundup 2000) were mixed	Precipitation was measured but not reported, Mean volume of leachate: 260 L	Leaching, combustion and extraction of soil samples Duration: about 2 years
	2 lysimeters of normal tillage field (surface: 0.5 m², depth 1.1 m)	Sandy loam soil, ploughed according to normal treatment Crops: spring barley and winter wheat Denmark	0.8 kg glyphosate / ha, (one application), unlabelled glyphosate (Roundup 2000)	Precipitation was measured but not reported, Mean volume of leachate: 375 L	Leaching and extraction of soil samples Duration: about 2 years

[1)] The concentration of glyphosate in Roundup was not reported.

5.3 Fate and behaviour in water

Studies show that glyphosate is stable to **hydrolytic degradation** in sterile water at pH 5, 7, and 9, and that chemical decomposition does not contribute to the degradation of glyphosate in water. No significant degradation products have been found in these studies. Therefore, no hydrolysis study for AMPA was conducted. Because of chemical structure similarity of glyphosate and AMPA and the general observation of the stability of AMPA in highly alkaline (*e.g.* 0.1 N NH_4OH solvent commonly used to extract glyphosate and AMPA from soil) and acidic aqueous

solutions (*e.g.* 6 N HCl elution solvent in AMPA crop method DFG 405), AMPA also could be characterized as stable toward hydrolysis.

In contrast to the lack of hydrolytic degradation in sterile water, glyphosate is rapidly degraded in water/sediment systems. Four **water sediment studies on glyphosate** were available from Glyphosate Taskforce member companies. These studies covered eight different sediment types and a range of water sediment ratio's and sediment characteristics. Sediment pH ranged from 6.6-8.1 and sediment organic carbon ranged from 0.11-7.2%. Three studies followed the BBA-guidance (part IV, 5-1 (1990)), one study was conducted according to Dutch guidance. Study duration ranged from 13 weeks to 120 days.

The results of these water/sediment studies show that, in addition to microbial degradation, a major contributor to the aquatic dissipation of glyphosate is adsorption to the sediment. They also demonstrated that from approximately 6 to 48% of the applied glyphosate is mineralized to carbon dioxide during 100 or 120 days of incubation. The principal metabolite of glyphosate in water/sediment system is AMPA. The maximum amount of AMPA detected ranged from 2 to 16% (water phase) and up to 27% (total system) of the total glyphosate applied. Hydroxymethyl-phosphonic acid (HMPA) was detected only in the water phase of one of the studies with maximum amount of about 10% of the dose after 61 days. Persistence endpoints for glyphosate ranged from 8.5 to 210.7 days (total system), 1.0 to 12.0 days (water phase), and 34.1 to 146.3 days (sediment phase). Modelling endpoints for glyphosate ranged from 13.8 to 329.9 days (total system), 6.8 to 21.8 days (water phase), and 34.1 to 303.3 days (sediment phase).

The water/sediment studies that were independently conducted with AMPA, when applied as test item, showed a very similar behaviour in water/sediments systems as glyphosate. Four additional **water sediment studies on AMPA** were available. These studies covered also eight sediment types, and a range of water sediment ratio's and sediment characteristics. Sediment pH ranged from 6.3-7.9 and sediment organic carbon ranged from 0.52-4.2%. Three studies followed SETAC guidance (part 1 8.2 (1995)) and one study was conducted according to the BBA-guidance (part IV, 5-1 (1990)). Study duration ranged from 100 to 119 days. These studies along with the results from the glyphosate water/sediment studies demonstrated that AMPA quickly dissipates from the water phase by both adsorption to the sediment and by degradation by the sediment micro-flora. The results showed that from 8 to 40% of the applied AMPA is mineralized to carbon dioxide. In one study other minor metabolites were detected in the water/sediment system. The major metabolite of AMPA which was found mainly in sediments was identified as 1-oxo AMPA (8.8–22.9% of the applied AMPA in both systems respectively). Another transient degradation product was detected mainly in the water phases of both aquatic systems (1.8-9.8% of applied AMPA) and was characterised as an acid labile compound in the study report. This metabolite was not identified in the study report but based on ready mineralization of AMPA to CO_2, one logical metabolic transformation expected is further oxidation of the carbon of AMPA and/or 1-oxo AMPA to form phosphonoformic acid. Phosphonoformic acid has been reported in the literature to be acid labile and readily degrades to carbon dioxide and phosphoric acid under mild acidic conditions.

The degradation and dissipation half-lives of glyphosate and AMPA were calculated in accordance with the kinetic approaches recommended in the latest guidance (FOCUS, 2006).

For FOCUS surface water modelling the geometric mean $DegT_{50,total\ system}$ of 61.2 days for glyphosate and of 83.7 days for AMPA are considered to be acceptable as half-lives for the water phase in combination with a conservative default $DegT_{50}$ of 1000 days for sediment .

The predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) were calculated for a number of glyphosate uses on various crops in the EU reflecting the representative GAP using the current versions of FOCUS STEPS 1-2 (version 2.1) for Step 1 and 2 and FOCUS SWASH (version 3.1) for Step 3. Step 3 calculations were carried out to provide more realistic estimates of the PEC_{sw} and PEC_{sed} for glyphosate only.

Depending on the crop and simulation model used, single and multiple applications at rates up to 4320 g glyphosate acid/ha were considered in Steps 1 to 3. In order to cover a wide range of uses, representative FOCUS crop scenarios were chosen. Several application scenarios were considered for the following representative crops: winter and spring cereals, potatoes and pome/stone fruit. Both single and multiple application scenarios representative for all intended uses were taken into account. **The overall maximum PEC_{sw} value of glyphosate at Steps 1, 2 and 3 was 101.2, 39.0 and 17.7 µg/L, respectively. The overall maximum PEC_{sw} value of AMPA at Step 1 and 2 was 41.0 and 16.9 µg/L.**

As stated before, HMPA was detected only in the water phase of one of the four available glyphosate water/sediment studies with maximum amount of about 10% of the dose after 61 days. However, the pattern of decline of HMPA was not clearly established within the experimental period of the study to allow a kinetic evaluation of HMPA. Therefore, the aquatic risk of HMPA was assessed based on molecular mass correction of the Step 1 and Step 2 results of glyphosate in surface water assuming a maximum occurrence of 10%. **The overall maximum PEC_{sw} value of HMPA at Step 1 and 2 was therefore estimated to be 6.7 and 2.6 µg/L.**

No risk assessment was conducted for 1-oxo AMPA or phosphonoformic acid since these transient degradation product were only seen in an AMPA water/sediment study and were never detected in any of the four available glyphosate water/sediment studies. In addition the maximum amount of AMPA found in the sediment phase (where 1-oxo-AMPA was mainly found) was less than 27%. Taking into account that 1-oxo-AMPA was only found at 23% of the applied AMPA in a water/sediment system, this metabolite does not qualify as a major aquatic metabolite of parent glyphosate anyway. In addition 1-oxo AMPA and phosphonoformic acid are considered logical and labile metabolic transformation products, expected in the pathway of mineralization of AMPA to CO_2. Due to minor changes in the molecular structures of AMPA, 1-oxo AMPA, phosphonoformic acid, and HMPA, from a structure activity relationship perspective, the environmental fate and behaviour and the aquatic risk assessments of these transitory degradation products of AMPA should be very similar to AMPA and HMPA.

5.4 Fate and behaviour in air

Glyphosate has low vapour pressure (1.31×10^{-5} Pa at 25°C) and significant concentrations are not expected to be found in air through volatilization. Two laboratory studies and one field study assessing the volatilization of glyphosate from soil and plant surfaces, reviewed during the 2001 EU review of glyphosate concluded that glyphosate can be classified as not volatile substance based on its Henry's law constant and on volatilization experiments from soil and plants with no significant rates. These conclusions were confirmed by two additional 24h-studies (one lab study at 20°C and one field study) conducted according to BBA guidance (Part IV 6-1 (1990)). In the unlikely events that glyphosate is present in air, direct photolysis in air will not occur (due to the lack of significant UV absorbance) but rapid photochemical oxidative degradation is expected (tropospheric half-life 1.6 h).

6. Effects on non-target species

Ecotoxicological studies described in this summary address data requirements specified in Commission Regulation 1107/2009 of 21 October 2009 amending Council Directive 91/414/EEC. Experimental details of ecotoxicological studies done with the formulated product MON 52276 that also satisfy data requirements specified in Annex IIA, Point 8, Ecotoxicological Studies were included in Document M-II; only the conclusions of the risk assessment have been reported here in summary form.

Based on the results of the current ecological risk assessment, it has been demonstrated that the supported uses of MON 52276 do not cause unacceptable effects on any of the species tested (birds, mammals, aquatics, bees and other arthropods, soil macro- and micro fauna and non-target plants). The following section summarizes the ecological risk assessment and conclusions for all taxa evaluated.

6.1 Effects on terrestrial vertebrates

An extensive regulatory bird and mammal toxicology database is available to assess acute and long-term effects of glyphosate, AMPA and the glyphosate-based formulation MON 52276 to terrestrial vertebrates. The bird and wild mammal risk assessments were carried out according to the current EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). This guidance utilizes a tiered assessment approach to assess the effects of plant protection products based on the requirements of Regulation 544/2011 and Regulation 545/2011 for active substances and plant protection products, respectively. Detailed descriptions of studies with birds and mammals are given under points 5 and 6, respectively, in the Annex II dossier of glyphosate. A list of the endpoints used in the ecological risk assessment is provided in Table 6.1-1.

Table 6.1-1: List of endpoints used in to assess effects on terrestrial vertebrates

Acute toxicity to mammals	LD_{50} >8000 mg a.s./kg bw
Acute toxicity to birds	LD_{50} = 4334 mg a.s./kg bw (based on extrapolation factor of 2.167)
Dietary toxicity to birds	LC_{50} > 5620 mg a.s./kg food
Reproductive toxicity to birds	NOEC = 201 mg a.s./kg bw/day
Reproductive toxicity to mammals:	NOEAEL = 2150 mg a.s./kg bw/day

[1] According to the current EFSA Guidance Document an extrapolation factor of 2.167 may be applied when at least 20 individual birds are tested at a limit dose.

The exposure of birds and mammals to glyphosate acid was estimated following application of MON 52276 on a field containing annual weeds with two scenarios:

1. A maximum single application rate of 2.16 kg a.s./ha that includes pre-planting, pre-emergence of crops and pre-harvest applications.

2. A maximum single application rate for orchards of 0.96 kg a.s./ha. As a worst case it is assumed that 1/3 of the area of an individual orchards or vineyard is treated, giving an overall application rate of 2.88 kg a.s./ha / 3 = 0.96 kg a.s./ha.

A repeat application of MON 52276 is only made to control new growth of weeds which would not have been exposed to the preceding application. Therefore, it is not appropriate to use a multiple application factor (MAF) for foliar residues in the case of this total herbicide.

Quantitative bird and mammal risk assessments for AMPA were not conducted because of AMPA's low toxicity profile and because of the low acute and chronic dietary field exposure levels. Low levels of AMPA have been measured in forage crop residue studies and numerous plant metabolism studies. Measured AMPA levels were <10% of the total radioactive residue in crop residue studies. Low exposure levels would also be predicted for prey items (e.g., insects) and similar to glyphosate, given a Log Pow < 3, AMPA does not possess bioaccumulation potential. Based on the combination of low exposure and low hazard, it can be concluded that ecological risk to birds and mammals under field exposure conditions will be low and a quantitative assessment is not required.

Acute toxicity to birds

Glyphosate acid and relevant glyphosate salts (as demonstrated by glyphosate IPA and K salts) have low acute oral toxicity to birds and no mortality was observed in the limit dose studies. Therefore, the acute endpoint for birds exposed to glyphosate acid was determined by extrapolation of the LD_{50} of >2000 mg a.s./kg bw. According to the current EFSA Guidance Document on Risk Assessment for Birds and Mammals an extrapolation factor of 2.167 may be applied when at least 20 individual birds are tested at a limit dose. Considering a limit dose of 2000 mg a.s./kg bw, and the fact that 50 birds were tested for glyphosate acid, an extrapolated LD_{50} value of 4334 mg a.s./kg bw was calculated.

No acute oral toxicity studies were conducted with MON 52276 since the active ingredient glyphosate shows low acute toxicity to birds. Additionally, glyphosate and MON 52276 have low acute toxicity in rat gavage studies with acute LD_{50} values ≥5000 mg/kg. Further, MON 52276 is applied as a spray and, accordingly, residues on food sources are better considered in terms of the individual active ingredients rather than the formulation[10].

Because of the lack of inherent acute oral toxicity of glyphosate and MON 52276 to birds, going beyond a screening level assessment (Table 6.1-2) was not required. The TER_A values for all MON 52276 application scenarios are greater than the relevant trigger of 10, indicating low acute risk to birds from glyphosate acid. For all limit tests on glyphosate and glyphosate salts, no effect were observed at the highest concentration tested, which provides as additional margin of safety.

Short-term and Long-term toxicity to birds

Derivation of the short-term toxicity exposure ratio is no longer a requirement according to *EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)*. Thus, no short-term risk assessment is presented. However, dietary LC_{50} values for glyphosate acid, glyphosate salts and MON 52276 demonstrate low short-term toxicity with all LC_{50} values >4640 mg/kg feed, achieving a no-mortality concentration at the highest concentration tested.

[10] European Commission, Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO 2021/VI/98, draft of June 2002.

In total, four one-generation avian reproduction studies have been performed. The later of these two studies, a mallard and a bobwhite reproduction studies, were not evaluated in the EU 2001 glyphosate evaluation. As it is anticipated that a formulation does not stay intact in the environment, evaluation of toxicity is based on the assessment of effects of the active substance. In the Glyphosate Monograph, the NOEC for the original bobwhite quail study [redacted] 1978) was concluded to be 200 mg/kg diet, rather than 1000 mg/kg feed, based on a statistically significant finding that was concluded not to be biologically significant by the study director. Although there was a small reduction in egg weight at 1000 mg/kg feed there was no significant impact on the biologically relevant endpoints of initial hatchling body weight, 14 day hatchling body weight, egg shell thickness and hatchling survival. Egg weight is not a standard endpoint in guideline avian reproduction studies and was a carryover from poultry performance studies. Consequently, the NOAEC concentration for this older glyphosate quail study ([redacted] 1978) was concluded by the Study Director to be 1000 mg a.e./kg diet.

Since no adverse effects were observed at the highest concentrations tested in the reproduction studies, the highest NOEC value (NOEC = 201 mg a.e./kg bw/day for bobwhite quail) has been used for the long-term risk assessment. This is supported by the current guidance document on Risk Assessment for Birds and Mammals (EFSA 2009), stating that in the case that more than one reproduction study was conducted on the same species, it is possible to merge the data sets as if it were one study. The corresponding study [redacted] 1999) was not reviewed in the glyphosate monograph; however, it has been widely used to support member state re-registrations and is summarised in Section IIA 8.1.4**Fehler! Verweisquelle konnte nicht gefunden werden.**. These two new one generation reproduction studies confirm that glyphosate does not pose unacceptable long-term risk to birds.

As elaborated in Annex 3 document, exposure of terrestrial vertebrates has been well characterized and there are several factors that limit long-term exposure. For example, glyphosate has been shown to rapidly dissipate on foliage and insects, does not bioaccumulate and treated vegetation will rapidly wilt and become unpalatable to herbivores.

Table 6.1-2 summarizes the long-term Tier 1 avian assessment using default DT_{50} values for insects and foliage. An additional margin of safety can be built into the assessment using the empirically derived DT_{50} values and time-weighted averages for foliage and insect residues. All TER_{LT} values are greater than the Annex VI trigger of 5. Therefore, no unacceptable long-term effects are to be expected from application of MON 52276 according to the proposed representative GAP.

Acute toxicity to terrestrial vertebrates other than birds

Because of low acute oral toxicity of glyphosate and MON 52276 to mammals, only a screening level assessment was required. Although acute oral toxicity studies were conducted with MON 52276, a LD_{50} value from a study conducted with glyphosate acid has been used in the acute assessment. The reason for this is because both glyphosate and MON 52276 have low acute toxicity in rat gavage studies, with acute LD_{50} values $\geq$5000 mg/kg, and since MON 52276 is applied as a spray, residues on food sources are better considered in terms of the individual active ingredients rather than the formulation[11].

[11] European Commission, Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO 2021/VI/98, draft of June 2002.

As shown in Table 6.1-2, all TER_A values for application of MON 52276 are greater than the relevant trigger of 10, indicating low acute risk to mammals from glyphosate acid.

Long-term toxicity to terrestrial vertebrates other than birds

There is no agreed EU endpoint for long term toxicity in mammals. In line with the acute oral toxicity of glyphosate acid to mammals, a substantial number of reproductive studies are available. Considering that in none of the studies available an ecological relevant effect was observed, an endpoint is proposed based on the multigenerational rat reproduction study by [redacted] 1997 (see IIA 5.6.1/03). For this ecotoxicological risk assessment the highest rate in the study (30000 mg/kg feed (2150 - 2532 mg/kg bw/d; male-female)), is used as the NOEAEL based on ecologically significant effects. For none of the studies available (see IIA 5.6), continuous feeding exhibited any effect on the propagation of two to three generations in the rat. The fertility and reproductive performance of each generation of parental animals and the clinical condition and survival of their offspring were not adversely affected. The only effect of treatment observed on four out of nine studies was a slight reduction in the bodyweight of the F1A pups at concentrations of 10000 to 30000 mg/kg feed in the glyphosate acid group with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the pre-mating period. This is not considered to be ecologically relevant.

Therefore, for glyphosate the lowest NOEAEL value of 2150 mg/kg bw/d is used for this risk assessment. Mammalian toxicological studies have shown that there is no evidence for cumulative glyphosate toxicity and that it is not genotoxic, oncogenic, teratogenic or a reprotoxin. Thus acute oral dosing and the reproduction study are considered to be appropriate for this risk assessment.

The mammalian toxicity endpoints for glyphosate that are most appropriate for acute and long-term ecological risk assessment are summarised in Table 10.3-1 of Annex IIIA, Section 6, Point 10. Further details can be found in the corresponding EU dossier for glyphosate, Annex IIA, Section 3, Point 5.

As an outcome of the low long-term oral toxicity of glyphosate and MON 52276 to mammals, only a screening level assessment, as presented in Table 6.1-2 was required. The TER_{LT} values for application of MON 52276 are greater than the relevant trigger of 5, indicating low long term risk to mammals from glyphosate acid. Although the assessment passed at the screening level it should be pointed out that an additional margin of safety exists because worst-case exposure assumptions were used in this assessment. As discussed under the long-term avian assessment, glyphosate has been shown to rapidly dissipate on foliage and insects and treated vegetation with rapidly wilt and be unpalatable.

Table 6.1-2: Toxicity/exposure ratios for terrestrial vertebrates

Application rate [kg a.s./ha]	Crop	Step	Indicator species	Time scale	TER	Annex VI Trigger
2.16	Annual weeds	Screening	Small granivorous bird	Acute	**>79.3**	10
		Tier 1	Small granivorous bird	Long-term	**15.4**	5
		Screening	small granivorous mammal	Acute	**>257**	10
		Screening	small granivorous mammal	Long-term	**285**	5
2.16	Cereals	Screening	Small omnivorous bird	Acute	**>12.6**	10
		Tier 1	Small insectivorous bird "passerine"	Long-term	**7.8**	5
			Small omnivorous bird "lark"	Long-term	**53.2**	
			Small granivorous/ insectivorous bird "bunting"	Long-term	**14.0**	
		Screening	small herbivorous mammal	Acute	**>31.3**	10
		Screening	small herbivorous mammal	Long-term	**38.9**	5
2.16	Oilseed rape	Screening	Small omnivorous bird	Acute	**>12.6**	10
		Tier 1	Small insectivorous bird "dunnock"	Long-term	**65.0**	5
			Small granivorous bird" finch"	Long-term	**15.4**	
			Small omnivorous bird "lark"	Long-term	**65.0**	
			Medium herbivorous/ granivorous bird "pigeon"	Long-term	**195**	
		Screening	small herbivorous mammal	Acute	**>31.3**	10
		Screening	small herbivorous mammal	Long-term	**38.9**	5
0.96	Orchards	Screening	Small insectivorous bird	Acute	**>96.5**	10
		Screening	Small insectivorous bird	Long-term	**21.7**	5
		Screening	small herbivorous mammal	Acute	**>61.1**	10
		Screening	small herbivorous mammal	Long-term	**58.4**	5
0.96	Vines	Screening	Small herbivorous bird	Acute	**>47.4**	10
		Screening	Small herbivorous bird	Long-term	**10.2**	5
		Screening	small herbivorous mammal	Acute	**>61.1**	10
		Screening	small herbivorous mammal	Long-term	**58.4**	5

6.2 Effects on aquatic species

The acute and chronic toxicity of glyphosate as the acid, the isopropylamine (IPA) salt, the potassium (K)-salt and its metabolites AMPA and HMPA to aquatic organisms was investigated in a series of laboratory studies with representative species from different trophic levels of the aquatic food chain, namely fish, aquatic invertebrates, algae and aquatic plants.

The representative joint GAP does not include direct applications to surface water. The main routes of exposure to aquatic organisms considered in this aquatic risk assessment are *via* spray drift, runoff and drainage where exposure to aquatic organisms could result as a consequence of the accidental entry of the compound into the environmental compartments occupied by organisms or as a consequence of run-off and drainage events.

Further details of how the predicted environmental concentrations for glyphosate acid in surface water (PEC_{sw}) are calculated, arising as a consequence of over-spraying, drift, drainage and run-off, are provided in MON 52276 Point IIIA 9.7. Table 6.2-1 outlines a brief summary of the FOCUS step 1 PECSW values for glyphosate acid and its metabolites following application of 4.32 kg glyphosate acid/ha (12 L MON 52276/ha).

Table 6.2-1: FOCUS step 1 PEC_{SW} values for glyphosate acid and its metabolites following application of 4.32 kg MON 52276/ha (equivalent to 12 L MON 52276/ha)

Test substance	FOCUS Step 1 Max PEC_{SW} [µg a.s./L]	PEC_{SED} [µg/kg]
Glyphosate acid	101.233	10500
AMPA	40.978	3320
HMPA	6.710	696

The FOCUS Step 1 PEC_{sw} values for glyphosate acid resulted in acceptable TER values for all aquatic species. The PEC_{sw} for all aquatic metabolites generated TER values exceeding the Annex VI trigger values for at least a factor of 10 for all aquatic species at Step 1. Therefore, no further surface water exposure assessment was performed for these metabolites.

Summary and full details of the tests on the active substance and the metabolites AMPA and HMPA, a major aquatic metabolite of glyphosate, are provided in the current EU Annex II summary documentation, Section IIA 8.2. Therefore, only studies representing the worst case for key species are presented in Table 6.2-2.

Table 6.2-2: Summary of the risk assessment for Glyphosate acid and its metabolites AMPA and HMPA exposure to aquatic species (assessment based most sensitive species of each group)

Use pattern*	Organism	Toxicity endpoint	FOCUS step	Max PEC_{SW} [µg/L]	TER	Risk assessment trigger
Glyphosate acid						
1 × 4320 g a.s./ha	Rainbow trout *(Oncorhynchus mykiss)*	96 hr LC_{50} = 38000 µg a.s./L	1	101.233	375	100
1 × 4320 g a.s./ha	Fathead minnow *(Pimephales promelas)*	255 d FFLC NOEC = 25700 µg a.s./L	1	101.233	254	10
1 × 4320 g a.s./ha	Rainbow trout *(Oncorhynchus mykiss)*	85 d ELS NOEC = 9630 µg a.s./L	1	101.233	95.1	10
1 × 4320 g a.s./ha	Zebra fish (*Danio rerio)*	168 hr NOEC = 3200 µg a.s./L	1	101.233	31.6	10

Use pattern*	Organism	Toxicity endpoint	FOCUS step	Max PEC_{SW} [µg/L]	TER	Risk assessment trigger
1 × 4320 g a.s./ha	*Daphnia magna*	48 hr EC_{50} = 40000 µg a.s./L	1	101.233	395	100
1 × 4320 g a.s./ha	*Daphnia magna*	21 d NOEC = 30000 µg a.s./L	1	101.233	296	10
1 × 4320 g a.s./ha	*Nitzschia palea*	96 hr E_bC_{50} = 4470 µg a.s./L	1	101.233	44.2	10
1 × 4320 g a.s./ha	*Skeletonema costatum*	72 hr E_bC_{50} = 11000 µg a.s./L	1	101.233	108.7	10
1 × 4320 g a.s./ha	Common duckweed (*Lemna gibba*)	14 d $EC_{50,\ frond\ count}$ = 12000 µg a.s./L	1	101.233	119	10
1 × 4320 g a.s./ha	*Myriophyllum aquaticum*	14 d EC_{50} = 12300 µg a.s./L	1	101.233	122	10
MON 52276						
1 × 4320 g a.s./ha	Common carp *(Cyprinus carpio)*	96 hr LC_{50} > 277000 µg a.s./L	1	101.233	> 2736	100
1 × 4320 g a.s./ha	*Daphnia magna*	48 hr EC_{50} = 209000 µg a.s./L	1	101.233	2065	100
1 × 4320 g a.s./ha	*Pseudokirchneriella subcapitata*	72 hr E_bC_{50} = 55000 µg a.s./L	1	101.233	543	10
1 × 4320 g a.s./ha	Common duckweed (*Lemna gibba*)	7 d $EC_{50,\ frond\ count}$ = 20570 µg a.s./L	1	101.233	203	10
1 × 4320 g a.s./ha	*Myriophyllum aquaticum*	14 d EC_{50} = 4440 µg a.s./L	1	101.233	43.9	10

Use pattern*	Organism	Toxicity endpoint	FOCUS step	Max PEC_{SW} [µg/L]	TER	Risk assessment trigger
AMPA						
1 × 4320 g a.s./ha	Rainbow trout *(Oncorhynchus mykiss)*	96 hr LC_{50} >100000 µg a.s./L	1	40.978	>2440	100
1 × 4320 g a.s./ha	Fathead minnow *(Pimephales promelas)*	28 d ELS NOEC = 12000 µg a.s./L	1	40.978	293	10
1 × 4320 g a.s./ha	*Daphnia magna*	48 hr EC_{50} = 690000 µg a.s./L	1	40.978	16839	100
1 × 4320 g a.s./ha	*Daphnia magna*	21 d NOEC = 15000 µg a.s./L	1	40.978	366	10
1 × 4320 g a.s./ha	*Desmodesmus subspicatus*	72 hr E_bC_{50} = 89800 µg a.s./L	1	40.978	2191	10
1 × 4320 g a.s./ha	*Myriophyllum aquaticum*	14 d EC_{50} = 70800 µg/L	1	40.978	1728	10
HMPA						
1 × 4320 g a.s./ha	*Daphnia magna*	48 hr EC_{50} > 100000 µg a.s./L	1	6.710	>14903	100
1 × 4320 g a.s./ha	*Pseudokirchneriella subcapitata*	72 hr E_rC_{50} > 115000 µg a.s./L	1	6.710	>17139	10
1 × 4320 g a.s./ha	Common duckweed (*Lemna gibba*)	7 d $EC_{50,\ frond\ count}$ >123000 µg/L	1	6.710	>18331	10

* The assumed use pattern represents an absolute worst case since the maximum cumulative yearly application rate is 4.32 kg a.s/ha but the relevant maximum single application rate is only 2.16 kg a.s./ha

The TER values calculated using worst-case PEC_{SW} values (FOCUS Step 1) for glyphosate acid and its metabolites AMPA and HMPA (see Section 5 for full calculations) exceed the Annex VI trigger values, indicating that the risk to aquatic organisms is acceptable following use of MON 52276.

Bioconcentration

A fish-bioconcentration study is not required, due to the low Log P_{OW}, which is below the trigger value of 3 (Log_{POW} = -3.2). However, a fish bioconcentration study has been conducted which achieved a bioconcentration factor of 1.1± 0.61, which is far below the Annex VI BCF trigger value of 1000. Therefore, a study is not necessary to determine bioaccumulation in aquatic non-target organisms.

Bioconcentration factor (BCF)		BCF = 1.1 ± 0.61; steady state after 120 ± 59 d log P_{ow} of glyphosate acid and its metabolites was < 3, accumulation potential in aquatic non-target organisms is hence considered to be low
Annex VI Trigger for the bioconcentration factor		100
Clearance time	CT_{50}	Not relevant
	CT_{90}	
Level of residues (%) in organisms after the 14-day depuration phase		Not relevant

6.3 Effect on bees and other arthropod species

Extensive acute and recent chronic testing bee has been performed with glyphosate, salts of glyphosate and MON 52276 in support of the ecological risk assessment for MON 52276. This section is divided into separate subsections that evaluate acute and chronic effects to bees and a third section that evaluates effects on other arthropods species.

Acute risk to bees

The acute risk to honeybees from use of MON 52276 was assessed using the maximum single application rate and the LD_{50} values to calculate hazard quotients (***EPPO 2010***) [i] as follows:

$$\text{Hazard Quotient} = \frac{\text{Maximum application rate (g a.s./ha)}}{\text{Acute LD}_{50}\ (\mu\text{g a.s./bee})}$$

Hazard quotients were calculated for oral exposure (Q_{HO}) and contact exposure (Q_{HC}) to MON 52276 with the highest individually applied dose of 2880 g a.s./ha and the toxicity endpoint of the formulation. A hazard quotient of less than 50 indicates a low risk to bees in the field. The results are shown Table 6.3-2. Hazard quotients for studies performed with glyphosate acid are also very low because oral and contact LD_{50} values >200 µg a.s./bee. A full summary of the acute bee effects data is summarized in section IIA.

Table 6.3-1: Acute risk to bees from exposure to MON 52276

Substance	Application rate [g a.s./ha]	LD_{50} [µg a.s./bee]	Hazard quotient
MON 52276	2880	Contact > 100	<28.8
		Oral > 77	<37.4

Both hazard quotients are less than 50, indicating that the active substances pose a low risk to bees. Therefore a low risk to bees is expected from the application of MON 52276.

Chronic risk to honeybees

The potential effect of glyphosate on the development of honey bee brood was determined in a bee brood feeding study performed in the field and in which colonies were exposed to glyphosate by feeding colonies treated sucrose solution. Conservative exposure doses for glyphosate were based on measured residues that were determined in a glasshouse residue study following application of 8 L MON 52276/ha, equivalent to 2.88 kg a.s./ha onto flowering *Phacelia tanacetifolia* and considering food requirements of bee colonies. Exposure estimates are regarded as conservative and worst case for foraging bees, since information was derived from an enclosed greenhouse and the bees could only forage on highly attractive treated *P. tanacetifolia* flowers. Residue findings were adjusted to the spray application rate of 2.16 kg a.s./ha because this rate is the maximum single application rate in field crops and cereals. The rate of 2.88 kg a.s./ha is reserved for spot treatments in orchards along the base of trees and does not represent a worst-case exposure. Three dose levels were tested in the bee brood study. The lowest dose was based on the mean pollen and nectar residue concentrations measured over the first three days following the spray application (75 mg glyphosate a.e./L) the mid-dose was based on the highest residue concentrations measured in pollen and nectar following the spray application (150 mg glyphosate a.e./L) and the highest dose was twice this latter dose (301 mg glyphosate a.e./L).

The chronic risk to honeybee colonies from use of MON 52276 was assessed by comparing the NOAEL determined in the bee brood feeding study with colony intake over the exposure period to calculate toxicity exposure ratios (***EPPO 2010***) [i] as follows:

$$\mathrm{T}ER = \frac{\text{NOAEL (mg a.s./kg food)}}{\text{Intake } (mg\ a.s./colony)}$$

Toxicity exposure ratios were calculated for the potential exposure of bee colonies foraging on MON 52276 treated crops and the toxicity endpoint of the bee brood feeding test. A TER of more than 1 indicates a low risk to bees in the field (EPPO 2010). The results are shown below.

As already described, the lowest dose was based on the mean pollen and nectar residue concentrations measured over the first three days following the spray application (75 mg glyphosate a.s./L), the mid-dose was based on the highest residue concentrations measured in pollen and nectar following the spray application (150 mg glyphosate a.s./L) and the highest dose was twice this latter dose (301 mg glyphosate a.s./L).

The risk assessment outlined in Table 6.3-2, is based on scenario 1 that represents the mean exposure levels from the worst-case green-house study with treated *Phacelia*.

Table 6.3-2: Chronic risk to honeybee colonies from exposure to glyphosate

Substance	NOAEL [mg a.s./kg food]	Glyphosate intake/colony [mg a.s.]	TER_{LT}
Glyphosate	266	75	3.5

The resulting TER_{LT} value for application of MON 52276 is greater than the relevant trigger of 1, indicating low risk to honeybee brood development from exposure to glyphosate.

No adverse toxicological or behavioural effects on adult bees or bee brood development were observed in any of the glyphosate treated colonies. The NOAEL (No Observed Adverse Effect Level) for brood development was the highest dose tested - 301 mg glyphosate a.s./L nominal (245 mg glyphosate a.s./kg nominal; 266 mg glyphosate a.s./kg actual measured), indicating low chronic risk to honeybee colonies from the application of MON 52276 according to the proposed GAP.

Other Arthropod species

To assess the effects of MON 52276 on terrestrial non-target arthropods other than bees, six different arthropod taxa (*Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Chrysoperla carnea*, *Aleochara bilineata*, *Poecilus cupreus*, and *Pardosa spp.*) were exposed to MON 52276. Several of the studies, covering a decade of testing, were performed under the guidance and a risk assessment scheme that preceded ESCORT II (Candolfi *et al.*, 2001). The new tests were chosen according to the recommendations of ESCORT II and represent different ecological groups. The tests cover different levels of exposure from laboratory trials on inert substrate to extended laboratory trials.

Risk assessments were conducted to examine the potential effects of MON 52276 on non-target arthropods following the guidance of ESCORT 2 (2001) [ii]. According to the guidance, a tiered approach is proposed, whereby Tier 1 testing and risk assessments should be carried out using dose-response data for the representative sensitive indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi*.

A summary of the endpoints for the tested arthropod species is provided below in Table 6.4-3.

Table 6.3-3: Effects on arthropod species other than bees

Species	Test substance	Substrate	Stage	Endpoint	Toxicity [g a.s./ha]
Parasitoids					
Aphidius rhopalosiphi	MON 52276	inert	adult	LR_{50}	<3600
Aphidius rhopalosiphi	MON 52276	whole plant	adult	LR_{50}	>4320
Predatory mites					
Typhlodromus pyri	MON 52276	inert	protonymphs	LR_{50}	<3600
Typhlodromus pyri	MON 52276	whole leaf	protonymphs	LR_{50}	>4320
Soil dwelling predators					
Aleochara bilineata	MON 52276	soil	adult	LR_{50}	>4320
Poecilus cupreus	MON 52276	inert	adult	LR_{50}	>3600
Pardosa ssp.	MON 52276	inert	adult	LR_{50}	>3600
Foliage dwelling predators					
Chrysoperla carnea	MON 52276	inert	adult	LR_{50}	>2160

The following equations were used to calculate the in-field and off-field HQs:

$$In\ field\ HQ = \frac{In\text{ - }field\ PER\,(g/ha)}{LR_{50}\ (\ g\ /\ ha\ )}$$ and

$$Off\text{ - }field\ HQ = \frac{PER_{off-field}\ (g/ha)}{LR_{50}\,(g/ha)} \times Correction\ factor$$

Due to the fact that in the two Tier 1 worst-case laboratory studies, no LR/LC_{50} was determined for *Typhlodromus pyri* and *Aphidius rhopalosiphi*, the risk assessment is based on Tier 2 extended laboratory studies. The HQ trigger for Tier 2 extended laboratory studies is 1.

The resulting HQ in-field and off-field values are presented in Table 6.4-4 and Table 6.4-5, respectively.

Table 6.3-4: In-field HQs for non-target arthropods (*T. pyri and A. rhopalosiphi;* Tier 2, 3D exposure scenario) exposed to MON 52276

Crop scenario	LR_{50} [kg a.s./ha]	In-field foliar		In-field soil		Trigger value
		PER [kg a.s./ha]	HQ	PER [kg a.s./ha]	HQ	
All crops	>4.32	2.16	<0.50	4.32	<1.0	1
Cereals, oilseed rape	>4.32	2.16	<0.50	2.16	<0.50	
Orchard crops, vines	>4.32	2.88	<0.67	4.32	<1.0	

Table 6.3-5: Off-field HQs for non-target arthropods (*T. pyri and A. rhopalosiphi;* Tier 2, 3D exposure scenario) exposed to MON 52276

Crop scenario	LR_{50} [kg a.s./ha]	Off-field foliar PER [kg a.s./ha]	Correction factor	Off-field foliar HQ	Trigger value
All crops	>4.32	0.0514	5	<0.101	1
Cereals, oilseed rape	>4.32	0.0598	5	<0.069	
Orchard crops, vines	>4.32	0.0579	5	<0.101	

PER = Predicted environmental rate; HQ = Off-field foliar PER *Correction factor / LR_{50}

For *Aphidius rhopalosiphi* and *Typhlodromus pyri*, the trigger value of HQ ≤ 1 demonstrates that no risk or unacceptable effects are expected from the use of MON 52276 considering in-field or off-field habitats.

In addition to the laboratory and extended laboratory studies conducted with *T. pyri* and *A. rhopalosiphi*, laboratory and extended laboratory studies are available with the foliage-dweller *Chrysoperla carnea* and the soil-dwelling predators *Aleochara bilineata, Poecilus cupreus* and *Pardosa spp*. The additional species tested represent different specific ecological compartments and therefore provide additional information for use in the risk assessment. The results of these additional worst-case laboratory studies indicate that no risk or unacceptable effects on foliage dwelling arthropods will be anticipated assuming in-field and off- field exposure scenarios.

In conclusion, no unacceptable effects on non-target arthropods will be anticipated for both in- and off-field habitats, resulting from the use of MON 52276 according to the proposed use pattern and no additional testing is required for MON 52276.

6.4 Effects on earthworms and other soil macro-organisms

Extensive acute and chronic testing has been performed with glyphosate, the IPA-salt of glyphosate (as representative for the other relevant salt types) and AMPA in support of the ecological risk assessment for MON 52276. This section is divided into three subsections that evaluate acute and chronic effects on earthworms and a final section that evaluates other soil macro-organisms. An overview of the data used in the assessment is summarized below.

Acute toxicity	Glyphosate acid:	LC_{50} = 5600 mg a.s./kg dry soil (*E. fetida*)
	Glyphosate IPA salt:	LC_{50} > 1000 mg gly-IPA/kg dry soil (*E. fetida*)
	MON 52276:	LC_{50} > 1250 mg a.s./kg dry soil (*E. fetida*)
	AMPA:	LC_{50} > 1000 mg/kg dry soil (*E. fetida*)
Reproductive toxicity	Glyphosate IPA salt:	NOEC = 472.8 mg a.s./kg dry soil (*E. fetida*)
	AMPA:	NOEC = 198.1 (*E. fetida*)

Reproductive toxicity to other soil non-target macro-organisms	Glyphosate IPA salt:	NOEC = 587 mg a.s./kg dry soil (*Folsomia candida*)
	Glyphosate IPA salt:	NOEC = 472.8 mg a.s./kg dry soil (*Hypoaspis aculeifer*)
	AMPA:	NOEC = 315 mg/kg dry soil (*Folsomia candida*)
	AMPA:	NOEC = 320 mg/kg dry soil (*Hypoaspis aculeifer*)

Acute toxicity to earthworms

The potential acute risk of MON 52276 and AMPA to earthworms was assessed by comparing the maximum instantaneous PEC_s with the 14-day LC_{50} value to generate acute TER values (Table 6.4-2). The TER_A was calculated as follows and are shown below:

$$TER_a = \frac{LC_{50}\,(mg/kg)}{PEC_S\,(mg/kg)}$$

Table 6.4-1: Acute TER values for earthworms exposed at the maximum application rate of 4320 g a.s./ha

Parent compound	Test substance	LC_{50} [mg a.s./kg dry soil]	Maximum $PEC_{soil,\ plateau}$ [mg/kg dry soil]	TER_A
Glyphosate	Glyphosate acid	5600	8.065	694
	Glyphosate IPA salt	>1000	8.065	>124
	MON 52276	>388	8.065	>48
	AMPA	>1000	5.345	>187

All the acute TER values are much higher than the Annex VI acute trigger value of 10, indicating that MON 52276 poses low acute risk to earthworms when applied according to the proposed use rates.

Chronic toxicity to earthworms

The potential long-term risk of MON 52276 and AMPA to earthworms was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC values and the maximum instantaneous PEC_s using the following equation and are presented below:

$$TER_{LT} = \frac{NOEC(mg/kg)}{PEC_s(mg/kg)}$$

Table 6.4-2: Long-term TER values for earthworms at the maximum application rate of 4320 g a.s./ha

Test substance	NOEC [mg a.s./kg dry soil]	Maximum instantaneous PEC_s [mg/kg dry soil]	TERLT
Glyphosate IPA salt	472.8	8.065	58.6
AMPA	198.1	5.345	37.1

The TER_{LT} values exceed the relevant Annex VI decision-making criteria of 5 for earthworms. Therefore, it can be concluded that chronic risk to earthworms and their ecological functions from the use of MON 52276 in all crops according to the proposed good agricultural practice will be low.

Chronic toxicity to *Hypoaspis* and *Folsomia*:

The potential long-term risk of MON 52276 and AMPA to two additional ecologically important soil macro-organisms was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC values and the maximum instantaneous PEC_s using the following equation and are presented below:

$$TER_{LT} = \frac{NOEC(mg/kg)}{PEC_s(mg/kg)}$$

Table 6.4-3: Toxicity data for soil macro organisms at the maximum application rate of 4320 g a.s./ha

Organism	Proposed toxicity endpoint[1] [mg/kg dry soil]	PEC_{SOIL} [mg/kg dry soil]	TER	Risk assessment trigger
Glyphosate acid/Glyphosate IPA				
Hypoaspis aculeifer	14 d NOEC = 472.8	8.065	58.6	5
Folsomia candida	28 d NOEC = 587	8.065	72.8	5
AMPA				
Hypoaspis aculeifer	14 d NOEC = 320	5.345	59.9	5
Folsomia candida	28 d NOEC = 315	5.345	58.9	5

[1] Since the 2001 EU evaluation on glyphosate new studies on the active substance have been performed and as a result these are proposed end-points which are used in the risk assessment.

The TER_{LT} values exceed the relevant Annex VI decision-making criteria of 5 for these additional soil macro organisms. Therefore, it can be concluded that chronic risk to soil macro-organisms and their ecological function from the use of MON 52276 in all crops according to the proposed good agricultural practice will be low.

6.5 Effects on soil micro-organisms

Standard soil micro-organism evaluations were performed for MON 52276 following the OECD 216 and 217 test guidelines. Based on laboratory testing with MON 52276, the Annex VI trigger value of > 25% effects (relative to the control group) after 28 days exposure was not exceeded at concentrations of 1× and 5× the maximum recommended annual use rate for 4.32 kg a.s./ha. As no significant effects on soil micro-organisms were observed at these rates, the use of MON 52276 at the proposed field rates poses no unacceptable long-term risk to non-target soil micro-organisms.

Additionally, the NOEC value of 160 mg/kg dry soil for the metabolite AMPA was derived in a separate soil micro-organism study and is approximately 30 times higher than the maximum $PEC_{soil, plateau}$ of 5.345 mg/kg for AMPA.

6.6 Effects on other non-target organisms (flora and fauna)

Based on the results of the current ecological risk assessment, it has been demonstrated that the proposed GAP uses of MON 52276 do not cause unacceptable effects on any of the species tested (aquatics, birds, mammals, bees, NTA, soil macro- and micro fauna). The following section summarizes the ecological risk assessment for non-target plants. The non-target plant risk assessment for glyphosate was based on data on seventeen plant species derived from studies performed with glyphosate, a model formulation containing glyphosate and Triton and a glyphosate wettable powder formulation. Each of these studies provided comparable ranges of EC_{50} values. Additionally, a study has been performed with MON 52276. The results from the MON 52276 are consistent with the overall results from the other studies. Additionally, glyphosate has shown not to have herbicidal activity pre-emergence of weeds and. AMPA has extremely low herbicidal activity, on average 22-fold less biological activity, compared to glyphosate. An overview of the non-target plant assessment and the conclusions of the assessment are summarized below.

Assessment of risk to non-target terrestrial plants

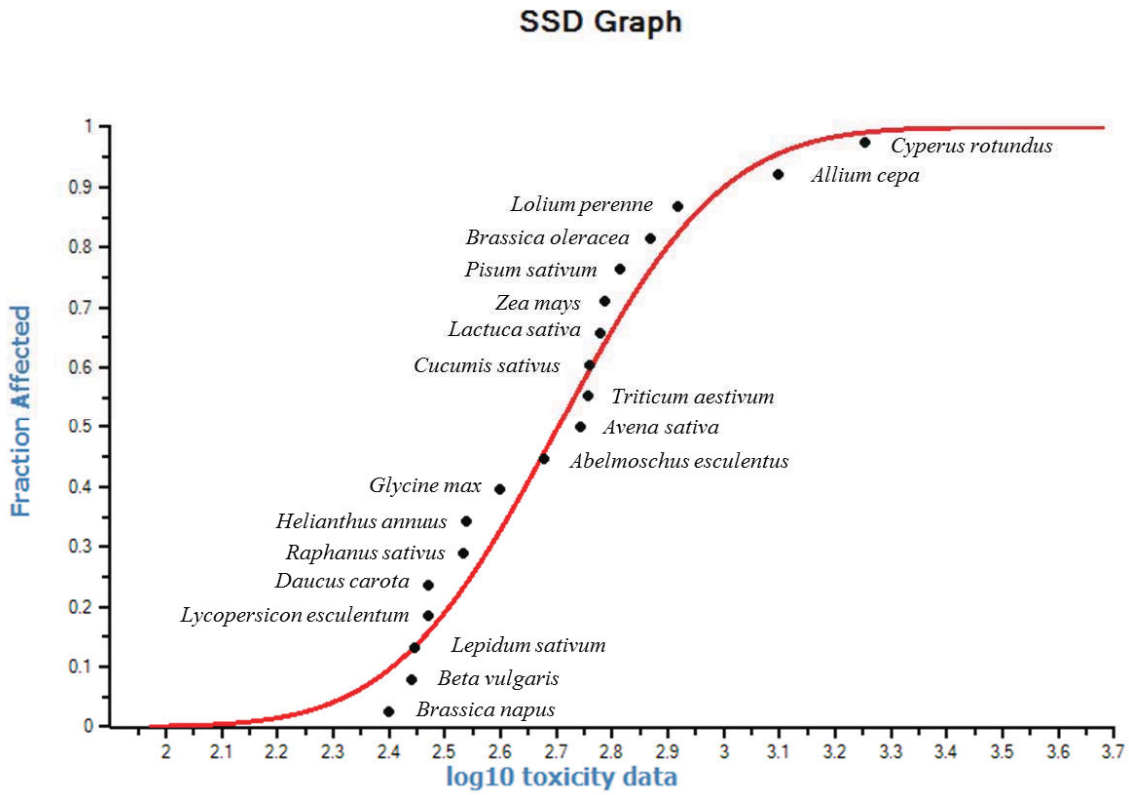
Non-target plant testing with glyphosate (technical) and glyphosate formulations evaluating potential effects following pre-emergent (soil) exposure and post-emergent (foliar) exposure indicated that the compound demonstrated no activity based on the results of a seedling emergence study.

For vegetative vigour, the ER_{50} for shoot fresh weight for the most sensitive monocotyledon species, wheat, was 242 g a.s./ha; with the ER_{50} for shoot fresh weight for the most sensitive dicotyledonous species, tomato, was 146 g a.s./ha. In a study conducted with the lead formulation MON 52276 it is clearly demonstrated that the toxicity is comparable to those achieved in other vegetative vigour studies.

PER_{drift} values at 1, and 5, meters were calculated based on the maximum application rate of 2.16 kg a.s./ha (equivalent to 6 L product/ha) for row crops in the EU. A maximum allowable single rate of 2.88 kg a.s./ha, was not assessed because this application rate is reserved for only ground directed applications that are typically made around the base of tree trunks.

The effect endpoints used in the terrestrial non-target plant risk assessment (*i.e.* ER_{50} for the 17 plant species for vegetative vigour studies (not including data from Mon 52276) were re-evaluated with a species sensitivity distribution from which an HC_5 was obtained. The HC_5 was calculated based on the recommendations of EUFRAM (EUFRAM, 2006 [iii]) using the software package ETX 2.0, developed by the RIVM, the Netherlands, based on Aldenberg and Jaworska (2000) [iv] and Aldenberg and Luttik (2002) [v].

Based on this methodology, the HC_5 value was estimated to be 206.35 g a.s./ha. An illustration of the species sensitivity distribution (SSD) from ETX 2.0 is shown below. For the MON 52276 vegetative vigour study, a comparable HC_5 value was estimated to be 220 g a.s./ha.



According to the Guidance Document on Terrestrial Ecotoxicology SANCO/10329/2002 rev.2 (final), 17 October 2002 [vi], risk to terrestrial plants is considered to be acceptable if the ER_{50} for less than 5% of the species is below the highest predicted exposure level. As this is the case for terrestrial non-target plants, for the refined risk assessment the TER values considering the HC_5 are compared to a trigger of 1.

Table 6.5-1 Glyphosate: TERs using an HC_5 based on a collection of non-target plant ER_{50}s

Buffer distance [meters]	Application rate [g a.s./ha]	Drift value[a] [%]	Drift reduction [%]	PER_{drift} [g a.s./ha]	HC_5 [g a.s./ha]	TER
Field crops, vegetables						
1	2160	2.77	0	59.832	206.35	**3.4**
5		0.57	0	12.312	206.35	**16.8**

[a] Drift estimates are based on 90th percentile values (BBA 2000).
Values in bold exceed trigger value of 1

In the environment, there is generally a large seed bank contained in the soil. This means that even if some individual plants of sensitive species were affected, populations would be able to recover within one season due to the soil reserve of viable seeds.

Further evidence supporting the fact that glyphosate formulations will not cause irreversible effects on non-target plants outside the treated field can be found in a study by Zwerger and

Pestemer (2000)[12] where four different species (oilseed rape (*Brassica napus*), oat (*Avena sativa*), *Chenopodium album* and *Alopecurus myosuroides*) were exposed to a glyphosate formulation (MON 52276, containing 360 g a.s./L with a different surfactant system) at different rates up to a maximum of 450 g a.s./ha to assess effects on the plants' life cycle. The results indicate that, although some effects could be seen on vegetative end-points (plant biomass) at rates higher than the spray drift rate at 1 m (i.e. 120 g a.s./ha), generative end-points, such as seed production, seed weight, germination capacity and seed viability, were not affected. Reproduction of the exposed species was therefore not at risk.

Furthermore, the use of modern technology for the reduction of drift (for example low pressure nozzles, anti-drift nozzles, air-assistance spraying systems) is recommended and will help decrease the potential toxicity from sprayed product to non-target plants on field margins.

Based on this assessment, a TER trigger ≥1 according to the Terrestrial Guidance Document is achieved without taking drift reduction measures or buffer zones into account. Good spray practices will also minimise exposure of non-target plants (and crops) to spray drift.

6.7 Effects on biological methods of sewage treatment

Based on the results of the studies summarized in the Annex 2 document, glyphosate and MON 52276 will not adversely impact sewage treatment processes.

Table 6.7-1. Effects on biological methods of sewage treatment

Respiration inhibition test (activated sludge)	Glyphosate acid: EC_{50} > 1000 mg a.s./L

6.8 Environmental risk mitigation

As discussed in the assessment, good spray practices will minimise exposure of non-target plants (and crops) to spray drift and minimise drift into aquatic environments.

7. Efficacy data and information

7.1 Effectiveness

7.1.1 Intended use

Glyphosate containing products are used in agriculture as foliar sprays, post emergence of weeds in a wide range of arable crops (seeded and transplanted). Uses include applications pre-planting, post-planting pre-emergence and post harvest of all crops. Pre-harvest uses in cereals, oilseeds and pulses are for dessication and annual and perennial weed control. Other uses include annual and perennial weed control in orchard crops and vines including olives, citrus and nuts and for grassland renovation. Non-crop uses include weed control in the amenity, forestry, industrial, aquatic and home and garden sectors.

[12] Zwerger, P. and Pestemer, W. (2000). Testing the phytotoxic effects of herbicides on higher terrestrial non-target plants using life-cycle test. *Z. PflKrankh. PflSchutz. Sonderh.* 17: 711-718.

7.1.2 Mode of action

Glyphosate is a herbicide used in agriculture and non-crop situations for the control of a wide range of monocot and dicot weeds in a range of situations. Glyphosate is a systemic non-selective foliar applied herbicide belonging to the group of the glycines. Glyphosate is classified by HRAC in Group G.

Glyphosate is taken up by green tissue of the leaves and stems of treated plants. It is transported systemically (*via* apoplastic and symplastic pathways) throughout the plant including the roots, rhizomes and stolons but especially to areas of metabolic activity in the plant (sinks), where it inhibits the shikimic acid pathway. Glyphosate binds to and blocks the activity of its target enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), an enzyme of the aromatic amino acid biosynthetic pathway. The inhibition of the enzyme prevents the plant from synthesizing the essential aromatic amino acids needed for protein biosynthesis. EPSPS is present in all plants, bacteria, and fungi, but not animals.

7.1.3 Effectiveness against weeds

The dossier gave an overview of the efficacy information concerning representative and supported uses already authorised in Member States according to the format provided in Appendix II of the SANCO/10387/2010 rev. 8 guidance document of October 28, 2010.

Considering that the substance was included into Annex I of Directive 91/414/EC and authorisations of plant protection products containing the substance (including the representative formulation in this submission) have already been evaluated according to the Uniform Principles of Annex VI of Directive 91/414/EEC, no other efficacy documentation was deemed to be necessary for the purpose oft he reregistration.

7.2 Information on the occurrence or possible occurrence of the development of resistance

Weed resistance is the inherited ability of a population to survive and reproduce following repeated exposure to a dose of herbicide normally lethal to the wild type (source: www.hracglobal.com January 2012)

Glyphosate has been commercialised in numerous formulations in well over 100 countries around the world since its first introduction as Roundup in 1974. It has become one of the most widely used broad spectrum herbicides in the world for non-selective control of weeds and unwanted plants in agricultural and non-agricultural situations at rates of up to 4320 g glyphosate acid equivalents./ha per application.

After more than three decades of widespread glyphosate use, resistance has been observed in biotypes of 21 weed species globally (source: www.weedscience.org January 2012). All these resistant biotypes can be effectively and economically managed through alternative control practices such as tank mixes with residual or selective herbicides, tillage or other means.

Globally, to date 21 species across 15 genera have been confirmed as having populations resistant to glyphosate. Table 7.2-1 summarizes the 15 genera, indicating the year resistance was first reported, the country and the cropping situation. Over half of the resistant species were first identified in traditional glyphosate use areas such as fallow ground applications, orchards, and vineyards. The first glyphosate resistant population was identified in 1996 in Australia, over 20 years after glyphosate was commercially introduced. (Note: www.weedscience.org provides a specific list of glyphosate resistant species and areas where resistant populations have been verified.)

Table 7.2-1. Genus, country and situation where glyphosate resistant weeds were first reported

Genus	First Reported	Country	Situation
Lolium (2 *spp.*)	1996/2001	Australia/Chile	Fallow/Orchards
Eleusine	1997	Malaysia	Orchards
Conyza (2 *spp.*)	2000/2003	USA/South Africa	RR soybeans/Vineyards
Plantago	2003	South Africa	Vineyards
Ambrosia (2 *spp.*)	2004	USA	RR soybeans
Parthenium	2004	Colombia	Orchards
Amaranthus (2 *spp.*)	2005	USA	RR soybeans/RR cotton
Sorghum (perennial)	2005	Argentina	RR soybeans
Digitaria (perennial)	2006	Paraguay	RR soybeans
Euphorbia	2006	Brazil	RR soybeans
Echinochloa	2007	Australia	Fallow
Urochloa	2008	Australia	Fallow
Kochia	2009	USA	Fallow/RR-maize/RR-soybeans
Poa	2010	USA	Turf
Chloris	2010	Australia	Fallow

Importantly, in order for a new weed species to be declared resistant, there are two criteria, as defined by the Weed Science Society of America (WSSA) that must be met: (1) the ability to survive application of rates of herbicide product that once were effective in controlling it (this is usually referred to as the X rate) and (2) resistance is heritable. The website www.weedscience.org is used by many weed scientists as the site where new species and new areas with resistant populations are first listed. A guideline as how to test and confirm for resistance is equally provided on this website.

Glyphosate Resistant Weeds in Europe

In Europe, resistance to glyphosate has been confirmed in only two genera and only in perennial crops. Resistance exists in *Lolium spp.* and *Conyza spp.* populations in perennial crops in six countries (France, Italy, Spain, Portugal, Greece and Czech Republic). In Spain, *Conyza bonariensis* first evolved resistance to glyphosate in 2004 in orchards. *Lolium rigidum* resistance was found in 2005 in asparagus, orchards, and vineyards in France. In 2006 a resistance case was

found in orchards in Spain, Valencia. Another resistant *Lolium rigidum* was found for the first time in vineyards and orchards in Italy in 2007.

In 2006 cases of resistance to glyphosate in *Conyza canadensis* were confirmed in Spanish orchards. In Spain, *Lolium multiforum and Lolium perenne* resistance to glyphosate was observed in orchards in 2006 and in 2009 a case of *Conyza sumatrensis* resistance was confirmed in orchards. In 2010 Greece and Portugal reported confirmed resistance of biotypes of *Conyza bonariensis* to glyphosate (Table 7.2-2).

Table 7.2-2 Confirmed glyphosate resistant weeds in Europe

Genus and Specie	Reported	Country	Situation
Conyza bonariensis	2004	Spain	Orchard
	2010	Greece	Orchards
	2010	Portugal	Orchards
Conyza Canadensis	2006	Spain	Orchards
	2007	Czech Republic	Railways
Conyza sumatrensis	2009	Spain	Orchards
Lolium multiflorum	2006	Spain	Orchards
Lolium rigidum	2005	France	Asparagus, Orchards and vineyards
	2006	Spain	Orchards
	2007	Italy	Orchards and vineyards

Glyphosate Resistant Mechanisms and Inheritance

Monsanto, a member of the Glyphosate Taskforce, in cooperation with leading academics from around the world has been investigating the mechanism of resistance in a number of glyphosate resistant species and biotypes. To date there are 5 confirmed mechanisms of resistance and one suspected (metabolism), as summarized in Table 5. This is unique among herbicides and herbicide groups where there are generally only one or two dominant mechanisms of resistance (i.e. target site and metabolism). This situation may in part explain why resistance to glyphosate was slow to develop and non-existent for more than 20 years following the first commercial sales of the herbicide.

Across all herbicide groups, **target-site** mutations and metabolism are the most common resistance mechanisms (Table 5). Target-site mutations are alterations in the amino acid sequence of a targeted protein such that the function of the protein in not altered, but the ability of the herbicide to bind to the protein is affected, thus limiting the capacity of the herbicide to kill the plant. Target-site mutations generally result in 'high levels' of resistance (i.e. 1000X) and is the common resistance mechanism for the ALS inhibitor and ACCase inhibitor groups. (Note: 1000 X means the biotype is not affected by a herbicide rate that is 1000 times greater than the rate that would normally kill a biotype). In contrast, glyphosate resistant weed species with target-site mutations demonstrate relatively 'weak' resistance (i.e. 2-3X). A rare second target-site mechanism is over expression of EPSPS, that has been identified as the primary resistance

mechanism in an *Amaranthus* species. This is the first instance of this type of target-site resistance found for a herbicide.

Several types of **non-target site mechanisms** have been defined for glyphosate (Table 7.2-3).These include; (1) reduced movement of glyphosate as a result of a hypersensitive effect (rapid tissue necrosis) of leaf tissue treated with glyphosate (*Ambrosia* sp.), (2) reduced translocation to rhizome/root tissue (*Sorghum* sp.), and (3) accumulation/sequestration of glyphosate in the vacuole preventing lethal concentrations getting into plastids, the site of the shikimic acid pathway (*Lolium* sp).

Table 7.2-3. Sites and mechanisms of resistance to glyphosate of weed species

Site of Resistance	Resistance Mechanism (RM)	No. Of Species exhibiting the RM
Target	Mutation	4
	Over Expression	2
Non-target	Extra cellular	1
	Translocation	2
	Intra cellular	2

In all cases studied to date, glyphosate resistance had been demonstrated to be inherited as dominant or semi-dominant with each mechanism conferred by a single gene locus. This is true for most herbicides.

The mechanism of action of a gene providing tolerance to glyphosate in glyphosate-tolerant crops is different from the genes identified as conferring target-site resistance. For example the commercial glyphosate tolerant gene from Monsanto, obtained from a bacterial strain of *Agrobacterium* sp., produces a EPSPS enzyme (CP4 EPSPS) that has less binding affinity to glyphosate than plant EPSPS. This fact combined with the high production of CP4 EPSPS in the crop accounts for the high level of tolerance found in crops containing the gene.

Resistance Best Management Practices

Public and private sector weed scientists have developed a set of best management practices to minimise the risk of the development of resistance for all herbicides. These practices are also applicable to managing populations that are already resistant to a herbicide. When glyphosate resistant weed biotypes have been identified, they have been effectively managed with other herbicides and/or farming practices. To date, the same is true for other herbicides.

For glyphosate, as for any herbicide, Good Agricultural Practices and Good Plant Protection Practices (EPPO 2003) are the basis of the weed resistance management strategy (EPPO 2002). In addition a proactive and reactive management strategy for glyphosate resistant weeds should be followed as described by HRAC (source: www.hracglobal.com) and Beckie (2011).

Successful management of resistance requires the use of both proactive and reactive management tactics and both are predicated upon the implementation of diversified weed management programs. Diversified programs incorporate the use of multiple herbicides with different mechanisms of action, with and without the concurrent use of mechanical and/or cultural control methods. There are several options for implementing a diversified program with no one option being clearly best across all farming situations. Proactive management tactics retard the onset of herbicide resistance.

The Herbicide Resistance Action Committee (HRAC) and Weed Science Society of America (WSSA) have both listed the following general guidelines on their respective websites. These represent a general consensus among public and private sector weed scientists on the key practices important for managing resistance and serve as the basis for farmer and distributer training programs.

General principles of herbicide resistance management

- Apply integrated weed management weed practices. Use multiple herbicide modes-of-action with overlapping weed spectrums in rotation, sequences or mixtures
- Use the full recommended herbicide rate and proper application timing for the hardest to control weed species present in the field
- Scout fields after herbicide application to ensure control has been achieved. Avoid allowing weeds to reproduce by seed or proliferate vegetatively.
- Monitor site and clean equipment between sites

The risk of resistance in Europe appears generally low and, where it occurs, should be relatively easy to manage according to the procedures and methods established successfully in France, Spain and other parts of the world. In case of confirmed resistance, a wide range of risk modifiers are available and to be selected as appropriate. These include:

- Communication of the required activities to farmers by label recommendations, literature and on-farm advice
- Non-chemical control measures, e.g. cultivation, mowing, mechanical weed control
- Modified use of glyphosate, e.g. frequency, timing, dose rate, tank mixtures
- Complementary or alternative herbicides from different classes
- Changes in agronomic practice, e.g. timing of agronomic activities, crop rotation, use of cover crops or green manures, ploughing, conservation tillage, husbandry systems.

The objective is to prevent the production of viable seed and to prevent the spread of viable plant parts.

7.3 Effect on the yield of treated plants or plant products in terms of quantity and/or quality

Considering that the substance was included into Annex I of Directive 91/414/EC and authorisations of plant protection products containing the substance (including the representative formulation in this submission) have already been evaluated according to the Uniform Principles of Annex VI of Directive 91/414/EEC, no specific crop safety information was deemed to be necessary for the purpose oft he renewal oft he EU-registration (See also 7.1.3).

7.4 Phytotoxicity to target plants (including different cultivars), or to target plant products

Considering that the substance was included into Annex I of Directive 91/414/EC and authorisations of plant protection products containing the substance (including the representative formulation in this submission) have already been evaluated according to the Uniform Principles of Annex VI of Directive 91/414/EEC, no specific crop safety information was deemed to be necessary for the purpose oft he renewal oft he EU-registration (See also 7.1.3).

7.5 Observations on undesirable or unintended side effects e.g. on beneficial and other non-target organisms, on succeeding crops, other plants or parts of treated plants used for propagating purposes (e.g. seed, cuttings, runners)

7.5.1 Impact on succeeding crops

- As discussed in IIIA-N 4.1.1 the results from confined rotational crop studies demonstrate that only very low levels of glyphosate or glyphosate metabolites are present in the soil and plant tissues of rotational crops planted after treatment of a primary crop with glyphosate. Uptake from soil is less than 1% of the applied dose. Also as discussed in IIIA-N 7.5.3, non-target plant testing with glyphosate and glyphosate formulations evaluating potential effects following pre-emergent (soil) exposure and post-emergent (foliar) exposure indicated that the compound demonstrated no activity in the seedling emergence study. No effects on succeeding crops are to be expected provided the following waiting times are respected between the application and the planting/sowing of a succeeding crop:

  Pre-drilling of seed (for instance stubble treatments, post-cultivation treatments or pre-plant treatments):

  The limiting factor is the time taken for glyphosate to be absorbed by and translocated into the weeds. Glyphosate is adsorbed by the soil, therefore residues in succeeding crops are not a concern. Typical recommendations : 2-3 days before planting

- Pre-planting of transplanted crops (plugs or bare root)

  The limiting factor is to ensure that moist plugs or bare roots do not come into contact with the treated vegetation (weeds) or with glyphosate in solution. Experience has shown that a waiting period of 3 days is sufficient after spraying.

- Post-drilling pre-emergence:

  The limiting factor is to treat before crop emergence. Typically there is no restriction on application after drilling except to avoid crop emergence

Considering that the substance was included into Annex I of Directive 91/414/EC and authorisations of plant protection products containing the substance (including the representative formulation in this submission) have already been evaluated according to the Uniform Principles of Annex VI of Directive 91/414/EEC, no additional data on succeeding crops was deemed to be necessary for the purpose oft he renewal oft he EU-registration.

7.5.2 Impact on adjacent crops

The effects of glyphosate acid and MON 52276 on vegetative vigour of a range of terrestrial non-target plants has been assessed in four glasshouse studies on non-target plants. A summary of the most sensitive species and the corresponding ER50 is provided in Table 7.5-1.

The effect endpoints used in the terrestrial non-target plant risk assessment (*i.e.* ER_{50} for the 17 plant species tested for vegetative vigour) were re-evaluated to construct a species sensitivity distribution from which an HC_5 was obtained. According to the Guidance Document on Terrestrial Ecotoxicology SANCO/10329/2002 rev.2 (final), 17 October 2002, the risk for terrestrial plants is assumed to be acceptable if the ER/EC_{50} for less than 5% of the species is below the highest predicted exposure level. As this is the case for terrestrial non-target plants, for the refined risk assessment the TER values considering the HC_5 are compared to a trigger of 1. Based on this assessment, a TER trigger of 1 according to the Terrestrial Guidance Document is achieved. Thus, no unacceptable risk to non-target terrestrial plants is to be expected for the use of MON 52276.

7.5.3 Impact on seed viability

Non-target plant testing with glyphosate and glyphosate formulations evaluating potential effects following pre-emergent (soil) exposure and post-emergent (foliar) exposure indicated that the compound demonstrated no activity in the seedling emergence study. A summary of the most sensitive species and the corresponding ER50 is provided in Table 7.5-1.

Table 7.5-1: Toxicity of glyphosate and formulation MON 52276 to non-target plants

Test substance Test type	Most sensitive species	Lowest ER_{50}	Reference/GLP
Glyphosate acid 21 d vegetative vigour	*Helianthus annuus* (sunflower)	ER_{50} (dry weight) = 295.9 g a.s./ha	IIA 8.12/01 236 GLY Harnish, 1994/yes
Glyphosate acid 21 d vegetative vigour	*Solanum lycopersicum* (tomato)	ER_{50} (dry weight) = 145.7 g a.s./ha	IIA 8.12/02 MSL-13320 Chetram, Lucash, 1994/yes Monograph reference 97-00102
Glyphosate acid (formulated product, WP) 28 d vegetative vigour	Oilseed rape (*Brassica napus*)	ER_{50} (visual damage) = 140 g a.s./ha	IIA 8.12/03 RJ2009B Everett *et al.*, 1996/yes
MON 52276 22 d vegetative vigour	Garden cress (*Lepidum sativum*)	ER_{50} (fresh weight) = 252 g a.s/ha	IIA 8.12/05 CEA.104 Blake, 2005
Glyphosate acid (formulated product, WP) 28 d Seedling emergence	Purple nutsedge (*Cyperus rotundus*) Oat (*Avena sativa*) Winter wheat (*Triticum aestivum*) Maize (*Zea mays*) Onion (*Allium cepa)* Sugar beet (*Beta vulgaris*) Lettuce (*Lactuca sativa*) Oilseed rape (*Brassica napus*) Cucumber (*Cucumis sativa*) Soybean (*Glycine max)* Okra (*Abelmoschus esculentus*) Rhubarb (*Rheum rhoponticum*)	ER_{50} (seedling emergence, seedling dry weight) > 4.48 kg a.s/ha	IIA 8.12/04 RJ2008B Everett *et al.*, 1996/yes

7.5.4 Impact on beneficial and other non target organisms

Based on the results of the current ecological risk assessment (please refer to IIIA-N 6.6), it has been demonstrated that the proposed GAP uses of MON 52276 do not cause unacceptable effects on any of the species tested (aquatics, birds, mammals, bees, NTA, soil macro- and micro fauna).

7.6 Conclusions

Glyphosate contained in MON 52276, has been tested in numerous field development trials which demonstrated effective herbicidal activity and crop safety. This has already been evaluated according to the Uniform Principles of Annex VI of Directive 91/414/EEC (national authorizations).

Glyphosate containing products are effectively used in agriculture as foliar sprays, post emergence to weeds, in a wide range of arable crops (seeded and transplanted). Uses include applications pre-planting, post-planting pre-emergence and post harvest of all crops. Pre-harvest uses in cereals, oilseeds and pulses are for desiccation and annual and perennial weed control. Other uses include annual and perennial weed control in orchard crops and vines including olives, citrus and nuts and for grassland renovation. Non-crop uses include weed control in the amenity, forestry, industrial, aquatic and home and garden sectors. Weed resistance development is documented but limited in scale and can easily be managed.

Glyphosate has no impact on seedling emergence or development of succeeding crops. No unacceptable risk to non-target terrestrial plants is to be expected for the use of MON 52276.

8. Overall Conclusions

8.1. Proposed decision

[i] EPPO/OEPP (2010) Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees Bulletin OEPP/EPPO Bulletin 40: 323-331.
[ii] Candolfi *et al.* (Eds) (2001). Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non-Target Arthropods. From the ESCORT 2 Workshop, March 2000. Publ. SETAC, Pensacola, USA.
[iii] Peter F. Chapman, Melissa Reed, Andy Hart, Tom Aldenberg, Keith Soloman, Jose Tarazona, Matthias Liess, Pamela Byrne, Methods of Uncertainty Analysis, Work Package 4, EUFRAM, September, 2006.
[iv] Tom Aldenberg, Joanna S. Jaworska (2000): Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicology and Environmental Safety, 40, pp.: 1-18
[v] Tom Aldenberg, Robert Luttik (2002): Extrapolation factors for tiny toxicity data sets from species sensitivity distributions with known standard deviation. In: Posthuma, L., Suter II G.W., Traas, T.P. (eds.). Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, Boca Raton, USA, pp. 103-118.
[vi] Anonymous (2002). Guidance Document on terrestrial ecotoxicology under council directive 91/414/EEC. SANCO/10329/2002. 17 October 2002.