

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

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

<sup>a</sup> Values given as 'smaller than' or 'larger than' were excluded.

<sup>b</sup> 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 allow 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)
<b>Algae</b>			
<i>Pseudokirchneriella subcapitata</i>	22 (11 – 45)		0.23
<b>Invertebrates</b>			
<i>Daphnia magna</i>	71 (50 – 100)		1.0
<b>Fish</b>			
<i>Oncorhynchus mykiss</i>	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 glyphosate 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 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 *Xenopus 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 ecotoxicological 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 (HC $p$ ). The Species Sensitivity Distribution from which the HC $p$  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 HC $p$ ) will

be. In Europe it is common to take the 5<sup>th</sup> 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.

<b>Taxonomic group</b>	<b>n</b>	<b>HC5 (90% confidence interval) In mg a.e./L</b>
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 ( $DT50_{\text{water}} < 10$  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	<i>Anabaena flos-aquae</i>	15	7	Malcolm Pirnie, 1987a
	<i>Chlamydomonas eugametos</i>	> 169	4	Hess, 1980
	<i>Chlorella pyrenoidosa</i>	590	4	Maule and Wright, 1984
	<i>Chlorella pyrenoidosa</i>	3.5	4	Ma et al., 2001ab
	<i>Chlorella vulgaris</i>	4.7	4	Ma et al., 2002a
	<i>Chlorococcum hypnosporum</i>	68	4	Maule and Wright, 1984
	<i>Navicula pelliculosa</i>	42	7	Malcolm Pirnie, 1987c
	<i>Scenedesmus obliquus</i>	10.2	4	Sáenz et al., 1997
	<i>Desmodesmus communis</i> var <i>communis</i>	7.2	4	Sáenz et al., 1997
	<i>Desmodesmus communis</i> var <i>communis</i>	70.5	4	Ma et al., 2003
	<i>Pseudokirchneriella subcapitata</i>	21.8	4	Bozeman et al., 1989
	<i>Pseudokirchneriella subcapitata</i>	485	3	NATEC, 1990
	<i>Pseudokirchneriella subcapitata</i>	13.8	7	Malcolm Pirnie, 1987b

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

<sup>a</sup> Glyphosate tested as isopropylamine (IPA) salt.

<sup>b</sup> Polyoxyethylene amine, a surfactant used in Roundup; toxicity values are given as mg POEA/L.

<sup>c</sup> 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	<i>Anabaena flos-aquae</i>	9.7	7	Malcolm Pirnie, 1987a
	<i>Chlamydomonas eugametos</i>	16.9	4	Hess, 1980
	<i>Navicula pelliculosa</i>	33.6	7	Malcolm Pirnie, 1987c
	<i>Scenedesmus obliquus</i>	2	4	Sáenz et al., 1997
	<i>Desmodesmus</i>	0.77	4	Sáenz et al.,



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

<sup>a</sup> Glyphosate tested as isopropylamine (IPA) salt.

<sup>b</sup> 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	<i>Lemna gibba</i>	10	7	Perkins, 1997
	<i>Lemna gibba</i>	25.5	7	Malcolm Pirnie, 1987e
	<i>Lemna gibba</i>	20.5	10	Sobrero et al., 2007
	<i>Myriophyllum sibiricum</i>	1.6	14	Perkins, 1997
Roundup	<i>Lemna gibba</i>	4.7	7	Perkins, 1997
	<i>Lemna gibba</i>	8.2	10	Sobrero et al., 2007
	<i>Lemna minor</i>	1.5	14	Hartman and Martin, 1984
	<i>Myriophyllum sibiricum</i>	1.2	14	Perkins, 1997
	<i>Myriophyllum sibiricum</i>	0.84	14	Roshon et al., 1999

	<i>Myriophyllum spicatum</i>	0.33	21	Sánchez et al., 2007
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### *Macrophytes, chronic toxicity data*

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

<sup>a</sup> 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	<i>Acartia tonsa</i>	35	2	Tsui and Chu, 2003
	<i>Ceriodaphnia dubia</i>	147	2	Tsui and Chu, 2003
	<i>Daphnia magna</i>	780	2	ABC Inc, 1978a
Glyphosate, IPA salt <sup>a</sup>	<i>Acartia tonsa</i>	49	2	Tsui and Chu, 2003
	<i>Ceriodaphnia dubia</i>	415	2	Tsui and Chu, 2003
	<i>Chironomus plumosus</i>	55	2	Folmar et al., 1979
	<i>Chironomus riparius</i>	5600	2	Buhl and Faerber, 1989
	<i>Chironomus tentans</i>	> 530	10	Beyers, 1993
	<i>Daphnia magna</i>	930	2	ABC Inc, 1981a
	<i>Hyalella azteca</i>	> 530	10	Beyers, 1993
Roundup	<i>Acartia tonsa</i>	1.8	2	Tsui and Chu, 2003
	<i>Anopheles quadrimaculatus larvae</i>	209	1	Holck and Meek, 1987
	<i>Ceriodaphnia dubia</i>	5.4	2	Tsui and Chu,

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

<sup>a</sup> Glyphosate tested as isopropylamine (IPA) salt.

<sup>b</sup> Polyoxyethylene amine, a surfactant used in Roundup; toxicity values are given as mg POEA/L.

<sup>c</sup> 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	<i>Daphnia magna</i>	100	21	ABC Inc. 1989c
	<i>Daphnia magna</i>	50	21	ABC Inc., 1982d
Glyphosate, IPA salt <sup>a</sup>				
Roundup	<i>Daphnia magna</i>	1.0	21	ABC Inc., 1989b
	<i>Tubifex tubifex</i>	> 27.5	28	Perkins, 1997

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

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

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

<sup>a</sup> Glyphosate tested as isopropylamine (IPA) salt.

<sup>b</sup> Polyoxyethylene amine, a surfactant used in Roundup; toxicity values are given as mg POEA/L.

<sup>c</sup> Aminomethylphosphonic acid, AMPA; toxicity values are given as mg AMPA/L.

<sup>d</sup> 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	<i>Oncorhynchus mykiss</i>	52	21	ABC Inc., 1989a
	<i>Pimephales promelas</i>	26	255	EG & G Bionomics, 1975
Roundup	<i>Oncorhynchus mykiss</i>	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	<i>Crinia insignifera</i> , newly emerged	83.6	2	Mann and Bidwell, 1999
	<i>Crinia insignifera</i> , adult	78	4	Bidwell and Gorrie, 1995
	<i>Litoria morei</i> , tadpole	121	2	Mann and Bidwell, 1999
	<i>Litoria moorei</i> , tadpole	81.2	2	Mann and Bidwell, 1999
	<i>Litoria moorei</i> , tadpole	111	4	Bidwell and Gorrie, 1995
	<i>Litoria moorei</i> , adult	> 180	4	Bidwell and Gorrie, 1995
Glyphosate, IPA salt <sup>a</sup>	<i>Litoria morei</i> , tadpole	> 466	2	Mann and Bidwell, 1999
	<i>Heleioporus eyrei</i> , tadpole	> 373	2	Mann and Bidwell, 1999
	<i>Limnodynastes dorsalis</i> , tadpole	> 400	2	Mann and Bidwell, 1999
	<i>Litoria moorei</i> , tadpole	> 343	2	Mann and Bidwell, 1999
Roundup	<i>Bufo americanus</i> <sup>b</sup> , larvae	1.7	4	Edginton et al., 2004
	<i>Bufo americanus</i> , larvae	1.9	16	Relyea, 2005
	<i>Crinia insignifera</i> , tadpole	3.1	2	Mann and Bidwell, 1999
	<i>Crinia insignifera</i> , tadpole	< 17	2	Bidwell and Gorrie, 1995
	<i>Crinia insignifera</i> , newly emerged	45	2	Mann and Bidwell, 1999
	<i>Crinia insignifera</i> , adult	42.5	2	Mann and Bidwell, 1999
	<i>Crinia insignifera</i> , adult	30	4	Bidwell and Gorrie, 1995
	<i>Heleioporus eyrei</i> , tadpole	5.4	2	Mann and Bidwell, 1999
	<i>Limnodynastes dorsalis</i> , tadpole	2.6	2	Mann and Bidwell, 1999
	<i>Litoria moorei</i> , tadpole	2.5	2	Mann and Bidwell, 1999
	<i>Litoria moorei</i> , tadpole	10	2	Mann and Bidwell, 1999
	<i>Litoria moorei</i> , tadpole	5.8	4	Bidwell and Gorrie, 1995
	<i>Litoria moorei</i> , adult	> 51	4	Bidwell and Gorrie, 1995

	<i>Rana clamitans</i> <sup>b</sup> , larvae	1.4	4	Edginton et al., 2004
	<i>Rana clamitans</i> , larvae	1.6	16	Relyea, 2005
	<i>Rana pipiens</i> <sup>b</sup> , larvae	1.1	4	Edginton et al., 2004
	<i>Rana pipiens</i> , larvae	1.8	16	Relyea, 2005
	<i>Xenopus laevis</i> , embryo	22.3	4	Perkins, 1997
	<i>Xenopus laevis</i> <sup>b</sup> , Larvae	0.88	4	Edginton et al., 2004

<sup>a</sup> Glyphosate tested as isopropylamine (IPA) salt.

<sup>b</sup> Tested at pH = 7.5 as Vision, a formulation containing POEA.



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