Ecotoxicology of glyphosate

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Scope

The purpose of this project is to make a review of the open literature, to evaluate the relevant papers and to write up a memorandum on this for the Danish Environmental Protection Agency. In this review the evaluations and conclusions aim at having an administrative cut, useful for the administration to approval of pesticides. A mini-review of the environmental, fate and effects of glyphosate will be presented with the main emphasis on field studies. Due to the limited time frame of the project the present document does not aim at reviewing all the literature in the area rather it is drawing the main lines in selected areas. The information reviewed here is based on search in the open literature using Science Citation Index (SCI), BIOSIS, ASFA and Life Science Collection as databases. Searches in the databases gave 9035 hits in BIOSIS and AGRIS (1982-1999), 2035 hits in SCIC (1989-1999) and 119 hits in ASFA. Within this project it was impossible to obtain and review all the literature due to the vast number of hits. Medical related references were eliminated and the search was primarily concentrated on literature published after 1991, as reviews were published at that time. The limitation does not indicate that articles prior to 1991 were not obtained, but they were rather obtained by cross-references. These limitations of the searches gave 2425 hits, of which 360 articles were ordered and reviewed, with main focus on field studies. Some papers were not retrieved in time due to the time span of the project.

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INTRODUCTION

Glyphosate is a herbicide widely used in agricultural land. To assess the toxicity of this compound to ecosystems an evaluation of the fate and ecological toxicity is undertaken. The review is based on the open literature and aimed at administrative needs in connection with re-evaluation of the approval of pesticides containing this compound. The review includes a short evaluation of the fate, but is mainly concerned with field studies on effects of glyphosate on selected soil and surface-water organisms.

CHEMICAL AND PHYSICAL PROPERTIES

Glyphosate (*N*-(phosphonomethyl)glycine) is a weak organic acid consisting of a glycine and phosphonomethyl moiety. The empirical formula is $C_3H_8NO_5P$ and the CAS no. 071-83-6.

$$H_2PO_3\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}COOH$$

Table 1. Physical and chemical properties of glyphosate (after: Mesink and Janssen 1994)

Parameter	Value
Physical state	Crystalline powder
Colour	White
Odour	None
Melting point	184.5 C
Boiling point	not applicable
Specific gravity	1.704
Vapour pressure	$<1*10^5$ Pa
Solubility in water	10 100 mg/l
Henry's law constant	$<7*10^{-11}$
Octanol water partitioning coefficient (Log Kow)	-2.8
Surface tension	0.072
Pka values	<2, 2.6, 5.6, 10.6
Molar absorptivity	0.086 litre/mol per cm
Flammability	Mot. Flammable
Explosiveness	not explosive
pH	2.5

ENVIRONMENTAL DISTRIBUTION, TRANSPORT AND FATE

Compartment distribution

Glyphosate is used in various formulas as a herbicide both in aquatic and terrestrial systems. Hence, glyphosate and metabolites thereof is present in soil, sediment and surface water. It may further potentially be present in air, due to spray drift, and in ground water due to migration/percolation. For example, Freedman (1990) cites on-site deposition rates of 22% to 86% relative to the amount of glyphosate applied from ground sprays, indicating that a large fraction may be dispersed to non-target sites.

Glyphosate is a man made compound, hence, in all compartments the background level must be assumed zero. In general concentrations found in the terrestrial and aquatic ecosystem depend among others on application rates and time since application. In Danish agriculture the recommended doses for application are up to 2400g glyphosate/hectare (Haldrup *et al.*1996).

General breakdown pathways

Glyphosate has two major breakdown pathways. It may be decomposed to aminomethyl-phosphonic-acid (AMPA) or sarcosine. Both of which may be further degraded (Fig 1). The breakdown of glyphosate can be chemical, photochemical or through biological processes. The major pathway depends on the environment.

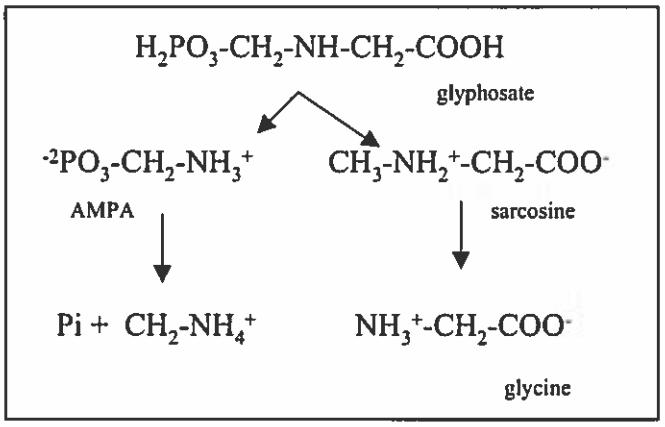

Fig. 1. Degradation routes for glyphosate (After Mesink and Janssen 1994)

Soil

Sources

Glyphosate can enter the terrestrial environment either due to direct intentional application, spills, drift or absorption from flooding water. The major route depends on the environment in question. For example, glyphosate is intentionally used in agroecosytems, but may unintentionally drift to adjacent ecosystems (Marrs and Frost 1997). The breakdown product AMPA may also be derived from other sources, these sources

will probably primarily distribute AMPA to the environment via sewage sludge (Andersen and Hansen 1997).

Dissipation

Degradation of glyphosate and metabolites in soil is dominated by microbial metabolism, with chemical and photochemical pathways being much less important (Sprankle *et al.*1975, Torstensson and Aamizepp 1977, Rueppel *et al.*1977, Torstensson 1985, Frantz *et al.*1997).

Glyphosate in soil usually shows a bi-phasic dissipation rate, with an initial faster disappearance of the freely dissolved part and a later slower degradation of the soil-bound fraction (Hance 1976, Nomura and Hilton 1977, Erbach *et al.*1998). Due to the slower second phase dissipation the DT_{90} (90% dissipation time) may be longer than anticipated from DT_{50} (50% dissipation time). The metabolite AMPA usually shows a peak soil concentration a few days after initial application and dissipate slower than glyphosate (Rueppel *et al.*1977, Ragab *et al.*1985).

In laboratory studies half-lives may range from days to several years (Rueppel *et al.*1977, Sprankle *et al.*1975, Nomura and Hilton 1977, Alferness and Iwata 1994, Erbach 1997, Jacobsen *et al.*1998). In two Hawaiian inceptisols (with volcanic ash as parent material) 375 mg glyphosate/kg degraded 0.8% and 1.2% within 60 days. Based on this the authors predicted half-live values as long as 22 years. In oxisols and vertisols soils half-lives occurred within 48 days (Nomura and Hilton 1977).

In field studies the soil dissipation times, measured as DT_{50}, mainly range from a few days to months, probably mainly dependent on biological activity, soil type and temperature (Torstensson *et al.*1989, Müller *et al.*1981, Heinonen-Tanski 1989) (Table 2).

Feng *et al.* (1990b) studied the fate of glyphosate after applying 2 kg glyphosate a.i./ha by aerial spray to a Canadian coastal rain forest. Within 1 year a continuous decline from 8.16-39.80 ppm (site dependent) to 0.25-2.89 ppm glyphosate was observed in top 5 cm. The sites consisted of alluvial sandy loam or sandy clay loam, vegetation of red alder (*Alnus rubra*) and salmonberry (*Rubus spectabilix*) and had a yearly rainfall of 210 to 480 cm.

Newton *et al.* (1984) studied the fate of 3.3 kg glyphosate a.e./ha in a loamy forest soil of a Canadian deciduous forest. The forest stand primarily consisting of red alder (*Alnus rubra*) and bitter cherry (*Prunus emarginata*) and with an understorey of vine maple (*Acer circinatum*), salmonberry (*Rubus spectabilix*) and swordfern (*Polstichum monitum*). Initial concentrations of exposed soil ranged from 0.2-1.24 mg a.e./kg (0-7.5 cm). The 50% dissipation was calculated to 40 days. For litter covered soils the initial concentrations were 0.12-3.05 mg/kg and the DT_{50} was calculated to 29 days. The rainfall was 230 cm a year.

Large variation may be found between soils. Torstensson and Stark (1979) observed the fate of glyphosate following field application of 2.4 kg glyphosate a.i./ha to 6 Swedish forest mull/brown soils (Table 2). They observed a reduction from 0.50- 2.07 (dependent on soil type) to 0.05-0.17 kg a.i./ha after 259 days, giving DT_{90} of 63 to 91 days. In iron podzols with low microbial activity 0.53 kg a.i./ha was reduced to 0.49 kg/ha over 287 days, whereas in a similar soil with higher microbial activity 0.45 kg/ha was reduced to 0.05 kg/kg during the same period.

Table 2. Dissipation of glyphosate from soils.

Soil type	pH	OM	Sand	Silt	Clay	Application Rate	Experiment duration (days)	Dissipation (%)	$DT_{(50)}$ / $DT_{(90)}$ (days)	References
Sandy loam (0-5 cm) Station 1-3 Station 7-9	 4.9 5.0	 30 31	 56 62	 24 25	 20 13	2.0 kg a.i/ha	 360 360	 82-94 82-94	 $45\text{-}60_{(50)}$ $45\text{-}60_{(50)}$	Feng *et al.*(1990b)
Sandy soil (0-5cm)	5.6	2.5	63	26	11	1.4 kg /ha	58- 1 year	70- 85		Heinonen-Tanski *et al.*(1985)
Loamy forest soil exposed soil litter covered soil	4- 4.7	3.8-5.2				3.3 kg a.e./ha	55d		 $40_{(50)}$ $29_{(50)}$	Newton *et al.*(1984)
Loam soil Silty soil	5.1 5.5	$44*_{OC}$ $1.5*_{OC}$				2.6 kg /ha	28-249 28-249	47- 95 10- 74		Müller *et al.*(1981)
Sandy loam (0-15cm)	5.4	2.7	81	8	11	4.3 mg a.i./ha	10-52-122	70-88-100		Ragab *et al.*(1985)
Podzol (5-10cm)	3.5	39.7	0	0	87	2.7 kg a.i./ha	7-28-78-365	20-30-95-99		Roy *et al.*(1989)
Clay loam Mull/Brown Podsol/Brown Mull/Brown Iron podsol Iron podsol	6.6 5.5 5.4 4.9 4.6 4.6	12 11 17 26 24 45				2 kg a.i./ha 2 kg a.i./ha 2 kg a.i./ha 2 kg a.i./ha 2/4 kg a.i./ha 2/4 kg a.i./ha	259 259 259 259 290[a] 296[b]	93 98 80 92 8 89- 90	$73_{(95)}$ $49_{(95)}$ $91_{(95)}$ $91_{(95)}$ $> 287_{(95)}$- $>287_{(95)}$ $21_{(95)}$- $49_{(95)}$	Torstensson and Stark (1979)

* OC: Organic carbon; a: Low microbial activity; b; Higher microbial activity than for a.

Mobility

Due to complexion in soil glyphosate reputedly is very immobile in soils (Torstensson 1985). Glyphosate and AMPA are primarily found in the upper 15 cm of the soil core, although leaching to lower layers has been reported (Feng *et al.*1990b, Roy *et al.*1989, Cheah *et al.*1997, Piccolo *et al.*1996, Smith *et al.*1996).

In a Canadian coastal rain forest aerial sprayed with 2 kg a.i./ha the upper 15cm of the soil column (alluvial sandy loam and sandy clay loam) retained more than 90% of the glyphosate (Feng *et al.*1990b). This upper layer consisted of 19 -35 % organic matter. Two samples showed more than 10% of the glyphosate in layers below 15 cm. In these studies the rainfall was 210 to 480 cm yearly.

Spraying Canadian boreal jack pine sandy forest soils with 2 kg glyphosate a.i./ha no glyphosate was observed in samples below 15 cm over 335 days (Roy *et al.*1989). Rainfall was 325 cm over 4 months (Table 2).

Summary of glyphosate in soil

Glyphosate dissipate with an initial fast decomposition followed by a slower dissipation rate. Due to the slower dissipation of the bound glyphosate the DT_{90} values much longer than anticipated from DT_{50}. Dissipation times found was mostly below 60 days but was in some cases several months or years, for example, with an 8% dissipation over 287 days. Soils with low microbial activity seem to have much longer glyphosate dissipations times than soils with high microbial activity. Most field studies found showed little mobility with most glyphosate present in the top 15 cm. There were very few performed with Danish soils. For the field studies concentration ranges and soil types were relevant for Danish practice and conditions, although in most cases the precipitation was much higher than normal for Danish conditions.

Water

Sources

The fate of glyphosate in water, especially in seawater, has been far less studied than in soil. Glyphosate can enter the aquatic environment either due to direct application, drift, runoff, or desorption from contaminated sediment. The major runoff usually occur in relation to the first storm event after application but may continue longer (Edwards *et al.*1980, Feng *et al.*1990b). For example, following successive treatments for three years (1.12 and 3.36 kg glyphosate/ha) to a watershed Edwards *et al.*(1980) detected glyphosate in the runoff in each of the years. The herbicide transport in the first runoff event after application accounted for 99% of the total runoff to a watershed. The total runoff was calculated to 0.1-0.5% (5.4 µg glyphosate/ha) of the total applied, although in one single event 1.8% of the applied was transported. In this study the yearly rainfall was 210 to 480 cm.

In the aquatic environment the breakdown of glyphosate is considered to be primarily microbial (Rueppel *et al.*1997). For example, no appreciable degradation of glyphosate was observed in distilled water, while rapid degradation occurred in the river water with a microbial activity (Zaranyika and Nyadoro 1993). For ground water glyphosate half-lives varied from 60 h for samples exposed to sunlight to 770 h for

those stored under dark and cold conditions (Mallet and Barcelo 1998). Anton *et al.*(1993) found little decomposition of glyphosate over 78 days when glyphosate was applied to tap water in open tanks illuminated by natural sunlight.

Dissipation from ponds

In laboratory studies degradation times from days to months have been recorded (Antón *et al.*1995, Zaranyika and Nyandro 1993, Wang et al.1994). For example, studying the fate and 100 mg /l or 100 µg/l of glyphosate in four river waters (contained in glass jars) the degradation was 46-62% and 54-89%, respectively, after 56 days (Wang *et al.*1994). Studying the fate of 150 ppm glyphosate contaminated river water in tanks with and without sediment the degradation after 72 days was 72% in tanks with sediment and 4%in tanks without sediment (Zaranyika and Nyandro 1993).

In field studies with pond water glyphosate usually dissipates from the water column in days to weeks (Table 3). In 4 plant infested forest ponds sprayed with 0.89 kg glyphosate a.e./ha (aerial application) the dissipation from the water was 95 – 100% within 38 days. The dissipation rate was slower in ponds with calcareous water and sediments, compared to less calcareous conditions (Goldborough and Bech 1989). The depths of these ponds were 0.5 to 1.0 m. In experimental field tanks sprayed with 0.89 mg a.e./ha, glyphosate dissipated much more rapidly from the water in tanks containing sediment than in those not containing sediment (Goldsborough and Bech 1989). The latter results should be regarded with care as the ponds without sediment probably was contaminated by incoming animals. Combined with the information from the laboratory study by Zaranyika and Nyandro (1993) the studies suggests that sediments act as primary sink for glyphosate in aquatic systems.

Dissipation from streams

Introducing 150 ppb solution into a US irrigation canal at a single spot (for 52 minutes) approximate 70 % of glyphosate added to the water was present 1.6 km down stream and 58 % present 14 km down stream (Comes *et al.*1976).

Bowmer *et al.*(1986) studied the fate of glyphosate following addition (3.6-3.9 g glyphosate/m^3) to a slower running Australian irrigation canal freed of submerged weeds. They found that the sediments attenuated loads of glyphosate only slowly. Attenuation being 13 and 27% each kilometre downstream, dependent on the canal, resulting in benthic uptake of 365 and 603 g/km.

Chen *et al.*(1989) observed a nearly total dissipation of glyphosate within 800 m in a plant grown Taiwan irrigation canal. In this canal 480 m^2 of the water was sprayed with 1.020 g glyphosate a.e./ha. In a canal without plants a spray of 130 m^2 also gave a total dissipation within 800 m.

Bowmer *et al.*(1986) found that only a minor proportion of glyphosate was adsorbed onto suspended solids, even in turbid irrigation water. In relation to this Feng *et al.*(1990a) reported that suspended sediment did not represent a major mechanism for export of aquatic residues from a watershed. The immediate dissipation from the water is primarily caused by adsorption to sediment, as the glyphosate in the sediment increases when the water level decreases (Feng *et al.*1990a).

Table 3. Dissipation of glyphosate in water.

Water type (% plant cover)	Size Width / length / depth	Average velocity (m/s)	Application Rate	Experiment (days (d) /distance (km))	Dissipation (%)	$DT_{(50)}$ / $DT_{(90)}$ (days)	References
Pond (F*) –plant (10 cm) (50 cm) +plant (95%) (10 cm) (50 cm)	1 m / 1 m / 1 m		1.2 a.e./ha	1- 2- 7- 14- 28 d 1- 2- 7- 14- 28 d	96- 96- 98- 99 – 100 (-10)-(-10)- 56- 75-100 90- 95- 99- 100 –100 5- 27- 85- 70- 50- 99		Chen *et al.*(1989)
Pond (F*) Birch Hike Spruce Manfor Microcosm –sediment +sediment	Area 0.2-0.7 ha Depth 0.9-1.5 m Area: 12m^2 D: 0,25m Volumen: 40 L 0.01 m^3 sediment		0.89 kg a.i./ha	11- 38 – 255 d 11- 38 – 255 d 11- 38 – 255 d 11 d 1 – 5 - 15 – 30 d 1- 5 - 15 – 30 d	1 – 100- 100 90 – 100 –100 90- 100- 100 95 325 (contaminated sample) 100- 45 - 95 - 95*	$1.5_{(50\%)}$d $1.9_{(50\%)}$ d $3.5_{(50\%)}$ d $2.0_{(50\%)}$ d $5.8_{(50\%)}$ d	Goldborough and Bech (1989)
Canal (F*)	3 m / 394 m / 0.6-1.2 m	0.15	3.9 mg/m^3 3.6 mg/m^3	0.25- 1.25- 3.25 km 0.25- 1.25 km	0 - 3 - 37 0- 27		Bowmer *et al.*(1986)
Canal (F*) -plant +plant (55%)		0.144 0.432	1.0 a.e./ha	0.1 - 0.8 km * compared to 15 m from spray	50- 99* 63- 60*		Chen *et al.*(1989)
Canal (F*) A B		0.506 0.368	150 ppb	0.3 - 1.6 – 8 – 14.4km	21- 28 91- 30–43-42		Comes *et al.*(1983)
Estuary (S*)			0.0017 kg a.e./ha 0.0016 kg a.e./ha 0.0016 kg a.e./ha (deposited)	1 d	30 17 15		Paveglio *et al.*(1996)

F: Fresh water; S: Salt water

Dissipation from marine water

Little information is available for seawater. A study was performed in a Washington bay (US) where 1.55-1.73 kg glyphosate a.e./ha was applied to coastal tide sediments grown with *Spartina* (Paveglio *et al.*1996). The sediment concentration immediately after spaying was 1.16 and 2.82 µg/g and during 119 days declined 51 and 72%, respectively. The herbicide present in the water phase was rapidly diluted by incoming water, declining 72% between the first and second high tide.

Dissipation from sediment

In a Canadian creek, Feng *et al.*(1990b) observed glyphosate residues in sediment more than 196 days after application to a forest watershed. Spraying the watershed with 2 kg glyphosate a.i./ha by aerial spray they found 6.34-6.80 µg/kg sediment at altitudes of 1600m and 0.44-0.58 µg/kg at 750m altitude. Samples taken between 196 and 364 days after application showed sediment residues of 0.14-1.92 µg glyphosate/kg.

Comes *et al.*(1976) applied 5.6 kg glyphosate/ha to the banks of two dried irrigation canals in Washington (US), down to 60 cm below normal water level. Filling these canals with water 158 and 172 days later caused no detectable glyphosate to elute in the water. The bank soil concentrations was 0.37 and 0.33 ppm glyphosate one day prior to water filling.

A similar experiment was performed by Browmer *et al.*(1986) in Australian irrigation canals. Applying 3.6 kg glyphosate/ha to dry sediment and then re-watering 4 days later caused 7% of the applied glyphosate to elute in the water.

In an open tank study concerning the fate of glyphosate in a Zimbabwean river sediment an application of 150 ppm to the water phase resulted in a sediment DT_{50} of more than 70 days (Zaranyika and Nyandro 1993). They recorded an initial fast degradation rate but the rate decreases asymptotically with time as observed in soil studies.

Summary of glyphosate in water

The fate of glyphosate in aquatic ecosystems is much less studied, in particular in marine systems. The main metabolic pathway is probably microbial-mediated, but photochemical decomposition may also play a significant role. In freshwater pond systems glyphosate usually dissipate from the water phase within days to weeks and adsorbs to the sediment. In the sediment glyphosate probably experience a fate similar to that in soil, although this is much less studied. In running water less glyphosate binds to the sediment and may be transported more than 14 kilometres. Very little information is present on fate of glyphosate in seawater. Few of the reported studies were performed in natural water systems. None of the above field studies were performed with Danish water systems but the concentration ranges, exposure regimes and flow rates were relevant for Danish practice and conditions.

INVERTEBRATES

Bioaccumulation

Tooby (1985) reported that bio-concentration factors are not existing for invertebrates. However, for fish, the maximum recorded bio-concentration factor is 1.6. This and the low log P_{ow} value (-4.58) of glyphosate suggest that glyphosate is not expected to accumulate in invertebrates in high concentrations.

Toxicity

Terrestrial invertebrates – laboratory effects

Eijsackers (1985) reported on experiments where 2 springtails (*Onychiurus quadriocellatus*, *Tomocerus flavescens*), 2 isopods (*Philoscia muscorum*, *Oniscus asellus*) and 3 carabid species (*Pterostichus oblongopunctatus*, *Abax ater, Notiophilus biguttatus*) were held on a compacted soil surface and exposed to Roundup *via* spraying in a Potter tower. Both isopods showed a consistent and significant decrease in longevity at the highest dose applied (5.96 ml/m^2) but not at 1.49 ml/m^2. This was also the case for one of the springtails (*Onychiurus quadriocellatus*), whereas the beetles seemed not to be affected. It should be noted that the concentrations used (corresponding to 60 and 14 kg/ha) are far higher than what is the recommended dose in Denmark (2.4 kg a.i./ ha). The same author also noted that *Notiophilus biguttatus* did not avoid glyphosate treated soil when offered a choice between treated and untreated soil. Other experiments reported by Eijsackers (1991) showed that litter fragmentation by soil arthropods was not negatively affected at a dose of 6 l glyphosate/ha. Eijsackers (1985) concluded on the basis of his own and earlier studies, that glyphosate seems not to be very toxic for the soil fauna, but that field experiments are lacking to validate this conclusion.

Kegel (1989) studied effects on 4 species of carabid beetles. The author found that the development of *Poecilus versicolor* larvae to adults was not negatively affected by 10 ml/m^2 Roundup. Similarly *Poecilus cupreus* was not affected at 1 ml/m^2 Roundup, which was the highest dose tested.

In a laboratory experiment simulating a field application of glyphosate, Whitehouse and Brown (1993) tested the toxicity to larvae of the Scarabaeid, *Phyllopertha horticola*, and adults of the carabid, *Pterostichus melanarius*. These authors applied glyphosate (0.6 kg a.i./ha) to trays with either bare soil, soil covered with turf, or soil covered with weeds. There were no significant effects on mortality in either of the two species for any of the habitat types.

Martin (1982) tested the effects of 1, 10 and 100 mg/kg "glyphosate" (probably the formulated herbicide) on the growth of juvenile earthworms (*Aporrectodea caliginosa*). It was reported that earthworms gained weight at all glyphosate treatments, but at a slightly lower rate than in the control treatment. The highest dose of 100 mg/kg is roughly corresponding to a dosage of 70 kg/ha, under the assumption that glyphosate is evenly distributed in the upper 5 cm of soil. In another study, using growth of the earthworm *A. caliginosa* as test parameter, Springett and Gray (1992) reported that

glyphosate (Roundup) applied in repeated low doses significantly reduced growth and retarded the sexual development of juveniles. These authors applied doses of 0.7, 1.4 and 2.8 g a.i./ha glyphosate at 2-week intervals over a total period of 100 days. Control animals reached a fresh weight (fw) of 650 mg within 60 days, whereas treated animals (all concentrations) reached a fw of approximately 450 mg during that time. Control animals reached sexual maturity after 25 days in the experimental system, whereas treated animals needed at least 55 days. The authors claimed that repeated applications of glyphosate is a common practise, and as such the experimental approach is interesting. However, the animals were cultured in a very small volume of soil, 10 cm^3, making the exposure extremely high and direct compared to a natural situation. It is therefore questionable how valid these results are for a normal field exposure situation.

Dalby *et al.* (1995) also investigated the toxicity of glyphosate to 4 earthworms species, *A. trapezoides*, *A. rosea*, *A. caliginosa* and *A. longa*, both in pot experiments where the herbicide was applied to the soil surface, in pot experiments with growing plants and with the herbicide applied to the canopy. The authors found that a single application at a dose of 33 kg/ha had no effect on mortality or growth in any of the tested species.

Ahn *et al.* (1997) investigated the susceptibility of the spider mite, *Tetranychus urticae*, to glyphosate sprayed onto leaves of kidney bean plants, where different stages of spider mites were living. Total amounts up to 4.74 mg a.i./leave were applied by spraying. Survival of *T. urticae* eggs, larvae and adults were not influenced by any of the tested dosages.

Another study of glyphosate toxicity to spider mites (*Tetranychus lintearius*) is reported by Searle *et al.* (1990). By using a slide dip method into solutions of glyphosate, different surfactants, and combinations thereof, they showed that glyphosate in itself at approximate field rates 3.6 g a.i./litre was "moderately toxic" to *T. lintearius*, causing 29 % mortality compared to 8 % in controls. Interestingly, glyphosate plus each of 4 sufactants at approximate field rates caused near 100 % mortality, significantly increasing the toxicity of glyphosate alone. The toxicity of surfactants alone was not tested in this study. It should be stressed that the slide dip method used in this study is likely to maximise the exposure of the mite to glyphosate; this would probably not be simulating the conditions in the field. It should be noted that additives, for example, cationic detergents, could cause toxic effects of formulations of Round Up.

Hislop and Pprokopy (1981) tested the susceptibility of a predator of spider mites in apple orchards, *Amblyseius fallacis*. Using the slide dip method it was found that immersion for 5 seconds in manufacturer-formulated glyphosate at the recommended field rate (1litre dissolved in 100 l water) caused 100 % mortality.

Summary of terrestrial invertebrates – laboratory effects

Most of the studies listed here have been performed under climatic and pedological conditions comparable to Danish conditions, and indicate that direct, deleterious effects of glyphosate on terrestrial invertebrates are unlikely to occur due to normal Danish agricultural practise.

Terrestrial invertebrate communities - Field effects

In a field study of hedgerows the effects of glyphosate on the spider and carabid beetle communities were assessed (Asteraki *et al.* 1992). In plots treated with glyphosate (5 l "Roundup"/ha) all flora was killed, leaving the soil bare for the rest of the study period. Especially the carabid community was seriously disrupted, probably because suitable habitats were destroyed and food availability for the omnivorous species was drastically reduced.

Mele and Carter (1999) studied the effects of herbicide application on earthworm communities in Australian conservation tillage agricultural soils (Table 4). They applied 0, 450 g a.i./ha and 900 g a.i./ha glyphosate to replicated plots of 20x3 m that were subsequently directly drilled to wheat. Earthworms were sampled at the end of the cool wet season (August-September) by hand sorting of soil samples. Earthworm densities in treated plots (both dosages) were significantly higher (about 50 %) than in similar control plots. Species composition was largely unaffected. The authors proposed that a stimulation of microbial populations by herbicide addition might have increased earthworm densities possibly due to the utilisation of the increased microbial population as a food source. The authors concluded that the role of lyphosate in returning plant residues to the soil suggests a minimal impact of chemical residues on earthworm population densities, at least in the short-term.

Gómez and Sagardoy (1985) studied the effects of glyphosate application on soil microarthropods in a sandy, semi-arid Argentinean agricultural soil. The soil was, at the outset of the experiment, covered with herbs and grasses, primarily *Solanum elaeagnifolium*. Application rates of 0, 2, 4 and 8 l/ha ("Glyphosate") were used and the microarthropod population was studied during the following 3 months. Glyphosate in any of the doses used had no significant effects on population densities of total microarthropods, Collembola or soil mites.

In a field litterbag study devoted to investigate the effects of glyphosate on decomposition and microarthropod functions Hendrix and Parmerlee (1985) found that normal field application rate and 10 times field rate of glyphosate to leaves of Johnson grass (*Sorghum halepense*) significantly decreased the decomposition rate. Glyphosate treatments were applied by dipping litterbags containing 3 g dried leaves for 30 seconds into solutions of commercial formulation of 0, 0.72 and 7.2 % a.i. The authors, using a conceptual model of the decomposition subsystem, suggested that 10 times herbicide treatment altered the system by (1) promoting the microbial utilisation of the herbicide or additive as a carbon source; (2) increasing the importance of microarthropod grazing relative to communition; (3) eliminating or reducing the importance of the predatory microarthropods; (4) increasing the rate of nutrient loss from the litter via microbial and microarthropod activity. The system thus became simplified with fewer recycling loops, accelerated soluble nutrient loss, and slower decay of carbon from the leaf tissue.

Gibb and Buhler (1998) studied the effect of glyphosate on the infectivity of the entomopathogenic nematode, *Steinernema carpocapsae*. Using grass turf cores sprayed with a recommended dose of Roundup, approximately 1.6 g/m (16 kg/ha), these authors could demonstrate that infectivity towards the greater wax moth, *Galleria mellonella*, was not influenced by glyphosate at this dosage.

In a study by Forschler *et al.* (1990) another entomopathogenic nematode, *Steinernema feltiae*, was used as test organism. Nematodes exposed to glyphosate in aqueous

suspensions, including a concentration equivalent to field dose, showed about 23 % inactivity compared to 4 % inactivity in demineralized water control. However, after having been rinsed in pure water, the same samples of nematodes showed no decreased infectivity to *Galleria mellonella*. The authors concluded that glyphosate may have caused some of the animals to become quiescent, but this had no adverse effect on overall survival or infectivity success.

Vega *et al.* (1993) reported that glyphosate in soil concentrations higher than 100 mg/kg (added to soil by mixing) significantly decreased the root gall, and egg mass indices and population of the root knot nematode, *meloidogyne incognita*, infecting soy bean, *Glycine max* and *Amaranthus spinosus*. Another study concerning the population development of an endoparasitic nematode, *Pratylenchus zeae*, on Rabi groundnut (*Arachis hypogaea*) in field plots sprayed treated with glyphosate, have been reported by Patra and Ray (1987). These authors found that glyphosate (0.75 and 1.0 kg a.i./ha), in some unknown way stimulated the population development of *P. zeae* compared to control plots.

Rovesti *et al.* (1988) found that the entomopathogenic nematode *Heterorhabditis bacteriophora* was not negatively influenced by glyphosate (Roundup) at recommended field rates. This concerned survival, mobility and infectivity to *G. mellonella* larvae. This was also valid for two similar entomopathogenic nematodes, *Steinernema carpocapsae* and *S. feltiae* (Rovesti and Deseö 1990).

One of the most important and relevant sources found during this literature search and review is beyond doubt "The Fallingsnow Ecosystem Project: Documenting the Consequences of Conifer Release Alternatives" (methods to facilitate the early growth of conifer seedlings)(Lautenschlager *et al.* 1998). This project is operational scale, replicated (4-10 ha/plot), and comprehensive, including the following ecosystem components: soils, microclimate, belowground fungi, vegetation, above and belowground insects, terrestrial gastropods, amphibians, small mammals, songbirds and moose. One of the treatments is helicopter applied glyphosate, post harvest, which may be compared with un-harvested and untreated, harvested plots. The project was initiated in 1993 and until now results have primarily been published in workshop reports and unpublished reports. There exist a workshop report (which it has not been possible to purchase in this project) with "popular summaries" (Wagner and Thompson 1998). Preliminary results have been summarised by Lautenschlager *et al.* (1998). These authors conclude that, "there are minimal differences among the herbicide and cutting alternatives tested, and that the initial differences observed between released and untreated plots are no longer common", i. e. 3 years after the study was initiated.

Summary of terrestrial invertebrates - field effects

The field studies of glyphosate effects on terrestrial invertebrates indicate that direct toxic effects are unlikely to occur under Danish conditions. However, at least three studies [Asteraki *et al.* (1992), Mele and Carter (1999), Hendrix and Parmerlee (1985)] suggest that indirect effects of glyphosate (or other herbicides) may occur through the herbicidal effect on plant cover and altered microfloral species composition. The studies mentioned are relevant for Danish climatic and pedological conditions.

Table 4. Terrestrial invertebrate communities.

Variable studied	Treatments	Effects	Reference
Earthworm density and species composition	0, 450 and 900 g/ha	Earthworm density was about 50 % higher in treated plots. No effect on species composition.	Mele and Carter (1999)
Microarthropod density (Collembola and mites)	0, 900, 1800 and 3600 g/ha	No effects	Gómez and Sagardoy (1985)
Organic matter loss, nutrient loss and microarthropod density and structure	Glyphosate applied by dipping litterbags with 3 g dried leaves for 30 s into solutions of commercial formulation of 0, 0.72 and 7.2 % a.i. (control, 1 and 10 times recommended dose)	Decomposition rates in both treatments (1 and 10 times) were about 30 % slower. The total number of microarthropods were about five times higher in treated litterbags compared to controls. Changes in guild structure of the microarthropod communities	Hendrix and Parmerlee (1985)
Infectivity of entomopathogenic nematodes (*Steinernema carpocapsae*) toward the wax moth, *Galleria mellonella*	0 and 7320 g/ha to the surface of soil cores	No effects on nematode infectivity	Gibb and Buhler (1998)
Infectivity of entomopathogenic nematodes (*Steinernema feltiae*) toward the wax moth, *Galleria mellonella*	Nematodes were exposed to solutions of 0, 0.18, 1.8 and 18 ppm a.i. for 24, 72 or 120 hrs.	No effects on nematode infectivity	Forschler *et al.* (1990)
Population growth of the endoparasitic nematode, *Pratylenhus zeae*	0, 0.75 and 1.0 kg a.i./ha (pre-emergence spraying)	2 times higher population growth in treated compared to control plots	Patra and Ray (1987)
Infectivity and viability of entomopathogenic nematodes (*Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *S. feltiae*	Nematodes were exposed to solutions of 0, 140, 280, 560, 1120, 2240 and 4500 ppm a.i. for 72 hrs.	No effects	Rovesti *et al.* (1988), Rovesti and Deseö (1990)

Aquatic invertebrates – laboratory effects

Roorda *et al.* (1978) used as test organism a mite with potential for integrated control of water hyacinth, and tested the direct susceptibility to glyphosate by dipping for 2 minutes. After 24 hours there was no mortality of 250, 500 and 1000 ppm solutions of the formulated compound, and very slight effects after 48 hours.

Toxicity of Roundup to aquatic invertebrates and fish was rigorously tested by Folmar *et al.* (1979). This paper reports acute and chronic toxicity of 4 invertebrate and 4 fish species (several life stages), including avoidance studies and stream drift of *Chironomid* larvae in artificial streams. Also the modifying effects of temperature, pH, and ageing of the water solutions were tested. The authors used reconstituted water with a hardness of 40 mg/L as $CaCO_3$, and varied the pH between 6.5 and 9.5, and tested temperatures between 12 and 22 °C. The test design was constructed to reveal dose-response relationships. It should also be stressed that all components of Roundup, i.e. technical grade glyphosate, the isopropylamine salt of glyphosate, the Roundup surfactant, and the formulated Roundup were tested. Based on these results the authors concluded that Roundup, at recommended rates, should not adversely affect resident populations of fish or invertebrates. However, spring applications in lentic situations, where dissolved oxygen levels are low or temperatures are elevated, could be hazardous to young-of-the-year-fishes.

Henry *et al.* (1994) investigated the effects of glyphosate, a detergent used with glyphosate, and a drift retardant used for aerial application of glyphosate, to 4 invertebrate species. The survival of caged *Chironomus* sp. (midge), *Hyalella azteca* (amphipod), *Stagnicola elodes* (pond snail), and *Nephelopsis obscura* (leech) was assessed in prairie pothole wetlands treated by air. There was no difference in survival of caged invertebrates between treated and reference wetlands after 21 days.

Summary of aquatic invertebrates – laboratory effects

The conclusions of the studies listed for aquatic invertebrates should also apply to Danish conditions. Glyphosate is unlikely to have any negative direct effects on aquatic invertebrates, at least with the doses normally used in Denmark.

Aquatic invertebrate communities - Field effects

Kreutzweiser *et al.* (1989) reported on drift responses of stream invertebrates to aerial applications of glyphosate (Roundup) in the Canadian "Carnation Creek Project" (Table 5). Roundup was applied aerial for conifer release to parts of the watershed and research was conducted to investigate the fate and effects of the recommended dose herbicide on various components of the watershed. Drifting invertebrates were collected in nets placed mid stream pre and post spraying. The application of glyphosate on or adjacent to small tributaries of Carnation Creek did not result in undue disturbance of stream invertebrates. Drift densities of most aquatic invertebrates did not increase in response to the herbicide application. The drift response of only two organisms, *Gammarus* sp., and the mayfly, *Paraleptophlebia* sp., may suggest a slight and ephemeral herbicide induced disturbance in and downstream of the treated areas. This disruption of drift patterns may have resulted from natural causes, but was coincident with glyphosate contamination and therefore cannot be dismissed as being unrelated to the herbicide applications. The authors

argued that the peak residue values of glyphosate seemed to be at a substantial margin of safety from the reported effective concentrations of glyphosate. In a parallel study at the Carnation Creek watershed Scrivener and Carruthers (1989) monitored the changes in benthic macro-invertebrate populations. Their results suggested that any herbicide impact could be similar in magnitude to those obtained after logging, and that they would be most pronounced after periods of freshet.

Hildebrand et al. (1980) reports another of the few existing field studies. In this study effects of Roundup on populations of *Daphnia magna* in a forest pond was studied. The authors reported that even a 100 times field dose had no effect on survival rates after 2, 4 or 8 days. However, the study design was of so poor quality (the authors sprayed small areas of pond surface with no control of the actual concentration in the water volumes studied) that these results are hardly of any relevance, nor should they be used in a risk assessment.

Gardner and Grue (1996) reported on field experiments in which glyphosate (Rodeo) was applied at the recommended rate (1 l/ha). Acute toxicity to *Daphnia magna* was assessed using *in situ* toxicity tests. Effects on the aquatic community (benthic and pelagic) was also assessed using sediment cores and activity traps suspended in the water column. The authors concluded that use of Rodeo was not associated with any adverse effects on survival of *Daphnia* or any significant changes of the benthic or pelagic invertebrate communities. Solberg and Higgins (1993) also used activity traps to assess the effect of Rodeo (recommended dose) on aquatic invertebrates in cattail controlled wetlands. These authors reported lower densities of invertebrates in treated wetlands in comparison to untreated. However, they were unable to determine if the differences in invertebrate abundance were caused by direct mortality from the glyphosate treatment or to movements of some invertebrates from treated to untreated areas within wetlands.

Källqvist *et al.* (1994), using 20 m^3 mesocosms, studied the effect of glyphosate on phytoplankton communities and productivity's in an oligotrophic Norwegian lake. The results indicate that structural changes of phytoplankton communities can occur at environmentally realistic conditions (1-10 µg/l). Such effects could potentially influence the phytoplankton grazer community, such as crustaceans, rotifers and other invertebrate larvae. However, in parallel studies (Hessen *et al.* 1994) there was no support for a strong indirect effect of glyphosate on zooplankton due to food reductions. Community changes and species shifts in the phytoplankton community could, however, explain a negative effect on rotifers.

Simenstad *et al.* (1996) evaluated the potential effects of glyphosate on mudflat benthic invertebrate communities by aerial applying Rodeo to three mudflat sites in Washington State, USA. The very large body of data obtained was rigorously treated and the authors concluded that the mudflat invertebrates showed no definitive differences in population trends that would indicate acute responses to the herbicide application over the 119 day study period.

Summary of aquatic invertebrates - field effects

The studies of glyphosate effects in aquatic field experiments show that direct and indirect effects on invertebrates have generally not been found. This corresponds with the results of aquatic laboratory experiments. The studies in which glyphosate has

been used in the control of freshwater plant growth may serve as examples of toxicity. However, such application is not of relevance to Danish conditions. The experiments conducted in streams are probably of greatest relevance to Danish conditions.

Table 5. Aquatic invertebrate communities.

Variable studied	Treatments	Effects	Reference
Drift response of stream invertebrates	2.0 kg a.i./ha (aerial)	No effects	Kreutzweiser et al (1989)
Changes in benthic macro-invertebrate populations	2.0 kg a.i./ha (aerial)	No effects	Scrivener and Carruthers (1989)
Changes in mudflat benthic invertebrate populations	2.7 kg a.i./ha (aerial)	No effects	Simenstad et al. (1996)
Effects on the benthic and pelagic invertebrate communities	0 and 0.450 kg a.i./ha	No effects	Gardner and Grue (1996)
Effects on pelagic invertebrate community	0 and 1.26 kg a.i./ha (aerial)	No effects	Solberg and Higgins (1993)
Changes in the zooplankton density and community structure	0, 1, 10 and 100 µg/l (in 20 m^3 mesocosm)	No effect	Hessen et al. (1994)

PLANTS

Glyphosate is an effective systemic broad-spectrum herbicide, which directly affects specific biosynthetic steps by inhibition of enzymatic pathways. The mode of action and metabolism in plants is quite well known and has been reviewed by e.g. Carlisle and Trevors (1988) and Freedman (1990). The primary mode of action is by blocking the synthesis of all cinnamate derivatives by inhibiting the 5-enolpyruvyl shikimate-3-phosphate (EPSP) synthase by which the essential aromatic aminoacids phenylalanine, tyrosine and tryptophane are synthesized. Glyphosate may also to some degree affect other enzymatic pathways, see e.g. (Ganson and Jensen 1988). Inhibition of EPSP synthase leads to the accumulation of high levels of shikimate, benzoic acids and benzoic acid derivatives (Lydon and Duke 1988, Lydon and Duke 1989). Therefore even small doses of glyphosate not causing visible injury can be detected in plants due to the accumulation of for instance shikimic acid (Stasiak *et al.* 1991).

Uptake and bioaccumulation

Glyphosate is metabolised by a minority of plants, e.g. *Equisetum arvense* (Marshall *et al.* 1987). The main product is aminomethylphosphonic acid (AMPA) and a num-

ber of more or less unknown metabolites (Marshall *et al.* 1987). Glyphosate is transported and distributed within the plant like photoassimilates, except in water stressed plants where the translocation is reduced compared to photoassimilates. This also reduces the phototoxicity of glyphosate in water stressed conditions. Since gly-phosate is not degraded in most plants it remains available for transfer to organisms such as herbivores and decomposers.

Feng and Thompson (1990) investigated the persistence of glyphosate in foliage of red alder and salmonberry at Vancouver Island, British Columbia, Canada. They sprayed 2.0 kg a.i./ha by aerial spray using Roundup in a spray volume of 252 l/ha. Immedi-ately after treatment they found foliar residues of glyphosate ranging from 261 µg a.i./g for red alder and 447 µg a.i./g for salmonberry ($P<0.10$). Glyphosate residues in leaf litter declined as a logarithmic function of time since application in both red alder and salmonberry. Based on regression analysis ($R^2 = 0,75$ for red alder and $R^2 = 0,76$ for salmonberry) a DT_{50} value of 10 days and DT_{90} values of 32-35 days were deter-mined for both red alder and salmonberry.

The foliar absorption of glyphosate from soil has been found to be very limited. This was investigated by Al Khatib *et al.* (1992) in a container experiment with alfalfa and pea. They applied a dose of 3277 and 32770 ppb to sand/silt loam (mixture 1:1 by volume). Seven days after application of glyphosate containing soil 0.9% of the glyphosate was detected in the leaves. In this soil, glyphosate soil residues coming into contact with leaves is only to a very little extent taken up, even at soil concentrations higher than expected under normal environmental and agricultural conditions.

In aquatic plants the translocation from shoot to root of glyphosate was significantly lower in the submersed species sago pondweed *Potamogeton pectinatus*, than generally seen in terrestrial plants. Of the amount applied 4.2% was recovered from the roots after 14 days and totally 86.6 % was recovered from the whole plant (Marquis *et al.* 1981).

Toxicity

Glyphosate is highly toxic to plants (Table 4). The effect is highest when leaves are directly exposed, when the plant is growing, and at high temperatures (Masiunas and Weller 1988). From the leaves it is taken up and transferred to all parts of the plant. However, it can also be taken up from water and soil (Salazar and Appleby 1982 and Al Khatib *et al.* 1992). Such pathways are relevant when the glyphosate is present in these environments in significant amounts, which may happen if it is not immediately degraded or adsorbed in soil or reach surface water by runoff. Sublethal levels of glyphosate affect amino acid production and other effects associated with the shikimic acid pathway, photosynthesis or increased ethylene concentrations, see e.g. (Stasiak *et al.* 1992).

Tolerance/resistance

Several mechanisms may explain why plant species show differential susceptibility to glyphosate. Among these are differences in interception, retention, absorption and physiologically mediated tolerance (metabolism) between species or strains of

species. Physiologically mediated tolerance to glyphosate can be selected for in plants. This way callus from chicory *Cichorium intybus* was made 25 times more tolerant to glyphosate than callus from the wild type (Sellin *et al.* 1992). After 15 years of successful use for weed control Powles *et al.* (1998) reported resistance to glyphosate in a population of weedy rigid ryegrass *Lolium rigidum* in Australia. This population proved to be resistant to glyphosate in pot dose-response experiments conducted outdoors, exhibiting 7 - 11-fold resistance as compared to a susceptible population. The presence of glyphosate resistance in a major weed species indicates a need for resistant management strategies taking into account the development of herbicide-resistance in weeds (Powles *et al.* 1998, Heap Ian 1997).

Boerboom *et al.* (1990) investigated the mechanism of glyphosate tolerance in 9 clones of the weedy species Birdsfoot trefoil *Lotus corniculatus*. It was found that the activity of the EPSP synthase was positively correlated with the tolerance of the clones. This highly indicates that the primary mechanism of glyphosate tolerance in this species is based on the level of EPSP synthase activity (Boerboom *et al.* 1990).

Marquis *et al.* (1979) found that the glyphosate tolerance of red fescue was not due to metabolism, but rather was related to its ability to regenerate roots and shoots from the crown of the plant, although the precise mechanism of resistance remained obscure (Marquis *et al.* 1979).

Toxicity to terrestrial plants through soil

In greenhouse Salazar and Appleby (1982) studied the influence of glyphosate applied to the soil surface prior to emergence or germination to three plant species. They used four soil types ranging from mineral to highly organic soils and applied 3.4 kg a.i./ha to bentgrass (*Agrostis tenuis* Sibth. cv. Highland) and 1.0 and 3.0 kg a.i./ha to red clover (*Trifolium pratense* L. cv. Kenstar) and alfalfa (*Medicago sativa* L. cv. Vernal). Four replicates were sampled in a repeated randomised complete block design. Bentgrass emergence was inhibited between 51 and 95 % when glyphosate was applied directly to the soil surface of the highly organic soil up to 5 days prior to emergence. The reduction was smaller in mineral and less organic soils (maximum inhibition 52%). The effect of glyphosate was also examined by applying glyphosate (1.0 and 3.0 kg a.i./ha) to a moist soil surface and placing alfalfa and red clover seeds on the sprayed surface 3, 6, 9 and 24 h later. Germination of alfalfa was reduced by approximately 50% at the low dose and 90% at the high dose and only showing small differences between the various times of application. Red clover germination was reduced by ca. 40% at the high dose. The growth of both species were reduced at both concentrations and again alfalfa was the most sensitive species with dry weight yield reductions to 34% (low dose) and 5% (high dose) of the control. The corresponding values for red clover were 69% and 34%. Salazar and Appleby (1982) concluded that glyphosate, at least under certain conditions and in some species can cause significant crop injury when used prior to planting or emergence.

Table 6. LD_{50}, I_{50}, NOEC and LOEC values for different terrestrial and aquatic plants. Non agricultural species are presented with scientific names in Italics and crop species are presented with their popular name without paying attention to special strains or sorts. The information on concentration factors may refer to different plant parts, which can only be deducted by use of the references.

Species	EC_{50} a.i. (Growth)	EC_{50} a.i. (EPSPS)	NOEC a.i.	LOEC a.i.	LD_{50} a.i.	Author
Betula papyrifera			0.04 kg/ha			Stasiak et al. (1992)
Lemna minor	2.0 mg/l			10 mg/l		Hartman and Martin (1984)
Lotus corniculatus	0.5 – 1.5 kg/ha	6.3 – 10.6 µM				Boerboom et al. (1990)
Myriophyllum spicatum			<1mg/l			Christoper and Bird (1992)
Populus tremuloides			0.04 kg/ha			Stasiak et al. (1991)
Prunus pensylvanica			0.04 kg/ha			Stasiak et al. (1991)
Tobacco	314 ppm 1858 µM					Strube et al. (1991)
Sugarcane			0.1 kg/ha			Richard (1991)
Potato				0.28 kg/ha		Masiunas and Weller (1988)
Beans					2µg/plant	Rahe et al. (1990)
Soybean			<5µg/plant			Morandi (1989)
Mustard					1µg/plant	Rahe et al. (1990)
Apple					23µg/plant	Rahe et al. (1990)
Wheat					0.9µg/plant	Rah et al. (1990)
Corn					2.5µg/plant	Rahe et al. (1990)

In Australian greenhouse and field experiments in loamy sand soil it was investigated whether glyphosate residues damaged tomatoes transplanted to the soil after spraying with Roundup. In the greenhouse it was found that glyphosate soil residues caused visible injury and an average dry weight reduction of 57% to tomatoes transplanted 15 days after application of 1.5 kg a.i./hectare. In the field, reductions was found with

planting up to 16 days after application - and at 9 days reductions of 50, 74 and 78% were recorded with glyphosate (360 g a.i./l) applied at 2, 4 and 8 l/ha respectively. A delay period of three weeks after application of glyphosate before sowing or transplanting could be considered safe (Cornish 1992). Considering long-term effects of soil glyphosate residues, Bromilov *et al.* (1996) found no deleterious effects on crop production after 20 years of combined herbicide application including glyphosate on a silty clay loam soil in Rothamsted UK.

Reduced defence

Sub-lethal doses of glyphosate induce increased sensitivity to some fungal diseases, e.g. Bergvinson and Borden (1992), Johal and Rahe (1984). The use of such fungal glyphosate synergists can reduce the necessary doses of glyphosate (Sharon *et al.* 1992). This may both have positive and negative implications. In weedy species reduced defence to specific parasitic fungi may be an agricultural and environmental advantage, due to better crop protection and reduced use of glyphosate. On the other hand crop species and forest trees exposed to sublethal doses of glyphosate may also become more vulnerable to unwanted fungal attacks. In sterilised soil higher doses of glyphosate are necessary to kill plants than in non-sterilised soils, because soil fungi attack and kill plants weakened by glyphosate, i.e., glyphosate increases the susceptibility of plants to fungal diseases (Johal and Rahe 1984). Rahe *et al.* (1990) found that the fungal genera *Pythium* and *Fusarium spp.* were the major glyphosate synergists of wheat and bean seedlings. Sharon *et al.* (1992) have shown that the defence reduction is directly associated with the influence of glyphosate on the shikimate pathway by suppression of a phytoalexine produced in the weedy legume *Cassia obtusifolia* as a defence response. This influence may prove useful in the production of selective mycoherbicides (Sharon *et al.* 1992).

Bergvinson and Borden (1992) found in three experiments that the lesions caused by the blue stain fungus *Ophiostoma clavigerum* were more severe in lodgepole pines *Pinus contorta* when these were pre-treated with glyphosate prior to the inoculation with the fungus. This was observed in an 80 year old stand of lodgepole pine *Pinus contorta* var. *latifolia* Engelm. 40 km north-east of Princeton, British Columbia. The dose was 1 ml of undiluted Roundup (360 mg a.i. /ml) administered to drilled holes just above the root collar with distilled water as controls. In the first experiment trees were treated the 15. of July, inoculated 3. of August and 4 weeks later the width and length of the lesions around the point of inoculation were 1.3 and 15.8 cm in the control and 1.7 and 27.9 cm in the treated. In the second experiment trees were treated the 19. of August, inoculated 25. of August and 4 weeks later the width and length of the lesions around the point of inoculation were 0.7 and 10.2 cm in the control and 0.9 and 21.4 cm in the treated. The mean vertical spread of fungal hyphae was investigated in the third experiment. In glyphosate treated trees *O. clavigerum* was spread vertically at more than 7 times the rate of the control, when measured four weeks after inoculation. As glyphosate inhibits the shikimic acid pathway and thereby the production of phenolic precursors used for plant defence, the impact of fungal infections is increased. This again increase effects of insect attacks, in this case the mountain pine beetle *Dendroctonus ponderosae* which is symbiotic with the blue stain fungus and like in the Dutch Elm disease the beetle is dependent upon the fungus weakening the tree (Bergvinson and Borden 1992). This experiment demonstrates that glyphosate increases the sensitivity to fungal attacks. However, the

concentration used and mode of application is not representative to an exposure likely to take place in association with use of glyphosate in forestry.

Plant community studies

In forestry glyphosate is used for control of competing vegetation especially when used in association with establishment of new cultures on clearcut areas. Often the event of clearcutting is more radical to the understorey vegetation than is the application of herbicides. Lund-Høie and Grønvold (1987) found that vegetation changes in such areas for most species lasted less than three years. In four species (20%) the decrease in coverage persisted after three years and 14 species (70%) showed an increased or unaltered cover after three years. Four of these were stimulated or unaffected even in the first year after application of glyphosate (Lund Høie and Grønvold 1987). In a comparison of the diversity in glyphosate treated and hand cutted clearcut areas the diversity of understorey plants was the highest in the glyphosate treated areas (Lund Høie and Grønvold 1987). This increase is explained by the resistance of some species to glyphosate (*Vaccinium* spp), especially *V. vitis-idaea* (Lund-Høie 1975) and to some extent *Vaccinium myrtillus* (Hoel 1984), as well as the increased possibilities for annuals *(Galeopsis tetrahit* and *Melampyrum pratense*) to establish where light- and root competition from deciduous trees (*Betula* and *Sorbus*) are eliminated (Lund Høie and Grønvold 1987).

In another study it was found that use of glyphosate in association with clearcuts in coniferous forests in 1-2 years term reduced the diversity of herb species, whereas in the 5 year term there was no difference between treatment and control blocks (Sullivan *et al.* 1996). Although not significantly different the shrubs tended to be affected in the treatment plots also in the longer term. It should be noted that these studies concerns regeneration of intensively managed coniferous forest and that they involve few replicates and treatments. In many studies grasses were not identified to species and only little information on fate or ecology (original forest species or weedy species) of single species were provided.

In sub-boreal spruce forests where willows *Salix spp.* are important as winter forage for ruminants control of willow may be performed by glyphosate. Pollack *et al.* (1990) found that glyphosate (2.1 kg a.i./ha) affected willows at least two years after application. In a Norwegian study, Hoel (1984) found that the deciduous tree species were still affected four years after application and that this part of the "weedy vegetation" was the most severely affected by normal application* of Roundup in forestry [* probably around 2 kg a.i./ha in clearcut areas in connection with establishment of conifer plantations.]. In such areas caution should be taken by choosing lesser application rates and/or later days of application to protect the larger herbivores in the long term. Kelpsas and Pfund (1990) found that control of grass with glyphosate in red cedar *Thuja plicata* forest may cause crown kill (10%) and growth reduction (25%) to seedlings if they are exposed. The rate of application was specified to 1 lb/A (pound per acre).

The removal of understorey vegetation by 1.75% glyphosate solutions (10 l/ha a.i.) was found to influence the soil temperature and organic matter content in the following period, whereas soil temperature was increased, soil organic matter content showed a decrease (Aust and Lea 1991). Probably the influence is mainly due to the removal of the vegetation.

Marrs *et al.* (1993) reports results from four bioassays, where seedlings grown in trays were exposed downwind of glyphosate applications (2.2 kg a.i./ha). Three experiments were done with *Lychnis flos-cuculi* seedlings including one with different surrounding grass structures, and the fourth tested the response of 15 species typical of semi-natural vegetation. The mortality of *Lychnis flos-cuculi* varied between experiments and appeared more or less unaffected by grassland structure, except immediately downwind of the sprayer. The multi-species experiment indicated a wide senstivity to spray drift, and one species was affected between 15 and 20 m downwind. Thus, seedlings of some species were affected at greater distances than established plants, indicating either greater capture of drift or a greater sensitivity.

Summary of toxicity to terrestrial plants

The above investigations treating the toxicity of glyphosate to plants in the terrestrial environment confirms that glyphosate is an effective herbicide when leaves are directly exposed. Glyphosate from soil containing glyphosate coming in to contact with leaves is only to a very little extent taken up even at doses higher than expected under normal environmental and agricultural conditions. Plants have a capacity to become tolerant to glyphosate but development of tolerance under natural conditions has only been observed very rarely. Glyphosate residues in soil can influence emergence, germination and production of plants sown or transplanted to soils, which recently (less than three weeks) have been treated with glyphosate in concentrations ranging from 0,7 – 3.0 kg a.i. /ha. There is no evidence of accumulated harmful effects to plants due to long-term normal agricultural application of glyphosate. There are no Danish investigations included in the above studies but in most cases concentration ranges and soil types included are relevant to Danish practice and conditions.

Toxicity to aquatic plants through water

The growth of safflower *Carthamus tinctorius* was reduced by 50% at a glyphosate concentration of about 0.6 – 1.2 g a.i./m^3(Bowmer *et al.* 1986). The phytotoxicity was not significantly reduced by adsorption of glyphosate onto suspended solids.

Hartman and Martin (1984) found that duckweed *Lemna minor* was less affected by glyphosate when a suspension of bentonite was present in the water to which glyphosate was added. With bentonite the otherwise lethal concentration (Table 6) only caused a reduction of less than 5%.

MICROORGANISMS AND ALGAE

Bioaccumulation

No information on bioaccumulation of glyphosate in microorganisms was found. However, many microorganisms in soil and water degrade glyphosate under both

aerobic and anaerobic conditions, hence it is not likely that glyphosate is subject to microbial bioaccumulation. Jacob *et al.* (1988) found that a specific strain of the soil bacteria *Pseudomonas* was able to eliminate 20 mM glyphosate an amount approximately 20-fold greater than that reported for any other microorganism at that time. The main product was aminomethylphosphonate, which was only to a small extend further degraded. Hallas *et al.* (1992) found that bacteria effectively (> 90%) degraded glyphosate (50 mg/l) in industrial wastewater (Hallas *et al.* 1992).

Toxicity to soil microorganisms

Few studies on field effects of glyphosate or Roundup and AMPA on soil microbial population have been reported.

Fungi

Garabito Garcia and Sandoval (1991) compared the recovery of soil microbial populations in three alkaline agricultural soils (Feozem, Castanozem and Chernozem) with pH approximately 7.5. The mean annual temperature was 26°C and the precipitation was 485 mm. They applied 6.01 L/ha of the formulation "Faena" (probably equal to approximately 2.2 kg a.i./ha). Studying bacteria, fungi and actinomycetes they found a difference between soil types and organism types. Actinomycete population size decreased significantly to app. 10% of the start value in all three soil types. The influence of the soil type apparently rather influenced the time at which the maximum decrease took place rather than it influenced the magnitude of the decrease. Bacteria and fungi were affected to a lesser and not significant extent than actinomycetes. Dependent on soil type both inhibition (to app. 30% of start value) and small stimulation was observed. In all cases recovery took place after approximately 6 weeks.

Abdel-Mallek *et al.* (1994) studied the inhibition of oxygen respiration and soil fungi count by glyphosate in Egyptian soil (Clay soil, pH 6.8, 3.07% organic matter, 1,2% total soluble salts). They applied 1.84 and 9.2 mg a.i./kg dry soil and studied the soil fungi (in duplicates) after 2, 4, 6, 8 and 10 weeks. They found that the rate of decay of three plant species was influenced by the above- mentioned concentrations, both stimulatory and inhibitory effects were revealed. The soil oxygen respiration was inhibited after 6 weeks at both concentrations. This reduction was 50 % or more. Compared to the control the count of soil fungi was reduced to 58% at the low dose and 68% at the high dose after 6 weeks and 68% at the low dose and 66% at the high dose after 10 weeks. These reductions were due to losses in the count of three species. In the 8.th week no statistically significant differences between treatment and controls were observed. The finding of effects at a concentration of 1.84 ppm be weight could indicate special circumstances, e.g. soil type. However, the description of this Egyptian soil only indicate a typical clay soil, but naturally it should be taken into account that the climate in Egypt is warmer than in Denmark.

Often glyphosate used as recommended will have no deleterious effects on soil microbial population (Gomez and Sagardoy 1985, Stratton and Stewart 1992, Heinonen Tanski et al. 1985). Wardle and Parkinson (1991) found no significant effects of glyphosate (field, and field dose). Soil microbial biomass, soil basal respiration, substrate-induced respiration (SIR), the ratio of the two latter and also the

inhibition of SIR by streptomycine or actidione were unaffected by glyphosate in a 45 day study. In the laboratory it was found that glyphosate was able to shift the directions of two species interactions among fungi (Wardle and Parkinson 1992).

In *in vitro* tests inhibition of soil fungi has often been observed (Chakravarty and Chatarpaul 1990; Chakravarty and Chatarpaul 1990; Sidhu and Chakravarty 1990; Estok et al. 1989; Wan et al. 1998; Powell and Bagyaraj 1984; Beyrle et al. ;1995; Bode et al. 1984; Mietkiwski et al. 1997; Rush and Gerik 989 and Kassaby and Hepworth 1987). The explanatory value of these for field effects is limited apart from providing an indication of a differential response among different species of fungi.

Chakravarty and Chatarpaul (1990) found no effects of glyphosate formulation (0.54 and 3.23 kg a.i. ha) on the ectomyorhiza formation of *Paxillus involutus*. After two months the count of fungi and bacteria was significantly lower at the 0,54 kg a.i./ha. The reductions were to 52% and 56% of control for fungi and bacteria respectively.

Bacteria

Some general physiological characteristics concerning bacteria have been found using laboratory cultures. Some bacteria possess aromatic amino acid biosynthese systems, which are sensitive to glyphosate (Fischer *et al.* 1986, Roisch and Lingens 1980). Exposed to glyphosate such bacteria will display growth inhibition, altered levels of aromatic-pathway enzymes and accumulation of early pathway metabolites (Fischer *et al.* 1986). Barton *et al.* (1982) found that another of the mechanisms involved in the toxicity of glyphosate to bacteria was an inhibition of the transport of both Fe^{2+} and Fe^{3+}. Apparently the ability to directly cleave the C-P bond is most abundant in gram-negative bacteria (Quinn *et al.* 1989) but also rarely found in gram-positive (Pipke *et al.* 1987, and Pipke *et al.* 1988). Krzysko Lupicka *et al.* (1997) found that most soil borne bacteria were unable to grow on glyphosate (10 mM), i.e. they did not degrade it. In their study three out of 26 strains isolated from soil were able to degrade glyphosate and AMPA, whereas glyphosate inhibited the growth of five strains. Quinn *et al.* (1988) found no evidence of glyphosate metabolism in glyphosate treated soil and that inhibition due to glyphosate could be partially reversed by addition of the end products of the aromatic amino acid biosynthetic pathway. However, all isolates were able to grow in the presence of 10 mM glyphosate without these supplements (Quinn *et al.* 1988).

Gomez and Sagardoy (1985) investigated the response of soil bacteria to Roundup application (0, 2, 4, 8, 40 l/ha) in a sandy soil. Only at the highest concentration which is ~10 times commonly recommended application rates they found a reduction in the number of *Acinetobacter*. From their experiments it can be stated that normal use of Roundup on a sandy soil is not likely to produce any significant alteration of that part of the soil ecosystem.

Martensson (1992) found that the ability of *Rhizobium* bacteria *in vitro* to induce nodules in leguminous plants was reduced in those previously exposed to glyphosate (10 mg a.i./l). Nodulation in treated plants ranged between 55 and 69% of control plants. This was especially shown for the ability of *Rhizobium trifolium* to induce nodulation in *Trifolium subterraneum* (Eberbach and Douglas 1989). However, Liu *et al.* (1991) found that many bacteria belonging to the *Rhizobiacea* were able to degrade glyphosate by a C-P lyase activity.

Table 7. Influence of the isopropylamine salt of glyphosate on some soil microbial processes.

	EC_{50}	EC_{90}	NOEC	Reference
Nitrification	1435 to 2920 ppm a.i.			Stratton (1990)
Nitrogenase activity	13 – 19 kg formulation/ha	>20 kg f./ha	0.2 – 4 kg f./ha	Santos and Flores (1995)
Respiration O_2	18 – 26 kg formulation/ha	>20 kg f./ha	0.2 – 4 kg f./ha	Santos and Flores (1995)

Kahru *et al.* (1996) analysed the toxicity of pure glyphosate to *Photobacterium phosphoreum* by use of BIOTOX™ test and found that EC_{50} and EC_{20} were 2.19 and 0.75 mg a.i./l respectively. They also compared the BIOTOX™ EC_{50} with a result obtained by use of MICROTOX™ which was 3.5 times higher (EC_{50} = 7.7 mg a.i./l) (Chang *et al.* 1981). Mixtures of herbicides including glyphosate showed both synergistic and antagonistic effects (Kahru *et al.* 1996).

In three species of bacteria possessing EPSP synthase Fischer *et al.* (1986) found NOEC values for glyphosate concentration in the growth media for *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* of 2.5 mM, 2.0mM and 2.0mM respectively.

Algae

Heinonen Tanski *et al.* (1985) found that the soil microbial population was practically unaffected by glyphosate at an application rate of 1.4 kg a.i./ha, except for algae, which showed a reduction trend in soil chlorophyll concentration in the late summer (21%) and autumn (43%) ($P<0.25$). The soil was fine sand with 11% clay, 2.5% organic matter and $pH_{(H2O)}$ was 5.6. The mineral contents were Ca 640; K 115; P 7.6 and Mg 50 (mg/l).

Maule and Wright (1984) tested the toxicity of glyphosate on a number of green algae and found that *Chlorococcum hypnosporum* was the most sensitive and *Chlorella pyrenoidosa* was the least sensitive measured by EC_{50} values. They conclude that glyphosate used as recommended will result in concentrations in the top-soil in the range of 1 – 4 ppm which is not likely to affect soil microalgae adversely.

Soil microorganism ecosystem studies

Microbial processes covers a number of different physiological activities including respiration, nitrogen metabolism, phosphorous mineralization, organic matter decomposition, enzymatic activities etc. which are all possible as objectives of effect studies of glyphosate on soil organisms. Generally the functioning of the soil microbial community is unaffected or even slightly stimulated by Roundup treatment

(Malkomes 1988, Wardle and Parkinson 1990). Repeated treatment can increase the degradation rate due to adaptation by the community to use glyphosate as a source of C, N and P (Dick and Quinn 1995). The adaptation can either be by changes in the composition of the community due alterations in species interactions (Wardle and Parkinson 1992), or by properties of the species to use glyphosate as a source (Dick and Quinn 1995).

In clearcut coniferous forest glyphosate (1.7 kg a.i./ha) stimulated the soil microbial biomass in the litter layer, whereas it had no effects in the underlying clay loam soil (Stratton and Stewart 1992). At field rates neither respiration nor number of bacteria, actinomycetes and fungi were affected. Rates 10 and 100 times the field application rate had a stimulatory effect on respiration in the underlying clay loam soil. It is concluded that glyphosate have no deleterious effects on soil microbial biomass and respiration when used under recommended conditions (Stratton and Stewart 1992).

Chakravarty and Chatarpaul (1990) found by adding glyphosate as the active ingredient in a formulation (0.54 and 3.23 kg a.i./ha) that it did not reduce soil microbial popu-lation or carbon dioxide formation in the long term (6 months) though a short term effect was seen.

Roslycky (1982) investigated the response of soil microbiota to glyphosate in 7 concentrations ranging from 0 to 1000 µg a.i./ml. Response curves were produced for number and O_2 respiration of fungi bacteria and actinomycetes. It was found that low concentrations (1 – 50 µg/ml) of glyphosate had little effect on the population size whereas the highest concentration (1000µg ml^{-1}) initially increased the populations of the two latter organism groups. High doses (1000µg/ml) suppressed the O_2 respiration by the microbiota (Roslycky 1982).

Most studies are typically covering a range of time from hours to weeks. Nicholson and Hirsch (1998) reports from a long-term study ca. 20 years that the pesticide applica-tion (including glyphosate since 1980) showed very small differences in the bacterial populations between treated and untreated plots. There were consistently higher num-bers in the treated plots, probably indicating the higher yield in these plots (Nicholson and Hirsch 1998).

Summary of the toxicity to soil microorganisms

In conclusion, both stimulatory and inhibitory effects of Roundup have been observed on soil microorganisms. Soil microalgae have not been found to be sensitive to normal agricultural use of glyphosate. In one study no effects on species level was observed, which is not in accordance with numerous *in vitro* tests. In another the reduction in the count of soil fungi could be identified to reductions in the count of three identified species or genera. Glyphosate can influence soil fungal community structure at concentrations as low as 1.84 ppm soil weight, but normally effects will only be observed at higher concentrations. Soil microbial population recovery times after exposure to glyphosate are typically a few weeks. The soil type in the Finnish study, which found effects on soil microalgae, appears representative of Danish sandy soils, whereas the climate probably is colder than the Danish.

Toxicity to aquatic microorganisms

Chan and Leung (1986) studied the effect of glyphosate (Roundup) on growth, respiration and enzyme activity in two species of aquatic bacteria in three experimental pools at the Lam Tseun River in Hong Kong. The surface areas were 25, 23 and 21 m^2 and mean depths were 0.8, 0.6 and 0.54 m, respectively. All pools had similar surroundings and supported abundant growth of water plants, alga genera. The number of colony forming units in a pond with initial 200 ppm a.i. glyphosate decreased from around $2\text{-}3*10^6$ ml^{-1} to a minimum of $1*10^3$ ml^{-1}. After six days at this minimum the bacterial population started to build up again.

The yield of the culture showed an EC_{90} for *Aeromonas hydrophila* of 1500 ppm glyphosate, whereas this concentration was 100% lethal to another aquatic bacteria *Pseudomonas chlororaphis.* The latter was strongly inhibited (~20% of control) at 50 ppm. The respiration and the dehydrogenase activity of *Aeromonas hydrophila* were not significantly inhibited by glyphosate at all the tested concentrations (0, 200, 800, 1500 ppm) whereas *Pseudomonas chlororaphis* was inhibited by the two highest concentrations. The protease activity was affected in both species, though most in *Pseudomonas chlororaphis.* The EPSPS activity was affected in both species .The different sensitivity of the two species tested, especially the inhibition of *P. chlororaphis* at 50 ppm indicates that the structure of the aquatic microflora community should be taken into account when considering safety margins.

In a mesocosm experiment Källquist *et al.* (1994) studied the effects of glyphosate on lake phytoplankton community structure at environmentally realistic concentrations (1 – 10 µg glyphosate/l). The study site was an oligotrophic Norwegian lake (total P = 7 mg/l, total N = 200 mg/l, Secchi depth = 6 m), medium rich in calcium (Ca = 19 mg/l) and slightly alkaline (pH between 7.5 and 8.3). The study was performed in enclosures of 4m depth and 2.5 m diameter. They were situated in Lake Omdalsvatn, 50 km north of Oslo. Five enclosures were used in the glyphosate part of the experiment. Two served as controls and to the remaining glyphosate were added in three concentrations (1µg/l, 10µg/l and 100µg/l). The Shannon-Wiener diversity index, which combines the species richness and the evenness in distribution of the individuals amongst the species, decreased signi-ficantly ($P<0.05$) from a stable range of values between 2.69 and 2.76 in the controls to 2.3 after two days in both of the two lower treatment concentrations. At the high concentration, which is of less interest when considering environmental realistic concentrations, similar effects as well as more profound effects on single species level could be observed. After 13 days the diversity in the treatment enclosures was still lower than in the control enclosures.

A large number of *in vitro* tests have been performed using aquatic microalgae and EC_{50}-values between 1 and 1100 ppm has been found for different species (Gardner et al 1997; Anton et al, 1993; Maule and Wright 1984, Christy et al. 1981; Saenz et al. 1997; Bozeman et al. 1989; Abdel-Hamid 1996). In *in vitro* tests Saenz *et al.* (1997) found that there was a difference in sensitivity between species for technical grade glyphosate, which was not found when the formulation (Ron-do) was used. For the green algae species *Scenedesmus quadricauda* they found that the use of Ron-do in the aquatic environment cause harmful effects on long-term development of the population. For *S. acutus* long term harmful effects to the population was not indicated.

The edaphic microalgae biomass (measured by chlorophyll a) of seawater was neither in the short term nor in the long term affected when 4.7 l RODEO/ ha* was used to control smooth cordgrass *Spartina alterniflora* in an estuary (Simenstad *et al.* 1996). [* this probably equals approximately 1.7 kg glyphosate a.i./ha.]

Summary of toxicity to aquatic microorganism

In conclusion aquatic microorganism show some sensitivity to environmental realistic concentrations of glyphosate at least in the short term. *In vitro* tests show that the response of different species to glyphosate measured by EC_{50} covers three orders of magnitude. The Norwegian lake study is of relevance to oligotrophic Danish lakes, however, the climate is different, which weakens the relevance.

SUMMARY

Fate in soil

Glyphosate dissipate with an initial fast decomposition followed by a slower dissipation rate. Due to the slower dissipation of the bound glyphosate the DT_{90} values much longer than anticipated from DT_{50}. Field dissipation times found was mostly below 60 days but was in some cases several months or years, for example, with an 8% dissipation over 287 days. Soils with low microbial activity seem to have much longer glyphosate dissipations times than soils with high microbial activity. Most field studies found showed little mobility with most glyphosate present in the top 15 cm. There were very few studie performed with Danish soils. For the field studies concentration ranges and soil types were relevant for Danish practice and conditions, although in most cases the precipitation was much higher than normal for Danish conditions.

Fate in water

The fate of glyphosate in aquatic ecosystems is much less studied, in particular in marine systems. The main metabolic pathway is probably microbial-mediated, but photochemical decomposition may also play a significant role. In freshwater pond systems glyphosate usually dissipate from the water phase within days to weeks and adsorbs to the sediment. In the sediment glyphosate probably experience a fate similar to that in soil, although this is much less studied. In running water less glyphosate binds to the sediment and may be transported more than 14 kilometres. Very little information is present on fate of glyphosate in seawater. Few of the reported studies were performed in natural water systems. None of the above field studies were performed with Danish water systems but the concentration ranges, exposure regimes and flow rates were relevant for Danish practice and conditions.

Toxicity to terrestrial invertebrates

Most of the studies listed have been performed under climatic and pedological conditions comparable to Danish conditions, and indicate that direct, deleterious effects of glyphosate on terrestrial invertebrates are unlikely to occur due to normal Danish agricultural practise.The field studies of glyphosate effects on terrestrial invertebrates indicate that direct toxic effects are unlikely to occur under Danish conditions. However, at least three studies [Asteraki *et al.* (1992), Mele and Carter (1999), Hendrix and Parmerlee (1985)] suggest that indirect effects of glyphosate (or other herbicides) may occur through the herbicidal effect on plant cover and altered microfloral species composition. The studies mentioned are relevant for Danish climatic and pedological conditions.

Toxicity to aquatic invertebrates

The conclusions of the studies listed for aquatic invertebrates should also apply to Danish conditions. Glyphosate is unlikely to have any negative direct effects on aquatic invertebrates, at least with the doses normally used in Denmark. The studies

of glyphosate effects in aquatic field experiments show that direct and indirect effects on invertebrates have generally not been found. This corresponds with the results of aquatic laboratory experiments. The studies in which glyphosate has been used in the control of freshwater plant growth may serve as examples of toxicity. However, such application is not of relevance to Danish conditions. The experiments conducted in streams are probably of greatest relevance to Danish conditions.

Toxicity to terrestrial plants

The investigations treating the toxicity of glyphosate to plants in the terrestrial environment confirms that glyphosate is an effective herbicide when leaves are directly exposed. Glyphosate from soil containing glyphosate coming in to contact with leaves is only to a very little extent taken up even at doses higher than expected under normal environmental and agricultural conditions. Plants have a capacity to become tolerant to glyphosate but development of tolerance under natural conditions has only been observed very rarely. Glyphosate residues in soil can influence emergence, germination and production of plants sown or transplanted to soils, which recently (less than three weeks) have been treated with glyphosate in concentrations ranging from 0,7 – 3.0 kg a.i. /ha. There is no evidence of accumulated harmful effects to plants due to long-term normal agricultural application of glyphosate. There are no Danish investigations included in the above studies but in most cases concentration ranges and soil types included are relevant to Danish practice and conditions.

Toxicity to soil microorganisms

Both stimulatory and inhibitory effects of Roundup have been observed on soil microorganisms. Soil microalgae have not been found to be sensitive to normal agricultural use of glyphosate. In one study no effects on species level was observed, which is not in accordance with numerous *in vitro* tests. In another the reduction in the count of soil fungi could be identified to reductions in the count of three identified species or genera. Glyphosate can influence soil fungal community structure at concentrations as low as 1.84 ppm soil weight, but normally effects will only be observed at higher concentrations. Soil microbial population recovery times after exposure to glyphosate are typically a few weeks. The soil type in the Finnish study, which found effects on soil microalgae, appears representative of Danish sandy soils, whereas the climate probably is colder than the Danish.

Toxicity to aquatic microorganism

Aquatic microorganism show some sensitivity to environmental realistic concentrations of glyphosate at least in the short term. *In vitro* tests show that the response of different species to glyphosate measured by EC_{50} covers three orders of magnitude. The Norwegian lake study is of relevance to oligotrophic Danish lakes, however, the climate is different, which weakens the relevance.

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