Aquatic ecotoxicity of glyphosate and formulated products containing glyphosate

Introduction

An estimation of the ecotoxicological impact of herbicides in surface water often is based on laboratory toxicity tests with standard and/or additional aquatic test species. Toxicity data of glyphosate, or any of the formulated products containing glyphosate, are usually expressed either as a concentration causing a specific effect (e.g. death or growth) in 50% of the tested organisms (Lethal Concentration or Effect Concentration, LC50 or EC50) or as the highest concentration tested not showing an effect compared to controls (No Observed Effect Concentration, NOEC). In this report all short term (acute) toxicity data are given as LC50/EC50's, whereas longer term (chronic) toxicity data are given as a NOEC.

In toxicity tests the compound can be administered as the technical product glyphosate, in the form of an acid. However, often a salt of glyphosate (e.g. the sodium salt or the isopropylamine salt = IPA salt) is used. Moreover, tests can be performed with a formulated product (e.g. Roundup; Vision) which usually contains a salt of glyphosate alongside one or more adjuvants which are added to increase the efficacy of the product.

The result (LC50/EC50 or NOEC) of a toxicity test often reflects the type of compound or formulation used in the test, and is e.g. given as mg/L of the acid compound, mg/L of the active ingredient (often a salt of glyphosate), or mg/L of the formulated product used in the test. Because of differences in molecular weight between the various glyphosate compounds and possible differences with regard to glyphosate content of formulated products, results for different compounds and formulations are sometimes difficult to compare. For this reason we converted all toxicity values into the same units, based on the molecular weight of the acid form of glyphosate (169.1). These 'acid equivalents' (= a.e.) constitute an unambiguous measure of the amount of glyphosate present, simply assuming that all glyphosate is present in the acid form.

Glyphosate is transformed in the environment into a number of substances, among which the metabolite aminomethylphosphonic acid (AMPA) is the most common. For reference, in this report some toxicity values are given for AMPA as well, expressed as mg AMPA/L.

The summary of toxicity data given in this report is not intended to be exhaustive but sufficient to get insight in the potential impact of glyphosate and/or its main formulated products on aquatic organisms. Moreover, we excluded toxicity data considered to be less reliable, e.g. because test duration was not given.

Comparison of toxicity data between glyphosate acid, the IPA salt of glyphosate and Roundup.

An important question at stake is to what extent the ecotoxicological effects of glyphosate are dependent on the type of compound or formulation used. For some of the tested species laboratory toxicity data are available for different forms of glyphosate, i.e. tested as the acid, the isopropylammonium (IPA) salt of glyphosate or as a formulated product Roundup. This enables a direct comparison between the toxicities of the various forms used. Acute and chronic toxicity data are treated separately.

Both for acute (Table 1) and chronic toxicity data (Table 2) the toxicity of glyphosate acid and the IPA salt of glyphosate are more or less similar, with the possible exception of the alga *Skeletonema costatum*, the fish *Oncorhynchus mykiss* and tadpoles of *Litoria morei*, where the IPA salt is at least four times less acutely toxic than the acid form of glyphosate. Overall, for the majority of species mentioned in Tables 1 and 2, Roundup appears to be more toxic than either the acid form of glyphosate or its IPA salt.

Table 1: Acute toxicity data for aquatic test species that allow a comparison between glyphosate acid, isopropylamine (IPA) salt and Roundup. All toxicity values are expressed as mg acid equivalents/L. Where several L(E)C50 values for a species are available the geometric mean (and range) values are given.

Species	**LC50 acid (mg a.e./L)**	**LC50 IPA salt (mg a.e./L)**	**LC50 Roundup (mg a.e./L)**
Algae			
Chlorella pyrenoidosa	45 (3.5 – 590)		59
Desmodesmus subspicatus		54	
Pseudokirchneriella subcapitata	44 (14 – 485)	41	1.5 (0.7 – 5.8)
Skeletonema costatum	1.2 (0.6 – 2.3)	5.9	1.8
Macrophytes			
Lemna gibba	17 (10 – 25)		6.2 (4.7 – 8.2)
Myriophyllum sibiricum	1.6		1.0 (0.8 – 1.2)
Invertebrates			
Acartia tonsa	35	49	1.8
Ceriodaphnia dubia	147	415	5.4
Chironomus plumosus		55	18
Daphnia magna	780	930	4.5 (3 – 7.4)
Fish			
Cyprinus carpio	620		4.9 (3.1 – 8.1)
Lepomis macrochirus[a]	120	180	4.6 (1.8 – 11)
Oncorhynchus gorbuscha[b]	23		4.3
Oncorhynchus keta[b]	22		3.4
Oncorhynchus kisutch[b]	36		6.8
Oncorhynchus tshawytsha[b]	30		7.2 (6.2 – 8.4)
Oncorhynchus mykiss[b]	22	180	6.7 (2.5 – 16)

Ictalurus punctatus		130	12 (12 – 13)
Pimephales promelas		97	4.0 (2.3 – 7.1)
Amphibians			
Litoria morei, tadpole	103 (81 – 121)	> 466	5.2 (2.5 – 10)
Heleioporus eyrei, tadpole	> 373		5.4
Crinia insignifera, adult	78		36 (30 – 42)

[a] Values given as 'smaller than' or 'larger than' were excluded.
[b] Where more values were given in a study (e.g. for separate tests in soft and hard water) the lowest value was taken.

Table 2: Chronic toxicity data for aquatic test species that alow a comparison between glyphosate acid, isopropylamine (IPA) salt and/or Roundup. All toxicity values are expressed as mg acid equivalents/L. Where several NOEC values for a species are available the geometric mean (and range) values are given.

Species	**NOEC acid (mg a.e./L)**	**NOEC IPA salt (mg a.e./L)**	**NOEC Roundup (mg a.e./L)**
Algae			
Pseudokirchneriella subcapitata	22 (11 – 45)		0.23
Invertebrates			
Daphnia magna	71 (50 – 100)		1.0
Fish			
Oncorhynchus mykiss	52		0.74

The increased toxicity of the Roundup (and Vision) formulation is commonly attributed to the adjuvants used in this product (polyoxyethylene amine or POEA). Some toxicity data for the adjuvant POEA, trade name MON 0818, can be found in Annex 1. Consequently, toxicity data for a glyphosate formulation containing POEA will provide a worst case ecotoxicological effect characterisation of glyphosate. This may be particularly relevant when assessing the acute risks of spray drift applications of Roundup (or Vision), since under these circumstances the aquatic organisms at risk are simultaneously exposed to both glyphosate and POEA. Whether this is also the case when addressing chronic risks, or the acute risks due to drainage emission, will depend on the fate characteristics of both glyphosate and the adjuvant.

Ecotoxicological effect characterisation for different taxonomic groups

As might be expected for a herbicide it appears that primary producers (algae and macrophytes) are amongst the most sensitive test species for glyphosate acid, the IPA salt and Roundup (see Tables 1 and 2 and Annex 1). The most sensitive species reported for glyphosate acid is the diatom *Skeletonema costatum* (geomean EC50 of 1.2 mg a.e./L; NOEC of 0.28 mg a.e./L). For the IPA salt of glysophate this again is the diatom *Skeletonema costatum* (EC50 of 5.9 mg a.e./L; no NOEC value available). The lowest toxicity values reported for

Roundup concern the EC50 of 0.33 mg a.e./L for the aquatic vascular plant *Myriophyllum spicatum* and the NOEC of 0.23 mg a.e./L for the green alga *Pseudokirchneriella subcapitata*. However, for the formulated product Roundup also relatively low L(E)C50 and NOEC values for some animal species are reported (e.g. the LC50 of 1.6 mg a.e./L for larvae the of the amphibian *Rana clamitans* and the NOEC of 0.74 mg a.e./L for the fish *Oncorhynchus mykiss*). Also for the formulated product Vision relatively low LC50's for some amphibians are reported (e.g. the LC50 of 0.88 mg a.e./L for larvae of *Xenopis laevis*).

As can be seen in the data presented in Annex 1, for the formulated products containing glyphosate and POEA (Roundup and Vision) a relatively large number of acute toxicity data are available for different species of primary producers (8), invertebrates (8), fish (12) and amphibians (8). Consequently the Species Sensitivity Distribution (SSD) approach can be applied for these taxonomic groups and formulated products of glyphosate to assess the potential ectoxicological risks. According to the HARAP Guidance Document (Campbell *et al*. 1999), toxicity data for at least 8 different species from the sensitive taxonomic group are recommended to construct SSDs. For fish, the HARAP Guidance Document recommends using a minimum of 5 toxicity data to construct SSDs specific for fish. This lower number of toxicity data for fish is chosen for, among other reasons, animal welfare considerations and because of the overall lower variability in fish toxicity data when e.g. compared with that of invertebrates. Although the guidance provided by the HARAP Guidance Document is more or less accepted within the context of pesticide registration (see e.g. EU 2002), other criteria may be used in other jurisdictions. For example, in setting Environmental Quality Standards within the context of the European Water Framework Directive (WFD), the construction of an SSD requires preferably more than 15 but at least 10 toxicity data for different species covering at least 8 taxonomic groups (EU 2003; Lepper 2002).

SSDs can be used to calculate the concentration at which a specified proportion of species are expected to suffer direct toxic effects. When compared with the first-tier effects assessment on the basis of standard test species, SSDs have the advantage of making more use of the available laboratory toxicity data for a larger array of species. They describe the range of sensitivity rather than focusing on a single value, they enable estimates to be made of the proportion of the species affected (within certain taxonomic groups) at different concentrations, and they can be shown together with confidence limits showing the sampling uncertainty due to the limited number of species tested. A statistical extrapolation technique, e.g. the method described in Aldenberg & Jaworska (2000), is often used to calculate the concentration at which a specified proportion of species (p) are expected to suffer direct toxic effects, referred to as the Hazardous Concentration (HC) to p% of the species (HCp). The Species Sensitivity Distribution from which the HCp is derived can be based on either acute or chronic toxicity data. However, the smaller the number of data available for the calculation, the larger the confidence interval around the SSD (and the HCp) will be. In Europe it is common to take the 5^{th} percentile of the SSD (median HC5), or the lower 90% confidence bound for it (lower limit HC5). When based on acute toxicity data the median HC5 is the concentration for which 95% of the tested species with 50% certainty have a higher L(E)C50 value, while the lower limit HC5 represents the concentration for which 95% of the species tested with 95% certainty have a higher LC50 or EC50.

Applying the SSD approach and calculating the HC5 on basis of acute toxicity data reported for Roundup and Vision (both formulations contain glyphosate and the adjuvant POEA) it again appears that primary producers (algae and aquatic vascular plants) are more sensitive than aquatic animals (Table 3). The median and lower limit HC5 values for primary producers are 0.146 and 0.015 mg a.e./L, respectively. The lowest median HC5 for animals is calculated for amphibians (0.753 mg a.e./L). However, the lowest lower limit HC5 for animals is calculated for aquatic invertebrates (0.111 mg a.e./L). The fish species tested appear to be relatively insensitive.

Table 3: Hazardous concentrations to 5% of the tested species (HC5) for different taxonomic groups of freshwater species based on acute toxicity data for Roundup (or Roundup and Vision in the case of amphibians) as presented in Annex 1. If more than one toxicity value was available for the same species and endpoint the geomean value was used.

Taxonomic group	**n**	**HC5 (90% confidence interval) In mg a.e./L**
Primary producers	8	0.146 (0.015 – 0.479)
Invertebrates	8	0.989 (0.111 – 3.067)
Fish	12	2.691 (1.797 – 3.430)
Amphibians	8	0.753 (0.291 – 1.233)

Following the criteria of the HARAP guidance document, the number of chronic toxicity data for aquatic species and Roundup is too low to apply the SSD approach and to calculate a chronic HC5. For glyphosate acid and the IPA salt of glyphosate, however, in total 10 chronic NOECs for different species of primary producers are available (Annex 1). Assuming that these chronic toxicity data for glyphosate acid and the IPA salt of glyphosate can be used in the same SSD, the calculated median chronic HC5 for primary producers was 0.425 mg a.e./L while the corresponding chronic lower limit HC5 was 0.062 mg a.e./L. For glyphosate acid and the IPA salt of glyphosate the number of chronic toxicity data for aquatic animal species was too low to apply the SSD approach (at least according to the HARAP guidance document).

Threshold concentrations for direct toxic effects of glyphosate and its formulated products

Van den Brink *et al.* (2006) showed that for herbicides and primary producers the lower limit of the acute HC5 and the median value of the chronic HC5 were protective of adverse effects in aquatic micro/mesocosms (classified as Effect class 1-2) even under a long-term exposure regime. The median HC5 estimate based on acute toxicity data of herbicides was protective of adverse effects in aquatic micro/mesocosms when a short-term exposure regime (pulse application in flow-through system; single application of a non-persistent ($DT50_{water}$ < 10 days) herbicide in stagnant test system) was studied (Van den Brink *et al.* 2006).

Assuming similar relationships between calculated HC5 values and “safe” concentrations for Roundup it can be concluded that a single short-term pulse exposure to concentrations lower than 0.146 mg a.e./L (median acute HC5 for primary producers) most probably does not affect populations of aquatic organisms. For glyphosate acid and the IPA salt of glyphosate the acceptable concentration for long-term exposure may be as high as 0.424 mg a.e./L (median chronic HC5 for primary producers). For long-term exposure to Roundup, however, it cannot be excluded that concentrations higher than 0.015 mg a.e./L (acute lower limit HC5 for primary producers) and lower than 0.146 mg a.e./L (median acute HC5 for primary producers) may lead to some toxic effects on algae or macrophytes. Most probably longer-term exposure to Roundup at concentrations lower than 0.111 mg a.e./L (lower limit acute HC5 of invertebrates) will not result in toxic effects on animal populations.

Ecotoxicity of the metabolite AMPA

Glyphosate is transformed in the environment into a number of substances, among which the metabolite aminomethylphosphonic acid (AMPA) is the most common. Available toxicity data are given in the tables in Annex 1. Acute toxicity data for algae, invertebrates and fish indicate that AMPA is less toxic than glyphosate acid. Similarly, chronic toxicity of AMPA towards algae is less than chronic toxicity of glyphosate acid. Available toxicity data therefore indicate that the toxicity of AMPA is less than the toxicity of glyphosate acid. AMPA is considered to be a non-relevant metabolite.

Annex 1: Summary of toxicity data

Toxicity values originally expressed as mg/L of the sodium salt of glyphosate were converted into mg a.e./L by multiplying by 0.88, toxicity values originally expressed as mg Roundup/L were converted into mg a.e./L by multiplying by 0.31 and toxicity values expressed as mg a.i./L of the isopropylamine salt were converted into mg a.e./L by multiplying by 0.74. Where toxicity data for POEA were expressed in acid equivalents as mg a.e./L in the original literature, they were converted into mg POEA/L by multiplying by 0.67.

Algae

For algae the distinction between acute and chronic toxicity data is in some cases somewhat superficial, since both types of data may be generated in a test with the same 4 or 7 days duration. For practical reasons and for better alignment with toxicity data for other species, EC50 values are denoted as acute toxicity values, whereas NOEC values are treated as chronic toxicity data.

Algae, acute toxicity data

Substance tested	Species	EC50 (mg a.e./L)	Test duration (days)	Reference
Glyphosate, acid	*Anabaena flos-aquae*	15	7	Malcolm Pirnie, 1987a
	Chlamydomonas eugametos	> 169	4	Hess, 1980
	Chlorella pyrenoidosa	590	4	Maule and Wright, 1984
	Chlorella pyrenoidosa	3.5	4	Ma et al., 2001ab
	Chlorella vulgaris	4.7	4	Ma et al., 2002a
	Chlorococcum hypnosporum	68	4	Maule and Wright, 1984
	Navicula pelliculosa	42	7	Malcolm Pirnie, 1987c
	Scenedesmus obliquus	10.2	4	Sáenz et al., 1997
	Desmodesmus communis var communis	7.2	4	Sáenz et al., 1997
	Desmodesmus communis var communis	70.5	4	Ma et al., 2003
	Pseudokirchneriella subcapitata	21.8	4	Bozeman et al., 1989
	Pseudokirchneriella subcapitata	485	3	NATEC, 1990
	Pseudokirchneriella subcapitata	13.8	7	Malcolm Pirnie, 1987b

	Pseudokirchneriella subcapitata	24.7	4	Tsui and Chu, 2003
	Skeletonema costatum	0.64	7	Malcolm Pirnie, 1987b
	Skeletonema costatum	2.27	4	Tsui and Chu, 2003
	Skeletonema costatum	1.2	4	EG & G Bionomics, 1978a
Glyphosate,IPA salt[a]	*Ankistrodesmus* sp.	305	4	Gardner et al., 1997
	Desmodesmus subspicatus	53.9	3	Dengler and Mende, 1994b
	Pseudokirchneriella subcapitata	41	4	Tsui and Chu, 2003
	Skeletonema costatum	5.9	4	Tsui and Chu, 2003
Roundup	*Chlorella pyrenoidosa*	58.6	7	Hernando et al., 1989
	Chlorella sorokiniana	0.93	4	Christy et al., 1981
	Pseudokirchneriella subcapitata	0.65	3	LISEC, 1989
	Pseudokirchneriella subcapitata	0.81	4	Thomas et al., 1990
	Pseudokirchneriella subcapitata	5.8	4	Tsui and Chu, 2003
	Skeletonema costatum	1.85	4	Tsui and Chu, 2003
POEA[b]	*Pseudokirchneriella subcapitata*	2.63	4	Tsui and Chu, 2003
	Skeletonema costatum	2.24	4	Tsui and Chu, 2003
AMPA[c]	*Desmodesmus subspicatus*	90	3	Dengler and Mende, 1994a

[a] Glyphosate tested as isopropylamine (IPA) salt.
[b] Polyoxyethylene amine, a surfactant used in Roundup; toxicity values are given as mg POEA/L;.
[c] Aminomethylphosphonic acid, AMPA; toxicity values are given as mg AMPA/L.

Algae, chronic toxicity data

Substance tested	Species	NOEC (mg a.e./L)	Test duration (days)	Reference
Glyphosate, acid	*Anabaena flos-aquae*	9.7	7	Malcolm Pirnie, 1987a
	Chlamydomonas eugametos	16.9	4	Hess, 1980
	Navicula pelliculosa	33.6	7	Malcolm Pirnie, 1987c
	Scenedesmus obliquus	2	4	Sáenz et al., 1997
	Desmodesmus	0.77	4	Sáenz et al.,

	communis var communis			1997
	Pseudokirchneriella subcapitata	45	3	NATEC, 1990
	Pseudokirchneriella subcapitata	10.6	7	Malcolm Pirnie, 1987b
	Skeletonema costatum	0.28	7	Malcolm Pirnie, 1987b
	Skeletonema costatum	< 0.6	4	EG & G Bionomics, 1978a
Glyphosate,IPA salt[a]	*Ankistrodesmus* sp.	14.8	4	Gardner et al., 1997
	Desmodesmus subspicatus	7.8	3	Dengler and Mende, 1994b
Roundup	*Chlorella sorokiniana*	0.62	4	Christy et al., 1981
	Pseudokirchneriella subcapitata	0.23	3	LISEC, 1989
AMPA[b]	*Desmodesmus subspicatus*	7.9	2	Dengler and Mende, 1994a as given by Giesy et al., 2000
	Desmodesmus subspicatus	0.96	2	Dengler and Mende, 1994a, as noted in Monograph

[a] Glyphosate tested as isopropylamine (IPA) salt.
[b] Aminomethylphosphonic acid, AMPA; toxicity values are given as mg AMPA/L.

Macrophytes

Macrophytes, acute toxicity data

Substance tested	Species	LC50 (mg a.e./L)	Test duration (days)	Reference
Glyphosate, acid	*Lemna gibba*	10	7	Perkins, 1997
	Lemna gibba	25.5	7	Malcolm Pirnie, 1987e
	Lemna gibba	20.5	10	Sobrero et al., 2007
	Myriophyllum sibiricum	1.6	14	Perkins, 1997
Roundup	*Lemna gibba*	4.7	7	Perkins, 1997
	Lemna gibba	8.2	10	Sobrero et al., 2007
	Lemna minor	1.5	14	Hartman and Martin, 1984
	Myriophyllum sibiricum	1.2	14	Perkins, 1997
	Myriophyllum sibiricum	0.84	14	Roshon et al., 1999

	Myriophyllum spicatum	0.33	21	Sánchez et al., 2007

Macrophytes, chronic toxicity data

Substance tested	Species	NOEC (mg a.e./L)	Test duration (days)	Reference
Glyphosate, acid	*Lemna gibba*	16.6	14	Malcolm Pirnie, 1987e
Glyphosate,IPA salt[a]				
Roundup	*Lemna minor*	17.4	14	Lockhart et al., 1989
	Potamogeton pectinatus	7.4	14	Hartman and Martin, 1985

[a] Glyphosate tested as isopropylamine (IPA) salt.

Invertebrates

Toxicity data from tests which did not clearly document test duration were excluded.

Invertebrates, acute toxicity data

Substance tested	Species	EC50 (mg a.e./L)	Test duration (days)	Reference
Glyphosate, acid	*Acartia tonsa*	35	2	Tsui and Chu, 2003
	Ceriodaphnia dubia	147	2	Tsui and Chu, 2003
	Daphnia magna	780	2	ABC Inc, 1978a
Glyphosate,IPA salt[a]	*Acartia tonsa*	49	2	Tsui and Chu, 2003
	Ceriodaphnia dubia	415	2	Tsui and Chu, 2003
	Chironomus plumosus	55	2	Folmar et al., 1979
	Chironomus riparius	5600	2	Buhl and Faerber, 1989
	Chironomus tentans	> 530	10	Beyers, 1993
	Daphnia magna	930	2	ABC Inc, 1981a
	Hyalella azteca	> 530	10	Beyers, 1993
Roundup	*Acartia tonsa*	1.8	2	Tsui and Chu, 2003
	Anopheles quadrimaculatus larvae	209	1	Holck and Meek, 1987
	Ceriodaphnia dubia	5.4	2	Tsui and Chu,

				2003
	Chironomus plumosus	18	2	Folmar et al., 1979
	Daphnia magna	3.0	2	Folmar et al., 1979
	Daphnia magna	7.4	2	EG & G Bionomics, 1980f
	Daphnia magna	4.0	2	EG & G Bionomics, 1980e
	Daphnia pulex	5.9	2	Hartman and Martin, 1984
	Daphnia pulex	7.9	4	Servizi et al., 1987
	Gammarus pseudolimnaeus	13	2	ABC Inc, 1982b
	Gammarus pseudolimnaeus	43	4	Folmar et al., 1979
	Procamburus clarkia	14.7	4	Holck and Meek, 1987
POEA[b]	*Acartia tonsa*	0.38	2	Tsui and Chu, 2003
	Ceriodaphnia dubia	0.77	2	Tsui and Chu, 2003
	Chironomus plimosus	13	2	Folmar et al., 1979
	Daphnia magna	2.0	2	ABC Inc., 1980b
	Daphnia pulex	4.1	2	Moore et al., 1987
	Daphnia pulex	2.0	4	Servizi et al., 1987
AMPA[c]	*Daphnia magna*	690	2	ABC Inc. 1991a

[a] Glyphosate tested as isopropylamine (IPA) salt.
[b] Polyoxyethylene amine, a surfactant used in Roundup; toxicity values are given as mg POEA/L.
[c] Aminomethylphosphonic acid, AMPA; toxicity values are given as mg AMPA/L.

Invertebrates, chronic toxicity data

Substance tested	Species	NOEC (mg a.e./L)	Test duration (days)	Reference
Glyphosate, acid	*Daphnia magna*	100	21	ABC Inc. 1989c
	Daphnia magna	50	21	ABC Inc., 1982d
Glyphosate,IPA salt[a]				
Roundup	*Daphnia magna*	1.0	21	ABC Inc., 1989b
	Tubifex tubifex	> 27.5	28	Perkins, 1997

[a] Glyphosate tested as isopropylamine (IPA) salt.

Fish

Fish, acute toxicity data

Substance tested	Species	LC50 (mg a.e./L)	Test duration (days)	Reference
Glyphosate, acid	*Cyprinodon variegates*	> 1000	4	EG & G Bionomics, 1978b
	Cyprinus carpio	620	4	Neskovic et al., 1996
	Jordanella floridae	> 30	4	Holdway and Dixon, 1988
	Lepomis macrochirus	> 24	2	Bionomics, 1973c
	Lepomis macrochirus	120	4	ABC Inc., 1978c
	Oncorhynchus gorbuscha	23, 190[d]	4	Wan et al., 1989
	Oncorhynchus keta	22, 148[d]	4	Wan et al., 1989
	Oncorhynchus kisutch	36, 174[d]	4	Wan et al., 1989
	Oncorhynchus ishawytsha	30, 211[d]	4	Wan et al., 1989
	Oncorhynchus mykiss	22, 197[d]	4	Wan et al., 1989
	Rasbora heteromorpha	168	4	HRC, 1977
Glyphosate,IPA salt[a]	*Hybognathus placitus*	> 648	4	Beyers, 1995
	Ictalurus punctatus	130	4	Folmar et al., 1979
	Lepomis macrochirus	140 – 220	4	Folmar et al., 1979
	Lepomis macrochirus	> 1000	4	ABC Inc., 1981b
	Oncorhynchus mykiss	> 1000	4	ABC Inc., 1981c
	Oncorhynchus mykiss	140 – 240	4	Folmar et al., 1979
	Pimephales promelas	97	4	Folmar et al., 1979
	Pimephales promelas	> 648	4	Beyers, 1995
Roundup	*Cyprinus carpio*	3.1	4	Liong et al., 1988
	Cyprinus carpio	8.1	4	Sun, 1987
	Cyprinus carpio	4.7	4	Tooby et al., 1980
	Gambusia affinis	5.3	2	Sun, 1987
	Ictalurus punctatus	12.1	4	EG & G Bionomics,

				1980a
	Ictalurus punctatus, fry	3.3	4	Folmar et al., 1979
	Ictalurus punctatus, adult	13	4	Folmar et al., 1979
	Lepomis macrochirus	1.8	4	ABC Inc., 1982a
	Lepomis macrochirus	5.0	4	Folmar et al., 1979
	Lepomis macrochirus	10.5	4	EG & G Bionomics, 1980b
	Oncorhynchus gorbuscha	9.6, 4.3[d]	4	Wan et al., 1989
	Oncorhynchus keta	5.9, 3.4[d]	4	Wan et al., 1989
	Oncorhynchus kisutch	6.8	4	Mitchel et al., 1987
	Oncorhynchus kisutch, fry	13	4	Servizi et al., 1987
	Oncorhynchus kisutch	8.4, 4.0[d]	4	Wan et al., 1989
	Oncorhynchus mykiss	2.5	4	ABC Inc., 1982c
	Oncorhynchus mykiss	6.8	4	EG & G Bionomics, 1980g
	Oncorhynchus mykiss	8.4	4	Morgan et al., 1991
	Oncorhynchus mykiss	8.4	4	EG & G Bionomics, 1980g
	Oncorhynchus mykiss	16.1	4	Hildebrand et al., 1982
	Oncorhynchus mykiss	4.7	4	Mitchell et al., 1987
	Oncorhynchus mykiss	4.7, 4.3[d]	4	Wan et al., 1989
	Oncorhynchus mykiss	10.4	4	Morgan and Kiceniuk, 1992
	Oncorhynchus nerka	8.3	4	Servizi et al., 1987
	Oncorhynchus tshawytsha	6.2	4	Mitchell et al., 1987
	Oncorhynchus tshawytsha	8.4	4	Wan et al., 1989
	Pimephales promelas	2.3	4	Folmar et al., 1979
	Pimephales promelas	7.1	4	EG & G Bionomics, 1980d
	Tilapia sp.	2.3	4	Liong et al., 1988
POEA[b]	*Lepomis macrochirus*	1.3	4	ABC Inc., 1980a

	Lepomis macrochirus	1.0 – 3.0	4	Folmar et al., 1979
	Ictalurus punctatus	13	4	Folmar et al., 1979
	Oncorhynchus garbuscha	2.8, 1.4[d]	4	Wan et al., 1989
	Oncorhynchus keta	2.4, 1.4[d]	4	Wan et al., 1989
	Oncorhynchus kisutch, fry	3.5	4	Servizi et al., 1987
	Oncorhynchus kisutch	3.2, 1.8[d]	4	Wan et al., 1989
	Oncorhynchus mykiss	4.2	4	ABC Inc., 1980c
	Oncorhynchus mykiss	0.65 – 7.4	4	Folmar et al., 1979
	Oncorhynchus mykiss, fry	3.2	4	Servizi et al., 1987
	Oncorhynchus nerka, fry	2.6	4	Servizi et al., 1987
	Oncorhynchus tshawytsha	2.8, 1.7[d]	4	Wan et al., 1989
	Pimephales promelas	1.0	4	Folmar et al., 1979
AMPA[c]	*Oncorhynchus mykiss*	520	4	ABC Inc., 1991b

[a] Glyphosate tested as isopropylamine (IPA) salt.
[b] Polyoxyethylene amine, a surfactant used in Roundup; toxicity values are given as mg POEA/L.
[c] Aminomethylphosphonic acid, AMPA; toxicity values are given as mg AMPA/L.
[d] Values for soft (creek) and hard (lake) water, resp.

Fish, chronic toxicity data

Substance tested	Species	NOEC (mg a.e./L)	Test duration (days)	Reference
Glyphosate, acid	*Oncorhynchus mykiss*	52	21	ABC Inc., 1989a
	Pimephales promelas	26	255	EG & G Bionomics, 1975
Roundup	*Oncorhynchus mykiss*	0.74	21	ABC Inc., 1989d

Amphibians

No chronic toxicity data were readily available from the open literature.

Amphibians, acute toxicity data

Substance tested	Species	LC50 (mg a.e./L)	Test duration (days)	Reference
Glyphosate, acid	*Crinia insignifera*, newly emerged	83.6	2	Mann and Bidwell, 1999
	Crinia insignifera, adult	78	4	Bidwell and Gorrie, 1995
	Litoria morei, tadpole	121	2	Mann and Bidwell, 1999
	Litoria moorei, tadpole	81.2	2	Mann and Bidwell, 1999
	Litoria moorei, tadpole	111	4	Bidwell and Gorrie, 1995
	Litoria moorei, adult	> 180	4	Bidwell and Gorrie, 1995
Glyphosate,IPA salt[a]	*Litoria morei, tadpole*	> 466	2	Mann and Bidwell, 1999
	Heleioporus eyrei, tadpole	> 373	2	Mann and Bidwell, 1999
	Limnodynastes dorsalis, tadpole	> 400	2	Mann and Bidwell, 1999
	Litoria moorei, tadpole	> 343	2	Mann and Bidwell, 1999
Roundup	*Bufo americanus*[b], larvae	1.7	4	Edginton et al., 2004
	Bufo americanus, larvae	1.9	16	Relyea, 2005
	Crinia insignifera, tadpole	3.1	2	Mann and Bidwell, 1999
	Crinia insignifera, tadpole	< 17	2	Bidwell and Gorrie, 1995
	Crinia insignifera, newly emerged	45	2	Mann and Bidwell, 1999
	Crinia insignifera, adult	42.5	2	Mann and Bidwell, 1999
	Crinia insignifera, adult	30	4	Bidwell and Gorrie, 1995
	Heleioporus eyrei, tadpole	5.4	2	Mann and Bidwell, 1999
	Limnodynastes dorsalis, tadpole	2.6	2	Mann and Bidwell, 1999
	Litoria moorei, tadpole	2.5	2	Mann and Bidwell, 1999
	Litoria moorei, tadpole	10	2	Mann and Bidwell, 1999
	Litoria moorei, tadpole	5.8	4	Bidwell and Gorrie, 1995
	Litoria moorei, adult	> 51	4	Bidwell and Gorrie, 1995

	Rana clamitans[b], larvae	1.4	4	Edginton et al., 2004
	Rana clamitans, larvae	1.6	16	Relyea, 2005
	Rana pipiens[b], larvae	1.1	4	Edginton et al., 2004
	Rana pipens, larvae	1.8	16	Relyea, 2005
	Xenopus laevis, embryo	22.3	4	Perkins, 1997
	Xenopus laevis[b]*,* Larvae	0.88	4	Edginton et al., 2004

[a] Glyphosate tested as isopropylamine (IPA) salt.
[b] Tested at pH = 7.5 as Vision, a formulation containing POEA.

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