IPCS

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

Environmental Health Criteria 159

Glyphosate

UNEP

nder the joint sponsorship of the United Nations Environment Programme,
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Environmental Health Criteria 159

GLYPHOSATE

First draft prepared by Dr H. Mensink and Dr P. Janssen, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands

Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization

World Health Organization
Geneva, 1994

The **International Programme on Chemical Safety (IPCS)** is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by the IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are kindly requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

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A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Case postale 356, 1219 Châtelaine, Geneva, Switzerland (Telephone No. 9799111).

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ENVIRONMENTAL HEALTH CRITERIA FOR GLYPHOSATE

A Task Group on Environmental Health Criteria for Glyphosate met at the Institute of Terrestrial Ecology, Monks Wood, United Kingdom, from 23 to 27 August 1993. Dr S. Dobson welcomed the participants on behalf of the host institution, and Dr M. Gilbert opened the Meeting on behalf of the three cooperating organizations of the IPCS (UNEP/ILO/WHO). The Task Group reviewed and revised the draft monograph and made an evaluation of the risks for human health and the environment from exposure to glyphosate.

The first draft of this monograph was prepared by Dr H. Mensink and Dr P. Janssen, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

Dr M. Gilbert was responsible for the overall scientific content of the monograph and for the organization of the meeting, and Dr P.G. Jenkins, IPCS, for the technical editing of the monograph.

The efforts of all who helped in the preparation and finalization of the monograph are gratefully acknowledged.

ABBREVIATIONS

a.i.	active ingredient
ALAT	alanine aminotransferase
AMPA	aminomethylphosphonic acid
AP	alkaline phosphatase
CHO	Chinese hamster ovary
CNS	central nervous system
HPLC	high-performance liquid chromatography
i.p.	intraperitoneal
IPA	isopropylamine
MATC	maximum acceptable toxicant concentration
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration

1. SUMMARY

1.1 Identity, physical and chemical properties, and analytical methods

Glyphosate is a weak organic acid consisting of a glycine and a phosphonomethyl moiety. The empirical formula is $C_3H_8NO_5P$. Glyphosate is usually formulated as a salt of the deprotonated acid of glyphosate and a cation, e.g., isopropylamine or trimethylsulfonium. The purity of technical grade glyphosate is generally above 90%. Technical grade glyphosate is an odourless white crystalline powder with a specific gravity of 1.704, a very low vapour pressure, and a high solubility in water. The octanol-water partition coefficient (log K_{ow}) is -2.8. Glyphosate is amphoteric and may exist as different ionic species, dependent on the actual pH.

Determination of glyphosate is in general laborious, complex, and costly. Derivatization with fluorogenic substances is the most common method and may be applied pre- or post-column. Determination is usually carried out with high performance liquid chromatography or gas liquid chromatography. Limits of determination for glyphosate in water, plants, soil and human urine, are 0.02-3.2 µg/litre, 0.01-0.3 mg/kg, 0.05-1 mg/kg and 0.1 mg/litre, respectively.

1.2 Sources of human and environmental exposure

Glyphosate is a post-emergent, systemic and non-selective herbicide that is used in both agricultural and non-agricultural areas all over the world. Glyphosate is applied to many crops and in various commercial formulations. The major formulation is Roundup in which glyphosate is formulated as the isopropylamine salt. Recommended application rates do not exceed 5.8 kg a.i./ha and are dependent on the type of use. Environmental exposure may occur because of deposition due to drift and accidental releases.

1.3 Environmental transport, distribution and transformation

The most important processes of dissipation that may be involved after application of glyphosate are complexation in water with ions, e.g., Ca^{2+} and Mg^{2+}, sorption to sediment, suspended particles in water, and soil, photodegradation in water, uptake by plants, and biodegradation.

Glyphosate dissipates from the water with DT_{50} values (dissipation) ranging from a few days to more than 91 days. Sediment or suspended particles are shown to be the major sink.

The adsorption coefficients ($K_{s/l}$) of glyphosate in laboratory experiments vary between 8 and 377 dm^3/kg for various soils and clay minerals. No data on the sorption of aminomethylphosphonic acid (AMPA), the major metabolite, under laboratory conditions are available.

R_f values of glyphosate do not exceed 0.2 in soil thin-layer chromatography experiments. Between less than 0.1% and 11% of the applied activity is recovered in the eluate of soil columns under leaching conditions simulating an extremely high rainfall. From field experiments it appears that AMPA is not likely to leach.

Glyphosate dissipates in field experiments from the soil with DT_{50} values between 3 and 174 days, mainly depending on edaphic and climatic conditions. Up to 1.8% of the applied dose dissipated from the soil due to run-off in some field experiments.

Under laboratory conditions, up to 45% of the applied activity may be absorbed by treated leaves, and this is followed by a substantial translocation.

Hydrolysis of glyphosate in sterile buffers is very slow with DT_{50} values >> 35 days. Photodegradation in water under natural conditions occurs with DT_{50} values $\leq$ 28 days. No substantial photodegradation in soil was recorded in a study lasting 31 days.

The time needed for 50% biodegradation of glyphosate in the whole system of a test with water and sediment is $\leq$ 14 days under aerobic conditions and 14-22 days under anaerobic conditions in the laboratory. The time needed for 50% biodegradation of glyphosate in the soil is 2-3 days under aerobic conditions.

The major metabolite in soil and water is AMPA. Maximum amounts of AMPA in soils are approximately 20% of the applied activity under aerobic conditions and 0.5% under anaerobic conditions. Maximum amounts of AMPA in sediments are 25% under both aerobic and anaerobic conditions.

Bioconcentration factors are low in laboratory tests with invertebrates and fish. Bluegill sunfish in a flow-through test

showed a depuration half-life of 35 days, after being exposed for 35 days. AMPA is recovered in bluegill sunfish up to 21 days after continuous exposure to glyphosate. Glyphosate has not been detected in fish living in directly sprayed water in field experiments. In one experiment, AMPA was detectable in carp up to 90 days after application. No biomagnification of glyphosate in litter by herbivorous and omnivorous small mammals in a forest brush ecosystem was indicated in a field experiment. Concentrations of up to 5 mg a.i./kg were measured in deermice immediately after spraying in this experiment.

A range of bacterial strains can degrade glyphosate. Bacteria capable of using the compound as sole phosphorus, sole carbon or sole nitrogen source have been identified. Growth is slow compared to growth on inorganic sources of P, C and N. There is evidence from the field that bacterial populations adapted to metabolise glyphosate. The presence of inorganic phosphate inhibits degradation of glyphosate with some, but not all, bacteria. Biodegradation of glyphosate may involve co-metabolism with other energy sources.

1.4 Environmental levels and human exposure

Data on the occurrence of glyphosate in environmental biota and abiota as part of regular monitoring programmes are very scarce. Data from field experiments in which common agricultural practice is simulated are used to indicate maximum environmental concentrations: < 1-1700 μg/litre surface water, 0.07-40 mg/kg dry weight soil, < 0.05-19 mg/kg dry weight sediment, 261-1300 mg/kg foliage, 5 mg/kg the viscera of deermice, 1.6-19 mg/kg wild berries, and 45 mg/kg lichens. The corresponding maximum concentrations of AMPA are: < 1-35 μg/litre (surface water), 0.1-9 mg/kg dry weight (soil), < 0.05-1.8 mg/kg dry weight (sediment), 1.7-< 9 mg/kg (foliage), 0.02-0.1 mg/kg (wild berries), and 2.1 mg/kg (lichens). The above-mentioned concentrations of glyphosate are generally found immediately after application. The concentration in lichens was found 270 days after application.

Measurements of daily human intake of glyphosate via food and drinking-water (total diet studies) are not available. The few data on occupational exposure indicate that exposure levels for workers applying glyphosate as the herbicide formulation Roundup are low.

1.5 Kinetics and metabolism in laboratory animals and humans

Technical glyphosate is only partially absorbed from the gastrointestinal tract. In studies with ^{14}C-labelled glyphosate, absorption percentages of 30-36% were found in several species. Dermal absorption is low. From the herbicide formulation Roundup, ≤ 5.5% of the glyphosate present is absorbed through the skin (contact time about 24 h). In body tissues, the highest concentrations, approximately 1% of the oral dose, are found in bone. Following a single oral dose, 62-69% is eliminated in the faeces without absorption. Of the absorbed glyphosate, 14-29% is excreted in urine and 0.2% or less in expired air. Biliary excretion following intravenous application was only 5-8%. In lactating goats, excretion in milk was shown to occur to a minor extent only (concentration ≤ 0.1 mg/kg whole milk at a dose level of 120 mg/kg diet). Biotransformation of glyphosate occurs to a very low degree only. The only metabolite, AMPA, accounts for 0.3% of the dose or less; the rest is unchanged glyphosate. Whole body clearance (99% of an oral dose) occurs in approximately 168 h.

1.6 Effects on laboratory mammals, and *in vitro* test systems

In experimental animals, technical glyphosate has very low acute toxicity by the oral and dermal administration routes; it is markedly more toxic by the intraperitoneal route than by other routes. Short-term feeding studies have been conducted in several species, but few effects were seen in most of these tests. In one 13-week study in mice with technical glyphosate, increased weights of several organs and growth retardation were observed at 50 000 mg/kg diet. In a 13-week study in rats no effect occurred (technical glyphosate dose levels up to 20 000 mg/kg diet). In another 13-week study, lesions of the salivary glands were found in rats and mice. In mice, the NOAEL was 3125 mg/kg diet; in rats, it was < 3125 mg/kg diet. These findings were not present in any other short-term or long-term studies conducted in different strains and species. The salivary lesions suggest that glyphosate may be acting as a weak adrenergic agonist.

Long-term toxicity was studied in mice and rats. Few effects were observed and, in almost all cases, at relatively high dose levels only. In mice, technical glyphosate produced growth retardation, hepatocyte hypertrophy or necrosis and urinary bladder epithelial hyperplasia at 30 000 mg/kg. In rats, the same test compound produced decreased growth, increased liver weights, degenerative lens changes and gastric inflammation at 20 000 mg/kg diet.

The available studies do not indicate that technical glyphosate is mutagenic, carcinogenic or teratogenic. Two multigeneration studies were carried out in rats. The main effects of technical glyphosate were decreased body weights of parent animals and pups and decreased litter size at 30 000 mg/kg diet. In one reproduction study, an increase in the incidence of unilateral renal tubular dilation in F_{3b} male pups at 30 mg/kg body weight was reported. The absence of a renal effect in pups at a higher dose level in the other reproduction study indicates that the reproducibility of this lesion is uncertain.

1.7 Effects on humans

The available controlled studies are limited to three irritation/sensitization studies in human volunteers, the results of which indicated no effect. Several cases of (mostly intentional) intoxications with technical glyphosate herbicide formulation Roundup have been reported. In a study on health effects in workers applying Roundup herbicide formulation, no adverse effects were found. Available data on occupational exposure for workers applying Roundup indicate exposure levels far below the NOAELs from the relevant animal experiments.

1.8 Effects on other organisms in the laboratory and field

Technical grade glyphosate is moderately to slightly toxic to aquatic microorganisms, with EC_{50} (3-4 days) values of 1.2-7.8 mg/litre, and 7-day NOEC values of 0.3-34 mg/litre. Formulations of glyphosate are slightly to highly toxic to aquatic microorganisms with 3-day EC_{50} values of 1.0 to > 55 mg product per litre. Cyanophyta (blue-green algae) are more sensitive to Roundup than true algae. Physiological processes that are affected include the greening process, respiration, photosynthesis, and the synthesis of aromatic amino acids.

Soil bacteria in culture have shown effects of glyphosate on nitrogen fixation, denitrification and nitrification. However, field studies after application of formulations have not shown significant effects. Closely related species of bacteria have been shown capable of degrading glyphosate.

Mycelial growth of ectomycorrhizal fungi in pure cultures is inhibited at concentrations of ≥ 29 µg Roundup/litre. Sensitive genera are *Cenococcum*, *Hebeloma* and *Laccaria*.

Glyphosate is slightly toxic to aquatic macrophytes with a 14-day NOEC value of 9 mg/litre, when dissolved in water. Roundup is also slightly toxic with 14-day NOEC values of 2.4-56 mg Roundup/litre, when dissolved in water. No data on acute toxicity are available. Phytotoxicity is much higher when sprayed deposits are not washed off.

Technical grade glyphosate is slightly to very slightly toxic to aquatic invertebrates with 2- to 4-day LC_{50} or EC_{50} values of $\geq$ 55 mg/litre, and a 21-day NOEC value of 100 mg/litre. Formulations of glyphosate are moderately to very slightly toxic to aquatic invertebrates with 2-day EC_{50} values of 5.3-5600 mg product/litre and 21-day MATC values of 1.4-4.9 mg product per litre. The higher toxicity of Roundup is mainly due to the presence of surfactants.

Technical grade glyphosate is moderately to very slightly toxic to fish, with 4-day LC_{50} values of 10 to > 1000 mg/litre, a 21-day NOEC value of 52 mg/litre, and an MATC value of > 26 mg/litre. Formulations of glyphosate are also moderately to very slightly toxic to fish with 4-day LC_{50} values of 2.4 to > 1000 mg product per litre, and 21-day NOEC values of 0.8-2.4 mg product/litre. The most sensitive species is the carp, when exposed to the formulation Sting. No treatment-related effects of Roundup on fish have been found under field conditions, with the exception of stress immediately after application of a recommended rate and avoidance of concentrations of $\geq$ 40 mg Roundup/litre.

Nodulation of sub-clover inoculated with *Rhizobium* is inhibited in a dose-related way in soil-free systems with nutrient solutions at concentrations of $\geq$ 2 mg a.i./litre. Seed germination of various forest species is not affected by glyphosate at the recommended application rates. The root length of red pine seedlings is decreased under laboratory conditions in a dose-related way at application rates of $\geq$ 0.54 kg a.i./ha. This decrease was not confirmed in a comparable field experiment.

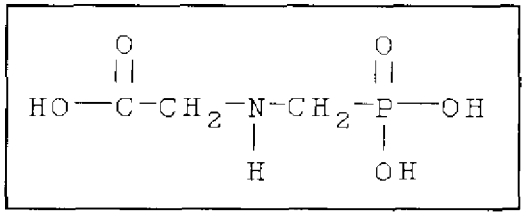
Technical grade glyphosate and Roundup are slightly toxic to bees when applied either orally or topically. The 2-day LD_{50} values are $\geq$ 100 μg (a.i. or product) per bee. The oral 2-day LD_{50} of Sting to bees is > 100 μg/bee. Roundup and Roundup D-pak are slightly toxic to earthworms with 14-day NOEC values of 500 and 158 mg product per kg dry weight, respectively. No adverse effects of Roundup were found on the fecundity and fertility of green lacewings, and there were no effects of Sting on the food uptake and mortality of the beetle *Poecilus*.

Technical grade glyphosate is slightly toxic to birds, with an LD_{50} of >3851 mg/kg body weight, an 8-day LC_{50} of >4640 mg/kg feed, and 112- to 119-day NOEC values of ≥ 1000 mg/kg feed. Roundup and an unknown formulation are also slightly toxic to birds, with an LD_{50} of > 2686 mg product/kg body weight and an 8-day LC_{50} of > 5620 mg product/kg feed. Generally no treatment-related effects of technical grade glyphosate or Roundup on mammals are found under laboratory conditions, except at very high application rates. Treatment-related effects on birds and mammals under field conditions appear to be primarily due to habitat changes after treatment with Roundup.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, AND ANALYTICAL METHODS

2.1 Identity

Glyphosate is the primary name of a weak organic acid that consists of a glycine moiety and a phosphonomethyl moiety. The chemical name is *N*-(phosphonomethyl)glycine according to IUPAC nomenclature. The CAS name is glycine, *N*-(phosphonomethyl)-, and its CAS registry number is 1071-83-6. The empirical formula is $C_3H_8NO_5P$, and the structural formula is as follows:



The relative molecular mass of glyphosate is 169.07. Technical grade glyphosate has a purity of ≥ 80%, but the purity generally exceeds 90%. Glyphosate usually is formulated as a salt of the deprotonated acid of glyphosate and a cation, e.g., isopropylamine. The CAS registry number of the salt of glyphosate and isopropylamine is 38641-94-0.

Surfactants and inerts may be added to formulations of glyphosate. The type of surfactant and its concentration may differ per formulation. A common surfactant in the major formulation Roundup is polyoxyethylene amine. Other known surfactants are ortho X-77 (Mitchell et al., 1987), LI-700, R-11 and Widespread (Monsanto, 1990a). Other additives in formulations may be sulfuric and phosphoric acids.

2.2 Physical and chemical properties

The physical and chemical properties of glyphosate are tabulated in Table 1. Glyphosate is an amphoteric compound of

which the ionic species and their pKa values are presented in Fig. 1. Due to its high polarity glyphosate is practically insoluble in, for instance, ethanol, acetone and benzene.

Table 1. Physical and chemical properties of glyphosate[a]

		Remarks
Physical state	crystalline powder	
Colour	white	
Odour	none	
Melting point[b]	184.5 °C	decomposition at 187 °C
Boiling point	n.a.	
Specific gravity (density)[c]	1.704	20 °C
Vapour pressure[d]	$< 1 \times 10^{-5}$ Pa	25 °C
Solubility in water[b,e]	10 100 mg/litre	20 °C
Henry's law constant	$< 7 \times 10^{-11}$	
Octanol-water partition coefficient (log K_{ow})[d]	-2.8	
Surface tension[d]	0.072 N/m	0.5% (w/v) at ~ 25 °C
pKa values[d,f]	< 2, 2.6, 5.6, 10.6	Sprankle et al. (1975)
Molar absorptivity[c]	0.086 litre/mol per cm	at 295 nm
Flammability[d]	not flammable	
Explosiveness[d]	not explosive	
pH[d]	2.5	1% solution

[a] data provided by Monsanto Ltd
[b] purity 96%
[c] purity 100%
[d] purity not reported
[e] pure glyphosate had been reported to have a water solubility of 11 600 mg/litre at 25 °C
[f] free acid
n.a. = not applicable

2.3 Formulations

Glyphosate can be applied in various formulations. A synopsis of these formulations, their concentrations of active ingredient, and the countries in which the use is permitted is presented in

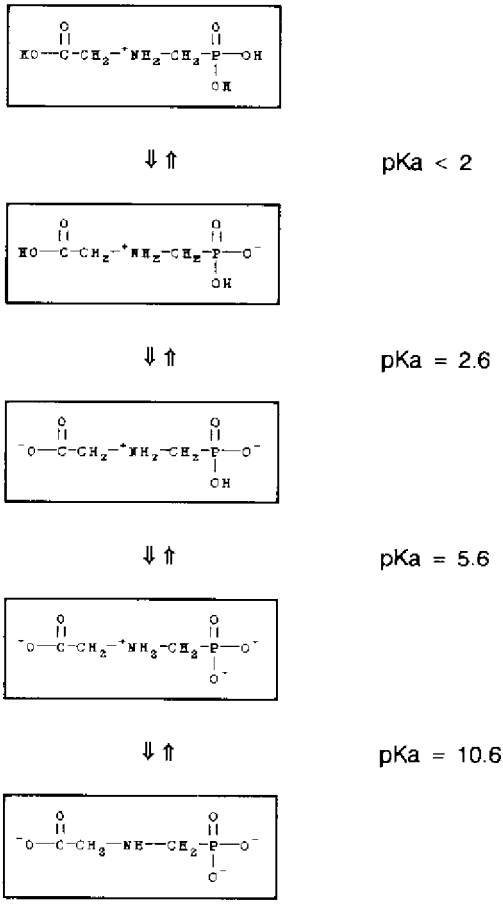


Fig. 1. Ionic species of glyphosate and their pKa values (Sprankle et al., 1975)

Table 2. This synopsis is not complete. Formulations may contain specific surfactants. The major formulation of glyphosate is Roundup containing 480 g/litre of the isopropylamine salt, which is equivalent to 360 g/litre of the free acid. Some other Roundup formulations that are characterized by other a.i. concentrations or other surfactants have been developed for specific applications.

Other formulations that have been developed for special equipment are Roundup Ultrabax for CDA equipment, Glyphosate Nomix for Nomix equipment, and EZ-JECT for tree injections. In Canada, Roundup was re-labelled as Vision in 1987 for use in forestry.

Table 2. Composition of various commercial formulations with glyphosate[a]

Name	Synonyms	Concentration a.i. (%)	Country
Roundup	Spasor,	48.0 (w/v);	Most countries
Sting	Vision,	41.0 (w/w)[b]	
	Swing, Arcade, Tomahawk	21.7 (w/w)	Belgium, Cameroon, France, Holland, Kenya, Malawi, Portugal, South Africa, Spain, United Kingdom
Armada	Frontier	16.6 (w/w)	Belgium, Cameroon, Ivory Coast, Gabon, Greece, Zaire
Dardo	Ricochet[g], Rival, Ultrasonic	12.2 (w/w)	Cameroon, Egypt, France, Greece, Israel, Italy, Portugal, Spain, United Kingdom
Squadron		20.2 (w/w)	Argentina, Australia, Columbia
Stirrup	Nomix, Expedite	18.3 (w/w)	France, United Kingdom
Wallop		20.8 (w/w)	Malaysia
Deploy Dry[c]		94.0 (w/w)	USA
Quotamaker[d]		75.0 (w/w)	USA
Landmaster II[e]		13.3 (w/w)	USA
Landmaster BW, Campaign[f]		12.9 (w/w)	USA
Roundup D-Pak		62.0 (w/w)	USA
Rodeo		53.8 (w/w)	USA
Ranger		28.6 (w/w)	USA
Roundup Lawn and Garden Conc.		18.0 (w/w)	USA
Roundup-Ready-To-Use		0.96 (w/w)	USA
Fusta		22.5 (w/w)	Spain

[a] all formulations produced by Monsanto Ltd; data provided by Monsanto Ltd
[b] based on the isopropylamine salt; equivalent to 36.0% (w/v) and 30.5% (w/w) of the free acid
[c] dry formulation of the monoammonium salt
[d] dry formulation of the sodium sesqui salt
[e] also contains 11.1% 2,4-D (isopropylamine salt)
[f] also contains 20.6% 2,4-D (isopropylamine salt)
[g] also contains simazine

Formulations may contain other active ingredients, e.g., simazine in Ricochet, 2,4-D in Landmaster, and MCPA in Fusta.

2.4 Conversion factors

1 ppm = 6.91 mg/m^3 at 25 °C and 101.3 kPa
1 mg/m^3 = 0.145 ppm

2.5 Analytical methods

2.5.1 *Sample handling and preparation*

The first preparative step before detection and measurement of glyphosate is generally extraction. As both glyphosate and its main metabolite aminomethylphosphonic acid (AMPA, see Fig. 2) show high polarity, and are therefore highly water soluble, they are difficult to extract with organic solvents. However, various methods have been developed. Some recently developed extraction methods for different media are summarized in Table 3.

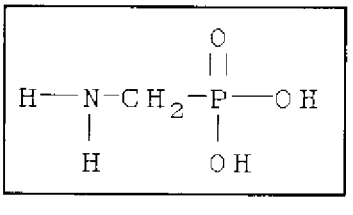


Fig. 2. The structural formula of AMPA

The second preparative step is the clean-up, which may include extraction, preconcentration by evaporation, ion-exchange chromatography or gel chromatography. Clean-up procedures may involve different combinations of chromatographic techniques. In a validation study in which plant tissues and water were analysed, a Chelex column was combined with anion-exchange clean-up (Cowell et al., 1986). No chromatography was included in the clean-up procedures for analysing glyphosate and AMPA in natural waters (Miles et al., 1986). In this procedure samples were successively filtrated, supplied with phosphate buffer, concentrated by evaporation, and filtrated, prior to derivatization.

Table 3. Sampling, preparation, and analysis of glyphosate

Medium	Sampling volume or weight	Preparations	Derivatization reagent	Analytical method	Limit of determination[a]	Recovery	Reference
Air	n.r.	collected onto an absorption liquid; evaporation to dryness	trifluoroacetic anhydride and trifluoroethanol	GC-MS and GC-EC	~ 0.3 µg/m^3	94%	Jauhiainen et al. (1991)
Cyanobacteria	100 ml	dry, resuspend in methanol/sodium acetate/triethylamine	PITC	HPLC with a radically compressed column	n.r.[b]	78%	Powell et al. (1990)
Plants	5 g	extraction with water/chloroform; preconcentration and clean-up on cation-exchange resin	trifluoroacetic anhydride and trifluoroethanol	GC-NPD	0.03 mg/kg	72-92%	Konar & Roy (1990)
Plants	25-50 g	extraction with water/chloroform; preconcentration and clean-up on anion-exchange and cation-exchange resin; evaporation to dryness		TLC with ninhydrin detection	0.01 mg/kg	n.r.	Bunyathyan & Gevorgyan (1984)
Water	250 ml	extraction with dichloromethane; adsorption on anion-exchange resin	*o*-phthalaldehyde	LC	3.2 µg/litre	89%	Wigfield & Lanouette (1990)

Table 3 (contd).

Medium	Sampling volume or weight	Preparations	Derivatization reagent	Analytical method	Limit of determination[a]	Recovery	Reference
Water	25 ml	extraction with dichloromethane/2-propanol; acidification with H_2SO_4; evaporation to dryness	FMOCCl	HPLC and TLC	0.02 µg/litre	80%	Gauch et al. (1989)
Water	1-1.5 litre	no extraction; preconcentration and clean-up with anion-exchange and cation-exchange resin		TLC with ninhydrin detection[c]	0.05 mg/litre	n.r.	Bunyathyan & Gevorgyan (1984)
Soil	5 g	extraction with deionized water/H_3PO_4; addition of Darco charcoal	trifluoroacetic anhydride/trifluoroethanol	GC-NPD	0.05 mg/kg	75%	Roy & Konar (1989)
Soil	2 g (sandy soil); 25 g (clayish soil)	extraction with KH_2PO_4 (sandy soil), KOH (clayish soil); no clean-up	FMOCCl	HPLC	1 mg/kg	80-119%	Miles & Moye, (1988b)
Soil, sediment, foliage	5 g (soil); 20 g (sed); 5 g (fol)	extraction with NH_4OH; adsorption on anion-exchange resin; further clean-up with Dowex cation-exchange resin	ninhydrin	LC	0.05-0.1 mg/kg (soil); 0.1 mg/kg (sed); 0.3 mg/kg (fol)[d]	73-79% (soil) 65-84% (sed) 81-84% (fol)	Thompson et al. (1989)

Table 3 (contd).

Urine and faeces of the rat	5-6 g	extraction with H_2O (only faeces); protein precipitation and lyophilization (only urine); clean-up with C_{18} column		HPLC (ion pair, strong anion and cation-exchange), LSC, ^{1}H NMR, ^{31}P NMR, GC/MS	n.r.	81-99%	Monsanto (1988a)
Urine (human male)	n.r.	adsorption on anion-exchange resin (SAX); elution of the resin with HCl; evaporation to dryness	trifluoroacetic anhydride/trifluoroethanol	GC-MS and GC-EC	0.1 mg/litre	n.r.	Jauhiainen et al. (1991)
Serum (human)	0.5 ml	extraction with trichloroacetic acid; adsorption on anion-exchange resin; elution with HCl; evaporation to dryness	*p*-toluene sulfonyl chloride	HPLC with UV detection	n.r.[e]	n.r	Tomita et al. (1991)

[a] In no study with a non-liquid medium was it reported whether the limit of determination was based on dry or fresh weight, except in the study of Thompson et al. (1989).
[b] The order of magnitude was reported to be picomol.
[c] The use of TLC with ninhydrin, copper nitrate and rhodamine B detection is reported for glyphosate in distilled water in Ragab (1978).
[d] The limits of determination in soil, sediment, and foliage are expressed per kg dry weight.
[e] Only the limit of detection was reported: 0.3 mg/litre (approximately 75% recovery).

PITC = phenylisothiocyanate; FMOCCl = 9-fluorenyl-methyl chloroformate; GC = gas chromatography; (HP)LC = (high-performance) liquid chromatography; TLC = thin layer chromatography; MS = mass spectroscopy; EC = electron capture detector; NPD = nitrogen-phosphorus detector; n.r. = not reported; LSC = liquid scintillation counting; NMR = nuclear magnetic resonance; sed = sediment; fol = foliage

Samples with urine and faeces of the rat were subjected to clean-up with a C_{18} column (Monsanto, 1988a). Prior to this extraction, proteins were precipitated and the samples were lyophilized; samples of faeces were, however, only extracted with water.

The third preparative step is derivatization. Derivatization with a fluorogenic reagent is common. Burns (1983), however, developed a preparation technique without derivatization. Derivatization prior to detection and measurement with HPLC can be pre-column (Miles et al., 1986; Lundgren, 1986; Miles & Moye, 1988a) or post-column (Moye et al., 1983; Tuinstra & Kienhuis, 1987). 9-Fluorenylmethyl chloroformate, phenylisothiocyanate and 1-fluoro-2,4-dinitrobenzene may be used as pre-column reagents, whereas ortho-phthalaldehyde-mercaptoethanol and ninhydrin may be used as post-column fluorogenic reagents. With post-column techniques, derivatives can be formed on-line, but it requires more equipment and experience. On the other hand, pre-column techniques are often more rapid and require less equipment and experience. In general the facilities required for derivatization with fluorogenic substances are very specific, and therefore not available in many laboratories (Konar & Roy, 1990). These authors proposed derivatization with a mixture of trifluoroacetic anhydride and trifluoroethanol prior to analysis with gas chromatography as a simpler, less laborious and more economical method. This proposal referred to the determination of glyphosate and AMPA in plant tissues. This and other recently developed techniques of clean-up and derivatization are summarized in Table 2. These techniques are intended to simplify and improve preparative techniques, which in general used to be complex and costly (Marcotte et al., 1977; Guinivan et al., 1982; Roseboom & Berkhoff, 1982; Moye et al., 1983; Moye & Deyrup, 1984; Deyrup et al., 1985; Miles et al., 1986; Lundgren, 1986; Miles & Moye, 1988b).

Sample preparation and derivatization, as developed by Powell et al. (1990) for cyanobacteria without deproteinization (see Table 3), should also be usable for plant and animal tissue. In this case, a simple maceration step prior to ethanol extraction should be included. Bunyathyan & Gevorgyan (1984) developed preparative techniques for different media prior to analysis with TLC. Only their procedures for plants and water are summarized in Table 3. The preparative technique for soil samples was comparable with that of Thompson et al. (1989), although samples of 25-50 g were required. Bunyathyan & Gevorgyan (1984) also developed a method for preparing 20-litre air samples prior to

TLC. They extracted the residues collected on a filter with water before clean-up on a cation-exchange resin.

2.5.2 *Analytical methods*

Various analytical methods for the determination of glyphosate have been described, including thin-layer chromatography (Young et al., 1977; Ragab, 1978; Bunyathyan & Gevorgyan, 1984), colorimetry (Glass, 1981), differential pulse polarography (Friestad & Brønstad, 1985), gas chromatography (Guinivan et al., 1982; Moye & Deyrup, 1984; Deyrup et al., 1985), high-performance liquid chromatography (Miles & Moye, 1988a; Powell et al., 1990), and ^{31}P NMR (Dickson et al., 1988). Some of these techniques, their analytical recoveries and limits of determination are listed in Table 3. The corresponding determination limits for AMPA, i.e. analysed with the same techniques, are listed in Table 4. Recoveries in the different media appear to be higher for glyphosate than for AMPA. This is probably due to optimization of the systems for glyphosate, as was done by Thompson et al. (1989).

Table 4. Limits of determination of AMPA

Medium	Limit of determination	Recovery	Reference
Plants	0.01 mg/kg	61-73%	Konar & Roy (1990)
Water	1.2 µg/litre	86%	Wigfield & Lanouette (1990)
Soil	0.01 mg/kg	66%	Roy & Konar (1989)
Soil	0.03-0.05 mg/kg	58-68%	Thompson et al. (1989)
Sediment	0.03 mg/kg	54-67%	Thompson et al. (1989)
Foliage	0.008 mg/kg	55-70%	Thompson et al. (1989)
Urine (human)	0.05 mg/litre	n.r.	Jauhiainen et al. (1991)
Serum (human)	n.r.[a]	n.r.	Tomita et al. (1991)

[a] n.r. = not reported; only the limit of detection was reported: 0.2 mg/litre (approximately 88% recovery)

TLC techniques are generally based on silica gel or cellulose plates; cellulose plates give a better separation (Dubelman, 1988). Ninhydrin and phosphate sensitive reagents may be used for

detection, although interference from co-extractives may occur. According to Dubelman (1988), fluorogenic reagents may be preferable in case of interference.

Fluorogenic derivatives can be determined in HPLC analysis with fluorescence detectors (Wigfield & Lanouette, 1990) and also with a spectrophotometer (Powell et al., 1990). In a GC analysis a nitrogen-phosphorus, electron capture or a flame photometric detector can be used.

3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

3.1 Anthropogenic sources

3.1.1 Production levels and processes

No data on the world production of glyphosate and its formulations are available. In addition, no data on losses to the environment during normal production and formulation or accidental losses have been reported.

The first phase of the production of glyphosate consists of refluxing a mixture of glycine (50 parts), chloromethylphosphonic acid (92 parts), an aqueous solution with 50% sodium hydroxide (150 parts), and water (100 parts) in a suitable reaction vessel. Another 50 parts of an aqueous solution with 50% sodium hydroxide are added to maintain the pH between 10 and 12, whereafter the reaction mixture is refluxed for another 20 h. The mixture is then cooled to room temperature and filtered. After adding 160 parts of concentrated hydrochloric acid, this mixture is again filtered. Glyphosate will slowly precipitate in the filtrate (IRPTC, 1991).

3.1.2 Uses

Glyphosate is a post-emergent, systemic and non-selective herbicide intended for use against deep-rooted perennial species, and also biennial and annual broad-leaved, grass and sedge species (WSSA, 1983; Monsanto, personal communication to the IPCS, 1991). Glyphosate is used in both agriculture and forestry. Fields of agricultural use include grassland renovation, horticulture, fructiculture, arable cultivation, and rice cultivation. Use in forestry includes the killing of fast growing competitors in conifer plantations or conservation areas, and the treatment of tree stumps. Glyphosate may also be used for weed killing in non-agricultural areas such as water systems, including irrigation and temporarily drained waters, parks, road verges and gardens.

The uses of glyphosate indicate that it can be applied in various crops for specific purposes. The major formulation Roundup may, for instance, be used in pre-plant treatments for seed bed preparations, and also against bracken infestations in forestry, against couchgrass (*Elytrigia repens*) infestations on

pastures, in direct treatments between rows of crops, or by direct wiping of the leaves of the weed, assuming the weeds are taller than the existing crop.

Glyphosate is used worldwide. In 1987, 35 160 ha of the area in British Columbia where vegetation management activities took place had been treated with Roundup. This was 94% of the total area where there were such activities (Ackurst, 1989).

The application rates of glyphosate are dependent on the formulation and type of use. In the Netherlands, recommended rates for the application of Roundup are 0.3-2.9 kg a.i./ha. In Canada the recommended application rates of Roundup are 1.1-1.7 kg a.i./ha for annual weeds and 1.2-5.8 kg a.i./ha for perennial weeds. The recommended application rates for Vision in Canadian forestry are 1.1-2.1 kg a.i./ha (Task Force on Water Quality Guidelines, 1991). Glyphosate is generally applied as a 0.5-5% solution in water by spraying, and as a 10-50% solution in water by wiping with, for instance, a rope-wick (Monsanto, personal communication to the IPCS, 1991).

The timing of application is dependent on the use. Application in late summer or autumn is recommended for use in forestry in Canada (Hildebrand et al., 1982). Application in agriculture may be pre- or post-harvest. In the Netherlands, for instance, glyphosate may be applied to cereals, potatoes and asparagus immediately (up to 7 days) before harvest, but only when the ripening is complete. Treatment of immature crops would result in higher residue levels, early crop desiccation and reduced yields.

Glyphosate may be applied in different ways. For large-scale treatments aerial application can be appropriate, small-scale treatments can be done with spraying equipment on the back or behind vehicles, or by wiping equipment.

Aerial applications will lead to losses due to wind-drift. Exposure of flora and fauna due to off-target deposits may take place. These downwind deposits depend on the meteorological conditions, the plant canopy structure and the application method, including the release height (Payne et al., 1989; Feng et al., 1990; Payne, 1992; Payne & Thompson, 1992). The non-volatile tank-mix fraction and the speed of the aircraft may influence the drop-size spectrum, and it can be expected that dispersal systems causing relatively small droplets and having a relatively low non-volatile fraction will cause the highest off-target deposits. Payne

(1992) assumed that the large differences in deposits in two comparable experiments were due more to different aircraft airspeeds than to different wind speeds. In these experiments the maximum deposits at a downwind distance of 50 m were 19 and 3 mg a.i./m^2 at aircraft airspeeds of 45 and 11–20 m/second, respectively. The application rate in both experiments was 2.1 kg a.i./ha. In other experiments with the same application rate, Payne & Thompson (1992) found that the meteorological conditions had a considerable impact on the off-target deposition up to 400 m downwind when spraying at different wind speeds (2.2–5.7 m/second) and turbulences. The deposits at a downwind distance of 400 m varied between 0.001 and 0.06 mg a.i./m^2, whereas they varied between 0.6 and 4 mg a.i./m^2 at a downwind distance of 50 m. Remarkably, the deposition was highest with an intermediate wind speed and intensity of turbulence. Payne et al. (1989) investigated the deposits for aerial applications of Roundup with different dispersal systems. When 2.1 kg a.i./ha was applied with a helicopter in a single crosswind swath over 100 ha, up to 13.4 mg a.i./m^2 was deposited on a downwind distance of 50 m. This maximum deposition was caused by a D8-46 hydraulic nozzle, whereas the highest depositions with a Thru Valve Boom and a Microfoil Boom were 2 and 0.4 mg a.i./m^2, respectively. These depositions were also found at a downwind distance of 50 m. At the time of application the windspeed 13 m above ground level was 0.4–0.5 m/second. Riley et al. (1991) modelled spray deposition of glyphosate using results from helicopter applications under semi-operational conditions. The study was designed to test the appropriateness of a New Brunswick "buffer zone" of 65 m to minimize the effects of spray drift. At a distance of 65 m, it was estimated that between 3.7% and 5.6% of the nominal spray rate was deposited.

3.1.3 Drinking-water

Appraisal

The low mobility of glyphosate in soil would indicate a minimal potential for the contamination of drinking-water from groundwater aquifers. The only possible source of drinking-water contamination is, therefore, surface waters. There have been no reported incidences of drinking-water contamination with glyphosate.

Conventional plants for processing of drinking-water would not remove glyphosate, but this could be achieved by coprecipitation after adding iron salts (AMA van der Linden, personal communication to

the IPCS, 1991). Ozone, increasingly used as an alternative to chlorine in drinking-water treatment, does effectively remove glyphosate through the hydroxyl radical ($HO^{\cdot}$) chain processes that occur in most ozonated waters (Yao & Haag, 1991; Haag & Yao, 1992).

4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION AND TRANSFORMATION

4.1 Transport and distribution between media

Appraisal

Following application, glyphosate selectively partitions to particulate matter suspended in surface water or to the soil substrate. This partitioning is usually rapid and occurred within 14 days in reported studies. The mechanism of sorption to soil is only partially understood. Glyphosate can adsorb to soils through phosphate binding sites. Competition with inorganic phosphate has been demonstrated in the laboratory but not measured in the field. Specific ions (Fe^{2+}, Fe^{3+} and Al^{3+}) complex glyphosate; metal complexes with humic acids in soil may be a main binding mechanism for glyphosate in soil. There is little reported information on desorption from soil; the data available suggest "strong" binding. This is supported by mobility studies which show little leaching of glyphosate below the upper few centimetres of the soil profile. The major metabolite, AMPA, is also retained in the upper soil layers.

There is very little information on the bioavailability of sediment-bound glyphosate to either aquatic or terrestrial organisms. Bioaccumulation and ecotoxicity studies have not, generally, been performed with added sediment.

Applied glyphosate can be translocated in plants. Glyphosate in plant foliage or leaf litter does not seem to represent a source of contamination of aquatic systems. Animals can ingest the herbicide residues in or on plants.

Dissipation of glyphosate from soil has been widely studied with very variable results (DT_{50} between 3 and 174 days). Biodegradation appears to be the major source of dissipation.

Run-off was minimal in experimental studies, but field results suggest that aquatic systems may be receiving glyphosate bound to soil particles following rainfall.

In this chapter the terms biodegradation and dissipation are used to distinguish between the decrease of the concentration in, for instance, the soil that is due to microbes transforming the

molecule to a smaller size (biodegradation) and the decrease of the concentration that might be due to microbial activity but also to other processes, e.g., sorption, leaching and run-off (dissipation).

4.1.1 Water

Glyphosate dissipates from the water with 50% dissipation times ranging from a few days to 2 weeks (Newton et al., 1984; Monsanto 1990a; see also Table 5). These DT_{50} values were deduced from both laboratory and field experiments in which sediment or suspended particles were shown to be the major sink.

In water with a near-neutral pH, the formation of an insoluble complex of Ca^{2+} with glyphosate was demonstrated in a laboratory experiment (Subramaniam & Hoggard, 1988). It was confirmed with X-ray powder diffraction and infrared spectra that this complex was not an ionic salt. At a near-neutral pH, the di-anionic species of glyphosate is dominant. Insoluble complexes have also been found with Mg^{2+}, Fe^{3+} and Cu^{2+}.

In a field experiment in a temperate coastal rainforest in British Columbia, Canada, the highest concentration of glyphosate in water was 162 µg/litre (Feng et al., 1990). This maximum was found in a directly sprayed tributary 2 h after an aerial application of Roundup at a rate of 2 kg a.i./ha. Concentrations in oversprayed tributaries without a high cover of overhanging riparian vegetation increased after the first rainfall. In oversprayed tributaries with a high cover of riparian vegetation almost no residues were found. Within 96 h after application the residues in all waters had declined below detection limits, indicating rapid dissipation. After rainstorms, peak concentrations of glyphosate were found in the sediments and on suspended particles of the oversprayed tributaries, with maximum concentrations of 7 mg a.i./kg dry weight and 0.06 µg a.i./litre unfiltered water, respectively. The amounts in the sediments of these waters were variable but declined over time. As 0.1-2 mg residue/kg dry weight sediment was found between 196 and 364 days after application, the residues appear to be persistent in sediments of oversprayed waters. Feng et al. (1990) concluded, therefore, that after rainstorms sediments appear to be the major sink.

In another field experiment in the same forest ecosystem, glyphosate dissipated rapidly from a small perennial, very slow flowing stream, in a site of 8 ha aerially sprayed with Roundup at

Table 5. Biodegradation[a] of technical grade glyphosate in water and sediments in the laboratory

Water type	Sediment type	Test type	Sediment (%)	Organic matter in sediment (%)	Temperature (°C)	pH of water	Experimental time (days)	Parameter	Time (days)	Reference
Pond water	silty clay loam	A	17	0.9	23-25	5.9-7.0	30	DT_{50}	14[b]	PTRL East Inc. (1990a)
Pond water	silty clay loam	An	16	0.9	20-27	5.7-6.5	365	DT_{50}	14[c,e]	PTRL East Inc. (1990b)
Surface water[d]	n.r.	A	9	n.r.	30	8.2-8.6	14	DT_{50}	< 14	Monsanto (1972a)
Lake water	sandy clay loam	An	33	1.4	30	6.6	42	DT_{50}	22[e]	Monsanto (1978a)

[a] Biodegradation in the whole system
[b] The biodegradation stopped after approximately 15 days
[c] The biodegradation stopped after approximately 150 days
[d] Three rivers and one lake in the USA
[e] Approximate value derived from data of the author(s)
A = aerobic; An = anaerobic; n.r. = not reported.

a rate of 3.3 kg a.i./ha (Newton et al., 1984). In water, 50% of the initial concentration had dissipated in 2 days. In sediment, maximum concentrations of approximately 0.6 mg a.i./kg were found 14 days after application. These were reduced to approximately 0.3 mg a.i./kg in 28 days, and to < 0.2 mg a.i./kg in 55 days after application. A comparable rapid dissipation from the water column was found for small forest ponds in a boreal forest in Manitoba, Canada, after applying Roundup at a rate of 0.9 kg a.i./ha (Goldsborough & Beck, 1989). The highest concentration in filtered water was 141 μg a.i./litre, within 6 h after application. The main mechanism of dissipation was probably sorption to the sediment. This was confirmed by additional experiments with polyethylene basins filled with unfiltered water and sediment that were placed in the spray zone. Without sediment, only a very small amount of the dose actually applied had dissipated after 30 days, whereas with sediment the initial concentrations in the water had decreased by 50%, approximately 6 days after application.

Comparable dissipation patterns were found in a field experiment (Monsanto, 1990a) in which Accord (30.5% a.i. w/w) was applied at a rate of 4.2 kg Accord/ha on three forestry sites with non-flowing pond water and flowing water. Concentrations of up to 1700 μg/litre filtered water were found in the pond water immediately after spraying. The initial concentrations in both pond and flowing water were reduced by 50% within 7 days. Concomitantly initial AMPA concentrations (maximally 35 μg/litre) were reduced by 50% within the same period. In flowing water the dissipation of both glyphosate and AMPA was even more rapid. Concentrations of glyphosate increased up to 19 mg/kg dry weight in the sediment of one pond 28 days after application. Concentrations of up to 1 mg/kg of both compounds were measured in the sediments of non-flowing ponds up to 400 days after application.

In field experiments in turbid Australian irrigation water, glyphosate adsorbed to suspended particles at different rates, apparently mainly depending on the application rate (Bowmer et al., 1986). At an initial concentration of 5 mg a.i./litre, 10–16% of the load adsorbed to suspended matter, whereas at an initial concentration of 0.05 mg a.i./litre, 53–71% adsorbed. In more saline water the degree of sorption was less, probably due to rapid flocculation. Maximum adsorbed amounts were approximately 7000 mg a.i./kg in less saline supply water, and approximately 2500 mg a.i./kg in more saline drainage water. When a supply

channel was emptied before spraying with 3.6 kg a.i./ha for control of aquatic weeds, and filled again with water 4 days after the treatment, the amount in the unfiltered water used for irrigation was 7% of the applied dose.

4.1.2 Soil sorption

Glyphosate is readily bound to many soils and clay minerals (Sprankle et al., 1975; Hance, 1976; Glass, 1987; Miles & Moye, 1988b). In laboratory experiments in which glyphosate was added to aqueous soil suspensions, the adsorption coefficient $K_{s/l}$ was 18-377 dm^3/kg in nine soils ranging from sandy loam to peat (Hance, 1976), and 33-76 dm^3/kg in three soils ranging from sandy loam to clay loam (Glass, 1987). These $K_{s/l}$ values indicate strong sorption. In both experiments the sorption could be described by the Freundlich equation. Glass (1987) found $K_{s/l}$ values for the clay minerals montmorillonite, illite and kaolinite of 138, 115 and 8 dm^3/kg, respectively.

The mechanism of sorption of glyphosate to soil is only partially understood. Several factors may be involved. The phosphonic moiety adsorbs weakly to unoccupied phosphate binding sites and can be displaced by phosphate (Hance, 1976). In laboratory experiments with nine soils the author showed that sorption was positively correlated with the unoccupied phosphate sorption capacity, and not correlated with the total phosphate sorption capacity, organic matter, clay, iron or aluminium content. No data are available that confirm competition of glyphosate and phosphate under field conditions, e.g., after application of artificial fertiliser. Miles & Moye (1988b) suggested that the main mechanism was probably by H-bonding and ion-exchange, as the degree of sorption in their experiments was not correlated with cation exchange capacity (CEC) values or surface areas. Contrary to the results of Miles & Moye (1988b) and of Hance (1976), sorption appeared to be correlated with CEC values and clay content in a sorption study with clay loam, silt loam and sandy loam (Glass, 1987).

The binding is also influenced by the presence of specific cations. Hensley et al. (1978) demonstrated that Fe^{2+}, Fe^{3+} and Al^{3+} inactivated glyphosate much more than Ca^{2+}, K^+ and Na^+. This was confirmed by Glass (1987) and Sprankle et al. (1975). Glass (1987) suggested that glyphosate was complexed by cations, released from cation-saturated clays via a cation-exchange with solution protons.

According to Heinonen-Tanski (1989), most of the soil-bound residues of glyphosate were recovered in the fulvic acid fraction (21-33%). Sorption of glyphosate to fulvic acids was also reported by Madhun et al. (1986), who added ^{14}C-glyphosate to an aqueous soil extract (ASE) of peat. In this study sorption was mainly on ASE fractions with a relative molecular mass ≤ 1000. Piccolo et al. (1992) studied the interaction of glyphosate with a pure iron-humic acid complex. Maximum adsorption values indicated that adsorption to the complex occurred to as great an extent as to whole soils. This suggested that humic acid complexes with polyvalent cations might represent a main binding substrate for glyphosate in soils. There was no desorption of bound residues of glyphosate following shaking with 0.01 mol $CaCl_2$/litre solution for 48 h, the maximum shaking time for the adsorption studies.

Desorption of glyphosate with ionized water from montmorillonite and illite needed three days before reaching an equilibrium in a study of Miles & Moye (1988b).

It can be concluded that sorption of glyphosate can be expected in the presence of available phosphate binding sites, the presence of iron and aluminium (oxides or hydroxides), and appropriate combinations of clay and organic matter.

4.1.3 Mobility in soils

In view of its $K_{s/l}$, glyphosate can be expected to be immobile or slightly mobile in many soils. This was confirmed by several experiments, both in the laboratory and in the field. In thin-layer chromatography studies with sandy loam, clay loam and sandy clay loam, the Rf values of ^{14}C-glyphosate were 0.14-0.20 (Sprankle et al., 1975). In comparable studies with silt loam, silty clay loam, and sandy loam Rf values were < 0.2 (Monsanto, 1972c). In a leaching study with columns of 30 cm and a high water flux of 51 cm over less than 2 days, < 0.1-6.6% of the applied activity was leached (Monsanto, 1978b). This experiment was performed with eight soils, ranging from sandy loam (organic matter content 0.7%) to volcanic ash (organic matter content 9.5%). More than 90% of the applied activity was recovered in the upper 0-14 cm layer.

Only one leaching study under laboratory conditions with respect to the mobility of AMPA has been reported. In this experiment with 30-day-old residues, < 0.1-1.6% of the applied activity was leached over 45 days (Monsanto, 1978b). The columns were 30 cm and the water flux over 45 days was low

(17 cm). The amount of AMPA that was recovered after 45 days in the upper 0-2 cm layer was low (0.5-12% of the applied activity), due to a high rate of mineralisation.

4.1.4 Dissipation from the soil in the field

Many field experiments on the dissipation of glyphosate from the soil have been performed. Some relevant studies are summarized in Table 6. They indicate DT_{50} values based on dissipation that range from 3 to 174 days depending on edaphic and climatic conditions. In a forest brush ecosystem in Oregon, USA, the DT_{50} value in loam was 29 days with and 40 days without litter (Newton et al., 1984). In field experiments in Sweden, Roundup was sprayed over reforestated sites (Torstensson et al., 1989). In the soils of these sites the DT_{50} values were $\leq$ 50 days, apparently depending on the soil respiration rate. The dissipation consisted of a fast first, and a much slower second phase, especially in sites in northern Sweden, which was possibly due to a longer frost period. In these sites 1-2% of the actually applied dose was recovered 1080 days after application. A comparable dissipation pattern was found in a field experiment on Finnish agricultural soils (Heinonen-Tanski et al., 1985). In this experiment 25% of the concentration in a sandy loam 2 days after the treatment was recovered one year after application. The application rate was 1.4 kg a.i./ha.

A study in a temperate coastal rain forest in British Columbia, Canada, showed that, 360 days after application, 6-18% of the initial levels was recovered (Feng et al., 1990). In this experiment Roundup was applied at a rate of 2 kg a.i./ha. The soils were alluvial sandy loam or sandy clay loam with highly organic surface horizons. Some of these soils were well drained, others were seasonally flooded. At each sampling time more than 90% of the recovered residues was in the upper 0-15 cm layer. Under all conditions the amount of glyphosate declined over time, whereas there was a transient increase of AMPA.

In other field experiments on boreal forest soils, comparable dissipation patterns were found. Stark (1983) reported DT_{90} values of 30-720 days, and Roy et al. (1989b) found a DT_{50} value of approximately 20 days on a sandy soil planted with jackpines (*Pinus banksiana*). In the field experiments of Roy et al. (1989b), glyphosate was detectable up to 335 days after application; almost all residues in the sandy soil were recovered in the organic top layer. In field experiments of Monsanto (1990a) in three forest

Table 6. Biodegradation and dissipation of glyphosate in soils

Soil type	Compound	Test type	Moisture content (%)	Temperature (°C)	pH	Organic matter (%)	Experimental duration (days)	DT_{50} (days)	Reference
Biodegradation									
Sandy loam	Tgg	L,A	14-16	25	7.3	2.8	360	2[b]	PTRL East Inc. (1991)
Silt loam	Tgg	L,A	12-14	25	7.5	1.0	360	2[b]	PTRL East Inc. (1991)
Dissipation									
Sandy loam	Tgg	G	11	32	5.7	1.0	112	130[b]	Monsanto (1972b); Rueppel et al. (1977)
Silt loam	Tgg	G	11	32	6.5	1.0	112	3[b]	Monsanto (1972b); Rueppel et al. (1977)
Silty clay loam	Tgg	G	11	32	7.0	6.0	112	25-27[b]	Monsanto (1972b); Rueppel et al. (1977)
Sand (humo-ferric podsol)	Ru	F	n.r.	n.r.	3.5-3.7	40	762	~ 20[a]	Roy et al. (1989b)
Sandy loam, sandy clay loam	Ru	F	n.r.	n.r.	4.2-4.9	15-31	360	45-60[b]	Feng & Thompson (1990)
Loam	Ru	F	n.r.	n.r.	4.0-4.7	3.8-5.2	55	29-40[b]	Newton et al. (1984)
Loamy sand	Ru	F	n.r.	n.r.	n.r.	0.8	370	3-4[b]	Monsanto (1983a)
Sandy clay loam	Ru	F	n.r.	n.r.	n.r.	7.0	370	122-174[b]	Monsanto (1983a)

[a] Based on data of the author(s) [b] Data reported by the author(s)

L = laboratory study; F = field study; G = greenhouse study; A = aerobic; An = anaerobic; Tgg = technical grade glyphosate; Ru = Roundup; n.r. = not reported

locations in the USA, the concentration course of glyphosate appeared to be rather irregular, especially during the first four months. However, 50% of the initial concentrations in the soil had mostly dissipated within 120 days. One clear exception was a site in Corvallis in which glyphosate increased up to 0.15 mg/kg dry weight, 180 days after application. On the same site AMPA increased up to 0.32 mg/kg, 346 days after application. The application rate in these experiments was 4.2 kg Accord/ha.

On a clay soil of a clear-cut boreal forest area, Roy et al. (1989b) found no dissipation of glyphosate due to run-off on a 8° slope. In a field experiment on agricultural soils without conventional tillage, the dissipation of glyphosate due to run-off on 6-16° slopes was < 1% of the applied dose when 1.1-3.4 kg a.i./ha was applied (Edwards et al., 1980). However, when 9.0 kg a.i./ha was applied, 1.8% of the applied dose dissipated due to run-off, mainly because of a rainstorm shortly after application.

4.1.5 Uptake and dissipation from plants

Uptake of ^{14}C-glyphosate by leaves of trembling aspen seedlings (*Populus tremuloides*) was initially rapid, after which it slowed down (Sundaram, 1990). The seedlings were exposed to Roundup that was dripped with a micro-applicator on some central leaves. The application rate was 0.35 kg a.i./ha leaf surface area. Most activity was washable from the leaves (61-77%), and 22-28% was recovered in the treated leaves within 48 h. As only 1-10% was recovered in the other parts of the seedlings, this indicated a rather low translocation after absorption. A rapid uptake of ^{14}C-glyphosate within a few hours was indicated for sugar beets (*Beta vulgaris*), when applied to a mature leaf (Gougler & Geiger, 1981). ^{14}C-glyphosate probably entered the phloem in a non-facilitated way. The subsequent transport through the phloem appeared to be according to an "intermediate permeability mechanism". When exposed for a longer time, plants may show substantial translocation of absorbed ^{14}C-glyphosate, as was shown for potatoes (*Solanum tuberosum*) by Smid & Hiller (1981). In the treated leaves of the potatoes 45% of the absorbed activity was recovered, whereas the rest was mainly translocated to the apical meristem and the roots. Up to 5% was recovered in the mother tuber. The degree of translocation was age-dependent, as older plants showed less translocation than younger plants.

The uptake of glyphosate by red raspberries (*Rubus strigosus*) was 9% of the amount that was deposited on the leaves after

spraying Roundup at a rate of 2 kg a.i./ha (Roy et al., 1989a). In the same field experiment the uptake was 14% by wild blueberries (*Vaccinium myrilloides*). Most glyphosate was recovered in the washings, which was also found under laboratory conditions. The initial absorbed amounts were 0.92–2.0 mg a.i./kg dry weight. The absorbed and washable amounts together were reduced by 50% within 13 days in the raspberries and within 20 days in the blueberries. AMPA was detectable up to 33 days after application. Metabolism occurred to only a minor extent as AMPA concentrations were less than 1.5% of the concurrent concentrations of glyphosate (similar results were reported by FAO/WHO, 1986b). In a field experiment by Feng & Thompson (1990) in a temperate coastal rainforest in British Columbia, Canada, the main target species for treatment with Roundup were red alder (*Alnus rubra*) and salmonberry (*Rubus spectabilis*). Immediately after spraying, the concentrations in leaf tissue were up to 448 mg a.i./kg dry weight. Glyphosate dissipated rapidly from the leaf litter with a DT_{50} value of 8–9 days. The leaf litter included leaves directly exposed on the trees and existing leaf litter from natural defoliation before treatment with Roundup. The authors assumed that leaf litter of these major brush species is an insignificant source of glyphosate input into streams or onto forest floor, because of the fast dissipation. A rapid dissipation of glyphosate from fresh foliage was also found in a field study (Monsanto, 1990a) in which initial concentrations of up to 1300 mg a.i./kg and 2.6 mg AMPA/kg decreased rapidly. A transient accumulation of glyphosate and AMPA was found in the leaf litter on some sites, but these amounts were reduced by approximately 90% within 100 days.

Glyphosate dissipated completely from wild berries (*Vaccinium vitis-idaea*, *Vaccinium myrtilus*) within one year in a field experiment in Finland in which Roundup was applied at a rate of 0.25–2.2 kg a.i./ha with a knapsack sprayer (Siltanen et al., 1981). Contrary to this dissipation pattern was that of glyphosate in reindeer lichens (*Cladonia rangiferina*) that were sampled in the same experimental plots. Around 270 days after application, dose-related concentrations of glyphosate and AMPA were recovered in lichens with maxima of 45 and 2.1 mg/kg for glyphosate and AMPA, respectively. Approximately 390 days after application of 0.8 kg a.i./ha, 6.4 and 0.3 mg/kg of glyphosate and AMPA were still detectable.

4.1.6 Ingestion by animals

As the concentration in the foliage may increase up to high amounts immediately after application, this implies the possibility of entry into the food chain through ingestion by herbivorous or omnivorous animals. This was confirmed by Sullivan & Sullivan (1979) who investigated the effects of glyphosate on the feed preference and daily chow consumption of black-tailed deer (*Odocoileus hemionus columbianus*). These herbivores did not avoid eating browse of alder (*Alnus rubra*) and alfalfa (*Medicago sativa*) that was treated with glyphosate at a rate of 2.2 kg/ha. Sometimes the treated alder browse was even preferred. Reindeer may be exposed to glyphosate, since reindeer lichens, which are an important food source, can take up a substantial amount of glyphosate (see above).

4.2 Abiotic degradation

Appraisal

Hydrolysis of glyphosate is very slow. Photodegradation in the field may occur.

4.2.1 Hydrolytic cleavage

Hydrolysis of glyphosate in sterile buffers is very slow. After 32 days ≤ 6.3% of the applied activity was recovered as AMPA, after applying ^{14}C-glyphosate at rates of 25 and 250 mg/litre to aqueous buffer solutions of pH 3, 6 and 9 (Monsanto, 1978b). These tests were performed at both 5 and 35 °C.

4.2.2 Photodegradation

Photochemical degradation in water may occur under both laboratory and field conditions, mainly depending on the type of light source. In sterile aqueous buffers of pH 5, 7, and 9, less than 1% of the applied dose was degraded (photodecomposition of ^{14}C-phosphonomethyl-labelled glyphosate) during 29-31 days, when exposed to sunlight (PTRL Inc., 1990).

Lund-Hoie & Friestad (1986) exposed Roundup to several light sources under different conditions. When exposed to UV light (λ = 254 nm) under laboratory conditions, concentrations of 1 and 2000 mg a.i./litre in deionized water showed DT_{50} values of 4 and

14 days, respectively. When exposed to sunlight under field conditions 1 mg a.i./litre in polluted water without sediment showed a much slower decomposition (DT_{50} > 63 days), possibly due to pollution preventing adequate UV penetration in the water. Polluted water with sediments showed a rapid dissipation from water, probably due to adsorption onto the sediments. In another field experiment 2 and 100 mg a.i./litre in deionized or polluted water without sediment showed DT_{50} values of ≤ 28 days, when exposed to sunlight. At the low concentration the dissipation in polluted water was more rapid than in deionized water. In the dark no dissipation occurred.

In laboratory experiments 1 mg/litre of glyphosate in sterilized natural and deionized water showed DT_{50} values of 4 to > 14 days when exposed to artificial light (350-450 nm) in photoreactors without sediment (Monsanto, 1978a). In these experiments Ca^{2+} acted as a photosensitizing agent.

Photodegradation by sunlight of glyphosate applied to a soil appeared to be an insignificant route of dissipation (PTRL Inc., 1989). In this study, ^{14}C-glyphosate mixed with unlabelled glyphosate was exposed for 31 days to natural sunlight, after application to a sandy loam at a rate of 4.5 kg a.i./ha. Extrapolated DT_{50} values that were based on first-order kinetics were 90 days in the sunlight and 96 days in the dark, indicating no substantial degradation due to photolysis. The temperature of the soil surface was 22-23 °C. Under unnatural light conditions glyphosate appeared not to be photodegraded substantially (Monsanto, 1972c; Rueppel et al., 1977; Monsanto, 1978a).

4.3 Biodegradation

Appraisal

Selected studies of the biodegradation of glyphosate have been considered; selection was on the basis of test conditions and modern methodologies. There is considerable variation in rate of breakdown in water, aquatic sediment and soil. Degradation occurs more rapidly in aerobic than anaerobic conditions. Half-times for biodegradation in the three media under laboratory conditions range between a few days and approximately 20 days. No data on biodegradation under anaerobic conditions are available.

The main route of biodegradation of glyphosate appears to be by splitting the C-N bond to produce AMPA. However, a second route with splitting of the C-P bond can also occur.

A range of bacterial strains can degrade glyphosate. Bacteria capable of using the compound as sole phosphorus, sole carbon or sole nitrogen source have been identified. Growth is slow compared to growth on inorganic sources of P, C or N. There is evidence from the field that bacterial populations adapt to the metabolism of glyphosate. Presence of inorganic phosphate inhibits degradation of glyphosate with some, but not all, bacteria. Biodegradation of glyphosate may involve co-metabolism.

The most relevant laboratory experiments in which the biodegradation in systems with water and sediment have been studied are summarized in Table 5. These studies indicate that the rate of biodegradation may vary substantially, depending on experimental conditions, e.g., the availability of oxygen, temperature and type of sediment. The time needed for 50% biodegradation of glyphosate in the whole system of a test with water and sediment is $\leq$ 14 days under aerobic and 14-22 days under anaerobic conditions in the laboratory.

In the experiments of PTRL East Inc. (1990a,b), less then 10% of the applied activity was recovered in the pond water over a period of 30 days under aerobic condition and 365 days under anaerobic conditions. During all experiments more than 50% of the applied activity was recovered in the sediment.

In experiments with water and their associated sediments the amount of a.i. declines over time with a generally transient increase of ^{14}C-AMPA, an increase of $^{14}CO_2$, and an increase of sediment-bound residues. An exception to this pattern of biodegradation can be observed in some aerobic and anaerobic experiments that were performed with pond water and a silty clay loam sediment (PTRL East Inc, 1990a,b). In this water/sediment system the biodegradation stopped after approximately 15 days under aerobic conditions and after approximately 150 days under anaerobic conditions. The glyphosate residues (a.i. plus AMPA) at both time points remained approximately 40% of the applied dose, which indicated substantial persistence in spite of the rapid initial degradation.

AMPA is the main metabolite of glyphosate found in both the water column and the sediment. Maximum amounts of AMPA under both aerobic and anaerobic conditions in the sediment were 25% of the applied activity (PTRL East Inc., 1990a,b). These maxima were found at 7-20 days after application. In the same experiments maximum amounts of sediment-bound residue were 9% of the applied activity under aerobic conditions and 4% under anaerobic conditions. These maxima were found at the end of the experiments. The amounts of evolved $^{14}CO_2$ in these studies gradually increased in most cases up to 24 and 35% of the applied activity after 30 days (aerobic), and 365 days (anaerobic), respectively. This indicates substantial differences in the mineralization rate. These differences are partly due to the availability of oxygen, since under anaerobic conditions the mineralization rate was slower than under aerobic conditions. This was also found by Monsanto (1972a, 1978a). In the aerobic experiments of Monsanto (1972a), four sediments that differed by up to two orders of magnitude in the total number of micro-organisms did not show substantial differences in mineralization rate.

Biodegradation studies with glyphosate in the soil under conditions where unequivocal interpretation is justified are scarce. Table 6 summarizes some relevant studies, indicating that the biodegradation rate may differ substantially, depending on the experimental conditions. The laboratory and greenhouse experiments in Table 6 were performed with moisture contents ($\geq$ 75% of the field capacity) that were adequate for optimal biodegradation.

In most laboratory experiments the biodegradation rate of glyphosate in soils appears to be rapid (see Table 6). Mostly biodegradation can be described with linear first-order kinetics.

Sometimes a non-linear first-order model taking into account spatial variability better describes the results observed (PTRL East Inc., 1991):

$$C = C_0 (1 + \beta t)^{-\alpha}$$

C in this equation is the concentration in the soil at time t, C_0 the initial concentration, and α and β are rate constants reflecting spatial variability.

The main metabolite under aerobic conditions of glyphosate in soil is AMPA. In aerobic laboratory experiments the maximum amounts in sandy loam and silt loam were 27 and 29%, respectively, of the applied activity. These maxima were reached 14 days after application (PTRL East Inc., 1991). From the data of PTRL East Inc. (1991), DT_{50} values for AMPA of approximately 50 days in sandy and silty loam can be derived. That AMPA is more persistent than glyphosate was also shown in a laboratory experiment with sandy loam (Monsanto, 1972b). The amounts of AMPA after 111 days were 10-17% of the applied activity. In this study, the temperature (32 °C) was higher than in the other studies discussed above.

Some minor unidentified metabolites were quantified in an aerobic laboratory experiment lasting 364 days with sandy loam and silt loam (PTRL East Inc., 1991). Two unknown metabolites did not exceed 3.5% of the applied activity, whereas some other unknown metabolites did not exceed 1.5% each. Rueppel et al. (1977) quantified some minor metabolites that did not exceed 1% of the applied activity. These metabolites were *N*-methylaminomethylphosphonic acid, glycine, *N*,*N*-dimethylaminomethylphosphonic acid, hydroxymethylphosphonic acid, and two unknown metabolites.

In aerobic laboratory experiments, the amounts of soil-bound residues immediately after application were 9-35% of the applied dose, after which they showed an irregular time-course during these experiments of approximately 112 days (Monsanto, 1972b). In general, the initial amounts were also the maximum amounts. In other laboratory experiments however, maximum amounts of soil-bound residues appeared to be reached after 14 days, whereafter they remained more or less constant or even decreased (PTRL East Inc., 1991). These maximum amounts were 7-9% of the applied activity, and were probably lower compared with other studies due to better extraction procedures.

Mineralization in the soil occurs under both aerobic and anaerobic conditions in the laboratory, although the rates may differ greatly, apparently mainly depending on the soil respiration rate and the temperature. When ^{14}C-phosphonomethyl-labelled glyphosate was applied to sandy loam and silt loam, 70-78% $^{14}CO_2$ evolved during an aerobic laboratory experiment of 360 days (PTRL East Inc., 1991). In this study the application rate was 4 mg a.i./kg dry weight. In an aerobic laboratory study with 15 Swedish forest soils, DT_{50} values based on $^{14}CO_2$ evolution varied

between 6 and 200 days. Mineralization was highly correlated with the soil respiration rate, but not with pH or organic matter content (Torstensson & Stark, 1981). This was confirmed by Torstensson & Stenström (1986) and Heinonen-Tanski (1989). Torstensson & Stenström (1986) reported that glyphosate was co-metabolized. In this case, co-metabolizing microorganisms are not supplied with energy by biodegrading glyphosate.

Establishing the correlation between soil respiration and mineralization requires both a standardized measurement of the respiration rate and an accurate measurement of the actual dose that reaches the soil (Torstensson & Stenström, 1986). In a laboratory experiment simulating temperatures under arctic conditions in forest soils, 51-71% of the applied activity was recovered as $^{14}CO_2$ 217 days after application of ^{14}C-glyphosate. In this study the mineralization rate was reduced 10-15 times during a temperature decrease of 10 °C over the first part of the study. The rate increased only 3.7-4 times with a temperature increase of 10 °C during the second part (Heinonen-Tanski, 1989).

Glyphosate in the soil appears to be degradable by micro-organisms in two ways (Jacob et al., 1988), as shown in Fig. 3. One route is via the formation of AMPA and a C_2 fragment which might be glyoxylate. This scheme for degradation was proposed by many researchers (Monsanto, 1972b; PTRL East Inc., 1991). In this route the splitting of the C-N bond is the first step. There is, however, another route of biodegradation via sarcosine (*N*-methyl-glycine) and orthophosphate, after which sarcosine is degraded to glycine and a one-carbon unit that eventually might form CO_2 via formaldehyde (Kishore & Jacob, 1987; Jacob et al., 1988). In this route the splitting of the C-P bond is the first step. In experiments with ^{14}C-glyphosate, isolated cultures of *Pseudomonas* sp. strain LBr were able to degrade glyphosate according to both routes (Jacob et al., 1988). Approximately 5% of the applied ^{14}C-glyphosate was not degraded via AMPA, but via sarcosine.

The growth rate of bacteria isolated from a sandy loam garden soil that was sprayed with Tumbleweed (a garden product) was less inhibited by technical grade glyphosate than the growth rate of bacteria from an unsprayed control (Quin et al., 1988). This indicated adaptation of the bacterial populations of the sprayed site. As addition of aromatic amino acids prevented growth inhibition in the population of the unsprayed site to a greater extent than in the population of the sprayed site, different

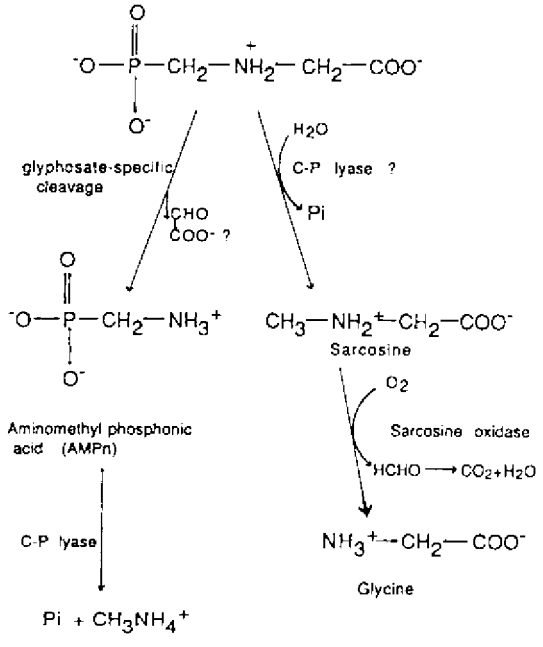


Fig. 3. Degradation routes of glyphosate in soil (Liu et al. 1991)

mechanisms of biochemical interference were indicated. The composition of the bacterial population on the unsprayed site was also different from the sprayed one. *Pseudomonas* sp. and lactose-fermenting bacteria could be identified in an inoculum from the sprayed soil able to use glyphosate as a sole source of phosphorus (Quinn et al., 1988). A different regulatory mechanism for biodegradation in unsprayed and sprayed sites was assumed: in the latter the aromatic amino acid pathway might be regulated by direct control of 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) by the end-products, whereas in the unsprayed site DAHP synthase might be indirectly regulated by prephenate. Also in other experiments bacteria were shown to use glyphosate as a sole P source (Kishore & Jacob, 1987; Pipke & Amrhein, 1988; Weidhase et al., 1990), thereby primarily degrading glyphosate to

orthophosphate and sarcosine, by splitting the C-P bond. In the study of Weidhase et al. (1990), 18.2% of the applied activity was recovered as sarcosine 8 h after application of ^{14}C-1-methyl-labelled glyphosate to a pure culture of *Pseudomonas* sp. GS. This biodegradation route of glyphosate via sarcosine was also demonstrated by Kishore & Jacob (1987). In their experiments with glyphosate as sole P source for *Pseudomonas* sp. PG2982, one hour after application of ^{14}C-labelled glyphosate, glycine, phosphate, and a one-carbon unit, possibly formaldehyde, were identified as metabolites. After one hour, the $^{14}CO_2$ evolution when the phosphonomethyl moiety was labelled was substantially higher, as compared with the 1- or 2-glycine-labelled moieties. The authors suggested that the so-called phosphate-starvation-inducible proteins, as identified by others, might be responsible for cleaving the C-P bond. In an experiment with pure cultures of a mutant of *Arthrobacter* sp. GLP-1 able to use glyphosate as a sole P source, 90% of the applied activity was released as orthophosphate at 240 h after application of ^{14}C-1-methyl-labelled glyphosate (Pipke & Amrhein, 1988). Orthophosphate inhibited further biodegradation of glyphosate. *Flavobacterium* sp. was found by Balthazor & Hallas (1986) to be able to degrade glyphosate in spite of the presence of orthophosphate. Liu et al. (1991) showed that 12 strains of bacteria from the family *Rhizobiaceae* could degrade glyphosate present in the medium as the sole phosphorus source; although growth of the bacteria was slower than with inorganic phosphate. Sarcosine was the intermediate breakdown product, indicating initial cleavage of the C-P bond, in *Rhizobium meliloti*, the strain used for detailed metabolic studies.

Carlisle & Trevors (1986a) deduced from their experiments that nitrate-reducing bacteria are involved in metabolizing glyphosate. Involvement of nitrifying bacteria in the biodegradation of glyphosate was also demonstrated by Murthy et al. (1989), when they investigated the treatment of waste water from a Roundup formulating factory.

Pseudomonas sp. may use glyphosate as a sole P or C source, as demonstrated by Weidhase et al. (1990). Only slight growth of the wild-type strain of the bacterium *Pseudomonas fluorescens* was observed with glyphosate as sole carbon or nitrogen source. The herbicide was metabolized to aminomethylphosphonate (Zboinska et al., 1992). Murthy et al. (1989) isolated a denitrifying bacterial species that was also able to use glyphosate as a C source. This species was isolated from activated sludge in a waste-water

treatment plant. A mutant of *Arthrobacter* sp. strain GLP-1 was able to utilize glyphosate as a sole N source, whereas this was not possible for the normal strain (Pipke & Amrhrein, 1988), probably due to the uptake of inorganic P released during biodegradation.

As the Biological Oxygen Demand and the Chemical Oxygen Demand of glyphosate are < 2 mg/g and 0.53 g/g, respectively, glyphosate cannot be considered as readily biodegradable (LISEC, 1990a,b). In suitable systems, however, glyphosate is biodegradable, as shown by Murthy et al. (1989), who investigated the biodegradation of glyphosate in waste-water treatment plants under different conditions in sequencing batch reactors on a laboratory scale. These reactors were fed with waste water from a Roundup manufacturing facility. Glyphosate was degraded completely within one cycle of 24 h, independent of whether there was an initial aerated or anoxic phase of 4 h. However, more glyphosate could be processed with an anoxic initial phase, probably due to better conditions for denitrification. Not only denitrifiers but also ammonifiers and nitrifiers appeared to be involved in the biodegradation of glyphosate. Only at the very high concentration of approximately 5000 mg a.i./litre was biodegradation repressed by non-glyphosate COD and inhibited by excess ammonia production.

Pseudomonas sp. strain LBr, *Flavobacterium* sp. and a denitrifying bacterial species were isolated from activated sludge as species with the ability to use glyphosate as a P source (Balthazor & Hallas, 1986; Jacob et al., 1988; Murthy et al., 1989). The denitrifier was also able to use glyphosate as a sole C source. *Flavobacterium* sp. degraded glyphosate to AMPA in both the presence and absence of PO_4^{3-} (Balthazor & Hallas, 1986). In this experiment the further degradation of AMPA appeared to be hampered in the presence of PO_4^{3-}.

Pseudomonas sp. strain LBr was capable of completely eliminating amounts of glyphosate up to 3212 mg/litre from a growth medium (Jacob et al., 1988).

Continuous exposure of an activated sludge treatment system in a pilot plant increased the ability of the sludge to metabolize glyphosate to AMPA (Hallas et al., 1992). In this trial an influent concentration of 50 mg a.i./litre was reduced to less than 5 mg a.i./litre under continuous-flow conditions with an average residence time of 10 min. The sludge was inoculated with immobilized bacteria capable of degrading glyphosate. The

effectiveness of the treatment was dependent on the presence of a nitrogen source and a non-glyphosate carbon source, and required a pH range of 6.0 to 8.0.

No data are available on the amounts of glyphosate that can be eliminated in conventional waste-water treatment plants under practical conditions. In waste water from glyphosate-producing plants, 28-45% is reported to be eliminated through biological treatment (Task Force on Water Quality Guidelines, 1991).

No data are available on the biodegradability of the surfactants in formulations. It is, however, probable that polyoxyethylene amine is biodegraded fairly rapidly in view of the biodegradability of structurally related compounds (Swisher, 1987).

4.4 Bioaccumulation

Appraisal

Glyphosate is not expected to bioaccumulate in view of its high water solubility and its ionic character. This was confirmed by several laboratory experiments with fish, crustaceans and molluscs and by field experiments.

In a static test, channel catfish (*Ictalurus punctatus*) were exposed to 0.94-0.99 mg ^{14}C-labelled a.i./litre (actual concentrations) for 10 days (ABC Inc, 1981d; Monsanto, 1981a). Of the absorbed amount, 76% was recovered in the viscera. More than 90% of the extractable residues in the viscera and the fillet was identified as glyphosate, whereas less than 2% was identified as AMPA. After 10 days of depuration 80% of the absorbed activity was eliminated. For exposed channel catfish the calculated bioconcentration factor based on the activity absorbed by the whole fish was 0.27. For depurated channel catfish the calculated bioconcentration factor was 0.052.

The marsh clam (*Rangia cuneata*) and crayfish (*Procambarus simulans*) were exposed in static tests lasting 28 days to synthetic uncontaminated sea water and a sandy loam sediment that was incorporated with 3 mg ^{14}C-labelled a.i./kg (ABC Inc., 1982d,e). These experiments were set up to assess the degree of bioconcentration of glyphosate when used in flooded rice levees and tidal water. The calculated bioconcentration factor for the edible parts of the clam increased during exposure up to 4.8,

whereas for the whole crayfish it increased up to 12. The highest concentrations in the edible parts of the clam and the whole crayfish were 0.3 mg ^{14}C-labelled residues/kg for both. After 28 days of depuration 48% of the accumulated residues were eliminated from the edible parts of the clam. The concentration in these parts was then 0.16 mg ^{14}C-residues/kg. The crayfish finally had eliminated 91% after 14 days of depuration. The concentration in the whole crayfish was then 0.02 mg ^{14}C-residues/kg. It must be stated that this test refers to the accumulation of ^{14}C and not glyphosate.

In a static test without sediment, in which rainbow trout (*Salmo gairdnerii*) were exposed to 2 mg a.i./litre (nominal concentration) for 12 h, the fillets of the fish contained 80 µg a.i./kg (in the original article the erroneous figure of 80 mg/kg was reported), and the eggs 60 µg a.i./kg (Folmar et al., 1979). This indicates a bioconcentration factor of 0.04 for the edible parts. Roundup was applied in this test.

In a flow-through test in which bluegill sunfish (*Lepomis macrochirus*) were exposed to 11-13 mg ^{14}C-labelled a.i./litre (actual concentrations) for 35 days, calculated daily bioconcentration factors based on the whole fish increased from < 0.1, 0.2 days after the start of the test, to 0.4-0.5 at the end (ABC Inc., 1989f). Maximum concentrations in the whole fish, viscera and fillet were 13, 7.6 and 4.8 mg ^{14}C-residues/kg, respectively. The time required to reach 90% of the steady state and the uptake rate constant were calculated to be 120 days and 0.02 mg/kg fish x (mg/litre water)$^{-1}$ x day^{-1}, respectively. During 21 days of depuration, the half-life of depuration was calculated to be 35. A slow decrease in tissue concentration during depuration was indicated. After the period of depuration 2.2 mg ^{14}C-residues/kg whole fish was still present. In an additional study to characterize the ^{14}C-residues, 95-97% of the residues in the water was glyphosate, whereas in the whole fish and tissues 28-91% of the recovered activity was glyphosate (ABC Inc., 1989g). In a whole fish sample 21 days after starting the test, 49% of the recovered activity was found to be AMPA. By treating homogenates with proteinase K it was indicated that a substantial amount of the absorbed residues was tightly associated with, or incorporated into, protein.

In a field experiment in a forest ecosystem in Oregon, USA, neither glyphosate nor AMPA were recovered in salmon fingerlings (*Oncorhynchus kisutch*) after aerial application of

Roundup at a rate of 3.3 kg a.i./ha (Newton et al., 1984). The fingerlings were released at the downstream edge of the sprayed site and analysed up to 55 days after treatment. Glyphosate was not recovered in carp (*Cyprinus carpio*) in a field experiment in which ponds were sprayed with Roundup at rates of 1.3-1.4 kg a.i./ha (Monsanto, 1980). In this experiment of approximately 90 days, AMPA was not recovered until 30 days after application. It then increased up to 0.21 mg/kg whole fish, remained constant for another 30 days, and then decreased to around the limit of determination (0.1 mg/kg) at the end of the experiment.

In a forest ecosystem in Oregon, USA, Roundup was aerially applied at a rate of 3.3 kg a.i./ha (Newton et al., 1984). Concentrations in mammals were of the same order of magnitude as the concentrations in litter and ground cover. The concentrations of glyphosate in the viscera of herbivorous small mammals decreased more slowly than in omnivorous and carnivorous small mammals, which was probably due to a higher ingestion of contaminated litter. The highest concentration was found in the viscera of omnivorous deermice (*Peromysces maniculatus*) immediately after spraying: 5 mg a.i./kg. Only small traces of AMPA were found in mammalian viscera.

4.5 Waste disposal

Small amounts of glyphosate can be disposed of by mixing with alkali and soil prior to burial in a pit or trench, whereas large amounts should be incinerated in units equipped with effluent gas scrubbing (IRPTC, 1991).

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Appraisal

The low toxicity, low volatility and low body absorption of glyphosate makes its application by backpack sprayer safe under field condition provided that the worker wears full protective clothing.

5.1 Environmental levels

A synopsis of concentrations of glyphosate is tabulated in Table 7. Measurements as part of regular monitoring programmes are very scarce; measurements in field experiments with recommended application rates simulating common agricultural practice are therefore included in Table 7. Only maximum amounts are tabulated as indicative values, since the rate at which they dissipate is not included here (see sections 4.1 and 4.3). Data on the occurrence of glyphosate and AMPA in sewage sludge are not available.

In biota the highest concentrations of glyphosate and AMPA were found in fresh foliage and reindeer lichen (*Cladonia rangiferina*). In abiota the highest concentrations of both compounds were found in the soil (see Table 7). The occurrence of glyphosate in the groundwater of Texas, USA, was reported by Hallberg (1989), but the measured concentration and the year of measurement were not specified.

Use of glyphosate as a herbicide may result in the presence of residues in crops and animal tissues destined for human consumption. Application as a herbicide may also be responsible for the presence of glyphosate in drinking-water. Direct measurements of glyphosate in foodstuffs (as part of food surveillance), drinking-water or total diets have not been carried out. The only information available comes from controlled residue studies. With technical glyphosate formulated as the isopropylamine salt in aqueous solution, numerous residue studies have been carried out in vegetables, grasses, oil seeds, mammalian products, poultry products and primary feed commodities. The results are summarized in the various reports of the FAO/WHO Joint Meeting on Pesticide Residues (FAO/WHO, 1986a, 1987, 1988). For detailed information on these studies the reader is referred to these reports. The appraisals made by the JMPR

Table 7. Maximum concentrations of glyphosate in environmental air, water, soil, sediment and biota

Sample	Compound	Location	Year[a]	Concentration	Reference
Netherlands					
Surface water	glyphosate	Drentsche Aa	1988-1989	0.5-1 µg/litre[b]	(Soppe, personal communication to the IPCS, 1991)
Surface water	AMPA	Drentsche Aa	1988-1989	6 µg/litre[b]	
Finland					
Loam soil (agric.)	glyphosate	Kettula	1978	17 mg/kg d.w.	Müller et al. (1981)
Loam soil (agric.)	AMPA	Kettula	1978	3.2 mg/kg d.w.	Müller et al. (1981)
Silt soil (agric.)	glyphosate	Kettula	1978	3.8 mg/kg d.w.	Müller et al. (1981)
Silt soil (agric.)	AMPA	Kettula	1978	0.4 mg/kg d.w.	Müller et al. (1981)
Wild berries	glyphosate	Laukaa, Konnevesi	1977	1.6-2.1 mg/kg[c]	Siltanen et al. (1981)
Wild berries	AMPA	Laukaa, Konnevesi	1977	0.02-0.07 mg/kg[c]	Siltanen et al. (1981)
Reindeer lichen	glyphosate	Laukaa, Konnevesi	1976	45 mg/kg[c]	Siltanen et al. (1981)
Reindeer lichen	AMPA	Laukaa, Konnevesi	1976	2.1 mg/kg[c]	Siltanen et al. (1981)
Canada					
Wild berries	glyphosate	Harker, Lamplugh	1985	8-19 mg/kg f.w.	Roy et al. (1989a)
Wild berries	AMPA	Harker, Lamplugh	1985	0.06-0.1 mg/kg f.w.	Roy et al. (1989a)
Surface water[d]	glyphosate	Carnation Creek	1984	162 µg/litre	Feng et al. (1990)
Surface water[e]	glyphosate	Carnation Creek	1984	< 1 µg/litre	Feng et al. (1990)
Surface water[d]	AMPA	Carnation Creek	1984	~ 3 µg/litre	Feng et al. (1990)

Table 7 (contd).

Canada (contd).					
Pond water	glyphosate	Manitoba	1986	141 µg/litre	Goldsborough & Beck (1989)
Pond water	AMPA	Manitoba	1986	2.2 µg/litre	Goldsborough & Beck (1989)
Sediment[d]	glyphosate	Carnation Creek	1984	6.8 mg/kg d.w.	Feng et al. (1990)
Suspended sediment	glyphosate	Carnation Creek	1984	0.06 µg/litre	Feng et al. (1990)
Soil	glyphosate	Carnation Creek	1984	40 mg/kg d.w.	Feng & Thompson (1990)
Soil	AMPA	Carnation Creek	1984	9 mg/kg d.w.	Feng & Thompson (1990)
Foliage (fresh)	glyphosate	Carnation Creek	1984	261-448 mg/kg d.w.	Feng & Thompson (1990)
Foliage (fresh)	AMPA	Carnation Creek	1984	< 9 mg/kg d.w.	Feng & Thompson (1990)
Topcrown foliage	glyphosate	Oregon Coast Range	1978	489 mg/kg[c]	Newton et al. (1984)
Topcrown foliage	AMPA	Oregon Coast Range	1978	2.1 mg/kg[c]	Newton et al. (1984)
Deermice (viscera)	glyphosate	Oregon Coast Range	1978	5.1 mg/kg[c]	Newton et al. (1984)
USA					
Pond water	glyphosate	Chassell, Corvallis, Cuthbert	1987	90-1700 µg/litre	Monsanto (1990a)
Pond water	AMPA	Chassell, Corvallis, Cuthbert	1987	2-35 µg/litre	Monsanto (1990a)
Stream water	glyphosate	Chassell, Corvallis, Cuthbert	1987	35-1237 µg/litre	Monsanto (1990a)
Stream water	AMPA	Chassell, Corvallis, Cuthbert	1987	< 1.0-10 µg/litre	Monsanto (1990a)
Pond sediment	glyphosate	Chassell, Corvallis, Cuthbert	1987	0.26-19 mg/kg d.w.	Monsanto (1990a)

Table 7 (contd).

Sample	Compound	Location	Year[a]	Concentration	Reference
USA (contd)					
Pond sediment	AMPA	Chassell, Corvallis, Cuthbert	1987	0.13-1.8 mg/kg d.w.	Monsanto (1990a)
Stream sediment	glyphosate	Chassell, Corvallis, Cuthbert	1987	0.11-0.69 mg/kg d.w.	Monsanto (1990a)
Stream sediment	AMPA	Chassell, Corvallis, Cuthbert	1987	< 0.05-0.38 mg/kg d.w.	Monsanto (1990a)
Soil (no litter on it)	glyphosate	Chassell, Corvallis, Cuthbert	1987	0.15-4.7 mg/kg d.w.	Monsanto (1990a)
Soil (no litter on it)	AMPA	Chassell, Corvallis, Cuthbert	1987	0.18-0.51 mg/kg d.w.	Monsanto (1990a)
Soil (litter on it)	glyphosate	Chassell, Corvallis, Cuthbert	1987	0.07-1.4 mg/kg d.w.	Monsanto (1990a)
Soil (litter on it)	AMPA	Chassell, Corvallis, Cuthbert	1987	0.14-0.68 mg/kg d.w.	Monsanto (1990a)
Foliage (fresh)	glyphosate	Chassell, Corvallis, Cuthbert	1987	650-1300 mg/kg[b]	Monsanto (1990a)
Foliage (fresh)	AMPA	Chassell, Corvallis, Cuthbert	1987	1.7-2.6 mg/kg[b]	Monsanto (1990a)

[a] Sampling invariably took place during the autumn period.
[b] Analytical procedure was unvalidated; the water was sampled at an inlet point of a drinking-water processing facility
[c] It was not reported whether values were based on dry or fresh weight
[d] Water unbuffered by vegetation
[e] Water buffered by vegetation

included the following more general statements. Pre-harvest (5-14 days) application of glyphosate (isopropylamine salt) in the cultivation of cereals results in significant residues in the grain and plant materials. Studies are available to show the fate of glyphosate in milling, baking and brewing. Residue levels in white flour were approximately 10-20% of the levels in wheat, while the bran residue levels were 2 to 4 times as high as those in the wheat. Glyphosate residues were not lost during baking, but residue levels decreased when bread was made from flour because of dilution. Glyphosate residue levels in malt and beer derived from field-treated barley were, respectively, about 25% and 4% of the original level in the barley. Some glyphosate is lost during washing, but most of the decrease can be attributed to dilution. The levels in groats (processed oats) were about 50% of the levels in the pre-harvest-treated oats. In all these cases, AMPA contributed only a small proportion (average < 2.5%) of the total residues (FAO/WHO, 1986a, 1987).

When administered to animals glyphosate is rapidly excreted without degradation. Residues in cattle, pig and poultry meat, eggs and milk were negligible after the animals were fed with a diet containing 100 mg/kg glyphosate and aminoglyphosate acid. The highest residues were in pig liver and kidney (up to 0.16 and 0.91 mg/kg, respectively) and cattle kidney (up to 1.4 mg/kg). Residues in animal feeds arising from pre-harvest glyphosate applications to cereals will result in only low residues in meat, milk and eggs (FAO/WHO, 1986a).

On the basis of these residue studies the JMPR has estimated the maximum residue levels that are likely to occur when glyphosate (as isopropylamine salt) is used in practice, and recommended these levels as Maximum Residue Limits (MRLs). These MRLs are presented in FAO/WHO (1986a, 1987, 1988).

5.2 General population exposure

Apart from the controlled residue studies mentioned above, no data are available.

5.3 Occupational exposure during manufacture, formulation or use

In the study of Monsanto (1977), worker exposure to Roundup was measured during herbicide mixing and application operations and upon re-entry of treated fields. The formulation was applied

on separate plots using three application devices, i.e. a broadcast boom sprayer, a handgun broadcast sprayer and a backpack/handgun sprayer. Exposure time during mixing was ≤ 5 min; during application this was about 45 to 60 min. Inhalational exposure during mixing and spraying was determined using a sampling device placed close to the applicator's face; the total air volume sampled was 15-20 times the daily ventilation volume. Dermal exposure during mixing and spraying was monitored by determination of deposition on gauze pads placed outside or inside the operator's clothing on different parts of the body. Operator exposure (inhalation and skin, determination method as above) upon re-entry was determined at 1, 3 and 7 days after application. In one treated plot, this was done by having operators walk through the plot for 28 to 44 min; in two other plots a dummy sampling device was used to determine exposure upon re-entry. Using the measured glyphosate residues total worker exposure was estimated. The results are presented in Table 8.

Table 8. Applicator/worker exposure to glyphosate[a]

Operation	Average dermal exposure (μg/h)	Average inhalation exposure (μg/h)[b]	Total exposure (μg/h)
Tank filling	805.1	17.9	823.0
Boom spraying	271.4	0.19	271.59
Handgun spraying	7957.0	2.47	7959.47
Backpack spraying	3619.0	0.92	3619.92
Field re-entry:			
1 day after treatment	2046.6	4.58	2051.18
3 days after treatment	2919.7	0.12	2919.82
7 days after treatment	15.9	0.12	16.02

[a] From: Monsanto (1977)
[b] Calculation based on an estimated breathing rate of 1.8 m^3/h

Monsanto (1990) conducted another collaborative study at three sites maintained by the USDA Forestry Service near Clayton, Georgia; the Savanah River Plant, South Carolina; and Edgefield, South Carolina. Glyphosate was being used to control vegetative

growth around pine seedlings planted in clear-cut forest areas. The workers were biologically monitored by analysis of collected composite urine specimens. Additionally, dermal/clothing deposition and simulated inhalation exposure were monitored by passive dosimetry with cotton cloth patches, hand rinses and air filters. At the three sites, exposure of workers to glyphosate using backpack sprayers while performing their duties under normal use conditions was monitored. The analytical results indicated that the majority of urine composite samples had unmeasurable residues of glyphosate. Deposition on air filters, patches and hands from measurement of washes, however, indicated a small amount of body exposure. It was concluded that penetration of clothing did not exceed 3.84% and thus clothing produced 96.2% protection. Body burden, as shown in urine samples, was extremely low and in most cases below the detection limit.

A new Monsanto study was conducted for the assessment of worker exposure to glyphosate during mist blower application of Roundup herbicide. Exposure was determined by a passive dosimetry technique while workers sprayed weeds around palm trees in a plantation in Malaysia. The workers were fitted with gauze patches at different locations on their clothing. Air sampling was performed in the breathing zone and the workers hands were washed at the end of the day. The passive dosimetry body dose estimates were calculated for a fully clothed worker with a long-sleeved shirt, long pants and rubber boots. The hand exposure would account for bare hands during the loading and spraying operations. Passive dosimetry estimates for the four replicates, corrected for clothing and dermal penetration, transport/storage/analytical recovery and normalized for body weight and amount of chemical handled, averaged 1.88 μg/kg body weight per kg a.i. This is little higher than the passive dosimetry estimates of forestry workers who applied Roundup with knapsack sprayers, which was 1.75 μg/kg body weight per kg a.i. Thus, it can be concluded that workers applying Roundup herbicide with mist blowers, experience some dermal exposure. In addition, inhalation exposure to glyphosate may be higher during mist blower application. However, Roundup demonstrated essentially no volatility. The only possible route would be via airborne particles. The actual amount of glyphosate absorbed through inhalation would be much lower than the estimated values because the measurement includes particles too large to be inhaled (Monsanto, 1991).

Jauhiainen et al. (1991) examined the exposure of workers to glyphosate during silvicultural clearing with brush saws equipped with herbicide sprayers. Measurements of air concentrations during spraying and urinary glyphosate levels both during and following spraying were carried out. Most of the air samples had glyphosate concentrations below 1.25 $\mu g/m^3$; the highest value observed was 15.7 $\mu g/m^3$. All urine samples taken had glyphosate concentrations below the limit of detection of 0.1 mg/litre (Jauhiainen et al., 1991).

Lavy et al. (1992) used two methods to monitor glyphosate exposure of workers planting conifer seedlings. Firstly, they estimated dermal exposure based on the rate of deposition on cotton gauze patches, surface area exposed, and a dermal penetration rate of 1.8%. This yielded dose estimates in the range of 0–875 or 0–1.7 $\mu g/kg$ body weight per h. Secondly, they attempted to measure urinary concentration levels but found no samples above the detection limit of 0.01 mg/litre. Based on the negative results with the urine samples, the authors concluded that the estimates based on patch deposition overestimated exposure by at least a factor of 11 for the most highly exposed workers (Lavy et al., 1992).

It should be noted that a dermal penetration rate of 1.8% was used in this calculation. In view of the discussion of the dermal penetration studies (section 6.1), a value of 5.5% is to be preferred. However, since the estimate based on 1.8% is already an overestimation of exposure, it is not considered necessary to adjust the estimate of Lavy et al. (1992) to 5.5%. In their final data assessment, the authors estimated exposure from the biological monitoring using postulated data, i.e. assuming a concentration in urine of half the lower limit of method validation. This yielded mean exposure values of 0.039 to 0.080 $\mu g/kg$ body weight per h (Lavy et al., 1992).

6. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Appraisal

Absorption from the gastrointestinal tract after oral intake is limited to 30-36% of the dose or less in various species, i.e. rats, rabbits, laying hens and lactating goats. Percutaneous absorption in Rhesus monkeys amounts to 5.5% only, and glyphosate is very poorly absorbed through excised human abdominal skin.

Radiolabelled glyphosate distributes widely in the body, but is primarily found in the bones where approximately 1% can be detected after oral administration.

Glyphosate is essentially not metabolized. This validates kinetic studies performed with radiolabelled compound.

After absorption, excretion of glyphosate occurs mainly in the urine. Biliary excretion is limited and elimination through exhaled air is very low. Total body clearance is 99% after 168 h.

6.1 Absorption

The absorption percentage in rats was reported to be 30–36% after single oral dosage at 10 and 1000 mg/kg body weight; calculations were based on excretion percentages in urine and faeces, and on the fact that biliary elimination is probably a minor route (Monsanto, 1988b; Brewster et al., 1991). From a similar study carried out in 1973, total absorption percentages of approximately 20% (male rats) and 45% (female rats) can be derived (Monsanto, 1973a). Comparable results were obtained in a recent single dose (5.6 or 56 mg/kg body weight) disposition study in F344/N rats (NTP, 1992), which indicated that 30% of the dose was absorbed.

In a 14-day oral study in rats with application of ^{14}C-glyphosate via the diet (dose levels 1, 10 and 100 mg/kg feed), the observed total excretion in urine was $\leq$ 10% and in faeces approximately 80–90%. Given the minor importance of the biliary elimination route, these data indicate absorption levels of about 15% or less (Monsanto, 1973c). The results of oral studies with

^{14}C-glyphosate in rabbits (Monsanto, 1973d), laying hens (Hazleton Lab. Inc., 1988a) and lactating goats (Hazleton Lab. Inc., 1988b) indicate gastrointestinal absorption percentages of approximately 30% or less.

Percutaneous absorption has been studied in Rhesus monkeys and in human tissue *in vitro*. After a single application of ^{14}C-glyphosate (isoproylamine salt) as the undiluted Roundup formulation to the shaven intact abdominal skin (contact time 24 h) of Rhesus monkeys, absorption amounted to only 1.8% of the dose. In this study, however, only 16% of the dose could be accounted for at the end of the study (Maibach, 1983). This low recovery strongly reduces the value of the study result (possibly skin absorption is seriously underestimated in this study). From an identical study with diluted Roundup formulation (1:29 with water), conducted by Wester et al. (1991), also in Rhesus monkeys (contact time 12 h), total absorption percentages of 3.7% (at low dose) or 5.5% (high dose) can be derived. Using both undiluted and diluted Roundup formulation, Wester et al. (1991) observed that percutaneous absorption of ^{14}C-glyphosate through human skin *in vitro* into human plasma as receptor fluid was $\leq$ 2% (contact-time up to 16 h). In another *in vitro* study with human skin, absorption of ^{14}C-glyphosate from three undiluted formulations (i.e. MON 0139, Roundup and Roundup spray mix) was studied (contact time 24 h); very low absorption percentages of 0.028 to 0.152% were found (Franz, 1983). With regard to these *in vitro* results it should be pointed out that this technique has not yet been fully validated and therefore direct extrapolation to *in vivo* human skin absorption should be undertaken cautiously.

The absorption after inhalational intake has not been determined.

6.2 Distribution

Concentrations of ^{14}C label in tissues were determined on day 7 after administration of a single oral dose (10 or 1000 mg/kg body weight) of ^{14}C-glyphosate to rats (Monsanto, 1988b). Although only a small proportion was absorbed, the isotope was widely distributed throughout the body, but was primarily found in bone. The principal results are presented in Table 9.

In rats tissue concentrations of ^{14}C label were determined on several occasions throughout a treatment period of 14 days and a post-dosing withdrawal period of 10 days (dietary administration

of ^{14}C-glyphosate at 1, 10 and 100 mg/kg diet). Maximum tissue levels were reached after 10 days or less, with highest concentrations (maximum 0.85 mg/kg at the 100 mg/kg dose level) in kidneys (Monsanto, 1973c). It should be noted, however, that in this study concentrations in bone or bone marrow were not measured. An increase of ^{14}C in excreta was observed during the withdrawal period after an initial rapid decrease; this indicated mobilization from storage in depot tissue (Monsanto, 1973c).

Table 9. Concentrations of ^{14}C label (as mg glyphosate-equivalents/kg fresh weight) in selected tissues of rats on day 7 after a single oral dose (rounded values) (Monsanto, 1988b)

	Dose: 10 mg/kg body weight		Dose: 1000 mg/kg body weight	
	male	female	male	female
Blood	0.0045	0.0027	0.33	0.17
Liver	0.030	0.014	1.9	1.3
Kidney	0.022	0.013	1.9	1.4
Spleen	0.012	0.0073	2.6	3.0
Lung	0.015	0.012	1.5	1.1
Thyroid	0.00080	0.00036	1.5	1.2
Nasal mucosa	0.0050	0.023	1.7	1.8
Stomach	0.0080	0.0037	2.4	2.4
Small intestines	0.022	0.018	1.9	1.6
Colon	0.034	0.016	11.0	9.2
Bone	0.55	0.31	30.6	19.7
Bone marrow	0.029	0.0064	4.1	12.5

In lactating goats concentrations of ^{14}C label in milk were measured after giving capsules containing a 9:1 mixture of ^{14}C-glyphosate and ^{14}C-aminomethylphosphonic acid (AMPA) to a dose level equivalent to 120 mg/kg diet (expressed as free acid) for 5 days. Concentrations in milk (as mg equivalents glyphosate/kg whole milk) ranged from 0.019 to 0.086 mg/kg during the test period; at day 5 after the last dose the concentration was 0.006 mg/kg (Hazleton Lab. Inc., 1988b; Monsanto, 1988d).

In a study on laying hens, carried out using a 9:1 mixture of ^{14}C-glyphosate and ^{14}C-AMPA, concentrations of radiolabel were measured in eggs collected during a 7-day period of dietary administration at 120 or 400 mg/kg diet. At 400 mg/kg, residues in egg white were 0.010-0.032 mg/kg (expressed as glyphosate-equivalents) and in egg yolk 0.096-0.753 mg/kg; at 120 mg/kg the corresponding concentration ranges were 0.003-0.017 and 0.002-0.24 mg/kg, respectively. At 120 mg/kg diet, no ^{14}C was detectable in egg white after 10 withdrawal days; in yolk 0.019 mg/kg was present at that time (at 400 mg/kg no withdrawal test was conducted) (Hazleton Lab. Inc., 1988a).

6.3 Metabolic transformation

Biotransformation of glyphosate occurs to a very low degree only. In rats it was shown that all of the ^{14}C in urine and faeces, after a single oral application of ^{14}C-glyphosate, was present as unchanged parent compound (Monsanto, 1973b). Also in rats, $\geq$ 97% of the ^{14}C in excreta, after a single oral dose, was shown to be unchanged compound. AMPA was the only metabolite, covering only 0.2-0.3% of the applied ^{14}C (Monsanto, 1988a). In laying hens also, AMPA was the only metabolite, accounting for only a minor part of the applied amount (Monsanto, 1988c).

6.4 Elimination and excretion

In the period of 0-5 days after a single oral application of ^{14}C-glyphosate (6.7 mg/kg body weight) to rats the total excretion in urine was 15% (males) and 35-43% (females) of the administered dose; total excretion in faeces was 85% (males) or 50-55% (females). Less than 1% of the radiolabel was expired as $^{14}CO_2$ (Monsanto, 1973a). In a more recent study (Monsanto, 1988b), the very low level of expiration as $^{14}CO_2$ was confirmed but no significant inter-sex difference in the level of ^{14}C in excreta was observed. The result of the latter study was that at both oral dose levels (10 and 1000 mg/kg body weight) elimination in faeces was 62-70% and excretion in urine was 14-18% (1000 mg/kg body weight) or 22-29% (10 mg/kg body weight); less than 0.2% of the dose was expired as $^{14}CO_2$. After single intravenous application (dose 10 mg/kg body weight) 75-79% appeared in urine and only 5-8% in faeces, a finding that shows that biliary elimination occurs to a limited degree only (Monsanto, 1988b).

Delayed excretion in rats during a 10-day post-dosing withdrawal period was observed after daily oral administration via

the diet for 14 days (Monsanto, 1973c); this suggests that some storage in tissue(s) occurs when uptake is prolonged. Tissue equilibrium was attained by day 10 of the dosing period and excretion equalled intake by day 6 of the dosing period.

In rabbits ≥ 80% was eliminated in faeces (with additional ^{14}C present in the gut) and 7-11% in urine within 5 days after administration of a single oral dose (6.7 mg/kg body weight) of ^{14}C-glyphosate. Less than 1% of the dose was expired as $^{14}CO_2$ (Monsanto, 1973d). In one oral study in lactating goats lasting 5 days, total excretion in urine varied from 20 to 24% and in faeces from 60 to 66% (Hazleton Lab. Inc., 1988b).

6.5 Retention and turnover

Total body clearance in the study of Monsanto (1973a) was 94-98% (males) or 82-84% (females) over a 48-h period after giving a single oral dose of ^{14}C-glyphosate; at 120 h post-dosing this was 99% (both sexes). In Monsanto (1988b), the kinetics of whole body elimination were estimated using the radioactivity (^{14}C) measured in urine and faeces after a single oral dose of ^{14}C-glyphosate (10 or 1000 mg/kg body weight). Because of the lack of biotransformation of glyphosate it is valid to base kinetics on total radioactivity. The elimination appeared to be biphasic. The half-life of the alpha elimination phase at 10 mg/kg body weight was 5.87 h (males) or 6.22 h (females); at 1000 mg/kg body weight this was 5.26 h (males) or 6.44 h (females). The half-life of the beta phase at 10 mg/kg body weight was 79 h (males) or 106 h (females); at 1000 mg/kg body weight this was 181 h (males) or 337 h (females). Pretreatment with unlabelled compound for 14 days (carried out at the low dose level) did not have an effect on whole body elimination. Seven days after dosing, < 0.05% of the dose was present in organs and < 0.5% in the remaining carcass. Highest concentrations were present in bone. It was estimated that 0.2-0.6% of the oral dose was associated with this site; after intravenous dosing this was approximately 1% (Monsanto, 1988b). Brewster et al. (1991) reported that in rats nearly all of the absorbed material had been eliminated from the body 168 h after oral administration of 10 mg/kg body weight; approximately 1% of the dose was still associated with the bone.

In a recent study on the disposition of glyphosate in F-344/N rats, 1% of a single oral dose (5.6 or 56 mg/kg) was found in the tissues 72 h after dosing; 20-30% of the administered radioactivity was eliminated via urine and 70-80% via the faeces (NTP, 1992).

7. EFFECTS ON LABORATORY ANIMALS AND *IN VITRO* TEST SYSTEMS

Appraisal

Glyphosate, administered by oral and dermal routes, has a very low acute toxicity. Both glyphosate and its concentrated formulations produce moderate to severe eye irritation, but only slight dermal irritation. Neither glyphosate nor tested formulations induce sensitization.

Short-term feeding studies have been conducted in several species. In CD-1 mice, increased liver, brain, heart and kidney weights, and growth retardation were reported at 50 000 mg/kg diet. At 10 000 mg/kg diet, an increase in relative liver weight was reported; however, there were no differences in absolute liver weights when this group was compared to controls. The relative increase represented only a 9% increase over the liver weight reported for controls and was not considered toxicologically significant. Additionally, there were no gross or histopathological changes in the liver at doses of 10 000 mg/kg or more. The Task Group considered the NOAEL to be 50 000 mg/kg diet.

In a 13-week study conducted in Charles River CD (Sprague-Dawley) BR rats, no treatment-related effects were observed at doses up to 20 000 mg/kg diet. The NOAEL was greater than the highest dose tested.

Two additional 13-week studies (one in rats and the other in mice) were conducted by NTP in which lesions of the salivary glands were observed in both species. The NOAEL in the rat study was < 3125 mg/kg diet and that in the mouse study was 3125 mg/kg diet. Other short-term and long-term studies conducted in different strains and species did not reveal similar lesions. The lesions indicate that glyphosate may be acting as a weak adrenergic agonist. The toxicological significance of the salivary gland lesions observed in the NTP studies is unknown.

In a 52-week study conducted in beagles, no compound-related effects were reported. The NOAEL was 500 mg/kg body weight per day. In a 7-day study with the Roundup formulation in female cattle, a NOAEL of 400 mg Roundup/kg body weight was reported. At higher dose levels, decreased feed intake and diarrhoea occurred.

In long-term feeding studies in both rats and mice, few toxic effects were observed. These effects were present at relatively high dose levels only. In mice, technical glyphosate produced growth retardation, hepatocyte hypertrophy or necrosis at 30 000 mg/kg diet only. At 5000 and 30 000 mg/kg diet an increase in epithelial hyperplasia of the urinary bladder was reported. The increased incidence of this lesion did not follow a dose-related trend and in the highest dose tested the incidence was actually lower than that reported at the medium dose level, in spite of a 6-fold increase in glyphosate. The observation at the medium dose (5000 mg/kg) is not considered a compound-related effect and the NOAEL is, therefore, considered to be 5000 mg/kg diet (814 mg/kg body weight).

Long-term feeding studies in rats resulted in decreased growth, increased liver weight and degenerative liver changes at 20 000 mg/kg diet only. At 8000 and 20 000 mg/kg diet, there was an apparent increase in the incidence of inflammation of the gastric mucosa in both sexes. The only statistically significant increase was observed in the medium-dose females (15%). This value was also outside the historical control range of 0-13%. This finding was not considered to be a treatment-related effect. There was no dose-related trend across all groups of treated females and there was no statistically significant difference in any treated male groups. The NOAEL was therefore 8000 mg/kg diet (410 mg/kg body weight).

Studies in rats and rabbits indicated that technical glyphosate is not teratogenic. Two multigeneration studies were conducted with technical glyphosate. In the first study, the only effect noted was an increased incidence of unilateral renal tubular dilation in F_{3b} male pups at 30 mg/kg body weight. In the second study, decreased body weights were reported for parents and pups and decreased litter size was associated with dose levels of 30 000 mg/kg diet. Decreased body weights reported for parents and pups at 10 000 mg/kg diet were not toxicologically significant. In parents, the decrease was only 2 to 4% below controls and for pups the decrease was 5.6 to 6.6% lower than controls. The findings in pups were also transient and did not occur consistently in all litters. The NOAEL was 10 000 mg/kg diet. The absence of a renal effect in pups at a higher dose level (1500 mg/kg body weight), though not invalidating earlier findings of unilateral renal tubular dilation in male F_{3b} pups, indicates that the reproducibility of this lesion and its toxicological significance are uncertain. It should be noted that in no other toxicological study was an effect on kidneys found.

Bioassays in mice and rats did not indicate that technical glyphosate was carcinogenic.

Glyphosate has been shown to have no genotoxic potential in a range of in vitro *and* in vivo *studies.*

7.1 Single exposure

Numerous acute toxicity studies have been performed to determine LD_{50} values of glyphosate and of herbicide formulations containing glyphosate as active ingredient. The results of these studies are summarized in Tables 10 (results for glyphosate) and 11 (results for formulations). These data show that glyphosate and its formulations have very low toxicity by the oral and dermal administration routes. By the intraperitoneal route glyphosate is markedly more toxic than by the other routes. General intoxication symptoms include breathing difficulties, ataxia and convulsions.

The mechanism of the toxic action of glyphosate has been studied in rats. Olorunsaga et al. (1979) observed dose-related reduced respiratory control ratios and increased phosphatase activity in mitochondria isolated from rat livers 5 h after single intraperitoneal doses ranging from 15 to 120 mg/kg body weight. This effect was also seen in rat liver mitochondria *in vitro* (Bababunmi et al., 1979; Olorunsaga, 1982a,b). The authors suggest that acute toxicity at lethal doses may occur as a result of the uncoupling of oxidative phosphorylation.

The acute toxicity in rats of the surfactant polyoxyethyleneamine, with which glyphosate is commonly formulated in Roundup, was compared to that of glyphosate in a study by Martinez et al. (1990). Both compounds exhibited pulmonary toxicity following either oral or intratracheal administration. The toxicity of the herbicide formulation was greater than can be accounted for on the basis of the dose response data from either compound alone (Martinez et al., 1990; Martinez & Brown, 1991).

A study was undertaken by Tai et al. (1990) to investigate the effects of glyphosate, surfactant, and their combination in Roundup on cardiovascular function in female beagles. They found that glyphosate alone at plasma levels ranging from 923 to 3450 mg/litre, which simulates the human ingestion situation, were shown to increase the myocardial contractility. The surfactant alone considerably reduced the cardiac output, the left

Table 10. Acute toxicity of glyphosate to experimental animals

Species (sex)	Product tested	LD_{50}/LC_{50}[a]	Reference
Oral studies			
Rat (m,f)	glyphosate techn., purity 97.8%	> 5000 mg/kg bw	FDRL (1988d)
Rat (m,f)	glyphosate techn., purity 96-99%	> 5000 mg/kg bw	Inveresk Research Int. (1989a)
Rat (m,f)	glyphosate techn., purity 96-99%	> 5000 mg/kg bw	NOTOX (1988)
Rat (m,f)	85.5% techn. glyphosate in water	> 5000 mg/kg bw	Bio/Dynamics Inc. (1988c)
Rat (m,f)	glyphosate, IPA salt, 65% in water	> 5000 mg/kg bw	Monsanto (1981b)
Rat (m,f)	glyphosate, EO salt[b]	2047 mg/kg bw	Knapek et al. (1986)
Goat (f)	glyphosate techn., purity 98.7%	3500 mg/kg bw	USDA (1987c)
Goat (f)	glyphosate, IPA salt, 65% in water	5700 mg/kg bw	USDA (1987b)
Dermal studies			
Rat (m,f)	glyphosate techn., purity 96-99%	> 2000 mg/kg bw	Inveresk Research Int. (1989c)
Rabbit (m,f)	glyphosate techn., purity 97.8%	> 5000 mg/kg bw	FDRL (1988b)
Rabbit (m,f)	85.5% techn. glyphosate in water	> 5000 mg/kg bw	Bio/Dynamics Inc. (1988a)
Rabbit (m,f)	glyphosate, IPA salt, 65% in water	> 5000 mg/kg bw	Monsanto (1981c)
Intraperitoneal studies			
Mouse (m)	glyphosate (not further specified)	545 mg/kg bw	
Mouse (f)	glyphosate (not further specified)	740 mg/kg bw	FAO/WHO (1986b)
Mouse (m,f)	glyphosate (not further specified)	134 mg/kg bw	FAO/WHO (1986b)
Rat (m)	glyphosate (not further specified	281 mg/kg bw	
Rat (f)	glyphosate (not further specified)	467 mg/kg bw	FAO/WHO (1986b)
Rat (m,f)	glyphosate (not further specified)	238 mg/kg bw	FAO/WHO (1986b)

[a] All values expressed as mg of product tested (as presented in "product tested" column)

[b] EO is an abbreviation of 5-ethoxy-oleinamine salt.

Table 11. Acute toxicity of glyphosate formulations to experimental animals

Species (sex)	Product tested[a]	LD_{50}/LC_{50}[b]	Reference
Oral studies			
Mouse (m,f)	Roundup	> 5000 mg/kg bw	Mitsukaido Labs (1986)
Rat (m,f)	Roundup	5000 mg/kg bw	Bio/Dynamics Inc. (1988e)
Rat (m,f)	"Compound No. 3607"	> 5000 mg/kg bw	Inveresk Research Int. (1988a)
Rat (m,f)	Roundup TX	> 5000 mg/kg bw	NOTOX (1987a,b)
Rat (m,f)	Alphee	> 5000 mg/kg bw	Bio/Dynamics Inc. (1987a)
Rat (m,f)	Sting TX	> 5000 mg/kg bw	NOTOX (1987f,g)
Rat (m,f)	Sting	2510 mg/kg bw	Younger Labs Inc. (1984)
Rat (m,f)	Sting	1950 mg/kg bw	Bio/Dynamics Inc. (1984b)
Rat (m,f)	MON 8780	> 5000 mg/kg bw	Bio/Dynamics Inc. (1985a)
Rat (m,f)	Agrichem Glyfosaat B	> 5000 mg/kg bw	NOTOX (1990e)
Rat (m,f)	"Glyfosaat 360 g/litre"	> 2000 mg/kg bw	NOTOX (1989c)
Rat (m,f)	Legend	> 2000 mg/kg bw	CIT (1991a)
Goat (f)	Roundup	4860 mg/kg bw	USDA (1983)
Dermal studies			
Rat (m,f)	"Compound No. 3607"	> 2000 mg/kg bw	Inveresk Research Int. (1988b)
Rat (m,f)	Roundup TX	> 4000 mg/kg bw	NOTOX (1987c)
Rat (m,f)	Sting TX	> 4000 mg/kg bw	NOTOX (1987h)
Rat (m,f)	MON 8780	> 5000 mg/kg bw	Bio/Dynamics Inc. (1985b)
Rat (m,f)	Agrichem Glyfosaat B or 2	> 4000 mg/kg bw	NOTOX (1990f)
Rat (m,f)	"Glyfosaat 360 g/litre"	> 2000 mg/kg bw	NOTOX (1989b)
Rat (m,f)	Legend	> 2000 mg/kg bw	CIT (1991b)
Rabbit (m,f)	Roundup	> 5000 mg/kg bw	Bio/Dynamics Inc. (1988f)
Rabbit (m,f)	Alphee	> 5000 mg/kg bw	Bio/Dynamics Inc. (1987b)
Rabbit (m,f)	Sting	> 5000 mg/kg bw	Bio/Dynamics Inc. (1984a)

Table 11 (contd).

Species (sex)	Product tested[a]	LD_{50}/LC_{50}[b]	Reference
Inhalation studies			
Rat (m,f)	Roundup (aerosol)	3180 mg/m^3	Monsanto (1983d)
Rat (m,f)	"Compound No. 3607"	> 4860 mg/m^3	Inveresk Research Int. (1989d)
Rat (m,f)	Aiphee	> 8900 mg/m^3	Monsanto (1987b)

[a] Composition of the formulations is given in Table 2, with the exceptions of Agrichem Glyphosaat B (or 2), "Glyfosaat 360 g/litre" and "Compound No. 3607", which all contain approximately 480 g/litre of the isopropylamine salt, and of MON 8780 (32.8% isopropylamine salt), and Legend (40% isopropylamine salt).

[b] All values given as mg formulation.

ventricular stroke work index and the mean arterial pressure. The joint effect of both glyphosate and the surfactant in Roundup formulation resulted in cardiac depression, which was mostly due to the surfactant since glyphosate itself increased myocardial contractility. The authors indicated that the probable cause of the observed increases in pulmonary vascular resistance index and pulmonary artery pressure was a direct vasoactive effect of glyphosate on the pulmonary artery.

7.2 Short-term exposure

7.2.1 Oral studies

In CD-1 mice, a 13-week feeding study was conducted with technical glyphosate (purity 98.7%) using dose levels of 5000, 10 000 and 50 000 mg/kg diet (equal to 940, 1890 and 9710 mg/kg body weight per day in males and 1530, 2730 and 14 860 mg/kg body weight per day in females). No effect on appearance or survival was observed. Growth retardation and increased weights of brain, heart and kidneys were observed at 50 000 mg/kg. Liver weights were increased at 50 000 and 10 000 mg/kg. Limited histopathology showed no adverse effects. The authors of the study concluded that the NOAEL was 10 000 mg/kg diet (1890 mg/kg body weight per day) (Bio/Dynamics Inc., 1979).

In a 13-week feeding study with technical glyphosate, Sprague-Dawley rats received 1000, 5000 or 20 000 mg/kg diet (equal to 63, 317 and 1267 mg/kg body weight per day in males and 84, 404 and 1623 mg/kg body weight per day in females). No effect on appearance, survival or growth occurred. Haematology, blood biochemistry and urinalysis, carried out at test end only, were also unaffected. Organ weights (determined for liver, kidneys and testes only) were not affected. Limited histopathology showed no adverse effect in any tissue. The NOAEL in this study was 20 000 mg/kg diet (1267 mg/kg body weight per day) (Monsanto, 1987a). Absence of toxicity was also found in another 13-week feeding study on rats using technical glyphosate and dose levels of 200 to 12 500 mg/kg diet (Tauchi, 1979 as cited by FAO/WHO, 1986b).

Two further 13-week studies in rodents were conducted on behalf of the US National Toxicology Program (1992). Both rats (F-344/N) and mice ($B6C3F_1$) were administered glyphosate (purity approximately 99%) in feed at levels of 3125, 6250, 12 500, 25 000 and 50 000 mg/kg diet.

In rats, reduced weight gains were observed at 25 000 mg/kg diet (males only) and at 50 000 mg/kg diet (males and females). No changes in feed consumption were found. Minor increases in the relative organ weight of liver, kidney and testes, and decreased thymus weight were observed in males only at several dose levels; these changes did not show a clear dose relation and therefore are not considered to be compound-related effects. Small increases in haematocrit and red cell blood counts were observed in male rats at $\geq$ 12 500 mg/kg diet. Clinical chemistry showed increased alkaline phosphatase (AP) and alanine aminotransferase (ALAT) at $\geq$ 6250 mg/kg diet in males and at $\geq$ 12 500 mg/kg diet in females. Bile acid levels in blood were increased at 25 000 mg/kg diet (males only) and at 50 000 mg/kg diet (males and females). Decreases in sperm count were observed in males at $\geq$ 25 000 mg/kg diet. The only histopathological lesions found were cytoplasmic alterations of the parotid and submandibular salivary glands, consisting of basophilic changes and hypertrophy of acinar cells. The parotid gland was more severely affected. The magnitude of the effect was dose-dependent, with focal lesions in less severe cases to diffuse involvement at higher doses. Lesions of a similar nature and magnitude were observed in both sexes. The sublingual gland was not detectably altered. Effects on the salivary glands were observed already at the lowest dose level tested of 3125 mg/kg diet (equal to 205 mg/kg body weight per

day for males and 213 mg/kg body weight per day for females). Thus, this study did not yield a NOAEL (NOAEL < 3125 mg/kg diet) (NTP, 1992).

In mice, reduced weight gains were observed at 50 000 mg/kg diet in both sexes. Increased organ weights were noted in heart, kidney, liver, thymus, lung and testis; these changes did not show a clear dose relation and therefore are not considered to be compound-related effects. Feed consumption levels were not changed significantly. Lesions in the parotid gland were observed, similar to those observed in rats. The sublingual gland and the submandibular salivary glands were not detectably altered. The effects on the parotid gland were observed in mice at 6250 mg/kg diet (equal to 1065 mg/kg body weight per day for males and 1411 mg/kg body weight per day for females), but were not seen at the lowest dose level tested of 3125 mg/kg diet (equal to 507 mg/kg body weight per day for males and 753 mg/kg body weight per day for females). The NOAEL in this study was 3125 mg/kg diet (507 mg/kg body weight per day) (NTP, 1992).

The salivary gland lesions could also be induced in rats by 14-day exposure at feed levels of 50 000 mg/kg diet. The salivary glands lesions induced by glyphosate were similar to those which could be induced by exposure to high subcutaneous doses of the β-adrenergic agonist isoproterenol and could be partially ameliorated with the β-adrenergic antagonist propanolol. This indicates that glyphosate may induce the salivary gland lesions by acting as a weak adrenergic agonist (NTP, 1992).

The short-term toxicity of technical glyphosate was also studied in dogs. Beagle dogs received technical glyphosate in gelatin capsules at dose levels of 0, 20, 100 or 500 mg/kg body weight per day for 52 weeks. No effect occurred with respect to clinical signs, body weight, feed consumption, ophthalmoscopy, haematology, blood biochemistry, urinalysis, gross pathology and histopathology. The only changes in treated groups relative to controls were increased pituitary weights (absolute and relative) in the medium- and high-dose males. Because there were no concomitant histological changes present in pituitaries and given the absence of an effect on this organ (and related organs) in all other studies, the toxicological significance of the increased pituitary weights is questionable. The NOAEL in this study was 500 mg/kg body weight per day (Monsanto, 1985).

A 7-day oral study was carried out with Roundup in female cattle weighing 160 to 272 kg. Brahman-cross heifers received

400, 500, 630 or 790 mg Roundup/kg body weight per day by nasogastric tube. At 790 mg/kg, 1/3 animals died before test end, showing laboured breathing and pneumonia caused by aspiration of rumen contents. Decreased feed intake was seen at 630 and 790 mg/kg; diarrhoea occurred at 500, 630 and 790 mg/kg body weight. Slight increases in a number of blood parameters, occurring at 790 mg/kg only, were probably due to extracellular fluid shifts and haemoconcentration secondary to diarrhoea. The NOAEL in this study was 400 mg Roundup/kg body weight per day (USDA, 1987a).

7.2.2 Dermal studies

Short-term dermal toxicity studies were carried out in rabbits with technical glyphosate and the formulation Roundup.

Technical glyphosate, moistened with saline, was applied under occlusion at dose levels of 100, 1000 and 5000 mg/kg body weight per day to the shaven intact or abraded skin of rabbits for 6 h/day, 5 days/week for 3 weeks. No effect on survival and growth occurred. Slight dermal irritation (barely perceptible erythema and oedema) was observed at 5000 mg/kg body weight only. No evidence of systemic toxicity was found (parameters: haematology and blood biochemistry in five animals of each sex per group, organ weights, gross pathology, limited histopathology) (IRDC, 1982). Absence of systemic effects was also found in the somewhat more limited 21-day study in rabbits (no haematology and blood biochemistry) with Roundup. Skin effects were more severe in this study: at both dose levels (76 and 114 mg/kg body weight per day, undiluted formulation applied) erythema and oedema were seen and, in addition, exudate and fissuring occurred at the abraded skin sites. After a 4-week recovery period the skin effects were no longer present (Bio/Dynamics Inc., 1975).

7.2.3 Inhalational studies

A 4-week inhalation study was carried out on rats with a 1:3 dilution of Roundup formulation. Test concentrations of 50, 160 and 360 mg/m^3 of the diluted formulation (equivalent to 17, 53 and 120 mg Roundup/m^3) were administered as an aerosol spray for 6 h/day, 5 days/week. The mass median aerodynamic diameter of the test material ranged from 1.8 to 2.7 μm with geometric standard deviations between 1.7 and 2.0. An increased incidence of irritation of the nasal turbinates (subacute inflammation), trachea (mononuclear cell infiltration) and lungs (perivascular

lymphoid infiltrates/aggregates) was observed among the high-dose females only. No signs of systemic toxicity were found (parameters: survival, growth, limited haematology and blood biochemistry, organ weights, limited histopathology) (Monsanto, 1983e).

7.3 Long-term toxicity and carcinogenicity

Only oral studies are available. Dietary studies using technical glyphosate were performed in mice and rats.

In Charles River CD-1 mice technical glyphosate was fed in the diet at concentrations of 0, 1000, 5000 or 30 000 mg/kg diet for 24 months (dose levels equal to 0, 157, 814 and 4841 mg/kg body weight per day for males and 0, 190, 955 and 5874 mg/kg body weight per day for females). No effect on survival or appearance was noted. Body weights were decreased in the males of the high-dose group. Haematology and organ weights showed no effects. Histopathology in liver revealed an increased incidence of central lobular hepatocyte hypertrophy among high-dose males (incidences: 9/49, 5/50, 3/50 and 17/50 in control, low-dose, medium-dose and high-dose males, respectively) and an increased incidence of central lobular hepatocyte necrosis also among high-dose males (incidences: 0/49, 2/50, 2/50 and 10/50). Increased incidences of epithelial hyperplasia in the urinary bladder were present in the medium-dose and high-dose males (incidences: 3/49, 3/50, 10/50 and 8/50). There were no statistically significant increases in the frequency of neoplastic lesions. The NOAEL in this study was 5000 mg/kg diet (814 mg/kg body weight per day) (Bio/Dynamics Inc., 1983a).

Two chronic feeding studies on rats were conducted with technical glyphosate, one in 1979-1981 and the other in 1988-1990. The first study, carried out using Charles River CD (Sprague-Dawley) BR rats, had dose levels of 0, 60, 200 and 600 mg technical glyphosate/kg diet (equal to about 0, 3, 10 and 32 mg/kg body weight per day, respectively). Survival, appearance, haematology, blood biochemistry, urinalysis and organ weights were not changed. The NOAEL was > 600 mg/kg diet (> 32 mg/kg body weight per day). Slight growth retardation during part of the study was noted in the high-dose males. The incidence of interstitial cell tumours in testes showed a statistically significant increase (incidences: 0/50, 3/50, 1/50 and 6/50; historical control range: 3-7%) (Bio/Dynamics Inc., 1981a). This finding, in itself constituting some evidence for a carcinogenic effect in rats, should

be judged in the light of the absence of an effect at much higher dose levels in the more recent 2-year study in rats (see below). This is also valid for the slight growth retardation (i.e. no effect on growth at much higher dose levels in the more recent study, see below). In the recent 2-year study, rats (same strain) were fed 2000, 8000 or 20 000 mg technical glyphosate/kg diet (equal to about 100, 410 and 1060 mg/kg body weight per day) for 24 months. There was no effect on survival or appearance. Growth was retarded in the high-dose females. Haematology and blood biochemistry showed no effects. In the high-dose males, the urine specific gravity (after 6 months only) and urine pH were increased. A statistically significant increased incidence of degenerative lens changes (basophilic degeneration of the posterior subcapsular lens capsule or mature cataracts) was found among the high-dose males (incidences 3/60, 4/60, 4/60 and 8/60 in the control, low-, medium- and high-dose groups, respectively. However this finding was within the historical control range of 0-33%. Liver weights were increased in the high-dose males only. Histopathology showed an increased incidence of inflammation of the gastric squamous mucosa in the medium- and high-dose groups (incidences in males: 2/58, 3/58, 5/59 and 7/59; females: 0/59, 3/60, 9/60, and 6/59; historical range: 0-13.3%). The incidence of pancreatic islet cell adenomas was increased (statistically significant) among low- and high-dose males (incidences: 1/58, 8/57, 5/60 and 7/59; historical control range of test laboratory 1.8-8.5%). The incidence in the control group was below the historical control range; the trend test for the observed increase was negative. No pancreatic carcinomas were found. The NOAEL in this study was 8000 mg/kg diet (410 mg/kg body weight per day) (Monsanto, 1990b).

Glyphosate has been tested in the US National Toxicology Program; pre-chronic studies have been completed (NTP, 1992).

7.4 Skin and eye irritation; sensitization

Many studies have been carried out with rabbits to examine the potential of glyphosate and its formulations to produce skin and eye irritation. The results of these studies are briefly summarized in Tables 12 (skin irritation) and 13 (eye irritation). Glyphosate and its formulations produce only mild skin irritation after undiluted single application. The result of the 21-day dermal study in rabbits with the formulation Roundup (Bio/Dynamics Inc., 1975; see subsection 7.2.2) shows that repeated application of undiluted formulation to the skin does lead to irritation. The

Table 12. Skin irritation tests on rabbits with glyphosate and its formulations

Product tested[a]	Contact time (h)	Draize score[b]	Classification[c]	Reference
Glyposate				
Glyphosate techn. 85% in water	4	0.8	slightly irritating	Bio/Dynamics Inc. (1988b)
Glyphosate techn., moistened powder	4	0	not irritating	FDRL (1988a)
Glyphosate techn., moistened powder	4	0	not irritating	Inveresk Research Int. (1989e)
Glyphosate, IPA salt 65% in water	24	0.2	not irritating	Monsanto (1981d)
Formulations				
Roundup, undiluted	4	1.9	slightly irritating	Bio/Dynamics Inc. (1988g)
"Compound No. 3607", undiluted	4	1.2	slightly irritating	Inveresk Research Int. (1988c)
Roundup TX, undiluted	4	0.7	slightly irritating	NOTOX (1987d)
Alphee, undiluted	4	1.0	slightly irritating	Bio/Dynamics Inc. (1987c)
Sting, undiluted	4	1.3	slightly irritating	Bio/Dynamics Inc. (1984c)
Sting TX, undiluted	4	3.6	moderately irritating	NOTOX (1987i)
Roundup L&G, undiluted	4	1.0	slightly irritating	Bio/Dynamics Inc. (1985c)
"Glyfosaat 360 g/litre", undiluted	4	0.3	not irritating	NOTOX (1989d)
Agrichem Glyfosaat B, undiluted	4	1.7	slightly irritating	NOTOX (1990d)
Agrichem Glyfosaat 2, undiluted	4	2.0	slightly irritating	NOTOX (1990b)
Legend, undiluted	4	0.7	slightly irritating	CIT (1991c)

[a] For glyphosate content of the tested formulations, see footnote a in Table 11.

[b] This score is the mean score per animal and was calculated using the data from the original report. The standard Draize scoring system was used; in calculating the mean response per animal, the maximum response observed for an animal was used.

[c] The classification is based on the mean Draize score per animal. Specification: score 0-0.5 not irritating; 0.6-2.0 slightly irritating; 2.1-5.0 moderately irritating; 5.1-8.0 severely irritating.

Table 13. Results of eye irritation tests in rabbits for glyphosate and its formulations.

Product tested[a]	Irritation index[b]	Classification[c]	Reference
Glyphosate			
Glyphosate techn. 85% in water, undiluted	45	strongly irritating	Bio/Dynamics Inc. (1988d)
Glyphosate, IPA salt, 65% in water	0	not irritating	Monsanto (1981e)
Glyphosate techn. (97.6%), undiluted	54	strongly irritating	FDRL (1988c)
Glyphosate techn. (96-99%), undiluted	29	irritating	Inveresk Research Int. (1989f)
Formulations			
Roundup, undiluted	54	strongly irritating	Bio/Dynamics Inc. (1990)
"Compound No. 3607", undiluted	13	irritating	Inveresk Research Int. (1989g)
Roundup TX, undiluted	31	strongly irritating	NOTOX (1987e)
Alphee, undiluted	6	slightly irritating	Bio/Dynamics Inc. (1987d)
Sting, undiluted	104	extremely irritating	Bio/Dynamics Inc. (1984d)
Sting TX, undiluted	19	irritating	NOTOX (1987j)
Roundup L&G, undiluted	19	irritating	Bio/Dynamics Inc. (1985d)
"Glyfosaat 360 g/litre", undiluted	33	strongly irritating	NOTOX (1989a)

Table 13 (contd).

Formulations (contd).			
Agrichem Glyfosaat B, undiluted	58	strongly irritating	NOTOX (1990c)
Agrichem Glyfosaat 2, undiluted	22	irritating	NOTOX (1990a)
Legend, undiluted	21	irritating	CIT (1991d)

[a] For glyphosate content of the tested formulations, see footnote a in Table 11.

[b] The irritation index represents a mean total score per animal for response in cornea, conjunctiva and iris, using the standard Draize scoring system. The irritation index was calculated using the data from the original report; in calculating the mean response per animal the maximum response observed for an animal was used. The calculation procedure is as follows:

-	score conjunctiva:	A. chemosis: 0-4 B. discharge: 0-3 C. erythema: 0-3	Calculation partial index for conjunctiva: 2 x (A + B + C) = 0-20
-	score iris:	0-2	Calculation partial index for iris: 5 x (0-2) = 0-10
-	score cornea:	A. opacity: 0-4 B. area: 0-4	Calculation partial index for cornea: 5 x A x B = 0-80

The total irritation index is the sum of the partial indices (0-110).

[c] The classification is based on the following scheme: index 0-5 not irritating; 5.1-12 slightly irritating; 12.1-30 irritating; 30.1-60 strongly irritating; 60.1-80 severely irritating; 80.1-110 extremely irritating.

eye-irritating potential is considerable for undiluted glyphosate and, with a few exceptions, also for the formulations. Single application generally produces moderate to severe reactions.

Sensitization studies have been carried out in guinea-pigs with glyphosate and its formulations. The results of these tests are summarized in Table 14. Neither glyphosate itself nor the tested formulations induced sensitization in any experiment.

Table 14. Results of sensitization tests in guinea-pigs for glyphosate and its formulations

Product tested[a]	Method	Result	Reference
Glyphosate			
Glyphosate (purity 99.7%)	Buehler	negative	Bio/Dynamics Inc. (1983c)
Glyphosate technical	Magnusson & Kligman[b]	negative	Safefarm Labs Inc. (1991b)
Glyphosate techn. (96-99%)	Magnusson & Kligman	negative	Inveresk Research Int. (1989b)
Formulations			
Roundup	Buehler	negative	Bio/Dynamics Inc. (1983b)
"Compound No. 3607"	Magnusson & Kligman	negative	Inveresk Research int. (1988d)
Roundup L&G	Buehler	negative	Bio/Dynamics Inc. (1987e)
Legend	Buehler	negative	Safefarm Labs Inc. (1991a)
Sting	Buehler	negative	Bio/Dynamics Inc. (1986)

[a] For glyphosate content of the tested formulations, see footnote a in Table 11.
[b] Method also called the maximization test.

The results of two dermal irritation studies and one dermal sensitization study performed with human volunteers exposed to Roundup are presented in subsection 8.1.

7.5 Reproductive toxicity, embryotoxicity and teratogenicity

Technical glyphosate has been tested for teratogenicity in rats and rabbits using the oral route. In addition, two oral multi-generation reproduction studies in rats have been reported.

7.5.1 Teratogenicity studies

Glyphosate (technical) was given to pregnant Charles River COBS CD rats by gavage at dose levels of 0, 300, 1000 and 3500 mg/kg body weight per day on days 6-19 of gestation. At 3500 mg/kg the following effects were observed: increased incidences of soft stools, diarrhoea, breathing rattles, red nasal discharge and reduced activity, increased mortality (6/25 dams dying before the end of the treatment period), growth retardation, increased incidence of early resorptions, decreases in total number of implantations and the number of viable fetuses, increased number of fetuses with reduced ossification of sternebrae. At the lower dose levels these effects were absent. The NOAEL in this study was 1000 mg/kg body weight per day (IRDC, 1980b).

In Dutch belted rabbits technical glyphosate was tested at dose levels of 0, 75, 175 and 350 mg/kg body weight per day (administration by gavage) from days 6-27 of gestation. In dams the incidences of diarrhoea and soft stools were increased in the high-dose group and, to a slight degree, also in the medium-dose group. The incidence of nasal discharge was increased in the high-dose group only. In the medium- and high-dose groups 2 and 10 dams, respectively, died during the study from unknown causes. The NOAEL in this study was 175 mg/kg body weight per day (IRDC, 1980c).

7.5.2 Reproduction studies

A three-generation feeding study in Charles River CD (Sprague-Dawley) BR rats was conducted in 1980-1981 and a two-generation study, using higher dose levels, was completed in 1990 with technical glyphosate. In the former study, dietary feeding levels were continuously adjusted to achieve dose levels of 0, 3, 10 and 30 mg/kg body weight per day. The only effect noted was an increased incidence of unilateral renal tubular dilation in the male pups (randomly selected) of the F_{3b} mating of the high-dose group (incidence 6/10 versus 0/10 in controls, not determined in intermediate groups, earlier litters not examined). The NOAEL in this study was < 30 mg/kg body weight (Bio/Dynamics Inc.,

1981b). The more recent two-generation study had dose levels of 0, 2000, 10 000 and 30 000 mg technical glyphosate/kg diet (equivalent to 0, 100, 500 and 1500 mg/kg body weight per day). In the high-dose group, the following effects were observed: soft stools and decreased body weights in parent animals, slightly decreased litter size and decreased pup weights (the latter seen at day 14 or 21 of lactation). The decreased body weights of parents and pups were seen to a slight degree in the medium-dose group also. No histological effect on kidneys was present in the F_{2b} male pups (15 and 23 pups examined in control and high-dose groups, respectively; first generation and F_{2a} pups not examined). The NOAEL in this study was 10 000 mg/kg diet (500 mg/kg body weight per day) (Monsanto, 1990c).

With regard to these reproduction studies using technical glyphosate, it should be noted that in both studies the number of pups submitted to histopathological examination was limited. These limitations make evaluation of the renal effect in pups, seen (at 30 mg/kg body weight) in the study of Bio/Dynamics Inc. (1981b), difficult.

7.6 Mutagenicity and related end-points

The results of several studies are summarized in Table 15. The results show that glyphosate is not mutagenic.

Table 15. Results of mutagenicity studies with glyphosate and its salts.

Test system	Test compound and concentrations	Result[c]	Reference
Ames test, *Salmonella typhimurium* TA98, TA100, TA1535 & TA1537, with and without metabolic activation	technical glyphosate (98.4%); 0.1-1000 µg/plate	-	Monsanto (1978c)
Ames test, *S. typhimurium* TA98, TA100, TA1535 & TA1537, with and without activation	technical glyphosate (98.4%); 10-5000 µg/plate	-	IET (1978)
Rec assay, *Bacillus subtilis*, strain H17 (rec^+) & M45 (rec^-), without activation	technical glyphosate (98.4%); 20-2000 µg/disc	-	IET (1978)
Reverse mutation assay in *Escherichia coli* strain WP2hcr, with and without activation	technical glyphosate (98.4%); 10-5000 µg/plate	-	IET (1978)
Forward mutation assay, CHO cells, *in vitro* (HGPRT-locus), with & without activation	technical glyphosate (98.7%); 0-20 mg/ml (- activation) or 5-25 mg/ml (+ activation)	-	Monsanto (1983b)
Cytogenetic study (chromosome aberrations) in rat bone marrow, *in vivo*	technical glyphosate (98.7%); 200-1000 mg/kg body weight, i.p.[b]; sampling after 6, 12 and 24 h	-	Monsanto (1983f)
Micronucleus test in erythrocytes of mice, *in vivo*	glyphosate (not specified); ½ LD_{50}, oral; sampling time not specified	-	Benova et al. (1989)
Dominant lethal test, mouse *in vivo*	technical glyphosate (98.7%); 200-2000 mg/kg body weight, oral	-	IRDC (1980a)

Table 15 (contd).

Test system	Test compound and concentrations	Result[c]	Reference
Recessive sex-linked lethal test, *Drosophila melanogaster*, *in vivo*	glyphosate (not specified); dose not given	-	Gopalan & Njagi (1981)
Unscheduled DNA repair assay rat hepatocytes, *in vitro*	technical glyphosate (98.7%); 0.0125-125 µg/ml	-	Monsanto (1983c)

[a] No higher concentrations tested because these would result in osmolalities much higher than physiological levels; these high osmolalities can produce non-specific chromosomal aberrations or sister chromatid exchange.

[b] In additional studies it was demonstrated that: (1) glyphosate produced no effect on viability and mitotic index of bone marrow cells of rats after i.p. doses of 200-1000 mg/kg body weight (Monsanto, 1983g); and (2) after giving ^{14}C-labelled glyphosate i.p. significant concentrations of ^{14}C reached the bone marrow (peak levels reached after 0.5 h remaining virtually constant up to 10 h after dosing) (Monsanto, 1983h).

[c] - = negative result

8. EFFECTS ON HUMANS

Appraisal

The formulation Roundup containing glyphosate is acutely toxic to humans when ingested intentionally or accidentally.

No controlled studies have been conducted, and therefore the human NOAEL level cannot not be derived.

No data are available to show the impact on workers exposed during the manufacture or formulation of glyphosate. No compound-related effects were observed in a test group of five applicators prior to and after exposure for one week.

The reported higher susceptibility of individuals older than 40 years to ingested Roundup intoxication is important and requires further investigation.

8.1 Cases of intentional and accidental exposure

Many cases of acute intoxication with herbicides containing glyphosate and surfactant (Roundup) have been reported; most of these were suicide attempts. Talbot et al. (1991) reviewed 93 cases of exposure to Roundup (Chinese names: lan-da, hao-ni-chun, nian-nian-chun) in Taiwan. The classification of the severity of acute poisoning with Roundup as given by these authors is presented in Table 16. Severe effects occurred only in the cases of intentional ingestion (80 of the 93 reported). Accidental exposures led to only mild effects. The typical symptoms were erosion of the gastrointestinal tract (66% of the self-poisonings), seen as sore throat, dysphagia and gastrointestinal haemorrhage. Other organs were affected less often (nonspecific leucocytosis 65%, lungs 23%, liver 19%, cardiovascular system 18%, kidney 14% and CNS 12%). Death (in 7/80 cases) occurred within hours after ingestion. The amount of undiluted Roundup ingested (rough estimates) in the lethal cases varied from 85 to 200 ml (corresponding to roughly 30 to 70 g glyphosate acid); but much larger amounts (500 ml Roundup, corresponding to 180 g glyphosate acid) were reported to have been ingested by some patients with mild to moderate symptoms. Overall, moderate symptoms were associated with estimated intakes of 20 to 500 ml, mild symptoms with 5 to 150 ml, no symptoms with 5 to 50 ml.

The authors pointed out that the patient's estimates of the amount ingested, and the conversion ratio used in their paper may be inaccurate (Talbot et al., 1991). Other reviews of cases of intoxication with Roundup have reported similar findings (Sawada & Nagai, 1987; Tominack et al., 1991). The data of Tominack et al. (1991) suggested that people over 40 years of age who ingest amounts greater than 150 ml Roundup are at greatest risk of a fatal outcome. These authors also pointed out that the surfactant contained in Roundup may be responsible for the clinical syndrome (as suggested by Sawada & Nagai, 1987), but that the available evidence on this point is, as yet, inconclusive.

Table 16. Classification of severity of acute poisoning with Roundup[a]

Classification	Description
Asymptomatic	no complaints and no abnormalities on physical or laboratory examination.
Mild	mainly gastrointestinal tract(GIT) symptoms (nausea, vomiting, diarrhoea, abdominal pain, mouth and throat pain) that resolved within 24 h. Vital signs were stable, and there was no renal, pulmonary or cardiovascular involvement.
Moderate	GIT symptoms lasting longer than 24 h, GIT haemorrhages, endoscopically verified oesophagitis or gastritis, oral ulceration, hypotension responsive to intravenous fluids, pulmonary dysfunction not requiring intubation, acid-base disturbance, evidence of transient hepatic or renal damage, or temporary oliguria.
Severe	pulmonary dysfunction requiring intubation, renal failure requiring dialysis, hypotension requiring treatment with pressor amines, cardiac arrest, coma, repeated seizures, or death.

[a] From: Talbot et al. (1991)

Further clinical experiences with patients exposed to Roundup either accidentally or through deliberate ingestion have been reported by Temple & Smith (1992). Symptoms resulting from dermal exposure incidental to the use of the product included periorbital oedema and chemosis of the eye, cardiovascular effects (tachycardia and elevated blood pressure), swelling and

paraesthesia at the site of dermal contact and prolonged skin irritation. Deliberate ingestion resulted in more severe effects, including lethality from apparent respiratory and cardiac arrest (Temple & Smith, 1992).

Two dermal irritation studies were carried out with volunteers. Application of 0.9 ml of a 9:1 dilution of Roundup formulation in water to the intact skin of the upper arm for 24 h produced no skin changes (Shelanski, 1973). Maibach (1986) tested undiluted Roundup (application of 0.1 ml to intact and abraded skin sites on the back for 24 h) and found erythema in only 1/24 subjects (23/24 no reaction) for the intact skin sites; for the abraded skin sites 4/24 subjects showed an equivocal reaction and 10/24 showed erythema (10/24 no reaction). The same author reported very briefly the absence of effect in a photoirritation study in humans using undiluted Roundup as test compound (application to abraded skin of upper arm for 24 h with irradiation with UVA light for 45 min) (Maibach, 1986).

A sensitization study was performed in 204 human volunteers with undiluted Roundup according to a modified Draize method. The summary report (no detailed report available) stated that there was no effect in any subject (Maibach, 1986). The same author reported absence of photosensitization by Roundup in volunteers.

8.2 Occupational exposure

The results of several studies focused primarily on the determination of the extent of exposure to glyphosate when the compound is used as herbicide are presented in section 5.3. The study of Jauhainen et al. (1991) included health examinations of a test group of five workers prior to and after an exposure period of 1 week. These examinations included haematology, clinical chemistry, ECG, pulmonary function tests, an interview for a health questionnaire and a general clinical examination (including recording of blood pressure, pulse rate and pressure craft of hands). A control group consisted of five workers. No compound-related effects were observed (Jauhainen et al., 1991). The other studies described in section 5.3 did not include a health evaluation of workers.

8.3 Subpopulations at special risk

The only information available on this point is some suggestive evidence referring to oral intoxications with Roundup; Tominack et al. (1991) suggested that people older than 40 years are at greater-than-normal risk after ingestion of Roundup.

9. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

In this chapter, concentrations or doses of formulations with glyphosate are always expressed as mg product per kg soil. Therefore, these figures may have been recalculated from the original data, in cases where the authors reported the data in, for instance, mg a.i./litre instead of mg Roundup per litre. For recalculation, the percentages of the active ingredient (free acid or the salt), given in Table 2, were used.

9.1 Laboratory experiments

9.1.1 Microorganisms

9.1.1.1 Water

Appraisal

The prokaryotic cyanobacteria are generally more sensitive to the effects of glyphosate than the eukaryotic true algae. Similar enzyme systems are inhibited in microorganisms to those thought to be responsible for the herbicidal properties of glyphosate in higher plants. The single semi-field study suggests very variable results but with no significant effect on populations or community structure.

The acute and chronic toxicities of technical grade glyphosate and its formulations to cyanobacteria, algae and diatoms are summarized in Table 17. Technical grade glyphosate is slightly toxic with 3- to 4-day EC_{50} values of 1.2–7.8 mg/litre, and 7-day NOEC values of 0.3–34 mg/litre. Formulations of glyphosate may be more toxic (3-day EC_{50} values of 1.0 to > 55 mg product/litre).

Toxicity to cyanobacteria and algae is dependent on the species or strain tested. Wängberg & Blanck (1988) exposed 16 species in pure cultures to Roundup for 14 days. The concentration at which growth was inhibited completely was 16 mg Roundup/litre for the most sensitive species (*Raphidonema longiseta* and *Anahaena* sp.) and 131 mg Roundup/litre for the least sensitive species (*Selenastrum capricornutum*). The prokaryotic *Cyanophyta* were significantly more affected by Roundup than the eukaryotic *Chlorococcales*.

Table 17. Aquatic microorganisms: acute and chronic toxicity of glyphosate and its formulations (EC_{50}, NOEC)

Organism	A	Test type	Compound	Test water	pH	Hardness (mg $CaCO_3$/ litre)	Temperature (°C)	Experimental duration (days)	Parameter[a]	Concentration (mg/litre)	Reference
Cyanobacteria											
Anabaena flos-aquae	A	S	Tgg	a.m.	7.5	285	24	7	NOEC	9.7[b,c]	Malcolm Pirnie Inc. (1987a)
Green algae											
Selenastrum capricornutum	-	S	Tgg	a.m.	7.0	n.r.	26	3.5-4	EC_{50}	7.8[b,e]	Bozeman et al. (1989)
S. capricornutum	A	S	Tgg	a.m.	7.5	285	24	7	NOEC	20[b,c]	Malcolm Pirnie Inc. (1987d)
S. capricornutum	-	S	St	a.m.	7.7-9.0	26	23	3	EC_{50}	1.0[b]	LISEC (1989b)
S. capricornutum	-	S	St	a.m.	7.7-9.0	26	23	3	EC_{50}	2.5[d]	LISEC (1989b)
S. capricornutum	-	S	St	a.m.	7.7-9.0	26	23	3	NOEC	0.2[b]	LISEC (1989b)
S. capricornutum	-	S	Ru	a.m.	7.7-10.0	26	24	3	EC_{50}	2.1[b]	LISEC (1989a)
S. capricornutum	-	S	Ru	a.m.	7.7-10.0	26	24	3	EC_{50}	8.0[d]	LISEC (1989a)
S. capricornutum	-	S	Ru	a.m.	7.7-10.0	26	24	3	NOEC	0.7[b]	LISEC (1989a)
Chlorella pyrenoidosa	-	S	Ru	a.m.	7.5	n.r.	25	2.1-7	EC_{50}	> 55[b]	Hernando et al. (1989)
C. pyrenoidosa	-	S	Ru	a.m.	7.5	n.r.	25	2.1-7	NOEC	< 55[b]	Hernando et al. (1989)

Table 17 (contd).

Diatoms											
Skeletonema costatum	-	S	Tgg	a.m.[h]	8.2-8.5	n.r.	20	4	EC_{50}	1.2[g]	E G & Bionomics (1978a)
S. costatum	-	S	Tgg	a.m.[h]	8.2-8.5	n.r.	20	4	EC_{50}	1.3[b]	E G & Bionomics (1978a)
S. costatum	-	S	Tgg	a.m.[h]	8.2-8.5	n.r.	20	4	NOEC	< 0.6[f]	E G & Bionomics (1978a)
S. costatum	A	S	Tgg	a.m.[h]	7.5	285	20	7	NOEC	0.3[b,c]	Malcolm Pirnie Inc. (1987b)
Navicula pelliculosa	A	S	Tgg	a.m.	7.5	285	20	7	NOEC	34[b,c]	Malcolm Pirnie Inc. (1987c)

[a] concentration of a formulation is expressed as mg of the formulation per litre
[b] based on biomass decrease
[c] based on actual concentrations
[d] based on inhibition of growth rate
[e] NOEC value not reported
[f] based on biomass and chlorophyll a decrease
[g] based on chlorophyll a decrease
[h] salinity was 30‰

A = actual concentrations are measured; - = nominal concentrations; S = static system; Tgg = technical grade glyphosate; Ru = Roundup; St = Sting; a.m. = artificial medium; n.r. = not reported

In *Pseudomonas chlororaphis* Roundup severely inhibited respiration at concentrations of ≥ 2623 mg/litre, whereas in *Aeromonas hydrophila* respiration was only slightly affected at these concentrations (Chan & Leung, 1986). The bacteria were exposed for 6 days.

Chan & Leung (1986) found that the activity of 5-enolpyruvyl-shikimic acid-3-phosphate synthase was inhibited strongly in aquatic bacteria at the lowest tested concentration of 656 mg Roundup/litre. This enzyme takes part in the biosynthetic sequence to phenylalanine, tryptophan, and tyrosine via the conversion of shikimate to chorismate, and the conversion of chorismate to anthralinate. In an *in vitro* experiment with cell-free extracts of *Aerobacter aerogenes*, technical grade glyphosate inhibited the conversion of shikimate to chorismate at concentrations of ≥ 0.2 mg a.i./litre (Amrhein et al., 1980).

In *Chlorella pyrenoidosa* Roundup affected the growth, the greening process and the photosynthetic metabolism at the lowest tested concentration of 55 mg/litre when exposed for 2.1 or 7 days (Hernando et al., 1989). Synthesis of chlorophyll a and b and carotenoids was then significantly inhibited. Roundup affected synthesis of chlorophyll a to a greater extent than that of the other pigments. *In vivo* and *in vitro* studies with isolated chloroplasts showed inhibition of the photosystems PS I and PS II at concentrations of ≥ 55 mg Roundup/litre. Hernando et al. (1989) suggested that glyphosate acted as an electron transport inhibitor and that the stronger inhibition of PS II compared with PS I was due to the surfactant polyoxyethyleneamine.

In periphytic algal communities that were collected in ponds of boreal forests, Roundup decreased the carbon fixation rate by 50% at concentrations of 243-479 mg/litre (Goldsborough & Brown, 1988). The algae were exposed for 4 h. Austin et al. (1991) cultured periphyton on glass plates suspended in artificial "stream-troughs" which were supplied with flowing water pumped from natural streams in British Columbia, Canada. The stream water was low in phosphorus and flowed out of an oligotrophic lake. Glyphosate was added to give nominal concentrations in the troughs of between 0.001 and 0.3 mg/litre. A further series of treatments added nutrients to troughs. The herbicide was not toxic to the periphyton. A transitory decrease in growth was followed by a stimulation of biomass in the glyphosate-treated troughs. Similar effects were seen with added nutrient. The authors considered the effect to be the result of

algae using glyphosate as a phosphate source. Communities of periphyton were similar in all treatments.

Various studies have indicated that glyphosate may affect aromatic amino acid synthesis in microorganisms, in addition to the greening process, respiration and photosynthesis (Amrhein et al., 1980; Chan & Leung, 1986; Pipke & Amrhein, 1988).

9.1.1.2 Soil

Appraisal

Soil bacteria in culture have shown effects of glyphosate on nitrogen fixation, denitrification and nitrification. However, field studies after application of formulations have not shown significant effects. Closely related species of bacteria have been shown to be capable of degrading glyphosate (see chapter 4). The lack of information on the bioavailability of glyphosate in soil makes it difficult to relate the evaluation of effects in culture to the actual exposure in the field.

Technical grade glyphosate inhibited the growth of bacteria isolated from a sprayed garden soil of sandy loam to a lesser extent than it did that of bacteria isolated from an unsprayed control (Quinn et al., 1988). In a mineral salt medium the growth rate of bacteria from the untreated site was reduced by 50% at 590 mg a.i./litre at 50 h after application.

In various studies, glyphosate, applied at the recommended rates, caused neither inhibition of processes that are part of the nitrogen cycle nor inhibition of enzymes involved in microbial activity.

Roundup did not affect dehydrogenase activity in sand and loamy sand when applied at rates of 4.9 and 24 kg a.i./ha (NATEC, 1990). In the same soils amended with lucerne (*Medicago sativa*), the same application rates did not change significantly the amounts of nitrate nitrogen, although in treated loamy sand there was a slight increase. These experiments, with a duration of 28 days, indicated a slight stimulation of nitrification in loamy sand. A stronger stimulation of nitrification was found in silt loam, silty clay loam and sandy loam up to 84 days after application of technical grade glyphosate at rates of 5 and 25 mg a.i./kg (ABC Inc., 1978d). This stimulation was not dose-related.

In this experiment no substance-related effects were found on nitrogen fixation and nitrite formation.

Nitrogen fixation was not affected significantly at an application rate of 13 mg a.i./kg dry weight whether the product applied was technical grade glyphosate or Roundup, or whether aerobic or anaerobic conditions were used (Carlisle & Trevors, 1986a). At concentrations ≥ 127 mg a.i./kg dry weight, nitrogen fixation was inhibited under anaerobic conditions, whereas this could not be verified under aerobic conditions due to very low acetylene reduction rates. In the same agricultural sandy loam, denitrification under anaerobic conditions was stimulated at concentrations of ≥ 13 mg a.i./kg dry weight, whether technical grade glyphosate or Roundup was applied. Denitrification was stimulated more strongly, when, in addition to the test compound, glucose was added. In the same soil, no substantial effects of either test compound on nitrification were found at 77 mg a.i./kg dry weight. Dose-related inhibition of nitrification was found at concentrations ≥ 230 mg a.i./kg dry weight for glyphosate and Roundup with respect to nitrate and nitrite production, respectively. For both substances the inhibition was transient in these studies. The temperature used was not reported.

No substantial effects on nitrification or denitrification were found in two agricultural soils from Southern Finland after application of an unknown formulation at a rate of 2.6 kg a.i./ha (Müller et al., 1981). A strong inhibition of nitrogenase activity was possibly an indirect effect due to a changed C/N ratio after the treatment. In this study lasting 28 days, pretreatment measurements were used as a control.

Roundup did not inhibit nitrification in three agricultural soils after treatment at a recommended application rate of 2.9 kg a.i./ha in 25-day experiments (Stratton, 1990). In a sandy loam, nitrification was stimulated after the application of 145 kg a.i./ha, whereas nitrification rates were inhibited by 50% at rates of 194-435 kg a.i./ha. Mineralization, expressed as the time-course of mg N/g dry weight (N = ammonium or nitrate nitrogen), in two agricultural sandy loam soils was stimulated significantly due to the treatment with 100 mg a.i./kg dry weight during an experiment lasting 70 days (Marsh et al., 1977).

Not only in agricultural soils but also in forest soils, the effects of Roundup on the nitrogen cycle appear not to be inhibitory. Preston & Trofymow (1989) found no significant effects on

nitrification or the immobilization of urea nitrogen in an organic fir forest floor and its underlying mineral horizon due to the application of 10 and 50 mg a.i./kg dry weight. This experiment lasted for 40 days.

In two agricultural sandy loam soils exposed to 100 mg a.i./kg dry weight for 210 days, transient slight effects on CO_2 evolution were observed by Marsh et al. (1977). These effects were both stimulatory (first 20 days) and inhibitory (until approximately 130 days) in both the soil from a permanent grassland and the soil from an arable field, but especially in the latter. Carlisle & Trevors (1986b) observed a dose-related increase in both O_2 consumption and CO_2 production in an agricultural sandy loam soil during a test of 10 days, with both technical grade glyphosate and Roundup. In general the increases were significant at concentrations greater than or equal to 127 mg a.i./kg. At the lowest dose (13 mg a.i./kg soil) no effects were observed except a significant increase in oxygen consumption due to Roundup. Oxygen consumption at doses greater than or equal to 127 mg a.i./kg was more strongly stimulated by Roundup than by technical grade glyphosate, possibly due to the isopropylamine or surfactant. Preston & Trofymow (1989) found no significant effect on CO_2 production in an organic fir forest floor and its underlying mineral horizon after the application of 10 and 50 mg a.i./kg dry weight. This experiment lasted 40 days.

Aerobic H_2 oxidation in an agricultural sandy loam was significantly inhibited by both technical grade glyphosate and Roundup, but only at the highest dose of 635 mg a.i./kg dry weight (Carlisle & Trevors, 1986b). Inhibition was stronger under anaerobic conditions, and was found at concentrations of $\geq$ 127 mg a.i./kg dry weight to be significant and dose related. Anaerobic inhibition might have been due to increased H_2 generation as a result of stimulated fermentation.

No effects of glyphosate on the activities of 1,3-β-glucanase and urease in a silt loam were found after application of Roundup at a rate of 12 mg a.i./kg dry weight (Lethbridge et al., 1981). Preston & Trofymow (1989) found no significant effect on urea hydrolysis in an organic fir forest floor and its underlying mineral horizon amended with 200 mg urea nitrogen per kg dry weight, due to treatment with Roundup at a rate of 50 mg a.i./kg dry weight.

The degradation of cellulose, starch, protein, or leaf litter was not inhibited at concentrations of 5 and 25 mg a.i./kg soil in three agricultural soils, with the exception of litter in silt loam at the highest dose (ABC Inc., 1978e). In this experiment lasting 84 days, technical grade glyphosate was applied.

Bacterial growth of *Rhizobium trifolii* in sterile solutions with Bergersen's broth was completely inhibited at solution concentrations of 10 and 20 mg a.i./litre, applied as an unknown formulation of glyphosate (Eberbach & Douglas, 1983). Only at 10 mg a.i./litre did the bacterial growth recover within 4 days. At lower concentrations no inhibition was found.

Mycelial growth of ectomycorrhizal fungi in pure cultures on agar was inhibited by 50% or more at concentrations of ≥ 1 mg a.i./litre for *Pisolithus tinctorius* and of ≥ 100 mg a.i./litre for *Cenococcum geophilum* and *Hebeloma longicaudum* (Estok et al., 1989). Ectomycorrhizal fungi commonly associated with pines (*Pinus* sp) were significantly inhibited at concentrations of ≥ 29 μg Roundup/litre (Chakravarty & Chatarpaul, 1990a). The most sensitive species were *Cenococcum graniforme*, *Hebeloma crustuliniforme* and *Laccaria laccata*. Roundup increased the susceptibility of sandy soil for *Gaeumannomyces gramminis*, a fungus causing "take-all disease" in wheat crops (Mekwatanakarn & Sivasithamparam, 1987). Application at a rate of 0.54 μg a.i./kg increased the survival and pathogenicity of the fungus significantly after 140 days of incubation at 25 °C. These authors concluded that Roundup affected microbial antagonists of the fungus.

9.1.2 Aquatic organisms

9.1.2.1 Plants

Appraisal

There is conflicting information on the effects of sediment on the phytotoxicity of glyphosate to aquatic plants; Lemna *showed reduced effects whilst* Carthamus *did not. Generally, glyphosate is thought to be largely unavailable to plants when added to soil and only effective as a herbicide when applied to foliage.*

The chronic toxicity to aquatic macrophytes when exposed to technical grade glyphosate or Roundup dissolved in water is

summarized in Tables 20 and 21. Glyphosate is slightly toxic with a 14-day NOEC value of 9 mg/litre. Roundup is also slightly toxic with 14-day NOEC values of 2.4-56 mg/litre. No data on acute toxicity for plants were available.

When Roundup was sprayed at a rate of 0.8 kg a.i./ha, the phytotoxicity to floating plants of the common duckweed (*Lemna minor*) was dependent on the extent of washed-off deposit (Lockhart et al., 1989). Phytotoxicity was highest when the sprayed deposits were not washed off within 6 h after application. Suspensions of 50 mg/litre of inorganic bentonite clay reduced the phytotoxicity of Roundup to common duckweed significantly (Hartman & Martin, 1984). When exposed for 14 days, concentrations up to 24 mg Roundup/litre had no effect on plant growth when sediment was added, whereas the growth was reduced by 50% at 5 mg Roundup/litre when no sediment was added.

Phytotoxicity of glyphosate to the safflower (*Carthamus tinctorius*) was not significantly reduced when the test compound was added to drainage water with suspended particles instead of distilled water (Bowmer et al., 1986). In these bioassays the inhibition of root elongation was measured when the plants were exposed to concentrations in unfiltered water of approximately 0.1-3 mg a.i./litre. The maximum concentration of adsorbed glyphosate was 2500 mg/kg.

Although it had inhibitory effects at high concentrations, Roundup had a stimulatory effect on the growth of common duckweed (*Lemna minor*) and tubers of sago pondweed (*Potamogeton pectinatus*) at lower concentrations (Hartman & Martin, 1985; Lockhart et al., 1989). Enhancement of growth of common duckweed and sago pondweed was found at 7-56 and 3 mg Roundup/litre, respectively. These stimulatory effects may refer to hormesis.

9.1.2.2 *Invertebrates*

Appraisal

The data from laboratory toxicity tests show that formulations are often more toxic than technical glyphosate to aquatic invertebrates. The surprising result that addition of clay particles to Daphnia test systems increased the toxicity of glyphosate is probably due to ingestion of herbicide bounds to the particles. Few studies have been

conducted in the presence of sediment; the reported toxicity of glyphosate is, therefore, difficult to relate to the field situation.

The acute and chronic toxicity of technical grade glyphosate and its formulations to aquatic invertebrates are summarized in Tables 18-21. Technical grade glyphosate is slightly to very slightly toxic, with LC_{50} values of ≥ 55 mg/litre and a 21-day NOEC value of 100 mg/litre. Formulations of glyphosate are moderately to very slightly toxic with 2-day EC_{50} values of 5.3-5600 mg product/litre and 21-day MATC values of 1.4-4.9 mg product/litre. The higher toxicity of Roundup to crustaceans is mainly due to the presence of surfactants (Servizi et al., 1987).

In a laboratory *in vitro* test with the gills of *Mytilus californianus*, a marine water mollusc, the active uptake of glycine was inhibited by 23 and 67%, respectively, when a mixture of ^{14}C-labelled and unlabelled glyphosate was applied at rates of 0.2-1.7 mg/litre (Swinehart & Cheney, 1987). This inhibition might be an indication for Mg^{2+}-moderated binding of glycine to the gill surface, whereas glyphosate can compete with glycine uptake by forming a metal complex.

Water fleas (*Daphnia magna*) were less sensitive to Roundup, when the water was aerated than when it was unaerated. The 2-day LC_{50} value decreased from 37 (with aeration) to 24 mg (without aeration) Roundup/litre (EG & G Bionomics, 1980f).

Addition of 50 mg/litre of inorganic bentonite clay decreased the 2-day EC_{50} value for mature water fleas (*Daphnia pulex*) from 16 to 7 mg Roundup/litre (Hartman & Martin, 1984). This higher toxicity might have been due to ingestion of particle-adsorbed glyphosate. Addition of bentonite also increased the toxicity in a chronic experiment with populations of *Daphnia pulex*. Immature water fleas were more sensitive to glyphosate than the adults under all conditions. Populations in all treatments had recovered within 14 days after the application (Hartman & Martin, 1984).

No avoidance of glyphosate was found when mayfly nymphs (*Ephemerella walkeri*) were tested at concentrations of 0.2-2 mg Roundup/litre, whereas avoidance was demonstrated at 24 mg/litre (Folmar et al., 1979).

Table 18. Aquatic organisms: acute toxicity of technical grade glyphosate in a static test system

Organism	A	Test water	pH	Hardness (mg $CaCO_3$/ litre)	Temperature (°C)	Experimental duration (days)	Parameter	Concentration (mg/litre)	Reference
Molluscs									
Crassostrea virginica, eggs	-	n.w.[a]	n.r.	n.r.	25	2	EC_{50}	> 10[b]	Bionomics (1973a)
Echinodermata									
Tripneustes esculentes	-	n.w.[a]	7.7-8.2	n.r.	20	4	EC_{50}	> 1000[c]	E G & Bionomics (1978d)
Crustaceans									
Daphnia magna, first instar	-	w.w.	7.8-8.0	> 250	19	2	LC_{50}	780	ABC Inc. (1978a)
Uca pugilator	-	a.m.[a]	n.r.	n.r.	21	4	LC_{50}	934	Bionomics (1973b)
Palaemonetes vulgaris	-	a.m.[a]	n.r.	n.r.	21	4	LC_{50}	281	Bionomics (1973b)
Mysidopsis bahia	-	n.w.[a]	6.4-8.3	n.r.	20	4	LC_{50}	> 1000	E G & Bionomics (1978c)
Insects									
Chironomus plumosus, fourth instar	-	r.w.	n.r.	40	22	2	EC_{50}	55[d]	Folmar et al. (1979)

Table 18 (contd).

Organism	A	Test water	pH	Hardness (mg $CaCO_3$/ litre)	Temperature (°C)	Experimental duration (days)	Parameter	Concentration (mg/litre)	Reference
Fish									
Ictalurus punctatus	-	r.w.	n.r.	40	22	4	LC_{50}	130	Folmar et al. (1979)
Salmo gairdnerii, 0.4 cm, 0.5 g	-	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	10-197	Wan et al. (1989)
Salmo gairdnerii, 0.4 cm, 0.6 g	-	r.w.	4.4-7.2	45	12	4	LC_{50}	86	ABC Inc. (1978b)
Oncorhynchus keta, 0.4 cm, 0.5 g	-	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	10-148	Wan et al. (1989)
O. kisutch, 0.4 cm, 0.5 g	A	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	27-174	Wan et al. (1989)
O. tshawytsha, 0.4 cm, 0.5 g	-	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	19-211	Wan et al. (1989)
O. gorbusha, 0.4 cm, 0.5 g	-	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	14-190	Wan et al. (1989)
Lepomis macrochirus	-	r.w.	n.r.	40	22	4	LC_{50}	140	Folmar et al. (1979)
L. macrochirus, 0.3 cm, 1.0 g	-	r.w.	6.6-7.0	45	21	4	LC_{50}	120	ABC Inc. (1978c)
Pimephales promelas	-	r.w.	n.r.	40	22	4	LC_{50}	97	Folmar et al. (1979)
Rasbora heteromorpha, 0.1-0.3 cm	-	r.w.	n.r.	25	21	4	LC_{50}	168	HRC (1977)
Cyprinodon variegatus, 0.7-1.0 cm	-	n.w.[e]	7.6-8.3	n.r.	20	4	LC_{50}	> 1000	E G & Bionomics (1978b)

[a] salinity 20-35‰
[b] based on abnormal development of oyster larvae
[c] based on immobility, drooping spines, and retracted podia
[d] based on immobilisation
[e] salinity 18‰

A = actual concentrations are measured; - = nominal concentration; a.m. = artificial medium; r.w. = reconstituted water; w.w. = well water; n.w. = natural surface water; n.r. = not reported

Table 19. Aquatic organisms: acute toxicity of formulations with glyphosate

Organism	A	Test type	Compound	Test water	pH	Hardness (mg $CaCO_3$/litre)	Temperature (°C)	Experimental duration (days)	Parameter	Concentration (mg/litre)[a]	Reference
Crustaceans											
Daphnia magna, first instar	-	S	Ru	r.w.	8.2-8.3	175	22	2	EC_{50}	5.3[b]	E G & G Bionomics (1980e)
D. magna, first instar	-	S	Ru	r.w.	7.7-8.1	175	22	2	EC_{50}	24-37[b]	E G & G Bionomics (1980f)
D. magna, first instar	-	S	Ru	r.w.	n.r.	40	22	2	EC_{50}	7.3[b]	Folmar et al. (1979)
D. magna, first instar	-	S	St	w.w.	8.3-8.5	225-275	20	2	EC_{50}	42[b]	ABC Inc (1984c)
D. magna, first instar	-	S	RuD	w.w.	7.9-8.6	255	20	2	EC_{50}	930[b]	ABC Inc (1981a)
D. pulex, mature	-	S	Ru	w.w.	7.6	282	15	2	EC_{50}	19[b]	Hartman & Martin (1984)
Gammarus pseudolimnaeus	A	CF	Ru	w.w	7.9-8.3	255	17	2	EC_{50}	42[b,c]	ABC Inc (1982b)
Insects											
Chironomus riparius, fourth instar	-	S	Rod	r.w.	7.6-7.8	42-44	22	2	EC_{50}	5600[b]	Buhl & Faerber (1989)
Chironomus plumosus, fourth instar	-	S	Ru	r.w.	n.r.	40	22	2	EC_{50}	44[b]	Folmar et al. (1979)

Table 19 (contd).

Organism	A	Test type	Compound	Test water	pH	Hardness (mg $CaCO_3$/litre)	Temperature (°C)	Experimental duration (days)	Parameter	Concentration (mg/litre)[a]	Reference
Fish											
Ictalurus punctatus, 0.7 cm, 3 g	-	S	Ru	r.w.	6.3-7.2	24-40	22	4	LC_{50}	52	E G & G Bionomics (1980a)
Ictalurus punctatus	-	S	Ru	r.w.	n.r.	40	22	4	LC_{50}	32	Folmar et al. (1979)
Salmo gairdnerii, 0.4 cm, 0.7 g	-	S	St	r.w.	6.8-7.3	40-45	12	4	LC_{50}	7.5	ABC Inc (1984a)
Salmo gairdnerii, 0.5 cm, 2.4 g	A	CF	Ru	w.w.	8.0-8.2	255	12	4	LC_{50}	8.2[c]	ABC Inc (1982c)
Salmo gairdnerii, 0.4 cm, 0.5 g	-	S	Ru	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	14-33	Wan et al. (1989)
Salmo gairdnerii, 0.3 cm, 0.3 g	-	S	Ru	r.w.	6.6-7.6	40	12	4	LC_{50}	36	E G & G Bionomics (1980c)
Salmo gairdnerii, 0.4 cm, 0.7 g	-	S	Ru	w.w	6.4-7.3	26-26	12	4	LC_{50}	22	E G & G Bionomics (1980g)
S. gairdnerii, 0.4 g	A	S	Ru	r.w	7.4-7.7	85	11	4	LC_{50}	22	EVS Consultants (1986a)
S. gairdnerii, 0.4 g	A	S	Ru	n.w	7.4-7.8	81	11	4	LC_{50}	15	EVS Consultants (1986a)
S. gairdnerii, 0.4 g	A	S	Ru	d.w	5.4-6.3	4.5	11	4	LC_{50}	26	EVS Consultants (1986a)

Table 19 (contd).

S. gairdnerii, 1.0 g	-	S	Ru	r.w	~ 7.2	40	12	4	LC_{50}	3.2	Folmar et al. (1979)
Salmo gairdnerii, 0.3 cm, 0.2 g	-	S	RuD	w.w	4.6-7.1	45	12	4	LC_{50}	> 1000	ABC Inc (1981c)
S. gairdnerii, 9.5 cm	-	S	Vis	n.r.	6.0	9.6	12.3	4	LC_{50}	34	Morgan & Kiceniuk (1992)
Lepomis macrochirus, 0.2 cm, 0.1 g	-	S	St	r.w.	6.8-7.5	40-45	22	4	LC_{50}	4.5	ABC Inc (1984b)
Lepomis macrochirus, 0.2 cm, 0.1 g	-	S	RuD	w.w.	4.9-7.1	45	22	4	LC_{50}	> 1000	ABC Inc (1981b)
Lepomis macrochirus, 0.2 cm, 0.2 g	A	CF	Ru	w.w.	8.0-8.2	255	22	4	LC_{50}	5.8[c]	ABC Inc (1982a)
Lepomis macrochirus, 0.4 cm, 0.3 g	-	S	Ru	r.w.	6.4-7.5	40	22	4	LC_{50}	46	E G & G Bionomics (1980b)
Pimephales promelas, 0.4 cm, 0.6 g	-	S	Ru	w.w.	6.7-7.7	39-44	22	4	LC_{50}	31	E G & G Bionomics (1980d)
Pimephales promelas	-	S	Ru	r.w.	n.r.	40	22	4	LC_{50}	5.6	Folmar et al. (1979)
Cyprinus carpio, 0.6 cm, 2.8 g	A	S	St	w.w.	7.2-7.9	40-48	22-23	4	LC_{50}	2.4	ABC Inc (1990)

Table 19 (contd).

Organism	A	Test type	Compound	Test water	pH	Hardness (mg $CaCO_3$/litre)	Temperature (°C)	Experimental duration (days)	Parameter	Concentration (mg/litre)[a]	Reference
Fish (contd).											
Oncorhynchus kisutch, 0.4 cm, 0.5 g	A	S	Ru	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	13-33	Wan et al. (1989)
O. kisutch, 11.8 g	A	S	Ru	d.w.	5.5-6.4	4.5	11	4	LC_{50}	22	EVS Consultants (1986c)
O. keta, 0.4 cm, 0.5 g	-	S	Ru	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	11-20	Wan et al. (1989)
O. tshawytsha, 0.4 cm, 0.5 g	-	S	Ru	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	17-33	Wan et al. (1989)
O. tshawytsha, 4.6 g	A	S	Ru	d.w.	5.8-6.7	4.5	11	4	LC_{50}	20	EVS Consultants (1986b)
O. gorbusha, 0.4 cm, 0.5 g	-	S	Ru	divers	6.3-8.2	5.3-148	n.r.	4	LC_{50}	14-33	Wan et al. (1989)
Oncorhynchus nerka, 3-6.5 cm, 0.2-3.8 g	A	S	Ru	n.w	7.7-8.0	84	4-5	4	LC_{50}	27-29	Servizi et al. (1987)

[a] All concentrations are expressed as mg of the formulation per litre
[b] Based on immobilisation
[c] Based on actual concentrations
A = actual concentrations are measured; - = nominal concentrations; CF = continous flow system; S = static system; Tgg = technical grade glyphosate; Ru = Roundup; Rod = Rodeo; St = Sting; RuD = Roundup D-Pak; d.w. = dechlorinated tap water; r.w. = reconstituted water; w.w. = well water; n.w. = natural surface water; a.m. = artificial medium

Table 20. Aquatic organisms: chronic toxicity of glyphosate (NOEC/MATC)

Organism	A	Test type	Test sub-stance	Test water	pH	Hardness (mg $CaCO_3$/ litre)	Tempera-ture (°C)	Experimen-tal duration (days)	Par-ameter	Concen-tration (mg/litre)	Reference
Macrophytes											
Lemna gibba	A	S	Tgg	a.m.	7.5	285	25	14	NOEC	9[a,b]	Malcolm Pirnie Inc. (1987e)
Crustaceans											
Daphnia magna, first instar	A	SS	Tgg	w.w.[c]	6.8-8.2	160-180	20	21	NOEC	100[a,d]	ABC Inc. (1989c)
Fish											
Salmo gairdneri, 0.5 cm, 1.3 g	A	CF	Tgg	w.w.	5.9-7.8	40-48	14-15	21	NOEC	52[a,e]	ABC Inc. (1989e)
Pimephales promelas, 1.5 g	A	CF	Tgg	w.w.	6.5-7.6	32-42	25	255	MATC	> 26[f]	E G & G Bionomics (1975)

[a] Based on actual concentrations
[b] Based on biomass decrease
[c] Mixed with natural surface water
[d] Based on survival and reproduction
[e] Based on survival, behaviour, and coloration
[f] Based on survival, growth and reproduction

A = actual concentrations are measured; CF = continous flow system; SS = semi-static system; S = static system; Tgg = technical grade glyphosate; w.w. = well water; a.m. = artificial medium

Table 21. Aquatic organisms: chronic toxicity of formulations with glyphosate

Organism	A	Test type	Test sub-stance	Test water	pH	Hardness (mg $CaCO_3$/ litre)	Tempera-ture (°C)	Experimen-tal duration (days)	Par-ameter	Concen-tration (mg/litre)[f]	Reference
Macrophytes											
Lemna minor	-	S	Ru	a.m.	n.r.	n.r.	25	14	NOEC	56[c,d]	Lockhart et al. (1989)
Lemna minor	-	S	Ru	a.m.	n.r.	n.r.	22	14	NOEC	2.4[c,e]	Hartman & Martin (1984)
Potamogeton pectinatus (tubers)	-	S	Ru	a.m.	n.r.	n.r.	22	14	NOEC	33[c,d]	Hartman & Martin (1985)
Crustaceans											
Daphnia magna, first instar	A	SS	St	w.w.[b]	7.2-8.2	174	20	21	MATC	1.4[g,i]	ABC Inc. (1989a)
Daphnia magna, first instar	A	SS	Ru	w.w.[b]	7.6-8.3	160-180	20	21	MATC	4.9[g,i]	ABC Inc. (1989b)

Table 21 (contd).

Fish											
Salmo gairdneri, 0.5 cm, 1.3 g	A	CF	St	w.w.	7.3-7.8	40-48	14-16	21	NOEC	0.8[a,h]	ABC Inc. (1989h)
Salmo gairdneri, 0.5 cm, 1.8 g	A	CF	Ru	w.w.	7.1-7.8	24-48	14-16	21	NOEC	2.4[a,h]	ABC Inc. (1989d)
Salmo gairdneri, 9.5 cm, 7.2 g	A	CF	Vis	n.r.	6.0	9.6	12.3	~ 60	NOEC	> 0.04[a,k]	Morgan & Kiceniuk (1992)

[a] Based on actual concentrations
[b] Mixed with natural surface water
[c] Roundup dissolved in test water
[d] Based on biomass decrease
[e] Based on reduction of frond formation
[f] All concentrations are expressed as mg of the formulation per litre
[g] Based on survival, reproduction and length of time to the first brood
[h] Based on survival, behaviour and coloration
[i] NOEC is 1.0 mg Sting/litre
[j] NOEC is 3.2 mg Roundup/litre (actual concentration)
[k] Based on mortality and growth

A = actual concentrations are measured; - = nominal concentrations; CF = continous flow system; SS = semi-static system; Ru = Roundup; St = Sting; Vis = Vision; w.w. = well water; a.m. = artificial medium; n.r. = not reported.

9.1.2.3 Vertebrates

Appraisal

The toxicity tests for fish are generally performed without sediment. As the bioavailability of glyphosate itself will be reduced under most conditions due to sorption onto sediment, no toxic effects are expected. Toxic effects, however, can be expected due to surfactants in some formulations. To a lesser extent, life-stage, pH, water hardness, temperature, and the presence of feed all influence toxicity. No adverse effects on the osmoregulatory mechanism were found.

The acute and chronic toxicity of technical grade glyphosate and its formulations to fish are summarized in Tables 18-21. Technical grade glyphosate is moderately toxic with 4-day LC_{50} values of 10 to > 1000 mg/litre, a 21-day NOEC value of 52 mg/litre, and an MATC value of > 26 mg/litre. Formulations of glyphosate have comparable toxicity with 4-day LC_{50} values of 2.4 to > 1000 mg product/litre, and 21-day NOEC values of 0.8-2.4 mg product/litre. Toxicity may vary substantially, depending on the species, the test compound and test conditions. In general, technical grade glyphosate is less toxic than the formulations. This difference is mainly due to a higher toxicity of surfactants in the formulations (Folmar et al., 1979; Servizi et al., 1987; Mitchell et al., 1987; Wan et al., 1989).

In laboratory experiments the major factors influencing the toxicity appear to be the tested species and its age, the presence of surfactants, the hardness, pH, temperature and the availability of ration. Wan et al. (1989) found that the toxicity of technical grade glyphosate to salmonids increased when hardness and pH decreased, whereas for Roundup and Accord CR the contrary was true, due to the presence of a 75% tallow amine surfactant in the formulations. This surfactant was most toxic in hard water with a relatively high pH. A higher toxicity of Roundup to salmonids at increasing hardness and pH was confirmed by Mitchell et al. (1987), but only partially by Servizi et al. (1987). It was also confirmed by Folmar et al. (1979), although they only investigated the effect of pH in reconstituted water with a moderate hardness. These authors demonstrated that the effects of a pH increase on the toxicity of Roundup and technical grade glyphosate were not only seen in rainbow trout (*Salmo gairdnerii*) but also in bluegill sunfish (*Lepomis macrochirus*). For both species Roundup became

more toxic as the pH increased from 6.5 to 7.5, whereas technical grade glyphosate became less toxic as the pH increased from 6.5 to 9.5. At pH values higher than 7.5, the toxicity of Roundup remained constant. In the investigations of Servizi et al. (1987), an antagonistic effect of glyphosate on the toxic action of a surfactant was found.

Increased toxicity of formulations due to surfactants was not only demonstrated for the tallow amine surfactant in Roundup but also for ortho X-77 in Rodeo (Mitchell et al., 1987). However, even in the presence of a surfactant, the acute toxicity of some formulations may be very slight, as was found by ABC Inc. (1980a,b). In these studies, 0.5% (v/v) of the surfactant X-77 was added to Roundup D-Pak, resulting in a 4-day LC_{50} value of this mixture for rainbow trout (*Salmo gairdnerii*) and for bluegill sunfish (*Lepomis macrochirus*) of 240 and 830 mg/litre, respectively.

Folmar et al. (1979) performed acute toxicity tests with various species in reconstituted water with a pH of 7.2 and a hardness of 40 mg $CaCO_3$/litre. In these tests it was demonstrated for rainbow trout (*Salmo gairdnerii*) and channel catfish (*Ictalurus punctatus*) that the sensitivity to Roundup increased in the following order: eyed eggs, 2-g fingerlings, sac fry, swim-up fry, and 1-g fingerlings. The 4-day LC_{50} values for the various life-stages of rainbow trout decreased from 39 mg Roundup/litre for eyed eggs to 3.2 mg/litre for small fingerlings. In an additional test, a 4-h exposure to concentrations of ≥ 12 mg Roundup/litre affected the survival of sac fry and swim-up fry significantly.

Holdway & Dixon (1988) also demonstrated that toxicity is dependent on the life-stage by applying technical grade glyphosate to larvae of flagfish (*Jordanella floridae*). At concentrations up to 30 mg a.i./litre, no 2- or 4-day-old larvae died, whereas 20% of the 8-day-old larvae died at the top dose. The effect was even more drastic when the larvae were not fed. This treatment killed 20% of the oldest larvae even at 3 mg a.i./litre. According to the authors, the effect of age might fit the idea of saltatory ontogeny, implying critical periods for organs and tissues.

While investigating the effect of temperature on toxicity, Folmar et al. (1979) demonstrated for rainbow trout (*Salmo gairdnerii*) and bluegill sunfish (*Lepomis macrochirus*) that toxicity increased with increasing temperatures. For the trout the 4-day LC_{50} values decreased from 34 mg Roundup/litre at 7 °C to

18 mg/litre at 17 °C. For the bluegill sunfish the 4-day LC_{50} values decreased from 18 mg/litre at 17 °C to 9.8 mg/litre at 27 °C.

Rainbow trout (*Salmo gairdneri*) showed the same sensitivity to Roundup, independent of whether the water was aerated or not (EG & G Bionomics, 1980g). In this experiment the 4-day LC_{50} under both conditions was 22 mg Roundup/litre.

The potential of coho salmon smolt (*Oncorhynchus kisutch*) to adapt to changes in water salinity encountered during migration was not influenced by the application of Roundup at actual concentrations up to 2.8 mg/litre (EVS Consultants, 1986d). The osmoregulatory mechanism, which is fully functional in smolts, was unaffected as indicated by plasma Na^+ concentrations, haematocrit values and the condition of the fish. During the experiment in which the fish were exposed for 10 days in fresh water and subsequently allowed to recover in fresh or sea water, no abnormal behaviour was observed.

No avoidance of glyphosate was found when rainbow trout were tested at concentrations up to 24 mg Roundup/litre (Folmar et al., 1979). Sublethal concentrations in acute toxicity tests with Roundup may cause loss of motility, complete loss of equilibrium, darkened pigmentation, or rapid respiration (EG & G Bionomics, 1980a,b,g; EVS Consultants, 1986a,b,c).

Rainbow trout (*Salmo gairdneri*) were exposed to glyphosate (as Vision) at 0, 6.25, 25 and 100 µg Vision/litre in a continuous flow system. There were no effects on growth or foraging behaviour, and no histopathological liver effects. Two out of three types of aggressive behaviour were also unaffected; the third, a warning "wig-wag" increased in frequency at the top-dose 1 month after treatment. After two months the frequency was equal to that of the control (Morgan & Kiceniuk, 1992).

9.1.3 Terrestrial organisms

9.1.3.1 Plants

Nodulation of sub-clover (*Trifolium subterraneum*) was inhibited by an unknown formulation with glyphosate in a dose-related way at concentrations of 2-20 mg a.i./litre (Eberbach & Douglas, 1989). In this experiment, 3-day-old seedlings were inoculated with *Rhizobium trifolii*. The seedlings were cultured

for 56 days in soil-free systems with nutrient solutions. In an additional experiment in which *Rhizobium* was exposed to the same concentrations, repeated washing of the inoculi prior to nodule initiation did not reduce the inhibition of the nodulation of the sub-clover after inoculation. This indicated that the effect on nodulation might be the result of damage to the bacteria rather than to carry-over of glyphosate from the bacteria to the plant.

Seed germination of various forest species was not affected by treatment with Roundup at concentrations up to 305 mg a.i. (free acid)/kg dry weight. Seed germination was affected at the highest tested dose of 1525 mg a.i. (free acid)/kg dry weight (Morash & Freedman, 1988). The effect on seed germination was confirmed by another experiment in which no differences were found between sprayed and unsprayed plots with respect to seedling composition and quantity. Morash & Freedman (1988) then incubated the soils of clear-cuts in a greenhouse. The application of Roundup in the field was at a rate of 2.3 kg a.i./ha.

Red pine seedlings (*Pinus resinosa*) were not affected by treatment with Roundup, with the exception of a dose-related decrease in root length (Chakravarty & Chatarpaul, 1990b). Non-affected growth parameters were shoot height, shoot weight, root weight, and mycorrhizal development. In this experiment lasting 60 days, Roundup was applied at rates of 0.54 and 3.2 kg a.i./ha. The conifer seedlings were inoculated with an ectomycorrhizal fungus (*Paxillus involutus*), 14 days after germination.

Glyphosate may affect various pathways of the secondary metabolism in the plant, although the actual targets in plants have not been located. The synthesis of aromatic amino acids, secondary hydroxyphenolic compounds, chlorophyll and δ-amino-levulinic acid were reported to be affected by glyphosate (Amrhein et al., 1980; Duke & Hoagland, 1981; Kitchen et al., 1981a,b). Aromatic amino acids are important for the synthesis of, for instance, some alkaloids, the phytohormone indole-3-acetic acid and phenolic compounds such as lignin and quinones.

With respect to the synthesis of aromatic amino acids, there are indications that the actual target is not shikimate kinase or anthranilate synthase, but probably 5-enolpyruvylshikimate-3-phosphate synthase or chorismate synthase (Amrhein et al., 1980). When applied to hypocotyls of buckwheat (*Fagopyrum esculentum*) and to cultured cells of smooth bedstraw (*Galium mollugo*), technical grade glyphosate inhibited the conversion of shikimate

to chorismate. This inhibition *in vivo* and *in vitro* was found at concentrations of ≥ 10 mg a.i./litre. In the cultured cells of *Galium*, the accumulation of shikimate was concomitant with a decrease of anthraquinones (Amrhein et al., 1980). Possibly the non-transportable carbon pool, to which carbon was found to be diverted in sugar beets (*Beta vulgaris*) due to glyphosate, was in fact shikimate (Gougler & Geiger, 1984). These effects on carbon metabolism were found at application rates equivalent to 5 kg a.i./ha leaves. Duke & Hoagland (1981) assumed that possibly chelation of divalent ions such as Ca^{2+} and Mg^{2+} that are involved in many metabolic pathways was the main cause of damage.

9.1.3.2 Invertebrates

Appraisal

Glyphosate has low toxicity for bees and earthworms.

Oral 2-day LD_{50} values of technical grade glyphosate and Roundup for bees were 100 µg a.i./bee and > 100 µg Roundup/bee, respectively. Contact 2-day LD_{50} values for these two substances were likewise > 100 µg a.i./bee and > 100 µg Roundup/bee (HRC, 1972). The oral 2-day LD_{50} of Sting was also > 100 µg Sting/bee (Altmann, 1984). In these experiments *Apis mellifera* was tested. The LD_{50} values indicate a slightly acute toxicity of technical grade glyphosate, Roundup, and Sting to honey-bees. Roundup was also slightly toxic to green lacewings (*Chrysoperla carnea*) when they were exposed by contact to 1 kg Roundup/ha (SFRSA, 1990). In this experiment the average number of eggs per female and the larval and pupal mortality were increased due to the treatment, resulting in an overall reduction in beneficial capacity of 41%. The beneficial capacity is a function of the larval and pupal mortality, and the average number of eggs per treated and untreated female. No effects on the food uptake and mortality of the beetle *Poecilus cupreus* Bonelli were observed 15 days after application of 6 kg Sting/ha (IFU, 1990).

When exposed to artificial soil contaminated with Roundup D-pak, earthworms (*Eisenia fetida*) were soft and/or slack, in a dose-related way, at concentrations ≥ 500 mg Roundup D-pak/kg dry weight (IBR, 1991a). No other adverse effects were found, indicating a 14-day NOEC of 158 mg/kg. A comparable experiment with Roundup also indicated slight toxicity for earthworms with a 14-day NOEC of 500 mg Roundup/kg dry

weight (IBR, 1991b). At higher concentrations thin, slack and lethargic worms with a dark skin were observed.

9.1.3.3 Vertebrates

Appraisal

Glyphosate has low toxicity to birds after acute oral or short-term dietary exposure. Mammals tested showed effects (body weight loss) only after high levels of dosing. Herbicide-treated foliage was not avoided by deer in the single study reported.

The acute, subacute and chronic toxicity of glyphosate and its formulations to birds is summarized in Table 22.

Male marsupials (*Sminthopsis macroura*) showed significant body weight loss after exposure to feed contaminated with concentrations of up to 5000 mg a.i/kg feed (Evans & Batty, 1986). No other treatment-related effects were found in the male marsupials. In female hopping-mice (*Notomys alexis* and *Notomys mitchelli*) fed similar doses, no treatment-related effects were found.

Glyphosate did not affect the daily chow consumption of black-tailed deer (*Odocoileus hemionus columbianus*) when these herbivores were exposed to browse treated with glyphosate at a rate of 2.2 kg/ha (Sullivan & Sullivan, 1979). The deer did not avoid the contaminated browse, and sometimes even preferred it. Irrespective of whether they were given treated alfalfa (*Medicago sativa*), treated alder (*Alnus rubra*) or untreated feed, the deer had the same daily chow consumption.

9.2 Field observations

9.2.1 Microorganisms

Appraisal

Some effects on microorganisms have been reported in field studies following application of glyphosate formulations. However, these have been minor and reversible in most cases. It is not possible to separate the direct toxic effects of the herbicide from changes in the habitat caused by its intended herbicidal action.

Table 22. Birds: acute and chronic toxicity of glyphosate and its formulations

Species	Compound	Sex	Age	Route	Experimental duration (days)	Parameter	Concentration	Reference
Colinus virginianus	Tgg	n.r.	n.r.	diet	8	LC_{50}	> 4640 mg/kg feed	Hazleton Lab. Inc. (1973a)
Colinus virginianus	Tgg	n.r.	14 days	oral		LD_{50}	> 3851 mg/kg b.w.	Wildlife Int Ltd. (1978c)
Colinus virginianus	Tgg	M,F	5 months	diet	119	NOEC	≥ 1000 mg/kg feed[a]	Wildlife Int Ltd. (1978b)
Colinus virginianus	Ru	n.r.	10 days	diet	8	LC_{50}	> 5620 mg/kg feed[b]	Wildlife Int Ltd. (1990b)
Anas platyrhynchos	Tgg	n.r.	n.r.	diet	8	LC_{50}	> 4640 mg/kg feed	Hazleton Lab. Inc. (1973b)
Anas platyrhynchos	Tgg	M,F	6 months	diet	112	NOEC	≥ 1000 mg/kg feed[a]	Wildlife Int Ltd. (1978a)
Anas platyrhynchos	Ru	n.r.	10 days	diet	8	LC_{50}	> 5620 mg/kg feed[b]	Wildlife Int Ltd. (1990a)
Poephilla guttata	Ru	M	mature	diet	7	LC_{50}	< 16 393 mg/kg feed[b]	Evans & Batty (1986)
Poephilla guttata	Ru	M	mature	diet	5	LC_{50}	> 8197 mg/kg feed[b]	Evans & Batty (1986)

[a] Based on reproduction impairment of one generation
[b] Concentrations expressed as mg of the formulation per kg body weight or feed

Tgg = technical grade glyphosate; Ru = Roundup; M = males; F = females; n.r. = not reported.

9.2.1.1 Water

In a pool located in Hong Kong, treatment with 656 mg Roundup/litre caused a substantial decrease in bacteria within 14 days after treatment. The number of colony-forming units had returned to the control level 30 days after treatment (Chan & Leung, 1986).

Diatom populations in the water and sediments of a pond and a stream showed a significantly different density of some species when aerially treated with Roundup at a rate of 2.2 kg a.i./ha (Sullivan et al., 1981). The authors concluded that these differences were probably due to seasonal and habitat variation, rather than to treatment with the herbicide.

Roundup may have affected the increase in ash-free dry weight and the chlorophyll *a* standing crop of periphyton in the first month after spraying with Roundup at a rate of 2.2 kg a.i./ha (Holtby & Baillie, 1989a). In this experiment lasting around 130 days, some tributaries in a watershed in British Columbia (Canada) were directly oversprayed.

9.2.1.2 Soil

When Roundup was applied to a sandy loam being prepared for conifer forestation, no significant changes in the soil respiration were found up to about 180 days after application of Roundup at a rate of 0.54 kg a.i./ha (Chakravarty & Chatarpaul, 1990a). Concomitantly the numbers of fungi and bacteria decreased significantly during the first 2 months, but after about 180 days the numbers had recovered. These results might indicate changes in microbial populations due to application of Roundup at recommended application rates.

Preston & Trofymow (1989) found no significant effects on the number of bacteria, actinomycetes, and nitrogen fixers in ferro-humic podsols covered with alder trees (*Alnus rubra*) in the Carnation Creek watershed, Canada, due to treatment with Roundup. The only consistent effect was a significant reduction of the number of fungi at one of the two treated sites. In this site the fungi appeared to have recovered at the end of the study. In this experiment of about 180 days, Roundup was hand-sprayed at a rate of 2 kg a.i./ha. In an additional comparable intensive field trial of one month, microflora populations appeared to have recovered from treatment after one month, with the exception of

the reduction of fungi in the litter of one of the treated sites. Actinomycetes and nitrogen fixers in the litter appeared to be reduced in numbers due to the treatment but they subsequently recovered. This reduction was not found in the underlying humus layer.

Stratton & Stewart (1992) studied microbial activity in forest soil and litter following the application of Roundup at 4.7 litres/ha (equivalent to 1.7 kg a.i./ha) to a coniferous forest previously clear-cut and replanted. Treated and untreated (covered with plastic sheeting during application) areas of forest were used to obtain soil and litter samples which were tested *in situ* over an 8 months period following spraying. Glyphosate had a generally stimulatory effect on microbial biomass in litter (up to 80% increase) but no significant effect in soil. There were no significant effects on the numbers of bacteria, fungi or actinomycetes in either soil or litter. Glyphosate generally stimulated respiration in both soil and litter; the degree of stimulation was very variable throughout the sampling period, ranging from a few percent to 100% increases in CO_2 evolution.

No substance- or dose-related effects on aerobic bacteria were observed in a sandy soil in a semi-arid region of Argentina up to 96 days after the application of Roundup (Gómez & Sagardoy, 1985). Doses of up to 2.8 kg a.i. (free acid)/ha were applied.

No substance-related effects on the growth of an ectomycorrhizal fungus were found when Roundup was applied at concentrations of up to 3.2 kg a.i./ha (Chakravarty & Chatarpaul, 1990b). In these experiments red pines (*Pinus resinosa*) were inoculated with the fungus *Paxillus involutus*. In the field 74-86% of the seedlings that were not inoculated were colonized by indigenous mycorrhizal fungi within two months.

9.2.2 Aquatic organisms

Appraisal

Little effect has been reported on aquatic invertebrates or fish exposed to glyphosate formulations sprayed in the field. Minor mortality in a single study of young trout may reflect the greater sensitivity of early life-stages.

9.2.2.1 Plants

No field data on toxicity to aquatic macrophytes are available.

9.2.2.2 Invertebrates

An increased drift of midge larvae (*Chironomus plumosus*) was found in artificial outdoor streams treated with 4.9 mg Roundup/litre (Folmar et al., 1979). No increased drift was found at 0.5 mg Roundup/litre. In streams in a coastal rainforest in British Columbia, Canada, only the drift densities of an amphipod (*Gammarus* sp) and mayflies (*Paraleptophlebia* sp) increased after treatment with Roundup at a rate of 2 kg a.i./ha (Kreutzweiser et al., 1989). Density peaks partly coincided with periods immediately following rainfall, which might indicate an effect due to glyphosate run-off, or an effect due to increased discharges.

During most streamflows, the abundance of benthic macroinvertebrates in a stream and at sites in tributary swamps was similar at untreated sites and at sites that had been treated with Roundup at a rate of 2.2 kg a.i./ha (Scrivener & Carruthers, 1989). However, after periods of frequent rainstorms leading to flooding, the abundance at the treated sites was 40–50% lower.

Water-fleas (*Daphnia magna*) did not show any mortality in a pond sprayed with Roundup at application rates of up to 220 kg a.i./ha (Hildebrand et al., 1980). In this experiment the water-fleas were exposed for 8 days in pens that were partly immersed in the water.

9.2.2.3 Vertebrates

Fingerlings (2.1 g, 5.8 cm) of rainbow trout (*Salmo gairdnerii*) that were exposed for 14 days to Roundup in flow-through pens in shallow streams did not show any mortality or substance-related effects at application rates of up to 220 kg a.i. (free acid)/ha (Hildebrand et al., 1982). In this experiment Roundup was sprayed manually on moderately flowing forest streams in British Columbia, Canada. In the same area an aerial application of 2.2 kg a.i. (free acid)/ha did not cause mortality or obvious signs of stress in rainbow trout fingerlings in flow-through pens, which were also exposed to Roundup for 14 days (Hildebrand et al., 1982). A direct aerial application of 2.1 kg a.i./ha on a tributary of the Carnation Creek watershed (British Columbia, Canada), however, killed 2.6% of the 120 fingerlings of coho salmon (*Oncorhynchus*

kisutch) within 24 h after application, whereas no mortality occurred at the unexposed sites (Holtby & Baillie, 1989b). Also some stress of the caged fingerlings was indicated within the first 2 h after application. Up to 2 years after application no consistent effects of Roundup on over-winter mortality, probability of entering and leaving the tributary, timing of spring emigration, and growth rates were found.

In artificial outdoor streams in Colorado, USA, rainbow trout (*Salmo gairdnerii*) were exposed to concentrations of up to 5 mg Roundup/litre for 12 h. No adverse effects on fecundity and gonadosomatic indices were found in this study by Folmar et al. (1979).

Rainbow trout (*Salmo gairdnerii*) in pens that were immersed in stream water avoided Roundup at concentrations of ≥ 40 mg Roundup/litre (Hildebrand et al., 1982).

9.2.3 Terrestrial organisms

Appraisal

Spray drift of herbicides will affect non-target plants. Adequate buffer zones have been defined for some application methods.

Changes in species diversity and population size and structure have been reported for terrestrial invertebrates and vertebrates following applications of glyphosate formulation in the field. Modifications in available food plants, insect populations associated with vegetation killed by the herbicide, and ground cover following intended effects of the spray probably account for these changes.

9.2.3.1 Plants

Red pine seedlings (*Pinus resinosa*) were not affected by treatment with Roundup in a field study. There was no dose-related decrease of the root length, as had been observed in a comparable laboratory experiment with the same doses (Chakravarty & Chatarpaul, 1990b). In this experiment lasting 154 days Roundup was applied at rates of up to 3.2 kg a.i./ha. The conifer seedlings were inoculated with an ectomycorrhizal fungus (*Paxillus involutus*) at 14 days after germination, and they were planted outdoor after being cultured in a greenhouse for up to 70 days after germination.

No plants were found as indicator species for damage due to drift of a formulation of glyphosate when this was applied with hydraulic ground sprayers (Marrs et al., 1989). In this study, native British species commonly found in nature reserves were exposed to spray drift, at several distances downwind from a zone sprayed with 0.5 and 2.2 kg a.i./ha. The effect of windspeed was investigated by spraying at speeds of 2.5 and 3.5 m/second. Death and severe growth suppression occurred at a distance of 2-6 m from the sprayer. Sublethal damage also occurred, mostly near to the sprayer, although for *Prunella vulgaris* damage occurred up to 20 m away. Epinasty was the most frequent symptom of damage. Most of the damaged plants recovered, however. Some of the species were consistently more sensitive, i.e. *Digitalis purpurea*, *Centaurea nigra*, *Prunella vulgaris* and *Lychnis flos-cuculi*. Marrs et al. (1989) concluded that, when spraying with ground sprayers, buffer zones around nature reserves should be 5-10 m.

9.2.3.2 *Invertebrates*

No significant and consistent effects on the number of nematodes and springtails were found in the upper 3 cm of ferro-humic podsols due to treatment with Roundup (Preston & Trofymow, 1989). The soils were covered with alder trees (*Alnus rubra*) and located in British Columbia, Canada. The only consistent effect was a significant reduction in the number of both oribatid and non-oribatid mites on one of the treated sites around 20 days after application. On this site the number of mites appeared to have returned to normal by the end of the study. In this experiment of around 180 days Roundup was hand-sprayed at a rate of 2 kg a.i./ha.

No substance- or dose-related effects on mites and springtails were observed in a sandy soil in a semi-arid region of Argentina up to 96 days after application of Roundup (Gómez & Sagardoy, 1985). Applied doses were up to 2.8 kg a.i. (free acid)/ha.

The numbers of herbivorous insects and ground invertebrates were significantly reduced up to 3 years after treatment with Roundup in a 4- to 5-year-old clear-cut planted with spruce seedlings (*Picea* sp) (Santillo et al., 1989b). No effects were found on predatory insects. The clear-cut was located in Maine, USA, and sprayed with 1.7 kg a.i./ha. During this experiment lasting 3 years, the vegetation did not recover completely, and, apparently, the effects on invertebrates were mainly due to habitat change. Unintentionally untreated areas in the sprayed site

showed a much lesser reduction of invertebrates. These areas may therefore be considered as potential sources for recolonization.

9.2.3.3 Vertebrates

Treatment of 4- to 5-year old clear-cuts in Maine, USA, planted with seedlings of spruce (*Picea* sp), with Roundup at a rate of 1.7 kg a.i./ha affected breeding bird populations up to 3 years after treatment (Santillo et al., 1989a). Total bird densities decreased with 36% due to reduced habitat complexity, as expressed in regenerated hardwood, vegetation height and foliage height diversity. The most sensitive species were the insectivorous common yellowthroat (*Geothlypis trichas*), Lincoln's sparrows (*Melospiza lincolnii*) and alder flycatchers (*Empidonax alnorum*). In less than 7-year-old clear-cuts in the Oregon coast range, Canada, planted with Douglas fir (*Pseudotsuga menziesii*), some breeding bird populations were affected due to two closely spaced treatments with Roundup at a rate of 0.8 kg a.i./ha each (Morrison & Meslow, 1984). Two years after the application, the densities of birds using shrubs for nesting and foraging had recovered, concomitant with the recovery of shrub vegetation. Sensitive species were the rufous-sided towhee (*Piplio erythrophthalmus*) and MacGillyvray's warbler (*Oporornis tolmiei*). On the other hand, the American goldfinch (*Carduelis tristis*) increased one year after application, apparently due to the treatment. Morrison & Meslow (1986) stated that the effectiveness of the treatment, which was not maximal in their study, is crucial to the degree to which bird populations are affected.

Small mammals may be affected by treatment with glyphosate, this depending mainly on the size of the treated area, the vegetation type and the extent to which the vegetation is damaged. Various experiments have been performed in and around clear-cuts planted with conifers in locations in the USA and Canada. Insectivorous shrews (*Blarina brivicauda*, *Sorex cinereus* and *Sorex hoyi*) and herbivorous voles were less abundant due to a treatment with Roundup at a rate of 1.7 kg a.i./ha (Santillo et al., 1989b). This significant reduction of the number of shrews was maintained for 3 years after application, whereas the population of voles recovered. No effects on the omnivorous deer mice (*Peromyscus maniculatus*) were observed. Deer mice also appeared to be unaffected in an experiment in which Roundup was applied at a rate of 3 kg a.i./ha (Sullivan, 1990). The population dynamics of both deer mice and the herbivorous Oregon voles (*Microtus oregoni*) appeared not to be influenced, although some partially

significant effects were observed that might have been due to the treatment. On the sprayed site there was an increase in the number of recruits of both species 3 years after application, and also an increased fecundity of deer mice and a higher survival of female voles 1 and 3 years after application. Sullivan (1990) observed no physiological changes that might have been due to ingestion. In a clear-cut treated with Roundup at a rate of 1.1 kg a.i./ha, only the population density of Southern Redbacked voles (*Clethrionomys gapperi*) was reduced by about 80% in a 1-year experiment (D'Anieri et al., 1987). In another clear-cut, no adverse effects on deer mice populations were evident after treatment with Roundup at a rate of 2.2 kg a.i./ha (Sullivan & Sullivan, 1981). Contrary to the results of Sullivan (1990), D'Anieri et al. (1987), Sullivan & Sullivan (1981) and Santillo et al. (1989b), a significant reduction in the population density of deer mice was observed by Ritchie et al. (1987) on a sprayed clear-cut of 38 ha at around 11 months after spraying with Roundup at a rate of 1.1–1.2 kg a.i./ha. However, no adverse effects on fertility or fecundity were indicated. Probably the effect on abundance was due to habitat change with respect to feed provision and cover. Possibly the effects on deer mice observed by Ritchie et al. (1987) were less confounded by immigration as the sprayed area was larger than in the studies in which effects on deer mice were lacking. However, when a site of 36 ha was sprayed twice with Roundup at a rate of 0.8 kg a.i./ha on each occasion, deer mice were not affected, possibly due to a relatively low effectiveness of the treatment (Anthony & Morrison, 1985). In the treated area, even an increase of Oregon voles was found after 1 year, concomitant with an increase of grass and other plants. The results indicated that small mammal populations were able to recover within 2 years after application of glyphosate, dependent on the recovery of shrubs.

10. EVALUATION OF HUMAN HEALTH HAZARDS AND EFFECTS ON THE ENVIRONMENT

10.1 Human health hazards

Results of direct measurements of glyphosate concentrations in foodstuffs (as part of food surveillance), drinking-water or total diets are not available.

Absorption from the gastrointestinal tract is limited to 36% or less and percutaneous absorption is 5.5% or less. Glyphosate is essentially not metabolized. Total body clearance is 99% in 7 days. Residues in livestock animals and their products (including milk) are minimal.

Summarized information on short- and long-term studies is given in Table 23 and on teratogenicity and reproduction studies in Table 24.

In animals, glyphosate has very low acute toxicity by the oral and dermal administration routes. The formulation Roundup is acutely toxic to humans when ingested intentionally or accidentally. No controlled studies are available and therefore the human NOAEL cannot be derived.

Animal studies show that glyphosate is not carcinogenic, mutagenic or teratogenic. Reproductive effects were only seen at dose levels producing maternal toxicity.

In experimental animals (13-week studies in rats and mice), an effect was observed in the parotid salivary glands, indicating that glyphosate may be acting as a weak adrenergic agonist. In rats, this occurred at feeding levels of $\geq$ 205 mg/kg body weight per day. The NOAEL in chronic feeding studies is $\geq$ 410 mg/kg body weight per day. A NOAEL of 175 mg/kg body weight per day observed in a rabbit teratogenicity study was chosen as the appropriate basis for toxicological evaluations in humans. Through application of a suitable safety factor, safe levels for humans can be estimated for use in the toxicological evaluation of any actual exposures. For technical glyphosate a safety factor of 100 is considered appropriate given the elaborate data sets available.

Table 23. Short-term and long-term studies on glyphosate

Species	Test compound	Dose levels (mg/kg diet)	Effects	NOAEL (mg/kg diet)
Short-term studies				
Mouse	technical glyphosate	5000, 10 000, 50 000	decreased growth and increased weights in brain, heart, kidneys (50 000)	10 000
Mouse	technical glyphosate	3125, 6250, 12 500, 25 000, 50 000	reduced weight gain (50 000) lesions of salivary glands (≥ 6250)	3125
Rat	technical glyphosate	1000, 5000, 20 000	no adverse effects	>20 000
Rat	technical glyphosate	200 to 12 500	no adverse effects	> 12 500
Rat	technical glyphosate	3125, 6250, 12 500, 25 000, 50 000	increased AP and ALAT (≥ 6250) increased haematocrit and red cell parameters (≥ 12 500), increased bile acids, decreased sperm counts (≥ 25 000), histological alterations in salivary glands (≥ 3125), reduced weight gain (≥ 25 000)	< 3125
Dogs	technical glyphosate	20, 100, 500	no adverse effects	≥ 500
Cattle	Roundup	400, 500, 630, 790	decreased feed intake (≥ 630) diarrhoea (≥ 500) increased blood parameters (790)	400
Long-term studies				
Mouse	technical glyphosate	1000, 5000, 30 000	decreased growth (30 000), increased incidence of hepatocyte hypertrophy and necrosis (30 000), increased incidence of urinary bladder epithelial hyperplasia (30 000)	5000

Table 23 (contd).

Species	Test compound	Dose levels (mg/kg diet)	Effects	NOAEL (mg/kg diet)
Long-term studies (contd).				
Rat	technical glyphosate	2000, 8000, 20 000	decreased growth (20 000), increased liver weights (20 000), increased incidences of degenerative lens changes (20 000) and of gastric inflammation (8000 and 20 000)	8000
Rat	technical glyphosate	60, 200, 600	slightly decreased growth (600)	[a]

[a] The slight effect at 600 mg/kg diet is considered marginal in the light of the absence of an effect on growth at higher dose levels (2000 and 8000) in a more recent 2-year study in rats.

Glyphosate and its concentrated formulations produce moderate to severe eye irritation, but only slight skin irritation. Neither glyphosate nor tested formulations induce sensitization.

Available studies on exposures of workers involved in application of the herbicide show that exposure is low when protective clothing is worn. The following data illustrate this point.

a) The highest estimated exposure (dermal and inhalation) of about 8000 μg/h, as reported in a study with spray applicators, corrected for incomplete absorption, equals about 50 μg/kg body weight per day (8-h working day for a 70-kg adult); between the latter level and the NOAEL of 175 mg/kg body weight per day, adjusted for incomplete absorption from the gastrointestinal tract (30-60%), i.e. 52-63 mg/kg body weight per day, there is a margin of safety of about 1100.

b) The highest exposure concentration found for forest brush saw workers was 15.7 $\mu g/m^3$; between this level and the NOAEL expressed as glyphosate from the 4-week inhalation study with Roundup of 16 mg/m^3 there is a margin of safety of 1000 (this is borne out by the absence of adverse findings in the workers' health examination in the study.

Table 24. Summary of teratogenicity and reproduction studies on glyphosate

Species	Test compound	Dose levels (mg/kg diet)	Effects	NOAEL[a] (mg/kg body weight)
Rat	technical glyphosate	300, 1000, 3500 mg/kg body weight, gestation days 6-19	mortality, clinical signs and decreased growth in dams, early resorptions, decreased numbers of implantations and visible fetuses, decreased ossification of fetal sternebrae (all at 3500 only); no fetal malformations	1000
Rabbit	technical glyphosate	75, 175, 350 mg/kg body weight, gestation days 6-27	diarrhoea and soft stools (350, slight at 175), nasal discharge (350)	175
Rat	technical glyphosate	3, 10, 30 mg/kg body weight given in diet, 3 gens	increased incidence of renal tubular dilation in F_{3b} male pups (30)	< 30[b]
Rat	technical glyphosate	2000, 10 000, 30 000 mg/kg diet, 2 gens	soft stools of parents (30 000), decreased litter size (30 000), decreased body weights of parents and pups (30 000 and 10 000)	100[b] (2000 mg/kg diet)

[a] Based on all observed effects (both in dams and offspring)

[b] There is some discrepancy in the results, and in the NOAELs, of the two reproduction studies carried out with technical glyphosate; the renal effects in the 3-generation study were not reproduced in the more recent 2-generation study with higher dose levels (for details, see section 7.5.2.).

10.2 Evaluation of effects on the environment

Following application, glyphosate will selectively partition to particulate matter suspended in surface water, aquatic sediment or to the soil substrate. This partitioning is usually rapid. The mechanism of sorption is only partially understood. There is little reported information on desorption from soil; the information available suggests "strong" binding. Mobility studies show little leaching of glyphosate below the upper few centimetres of the soil profile. The major metabolite, AMPA, also appears not to leach through soil.

Given this environmental distribution, organisms living in aquatic sediment or soil would be expected to come into closest contact with residues of the herbicide.

There is very little information on the bioavailability of sediment- or soil-bound glyphosate to either aquatic or terrestrial organisms. Few bioaccumulation or ecotoxicity studies have been performed with sediment present.

Comparison of exposure concentrations and toxic effects is, therefore, difficult since the relevant organisms have not been tested and exposure of tested organisms is not by a realistic route.

10.2.1 Exposure levels and toxic effects

Exposure concentrations have been calculated from experimental application of Roundup in the field (see Table 7 in chapter 5). The methodology is presented in Fig. 4. No monitoring results of environmental concentrations following actual use in agriculture or forestry are available.

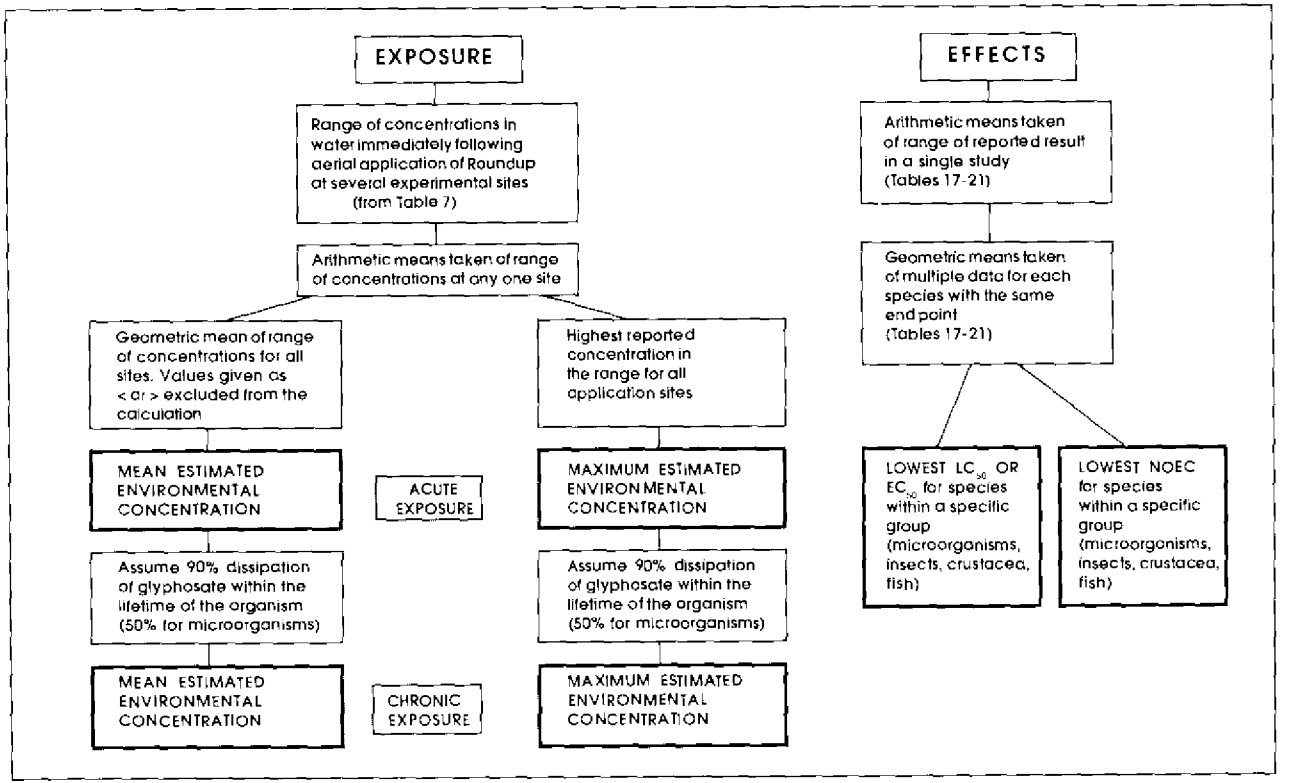
The lowest $LC(EC)_{50}$ and NOEC values for microorganisms, invertebrates and fish have been taken from the toxicity tests reported in chapter 9 (see Fig. 4). These all refer to organisms living in the open water and are, therefore, of questionable significance for a compound which partitions to sediment. There is no information on species living in aquatic sediment and little information available on soil-living organisms, with the exception of microorganisms.

10.2.2 Hazard evaluation for aquatic organisms

Tables 25 and 26 compare the estimated mean and maximum exposure concentrations, following aerial application of Roundup, to the lowest reported $LC(EC)_{50}$ and NOEC concentrations for acute and chronic exposure of aquatic organisms. The ratio between exposure and effect concentrations has been calculated. These tables are meant as a guide to establishing possible hazard and are not intended to estimate the degree of effect likely to be seen in the field. The "possible hazard" classification is a simple one using different classification phrases for order of magnitude segments of the ratios.

The toxicity of formulations to aquatic organisms is greater than for technical glyphosate in many cases. This increased

Figure 4. Methodology for deriving estimated environmental exposure concentration and effects concentration[a]



[a] Tables 25 and 26 use concentrations in terms of active ingredient and formulation, respectively

Table 25. Indications of environmental hazard for aquatic organisms by technical grade glyphosate

Effect	Organisms	Estimated exposure concentration (mg a.i./litre)	Toxicity data (mg a.i./litre)	End-point	Ratio of exposure to toxicity concentrations	Possible hazard
Mean estimated exposure concentration						
Acute	microorganisms	0.1	EC_{50} = 1.2	mortality	0.082	small
Acute	insects	0.1	EC_{50} = 55	mortality	0.0018	negligible
Acute	crustaceans	0.1	EC_{50} = 281	mortality	0.00035	negligible
Acute	fish	0.1	LC_{50} = 94	mortality	0.0010	negligible
Chronic	algae	0.05	NOEC = 0.3	growth	0.17	present
Chronic	crustaceans	0.01	NOEC = 100	reproduction	0.00010	negligible
Chronic	fish	0.01	NOEC = 52	behaviour	0.00019	negligible
Maximum estimated exposure concentration						
Acute	microorganisms	1.7	EC_{50} = 1.2	mortality	1.4	large
Acute	insects	1.7	EC_{50} = 55	mortality	0.031	small
Acute	crustaceans	1.7	EC_{50} = 281	mortality	0.0060	negligible
Acute	fish	1.7	LC_{50} = 94	mortality	0.018	small
Chronic	algae	1.7	NOEC = 0.3	growth	5.7	large
Chronic	crustaceans	0.17	NOEC = 100	reproduction	0.0017	negligible
Chronic	fish	0.17	NOEC = 52	behaviour	0.003	negligible

Table 26. Indications of environmental hazards for aquatic organisms by Roundup

Effect	Organisms	Estimated exposure concentration (mg Roundup/litre)	Toxicity data (mg Roundup/litre)	End-point	Ratio of exposure to toxicity concentrations	Possible hazard
Mean estimated exposure concentration						
Acute	microorganisms	0.32	EC_{50} = 2.1	mortality	0.15	present
Acute	crustaceans	0.32	EC_{50} = 10	mortality	0.032	small
Acute	insects	0.32	EC_{50} = 44	mortality	0.0073	negligible
Acute	fish	0.32	LC_{50} = 13	mortality	0.025	small
Chronic	microorganisms	0.16	NOEC = 0.7	growth	0.23	present
Chronic	crustaceans	0.032	NOEC = 3.5	reproduction	0.0091	negligible
Chronic	fish	0.032	NOEC = 2.4	behaviour	0.013	negligible
Maximum estimated exposure concentration						
Acute	microorganisms	5.6	EC_{50} = 2.1	mortality	2.7	large
Acute	crustaceans	5.6	EC_{50} = 10	mortality	0.56	present
Acute	insects	5.6	EC_{50} = 44	mortality	0.13	present
Acute	fish	5.6	LC_{50} = 13	mortality	0.43	present
Chronic	microorganisms	5.6	NOEC = 0.7	growth	8.0	large
Chronic	crustaceans	0.56	NOEC = 3.5	reproduction	0.16	small
Chronic	fish	0.56	NOEC = 2.4	behaviour	0.23	small

toxicity is due to surfactants present in the product. No account has been taken of possible degradation of surfactants in the ratios presented, since no data are available. The ratios, therefore, may overestimate the toxicity of glyphosate. If the compound is bound to sediment in the environment, this could also reduce its toxic effect. Since no clear evidence is available to demonstrate this reduced toxicity, no account has been taken of partitioning to particulates. This will also tend to overestimate toxic effect of glyphosate.

As can be seen from the tables, possible hazard for most aquatic organisms is small or negligible. Fish and aquatic invertebrates would not be affected by glyphosate use. Only microorganisms, with both acute and chronic exposure, appear to be susceptible to effects of the herbicide. The comparisons made in the table do not allow estimates of the degree of toxic effects likely to be seen in the field. From the field evidence available, populations and communities of algae are not likely to be affected after application of glyphosate formulation. Transitory changes in number and functioning of aquatic microorganisms are possible after use of the herbicide.

Since data are not available, evaluation of the hazard of bound residues of glyphosate to sediment-living organisms is not possible.

Minimal bioaccumulation of glyphosate has been reported in both laboratory experiments and in the field. The physico-chemical properties of the compound are consistent with this conclusion.

10.2.3 Hazard evaluation for terrestrial organisms

Limited test data show low toxicity of glyphosate and its formulations to honey-bees, earthworms, birds and mammals. These data suggest low risk for these organisms from use of the herbicide. Field studies have been conducted and support the view that glyphosate does not affect soil microorganisms in the long term.

Marked changes in populations of birds and small mammals have been seen in field studies following application of glyphosate. These seem to result from the changes in habitat, vegetation cover, food organisms, etc., resulting from the intended herbicidal effect of the compound.

11. RECOMMENDATIONS FOR PROTECTION OF HUMAN HEALTH

a) Protective clothing is necessary to ensure the safety of herbicide applicators.

b) A market-basket survey would be useful to determine the possible exposure of the general population.

12. FURTHER RESEARCH

a) Further research is required to determine whether β-adrenergic effects observed in rodents have any implications for human health.

b) The role of adjuvants in the toxicity of glyphosate formulations needs to be investigated further in laboratory mammals and organisms in the environment.

c) A controlled study on exposure of agricultural workers is needed.

d) The bioavailability of sediment- and soil-bound glyphosate in the environment should be studied.

e) Studies on the environmental behaviour and fate of adjuvants are required.

f) Further toxicity studies of sediment-living organisms are needed.

g) The effects of phosphate fertilizers on the binding of glyphosate to soils should be investigated.

h) Analytical techniques that are less costly but still adequate should be developed.

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RESUME

1. Identité, propriétés physiques et chimiques et méthodes d'analyse

Le glyphosate, ou N-(phosphonométhyl)glycine, est un acide organique faible. Sa formule brute est $C_3H_8NO_5P$. Il est généralement présenté sous la forme du sel de l'acide correspondant déprotoné et d'un cation comme l'isopropylammonium ou le triméthylsulfonium. La pureté du glyphosate de qualité technique est généralement supérieure à 90%. Le glyphosate de qualité technique se présente sous la forme d'une poudre cristalline blanche inodore dont la densité est de 1,704. Sa tension de vapeur est très faible et il est très soluble dans l'eau. Le coefficient de partage octanol-eau (log K_{ow}) est égal à -2,8. Le glyphosate est amphotère et il peut exister sous différentes formes ioniques, selon le pH du milieu.

Le dosage du glyphosate est généralement une opération laborieuse, complexe et coûteuse. La méthode la plus courante consiste à en préparer un dérivé avec une substance fluorigène, avant ou après passage sur colonne. Le dosage s'effectue en général par chromatographie liquide à haute performance ou chromatographie gaz-liquide. Les limites de détection dans l'eau, les plantes, le sol et l'urine humaine sont respectivement de 0,02-3,2 µg/litre, 0,01-0,3 mg/kg, 0,05-1 mg/kg et 0,1 mg/litre.

2. Sources d'exposition humaine et environnementale

Le glyphosate est un herbicide non sélectif utilisé en traitement endothérapique après l'émergence; il est utilisé partout dans le monde sur des terrains agricoles ou non. On l'épand en plusieurs formulations commerciales sur de nombreuses récoltes. La plus courante est le Roundup qui consiste en un sel d'isopropylammonium. La dose d'emploi recommandée ne dépasse 5,8 kg de matière active par hectare et dépend de l'usage auquel on le destine. L'environnement peut être contaminé par suite du dépôt d'embruns ou de la libération accidentelle du produit.

3. Transport, distribution et transformation dans l'environnement

Les principaux processus de dissipation qui interviennent après l'épandage de cet herbicide sont les suivants: formation de

complexes avec certains ions présents dans l'eau comme Ca^{2+} et Mg^{2+}, sorption aux sédiments ainsi qu'aux particules en suspension dans l'eau et le sol, photodécomposition dans l'eau, fixation par les plantes et biodégradation.

Le glyphosate disparaît de l'eau avec des valeurs du TD_{50} (temps de dissipation) qui vont de quelques jours à plus de 91 jours. Le principal milieu récepteur est constitué par les sédiments ou les particules en suspension.

En laboratoire, les coefficients d'adsorption ($K_{s/l}$) du glyphosate varient de 8 à 377 dm^3/kg pour différents sols et substances argileuses. On ne dispose d'aucune donnée sur la sorption de l'acide aminométhylphosphonique (AMPA) qui en est le principal métabolite, dans les conditions du laboratoire.

La valeur du R_f ne dépasse pas 0,2, selon certaines mesures par chromatographie sur couche mince de terre. Dans des conditions de lessivage reproduisant des précipitations extrêmement fortes, on récupère dans l'éluat d'une colonne de terre, entre 0,1 et 11% de l'activité appliquée initialement. L'expérimentation sur le terrain montre qu'il n'y a probablement pas lessivage de l'AMPA.

L'expérimentation sur le terrain montre qu'en ce qui concerne la dissipation du glyphosate dans le sol, les valeurs du TD_{50} varient de 3 à 174 jours, principalement en fonction des conditions édaphiques et climatiques. Certaines expériences sur le terrain ont montré que le ruissellement pouvait entraîner jusqu'à 1,8% de la dose appliquée sur la sol.

Au laboratoire jusqu'à 45% de l'activité appliquée peut être absorbée par le feuillage après traitement, après quoi il y a une migration importante dans la plante.

L'hydrolyse du glyphosate en tampon stérile est très lente, les valeurs du TD_{50} étant $>>$ à 35 jours. En ce qui concerne la photodécomposition dans l'eau dans les conditions naturelles, les valeurs du TD_{50} sont $\leq$ à 28 jours. Lors d'une étude qui s'est prolongée pendant 31 jours, on n'a pas enregistré de photodécomposition notable dans le sol.

Le temps nécessaire à la biodégradation de 50% d'une quantité donnée de glyphosate dans l'ensemble d'un système d'épreuve en présence d'eau et de sédiments était $\leq$ à 14 jours en aérobiose et compris entre 14 et 22 jours en anaérobiose. Dans le sol, le temps

de demi-biodégradation du glyphosate est de 2 à 3 jours en aérobiose.

Le principal métabolite qui se forme dans le sol et dans l'eau est l'AMPA. La quantité maximale d'AMPA présente dans le sol est d'environ 20% de l'activité appliquée en aérobiose et de 0,5% de cette activité en anaérobiose. Ce chiffre atteint 25% dans les sédiments dans les deux types de conditions.

Les épreuves de laboratoire montrent que chez les invertébrés et les poissons, le facteur de bioconcentration est faible. Lors d'une épreuve en aquarium à écoulement continu, on a constaté que chez *Lépomis macrochirus* le temps de demi-épuration était de 35 jours après une exposition de même durée. Après exposition continue à du glyphosate, on retrouve de l'AMPA chez ce même poisson pendant des périodes allant jusqu'à 21 jours. Des mesures sur le terrain n'ont pas permis de déceler la présence de glyphosate chez les poissons vivant dans des eaux sur lesquelles cet herbicide avait été directement pulvérisé. Lors d'une expérience, on a décelé de l'AMPA chez les carpes jusqu'à 90 jours après l'épandage. Une autre expérience menée sur le terrain a montré qu'il n'y avait pas de bioamplification du glyphosate dans les portées de petits mammifères herbivores ou omnivores vivant en brousse. On a notamment mesuré des concentrations allant jusqu'à 5 mg de matière active par kg chez des souris du genre *Peromyscus*, immédiatement après l'épandage.

Plusieurs souches de bactéries peuvent décomposer le glyphosate. On a identifié des bactéries qui sont capables d'utiliser de composé comme seule source de phosphore, de carbone ou d'azote. La croissance est alors plus lente que lorsque elles utilisent des sources inorganiques de P, de C et de N. On est fondé à penser, d'après les observations effectuées sur le terrain, que certaines populations bactériennes se sont adaptées à la métabolisation du glyphosate. La présence de phosphate inorganique inhibe la décomposition du glyphosate par certaines bactéries mais pas toutes. La biodécomposition du glyphosate peut comporter un co-métabolisme avec d'autres sources d'énergie.

4. Concentrations dans l'environnement et exposition humaine

Il n'existe que de très rares données provenant de programmes de surveillance systématique et concernant la présence de glyphosate dans la faune et la flore ainsi que dans le milieu

abiotique. Pour avoir une idée des concentrations maximales dans l'environnement, on fait appel aux données fournies par des essais sur le terrain au cours desquels on simule des épandages à usage agricole; ces concentrations sont les suivantes: < 1-1700 µg/litre dans les eaux de surface, 0,07-40 mg/kg de poids sec dans le sol, < 0,05-19 mg/kg de poids sec dans les sédiments, 261-1300 mg/kg dans les feuilles, 5 mg/kg dans les viscères des souris du genre *Peromyscus*, 1,6-19 mg/kg dans les baies sauvages et 45 mg/kg dans les lichens. Les concentrations maximales correspondantes d'AMPA sont les suivantes: < 1-35 µg/litre (eaux de surface), 0,1-9 mg/kg de poids sec (sol), < 0,05-1,8 mg/kg de poids sec (sédiments), 1,7-< 9 mg/kg (feuilles), 0,02-0,1 mg/kg (baies sauvages) et 2,1 mg/kg (lichens). Les concentrations ci-dessus de glyphosate sont celles que l'on observe en général immédiatement après l'épandage. En ce qui concerne les lichens, la concentration mentionnée a été observée 270 jours après l'épandage.

On ne dispose pas de mesures de la dose journalière ingérée par l'homme avec les aliments et l'eau de boisson (études de rations totales). Les quelques données disponibles au sujet de l'exposition professionnelle indiquent que celle-ci est faible pour les ouvriers qui épandent du glyphosate comme désherbant sous forme de Roundup.

5. Cinétique et métabolisme chez les animaux de laboratoire et l'homme

Le glyphosate technique n'est que partiellement résorbé au niveau des voies digestives. Lors d'études effectuées sur du glyphosate marqué au carbone-14, on a observé des pourcentages d'absorption de 30 à 36% chez plusieurs espèces. L'absorption par voie percutanée est faible. Dans le cas de l'herbicide Roundup, le glyphosate qu'il contient est absorbé dans une proportion ≤ à 5,5% à travers la peau (durée de contact environ 24 heures). En ce qui concerne les tissus de l'organisme, la concentration maximale, correspondant à environ 1% de la dose ingérée, se retrouve dans les eaux. Après administration d'une seule dose par voie orale, le produit est éliminé à hauteur de 62 à 69% dans les matières fécales sans absorption. Après absorption, 14 à 29% de la dose passe dans l'urine et 0,2% au maximum dans l'air expiré. Après administration par voie intraveineuse, le taux d'excrétion dans les voies biliaires n'a été que de 5 à 8%. Chez des chèvres en lactation, on a montré que le glyphosate n'était excrété dans le lait qu'en faible proportion (concentration ≤ à 0,1 mg/kg de lait entier pour une dose ingérée de 120 mg/kg de nourriture). Le glyphosate n'est

métabolisé que dans une très faible proportion. Son seul métabolite, l'AMPA, correspond à 0,3% de la dose ou même moins; le reste correspond au produit initial. Il faut environ 168 heures pour que le glyphosate soit éliminé en totalité de l'organisme (99% d'une dose orale).

6. Effets sur les mammifères de laboratoire et les systèmes d'épreuve *in vitro*

Chez l'animal de laboratoire, le glyphosate technique ne présente qu'une très faible toxicité aiguë lorsqu'il est administré par la voie orale ou percutanée; il est nettement plus toxique par la voie intrapéritonéale que par les autres voies d'administration. Des études d'alimentation de brève durée ont été effectuées sur plusieurs espèces, mais la plupart de ces épreuves n'ont guère révélé d'effets. Lors d'une épreuve de 13 semaines sur des souris au cours de laquelle on a utilisé du glyphosate technique, on a constaté une augmentation du poids de plusieurs organes ainsi qu'un retard de croissance à la dose de 50 000 mg/kg de nourriture. Lors d'une étude de même durée sur le rat, on n'a pas observé d'effet (les doses de glyphosate technique utilisées allaient jusqu'à 20 000 mg/kg de nourriture). Lors d'une autre étude de 13 semaines, on a observé des lésions au niveau des glandes salivaires chez des rats et des souris. Chez les souris, la dose sans effet létal observable était de 3125 mg/kg de nourriture; chez le rat, elle était < à 3125 mg/kg de nourriture. Aucun de ces effets n'a été observé lors d'études à court ou à long terme effectuées sur diverses souches et espèces. Les lésions au niveau des glandes salivaires incitent à penser que le glyphosate pourrait se comporter comme un agoniste adrénergique de faible activité.

La toxicité à long terme a été étudiée sur des souris et des rats. Peu d'effets ont été observés et dans presque tous les cas, uniquement à des doses relativement élevées. Chez les souris, le glyphosate technique a produit un retard de croissance, une hypertrophie ou une nécrose des hépatocytes ainsi qu'une hyperplasie de l'épithélium vésical à la dose de 30 000 mg/kg. Chez les rats, le même composé a entraîné une réduction de la croissance, une augmentation du poids du foie, une dégénérescence du cristallin et une inflammation de la muqueuse gastrique à la dose de 20 000 mg/kg de nourriture.

Les études dont on connaît les résultats ne concluent pas à l'existence d'un pouvoir mutagène, cancérogène ou tératogène du glyphosate technique. Deux études ont été effectuées sur plusieurs

générations de rats. Les principaux effets du glyphosate technique consistaient en une réduction du poids corporel des géniteurs et des ratons ainsi qu'une diminution de la taille des portées, à la dose de 30 000 mg/kg de nourriture. Dans une étude portant sur la reproduction, on a constaté une augmentation dans l'incidence de la dilatation unilatérale des tubules rénaux chez les ratons mâles de la génération F_{3b}, à la dose de 30 mg/kg de poids corporel. Toutefois la reproductibilité de cette lésion reste incertaine du fait qu'elle n'a pas été observée chez les ratons soumis à une dose plus élevée, dans la deuxième de ces études.

7. Effets sur l'homme

Les études contrôlées dont on dispose se limitent à trois études sur l'irritation et la sensibilisation provoquées par le glyphosate chez des volontaires humains, et qui ont toutes donné des résultats négatifs. Plusieurs cas d'intoxication (la plupart du temps volontaires) avec un herbicide composé de glyphosate technique, le Roundup, ont été signalés. Une étude, consacrée à des travailleurs qui épandaient du Roundup, n'a pas révélé d'effets indésirables. Les données disponibles sur l'exposition professionnelle d'ouvriers appliquant du Roundup montrent que le niveau d'exposition est très inférieur à la dose sans effet létal observable qui ressort de l'expérimentation animale.

8. Effets sur les êtres vivants dans leur milieu naturel

Le glyphosate de qualité technique est légèrement à modérément toxique pour les microorganismes aquatiques avec une CE_{50} (3 à 4 jours) allant de 1,2 à 7,8 mg/litre et une concentration sans effets observables à 7 jours allant de 0,3 à 34 mg/litre. Sous ses différentes formulations, le glyphosate est légèrement à fortement toxique pour les microorganismes aquatiques avec des valeurs de la CE_{50} à 3 jours allant de 1,0 à plus de 55 mg de produit par litre. Les cyanophycées (algues bleues) sont plus sensibles au Roundup que les algues proprement dites. Les processus physiologiques affectés sont notamment le verdissement, la respiration, la photosynthèse et la synthèse des acides aminés aromatiques.

Sur les bactéries terricoles en culture, le glyphosate agit au niveau de la fixation de l'azote, de la dénitrification et de la nitrification. Cependant des observations effectuées sur le terrain après épandage de diverses formulations de glyphosate n'ont pas révélé la présence d'effets sensibles. Des bactéries appartenant à

des espèces étroitement apparentées aux bactéries précitées se sont révélées capables de dégrader le glyphosate.

Chez les champignons ectomycorhiziens, la croissance du mycélium en culture pure est inhibée par des concentrations ≥ à 29 mg de Roundup par litre. Les genres sensibles à cette inhibition sont *Cenococcum*, *Hebeloma* et *Laccaria*.

Le glyphosate est légèrement toxique pour les macrophytes aquatiques avec une valeur de la concentration sans effets observables à 14 jours de 9 mg/litre, en solution dans l'eau. le Roundup est également légèrement toxique avec, pour cette concentration, des valeurs allant de 2,4 à 56 mg/litre, également en solution dans l'eau. On ne dispose d'aucune donnée sur la toxicité aiguë. La phytotoxicité est beaucoup plus importante en l'absence de lessivage des dépôts d'herbicide.

Le glyphosate de qualité technique est très légèrement à légèrement toxique pour les invertébrés aquatiques avec des valeurs de la CL_{50} ou de la CE_{50} à 2-4 jours ≥ 55 mg/litre et une valeur de la concentration sans effets observables à 21 jours de 100 mg/litre. Les diverses formulations de glyphosate sont très légèrement à modérément toxiques pour les invertébrés aquatiques pour des valeurs de la CE_{50} à 2 jours s'étageant entre 5,3 et 5600 mg de produit par litre et des valeurs de la MATC à 21 jours allant de 1,4 à 4,9 mg de produit par litre. La toxicité plus forte du Roundup est essentiellement due à la présence d'agents tensioactifs.

Le glyphosate de qualité technique est très légèrement à modérément toxique pour les poissons avec des valeurs de la CL_{50} à quatre jours allant de 10 à > 1000 mg/litre, une valeur de la concentration sans effets observables à 21 jours de 52 mg/litre et une valeur de la MATC de > 26 mg/litre. Les diverses formulations du glyphosate sont également très légèrement à modérément toxiques pour les poissons avec des valeurs de la CL_{50} à quatre jours de 2,4 à > 1000 mg de produit par litre et des valeurs de la concentration sans effets observables à 21 jours allant de 0,8 à 2,4 mg de produit par litre. C'est la carpe qui s'est révélée être l'espèce la plus sensible, après exposition à une formulation de glyphosate appelée Sting. Sur le terrain, on n'a pas constaté d'effets sur les poissons qui soient attribuables au traitement par le Roundup, à l'exception d'un stress constaté immédiatement après l'épandage du produit à la dose recommandée en évitant que celle-ci ne dépasse 40 mg de Roundup par litre.

On constate que le glyphosate inhibe, dans une proportion qui dépend de la dose, la formation de nodosités par le trèfle souterrain inoculé par du *Rhizobium*, en culture hors-sol et en présence de solutions nutritives contenant une concentration de matière active ≥ 2 mg/litre. La germination de diverses espèces forestières n'est pas affectée par la présence de glyphosate aux doses d'emploi recommandées. Avec des doses d'emploi ≥ 0,54 kg de matière active par hectare, on constate au laboratoire qu'il y a réduction, proportionnée à la dose, de la longueur des racines des jeunes pousses de pins sylvestres. Cette diminution n'a pas été confirmée lors d'une étude du même genre sur le terrain.

Le glyphosate de qualité technique et le Roundup sont légèrement toxiques pour les abeilles en application orale ou topique. Les valeurs de la DL_{50} à deux jours sont ≥ 100 µg (de matière active ou de produit) par abeille. La DL_{50} par voie orale à deux jours du Sting pour les abeilles est > 100 µg/abeille. Le Roundup et le Roundup D-pack sont légèrement toxiques pour les lombrics avec des valeurs de la concentration sans effets observables à 14 jours respectivement égales à 500 et 158 mg de produit par kg de poids sec. Aucun effet nocif, attribuable au Roundup, n'a été observé sur la fécondité et la fertilité d'un certain nombre d'insectes appartenant au groupe des névroptères et le Sting n'a pas non plus produit d'effets sur la consommation de nourriture ou la mortalité des insectes du genre *Poecilus*.

Le glyphosate de qualité technique est légèrement toxique pour les oiseaux avec une DL_{50} > 3851 mg/kg de poids corporel, une CL_{50} à huit jours > 4640 mg/kg et des valeurs de la concentration sans effets observables à 112-119 jours, ≥ 1000 mg/kg de nourriture. On a constaté que le Roundup et une autre formulation dont le nom n'est pas connu était également toxique pour les oiseaux, avec un DL_{50} > 2686 mg de produit par kg de poids corporel et une CL_{50} à huit jours > à 5620 mg de produit par kg de nourriture. Généralement, on ne constate, sur les mammifères de laboratoire, aucun effet qui soit attribuable au traitement par le glyphosate de qualité technique ou le Roundup. Les effets attribués au traitement par cet herbicide et constatés chez les oiseaux et les mammifères dans leur milieu naturel, semblent être dus principalement aux modifications du biotope consécutives au traitement herbicide.

RESUMEN

1. Identidad, propiedades físicas y químicas y métodos analíticos

El glifosato es un ácido orgánico débil formado por una molécula de glicina y otra de fosfonometilo. La fórmula empírica es $C_3H_8NO_5P$. Normalmente se formula como una sal del ácido del glifosato en el que se ha sustituido un protón por un catión, por ejemplo la isopropilamina o el trimetilsulfonio. La pureza del glifosato de calidad técnica suele ser superior al 90%. Este es un polvo cristalino blanco e inodoro con un peso específico de 1,704, una presión de vapor muy baja y una solubilidad en agua alta. El coeficiente de reparto octanol/agua (log K_{ow}) es -2,8. El glifosato es anfótero y se puede encontrar formando compuestos iónicos diversos, en función del Ph del medio.

Su determinación es en general laboriosa, compleja y costosa. El método más habitual es la transformación con sustancias fluorogénicas en derivados más fácilmente detectables y se puede utilizar antes o después de la columna. La determinación se suele llevar a cabo mediante cromatografía líquida de alto rendimiento o cromatografía gas-líquido. Los límites de determinación del glifosato en el agua, las plantas, el suelo y la orina humana son de 0,02-3,2 µg/litro, 0,01-0,3 mg/kg, 0,05-1 mg/kg y 0,1 mg/litro, respectivamente.

2. Fuentes de exposición humana y ambiental

El glifosato es un herbicida que actúa después del brote de manera sistémica y no selectiva, y se utiliza en zonas agrícolas y no agrícolas de todo el mundo. Se aplica a numerosos cultivos con formulaciones comerciales diferentes. La más importante es el Roundup, en el que el glifosato aparece en forma de la sal de isopropilamina. Las dosis de aplicación recomendadas no superan los 5,8 kg de a.i./ha y dependen del tipo de uso. Se puede producir exposición ambiental como consecuencia de la deposición debida a corrientes o escapes accidentales.

3. Transporte, distribución y transformación en el medio ambiente

Las más importantes vías de desaparición del glifosato tras su aplicación son la formación en el agua de complejos con iones, por ejemplo con el Ca^{2+} y el Mg^{2+}, la sorción al sedimento, las

partículas suspendidas en el agua y el suelo, la fotodegradación en el agua, la fijación en las plantas y la biodegradación.

El glifosato desaparece del agua con unos valores de TD_{50} que oscilan entre varios días y más de 91 días. Se ha comprobado que se deposita sobre todo en las partículas del sedimento o suspendidas.

Los coeficientes de adsorción ($K_{s/l}$) del glifosato en experimentos de laboratorio varían entre 8 y 377 dm^3/kg para diferentes suelos y minerales arcillosos. No se dispone de datos, en condiciones de laboratorio, sobre la sorción del ácido aminometilfosfónico (AAMF), su principal metabolito.

En los experimentos de cromatografía en capa fina, los valores R_f del glifosato no son superiores a 0,2 en el suelo. En el eluato de columnas de suelo obtenido en condiciones de lixiviación simulando una precipitación muy intensa se recupera una cantidad que oscila entre menos del 0,1% y el 11% de la dosis aplicada. De los estudios sobre el terreno se desprende que no es probable la lixiviación del AAMF.

En los experimentos sobre el terreno el glifosato desaparece del suelo con un TD_{50} que varía entre 3 y 174 días, principalmente en función de las condiciones edáficas o climáticas. En algunos experimentos sobre el terreno desaparecía del suelo, debido a la escorrentía, hasta el 1,8% de la dosis aplicada.

En condiciones de laboratorio, las hojas tratadas podrían absorber hasta el 45% de la cantidad aplicada, produciéndose a continuación un importante desplazamiento.

La hidrólisis del glifosato en tampones estériles es muy baja, con valores de $TD_{50} >> 35$ días. En condiciones naturales, la fotodegradación en agua se produce con valores de $TD_{50} \leq 28$ días. En el curso de un estudio de 31 días no se registró una fotodegradación importante en el suelo.

El tiempo necesario para la biodegradación del 50% del glifosato en el sistema completo de una prueba con agua y sedimento es ≤ 14 días en condiciones aerobias y de 14 a 22 días en condiciones anaerobias de laboratorio. El tiempo necesario para la biodegradación del 50% del glifosato en el suelo es de 2-3 días en condiciones aerobias.

El metabolito principal en el suelo y el agua es el AAMF. Las cantidades máximas de AAMF en el suelo son de aproximadamente el 20% de la dosis aplicada en condiciones aerobias, y del

0,5% en condiciones anaerobias. Las cantidades máximas de AAMF en el sedimento son del 25%, tanto en condiciones aerobias como anaerobias.

De las pruebas de laboratorio se desprende que los factores de bioconcentración en invertebrados y peces son bajos. Tras una exposición al glifosato de 35 días, *Lepomis macrochirus* mostró en una prueba en corriente un periodo de semidepuración de 35 días. Se recuperó AAMF en *Lepomis macrochirus* hasta 21 días después de una exposición continuada. No se detectó glifosato en peces que vivían en agua directamente rociada en experimentos sobre el terreno. En un experimento se detectó AAMF en carpas hasta 90 días después de la aplicación. En otro experimento sobre el terreno no se observó bioampliación del glifosato en el lecho de pequeños mamíferos herbívoros y omnívoros de un ecosistema de matorral boscoso. En este mismo experimento, inmediatamente después del rociado se determinaron concentraciones de hasta 5 mg de a.i./kg en ratones de pies blancos (*Peromyscus leucopus*).

Existen diversas bacterias que pueden degradar el glifosato. Se han identificado cepas capaces de utilizar este compuesto como única fuente de fósforo, de carbono o de nitrógeno. El crecimiento es lento si se compara con el obtenido de fuentes inorgánicas de P, C y N. Hay pruebas en el medio ambiente de la existencia de poblaciones bacterianas que se han adaptado para metabolizar el glifosato. La presencia de fosfato inorgánico inhibe la degradación de este compuesto por algunas bacterias, pero no por todas. La biodegradación del glifosato podría tener un metabolismo común con el de otras fuentes de energía.

4. Niveles ambientales y exposición humana

Los datos sobre la presencia de glifosato en la biota y la abiota del medio ambiente como parte de programas de vigilancia regular son muy escasos. Se utilizan datos obtenidos en experimentos sobre el terreno en los que se simula la práctica agrícola normal para indicar las concentraciones máximas en el medio ambiente: < 1-1700 µg/litro de agua superficial, 0,07-40 mg/kg de peso seco de suelo, < 0,05-19 mg/kg de peso seco de sedimento, 261-1300 mg/kg de follaje, 5 mg/kg de vísceras de ratón de pies blancos, 1,6-19 mg/kg de bayas silvestres y 45 mg/kg de líquenes. Las concentraciones máximas correspondientes de AAMF son las siguientes: < 1-35 µg/litro (agua superficial), 0,1-9 mg/kg de peso seco (suelo), < 0,05-1,8 mg/kg de peso seco (sedimento), 1,7-< 9 mg/kg (follaje), 0,02-0,1 mg/kg (bayas silvestres) y 2,1 mg/kg

(líquenes). Las concentraciones de glifosato mencionadas más arriba se suelen encontrar inmediatamente después de la aplicación. La concentración en los líquenes se determinó 270 días después de dicha aplicación.

No se dispone de mediciones de la ingestión humana diaria de glifosato a través de los alimentos y el agua de bebida (estudios completos de alimentación). Los escasos datos disponibles sobre la exposición ocupacional ponen de manifiesto que los niveles de exposición para los trabajadores que aplican el glifosato en la formulación del herbicida Roundup son bajos.

5. Cinética y metabolismo en animales de laboratorio y en el ser humano

La absorción del glifosato de calidad técnica en el tracto intestinal es sólo parcial. En estudios con glifosato marcado con ^{14}C, se encontró en varias especies un porcentaje de absorción del 30% al 36%. La absorción cutánea es baja. De la formulación del herbicida Roundup, a través de la piel sólo se absorbe $\leq$ 5,5% del glifosato presente (tiempo de contacto de unas 24 horas). En los tejidos del organismo, la concentración más alta, aproximadamente el 1% de la dosis oral, se encuentran en los huesos. Tras una dosis oral única, se eliminó en las heces sin absorción el 62-69%. Del glifosato absorbido, un 14-29% se excretó en la orina y el 0,2% o menos en el aire expirado. La excreción biliar posterior a la administración intravenosa fue sólo del 5-8%. Se observó que la excreción en la leche de cabras lactantes se producía sólo en escasa proporción (concentración $\leq$ 0,1 mg/kg de leche entera a un nivel de dosis de 120 mg/kg de alimentos). La biotransformación del glifosato se da únicamente en un grado muy bajo. El único metabolito, el AAMF, representa el 0,3% de la dosis o menos; el resto es glifosato inalterado. La eliminación de todo el organismo (99% de una dosis oral) se produce aproximadamente en 168 horas.

6. Efectos en animales de laboratorio y en sistemas de prueba *in vitro*

El glifosato de calidad técnica administrado por vía oral y cutánea a animales de experimentación tiene una toxicidad aguda muy baja; por vía intraperitoneal es notablemente más tóxico que por cualquier otra. Aunque se han realizado estudios de alimentación de corta duración en varias especies, en la mayor parte de estas pruebas se han observado pocos efectos. En un estudio de 13 semanas realizado en ratones con glifosato de calidad

técnica, a una concentración de 50 000 mg/kg de alimento, se observó aumento de peso de varios órganos y un retraso del crecimiento. En un estudio de 13 semanas en ratas no se advirtieron efectos (con dosis de glifosato de calidad técnica de hasta 20 000 mg/kg de alimento). En otro estudio de 13 semanas se detectaron lesiones en las glándulas salivales de ratas y ratones. En ratones, el NOAEL fue de 3125 mg/kg de alimento; en ratas fue < 3125 mg/kg de alimento. Estos resultados no se obtuvieron en ningún otro estudio de corta o larga duración realizado en diferentes razas y especies. Las lesiones de las glándulas salivales parecen indicar que el glifosato puede actuar como agonista adrenérgico débil.

Se estudió la toxicidad a largo plazo en ratones y ratas. Se observaron escasos efectos y, en la mayor parte de los casos, sólo a dosis relativamente altas. Con dosis de 30 000 mg/kg de glifosato de calidad técnica se produjo en los ratones retraso del crecimiento, hipertrofia o necrosis de los hepatocitos e hiperplasia epitelial de la vejiga urinaria. La misma prueba en ratas con dosis de 20 000 mg/kg de alimento provocó una disminución del crecimiento, aumento del peso del hígado, cambios degenerativos del cristalino e inflamación gástrica.

De los estudios disponibles no se desprende que el glifosato de calidad técnica tenga actividad mutagénica, carcinogénica o teratogénica. Se realizaron con este compuesto dos estudios en varias generaciones de ratas. Los principales efectos del glifosato de calidad técnica con dosis de 30 000 mg/kg de alimento fueron una disminución del peso corporal de los padres y las crías y la reducción del tamaño de la camada. Se ha informado que en un estudio de reproducción con dosis de 30 mg/kg de peso corporal se produjo un aumento del número de casos de dilatación tubular renal unilateral en crías macho de la F_{3b}. La ausencia de efectos renales en las crías con dosis más elevadas en el otro estudio de reproducción pone de manifiesto que la reproducibilidad de la lesión es incierta.

7. Efectos en el ser humano

Sólo se dispone de tres estudios controlados sobre irritación/sensibilización en voluntarios, cuyos resultados indican la ausencia de efectos. Se ha informado de varios casos de intoxicación (la mayor parte intencionados) con la formulación Roundup de herbicida a base de glifosato de calidad técnica. No se detectaron efectos adversos tras realizar un estudio para

determinar el estado de salud de los trabajadores que aplican la formulación del herbicida Roundup. Los datos disponibles sobre exposición en el trabajo de quienes aplican el Roundup indican que los niveles de exposición están muy por debajo del NOAEL obtenido en los experimentos correspondientes con animales.

8. Efectos sobre otros organismos en el laboratorio y en el medio ambiente

El glifosato de calidad técnica tiene una toxicidad de moderada a ligera para los microorganismos acuáticos, con valores de CE_{50} (3-4 días) de 1,2-7,8 mg/litro y valores de NOEC (7 días) de 0,3-34 mg/litro. Las formulaciones de glifosato son entre ligeramente tóxicas y muy tóxicas para los microorganismos acuáticos, con valores de CE_{50} en tres días de 1,0 a > 55 mg de producto por litro. Las cianofíceas (algas verdeazuladas) son más sensibles al Roundup que las algas verdaderas. Afecta a diversos procesos fisiológicos, entre ellos la formación del color verde, la respiración, la fotosíntesis y la síntesis de aminoácidos aromáticos.

En cultivos de bacterias del suelo se ha comprobado la influencia del glifosato sobre la fijación del nitrógeno, la desnitrificación y la nitrificación. Sin embargo, en estudios sobre el terreno no se han observado efectos significativos tras la aplicación de varias formulaciones. Diversas bacterias estrechamente relacionadas han demostrado que son capaces de degradar el glifosato.

A concentraciones $\geq$ 29 μg de Roundup/litro se inhibe el crecimiento de los micelios de las ectomicorrizas en cultivos puros. Son géneros sensibles *Cenococcum*, *Hebeloma* y *Laccaria*.

Cuando se disuelve en agua, el glifosato es ligeramente tóxico para las macrofitas acuáticas, con un valor de NOEC en 14 días de 9 mg/litro. El Roundup disuelto en agua es también ligeramente tóxico, con valores de NOEC en 14 días de 2,4-56 mg de Roundup/litro. No se dispone de datos acerca de su toxicidad aguda. La fitotoxicidad es mucho más elevada cuando el agua no arrastra los depósitos del rociado.

La toxicidad del glifosato de calidad técnica para los invertebrados acuáticos varía entre ligera y muy ligera, con unos valores de la CL_{50} o la CE_{50} en 2 a 4 días de $\geq$ 55 mg/litro, y un valor de NOEC en 21 días de 100 mg/litro. Las formulaciones de glifosato tienen una toxicidad entre moderada y muy ligera para

los invertebrados acuáticos, con valores de CE_{50} en 2 días de 5,3-5600 mg de producto/litro y valores de MATC en 21 días de 1,4-4,9 mg de producto por litro. La toxicidad más elevada del Roundup se debe fundamentalmente a la presencia de surfactantes.

La toxicidad del glifosato de calidad técnica para los peces es entre moderada y muy ligera, con valores de CL_{50} en 4 días de 10 a > 1000 mg/litro, una NOEC en 21 días de 52 mg/litro, y un valor MATC de > 26 mg/litro. Las formulaciones del glifosato tienen también una toxicidad entre moderada y muy ligera para los peces, con valores de CL_{50} en 4 días de 2,4 a > 1000 mg de producto por litro y valores de NOEC en 21 días de 0,8-2,4 mg de producto/litro. La especie más sensible es la carpa, cuando se la expone a la formulación Sting. No se han observado en los peces efectos relacionados con el tratamiento de Roundup en el medio ambiente, salvo cierta tensión inmediatamente después de la aplicación de una dosis recomendada y evitando concentraciones ≥ 40 mg de Roundup/litro.

En sistemas de cultivo sin suelo con soluciones nutrientes en concentraciones ≥ 2 mg de i.a./litro se produce una inhibición dependiente de la dosis de la nodulación del trébol subterráneo inoculado con *Rhizobium*. El glifosato en las dosis de aplicación recomendadas no afecta a la germinación de las semillas. La longitud de las raíces de los plantones de pino rojo disminuye en condiciones de laboratorio en función de la dosis con unas concentraciones de aplicación ≥ 0,54 kg de i.a./ha. Esta reducción no se confirmó en un experimento comparable sobre el terreno.

El glifosato de calidad técnica y el Roundup son ligeramente tóxicos para las abejas cuando se aplican por vía oral o tópica. Los valores de la DL_{50} en 2 días son ≥ 100 µg (i.a. o producto) por abeja. La DL_{50} en 2 días por vía oral de Sting para las abejas es > 100 µg/abeja. Roundup y Roundup D-pak son ligeramente tóxicos para las lombrices de tierra, con valores NOEC en 14 días de 500 y 158 mg de producto por kg de peso seco, respectivamente. No se observaron efectos adversos del Roundup sobre la fecundidad y fertilidad de especies de la familia *Chrysopidae*, y tampoco se detectaron efectos de Sting en la ingestión de alimentos y la mortalidad del escarabajo *Poecilus*.

El glifosato de calidad técnica es ligeramente tóxico para las aves, con una DL_{50} > 3851 mg/kg de peso corporal, una CL_{50} en 8 días de > 4640 mg/kg de alimento, y valores NOEC en 112-119 días de ≥ 1000 mg/kg de alimento. Roundup y una formulación

desconocida son también ligeramente tóxicos para las aves, con una DL_{50} de > 2686 mg de producto/kg de peso corporal y una CL_{50} en 8 días de > 5620 mg de producto/kg de alimento. En condiciones de laboratorio no se han observado en general sobre los mamíferos efectos relacionados con el tratamiento de glifosato de calidad técnica o Roundup, salvo con dosis de aplicación muy elevadas. Los efectos relacionados con el tratamiento en las aves y mamíferos del medio ambiente parecen deberse fundamentalmente a cambios de hábitat después del tratamiento con Roundup.

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