Proposed EQS for Water Framework Directive Annex VIII substances: glyphosate *(For consultation)*

by
Water Framework Directive - United Kingdom Technical Advisory Group (WFD-UKTAG)

Publisher: **Water Framework Directive - United Kingdom Technical Advisory Group (WFD-UKTAG)**
SNIFFER
25 Greenside Place
Edinburgh
EH1 3AA
Scotland
www.wfduk.org

This report is the result of research commissioned and funded by the Environment Agency and the Scotland and Northern Ireland Forum for Environmental Research (SNIFFER).

Author(s):
D Maycock, M Crane, C Atkinson and I Johnson

Research performed:
2010

Dissemination Status:
Publicly available

Keywords:
Glyphosate, Water Framework Directive, specific pollutants, predicted no-effect concentrations, freshwater, saltwater

Research Contractor:
WRc plc, Frankland Road, Blagrove, Swindon, Wiltshire, SN5 8YF. Tel: +44 1793 865000

Environment Agency's Project Manager:
Stephanie Cole, Evidence Directorate

Collaborators:
Environment Agency
Scottish Environment Protection Agency (SEPA)
Northern Ireland Environment Agency (NIEA)

Environment Agency Science Project Number:
SC080021/5a(x)

Use of this report

The development of UK-wide classification methods and environmental standards that aim to meet the requirements of the Water Framework Directive (WFD) is being sponsored by the UK Technical Advisory Group (UKTAG) for WFD on behalf of its member and partners.

This technical document has been developed through a project managed by the Environment Agency and has involved members and partners of UKTAG. It provides background information to support the ongoing development of the standards and classification methods.

While this report is considered to represent the best available scientific information and expert opinion available at the time of its completion, it does not necessarily represent the final or policy positions of UKTAG or any of its partner agencies.

Executive summary

The UK Technical Advisory Group (UKTAG) has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for glyphosate using the methodology described in Annex V of the Directive.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for glyphosate and its primary salt, isopropylamine (IPA), along with any data that relate impacts under field conditions to exposure concentrations. The data used in the report includes confidential information that has been supplied by Monsanto Europe S.A. and Syngenta Limited. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data. Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD.

Where possible, PNECs have been derived for freshwater and saltwater environments, and for long-term/continuous exposure and short-term/transient exposure. If they were to be adopted as EQSs, the long-term PNEC would normally be expressed as an annual average concentration and the short-term PNEC as a 95th percentile concentration. The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

In this report all references to glyphosate refer to the parent acid. Concentrations are expressed on the basis of the acid equivalent (a.e.). The acid equivalent represents the original acid portion of the molecule and allows for comparisons between studies using either glyphosate or glyphosate salts.

Properties and fate in water

Glyphosate-based herbicides have a broad spectrum of activity towards plants. In contrast, activity towards animals is believed to be weak because the mode of action for glyphosate is a biochemical pathway found only in plants and some micro-organisms. Glyphosate inhibits plant growth by inhibiting the production of essential aromatic amino acids through competitive inhibition of synthesis of the enzyme enolpyruvylshikimate phosphate (EPSP). This is a key enzyme in the shikimic acid pathway for the synthesis of chorismate, which is the precursor for the essential amino acids phenylalanine, tyrosine and tryptophan.

Glyphosate is a weak organic acid and is usually formulated as a salt of the deprotonated acid of glyphosate and a cation, e.g., isopropylamine. Glyphosate is sold in many different formulations for uses ranging from agriculture and forestry, to ready-to-use products for the home and garden. To work effectively glyphosate must be mixed with a surfactant that facilitates its uptake by the plant.

Although readily soluble in water glyphosate is readily ionized and the anion will be strongly adsorbed to sediments and soils of pH > 3.5. Glyphosate degrades to natural products such as CO_2 and phosphate ions with degradation in terrestrial and aquatic systems occurring predominantly via microbial processes.

Bioconcentration of glyphosate in aquatic organisms is low. Glyphosate is not suspected of being an endocrine-disrupting chemical.

Availability of data

Long-term laboratory data are available for eight different freshwater taxonomic groups: algae, amphibians, crustaceans, fish, macrophytes, molluscs, nematodes and nematomorpha. Freshwater short-term toxicity data are also available for eight taxonomic groups: algae, amphibians, crustaceans, fish, insects, macrophytes, molluscs and protozoa. The toxicity of glyphosate occurs over a wide concentration range. The available short-term and long-term toxicity test data indicate that for glyphosate and its salt aquatic plants are the most sensitive taxa of those tested. For marine organisms, single species short-term toxicity data are available for six different taxonomic groups (algae, crustaceans, echinoderms, fish, molluscs and protozoans). However, no long-term toxicity data are available for the minimum of three saltwater taxa (algae, crustaceans and fish) required under Annex V of the WFD. Laboratory data are supplemented by freshwater and saltwater mesocosm and field data which indicate that glyphosate may not be as toxic in natural settings as in laboratory tests, due to rapid dissipation.

Derivation of PNECs

Long-term PNEC for freshwaters

Long-term data are available for eight taxonomic groups (algae, amphibians, crustaceans, fish, macrophytes, molluscs, nematodes and nematomorpha) for glyphosate and glyphosate IPA salt. Based on the information available algae and macrophytes appear to be the most sensitive taxa to glyphosate.

Using the assessment factor method to derive a $PNEC_{freshwater}$ requires that an assessment factor of 10 is applied to the lowest reliable NOEC or EC10 (332 µg l^{-1} for *Myriophyllum sibiricum*). This results in:

$PNEC_{freshwater\_lt}$ = 332 µg l^{-1}/AF (10) = 33 µg l^{-1} glyphosate (rounded)

However, there are sufficient freshwater ecotoxicity data to allow a PNEC to be derived by the probabilistic method from the HC5 of an SSD. As a result of this the PNEC derived by the assessment factor (AF) method is not recommended for adoption as an EQS.

Since there are no obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group, the draft WFD Technical Guidance approach of using a combined freshwater and saltwater dataset for the freshwater and marine effects assessment has been used. An HC5 of 586.8 µg l^{-1} was derived using a log-normal species sensitivity distribution. An assessment factor of three applied to the HC5 results in:

$PNEC_{freshwater\_lt}$ = 586.8 µg l^{-1}/AF (3) = 196 µg l^{-1} glyphosate (rounded)

Short-term PNEC for freshwaters

Short-term toxicity data are available for eight taxonomic groups: algae, amphibians, crustaceans, fish, insects, macrophytes, molluscs and protozoa. Using the assessment factor method to derive a $PNEC_{freshwater}$ requires that an assessment factor of 10 is applied to the lowest reliable L(E)C50 (844 µg l^{-1} for *Myriophyllum sibiricum*). This results in:

$PNEC_{freshwater\_st}$ = 844 µg l^{-1}/AF (10) = 84 µg l^{-1} glyphosate (rounded)

However, there are sufficient freshwater ecotoxicity data to allow a PNEC to be derived by the probabilistic method from the HC5 of an SSD. As a result of this the PNEC derived by the assessment factor (AF) method is not recommended for adoption as an EQS.

The freshwater and saltwater datasets have been combined since there is no apparent difference in sensitivity between freshwater and marine taxa. Based on the 30 fresh- and saltwater species L(E)C50s (using geometric means where applicable) the median (i.e. 50 per cent confidence) 5th percentile cut-off value of 1988.3 µg l^{-1} glyphosate is calculated. According to the draft WFD Technical Guidance the AF should normally be 10. This PNEC is essentially the same as the long-term combined freshwater and saltwater PNEC of 196 µg l^{-1}. Examination of the dataset indicates that the acute to chronic ratio is at least 2.5 suggesting that an AF of 10 is too stringent and that a lower assessment factor is more appropriate, and therefore, an assessment factor of 5 is recommended. This results in:

$PNEC_{freshwater\_st}$ = 1988.3 µg l^{-1}/AF (5) = 398 µg l^{-1} glyphosate (rouned)

Long-term PNEC for saltwaters

Since long-term single species toxicity data are only available for algae and eelgrass, a combined freshwater and saltwater dataset for the marine effects assessment was used to derive the PNEC. The saltwater toxicity data do not differ markedly from the range of values obtained for corresponding freshwater species. If a combined dataset is used, the draft WFD Technical Guidance recommends that the AF of 1-5 applied to the HC5 estimated from the SSD should only be applied for coastal and territorial waters if the data set used to establish the SSD comprises long-term NOECs or EC10s for at least two additional typically marine taxonomic groups, other than fish, crustaceans and algae. If such data is unavailable then an additional AF of 10 should be applied to deal with residual uncertainty. However, it can be argued that the additional AF of 10 is not required for glyphosate since aquatic plants are the most sensitive taxa and data for echinoderms and molluscs are not expected to show lower toxicity values. This results in:

$PNEC_{saltwater\_lt}$ = 586.8 µg l^{-1}/AF (3) = 196 µg l^{-1} glyphosate (rounded)

Short-term PNEC for saltwaters

Reliable short-term saltwater toxicity data are available for algae, invertebrates and fish and there is no evidence to suggest that other saltwater species (particularly those that are exclusively saltwater in distribution) would be more sensitive. Therefore, the freshwater and saltwater datasets have been combined. No additional AF is required for the saltwater short-term EQS as there are data for two additional two marine taxonomic groups (molluscs and echinoderms). This results in:

$PNEC_{saltwater\_st}$ = 1988.3 µg l^{-1}/AF (5) = 398 µg l^{-1} glyphosate (rounded)

PNECs for sediment

The TGD trigger value of a log Koc or log Kow of ≥3 is met, as the reported log Koc for glyphosate is 2.9 – 4.8 (EC 2002).

No long–term sediment studies were available. Short-term data are available for studies carried out using various glyphosate formulations. These results suggest a wide range in toxicity, which may be explained by differences in organic carbon and the partitioning behaviour of glyphosate in sediment. Because of the uncertainties, short

exposure periods, use of different glyphosate formulations and the wide range in toxicity values in the empirical data no $PNEC_{sediment}$ can be recommended.

PNECs for secondary poisoning

The EU Technical Guidance Document (TGD) bioconcentration factor (BCF) trigger of 100 in whole fish is not exceeded by glyphosate so there is no need to derive PNECs for secondary poisoning.

Summary of proposed PNECs

Receiving medium/ exposure scenario	TGD deterministic approach (AFs) glyphosate	TGD probabilistic approach (SSDs) glyphosate	Existing EQS
Freshwater/long-term	33 µg l^{-1}	196 µg l^{-1}	-
Freshwater/short-term	84 µg l^{-1}	398 µg l^{-1}	-
Saltwater/long-term	33 µg l^{-1}	196 µg l^{-1}	-
Saltwater/short-term	84 µg l^{-1}	398 µg l^{-1}	-
Sediments	Insufficient data	-	-
Secondary poisoning	Not required	-	-

Analysis

The proposed PNECs for glyphosate in both freshwater and saltwater are potentially in the range 33 to 398 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50%. Using this criterion, it is evident that current analytical methodologies (non-standard) employing coupling ion chromatography (IC) separation with inductively coupled plasma mass spectrometry (ICP–MS) detection), capable of achieving detection limits as low as 700 ng l^{-1}, or large-volume injection in a coupled-column LC system using fluorescence detection (LC–LC–FD), capable of achieving detection limits as low as 20 ng l^{-1}, should offer adequate performance to analyse for glyphosate.

Implementation issues

There are no known issues to prevent adopting the proposed PNECs as EQSs for glyphosate.

Contents

1 Introduction

The UK Technical Advisory Group (UKTAG) supporting the implementation of the Water Framework Directive (2000/60/EC)[1] is a partnership of UK environmental and conservation agencies. It also includes partners from the Republic of Ireland. UKTAG has commissioned a programme of work to derive Environmental Quality Standards (EQSs) for substances falling under Annex VIII of the Water Framework Directive (WFD). This report proposes predicted no-effect concentrations (PNECs) for glyphosate using the methodology described in Annex V of the Directive.

The PNECs described in this report are based on a technical assessment of the available ecotoxicity data for glyphosate and its salts, along with any data that relate impacts under field conditions to exposure concentrations. The data used in the report includes confidential information that has been supplied by Monsanto Europe S.A. and Syngenta Limited. The data have been subjected to rigorous quality assessment such that decisions are based only on scientifically sound data.[2] Following consultation with an independent peer review group, critical data have been identified and assessment factors selected in accordance with the guidance given in Annex V of the WFD. The feasibility of implementing these PNECs as EQSs has not been considered at this stage. However, this would be an essential step before a regulatory EQS can be recommended.

This report considers glyphosate and its primary salt, isopropylamine (IPA). Data were also available for the trimesium salt of glyphosate. Glyphosate trimesium has not been manufactured or sold globally since 2004. It is very unlikely that any significant volume of product containing the trimesium salt of glyphosate will enter the UK (pers. comm.[3]). Taking into account "sell out periods" and going significantly beyond the regulatory storage stability period (two years) the likelihood of glyphosate trimesium being released to the environment is considered negligible. Therefore, this data has not been considered when deriving the PNEC.

In the UK there are currently 234 registered glyphosate formulations of which 43 are approved for aquatic use, i.e. use in or near water (The Pesticides Safety Directorate)[4]. Formulations may vary according to (i) the salt that is used in the formulation, (ii) adjuvants (e.g. surfactants, emulsifiers) and (iii) concentration of the parent acid in the product. This document is not intended to support the aquatic/waterside approval or use of proprietary herbicides containing glyphosate.

In this report all references to glyphosate refer to the free acid. Concentrations are expressed on the basis of the acid equivalent (a.e.). The acid equivalent represents the original acid portion of the molecule and allows for comparisons between studies using either glyphosate or glyphosate salts.

1.1 Properties and fate in water

Glyphosate is a weak organic acid. The parent acid of glyphosate is effective as a herbicide but does not produce a stable formulation or mix well with other products. Glyphosate is usually formulated as a salt of the deprotonated acid of glyphosate and a cation. The most frequently

[1] *Official Journal of the European Communities* L327:1–72 (22/12/2000). Can be downloaded from http://www.eu.int/comm/environment/water/water-framework/index_en.html
[2] Data quality assessment sheets are provided in Annex I.
[3] Association of European Glyphosate Producers (Cheminova, Monsanto, Syngenta)
[4] http://www.pesticides.gov.uk/databases.asp [accessed 25/08/2009]

used salt is isopropylamine but there are formulations that use potassium or diammonium. The cation is bound to the glyphosate molecule by a relatively weak electrostatic charge and because of this, once the product is added to a spray tank the formulated salt can easily be replaced by other positively charged salts present in the water used as a carrier. Therefore, the glyphosate that reaches the leaf surface is often not associated with the salt with which it is formulated. Glyphosate is sold in many different formulations primarily for the post-emergence control of annual and perennial weeds in various applications ranging from agriculture and forestry, to ready-to-use products for the home and garden (WHO 1994). To work effectively, glyphosate must be mixed with a surfactant that facilitates its uptake by the plant.

Although readily soluble in water glyphosate is readily ionized and the anion will be strongly adsorbed to sediments and soils of pH > 3.5. Glyphosate degrades to natural products such as CO_2 and phosphate ions with degradation in terrestrial and aquatic systems occurring predominantly via microbial processes. It is rapidly removed from water to sediments and suspended particulate matter.

Aminomethylphosphonic acid (AMPA) is the principal metabolite produced from glyphosate degradation in water (EC 2002).

Glyphosate is not suspected of being an endocrine-disrupting chemical.

2 Results and observations

2.1 Identity of substance

Table 2.1 gives the chemical name and Chemical Abstracts Service (CAS) number for glyphosate and its primary salt.

Table 2.1 Species covered by this report

Name	CAS Number
Technical material	
Glyphosate	1071-83-6
Salt	
Glyphosate isopropylamine	38641-94-0

2.2 PNECs proposed for derivation of quality standards

Table 2.2 lists proposed PNECs obtained using the methodology described in the Technical Guidance Document (TGD) issued by the European Chemicals Bureau (ECB) on risk assessment of chemical substances (ECB 2003).

Section 2.6 summarises the effects data identified from the literature for glyphosate. The use of these data to derive the values given in Table 2.2 is explained in Section 3.

Table 2.2 Proposed overall PNECs as basis for quality standard setting

PNEC	TGD deterministic approach (AFs)	TGD probabilistic approach (SSDs)	Existing EQS
Freshwater short-term	84 µg l^{-1}	398 µg l^{-1}	-
Freshwater long-term	33 µg l^{-1}	196 µg l^{-1}	-
Saltwater short-term	84 µg l^{-1}	398 µg l^{-1}	-
Saltwater long-term	33 µg l^{-1}	196 µg l^{-1}	-
Sediment	Insufficient data	–	-
Secondary poisoning	Not required	–	-

AF = assessment factor
SSD = species sensitivity distribution

2.3 Hazard classification

Table 2.3 gives the R-phrases (Risk-phrases) and labelling for glyphosate and its salts.

Table 2.3 Hazard classification

R-phrases and labelling	Reference
R41, 51/53 S2, 26, 39, 61	ECB 2005

2.4 Physical and chemical properties

Table 2.4 summarises the physical and chemical properties of glyphosate and its salt.

2.5 Environmental fate and partitioning

Table 2.5 summarises the information obtained from the literature on the environmental fate and partitioning of glyphosate and its environmentally significant salt.

Glyphosate is an amphoteric compound and may exist as different ionic species dependent on pH. Glyphosate has a net negative charge in the pH range 5 to 9 that increases with pH (HSDB 2006). Glyphosate is highly soluble in water but due to its high polarity, it is practically insoluble in organic solvents such as acetone. The different glyphosate salts exhibit differences in solubility with glyphosate isopropylamine being considerably more soluble in water than other salts. Once in an aqueous environment, salts of glyphosate dissociate to glyphosate(anion). The form of glyphosate at a given pH is dictated by the acid dissociation constants (pKa, see Table 2.5). Glyphosate has a negative log K_{ow} value and does not partition to organic substances but tends to remain in water.

Glyphosate is a weak organic acid. In most cases, glyphosate will dissipate rapidly from points of entry into natural water bodies through adsorption to organic substances and inorganic clays, degradation and dilution. Residues adsorbed to suspended particles are precipitated into bottom sediments where they can persist until degraded microbially, with a half-life that ranges from 12 days to 10 weeks, the longest half-lives being in water with the highest alkalinity (Goldsborough and Brown 1993).

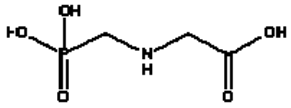
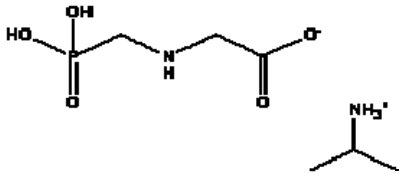
Field experiments suggest that glyphosate dissipates rapidly. Feng *et al.* (1990) intentionally over-sprayed a stream and recorded a maximum glyphosate concentration 2 hours after the aerial application (162 µg l^{-1}: 2 kg a.i. h^{-1} Roundup®). Concentrations in over-sprayed tributaries without a high cover of overhanging riparian vegetation increased after the first rainfall. In over-sprayed tributaries with a high cover of riparian vegetation almost no residues were found. Within 96 hours after application the residues in all water had declined below the detection limits. After rainstorms, peak concentrations of glyphosate were found in the sediments and on suspended particles of the over-sprayed tributaries, with maximum concentrations of 7 mg a.i. kg^{-1} dw in sediment and 0.06 µg a.i. l^{-1} in unfiltered water, respectively. The amounts in the sediments of these waters were variable but declined over time (0.1 – 2 mg residue kg^{-1} dw sediment 196 and 264 days after application).

Newton *et al.* (1994) determined the aquatic fate of glyphosate in a forest field study. Eight-hectare residual stands of low-quality hardwoods were treated with 4.12 kg ha^{-1} glyphosate (acid equivalent) applied aerially in late summer. Glyphosate residues in pond water peaked at 1-2 mg l^{-1} after application and declined to 10 µg l^{-1} within 2 – 10 days. Stream water received similar

initial concentrations (1.2 mg l^{-1}) but these declined more rapidly, reaching 48 µg l^{-1} within the first day. Sediment bound glyphosate levels reached 0.69 mg kg^{-1} in streams, and became non-detectable by 335 d post-treatment. AMPA appeared at low levels in all degrading matrices, including sediments, soon after deposition of glyphosate.

Bioconcentration of glyphosate in aquatic organisms is low. In carp (*Cyprinus carpio*) and tilapia (*Oreochromis mossambicus*) whole body BCFs ranged from 10.0 to 42.3 and 12.0 to 65.5, respectively depending on the period of exposure. Maximum accumulation was achieved after 5 to 7 days (Wang *et al*. 1994). These estimates of bioconcentration were based on total radioactivity and not on the identification of glyphosate residues.

Table 2.4 Physical and chemical properties of glyphosate and its environmentally significant salt

Property	Reference	Glyphosate	Glyphosate isopropylamine
CAS number	ECB 2005 EC 2002	1071-83-6	38641-94-0
Substance name	ECB 2005 (IUPAC)	*N*-(phosphonomethyl)-glycine	N-(phosphonomethyl)glycine, compound with 2-propylamine (1:1)
Molecular formula	HSDB 2006 EC 2002	$C_3H_8NO_5P$	$C_3H_8NO_5P.C_3H_9N$
Molecular structure	Chemfinder 2005		
Molecular weight	HSDB 2006 EC 2002	169.1	228.22
Colour/form	HSDB 2006 EC 2002	White solid Colourless crystals	White powder
Odour	HSDB 2006	Odourless	Odourless
Melting point (°C)	EC 2002 HSDB 2006	189.5°C 230°C	– Occurs in two steps at 143-164°C and 189-223°C
Boiling point (°C)	HSDB 2006 BCPC 2006	Decomposes above 200°C	Decomposes without boiling
Vapour pressure	HSDB 2006 EC 2002	9.8 x 10^{-8} mmHg at 25°C 1.31 x 10^{-5} Pa (25°C, acid)	1.58 × 10^{-8} mmHg at 25°C –
Density/ specific gravity	HSDB 2006 EC 2002 BCPC 2006	1.705 g cm^{-3} at 20°C	1.482 g cm^{-3} at 20°C
Henry's Law constant	HSDB 2006 BCPC 2006	2.1 x 10^{-12} atm m^3 mol^{-1} at 25°C 2.1 x 10^{-7} Pa m^3 mol^{-1} (calc)	4.6 x 10^{-10} Pa m^3 mol^{-1} at 25°C (calc)
Water solubility	IUCLID 2000	11.6 g l^{-1} at 25°C, pH 2.5	-

Property	Reference	Glyphosate	Glyphosate isopropylamine
	HSDB 2006 EC 2000	10.5 g l^{-1} at 20°C, pH 1.9	1.05 x 10^{6} mg l^{-1} at 25°C -
Solubility in organic solvents	HSDB 2006 EC 2002	Insoluble in common organic solvents, e.g. acetone, ethanol and xylene	Insoluble in common organic solvents

Table 2.5 Environmental fate and partitioning of glyphosate

Property	Glyphosate
Abiotic fate	According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere, glyphosate, which has a vapour pressure of 9.8 $x10^{-8}$ mm Hg at 25°C, is expected to exist solely in the particulate-phase in the ambient atmosphere. Particulate-phase glyphosate may be removed from the air by wet and dry deposition (HSDB 2006).
Hydrolytic stability	Glyphosate was stable to hydrolysis at pH 5, 7 and 9 at 5 - 35°C (HSDB 2006).
Photostability	Minimal photodegradation at pH 5, 7, or 9. The photolytic half-life of glyphosate in deionized water exposed outdoors to sunlight was ~ 5 weeks at 100 mg l^{-1} and 2 weeks at 2000 mg l^{-1}. The degradation product was aminomethylphosphonic acid (AMPA)(HSDB 2006) DT_{50} = 33 days at pH 5; 69 days at pH 7 and 77 days at pH 9 (xenon lamp, EC 2002).
Volatilisation	Volatilisation from water surfaces is not expected to be an important fate process because glyphosate exists as a zwitterion in water and ionic species do not volatilize (HSDB 2006).
Distribution in water/sediment systems	Glyphosate is expected to adsorb to suspended solids and sediment, based upon log Koc values of 3.42 – 3.69 (HSDB 2006). After 1 day: 47-64% in water, 31-44% in sediment; after 100 days: 3% in water, 29-44% in sediment (EC 2002).
Degradation in soil	Glyphosate is expected to have slight mobility in soils based upon reported Koc values. Mobility in soil is also affected by pH, phosphate levels and soil type. Glyphosate binds to organic matter and clay in soil and may also form insoluble complexes with metal ions. Half-life in soil is normally < 60 days (HSDB 2006). Microbial degradation seems to be the main environmental degradation pathway. In Swedish forest soils sprayed with Roundup, DT50 was <50 days and was dependent on the soil respiration rate (Torstensson *et al*. 1989).
Biodegradation in water	Not readily biodegradable (EC 2002). Field and laboratory studies have reported microbial degradation of glyphosate to AMPA and CO_2 with DT_{50} 2-14 days (Giesy *et al.* 2000).
Octanol–water coefficient (log Kow)	-3.40 (HSDB 2006) <3.2 (pH 2-5, 20°C) (BCPC 2006)
Log Koc	3.42 – 3.69 (HSDB 2006) 2.9 – 4.8 (4.25 sediment) (EC 2002)
Dissociation constant pKa	pKa1=2.3 at 20°C (phosphate acid) pKa2=5.7 at 20°C (secondary amine) pKa3=10.2 at 25°C (carboxylic acid (HSDB 2006))
Bioaccumulation BCF values	BCF values of 10 to 65.5 measured in fish suggest bioconcentration in aquatic organisms is low (Wang *et al*. 1994).

BCF = bioconcentration factor

2.6 Effects data

A summary of the mode of action of this substance can be found in Section 2.6.5.

Data collation followed a tiered approach. Initially, data were retrieved from the US Environmental Protection Agency (US EPA) ECOTOX database.[5] Further data sources used included:

- ScienceDirect®;[6]
- Hazardous Substances Data Bank (HSDB®) database of the US National Library of Medicine;[7]
- USDA, Forest Service, Human Health and Ecological Risk Assessment Final Report – Glyphosate (USDA 2003);[8]
- OPP Pesticide Ecotoxicity Database - an online US EPA database held by the Office of Pesticide Programs that summarises ecotoxicological data used by the EPA for ecotoxicological assessments. This consists primarily of the endpoint data submitted in support of registration and re-registration of pesticide products (OPP 2007)[9].
- US EPA Re-registration Eligibility Report (RED) for glyphosate (US EPA 1993 – referred to in this report as US EPA RED (1993);
- European Commission Plant Protection Products (PPP) review report on glyphosate (EC 2002);
- World Health Organisation Environmental Health Criteria (EHC) Monographs – 159 Glyphosate (WHO 1994)[10]

The last literature search was carried out in October 2009 and a further assessment was carried out in early 2012 to identify any additional data that was relevant to PNEC derivation. Only the most pertinent data have been used in the production of this report. All concentrations of glyphosate in this report are expressed relative to glyphosate acid (as a.e. (acid equivalent)). It is reasonable to assume that published toxicity values are similarly expressed unless otherwise stated; errors will result if this convention has not been observed. Where uncertainty exists as to the acid equivalent value this has been made evident in the text.

The data used in the report also includes confidential information that has been supplied by Monsanto Europe S.A. and Syngenta Ltd.

2.6.1 Toxicity to freshwater organisms

Freshwater toxicity data on glyphosate and its salts are available for various taxonomic groups including algae, invertebrates and fish as required for the application of the approach specified in the EU Technical Guidance Document (TGD) (ECB 2003). Long-term data are available for eight taxonomic groups: algae, amphibians, crustaceans, fish, macrophytes, molluscs, nematodes and

[5] http://www.epa.gov/ecotox/
[6] http://www.sciencedirect.com/
[7] http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
[8] http://www.fs.fed.us/foresthealth/pesticide/risk.shtml
[9] Http://www.ipmcenters.org/Ecotox/index.cfm
[10] http://www.inchem.org

nematomorpha. Freshwater short-term toxicity data are available for eight taxonomic groups: algae, amphibians, crustaceans, fish, insects, macrophytes, molluscs and protozoa (Table 2.6).

Table 2.6 Summary of available freshwater data for glyphosate (technical and glyphosate IPA salt)

Type of data	Taxonomic groups for which information is available
Long-term	Algae, amphibians, crustaceans, fish, macrophytes, molluscs, nematodes and nematomorpha
Short-term	Algae, amphibians, crustaceans, fish, insects, macrophytes, molluscs and protozoa

Diagrammatic representations of the available freshwater data (cumulative distribution functions) for glyphosate, including glyphosate IPA salt, are presented in Figure 2.1 for long-term data and Figure 2.2 for short-term data. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. However, they are not species sensitivity distributions and have not been used to derive glyphosate PNECs.

The lowest critical freshwater data are presented in Table 2.8 for long-term toxicity data and Table 2.9 for short-term toxicity data. These tables do not contain all the available toxicity data but only those which are considered most relevant to the derivation of PNECs.

Figures 2.1 and 2.2 indicate that the toxicity of glyphosate occurs over a wide concentration range. The available short-term and long-term toxicity test data show that, for glyphosate and its salts, aquatic plants are the most sensitive taxa of those tested. This is consistent with the use of the substance as a herbicide. It should be noted that at very low concentrations algal populations may be enhanced. However, it is not clear whether this is a hormetic effect or a stimulation of algal growth due to the utilization of glyphosate as a nutrient source by algae.

The data were also evaluated to assess whether measured differences in toxicity were due to different physical formulation effects (e.g., the use of the same chemical formulation but including either technical grade material or a salt with a surfactant). A number of studies have been conducted which assess the toxicity of the different forms and components of technical-grade chemical (glyphosate acid, glyphosate IPA salt), formulations (Roundup) and the surfactant (see Table 2.7). The majority of species show lower sensitivity to the technical product or its salts than glyphosate formulated with surfactant. The data also show that the IPA salt is generally less toxic than glyphosate acid. The exception to this is the result from the study by Bringolf *et al*. (2007) who found that the glyphosate IPA salt was 10-fold more toxic to the freshwater mussel, *Lampsilis siliquiodea*, than technical grade glyphosate. Bringolf *et al*. (2007) used a technical grade glyphosate IPA salt with purity > 95%. They concluded that the liberation of ammonia from the amine group of the IPA upon addition to the water was a possible contributory factor to the toxicity observed. The concentration of ammonia in the technical-grade glyphosate IPA treatments (up to 180 µg N l^{-1}) was correlated strongly (r^2 = 0.994) with glyphosate IPA concentration. The purity of the IPA salt in the other studies ranged between 56.8 and 60.5%. According to The Pesticide Manual (BCPC 2006) glyphosate IPA is a wet cake that contains approximately 62% w/w IPA salt and approximately 35% water as the major impurity.

Much of the toxicity data in the peer-reviewed literature focuses on commercial formulations of glyphosate with particular attention given to Roundup. Roundup is formulated with the surfactant POEA (polyethoxylated tallowamine). Two aquatic fish toxicity studies (Folmar *et al*.1979, Wan *et al*. 1989) have been conducted on glyphosate, the POEA surfactant, and a Roundup formulation

which allow a quantitative assessment of the relative toxicities of glyphosate and POEA. Both of these studies indicate that POEA is substantially more toxic than glyphosate and that POEA surfactant is the primary toxic agent of concern. Tsui and Chu (2003) investigated the relative toxicity contributions of glyphosate salt and POEA to the overall toxicity of Roundup using microalgae, protozoa and invertebrates. They found that POEA accounted for up to 86% of the relative toxicity found in organisms exposed to glyphosate formulation and that POEA exhibits higher toxicity in alkaline media.

Surfactants interact with biological membranes and can be expected to alter the structure, physical properties and function. The degree to which this occurs depends on the physical and chemical properties of the surfactant. Possible mechanisms by which surfactants might exert biological effects or affect the toxicity of glyphosate include: decreasing surface tension; perturbing membrane permeability or transport function of membranes or other diffusion barriers (e.g. leaf cuticle). There is no evidence for specific mechanisms of interactions between glyphosate and surfactants (Diamond and Durkin 1997).

Tsui and Chu (2003) also observed that glyphosate acid and glyphosate IPA salt generally lowered the pH of the test media. This lowering of pH was greater with glyphosate acid and may account for the lower LC50s seen in the tests with glyphosate acid compared to those carried out with glyphosate IPA salt.

Folmar *et al*. (1979) also found glyphosate to be less toxic to fish at higher pH compared to lower pH. 96-hour LC50s were 140000 and 240000 µg a.e. l^{-1} at pH 6.5 and 9.5, respectively, for both rainbow trout and bluegills. Wan *et al*. (1989) found that increasing water hardness and pH value appear to be key contributors to the variation seen in 96-hour LC50 values for juvenile salmonids.

Commercial glyphosate formulations are mixtures of compounds and each constituent will follow its own environmental fate pathways depending on its chemical and physical properties. This can result in temporal differences in the arrival of components of the formulation reaching a water course once applied. Given the disparity in toxicity values between glyphosate and the formulations containing surfactants only data on glyphosate and glyphosate IPA salt have been used for the derivation of PNECs.

Once the product is added to the spray tank, the salt ion is easily dissociated from the glyphosate parent molecule. Thus, the glyphosate that reaches the leaf surface is often not associated with the cation with which it was formulated. Furthermore, it should be noted that statutory monitoring of glyphosate will result in determination of glyphosate in the acid form and not as any preparation.

Table 2.7 Comparison of acute toxicity to different taxa of glyphosate, glyphosate IPA salt, glyphosate formulation Roundup®, and the surfactant POEA

Species	Taxa	Endpoint	Value (µg a.e. l^{-1})				Reference
			Glyphosate acid	Glyphosate IPA salt	Roundup®	POEA	
Pseudokirchneriella subcapitata	ALG	96 h EC50	24700	41000	5810	3920	Tsui and Chu 2003
Ceriodaphnia dubia	CRU	48 h LC50	147000	415000	5390	1150	
Skeletonema costatum	ALG	96 h EC50	2270	5890	1850	3350	
Acartia tonsa	CRU	48 h LC 50	35300	49300	1770	570	
Lampsilis siliquiodea	MOL	96 h EC50	> 200000	7200	5900	3800	Bringolf *et al*. 2007
Litoria moorei	AMP	48 h LC50	81200	>343000	2900	-	Mann and Bidwell 1999
Rana clamitans	AMP	96 h LC50	-	>28600	2000	2200	Howe *et al*. 2004
Oncorhynchus mykiss (formerly Salmo gairdneri)	FIS	96 h LC50	140000	-	7600	7400	Folmar *et al*. 1979
Lepomis macochirus	FIS	96 h LC50	140000	-	4200	1300	Folmar *et al*. 1979
Oncorhynchus mykiss	FIS	96 h LC50	197000	-	4340	1700	Wan *et al.* 1989

Figure 2.1 Cumulative distribution function of freshwater long-term data (μg a.e. l^{-1}) for glyphosate (technical and glyphosate IPA salt)

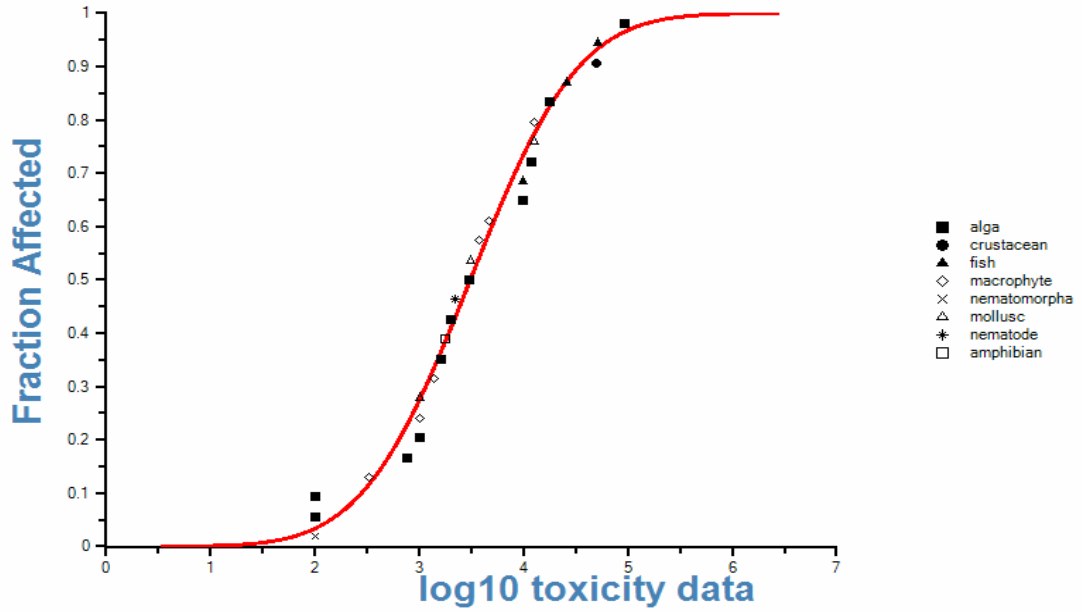


Figure 2.2 Cumulative distribution function of freshwater short-term data (μg a.e. l^{-1}) for glyphosate (technical and glyphosate IPA salt)

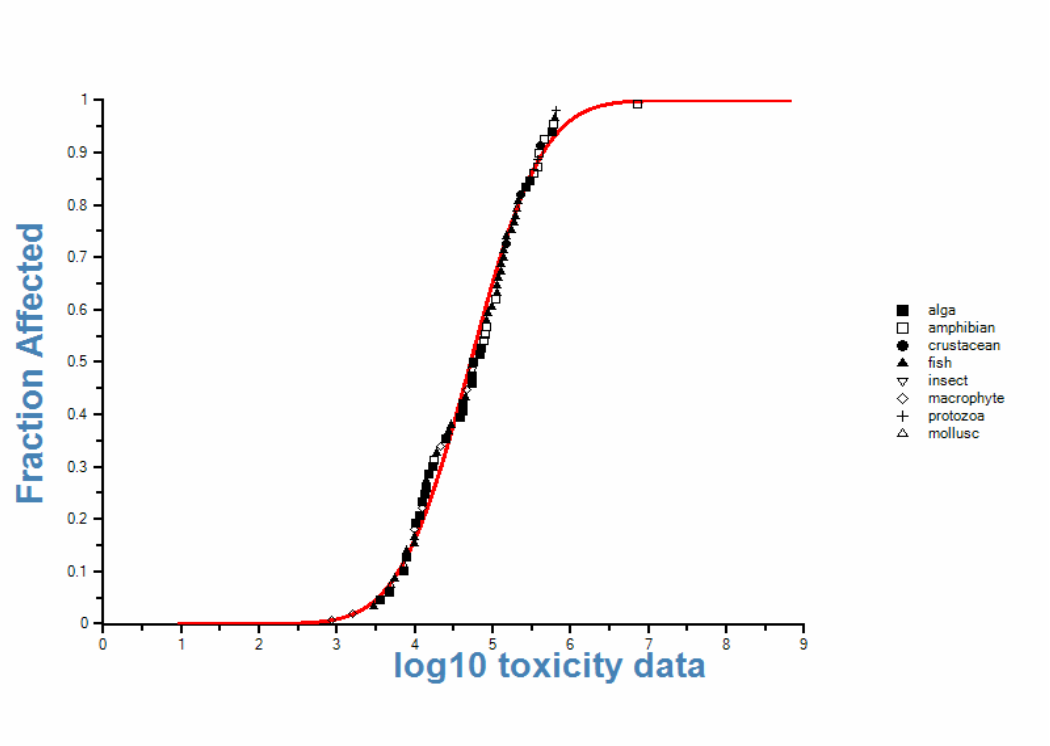


Table 2.8 Most sensitive long-term aquatic toxicity data for freshwater organisms exposed to glyphosate (technical and glyphosate IPA salt)

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. (μg a.e. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
Glyphosate acid, purity 97.5%	*Scenedesmus acutus*	Green alga	ALG	Growth rate	NOEC	72 hours	100	s	n	T = 24±2°C	3	Vendrell *et al*. 2009
Glyphosate acid, purity 97.5%	*Chlorella vulgaris*	Green alga	ALG	Growth rate	NOEC	72 hours	100	s	n	T = 24±2°C	3	Vendrell *et al*. 2009
99.5% a.i. IPA salt	*Scenedesmus quadricauda*	Green alga	ALG	Growth	NOEC[3]	96 hours	1600	s	n	T = 22°C	2	Saenz *et al.* 1997
Glyphosate acid, purity 97.5%	*Scenedesmus subspicatus*	Green alga	ALG	Growth rate	EC10	72 hours	1600	s	n	T = 24±2°C	3	Vendrell *et al*. 2009
Glyphosate acid, purity 97.5%	*Chlorella saccharophila*	Green alga	ALG	Growth rate	EC10	72 hours	3000	s	n	T = 24±2°C	3	Vendrell *et al*. 2009
99.5% a.i. IPA salt	*Scenedesmus acutus*	Green alga	ALG	Growth	NOEC[3]	96 hours	4000	s	n	T = 22°C	2	Saenz *et al.* 1997
Glyphosate acid 95.6% purity	*Pseudokirch-neriella subcapitata*	Green alga	ALG	Growth rate & biomass	NOEC	120 hours	10000	s	y	T = 24±1°C pH3.5 – 8.9	2	Smyth *et al*. 1995 (Confidential data)
Glyphosate acid 95.6% purity	*Anabaena flos-aquae*	Blue-green alga	ALG	Growth rate & biomass	NOEC	120 hours	12000	s	y	T = 24±1°C pH3.5 – 8.2	2	Smyth *et al*. 1996d (Confidential data)
Glyphosate acid 95.6% purity	*Navicula pelliculosa*	Diatom	ALG	Growth	NOEC	120 hours	19000	s	y	T = 24±1°C pH3.7 – 8.7	2	Smyth *et al*. 1996b (Confidential data)
Glyphosate acid > 95% purity	*Pseudokirch-neriella subcapitata*	Green alga	ALG	Growth rate	EC10	72 hours	92500	s	n	T = 22°C pH 8	2	Cedergreen and Streibig 2005
Higher plants												
Glyphosate acid 97% purity	*Myriophyllum sibiricum*	Water milfoil	MAC	Growth rate	NOEC	14 days	332	s	m	T = 25°C	2	Roshon 1997

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. (μg a.e. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Glyphosate acid 95.6% purity	*Lemna gibba*	Duckweed	MAC	Growth	NOEC	14 days	1400	ss	y	T = 25±1°C pH 3.5 – 5.8	1	Smyth *et al.* 1996a (Confidential data)
Glyphosate acid purity not stated	*Potamogeton pectinatus*	Sago pondweed	MAC	Growth	MATC	28 days	3162	s	n	T = 20-23°C	2	Fleming *et al*. 1991
Glyphosate acid > 95% purity	*Lemna minor*	Duckweed	MAC	Growth rate	EC10	7 days	3780	s	n	T = 24°C pH 5	2	Cedergreen and Streibig 2005
Glyphosate acid 95% purity	*Lemna gibba*	Duckweed	MAC	Growth rate	EC10	10 days	4600	s	y	T = 24±2°C pH 6.5 - 7.8	2	Sobrero *et al*. 2007
Glyphosate acid 96.6% purity	*Lemna gibba*	Duckweed	MAC	Growth	EC10	14 days	12690	s	y	T = 25±2°C	2	Hughes 1987a (Confidential data)
Invertebrates												
Glyphosate acid 95% purity	*Chordodes nobilii*	Horsehair worm	NEMA	Embryo/larval viability	LOEC	96 hours	100	ss	y	T = 23±1°C pH 7 – 7.5	3	Achiorno *et al*. 2008
Glyphosate acid purity not stated	*Caenorhabditis elegans*	Roundworm	NEM	reproduction	MATC	72 hours	2214	s	n	T = 20°C	2	Ruan *et al*. 2009
IPA salt > 95% purity	*Lampsilis siliquoidea*	mussel	MOL	Survival	NOEC	28 days	3100	ss	y	T = 21.1 – 21.8°C pH 8.2 – 8.76 DO > 83% saturation	2	Bringolf *et al*. 2007
Glyphosate acid 97% purity	*Pseudosuccinea columella*	Snail	MOL	Hatching success (3rd generation)	MATC	12 days[4]	3162	ss	n	T = 25±2°C pH 6.8 – 7.2	2	Tate *et al*. 1997
Glyphosate acid 98% purity	*Lampsilis siliquoidea*	mussel	MOL	Growth	NOEC	21 days	12500	ss	y	T = 21.1 – 21.8°C pH 8.2 –	2	Bringolf *et al*. 2007

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. (µg a.e. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
										8.76 DO > 83% saturation		
Glyphosate acid 99.7% purity	*Daphnia magna*	Waterflea	CRU	Reproduction	NOEC	21 days	50000	f	y	T = 20°C pH = 6.1 – 8.1 DO = 6.4 - 9.0 mg l^{-1}	1	McAllister 1982 (Confidential data)
Vertebrates (Fish and amphibians)												
Glyphosate IPA salt, purity 56.8%	*Rana pipiens*	Northern Leopard frog	AMP	Survival/ development/ length	NOEC	42 day	>1800	ss	y	T = 20±1°C pH = 7.8 –8.3	2	Howe *et al*. 2004
Technical grade 62% purity	*Cyprinus carpio*	Carp	FIS	Histopath-ologic changes	Effect	14 days	10000	ss	n	T = 20°C	2	Neskovic *et al.* 1996
Glyphosate acid 87.3% purity	*Pimephales promelas*	Fathead minnow	FIS	Survival/ Growth/ Reproduction	NOEC	254 days	> 25700	f	y	T = 25±1°C pH = 6.5 – 7.6 DO = 8.1 mg l^{-1}	1	E G & G Bionomics, 1975 (Confidential data)
Glyphosate acid 97.67% purity	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival/ growth	NOEC	21 day	52000	f	y	T = 14-15°C pH = 5.9 – 7.8 DO = 7.6 - 8.4 mg l^{-1}	1	Bowman 1989 (Confidential data)

[1] Exposure: s = static; ss = semi-static; f = flow-through
[2] Toxicant analysis: y = measured; n = not measured; u = unknown
[3]NOEC calculated as LOEC/2 as size of effect at LOEC estimated as >10 < 20 % relative to control.
[4]1st and 2nd generations continuously exposed prior to 12-day hatching period of 3rd generation snails (time period not stated)
ALG = alga, AMP = amphibians; CRU = crustacean, FIS = fish, MAC = macrophyte; MOL = molluscs; NEM = nematoda; NEMA = nematomorpha
NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
MATC = maximum allowable toxicant concentration (= geomean of NOEC and LOEC)
EC10 = concentration effective against 10% of the organisms tested
DO = dissolved oxygen, T = temperature

Table 2.9 Most sensitive short-term aquatic toxicity data for freshwater organisms exposed to glyphosate (technical and glyphosate IPA salt)

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
Glyphosate acid, purity 95%	*Chlorella pyrenoidosa*	Green algae	ALG	Growth	EC50	96 hours	3530	s	n	T = 25°C	2	Ma *et al.* 2001
Glyphosate acid, purity 95%	*Chlorella vulgaris*	Green algae	ALG	Growth	EC50	96 hours	4696	s	n	T = 25°C	2	Ma *et al.* 2002
99.5% a.i. IPA salt	*Scenedesmus quadricauda*	Green alga	ALG	Growth	EC50	96 hours	7200	s	n	T = 22°C	2	Saenz *et al.* 1997
Glyphosate acid, technical grade purity not stated	*Pseudokirch-neriella subcapitata*	Green alga	ALG	Growth inhibition	EC50	96 hours	7800	s	n	T = 24±2°C	2	St-Laurent *et al*. 1995
99.5% a.i. IPA salt	*Scenedesmus acutus*	Green alga	ALG	Growth	EC50	96 hours	10200	s	n	T = 22°C	2	Saenz *et al.* 1997
Glyphosate acid 95.6% purity	*Navicula pelliculosa*	Diatom	ALG	Growth	EC50	120 hours	17000	s	y	T = 24±1°C pH3.7 – 8.7	2	Smyth *et al*. 1996b (Confidential data)
Glyphosate acid 95.6% purity	*Pseudokirch-neriella subcapitata*	Green alga	ALG	Growth rate	EC50	120 hours	21000	s	y	T = 24±1°C pH3.5 – 8.9	2	Smyth *et al*. 1995 (Confidential data)
Glyphosate acid ≥97% purity	*Pseudokirch-neriella subcapitata*	Green alga	ALG	Growth (biomass)	EC50	96 hours	24700	s	y	T = 25±1°C pH 7.5	1	Tsui and Chu 2003
Glyphosate acid 95.6% purity	*Anabaena flos-aquae*	Blue-green alga	ALG	Growth rate	EC50	120 hours	38000	s	y	T = 24±1°C pH3.5 – 8.2	2	Smyth *et al*. 1996d (Confidential data)
Glyphosate acid 96.7% purity	*Chlorococcum hypnosporum*	Green alga	ALG	Growth rate	EC50	96 hours	68000	s	y	T=25°C	2	Maule and Wright 1984

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Glyphosate acid, 95% purity	*Pseudo-kirchneriella subcapitata*	Green algae	ALG	Growth inhibition	EC50	96 hours	129000	s	n	T = 20±2°C	2	Pereira *et al*. 2009
Glyphosate acid > 95% purity	*Pseudokirch-neriella subcapitata*	Green alga	ALG	Growth rate	EC50	72 hours	270000	s	n	T = 22°C pH 8	2	Cedergreen and Streibig 2005
Higher plants												
Glyphosate acid, 97% purity	*Myriophyllum sibiricum*	Water milfoil	MAC	Root length	EC50	14 days	844	s	m	T = 25°C	2	Roshon 1997
Glyphosate acid 95.6% purity	*Lemna gibba*	Duckweed	MAC	Growth (no. of fronds)	EC50	14 days	12000	ss	y	T = 25±1°C pH 3.5 – 5.8	1	Smyth *et al.* 1996a (Confidential data)
Glyphosate acid 95% purity	*Lemna gibba*	Duckweed	MAC	Growth rate	EC50	10 days	20500	s	y	T = 24±2°C pH 6.5 - 7.8	2	Sobrero *et al*. 2007
Glyphosate acid 96.6% purity	*Lemna gibba*	Duckweed	MAC	Growth	EC50	14 days	25500	s	y	T = 25±2°C	2	Hughes 1987a (Confidential data)
Glyphosate acid > 95% purity	*Lemna minor*	Duckweed	MAC	Growth rate	EC50	7 days	46900	s	n	T = 24°C pH 5	2	Cedergreen and Streibig 2005
Invertebrates												
IPA salt > 95% purity	*Lampsilis siliquoidea*	Mussel (glochidia)	MOL	Immobility	EC50	48 hours	5000	ss	y	T = 21.1 – 21.8°C pH 8.2 – 8.76 DO > 83% saturation	2	Bringolf *et al*. 2007
IPA salt > 95% purity	*Lampsilis siliquoidea*	Mussel (juveniles)	MOL	Immobility	EC50	96 hours	7200	ss	y	T = 21.1 – 21.8°C pH 8.2 – 8.76 DO > 83% saturation	2	Bringolf *et al*. 2007

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Glyphosate acid, technical grade purity not stated	*Chironomus plumosus*	Chironomid	INS	Immobility	EC50	48 hours	55000	s	n	T = 22°C pH 7.2	2	Folmar *et al.* 1979
Glyphosate acid 95.6% purity	*Daphnia magna*	Waterflea	CRU	-	EC50	48 hours	134000	s	u	-	C/4	OPP 2007
Glyphosate acid ≥ 97% purity	*Ceriodaphnia dubia*	Waterflea	CRU	Survival	LC50	48 hours	147000	s	y	T = 25±1°C pH 8.07	1	Tsui and Chu 2003
Glyphosate acid 98% purity	*Lampsilis siliquoidea*	Mussel (juveniles)	MOL	Immobility	EC50	96 hours	>200000	ss	y	T = 21.1 – 21.8°C pH 8.2 – 8.76 DO > 83% saturation	2	Bringolf *et al*. 2007
IPA salt 56.8% purity	*Tetrahymena pyriformis*	Ciliate	PRO	Growth inhibition	EC50	40 hours	386000	s	y	T = 27±1°C pH 7.4	1	Tsui and Chu 2003
Glyphosate acid, purity 95%	*Daphnia magna*	Waterflea	CRU	Immobility	EC50	48 hours	>2000000	s	n	T = 20±2°C	2	Pereira *et al*. 2009
Vertebrates (fish and amphibians)												
Glyphosate acid 95% purity	*Jordanella floridae*	Flagfish	FIS	Survival	LC20	96 hours	2940	s	y	T = 25.3°C pH 7.96 DO = 8.3 mg l^{-1}	3	Holdway and Dixon 1988
Glyphosate acid 94% purity	*Cyprinus carpio*	Carp	FIS	Survival	LC50	48 hours	5500	s	n	T = 22°C	3	Wang *et al.* 1994
Glyphosate acid 94% purity	*Oreochromis mossambicus*	Tilapia	FIS	Survival	LC50	48 hours	7900	s	n	T = 22°C	3	Wang *et al.* 1994

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. ($\mu g\ l^{-1}$)	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Technical grade 88.5 – 95.4%	*Oncorhynchus mykiss*	Rainbow trout	FIS	Survival	LC50	96 hours	10000	s	y	T = 14°C pH 6.3	2	Wan *et al.* 1989
Glyphosate acid 95% purity	*Jordanella floridae*	Flagfish	FIS	Survival	LC20	96 hours	29600	s	y	T = 25.3°C pH 7.96 DO = 8.3 mg l^{-1}	2	Holdway and Dixon 1988
Glyphosate acid 95.6% purity	*Lepomis macrochirus*	Bluegill sunfish	FIS	Survival	LC50	96 hours	45000	s	u	-	C/4	OPP 2007
Glyphosate acid 87.3% purity	*Pimephales promelas*	Fathead minnow	FIS	Survival	LC50	24 hours	84900	s	n	T = 19°C pH 3.7 -7.0	2	E G & G Bionomics, 1975 (Confidential data)
Glyphosate acid technical grade purity not stated	*Pimephales promelas*	Fathead minnow	FIS	Survival	LC50	96 hours	97000	s	n	T = 22°C pH 7.2	2	Folmar *et al*. 1979
Glyphosate IPA salt, purity 56.8%	*Rana clamitans*	Green frog	AMP	Survival	LC50	96 hours	>28700	ss	y	T = 20±1°C pH 7.8 –8.3	2	Howe *et al*. 2004
Technical grade IPA salt	*Crinia insignifera*	Australian frog	AMP	Survival	LC50	96 hours	78000	s	u	-	S/4	OPP 2007
Technical grade glyphosate acid	*Litoria moorei*	Australian tree frog	AMP	Survival	LC50	48 hours	81200	ss	y	T = 23.4 – 25.4°C pH 2.9 - 7.7	1	Mann and Bidwell 1999

[1] Exposure: s = static; ss = semi-static
[2] Toxicant analysis: y = measured; n = not measured; u = unknown
ALG = alga, AMP = amphibian, CRU = crustacean, FIS = fish, INS = insect, MAC = macrophyte, MOL = mollusc, PRO = protozoan
LC/EC50 = concentration necessary to cause mortality to/effect in 50% of test organisms

OPP 2007 – this is an online US EPA database held by the Office of Pesticide Programs that summarises ecotoxicological data used by the EPA for ecotoxicological assessments. It consists primarily of the endpoint data submitted in support of registration and reregistration of pesticide products.

Data are classified by the US EPA as ‘core’ if all essential information was reported and the study was performed according to recommended US EPA or American Society for Testing Materials (ASTM) methodology. Minor inconsistencies with standard recommended procedures may be apparent, but the deviations do not detract from the study's soundness or intent. Studies within this category fulfil the basic requirements of current FIFRA guidelines and are acceptable for use in a risk assessment (equivalent Klimisch code 1). Data not meeting this requirement are classified as either supplemental (Klimisch code 2) or invalid (Klimisch code 3). Where this data has been reported in Tables 2.9 and 2.11 the following notation has been used to identify the US EPA classification: C = core and S = supplemental.

The Klimisch codes assigned in the tables reflect the quality assessment given by the authors of this report therefore, the data reported from this source is Klimisch code 4 (secondary literature). However, since the acceptability of each study is stated, according to the criteria given above, it was felt that this should be reported as it aids interpretation of the overall dataset. These data are considered in the round and influence the AF, however, under previous peer review it was considered inappropriate to set the PNEC on a value for which the full study details were not available. It is not unknown for the details to be incorrectly recorded on the database and therefore since we have studies available on which to base the PNEC these are to be preferred.

2.6.2 Toxicity to saltwater organisms

Single species short-term toxicity data for glyphosate and glyphosate IPA salt for saltwater organisms are available for six different taxonomic groups: algae, crustaceans, echinoderms, fish, molluscs and protozoans. Long-term toxicity data are available for algae and eelgrass (*Zostera*).

Diagrammatic representations of the available saltwater data (cumulative distribution functions) for glyphosate, including glyphosate IPA salt, are presented in Figure 2.3 for long-term data and Figure 2.4 for short-term data. These diagrams include all data regardless of quality and provide an overview of the spread of the available data. However, they are not species sensitivity distributions and have not been used to derive glyphosate PNECs.

The lowest critical saltwater data are presented in Table 2.10 for long-term toxicity data and Table 2.11 for short-term toxicity data. These tables do not contain all the available toxicity data but only those which are considered most relevant to the derivation of PNECs.

Figure 2.3 Cumulative distribution function of all saltwater long-term data (µg a.e. l^{-1}) for glyphosate (technical and glyphosate IPA salt)

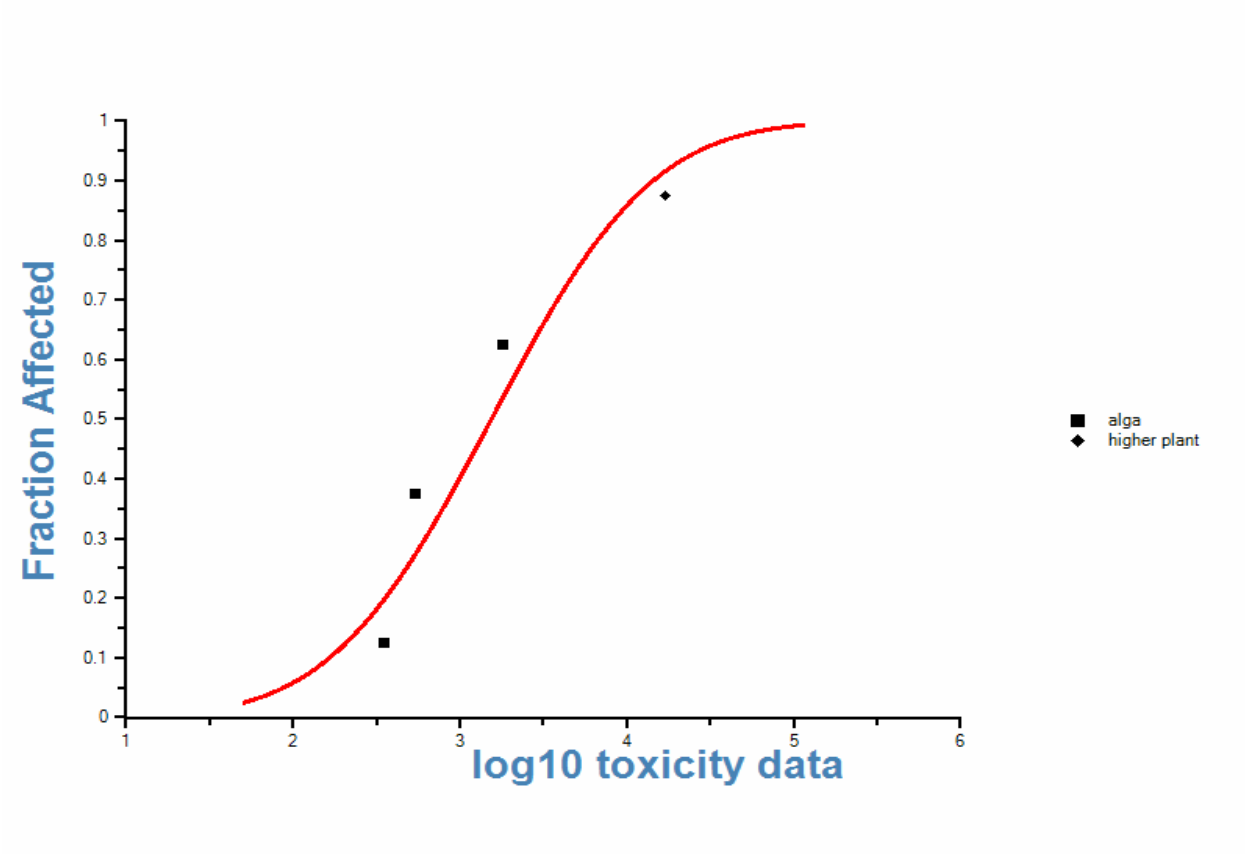


Figure 2.4 Cumulative distribution function of saltwater short-term data (μg a.e. l^{-1}) for glyphosate (technical and glyphosate IPA salt)

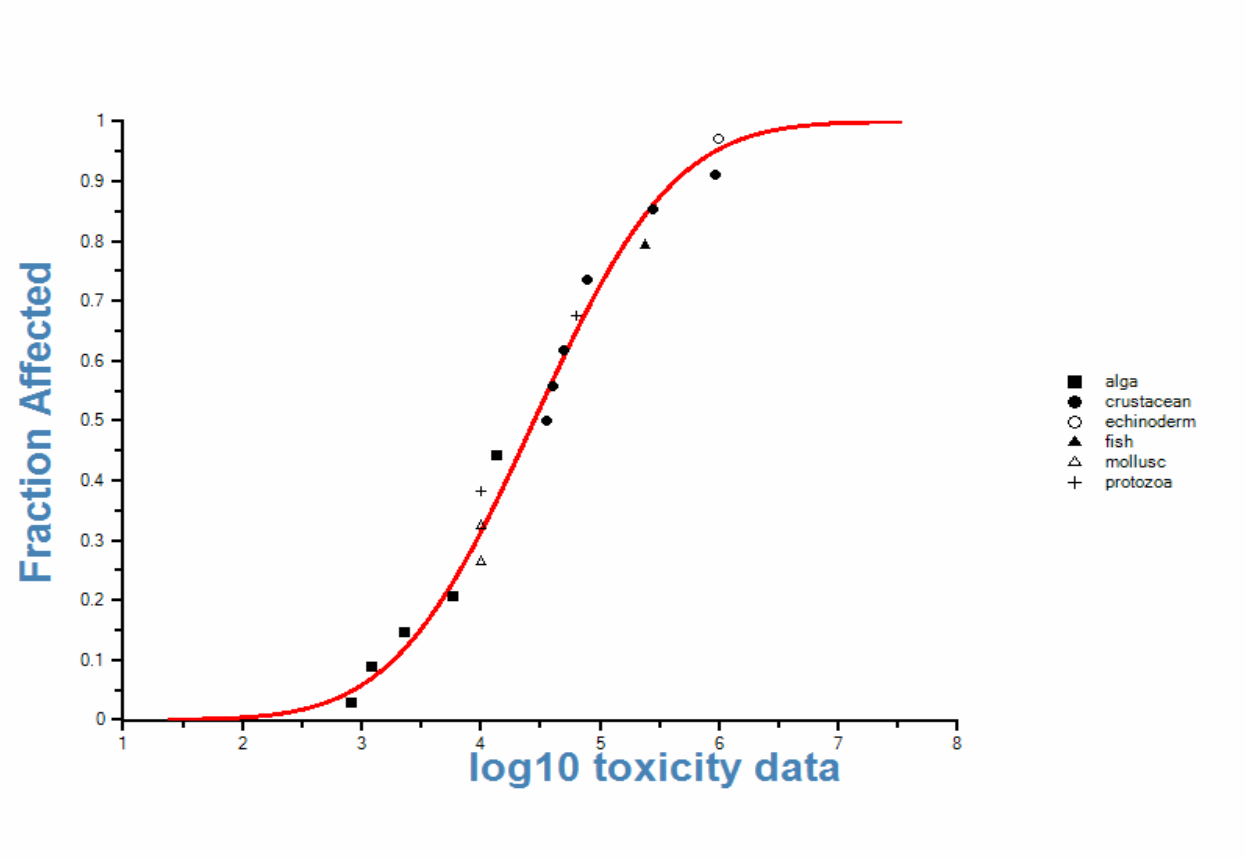


Table 2.10 Most sensitive long-term aquatic toxicity data for saltwater organisms exposed to glyphosate

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. (μg a.e. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
Glyphosate acid 96.6% purity	*Skeletonema costatum*	Diatom	ALG	Growth (cell density)	EC10	168 hours	348	s	y	T = 20°C Salinity 30‰	3	Hughes 19878b (Confidential data)
Glyphosate purity not stated	*Skeletonema costatum*	Diatom	ALG	Growth (cell numbers and chlorophyll *a*)	EC10	96 hours	534	s	n	T = 20±1°C Salinity 30‰ pH 8.2 - 8.5	2	EG & G Bionomics 1978 (Confidential data)
Glyphosate acid 95.6% purity	*Skeletonema costatum*	Diatom	ALG	Growth (cell density)	NOEC	72 hours	1800	s	y	T = 20±1°C Salinity 30.5‰ pH 7.1 - 8.8	1	Smyth *et al.* 1996c (Confidential data)
Higher plants												
Glyphosate acid purity not stated	*Zostera marina*	Eelgrass	-	Growth rate	NOEC	72 hours	>16900	s	n	T = 10°C Salinity 12 - 15‰	2	Nielsen and Dahllöf 2007

[1] Exposure: s = static
[2] Toxicant analysis: y = measured; n = not measured
ALG = alga,
NOEC = no observed effect concentration
EC10 = concentration necessary to cause an effect in 10% of test organisms

Table 2.11 Most sensitive short-term aquatic toxicity data for saltwater organisms exposed to glyphosate (technical and glyphosate IPA salt)

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. (μg a.e. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Algae												
Glyphosate acid 96.6% purity	*Skeletonema costatum*	Diatom	ALG	Growth (cell density)	EC50	7 days	831	s	y	T = 20°C Salinity 30‰	3	Hughes 1987b (confidential data)
Glyphosate purity not stated	*Skeletonema costatum*	Diatom	ALG	Growth (chloro-phyll *a*)	EC50	96 hours	1200	s	n	T = 20±1°C Salinity 30‰ pH 8.2 - 8.5	2	EG & G Bionomics 1978 (Confidential data)
Glyphosate acid ≥ 97% purity	*Skeletonema costatum*	Diatom	ALG	Growth inhibition	EC50	96 hours	2270	s	y	T=20±1°C Salinity 30‰	1	Tsui and Chu 2003
56.8% a.i. IPA salt	*Skeletonema costatum*	Diatom	ALG	Growth inhibition	EC50	96 hours	5890	s	y	T=20°C Salinity 30‰ pH 8	1	Tsui and Chu 2003
Glyphosate acid 95.6% purity	*Skeletonema costatum*	Diatom	ALG	Growth (growth rate)	EC50	72 hours	18000	s	y	T = 20±1°C Salinity 30.5‰ pH 7.1-8.8	1	Smyth *et al.* 1996c (Confidential data)
Invertebrates												
Glyphosate acid purity ≥ 97%	*Euplotes vannus*	Ciliate	PRO	Growth inhibition	EC50	48 hours	10100	s	Y	T=20±1°C Salinity 30‰ pH 8	1	Tsui and Chu 2003
Glyphosate acid purity not stated	*Ruditapes decussatus*	Clam	MOL	Survival	NOEC	96 hours	>10000	ss	n	T=22°C Salinity 30‰	2	Elandalloussi *et al*. 2008
Glyphosate acid purity 96.7%	*Crassostrea virginica*	Virginia oyster	MOL	Survival	LC50	48 hours	>10000	s	n	T=25°C Salinity 20 - 35‰	4	WHO 1994
Glyphosate acid purity ≥ 97%	*Arcartia tonsa*	copepod	CRU	Survival	LC50	48 hours	35300	s	y	T=20±2°C Salinity 30‰ pH8	1	Tsui and Chu 2003

Chemical formulation	Scientific name	Common name	Taxonomic group	Effect	Endpoint	Test duration	Conc. (μg a.e. l^{-1})	Exposure[1]	Toxicant analysis[2]	Comments	Reliability (Klimisch Code*)	Reference
Glyphosate acid purity 95.6%	*Americamysis bahia* < 24 h old	Shrimp	CRU	Survival	LC50	96 hours	40000	s	u	-	C/4	OPP 2007 (Brixham Laboratory, UK)
56.8% a.i. IPA salt	*Arcartia tonsa*	Copepod	CRU	Survival	LC50	48 hours	49300	s	Y	T=20±2°C Salinity 30‰ pH8	1	Tsui and Chu 2003
56.8% a.i. IPA salt	*Euplotes vannus*	Ciliate	PRO	Growth	EC50	48 hours	64090	s	y	T=20±1°C Salinity 30‰ pH 8	1	Tsui and Chu 2003
Glyphosate acid, technical grade purity not stated	*Tripneustes esculentes*	Edible sea egg	ECH	Immobility	EC50	96 hours	> 1000000	s	n	T=20°C Salinity 20 - 25‰ pH 7.7-8.2	4	EG &G Bionomics 1978 cited in WHO 1994
Fish												
Glyphosate acid 95.6% purity	*Cyprinodon variegatus*	Sheepshead minnow	FIS	Survival	LC50	96 hours	240000	s	u	-	C/4	OPP 2007

[1] Exposure: s = static
[2] Toxicant analysis: y = measured; n = not measured; u= unknown
ALG = alga, CRU = crustacean, ECH = echinoderm, FIS = fish, MOL = mollusc, PRO = protozoa
NOEC = no observed effect concentration
LC/EC50 = concentration necessary to cause mortality to/effect in 50% of test organisms

2.6.3 Toxicity to sediment dwelling organisms

An environmental assessment report written by the US Fish and Wildlife Service gives only brief details of studies undertaken by Kubena *et al.* (1996) and Kubena (1996). Ten day bioassays were carried out with *Leptocheirus plumulosus* and *Eohaustorius estuarius* in 1 litre test chambers to which Willapa Bay (Lewis Unit) sediment spiked with Rodeo® and R-11® Spreader Activator was added and covered with seawater from Puget Sound. For these 10 day bioassays, glyphosate and octylphenol polyethoxylates up to the maximum concentrations tested (2066 and 165 mg kg^{-1}, respectively) did not affect amphipod survival (Kubena 1998). Kubena *et al.* (1996) conducted 96 hour bioassays with Pacific oysters (*Crassostrea gigas*) in which Willapa Bay sediment spiked with Rodeo® and surfactant (X-77® Spreader, R-11® Spreader Activator or LI-700®) was added to 2.8 ml chambers with seawater from Puget Sound. For these bioassays, glyphosate and nonylphenol polyethoxylates, octylphenol polyethoxylates or phosphatidylcholine concentrations at 5122 mg kg^{-1} and 256, 410, or 224 mg kg^{-1}, respectively, reduced survival of larval oysters. Tsui and Chu (2004) cite 96-hour LC50 values of Rodeo in sediment to be 3988 and 13368 mg kg^{-1} for *C. gigas* and *E. estuarius*, respectively, from the same studies.

Tsui and Chu (2004) in their own study, spiked Roundup Bioactive and Roundup into a clean sediment which was amended with appropriate amounts of peat moss to study the effect of different organic carbon levels (0, 0.4, 1.2 and 2.1%) on sediment toxicity, with *Ceriodaphnia dubia* exposed to overlying water or pore water prepared from the contaminated sediments. Sediment-porewater partitioning of glyphosate was found to be influenced by sediment organic carbon (i.e, glyphosate adsorption increased with increasing sediment organic carbon). From the pore water toxicity tests, Roundup Biactive (48 h LC50 = 340 mg kg^{-1}) and Roundup (48 h LC50 = 244 mg kg^{-1}) were similarly toxic in the sediment tests at 0% organic carbon. An increase in organic carbon significantly decreased the toxicity of Roundup in sediment to 312, 594 and 746 mg kg^{-1} at 0.4, 1.2 and 2.1% total organic carbon, respectively. The results for Roundup Bioactive are less conclusive. The 48 hour LC50 values did not differ significantly from each other at any of the organic carbon levels. Adsorption of glyphosate to the sediment may have been influenced by the unknown surfactant in this formulation.

Both Tsui and Chu (2003) and Hartman and Martin (1984) report increased toxicity of glyphosate formulations to *Ceriodaphnia dubia* and *Daphnia pulex*, respectively, in the presence of suspended sediment. In the Tsui and Chu (2003) study, although the addition of kaolin clay at 150 and 200 mg l^{-1} was significantly more toxic than at 0 – 100 mg l^{-1} at 48 hours the addition of suspended particles alone at these higher levels was accompanied by unacceptable levels of control mortality making it impossible to separate toxicity due to glyphosate. Hartman and Martin (1984) used a single suspended sediment concentration of 50 mg kaolin clay l^{-1}, which had no effect on control mortality and increased the toxicity of glyphosate more than twofold.

2.6.4 Endocrine-disrupting effects

A report submitted to the USDA forest service concluded that extensive testing in experimental animals and wildlife provided reasonably strong evidence that glyphosate is not an endocrine disruptor (SERA 2002). The list of purported endocrine disruptors compiled by the Institute of Environment and Health (IEH 2005) lists glyphosate as a substance with no evidence of potential endocrine-disrupting effects.

Two more recent studies have shown that high concentrations of glyphosate can damage cortisol response to stress in fish, induce unexplained changes in plasma estradiol and can affect reproduction (Cericato *et al*. 2008, Soso *et al*. 2007). These studies were carried out using formulation products. No details are available on the surfactant present in the formulations and it is possible that these substances may have contributed to the effects seen.

2.6.5 Mode of action of glyphosate

Plants

Glyphosate is a broad-spectrum, systemic, post-emergence herbicide. Glyphosate's primary action is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP). This is a key enzyme in the shikimic acid pathway for the synthesis of chorismate, which is the precursor for the essential amino acids phenylalanine, tyrosine and tryptophan (Giesy *et al.* 2000). These acids are used by plants in protein synthesis and to produce many secondary plant products (e.g., growth promotors, growth inhibitors, phenolics, lignin). Decreased rates of synthesis of protein, indole acetic acid (a plant hormone) and chlorophyll have also been noted. Death of the plant is slow and is first observed as a cessation of growth, followed by chlorosis and then necrosis of plant tissues (Solomon and Thompson 2003).

Aquatic animals

Activity toward animals is weak because the mode of action for glyphosate is a biochemical pathway apparently unique to plants and some micro-organisms (Giesy *et al.* 2000). No studies were available that examined the mode of action of glyphosate in aquatic organisms.

2.6.6 Mesocosm and field studies

Several mesocosm and field studies have been conducted in relation to the use of glyphosate for the control of aquatic weeds. For the majority of these studies glyphosate is applied mixed with a surfactant.

Freshwater mesocosm and field studies

Austin *et al. (*1991) studied the effects of glyphosate (Vision®) on periphyton in artificial streams. An increase in periphyton was noted at concentrations of 1.9 to 287.4 µg a.e. l^{-1}. The authors suggest that the algae used glyphosate as a phosphorus source and that glyphosate could contribute to eutrophication of nutrient poor but oxygen-rich waterways.

Gardner and Grue (1996) applied a mixture of glyphosate (Rodeo and the surfactant LI700) to a freshwater wetland in central Washington State, USA, for the control of purple loosestrife, with the purpose of studying the effects on non-target organisms. The study took place over 5 weeks. *In situ* toxicity tests were conducted using duckweed, *Lemna gibba*, *Daphnia* (*D. magna*) and rainbow trout (*Oncorhynchus mykiss*) placed in test chambers/cages. In addition, samples of benthic and water column invertebrates were monitored 24 hours and 7 days post-spray using activity traps and sediment cores. Measured glyphosate concentrations in water samples taken 5 cm below the water surface immediately following the spray (at the recommended application rate of 1 l ha^{-1} Rodeo) ranged from 10 µg l^{-1} to 100 µg $^{-1}$. The effects on trout survival could not be assessed due to low survival (0 – 22%) in both the controls and treated areas. There were no significant differences in survival of *Daphnia* following the application of Rodeo. However, there were a significantly lower number of duckweed fronds alive after 48 h exposure in the treated areas compared to controls. The phytotoxicity observed most likely resulted from direct exposure to the herbicide spray rather than exposure to glyphosate in the water. With respect to the water column invertebrate assemblages, there was a significant increase in copepods detected 7 days post-spray in the treated areas. A significant increase in the average number of branchiopods at the control sites 7 days post-spray was not replicated in the treated areas, suggesting that the herbicide may have depressed branchiopod populations. No effects were seen in benthic invertebrates.

Cole *et al. (*1997) sampled six species of amphibians on red alder sites 1 year before and 1 and 2 years after the following treatments: i) control (uncut), ii) clearcut and broadcast burned and iii) clearcut, broadcast burned and then sprayed with the herbicide glyphosate. All sites included uncut riparian buffer strips. Based on capture rates, glyphosate spraying had no effect on populations of the species monitored. The reduction in ensantina salamanders (*Ensantina eschscholtzii*) and Pacific giant salamanders (*Dicamptodon tenebrosus*) were attributed to the loss of red alder habitat rather than the method of removal.

Perschbacher *et al.* (1997) found no effects on plankton productivity, zooplankton populations or water quality after simulated direct spraying of 500 l outdoor pond mesocosms without a mud substrate, with Roundup at an application rate of 0.43 a.i. kg ha^{-1}.

Linz *et al.* (1999) assessed the response of invertebrates to a major reduction in cattail coverage caused by glyphosate applied to wetlands in North Dakota, USA, one and two years post-treatment. Six wetlands were aerially-treated with 5.8 l ha^{-1} glyphosate (Rodeo) mixed in aqueous solution containing X-77 surfactant and a further four wetlands served as references. Numbers of Crustacea, Hydracarina, Oligochaeta, Copepoda, Ostracoda and Cladocera were similar between treated and reference wetlands, while abundance of Gastropoda was greater in the treated wetlands. Insect abundance was greater in treated wetlands, with activity traps yielding highest numbers in July. Corixidae and Chironomidae were more abundant in treated wetlands, whereas Chaoboridae were consistently more plentiful in the reference wetlands. The authors concluded that these results were secondary effects due to the reduction in cattail coverage rather than a direct result of glyphosate application.

Relyea (2005b) simulated a direct overspray of a small wetland using 1200 l polyethylene tanks filled with 1000 litres of well water. Roundup® was applied to give a nominal concentration of 3.8 mg a.i. l^{-1}. The tanks had been stocked with algae and 25 species of aquatic animals including six species of larval amphibians. These species naturally coexist and the densities of each species were based within the range of natural densities. The experiment was a randomized design with five pesticide treatments (carbaryl, malathion, 2,4-D and glyphosate plus control) that were replicated six times for a total of 30 experimental units. The organisms were exposed for 2 weeks after which time the experiment was terminated. Compared to the control tanks species richness was 22% lower in the Roundup treated tanks. The glyphosate formulation showed no effect on zooplankton, insect predators or snails. However, periphyton biomass was 40% greater. 100% mortality occurred in leopard frogs (*Rana pipiens*) and gray tree frogs (*Hyla versicolor*) and there was 98% mortality of wood frogs (*R. sylvatica*). Roundup did not have a significant effect on toads, spring peepers, and the spotted salamanders, although few toads survived in the control treatments making it difficult to assess the effects of the herbicide. Relyea (2005c) conducted a subsequent study with Roundup at the same application rate in outdoor tanks in the absence of predators in order to eliminate this potential confounding factor. In this study either soil or loam was added, as the presence of sediment is known to adsorb glyphosate and thus reduce concentrations in the water column. The presence of a substrate and the absence of predators did not alter mortality rates substantially from those seen in the first study. After 3 weeks mortality rates were 96 – 100% for the three larval amphibian species exposed. No water samples were taken for analytical confirmation of glyphosate concentrations in either study. These results would appear to be consistent with the 96-hour EC50s reported for other amphibian species (Mann and Bidwell 1999, Howe *et al*. 2004) exposed to Roundup. Howe *et al*. (2004) also demonstrated that the surfactant in Roundup was also toxic to amphibians. The evidence shows that glyphosate has some impact on larval amphibian development; however it is minimal when compared to the affect of its formulation surfactants.

Seasonal variation in the response of riverine microbial communities to an environmentally relevant exposure to glyphosate (10 µg l^{-1}) was assessed on natural communities collected in spring and summer, using two14-day microcosm studies (Pesce *et al*. 2009). Glyphosate concentrations remained relatively constant until day 6 in both the spring (9°C) and summer (14°C) microcosms. Between day 6 and day 14 glyphosate concentrations decreased by approximately 30% in spring

microcosms and to below the quantification limit (0.5 µg l^{-1}) in summer microcosms. pH values fluctuated between 8.4 and 8.8.

In spring the algal community was composed of pinnate diatoms (*Navicula* (86%), *Gomphonema* and *Nitzschia* species), and remained relatively constant throughout the 14-day exposure period with no significant differences in genera distribution between the control and treated microcosms. The summer algal community consisted of diatoms, as in spring, together with *Asterionella* sp. *Cyclotella* sp. and Chlorophycea (*Oocystis* and *Scenedesmus* species). Significant differences were observed between control and treated microcosms. *Asterionella, Cyclotella*, and *Oocystis* disappeared between day 0 and day 3. From day 3 there was a high predominance of pinnate diatoms (80-95%) and *Scenedesmus*. Community structure was highly variable in the control microcosms

In summary, these mesocosm and field data support the results of laboratory single species tests indicating that aquatic invertebrates are not sensitive to glyphosate formulations when compared with algae. The amphibian studies indicate that whilst laboratory tests and semi-field studies indicate high sensitivity to a number of glyphosate formulations these are not easily monitored in field situations.

Saltwater mesocosm and field studies

Garnett *et al.* (1992) investigated the use of glyphosate to control *Spartina* in three separate trials around the coasts of Britain. Two treatments were applied: glyphosate (1.8 kg a.e. ha^{-1}) and glyphosate (1.8 kg a.e. ha^{-1}) plus an additive (2% of spray solution). The additive contained 50% w/w nonylphenol ethylene oxide condensate and 50% w/w primary alcohol ethylene oxide condensate. In the Lindisfarne National Nature Reserve, Northumberland, they found significant depressions in populations of the gastropod *Hydrobia ulvae* and juvenile bivalves, *Macoma balthica*, 1 day after spraying compared to 1 day before treatment. The decrease was greatest in plots treated with glyphosate plus the additive. These populations had increased to levels similar to or greater than prespray densities 1 year later. The gastropods *Littorina saxatilis* and *Littorina littoralis*, showed no significant changes and *Tubifex* spp. responses were inconsistent. In the Dee estuary (Cheshire), changes in nematodes, the amphipod *Corophium arenarium*, the oligocheate *Lumbricillus* spp. and the polychaete *Spiophanes* spp. were not interpretable due to the high degree of natural variation in the untreated replicates. In the Dyfi Estuary (Dyfed) counts of *Corophium volutator* declined after treatment but had recovered 7 weeks after spraying.

Simenstad *et al.* (1996) conducted a study to evaluate the potential effects on mudflat benthic communities of glyphosate control of smooth cordgrass in Willapa National Wildlife Refuge in Washington State, USA. Measurement of the concentration of glyphosate in the first tidal inundation after spray application from a helicopter was 25.6 µg l^{-1} (application rate 4.7 l ha^{-1} Rodeo; 0.9 l ha^{-1} X-77 surfactant). Benthic microflora and invertebrates were sampled before and 1, 14, 28 and 119 days after spraying. Natural variability in the aquatic communities and inherent differences in the treatment sites precluded strong inferential tests of acute effects. However, there were no indications of either short- or long-term effects on the mudflat communities.

Nielsen and Dahllöf (2007) investigated the effect of glyphosate on a natural phytoplankton population from Roskilde Fjord six times during one year to account for seasonal species variation. Surface water sampled from the fjord was filtered through a 45 µm filter to remove larger zooplankton. In December and January the density was low and the phytoplankton was dominated by dinoflagellates. In April the density had increased tenfold mainly due to diatoms and euglenoids. In June the phytoplankton was totally dominated by the mixotrophic ciliate *Mesodinium rubrum*. By October density was decreasing and dinoflagellates were again dominant. Triplicate samples, from each time period, were exposed to 16.9, 169 and 1690 µg glyphosate acid l^{-1}. Primary production was used as the endpoint for effects on phytoplankton using incorporation of $H^{14}CO_3^-$, measured following 48 hours exposure. No significant effects were found on ^{14}C incorporation at any time of year.

3 Calculation of PNECs as a basis for the derivation of quality standards

3.1 Derivation of PNECs by the TGD deterministic approach (AF method)

3.1.1 PNECs for freshwaters

PNEC accounting for the annual average concentration

For the freshwater environment, data are available for the 'base set' of toxicity tests (i.e., tests with algae, crustaceans and fish) and therefore the EU TGD assessment factor (AF) method can be applied (ECB 2003). Reliable long-term data were available for eight taxonomic groups (algae, amphibians, crustaceans, fish, macrophytes, molluscs and nematodes and nematomorpha) for glyphosate and glyphosate IPA salt. Based on the information available algae and macrophytes appear to be the most sensitive taxa to glyphosate. Table 2.8 summarises the most sensitive long-term freshwater toxicity data that were found.

Algae

Data were available for a number of algal species. The life cycle of aquatic micro-organisms is short, ranging from hours to days, meaning that even relatively short exposure periods are representative of multi-generational studies. Current recommended algal study guidelines stipulate an exposure period of 72- to 96-hours. Three of the studies were carried out in support of pesticide registration under US EPA FIFRA (Federal Insecticide, Fungicide and Rodenticide) guidelines and extend over a five day period (120 hours) although NOEC and EC50 values are also reported for 72- and 96-hours. Responses varied with NOEC and EC10 values ranging from 100 to 92500 µg a.e. l^{-1} with considerable differences noted for the same species. The most sensitive results come from a study that has been assessed as unreliable (Vendrell *et al*. 2009).

Vendrell *et al*. (2009) tested the toxicity of glyphosate (analytical standard, purity 97.5%) on four species of freshwater green algae, *Scenedesmus acutus*, *S. subspicatus*, *Chlorella vulgaris* and *C. saccharophila.* The study exposed the algae to various glyphosate concentrations using microplate bioassays and growth was measured at 450 nm wavelength using a microplate reader. It should be noted that this is a non-standard methodology which uses high inoculum levels and is not readily comparable with OECD guideline criteria. After 72 hours growth rates were determined and compared using standard statistical methods (ANOVA followed by multiple range tests) and EC10 and EC50 values were estimated by the linear regression of probit of percentage growth on log concentration of glyphosate. The results suggest that the data did not provide a good fit to the model. The two EC10 values reported for *C. saccharophila* and *S. subspicatus* of 3000 and 1600 µg l^{-1}, respectively, both had negative value lower 95% confidence intervals and for the other two species, EC10 values and their confidence limits at 95% were negative. Glyphosate concentrations that were reported to cause significant differences in growth rates compared to control values ranged from 1560 to 100000 µg l^{-1} for *C. saccharophila*, from 100 to 100000 µg l^{-1} for *C. vulgaris*, from 100 to 50000 µg l^{-1} for *S. acutus* and 390 to 50000 µg l^{-1} for *S. subspicatus*. Very little

information is provided as to the % inhibition seen at the different concentrations. The authors note that growth reduction was particularly high at or above 25000 µg glyphosate l^{-1} for all four species. The tabulated data provided in the paper shows that for all four species growth rates are very similar between 100 and 12500 µg l^{-1} glyphosate with no clear concentration-response. Without access to the raw data it is not possible to assess whether these results represent a statistically significant or biologically significant difference at these lower concentrations.

Saenz *et al*. (1997) report the effects of glyphosate IPA salt and the commercial formulation Ron-do® on population growth and chlorophyll-*a* content in the green algae, *S. acutus* and *S. quadricauda*. The study used what is described as technical grade isopropylamine salt with a high purity of 99.5%. This has led to some reviewers suggesting that the test substance was in fact the parent substance, glyphosate acid. Whilst IPA salt of this purity is unusual, in that most tests use IPA salt with purity ranging between 56 to 65%, one other study (Bringolf *et al*. 2007) that also used technical grade glyphosate IPA salt with purity given as > 95% has been identified. Ron-do® contains 48% active ingredient, as glyphosate IPA salt and 15% surfactant oxide-coco-amide-propyl dimethyl amine. The results reported in Saenz *et al*. (1997) do not follow the general rule that has been seen whereby aquatic plants are more sensitive to glyphosate formulations relative to glyphosate IPA salt (see Section 2.6.1). This rule is primarily based on effects seen with Roundup which contains the toxic surfactant POAE. The effects of the surfactant in the formulation used by Saenz *et al*. (1997) are unknown and may account for the atypical result.

There is a concern with the Saenz *et al*. (1997) study in so much as there is an inconsistency between the text and the results given in tabular form. According to the text, Dunnett's procedure was used to calculate the NOEC values. Using this method of calculation one would expect the NOEC to correspond with one of the test concentrations used in the study. Both NOECs are considerably lower than the lowest test concentration used and are unlikely to be EC10 values. The NOEC values were given as 2000 µg $l^{-1}$for *S. acutus* and 770 µg l^{-1} for *S. quadricauda*. According to the text for *S. acutus* 10, 76 and 89% growth inhibition was seen at 8000, 12000 and 16000 µg l^{-1}, respectively and for *S. quadricauda* 15, 60, 75 and 80% inhibition was seen at 3200, 12500, 25000 and 50000 µg l^{-1}, respectively. These values are also consistent with the figures presented in the paper. In contrast, the 96-hour EC50 tabulated values are consistent with the text and figures presented in the paper. According to the TGD, a LOEC may be used to derive a NOEC as follows: - LOEC > 10 and < 20% effect: NOEC can be calculated as LOEC/2. This approach has been adopted in this report.

Two results are available for green alga, *Pseudokirchneriella subcapitata*. In unbuffered water, in which the pH was allowed to drop with increasing concentrations of glyphosate, the 5-day growth rate NOEC was 10000 µg a.e. l^{-1} (Smyth *et al*. 1995). In buffered water in which the pH was held at pH 8, the 72-hour EC was 92500 µg a.e. l^{-1} (Cedergreen and Streibig 2005). Glyphosate and the IPA salt of glyphosate generally lower the pH of the test media, the effect of which is stronger in the former (Tsui and Chu 2003). Small pH changes have the potential to be either stimulatory or inhibitory due to increases or decreases in CO_2 and trace nutrient availability, depending on the species and growth medium used in the tests. The recommended pH of the nutrient medium at the start of the test is pH 7.5 (EPA 1996) and pH 8.1 (OECD 2006). The pH values at the start of the exposure in the unbuffered system (Smyth *et al.* 1995) were pH 6.6 in the 10000 µg a.e. l^{-1} treatment and pH 5.9 in the next highest test concentration.

Smyth *et al*. (1996b) reported a 120-hour growth rate NOEC of 18000 µg a.e. l^{-1} (nominal) for a freshwater diatom *Navicula pelliculosa*. All significant differences identified in the four lowest nominal test concentrations (1800 to 10000 µg a.e. l^{-1}), were due to growth enhancement. Throughout the course of the study no significant differences were determined in any of the analyses for the 18000 µg a.e. l^{-1} test concentration. The 120-hour growth rate ErC50 was calculated as 17000 µg a.e. l^{-1}. This result which is below the NOEC is most probably a statistical quirk due to stimulatory effects at the lower concentrations influencing the estimation. The mean measured glyphosate concentration is reported in Table 2.8.

Macrophytes

The most sensitive result is a 14-day NOEC for water milfoil, *Myriophyllum sibiricum*, of 332 µg a.e. l^{-1} (Roshon 1997). No significant differences between control plants and those treated with 332 µg a.e. l^{-1} were observed for the following endpoints: area under the growth curve (shoot height), increase in shoot length, root length, chlorophyll *a* (apical dry weight) and carotenoid content (apical dry weight). The IC25 values for these same endpoints ranged from 559 µg a.e. l^{-1} (chlorophyll *a*) to 1283 µg a.e. l^{-1} (shoot length). The lowest IC50 of 844 µg a.e. l^{-1} was for root length.

Three results are available for duckweed, *Lemna gibba*, ranging from 1400 to 12690 µg a.e. l^{-1}. Smyth *et al*. (1996a) report a 14-day growth (frond number) NOEC of 2900 µg a.e. l^{-1} (measured) and a 14-day growth (weight increase) NOEC of 5600 µg a.e. l^{-1} (measured). However, they also noted other symptoms of toxicity. From day 2 onwards plants in the nominal concentrations ≥ 23000 µg a.e. l^{-1} exhibited, concentration-related symptoms, which included pale frond colouration, emergence of stunted new fond growths and reduced root growth. The plants also floated in an unnatural manner. These symptoms were noted at 12000 µg a.e. l^{-1} from day 5 and at 5600 µg a.e. l^{-1} from day 9. On day 14, a small number of fronds (< 5%) were observed to be showing pale colouration in all three nominal 3000 µg a.e. l^{-1} test replicates. For this reason the authors recommended an overall study NOEC of 1400 µg a.e. l^{-1}.

The study by Sobrero *et al*. (2007) produced a 10-day growth rate EC10 of 4600 µg a.e. l^{-1} (nominal). The pH in this study was higher, (pH = 6.5 – 7.8) compared to Smyth *et al*. (1996a) where pH ranged between 3.5 and 5.8.

Hughes (1987a) reported a 14-day growth (frond number) EC25 and EC50 of 18000 and 25500 µg a.e. l^{-1}, respectively based on measured concentrations. A stimulatory effect was seen at the lowest two concentrations (4280 and 9020 µg a.e. l^{-1}, producing 1.8 and 3.6% stimulation, respectively) and these data were omitted from the regression analysis. OECD guidance (Guideline 201) suggests that low concentration stimulation can usually be ignored in EC50 calculations unless it is extreme. Where an ECx value for low x is to be calculated, special procedures may be needed if available curve fitting software cannot accept minor stimulation. An EC10 was recalculated using the Toxicity Relationship Analysis Program (TRAP) from the U.S. EPA National Health and Environmental Effects Research Laboratory (NHEERL). The threshold least squares nonlinear regression analysis model was the analysis option selected from the TRAP program to calculate EC values as it provided the best data fit (p= 0.988). This resulted in an EC10 value of 12690 (95% CI: 5040 – 31960) µg a.e. l^{-1} and an EC50 value of 23160 µg a.e. l^{-1}.The effect of glyphosate addition on the pH of the test medium is not reported.

Invertebrates

The most sensitive result is a 96-hour larval viability LOEC of 100 µg a.e. l^{-1} for the horsehair worm, *Chordodes nobilii*. This species belongs to the Nematomorpha, which is a group of worm-like animals similar to nematodes. Adults are free-living and reproduction takes place in freshwater environments, where preparasitic larvae undergo development. All species have a parasitic juvenile stage. Bioassays were performed with embryos and larvae (preparasitic stage) and, therefore, cover a sensitive period of development. The exposure period was equivalent to approximately 10% of the embryonic phase. Test organisms were exposed to glyphosate at concentrations between 0.01 and 8.0 mg a.e. l^{-1}. Embryo development was unaffected but there was a significant decrease in the infective capacity of larvae derived from eggs that had been exposed to ≥ 0.1 mg l^{-1}. A similar result was obtained for directly exposed larvae with an exposure period of 48-hours. A concurrent study was performed using a glyphosate formulation described as 'like' Roundup (35.2% w/v). No differences in toxicity were detected between the active ingredient and formulated product. A bioassay with adults was only performed with the formulated product. After 96-hours exposure, followed by a 48-hour recovery period in clean media, 50% ± 12.91% (n = 4) mortality was observed in horsehair worms exposed to 1.76 mg a.e.l^{-1}. This study is not considered to meet relevancy criteria for study selection. The most sensitive endpoint is not based on direct toxicity but

an indirect effect on the ability to infect its host. Little comparative data is available with which to assess the result obtained. Generally, these larvae are passive invaders of hosts, ingested rather than actively seeking a host, and host-organism-specific. There are no data to support the conclusions made by the authors that the effects seen are indicators of a decline in its ecological role. It is possible that a reduced number of worms within a host may lead to increased reproductive output due to decreased competition.

Ruan *et al*. (2009) exposed the nematode *Caenorhabditis elegans* to glyphosate at concentrations between 7 and 7000 µg l^{-1}. Exposures were carried out for 72-hours with the addition of food and for 24-hours in the absence of food starting with L4 larvae. *C. elegans* has a short life cycle of approximately 55 hours for the development from eggs to adults, therefore the exposure time period can be regarded as chronic. After exposure for 72-hours the brood size declined in a concentration-dependent manner, but only the brood size of the group exposed to the highest test concentration reached statistical significance. The decrease in brood size at this concentration was 20% compared with control values. Generation time was extended but did not reach statistical significance. There was a 10-fold difference between the highest test concentration and the next lowest concentration in the series. Given that the effect seen was 20% the true NOEC is likely to lie somewhere between 700 and 7000 µg l^{-1}, therefore, the geometric mean (MATC) has been used as a surrogate. This is more conservative than using a LOEC/2 which is an alternative that is used under the TGD when the LOEC is the lowest test concentration in a series. Use of the MATC is a convention practiced by other jurisdictions (US EPA, Environment Canada) and is considered appropriate in this instance.

The acute and chronic toxicity of various forms of glyphosate to early life stages of the freshwater mussel, *Lampsilis siliquoidea*, was determined by Bringolf *et al*. (2007). A 28-day survival NOEC of 3100 µg a.e. l^{-1} was determined for two-month post-transformation juveniles exposed to technical grade glyphosate IPA salt. No effect on survival was found for one-month post-transformation juveniles exposed to glyphosate acid at concentrations up to 200000 µg a.e. l^{-1}. However, significant effects on juvenile growth were seen at concentrations > 12500 µg a.e. l^{-1}. Whilst the liberation of ammonia from the amine group of the IPA upon addition to the water was considered a possible contributory factor to the toxicity observed the amount of ammonia present was considerably below the 96-h LC50 values reported for juvenile mussels (Augspurger *et al*. 2003).

The effects of glyphosate on the aquatic snail, *Pseudosuccinea columella*, intermediate host of the sheep liver fluke, are equivocal making it difficult to assess the relevance of the findings. Tate *et al*. (1997) exposed snails to concentrations of 100, 1000, and 10000 µg a.e. l^{-1} for three generations. No marked effects were noted on the first or second generations. In the third generation, snail embryos exposed to 1000 a.e. µg l^{-1} developed much faster than those exposed at 100 or 10000 a.e. µg l^{-1} and faster than control snails. Hatching, however, was inhibited at 10000 a.e. µg l^{-1}. In a follow up study, Tate *et al*. (2000) noted effects on concentrations of amino acids in snails (specifically alanine, glycine, glutamic acid and threonine) at the same concentrations. The mechanism for the effect of glyphosate on amino acid and protein metabolism is not known. As the concentration interval between the LOEC and NOEC is 10-fold a MATC has been calculated.

A flow-through 21-day life cycle toxicity test (McAllister, 1982) was conducted to determine the toxicity of glyphosate to *Daphnia magna*. No significant decreases in survival or growth (length) of adult daphnids were observed. Length of daphnids in the lowest and highest test concentrations, (26000 and 378000 µg a.e. l^{-1}, respectively) were significantly longer than controls. Reproduction was significantly decreased at the three highest concentrations but significantly increased at the lowest test concentration compared to controls. The increased reproduction at the lowest test concentration, as well as the reproduction in the control, was within the range of production obtained in the controls of nine previous *Daphnia magna* chronic studies conducted at ABC Laboratories and was discounted as an effect of exposure to glyphosate.

Vertebrates

The most sensitive result is an unbounded 42-day survival/development NOEC of 1800 µg a.e. l^{-1} for the northern leopard frog, *Rana pipiens*, (Howe *et al*. 2004). Tadpoles, Gosner stage 25, were exposed to 600 and 1800 µg a.e. l^{-1}, as glyphosate IPA salt, in a static renewal system for 42-days followed by rearing in clean water. The rate of development was assessed by recording the number of days taken to reach Gosner stage 42 (forelimb emergence) for each surviving tadpole from the first day of exposure. There was no significant difference in the time taken to forelimb emergence between tadpoles exposed to glyphosate IPA salt and controls.

Available studies indicate that glyphosate has low toxicity to fish. The most sensitive result is a 14-day effect for histologic changes in the gills, kidneys, and liver of carp, *Cyprinus carpio*, exposed to 10000 µg a.e. l^{-1} (Neskovic *et al*. 1996). In this study, carp were exposed to technical grade glyphosate but the purity was only 62%, much lower than that used in other studies with technical grade material. The authors also report a 96-hour LC50 value for the technical grade glyphosate in carp as 620000 (95% CI 607000-638000) µg l^{-1}, which is higher than values for more highly purified forms of glyphosate (see Table 2.9) in trout and bluegill sunfish. The sub-lethal studies were conducted over 14-days of exposure to concentrations of 2500, 5000, 10000 µg a.e. l^{-1}. At 10000 a.e. µg l^{-1} abnormal histopathologic changes were noted in the gills and liver. At 5000 µg l^{-1} abnormal histopathologic changes were noted only in the gills. These changes were accompanied by increased alkaline phosphatase activity. While these effects cannot be directly associated with potential longer term effects on fish populations, the histologic changes in the gills and liver are potentially adverse.

The most comprehensive long-term study on fish is the 254-day life-cycle study on fathead minnow, *Pimephales promelas*, (E G & G Bionomics 1975). No effect on mortality or reproduction was observed at a concentration of 25700 µg a.e. l^{-1} using 87.3% pure technical grade glyphosate.

PNEC accounting for the annual average concentration

The lowest reliable long-term NOEC is the 14-day growth NOEC of 332 µg a.e. l^{-1} for the macrophyte, *Myriophyllum sibiricum*, (Roshon 1997). As long-term NOECs for at least three freshwater species representing three trophic levels (i.e. algae, crustaceans and fish) are available, the appropriate assessment factor in accordance with the TGD is 10. This results in:

$PNEC_{freshwater\_lt}$ = 332 µg l^{-1}/AF (10) = 33 µg l^{-1} glyphosate (rounded)

Since glyphosate has been shown to dissipate rapidly it is unlikely that aquatic organisms would be exposed to glyphosate for sufficiently long to result in chronic toxicity unless field application is repeated, allowing a continuous release of herbicide into the water body, or there is a continuous discharge from point sources. The PNEC calculated above is sufficiently protective of the effects seen and is likely to be conservative under most natural conditions. Also worth noting is the fact that natural water has higher buffering capacity than test media in resisting pH change.

PNEC accounting for a maximum allowable concentration

Freshwater short-term toxicity data are available for eight taxonomic groups (algae, amphibians, crustaceans, fish, insects, macrophytes, molluscs and protozoans). Table 2.9 summarises the most sensitive short-term freshwater toxicity data found for glyphosate and glyphosate IPA salt.

As expected, glyphosate has the greatest effects on algae and macrophytes. Data are also available for crustaceans, insects, molluscs, fish, and amphibians, which are considerably less sensitive than plants, with reliable toxicity values ranging from 7200 to 2000000 µg a.e. l^{-1} (Table 2.10).

Based on guidance in the TGD on effects assessment for intermittent releases [Section 3.3.2 of Part II of the TGD document (ECB 2003)] and the fact that there is a considerable acute toxicity database for freshwater organisms, an assessment factor of 10 rather than 100 should be applied to the lowest reliable acute data for the submersed macrophyte, *Myriophyllum sibiricum,* (Roshon 1997). This results in:

$PNEC_{freshwater\_st}$ = 844 µg l^{-1}/AF (10) = 84 µg l^{-1} glyphosate (rounded)

3.1.2 PNECs for saltwaters

The effects database for marine species is considerably smaller than that for freshwater organisms. Short-term toxicity data are available for six different taxonomic groups (algae, crustaceans, echinoderms, fish, molluscs and protozoans) and long-term data are only available for the alga *Skeletonema costatum* and the higher plant *Zostera marina*.

Three results are available for the diatom, *Skeletonema costatum*, ranging from 348 to 1800 µg a.e. l^{-1}. The most sensitive result is a 7-day cell density EC10 for the diatom, *Skeletonema costatum*, of 348 µg a.e. l^{-1} glyphosate, purity 96.6% (Hughes1987b). Hughes (1987b) cultured and tested *S. costatum* in a medium based on artificial seawater. Compared with the medium used for culturing the alga, the additions for testing excluded EDTA and contained 50% lower metals, nutrient salts and vitamins. *Skeletonema* is known to grow poorly when cultured in artificial seawater medium (Missimer *et al.* 1989). The ISO (2006) standard method states 'For *Skeletonema* the use of natural seawater may be necessary for the long-term maintenance of cultures and may also be necessary for the test medium because a synthetic seawater medium may not always support sufficient growth to meet the test quality criteria.' The control growth rates obtained by Hughes (1987b) do not meet the test validity criterion now required by ISO (2206) for a control growth rate of at least 0.9 d^{-1}. No information on pH values in the test medium is reported. For these reasons this study was considered unreliable.

The study by EG & G Bionomics (1978) reported 96-hour EC50 values of 1200 and 1300 µg a.e. l^{-1} for decreases in *in vivo* chlorophyll *a* and decreased cell numbers, respectively. Subsequently, the data were re-analysed (Monsanto Company). Concentration-response curves were modelled with logistic regression using a standard 3-parameter logistic regression from Hill and Graphpad (version 5.0). A 96-hour EC10 value of 534 µg a.e. l^{-1} for both endpoints was calculated by inverse prediction (model fit $R^2 > 0.99$). Details on the test medium are not given but are reported to conform to US EPA guidelines. It is not possible from the data to calculate the control growth rate. However, concurrent tests were performed with a reference toxicant which were reported to meet validity criteria and, therefore, this study was considered reliable with restrictions (Klimisch code 2).

A more recent study (Smyth *et al*. 1996c) used a medium based on natural seawater and obtained a control growth rate of 1.423 over days 0 to 3 (the ISO test period) and 1.113 over days 0 to 4. Therefore this study meets ISO validity criterion. The study report gives endpoint data for 72-hour, 96-hour and 120-hour biomass and growth rate EC50s and NOECs. In line with current guidance the preferred endpoint that should be used is the 72-hour NOEC for growth rate of 1800 µg a.e. l^{-1}.

The only other long-term result for a marine species is the unbounded 72-hour growth NOEC for eelgrass, *Zostera marina*, of 16900 µg a.e. l^{-1}. A stimulatory effect based on the relative growth rate (weight) was observed at 1690 µg a.e. l^{-1}.

PNEC accounting for the annual average concentration

The most sensitive long-term result for a marine species is an EC10 of 534 µg a.e. l^{-1} for *S. costatum*, (EG & G Bionomics 1978). According to the draft WFD Technical Guidance in the

absence of any long-term data from saltwater species representing crustaceans or fish an assessment factor of 1000 should be applied to this critical datum. However since algae are expected to be the most sensitive species based on the mode of action of glyphosate (see Section 2.6.5) an assessment factor of 10 is recommended. This would result in:

$PNEC_{saltwater\_lt}$ = 534 µg l^{-1}/AF (10) = 53 µg l^{-1} glyphosate (rounded)

Note that this value is higher than the $PNEC_{freshwater\_lt}$ value of 33 µg l^{-1} glyphosate (rounded)

PNEC accounting for a maximum allowable concentration

Saltwater short-term toxicity data are available for six taxonomic groups (algae, crustaceans, echinoderms, fish, molluscs and protozoa (Table 2.11). As expected algae are the most sensitive taxa. Echinoderms, an exclusively marine group, would appear to be unaffected by glyphosate.

The most sensitive result is an EC50 of 1200 µg a.e. l^{-1} for *S. costatum*, (EG & G Bionomics 1978). Based on the draft WFD Technical Guidance as there is at least one short-term L(E)C50 from each of three trophic levels of the base set (fish, crustaceans and algae) and glyphosate has a known mode of action for which the most sensitive taxa are represented, an assessment factor of 10, should be applied to the most sensitive datum. This would results in:

$PNEC_{saltwater\_st}$ = 1200 µg l^{-1}/AF (10) = 120 µg l^{-1} glyphosate (rounded)

Note that this value is higher than the $PNEC_{freshwater\_st}$ value of 84 µg l^{-1} glyphosate (rounded)

The marine database is too small to draw firm conclusions on possible differences between freshwater and saltwater toxicity, particularly for chronic effects, but results for freshwater and saltwater algae are comparable. The short-term marine database does include endpoints for molluscs, crustaceans and echinoderms and these are comparable to the freshwater data for invertebrates.

Since there are no obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group, the technical guidance approach of using a combined freshwater and saltwater dataset for the freshwater and marine effects assessment should be used (EC 2010). Therefore, the proposed freshwater PNECs should be considered in deriving corresponding values for saltwater bodies without an additional assessment factor.

3.2 Derivation of PNECs by the TGD probabilistic approach (SSD method)

Given the large body of both long-term and short-term data that is available for a range of taxonomic groups (including those considered most sensitive to glyphosate) it is proposed that PNECs are derived where possible by a probabilistic approach. This approach is consistent with the draft WFD Technical Guidance (EC 2010) providing that the necessary acceptability criteria are satisfied.

In selecting the endpoints for PNEC derivation using the probabilistic approach, the most sensitive (i.e. lowest) result was selected for *P. subcapitata* and *L. siliquoidea* rather than a species geomean. In both cases test conditions were regarded as sufficiently dissimilar to allow for this approach. For *P. subcapitata*, the result of 92500 µg a.e. l^{-1} was achieved after buffering the test media to maintain a pH value of 8. There is some evidence to suggest that pH level affects glyphosate toxicity. The pH-dependent dissociation of glyphosate determines the speciation of

glyphosate in aquatic systems. The toxicity of each form of glyphosate is not known. The addition of glyphosate to test media decreases pH with increasing concentration. Where test organisms are known to be sensitive to pH it is not possible to differentiate between possible effects due to pH sensitivity and effects due to glyphosate toxicity. The results for *L. siliquoidea* are from exposure to technical grade glyphosate IPA salt and glyphosate acid. As the test substance is not the same it is inappropriate to combine the results. As a precautionary measure the most sensitive result has been selected. Where a number of studies reported data for a species derived under the same or very similar experimental conditions then these data were combined by calculating a geometric mean.

3.2.1 Freshwater long-term PNEC

Based on the full data set of 17 freshwater species NOECs (using geometric means where applicable) and use of the program ETX 2.0 (Van Vlaardingen et al., 2004) for deriving an SSD, the median (i.e. 50 per cent confidence) 5th percentile cut-off value of 621.3 µg l^{-1} glyphosate has been calculated. The SSD meets all of the goodness-of-fit criteria (Table 3.1).

3.2.2 Saltwater long-term PNEC

There is an insufficient data set and therefore it is not possible to derive a marine PNEC based exclusively on marine data using an SSD approach.

3.2.3 Combined Freshwater and Saltwater long-term PNEC

Since there are no obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group, the draft WFD Technical Guidance approach of using a combined freshwater and saltwater dataset for the freshwater and marine effects assessment should be used. Based on the 19 fresh- and saltwater species NOECs (using geometric means where applicable) (Table 3.2) and use of the program ETX 2.0 (Van Vlaardingen *et al*., 2004) for deriving an SSD (see Figure 3.1), the median (i.e. 50 per cent confidence) 5th percentile cut-off value of 586.8 µg l^{-1} glyphosate is calculated. The SSD meets all of the goodness-of-fit criteria (Table 3.1).

Table 3.1 – 5% Hazardous Concentration (HC5) values and SSD goodness-of-fit

Dataset	Model	n	Hazard Concentration HC_5 (µg l^{-1})			SSD goodness-of-fit (acceptance at 1% and 5% significance)		
			Median	Lower limit	Upper limit	Anderson-Darling	Kolmogorov-Smirnov	Cramer von Mises
FW only	Log-normal	17	621.3	213.3	1258.9	✓	✓	✓
FW and SW combined	Log-normal	19	586.8	215.4	1157.2	✓	✓	✓
FW and SW alga and higher plants	Log-normal	11	479.8	118.4	1087.5	✓	✓	✓

According to the latest guidance (ECHA 2008, EC 2010), an assessment factor of 1–5 should be applied in order to derive the PNEC from the 5th percentile of the SSD. The size of this assessment factor needs to be justified by taking into account aspects such as:

- The overall quality of the database
- The representivity of taxonomic groups
- Knowledge of the mode of action
- Uncertainties around the HC5
- Comparison with field and mesocosm studies

The minimum requirement of 10 NOEC values, preferably more than 15 NOEC values, for different species covering at least eight taxonomic groups is not met since only seven taxonomic groups are represented (Table 3.2). The missing taxonomic group is insects. Folmar *et al*. (1979) conducted experiments to determine whether mayfly nymphs avoided the glyphosate formulation Roundup. The nymphs avoided Roundup at concentrations of10000 µg a.e. l^{-1} but not at 1000 µg a.e. l^{-1}. In a separate study Folmar *et al*. (1979) assessed the impact of glyphosate IPA salt on the stream drift of chironomid larvae. Experiments were conducted in eight artificial streams. Exposure concentrations were calculated to be 20, 200 and 2000 µg a.e. l^{-1}. Exposure to glyphosate IPA salt did not stimulate drift at any of the test concentrations. The 48-hour EC50 for *Chironomus plumosus* is 55000 µg a.e. l^{-1} (Table 2.9). Although limited this data suggests that insects are unlikely to be sensitive to exposure to glyphosate and are virtually absent from marine waters. Therefore, the absence of representation in the SSD is not considered germane to the PNEC calculation. Glyphosate's specific mode of action means that algae and higher plants are expected to be the most sensitive species and this is confirmed by the data. These species are well represented within this dataset. The number of chronic NOEC values (*n* = 19) meets the general requirement for the number of input data.

The difference between the median HC5 and the 90% lower confidence limit is a factor of 2.7 and the difference between the median HC5 and the 90% upper confidence limits is a factor of 2. The overall spread of the HC5 estimate is a factor of 5.4.

The WFD Technical Guidance recommends constructing an SSD using only those taxa that are expected to be particularly sensitive where substances have a particular mode of action. In the case of glyphosate, a herbicide, this means data for higher plants and algae. Using data for only these groups the resulting SSD meets all of the goodness-of-fit criteria and results in an HC5 of 479.8 µg l^{-1} (Table 3.1), which is not dissimilar to the HC5 of 586.6 µg l^{-1} that is derived when using data for all taxa. This demonstrates that there is no significant break in the distribution between sensitive and other species (see Figure 3.1). Therefore according to the guidance the SSD based on the full dataset should be used in deriving the PNEC.

The results of mesocosm and field studies indicate that glyphosate may not be as toxic in natural settings as in laboratory tests, due to rapid dissipation.

Based on the considerations above an assessment factor of 3 is considered to be appropriate for the derivation of the PNEC for glyphosate from the HC5. This results in:

$$\mathbf{PNEC_{freshwater\_lt} = 586.8\ \mu g\ l^{-1}/AF\ (3) = 196\ \mu g\ l^{-1}\ glyphosate\ (rounded)}$$

If a combined dataset is used, the technical guidance recommends that the AF of 1-5 applied to the HC5 estimated from the SSD should only be applied for coastal and territorial waters if the data set used to establish the SSD comprises long-term NOECs or EC10s for at least two additional typically marine taxonomic groups, other than fish, crustaceans and algae. If such data is unavailable then an additional AF of 10 should be applied to deal with residual uncertainty. However, it can be argued that the additional AF of 10 is not required since aquatic plants are the most sensitive taxa and data for echinoderms and molluscs are not expected to show lower toxicity

values. Although no data are available for marine macrolgae such as *Fucus* information is available for marine microalagae *Skeletonema* and the eelgrass *Zostera*. This results in:

$PNEC_{saltwater\_lt}$ = 586.8 µg l^{-1}/AF (3) = 196 µg l^{-1} glyphosate (rounded)

The resultant PNEC of 196 µg l^{-1} is protective of the most sensitive species in both media (*M. sibiricum* and *S. costatum*) after long-term exposure to glyphosate.

Figure 3.1 Log-normal SSD fitted to key long-term fresh- and saltwater data (Table 3.2) (µg a.e. l^{-1}) for glyphosate

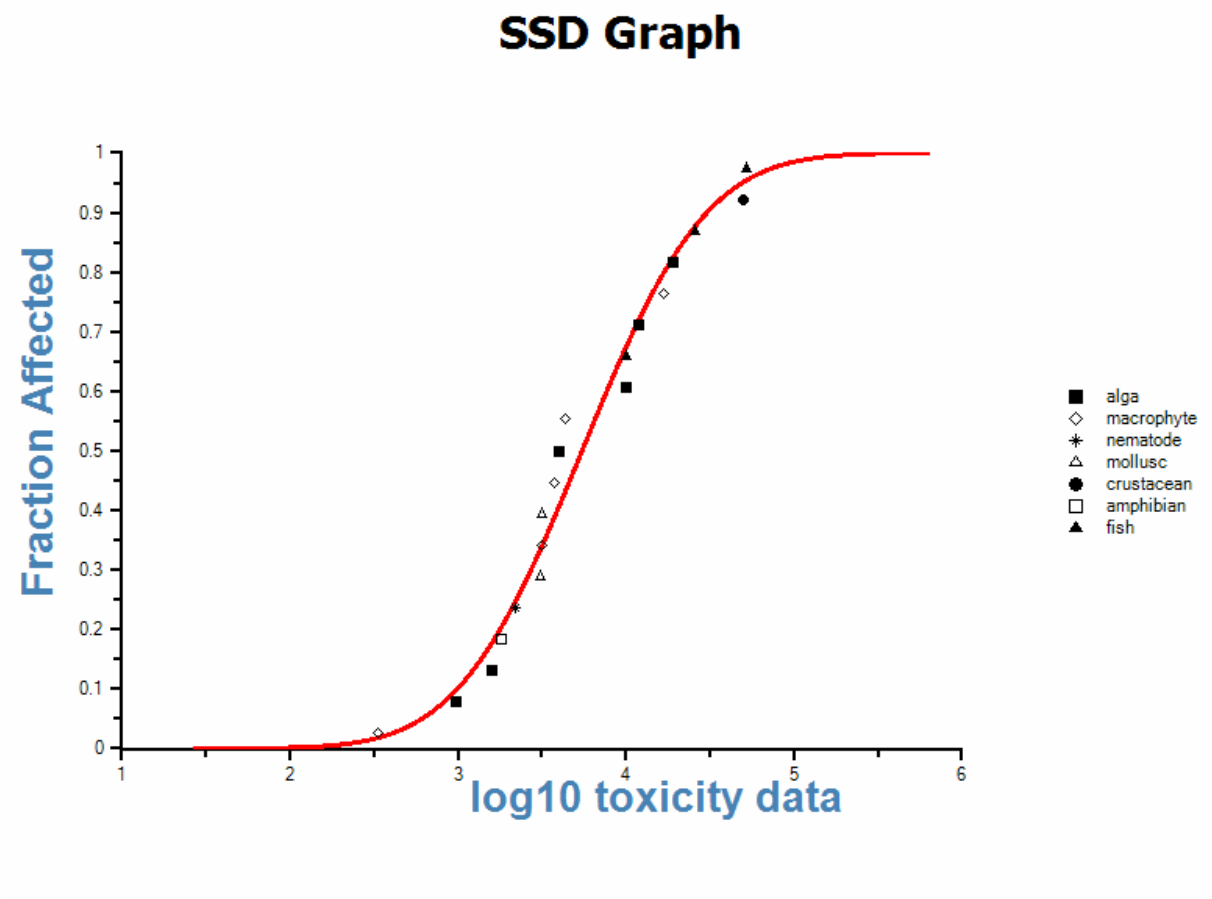


Table 3.2 Comparison of long-term glyphosate ecotoxicological dataset against minimum requirements for SSD derivation according to EU Technical Guidance (Refer to Table 2.8 and 2.10 for study detail)

Minimum SSD Requirement	N° of reliable endpoints in dataset[1]	Species	NOEC/EC10/MATC (µg l^{-1})
Fish	1	*Oncorhynchus mykiss*	52000
2nd chordate family (fish, amphibian etc)	2	*Cyprinus carpio* *Pimephales promelas*	10000 25700
Crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.)	1	*Daphnia magna*	50000
Insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc)	-	-	-
Family in a phylum other than arthropoda/chordate (e.g. Rotifera, Annelida, Mollusca, etc)	3	*Caenorhabditis elegans* *Lampsilis siliquoidea* *Pseudosuccinea columella*	2214 3100 3162
Family in any insect order or phylum not represented	1	*Rana pipiens*	1800
Algae	6	*Skeletonema costatum*Ψ *Scenedesmus quadricauda* *Scenedesmus acutus* *Pseudokirchneriella subcapitata* *Anabaena flos-aquae* *Navicula pelliculosa*	980 1600 4000 10000 12000 19000
Higher plant	5	*Myriophyllum sibiricum* *Potamogeton pectinatus* *Lemna minor* *Lemna gibba*Ψ *Zostera marina*	332 3162 3780 4340 16900
Total NOECs	**19**		
Taxa Criteria Met	**7/8**		

ΨDerived as a geometric mean of multiple results from the same species.
[1]Reliability defined as a score of 1 or 2 against Klimisch *et al*. (1997) criteria

3.2.4 Combined Freshwater and Saltwater short-term PNEC

Sufficient reliable short-term data are available to construct an SSD. The freshwater and saltwater datasets have been combined since there is no apparent difference in sensitivity between freshwater and marine taxa. Based on the 30 fresh- and saltwater species L(E)C50s (using geometric means where applicable) (Table 3.3) and use of the program ETX 2.0 (Van Vlaardingen *et al*., 2004) for deriving an SSD, the median (i.e. 50 per cent confidence) 5th percentile cut-off

value of 1988.3 µg l^{-1} glyphosate is calculated with a lower 90% confidence interval (CI) of 813.4 µg l^{-1} and an upper 90% CI of 3848.4 µg l^{-1}. The assumption that the input data are normally distributed is accepted at the highest level (P = 0.01) using the Kolmogorov-Smirnov, Cramer van Mises and Anderson-Darling Goodness-of-fit tests for normality.

The difference between the median HC5 and the 90% lower confidence limit is a factor of 2.4 and the difference between the median HC5 and the 90% upper confidence limits is a factor of 1.9. The overall spread of the HC5 estimate is a factor of 4.7.

According to the draft WFD Technical Guidance the AF should normally be 10. No additional AF is required for the saltwater short-term EQS as there are additional data for two marine taxonomic groups (molluscs and echinoderms. This results in:

$PNEC_{fresh- \text{ and } saltwater\_st}$ = 1988.3 µg l^{-1}/AF (10) = 199 µg l^{-1} glyphosate (rounded)

This PNEC is very similar to the long-term combined freshwater and saltwater PNEC of 196 µg l^{-1}. Examination of the dataset indicates that the acute to chronic ratio is at least 2.5 suggesting that an AF of 10 is too stringent and that a lower assessment factor is more appropriate. Therefore, an assessment factor of 5 is recommended. This results in:

$PNEC_{fresh- \text{ and } saltwater\_st}$ = 1988.3 µg l^{-1}/AF (5) =398 µg l^{-1} glyphosate (rounded)

Figure 3.2 Log-normal SSD fitted to key short-term fresh- and saltwater data (Table 3.3) (µg a.e. l^{-1}) for glyphosate

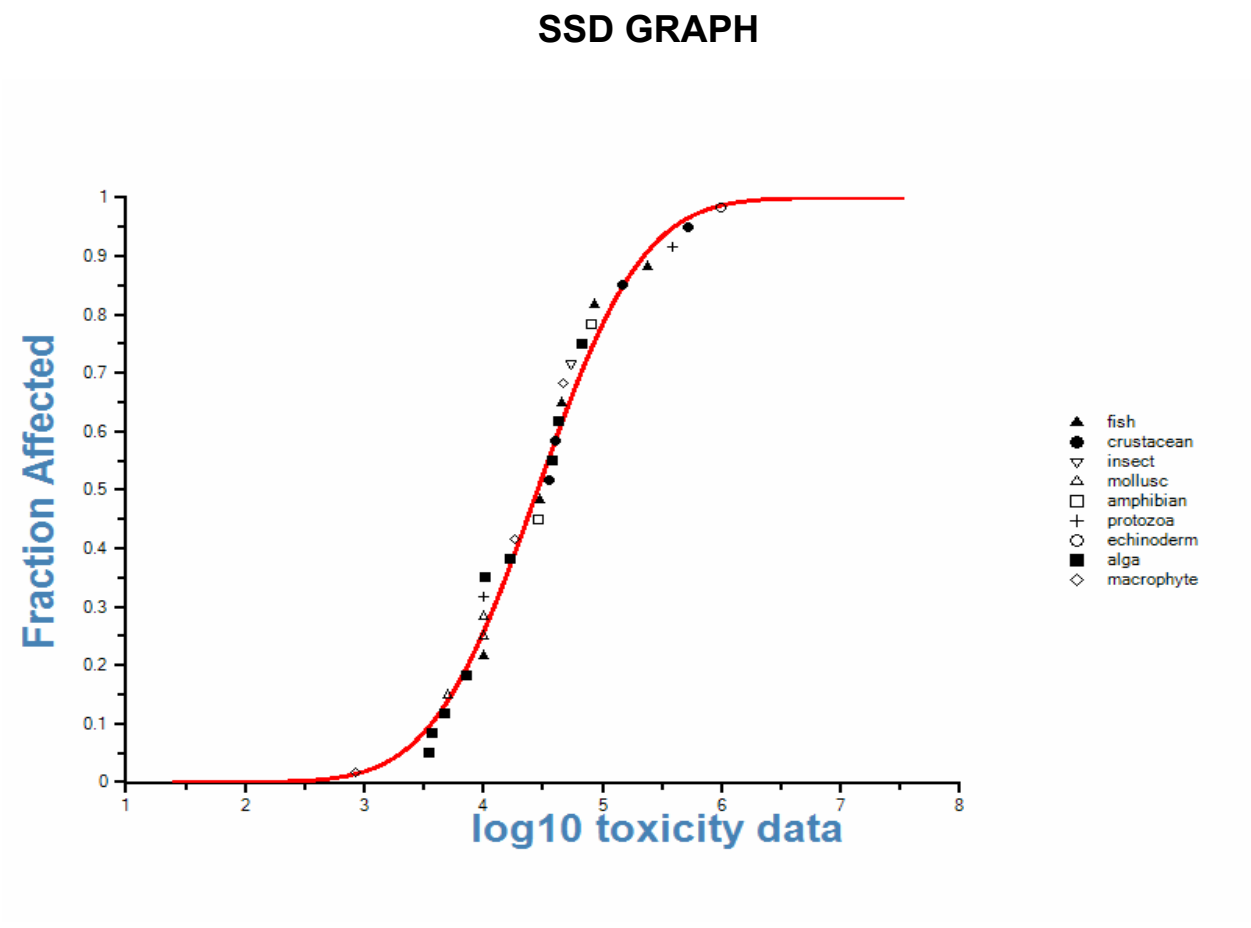


Table 3.3 Comparison of short-term glyphosate ecotoxicological dataset against minimum requirements for SSD derivation according to EU Technical Guidance (Refer to Table 2.9 and 2.11 for study detail)

Minimum SSD Requirement	N° of reliable endpoints in dataset[1]	Species	L(E)C50 (µg l^{-1})
Fish	1	*Oncorhynchus mykiss*	10000
2nd chordate family (fish, amphibian etc)	4	*Jordanella floridae* *Lepomis macrochirus* *Pimephales promelas* *Cyprinodon variegatus*	29600 45000 84900 240000
Crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.)	4	*Arcartia tonsa* *Americamysis bahia* *Ceriodaphnia dubia* *Daphnia magna*Ψ	35300 40000 147000 517687
Insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc)	1	*Chironomus plumosus*	55000
Family in a phylum other than arthropoda/chordate (e.g. Rotifera, Annelida, Mollusca, etc)	3	*Lampsilis siliquoidea* *Ruditapes decussates* *Crassostrea virginica*	5000 10000 10000
Family in any insect order or phylum not represented	2	*Rana clamitans* *Litoria moorei*	28700 81200
Family in any insect order or phylum not represented	2	*Euplotes vannus* *Tetrahymena pyriformis*	10100 386000
Exclusive marine species	1	*Tripneustes esculentes*	1000000
Algae	9	*Chlorella pyrenoidosa* *Skeletonema costatum*Ψ *Chlorellla vulgaris* *Scenedesmus quadricauda* *Scenedesmus acutus* *Navicula pelliculosa* *Anabaena flos-aquae* *Pseudokirchneriella subcapitata*Ψ *Chlorococcum hypnosporum*	3530 3660 4696 7200 10200 17000 38000 42638 68000
Higher plant	3	*Myriophyllum sibiricum* *Lemna gibba*Ψ *Lemna minor*	844 18443 46900
Total NOECs	**30**		
Taxa Criteria Met	**8/8**		

ΨDerived as a geometric mean of multiple results from the same species.
[1]Reliability defined as a score of 1 or 2 against Klimisch *et al*. (1997) criteria

3.3 Derivation of existing EQSs

There are no existing EQS values for glyphosate.

3.4 Derivation of PNECs for sediment

The TGD trigger value of a log Koc or log Kow of ≥3 is met, as the reported log Koc for glyphosate is 2.9 – 4.8 (EC 2002).

No long–term sediment studies were available. Short-term data are available for studies carried out using various glyphosate formulations. Tsui and Chu (2004) report 96-h LC50 values of Rodeo in sediment to be 3988 and 13368 mg kg^{-1} for *C. gigas* and *E. estuarius*, respectively, from Kubena (1998). The 10-day survival NOEC for the amphipods *Leptocheirus plumulosus* and *Eohaustorius estuarius* was greater than 2066 mg kg^{-1} (Kubena 1998), and the survival of oyster larvae (*Crassostrea gigas*) was reduced at 5122 mg kg^{-1} (Kubena 1996). In their own study Tsui and Chu (2004) reported 48-h LC50 values for *C. dubia* exposed to Roundup Biactive and Roundup of 340 mg kg^{-1} and 244 mg kg^{-1} respectively. These results suggest a wide range in toxicity, which may be explained by differences in organic carbon and the partitioning behaviour of glyphosate in sediment.

Because of the uncertainties, short exposure periods, use of different formulations and wide range in toxicity values in the empirical data no $PNEC_{sediment}$ can be recommended.

3.5 Derivation of PNECs for secondary poisoning of predators

3.5.1 Mammalian and avian toxicity data

Several reviews have been published regarding glyphosate (US EPA IRIS 1990, WHO 1994, EXTOXNET PIP 1996, EC 2002, NIOSH 2006). All of these data sources were consulted, but the primary sources were the most recent publications (see Table 3.4). Additional literature searches were performed from 2002 to the present day in an attempt to locate any lower effect data since 2002. However, none were found.

For avian data, due to the lack of relevant data in most of the reviews mentioned above, the IUCLID and WHO/FAO reviews were considered to be the most sound and scientifically accurate data. As for the mammalian data, a comprehensive literature search was also performed from 2000 to 2007 in an attempt to locate any lower effect data since 2000.

Table 3.4 Most sensitive mammalian and bird oral toxicity data relevant for the assessment of secondary poisoning

Type of study, reference & result	Details
Sub-chronic toxicity to mammals	
Bio Dynamics (1979) Cited in IPCS INCHEM (1994) **Sub-chronic NOAEL = 1890 mg kg^{-1} bw d^{-1}**	CD-1 mice received glyphosate for 13 weeks at doses of 0, 940, 1890 and 9710 mg kg^{-1} bw d^{-1} in males and 0, 1530, 2730 and 14 860 mg kg^{-1} bw d^{-1} in females. The NOAEL was based on increased liver, brain, heart and kidney weights and growth retardation.

Type of study, reference & result	Details
NTP (unknown) Cited in NIOSH (2006) **Sub-chronic LOAEL = 5000 mg kg^{-1} bw d^{-1}**	Mice received glyphosate in their diet for 13 weeks at unspecified doses. The LOAEL was based on changes in structure or function of salivary glands, liver weight and bladder weight. The types of changes observed were not specified and the original reference could not be located.
Federal Register (2004) Cited in NIOSH (2006) **Sub-chronic LOAEL = 4500 mg kg^{-1} bw d^{-1}**	Mice received glyphosate in their diet for 90 days at unspecified doses. The LOAEL was based on weight loss and decreased weight gain. The original reference could not be located.
Teratology (2000) Cited in NIOSH (2006) **Sub-chronic LOAEL = 3500 mg kg^{-1} bw d^{-1}**	Rats received glyphosate in their diet for 14 days at unspecified doses. The LOAEL was based on weight loss and decreased weight gain.
Anon Cited in EC (2002) **Sub-chronic NOAEL = 150 mg kg^{-1} bw d^{-1}**	Rats received glyphosate orally for 90 days at doses not specified. The NOAEL was based on unspecified effects on the liver, gastrointestinal mucosa and salivary glands. The original reference was not stated.
Huanjing Kexue (1984) Cited in NIOSH (2006) **Sub-chronic LOAEL = 250 mg kg^{-1} bw d^{-1}**	Rats received glyphosate in their diet for 90 days at unspecified doses. The LOAEL was based on somnolence, unspecified changes to food intake, hypermotility and diarrhoea.
Monsanto (A) Cited in IUCLID (2000) **Sub-chronic NOAEL = 1000 mg kg^{-1} bw d^{-1}**	Sprague Dawley rats received glyphosate in their diet for 90 days at doses of 0, 50, 250 or 1000 mg kg^{-1} bw d^{-1}. The study was conducted under GLP. The NOAEL was based on unspecified changes to body weights, food consumption and incidence of ophthalmic and unspecified clinical observations.
Monsanto (1987) Cited in IPCS INCHEM (1994) **Sub-chronic NOAEL = 1267 mg kg^{-1} bw d^{-1}**	Sprague-Dawley rats received glyphosate in their diet for 13 weeks at doses of 0, 63, 317 or 1267 mg kg^{-1} bw d^{-1} in males and 0, 84, 404 or 1623 mg kg^{-1} bw d^{-1} in females. The NOAEL was based on the absence of treatment-related effects.
NTP (unknown) Cited in NIOSH (2006) **Sub-chronic LOAEL = 2000 mg kg^{-1} bw d^{-1}**	Rats received glyphosate in their diet for 13 weeks at unspecified doses. The LOAEL was based on changes in liver weight, erythrocyte (red blood cell) count and levels of transaminases. The types of changes observed were not specified and the original reference could not be located.
Chronic toxicity to mammals	
Hogan (1983) Cited in IPCS INCHEM (1986) **Chronic NOAEL = 0.5% diet mg kg^{-1} bw d^{-1}** **Equivalent to 814 mg kg^{-1} bw d^{-1} (males) and 955 mg kg^{-1} bw d^{-1} (females)**	Male and female Charles River CD-1 mice (50/sex/group) received technical glyphosate in their diet for 24 months at doses of 0, 0.1, 0.5 or 3% (0, 100, 500 or 3000 mg/kg diet approximately 0, 50, 250 or 1500 mg kg^{-1} bw d^{-1}). The NOAEL was based on decreased body weight gain, hepatocyte hypertrophy, centrilobular hepatocyte necrosis and chronic interstitial nephritis.

Type of study, reference & result	Details
Anon Cited in EC (2002) **Chronic NOAEL = 31 mg kg^{-1} bw d^{-1}**	Rats received glyphosate for 2 years at unspecified doses. The NOAEL was based on increased liver weight, clinical chemistry and histological effects, increased salivary gland weight and histological changes in stomach mucosa, bladder epithelium and the eye (cataracts). The original reference was not stated.
Lankas (1981) Cited in IPCS INCHEM (1986) **Chronic NOAEL = >31 mg kg^{-1} bw d^{-1}**	Male and female Charles River Sprague-Dawley rats (50/sex/group) received technical glyphosate in their diet for 26 months at doses of 0, 3, 10 or 31 mg kg^{-1} bw d^{-1} (males) and 0, 3.4, 11 or 34 mg/kg bw/day mg kg^{-1} bw d^{-1} (females). The NOAEL was based on the lack of any treatment related effects at the top dose.
Monsanto (1985) Cited in IPCS INCHEM (1994) and US EPA IRIS (1990) **Chronic NOAEL = 500 mg kg^{-1} bw d^{-1}**	Beagle dogs (6/sex/group) received glyphosate in their diet for 52 weeks at doses of 0, 20, 100 or 500 mg kg^{-1} bw d^{-1}. The NOAEL was based on increased pituitary weights. There were no other treatment-related effects.
Effects on reproduction of mammals	
Beuret *et al*., (2005) Cited in NIOSH (2006) **Sub-chronic LOAEL = 20 mg kg^{-1} bw d^{-1}**	Rats received glyphosate in their diet for 21 days at unspecified doses. The LOAEL was based on unspecified changes in food intake, fluid intake, and liver weight, excessive lipid peroxidation, weight loss and decreased weight gain.
Schroeder (1981) Cited in IPCS INCHEM (1986) **Reproductive NOAEL = 30 mg kg^{-1} bw d^{-1}**	Male and female Sprague-Dawley rats (12/sex/group) received glyphosate in their diet for 60 days at doses of 0, 3, 10 or 30 mg kg^{-1} bw d^{-1}. Administration was continued for three generations. The NOAEL was the highest dose tested and no treatment-related effects were observed at this dose.
Monsanto (E) Cited in IUCLID (2000) **Reproductive NOAEL = 30 mg kg^{-1} bw d^{-1}**	Male and female Sprague-Dawley rats received glyphosate in their diet for 30 weeks in a three-generation study at doses of 0, 3, 10 or 30 mg kg^{-1} bw d^{-1}. The NOAEL was based on no treatment-related effects on foetal, pup or adult survival; parental or pup body weights; food consumption; or mating, fertility or gestation.
Monsanto (D) Cited in IUCLID (2000) **Reproductive NOAEL = 500 mg kg^{-1} bw d^{-1}**	Male and female Sprague-Dawley rats received glyphosate in their diet for 51 weeks at doses of 0, 2000, 10 000 or 30 000 mg kg^{-1} diet (approximately 0, 100, 500 or 1500 mg kg^{-1} bw d^{-1}). The study was conducted according to GLP. The NOAEL was based on reduced body weight gain and soft stools in the adults that occurred at the highest dose. The body weights of high dose pups were reduced during the last week of lactation.

Type of study, reference & result	Details
Anon Cited in EC (2002) **Developmental NOAEL = 300 mg kg^{-1} bw d^{-1}**	Rats received glyphosate for an unspecified duration at unspecified doses. The NOAEL was based on a decreased number of viable foetuses, reduced foetal weight, retarded ossification and increased incidence of skeletal and visceral anomalies. However, these effects were confined to doses that were maternally toxic.
Anon Cited in US EPA IRIS (1990) **Reproductive NOAEL = 350 mg kg^{-1} bw d^{-1}**	Female rabbits (16/group) received glyphosate via oral gavage at doses of 0, 75, 175 or 350 mg kg^{-1} bw d^{-1}. The length of exposure was not stated. The NOAEL was based on a slight increase in the incidence of soft stools and diarrhoea and nasal discharge. The original reference was not cited.
Acute toxicity to birds	
Anon Cited in WHO/FAO (1996) **Acute oral LD50 = >3851 mg kg^{-1} bw** **Short-term LC50 = >4650 mg kg^{-1} diet**	An acute oral LD50 and short-term LC50 of >3851 mg kg^{-1} bw and >4650 mg kg^{-1} diet, respectively, were reported in birds (species unspecified). As a result, the authors state that glyphosate has low toxicity to birds after acute exposure. No further information was available and the original reference was not cited.
Reproductive toxicity to birds	
Monsanto (B) Cited in IUCLID (2000) **Parental and offspring NOAEL = 1000 mg kg^{-1} diet**	Male and female Mallard ducks (*Anas platyrhynchos*) received glyphosate in their diet for 22 weeks at doses of 0, 50, 200 or 1000 mg kg^{-1} diet. The study was not conducted to GLP, but the OECD Guideline 206 was followed. The parental NOAEL was based on no treatment-related changes to adult survival, behaviour, body weight or food consumption. The offspring NOAEL was based on no changes in the numbers of eggs laid, eggs cracked, viable embryos, live 3-week embryos, normal hatchlings and 14-day survivors, as well as hatchling body weight, 14-day body weight, egg weight and eggshell thickness.
Monsanto (C) Cited in IUCLID (2000) **Parental and offspring NOAEL = 1000 mg kg^{-1} diet**	Male and female Bobwhite quails (*Coilinus virginianus*) (6/sex/group) received glyphosate in their diet for 22 weeks at doses of 0, 50, 200 or 1000 mg kg^{-1} diet. The parental NOAEL was based on no treatment-related effects on behaviour, body weight or food consumption of adult birds. The offspring NOAEL was based on no changes in the numbers of valid eggs laid and cracked, viable embryos, live 3-week embryos, normal hatchling body weight and 14-day survivors, as well as eggshell thickness, hatchling body weight and 14-day hatchling body weight.

3.5.2 PNECs for secondary poisoning of predators

The TGD BCF trigger of 100 in whole fish is not exceeded by glyphosate, so there is no requirement to derive PNECs for secondary poisoning.

4 Analysis and monitoring

As both glyphosate and its main metabolite AMPA show high polarity, and are, therefore, highly water soluble, they are difficult to extract with organic solvents. Glyphosate in water can be determined using direct aqueous injection high-pressure liquid chromatography (HPLC) with post-column derivatisation. In this method, a small aliquot of filtered water is injected into a reverse-phase HPLC column. The post-column reactions involve oxidation of glyphosate to glycine, which then reacts with o-phthalaldehyde to form an isoindole that is measured fluorometrically. The detection limit for this method is 6 µg l^{-1} for tap-water and 9 µg l^{-1} for groundwater (USEPA 1988). More recent studies in the scientific literature report lower detection rates.

Guo *et al.* (2005) report quantitative determination of trace glyphosate and phosphate in waters by coupling ion chromatography (IC) separation with inductively coupled plasma mass spectrometry (ICP–MS) detection. The separation of glyphosate and phosphate on a polymer anion-exchange column (Dionex IonPac AS16, 4.0mm×250 mm) was obtained by eluting them with 20mM citric acid at 0.50 ml min^{-1}, and the analytes were detected directly and selectively by ICP–MS at m/z = 31. Based on a 500 µl sample injection volume, the detection limits were 0.7 µg l^{-1} for both glyphosate and phosphate, and the calibrations were linear up to 400 µg l^{-1}. Spiked recoveries of 97.1–107.0% were achieved with relative standard deviations of ≤7.4% (n = 3). Compared to other reported methods for glyphosate and phosphate, the developed IC–ICP–MS method was considered sensitive and simple, and did not require any chemical derivatization, sample pre-concentration and mobile phase conductivity suppression.

Hildalgo *et al.* (2004) describe a method for analysing environmental waters based on precolumn derivatization with the fluorescent reagent 9-fluorenylmethylcloroformate (FMOC) followed by large-volume injection in a coupled-column LC system using fluorescence detection (LC–LC–FD). The limits of quantification (LOQ) of glyphosate and AMPA were 0.1 µg l^{-1}. Additionally, the use of Amberlite® IRA-900 for preconcentration of glyphosate, prior to the derivatization step reduced the LOQ of glyphosate to 0.02 µg l^{-1}.

For water, proposed PNECs derived for glyphosate are in the range 33 to 398 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50 per cent. Using this criterion, it is evident that current analytical methodologies should offer adequate performance to analyse for glyphosate.

5 Conclusions

5.1 Availability of data

Long-term laboratory data are available for eight different freshwater taxonomic groups: algae, amphibians, crustaceans, fish, macrophytes, molluscs, nematodes and nematomorpha. Freshwater short-term toxicity data are also available for eight taxonomic groups: algae, amphibians, crustaceans, fish, insects, macrophytes, molluscs and protozoa. The toxicity of glyphosate occurs over a wide concentration range. The available short-term and long-term toxicity test data indicate that for glyphosate and its salt aquatic plants are the most sensitive taxa of those tested. For marine organisms, single species short-term toxicity data are available for six different taxonomic groups (algae, crustaceans, echinoderms, fish, molluscs and protozoans). However, no long-term toxicity data are available for the minimum of three saltwater taxa (algae, crustaceans and fish) required under Annex V of the WFD. Since there are no obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group, the technical guidance approach of using a combined freshwater and saltwater dataset for the freshwater and marine effects assessment should be used. Laboratory data are supplemented by freshwater and saltwater mesocosm and field data which indicate that glyphosate may not be as toxic in natural settings as in laboratory tests, due to rapid dissipation.

5.2 Derivation of PNECs

The proposed PNECs are described below and summarised in Table 5.1.

5.2.1 Long-term PNEC for freshwaters

Using the assessment factor method to derive a $PNEC_{freshwater}$ requires that an assessment factor of 10 is applied to the lowest reliable NOEC or EC10 (332 µg l^{-1} for *Myriophyllum sibiricum*). This results in:

$PNEC_{freshwater\_lt}$ = 332 µg l^{-1}/AF (10) = 33 µg l^{-1} glyphosate

However, there are sufficient freshwater ecotoxicity data to allow a PNEC to be derived by the probabilistic method from the HC5 of an SSD. As a result of this the PNEC derived by the assessment factor (AF) method is not recommended for adoption as an EQS.

Since there are no obvious differences in the sensitivity of freshwater or saltwater species of the same taxonomic group, the draft WFD Technical Guidance approach of using a combined freshwater and saltwater dataset for the freshwater and marine effects assessment has been used. An HC5 of 586.8 µg l^{-1} was derived using a log-normal species sensitivity distribution. An assessment factor of three applied to the HC5 results in:

$PNEC_{freshwater\_lt}$ = 586.8 µg l^{-1}/AF (3) = 196 µg l^{-1} glyphosate (rounded)

5.2.2 Short-term PNEC for freshwaters

Using the assessment factor method to derive a $PNEC_{freshwater}$ requires that an assessment factor of 10 is applied to the lowest reliable L(E)C50 (844 µg l^{-1} for *Myriophyllum sibiricum*). This results in:

$PNEC_{freshwater\_st}$ = 844 µg l^{-1}/AF (10) = 84 µg l^{-1} glyphosate

However, there are sufficient freshwater ecotoxicity data to allow a PNEC to be derived by the probabilistic method from the HC5 of an SSD. As a result of this the PNEC derived by the assessment factor (AF) method is not recommended for adoption as an EQS.

The freshwater and saltwater datasets have been combined since there is no apparent difference in sensitivity between freshwater and marine taxa. Based on the 30 fresh- and saltwater species L(E)C50s (using geometric means where applicable) the median (i.e. 50 per cent confidence) 5th percentile cut-off value of 1988.3 µg l^{-1} glyphosate is calculated. According to the draft WFD Technical Guidance the AF should normally be 10. This PNEC is essentially the same as the long-term combined freshwater and saltwater PNEC of 196 µg l^{-1}. Examination of the dataset indicates that the acute to chronic ratio is at least 2.5 suggesting that an AF of 10 is too stringent and that a lower assessment factor is more appropriate, and therefore, an assessment factor of 5 is recommended. This results in:

$PNEC_{freshwater\_st}$ = 1988.3 µg l^{-1}/AF (5) = 398 µg l^{-1} glyphosate (rounded)

5.2.3 Long-term PNEC for saltwaters

Since long-term single species toxicity data are only available for algae and eelgrass, a combined freshwater and saltwater dataset for the marine effects assessment was used to derive the PNEC. The saltwater toxicity data do not differ markedly from the range of values obtained for corresponding freshwater species. If a combined dataset is used, the technical guidance recommends that the AF of 1-5 applied to the HC5 estimated from the SSD should only be applied for coastal and territorial waters if the data set used to establish the SSD comprises long-term NOECs or EC10s for at least two additional typically marine taxonomic groups, other than fish, crustaceans and algae. If such data is unavailable then an additional AF of 10 should be applied to deal with residual uncertainty. However, it can be argued that the additional AF of 10 is not required since aquatic plants are the most sensitive taxa and data for echinoderms and molluscs are not expected to show lower toxicity values. This results in:

$PNEC_{saltwater\_lt}$ = 586.8 µg l^{-1}/AF (3) = 196 µg l^{-1} glyphosate (rounded)

5.2.4 Short-term PNEC for saltwaters

Reliable short-term saltwater toxicity data are available for algae, invertebrates and fish and there is no evidence to suggest that other saltwater species (particularly those that are exclusively saltwater in distribution) would be more sensitive. Therefore the freshwater and saltwater datasets have been combined. No additional AF is required for the saltwater short-term EQS as there are an additional two marine taxonomic groups (molluscs and echinoderms). This results in:

$PNEC_{saltwater\_st}$ = 1988.3 µg l^{-1}/AF (5) = 398 µg l^{-1} glyphosate

5.2.5 PNEC for sediments

The TGD trigger value of a log Koc or log Kow of ≥3 is met, as the reported log Koc for glyphosate is 2.9 – 4.8 (EC 2002).

No long–term sediment studies were available. Short-term data are available for studies carried out using various glyphosate formulations. These results suggest a wide range in toxicity, which may be explained by differences in organic carbon and the partitioning behaviour of glyphosate in

sediment. Because of the uncertainties, short exposure periods, use of different glyphosate formulations and wide range in toxicity values in the empirical data no $PNEC_{sediment}$ can be recommended.

5.2.6 PNEC for secondary poisoning

The EU Technical Guidance Document BCF trigger of 100 in whole fish is not exceeded by glyphosate, so there is no requirement to derive PNECs for secondary poisoning.

Table 5.1 Summary of proposed PNECs

Receiving medium/ exposure scenario	TGD deterministic approach (AFs) glyphosate	TGD probabilistic approach (SSDs) glyphosate	Existing EQS (µg l^{-1})
Freshwater/long-term	33 µg l^{-1}	196 µg l^{-1}	-
Freshwater/short-term	84 µg l^{-1}	398 µg l^{-1}	-
Saltwater/long-term	33 µg l^{-1}	196 µg l^{-1}	-
Saltwater/short-term	84 µg l^{-1}	398 µg l^{-1}	-
Sediments	Insufficient data	-	-
Secondary poisoning	Not required	-	-

5.3 Analysis

The proposed PNECs for glyphosate in both freshwater and saltwater are in the range 33 to 398 µg l^{-1}. The data quality requirements are that, at a third of the EQS, total error of measurement should not exceed 50%. Using this criterion, it is evident that current analytical methodologies (non-standard) employing coupling ion chromatography (IC) separation with inductively coupled plasma mass spectrometry (ICP–MS) detection), capable of achieving detection limits as low as 700 ng l^{-1}, or large-volume injection in a coupled-column LC system using fluorescence detection (LC–LC–FD), capable of achieving detection limits as low as 20 ng l^{-1}, should offer adequate performance to analyse for glyphosate.

5.4 Implementation issues

5.4.1 Stimulatory effects

Glyphosate exhibits similar toxicity to both algae and macrophytes. A greater complication in the characterization of ecological effects may involve the enhancement of algal populations at low concentrations of glyphosate. It is unclear whether this is a hormetic effect or simply a stimulation of algal growth due to the utilization of glyphosate as a nutrient source by algae (Austin *et al.* 1991, Hughes 1987ab, Smyth *et al.* 1996b).

The toxicity of glyphosate is also dependent on water quality parameters such as temperature, pH and hardness (Folmar *et al.* 1979, Wan *et al.* 1989) but it has not been possible to develop PNECs that reflect their influence.

References & Bibliography

Achiorno C L, de Villalobos C and Ferrari L (2008) Toxicity of the herbicide glyphosate to *Chordodes nobilii* (Gordiida, Nematomorpha). *Chemosphere*, **71,** 1816-1822.

APHA (1985) Standard methods for the examination of water and waste water. American Public Health Association, 15th Edition, 1268 pp.

ASTM (1980) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Report E-790-80. American Society for Testing and Materials, Philadelphia.

ASTM (1993) Standard guide for conducting static, 96 hour toxicity test with microalgae. Practice E-1218-90. American Society for Testing and Materials, Philadelphia.

ASTM (1998) Standard guide for conducting static, axenic, 14-day phytotoxicity tests in test tubes with the submersed aquatic macrophyte *Myriophyllum sibiricum* Komarov. Report E-1913-97. American Society for Testing and Materials, Philadelphia.

Augspurger T, Keller AE, Black MC, Cope WG, Dwyer JF. (2003) Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure, Environmental Toxicology and Chemistry, **22**, 2569-2575.

Austin, A P, Harris G E and Lucey W P (1991) Impact of an Organophosphate Herbicide (Glyphosate) on Periphyton Communities Developed in Experimental Streams. *Bulletin of Environmental Contamination and Toxicology,* **47,** 29-35.

BCPC (2006) The Pesticide Manual, 14th Edition. British Crop Protection Council. pp1350.

Beuret, C.J., Zirulnik, F. and Gimenez, M.S. (2005). Effect of herbicide glyphosate on liver lipoperoxidation in pregnant rats and their foetuses. *Reprod. Toxicol*. **19**, p. 501-504.

Bio/Dynamics Inc. (1979). A three month feeding study of glyphosate (Roundup technical) in mice. (Project No. 77-211) - Final report. E. Millstone, NJ, Bio/Dynamics Inc., Division of Biology and Safety Evaluation (Unpublished report submitted by Monsanto Ltd.). (Cited in IPCS INCHEM 1994)

Bowman J H (1989) Flow-through toxicity of glyphosate to Rainbow Trout (*Salmo gairdneri*) for a 21-day duration period. Analytical Bio-Chemistry Laboratories, Inc. Missouri, USA. Final Report #37695 (Confidential data supplied by Monsanto Europe S.A.).

Bringolf R B, Cope W G, Mosher S, Barnhart M C and Shea D (2007) Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae). *Environmental Toxicology and Chemistry,* **26,** 2094-2100.

Cedergreen N and Streibig J C (2005) the toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard. *Pesticide Management Science*, **61,** 1152-1160.

Cericato, L., Neto, J.G.M., Fagundes, M., Kreutz, L.C., Quevedo, R.M., Finco, J., Da Rosa, J.G.S., Koakoski, G., Centenaro, L., Pottker, E., Anziliero, D. and Barcellos, L.J.G. (2008). Cortisol response to acute stress in jundia *Rhamdia quelen* acutely exposed to sub-lethal concentrations of agrichemicals. *Comp. Biochem. Physiol*. **148C**, 281-286.

Chemfinder (2005) *ChemFinder database.* Cambridge, MA: CambridgeSoft Corp. Available from: http://chemfinder.cambridgesoft.com/ [Accessed 21 November 2006]

CMTTAO (1975) Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. Ecol. Res. Ser. Publ. No. EPA-660/3-75-009. Committee on Methods for Toxicity Tests with Aquatic Organisms, United States Environmental Protection Agency, 61pp.

Cole E C, McComb W C, Newton M, Chambers C L and Leeming J P (1997) Response of amphibians to clearcutting, burning, and glyphosate application in the Oregon coast range. *Journal of Wildlife Management,* **61,** 656-664.

Diamond G L and Durkin P R (1997) Effects of surfactants on the toxicity of glyphosate, with specific reference to RODEO. Syracuse Research Corporation, Syracuse, NY, USA. Report No. SERA TR 97-206-1b.

EC(2010) Chemicals and the Water Framework Directive: Technical guidance for deriving environmental quality standards. Draft version 6.0 of 23 February 2010. DG Environment, Brussels.

ECHA (2008) Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose [concentration]-response for environment. May 2008. European Chemicals Agency, Helsinki, Finland.

E G & G Bionomics (1975) Chronic toxicity of glyphosate to the fathead minnow (*Pimephales promelas*, Rafinesque). E G & G Bionomics, Massachusetts, USA (Confidential data supplied by Monsanto Europe S.A.).

Elandalloussi L M, Leite R B, Rodrigues P M, Afonso R and Cancela M L (2008) Effect of the herbicide Roundup® on Perkinsus *olsensi* in vitro proliferation and in vivo survival when infecting a permissive host, the clam *Ruditapes decussates. Bulletin of Environmental Contamination and Toxicology*, **80,** 512-515.

European Chemicals Bureau (ECB) (2003) *Technical Guidance Document in Support of Commission Directive 93/67/EEC on risk assessment for new and notified substances: Commission Directive (EC) No. 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market*. Parts I–IV. Luxembourg: Office for Official Publications of the European Communities. Available from: http://ecb.jrc.it/tgdoc [Accessed 21 November 2006].

European Chemicals Bureau (ECB) (2005) European Substances Information System (ESIS). Available from: http://ecb.jrc.it/esis/ [Accessed 21 November 2006]

European Commission (EC) (2002) *Review report for the active substance glyphosate. Finalised in the Standing Committee on Plant Health at its meeting on 2 October 2001 in view of the inclusion of glyphosate in Annex I of Directive 91/414/EEC*. 7599/VI/97-Final. Brussels: European Commission Directorate-General Health & Consumer Protection. Available from: http://europa.eu.int/comm/food/plant/protection/evaluation/exist_subs_rep_en.htm [Accessed 21 November 2006].

European Union (1997) *European Union Draft Assessment Report: Glyphosate*. Public Version. Prepared by the rapporteur Member State Greece. Parma, Italy: European Food Safety Authority, Pesticide Risk Assessment Peer Review Unit (PRAPeR).

EXTONET PIPS (1996) Extension Toxicology Network Pesticide Information Profile. http://extonet.orst.edu/pips/glyphosa.htm. [Accessed May 2007]

Federal Register (2004) (United States Government Printing Office, Supt of Documents, Washington, D.C., 20402). Volume 1.

Feng J C, Thompson D G and Reynolds P E (1990) Fate of Glyphosate in a Canadian Forest Watershed. 1. Aquatic Residues and Off-Target Deposit Assessment. *Journal of Agricultural and Food Chemistry,* **38,** 1110-1118.

Fleming W J, Ailstock M S, Momot J J and Norman C M (1991) Response of Sago Pondweed, a submerged aquatic macrophyte, to herbicides in three laboratory culture systems. *Plants for toxicity assessment: Second Volume*, ASTM STP 1115, J W Gorsuch, W R Lower, W Wang and M A Lewis, Eds., American Society for Testing and Materials, Philadelphia, pp267-275.

Folmar L C, Sanders H O and Julin A M (1979) Toxicity of the Herbicide Glyphosate and Several of Its Formulations to Fish and Aquatic Invertebrates. *Archives of Environmental Contamination and Toxicology,* **8,** 269-278.

Gardner S C and Grue C E (1996) Effects of Rodeo® and Garlon® 3A on nontarget wetland species in central Washington. *Environmental Toxicology and Chemistry,* **15,** 441-451.

Garnett R P, Hirons G, Evans C and O'Conner D (1992) The control of *Spartina* (cord-grass) using glyphosate. *Aspects of Applied Biology,* **29,** 359-364.

Giesy J P, Dobson S and Solomon K R (2000) Ecotoxicological risk assessment for Roundup® Herbicide. *Reviews of Environmental Contamination and Toxicology*, **167**, 35-120.

Goldsborough L G and Brown D J (1993) Dissipation of glyphosate in small forest ponds. *Archives of Environmental Contamination and Toxicology*, **18**, 537-544.

Guo Z X, Cai Q T and Yang Z G (2005) Determination of glyphosate and phosphate in water by ion chromatography - inductively coupled plasma mass spectrometry detection. *Journal of Chromatography A,* **1100,** 160-167.

Hartman W A and Martin D B (1984) Effect of suspended bentonite clay on the acute toxicity of glyphosate to *Daphnia pulex* and *Lemna minor*. *Bulletin of Environmental Contamination and Toxicology*, **33**, 355-361.

Hazardous Substances Data Bank (HSDB) (2006) *Toxicology Data Network (TOXNET®): Hazardous Substances Data Bank (HSDB®)* [online]. Bethesda, MD: Division of Specialized Information Services (SIS) of the US National Library of Medicine (NLM). Available from: http://toxnet.nlm.nih.gov/ [Accessed 14 February 2007].

Hidalgo C, Rios C, Hidalgo M, Salvado V, Sancho J V and Hernandez M (2004) Improved coupled-column liquid chromatographic method for the determination of glyphosate and aminomethylphosphonic acid residues in environmental waters. *Journal of Chromatography A,* **1035,** 153-157.

Hogan, G.K. (1983). A chronic feeding study of glyphosate in mice. Unpublished report from Bio-/Dynamics, Inc. (Project No. 2062/22). E. Millstone, NJ, USA. Submitted to WHO by Monsanto Europe, S.A., Brussels, Belgium. (Cited in IPCS INCHEM 1986).

Holdway DA and Dixon DG (1988) Acute toxicity of permethrin or glyphosate pulse exposure to larval white sucker (*Catostomus commersoni*) and juvenile flagfish (*Jordanella floridae*) as modified by age and ration level. *Environmental Toxicology and Chemistry,* **7**, 63-68.

Howe C M, Berrill M, Pauli B D, Helbing C C, Werry K and Veldhoen N (2004) Toxicity of glyphosate-based pesticides to four North American frog species. *Environmental Toxicology and Chemistry,* **23,** 1928-1938.

Huanjing Kexue (1984). Environmental Sciences. (China International Book Trading Corp., POB 2820, Beijing, People Republic of China).

Hughes J S (1987a) Volume V: The toxicity of glyphosate technical to *Lemna gibba*. Malcolm Pirnie Inc., White Plains, NY 10602, USA. Laboratory Project ID 1092-02-1100-5 (Confidential data supplied by Monsanto Europe S.A.).

Hughes J S (1987b) Vol III: The toxicity of glyphosate technical to *Skeletonema costatum*. Malcolm Pirnie Inc., White Plains, NY 10602, USA. Laboratory Project ID 1092-02-1100-3 (Confidential data supplied by Monsanto Europe S.A.).

Institute for Environment and Health (IEH) (2005) *Chemicals purported to be endocrine disruptors. A compilation of published lists.* (Web Report W20). Leicester: IEH. Available from: http://www.silsoe.cranfield.ac.uk/ieh/publications/endocrine.html [Accessed 11 March 2007].

International Programme on Chemical Safety (IPCS INCHEM) (1986). Glyphosate (Pesticide residues in food: 1986 evaluations Part II Toxicology.

International Uniform Chemical Information Database (IUCLID) (2000) *IUCLID dataset for glyphosate.* Ispra, Italy: European Chemicals Bureau, European Commission.

IPCS INCHEM (1994). Environmental Health Criteria 159, Glyphosate. Available from www.inchem.org/documents/ehc/ehc/ehc159.htm. [Accessed March 2007].

ISO (2006). ISO 10253:2006 Water quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum.* Internation Standards Organisation.

Kubena K M (1998) Rounding up the facts about Rodeo: An evaluation of non-target effects of estuarine invertebrates and juvenile salmon. MSc, School of Fisheries, University of Washington, Seattle. cited in US Fish and Wildlife Service (1997) Control of Smooth Cordgrass (*Spartina alterniflora*) on Willapa National Wildlife Refuge. Environmental Assessment.

Kubena K M, Grue C E and DeWitt T H (1996) Development of concentration response curves for oyster pediveliger larvae (*Crassostrea gigas*) exposed to formulations of Rodeo®, a herbicide used to control smooth cordgrass. Paper presented at the Society of Environmental Toxicology and Chemistry. 17th Annual Meeting. Washington DC. Nov 17-21, 1996. cited in US Fish and Wildlife Service (1997) Control of Smooth Cordgrass (*Spartina alterniflora*) on Willapa National Wildlife Refuge. Environmental Assessment.

Lankas, G.R. (1981). A lifetime study of glyphosate (Roundup technical) in rats. Unpublished report from Bio-/Dynamics, Inc. (Project No. 410/77). E. Millstone, NJ, USA. Submitted to WHO by Monsanto Europe, S.A., Brussels, Belgium. (Cited in IPCS INCHEM 1986)

Linz G M, Bleier W J, Overland J D and Homan H J (1999) Response of invertebrates to glyphosate-induced habitat alterations in wetlands. *Wetlands,* **19,** 220-227.

Ma J, Liang W, Xu L, Wang S, Wei Y and Lu J (2001) Acute Toxicity of 33 Herbicides to the Green Alga *Chlorella pyrenoidosa*. *Bulletin of Environmental Contamination and Toxicology,* **66**, 536-541.

Ma J, Ligen X, Wang S, Zheng R, Jin S, Huang S, Huang Y (2002) Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*. *Ecotoxicology and Environmental Safety*, **51**, 128-132.

Mann R M and Bidwell J R (1999) The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. *Archives of Environmental Contamination and Toxicology,* **36,** 193-199.

Maule A and Wright S J L (1984) Herbicide effects on the population growth of some green algae and cyanobacteria. *Journal of Applied Bacteriology,* **57**, 369-379.

Mayer F L and Ellersieck M R (1986) Manual of acute toxicity: interpretation and database for 410 chemicals and 66 species of freshwater animals. Resource Publication 160. Washington DC: US Department of the Interior Fish and Wildlife Service.

McAllister W A (1982) Chronic toxicity of glyphosate to *Daphnia magna* under flow-through test conditions. Analytical Biochemistry Laboratories, Columbia, Missouri, USA. Study No. AB-82-036 (Confidential data supplied by Monsanto Europe S.A.).

Missimer CL, Lemarie DP, Rue WJ. 1989. Evaluation of a chronic estimation toxicity test using *Skeletonema costatum.* Aquatic Toxiciology and Hazard Assessment. 12th Vol. ASTM. STP.1027. Eds: Cowgill UM, Williams LR. American Society for Testing and Materials. Philadelphia, ISA. Pp 345-354.

Monheit S, Leavitt J R, Trumbo J (2005) The Ecotoxicology of Surfactants used with Glyphosate Based Herbicides. Noxious Times, 6, 6-12.
http://www.cdfa.ca.gov/phpps/ipc/noxioustimes/noxtimes_hp.htm

Monsanto (A) Report ML 86-351. 90-Day Study of Glyphosate Administered in Feed to Sprague-Dawley Rats. (Cited in IUCLID)

Monsanto (B) Report WL-78-53. One-Generation Reproduction Study – Mallard Duck: Glyphosate Technical. (Cited in IUCLID)

Monsanto (C) Report WL-78-53. One-Generation Reproduction Study – Bobwhite Quail: Glyphosate Technical. (Cited in IUCLID)

Monsanto (D) Report WL-88-106. Two-Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats. (Cited in IUCLID)

Monsanto (E) Report WL-77-417. A Three-Generation Reproduction Study in Rats with Glyphosate (Cited in IUCLID).

Monsanto (1985). 12-month study of glyphosate administered by gelatine capsule to Beagle dogs (Project No. ML-83-137). St. Louis, Missouri, Monsanto Environmental Health Laboratory (Unpublished report No. MSL-5069 submitted by Monsanto Ltd). (Cited in IPCS INCHEM 1994)

Monsanto (1987). 90-day study of glyphosate administered in feed to Sprague-Dawley rats (Project No. ML-86-351/EHL 86128). St. Louis, Missouri, Monsanto Environmental Health Laboratory (Unpublished report No. MSL-7575 submitted by Monsanto Ltd). (Cited in IPCS INCHEM 1994)

Neskovic N K, Poleksic V, Elezovic, I, Karan V and Budimir M (1996) Biochemical and histopathological effects of glyphosate on carp, *Cyprinus carpio* L. *Bulletin of Environmental Contamination and Toxicology,* **56,** 295-302.

Newton M, Horner L M, Cowell J E, White D E, Cole E C (1994) Dissipation of glyphosate and aminomethylphosphonic acid in North American Forests. *Journal of Agricultural and Food Chemistry*, **42,** 1795-1802.

Nielsen L W and Dahllöf (2007) Direct and indirect effects of the herbicides Glyphosate, Bentazone and MCPA on eelgrass (*Zostera marina*). *Aquatic Toxicology*, **82,** 47-54.

NIOSH (2006). The Registry of Toxic Effects of Chemical Substances – Glycine, N-(phosphonomethyl)-. Available http://www.cdc.gov/niosh/rtecs/mc106738.html [Accessed May 2007]

NTP (unknown). National Toxicology Program Technical Report Series. (Research Triangle Park, NC 27709) No. 206. (Cited in NIOSH 2006).

OPP (2007) Office of Pesticides Programs Pesticide Ecotoxicity database. http://www.ipmcenters.org/Ecotox/index.cfm

Pereira J L, Antunes S C, Castro B B, Marques C R, Gonçalves A M M, Gonçalves F and Pereira R (2009) Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: commercial formulation versus active ingredient. *Ecotoxicology*, **18,** 455-463.

Perschbacher P W, Stone N, Ludwig G M and Guy C B (1997) Evaluation of effects of common aerially-applied soybean herbicides and propanil on the plankton communities of aquaculture ponds. *Aquaculture,* **157,** 117-122.

Peterson H G, Boutin C, Martin P A, Freemark K E, Ruecker N J and Moody M J (1994) Aquatic Phyto-Toxicity of 23 Pesticides Applied at Expected Environmental Concentrations. *Aquatic Toxicology,* **28,** 275-292.

Pettersson M and Ekelund N G A (2006) Effects of the herbicides roundup and avans on *Euglena gracilis*. *Archives of Environmental Contamination and Toxicology,* **50,** 175-181.

Relyea R A (2005a) The lethal impacts of roundup and predatory stress on six species of North American tadpoles. *Archives of Environmental Contamination and Toxicology,* **48,** 351-357.

Relyea R A (2005b) The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications,* **15,** 618-627.

Relyea R A (2005c) The lethal impact of roundup on aquatic and terrestrial amphibians. *Ecological Applications,* **15,** 1118-1124.

Roshon R D (1997) A toxicity test for the effects of chemicals on the non-target submersed aquataic macrophyte, *Myriophyllum sibiricum* Komarov. PhD thesis. University of Guelph, Guelph, Ontario, Canada.

Ruan Q-L, Ju J-J, Li Y-H, Liu R, Pu Y-P, Yin L-H and Wang D-Y (2009) Evaluation of pesticide toxicities with differing mechanisms using *Caenorhabditis elegans*. *Journal of Toxicology and Environmental Health Part, A*, **72,** 746-751.

Saenz M E, DiMarzio W D, Alberdi J L and Tortorelli M D (1997) Effects of technical grade and a commercial formulation of glyphosate on algal population growth. *Bulletin of Environmental Contamination and Toxicology,* **59,** 638-644.

SERA (2002) Syracuse Environmental Research Associates, Inc. Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Tricloopyr, and Hexazinone: Final Report: SERA TR 01-43-08-04a. Submitted to USDA Forest Service, Riverdale, MD, USA.

Schroeder, R.E. (1981). Three generation reproduction study with glyphosate technical in albino rats. Unpublished report from Bio-/Dynamics, Inc. (Project No. 2063/77). E. Millstone, NJ, USA. Submitted to WHO by Monsanto Europe, S.A., Brussels, Belgium. . (Cited in IPCS INCHEM 1986)

Simenstad CA, Cordell JR, Tear L, Weitkamp LA, Paveglio FL, Kilbride KM, Fresh KL and Grue CE (1996) Use of Rodeo® and X-77® Spreader to control smooth cordgrass (*Spartina alterniflora*) in a southwestern Washington estuary: 2. Effects on benthic microflora and invertebrates. *Environmental Toxicology and Chemistry*, **15,** 969-978.

Smyth D V, Kent S J, Morris D S, Morgan D J, Magor S E (1995) GLYPHOSATE ACID: Acute toxicity to the green alga (*Selenastrum capricornutum*). Brixham Environmental Laboratory, Brixham, Devon, UK. Study Report No. BL5550/B (Confidential data supplied by Syngenta Limited).

Smyth D V, Kent S J, Morris D S, Cornish S K and Shillabeer N (1996a) GLYPHOSATE ACID: Toxicity to duckweed (*Lemna gibba*). Brixham Environmental Laboratory, Brixham, Devon, UK. Study Report No. BL5662/B (Confidential data supplied by Syngenta Limited).

Smyth D V, Kent S J, Morris D S, Johnson P A and Shillabeer N (1996b) GLYPHOSATE ACID: Toxicity to the freshwater diatom *Navicula pelliculosa*. Brixham Environmental Laboratory, Brixham, Devon, UK. Study Report No. BL5673/B (Confidential data supplied by Syngenta Limited).

Smyth D V, Kent S J, Morris D S, Shearing J M and Shillabeer N (1996c) GLYPHOSATE ACID: Toxicity to the marine alga *Skeletonema costatum.* Brixham Environmental Laboratory, Brixham, Devon, UK. Study Report No. BL5684/B (Confidential data supplied by Syngenta Limited).

Smyth D V, Shillabeer N, Morris D S, Wallace S J (1996d) GLYPHOSATE ACID: Toxicity to blue-green alga *Anabaena flos-aquae*. Brixham Environmental Laboratory, Brixham, Devon, UK. Study Report No. BL5698/B (Confidential data supplied by Syngenta Limited).

Sobero M C, Rimoldi F and Ronco A E (2007) Effects of the glyphosate active ingredient and a formulation on *Lemna gibba* L. at different exposure levels and assessment end-points. *Bulletin of Environmental Contamination and Toxicology*, **79,** 537-543.

Solomon K R and Thompson D G (2003) Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews,* **6,** 289-324.

Soso AB, Barcellos LJG, Ranzani-Paiva MJ, Kreutz LC, Quevedo RM, Anziliero D, Lima M, Bolognesi da Silva L, Ritter F, Bedin AC and Finco JA (2007) chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundiá (*Rhamdia quelen*). *Environmental Toxicology and Pharmacology*, **23,** 308-313.

Syracuse Research Corporation (SCR) (2005) PhysProp physical properties database [online]. Syracuse, NY: SRC. Available from: http://www.syrres.com/esc/physdemo.htm [Accessed 11 March 2007].

Tate T M, Spurlock J O and Christian F A (1997) Effect of glyphosate on the development of *Pseudosuccinea columella* snails. *Archives of Environmental Contamination and Toxicology*, **33,** 286-289.

Tate T M, Jackson R N and Christian F A (2000) Effects of glyphosate and dalapon on total free amino acid profiles of *Pseudosuccinea columella* snails. *Bulletin of Environmental Contamination and Toxicology*, **64,** 258-262.

Teratology (2000). The International Journal of Abnormal Development. (Alan R. Liss, Inc., 41 E. 11th Street, New York, NY 10003). Volume 1.

Torstensson N T, Lundgren L M, Stenström J (1989) Influence of climatic and edaphic factors on persistence of glyphosate and 2, 4-D in forest soils. *Ecotoxicology and Environmental Safety*, **18,** 230-239.

Tsui M T K and Chu L M (2003) Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere,* **52,** 1189-1197.

Tsui M T K and Chu L. M (2004) Comparative toxicity of glyphosate-based herbicides: Aqueous and sediment porewater exposures. *Archives of Environmental Contamination and Toxicology,* **46,** 316-323.

US Department of Agriculture (USDA) (2003) *Glyphosate formulations – human health and ecological risk assessment*. Final Report. Prepared for USDA Forest Service by Syracuse Environmental Research Associates. Report No. SERA TR 02-43-09-04a http://www.fs.fed.us/foresthealth/pesticide/pdfs/04a03_glyphosate.pdf [Accessed 19 October 2009].

US Environmental Protection Agency (US EPA) (1993) Reregistration Eligibility Decision (RED) glyphosate. EPA 738-R-05-002. Washington, DC: Office of Prevention, Pesticides and Toxic Substances, US EPA. Available from: http://www.epa.gov/oppsrrd1/REDs/old_reds/glyphosate.pdf [Accessed 11 March 2007].

US Environmental Protection Agency (US EPA) (1988) Methods for the determination of organic compounds in drinking water. EPA-600/4-88/039

US EPA IRIS (1990). United States Environmental Protection Agency Integrated Risk Information System. Glyphosate.

Vlaardingen PLA, Traas TP, Wintersen AM and Aldenberg T (2004) *ETX* 2.0. *A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data*. Report no. 601501028/2004. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM).

Vendrell E, Gómez de Barreda Ferraz D, Sabater C and Carrasco J M (2009) Effect of glyphosate on growth of four freshwater species of phytoplankton: a microplate bioassay. *Bulletin of Environmental Contamination and Toxicology*, **82,** 538-542.

Wan WT, Watts RG and Moul DJ (1989) Effects of different dilution water types on the acute toxicity of juvenile pacific salmonids and rainbow trout of glyphosate an its formulated products. *Bulletin of Environmental Contamination and Toxicology*., **43,** 378-385.

Wang Y S, Jaw C G and Chen Y L (1994) Accumulation of 2,4-D and Glyphosate in Fish and Water Hyacinth. *Water Air and Soil Pollution,* **74,** 397-403.

World Health Organization (WHO) (1994) *Environmental Health Criteria 159: glyphosate* Geneva: WHO. Available from: http://www.inchem.org/documents/ehc/ehc/ehc159.htm [Accessed 11 March 2007]

List of abbreviations

AA	annual average
a.e.	acid equivalents
AF	assessment factor
a.i.	active ingredient
AMPA	aminomethylphosphonic acid
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstracts Service
DO	dissolved oxygen
Dw	Dry weight
EC50	Concentration effective against 50 per cent of the organisms or animals tested
ECD	electron capture detection
EHC	Environmental Health Criteria
EQS	Environmental Quality Standard
FD	Fluorescence detector
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act [US]
GC	gas chromatography
GLP	Good Laboratory Practice (OECD)
IC	Ion chromatography
LC50	Concentration lethal to 50 per cent of the organisms or animals tested
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
lt	long-term
MAC	maximum allowable concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
OECD	Organization for Economic Co-operation and Development
PNEC	predicted no-effect concentration
POEA	Polyethoxylated tallowamine surfactant
RED	Reregistration Eligibility Document
SSD	species sensitivity distribution
st	short-term
TGD	Technical Guidance Document

UKTAG	UK Technical Advisory Group
US EPA	US Environmental Protection Agency
WFD	Water Framework Directive
ww	Wet weight

ANNEX I Data quality assessment sheets

Identified and ordered by alphabetical order of references.

Data relevant for PNEC derivation were quality assessed in accordance with the so-called Klimisch Criteria (Table A1).

Table A1 Klimisch Criteria*

Code	Category	Description
1	Reliable without restrictions	Refers to studies/data carried out or generated according to internationally accepted testing-guidelines (preferably GLP**) or in which the test parameters documented are based on a specific (national) testing guideline (preferably GLP), or in which all parameters described are closely related/comparable to a guideline method.
2	Reliable with restrictions	Studies or data (mostly not performed according to GLP) in which the test parameters documented do not comply totally with the specific testing guideline, but are sufficient to accept the data or in which investigations are described that cannot be subsumed under a testing guideline, but which are nevertheless well-documented and scientifically acceptable.
3	Not reliable	Studies/data in which there are interferences between the measuring system and the test substance, or in which organisms/test systems were used that are not relevant in relation to exposure, or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert assessment.
4	Not assignable	Studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature.

* Klimisch H.-J, Andreae M and Tillmann U, 1997 A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*, **25**, 1–5.
** OECD Principles of Good Laboratory Practice (GLP). See:
http://www.oecd.org/department/0,2688,en_2649_34381_1_1_1_1_1,00.html

Reference	Achiorno *et al*. 2008

Information on the test species	
Test species used	*Chordodes nobilii*
Source of the test organisms	Adult *C. nobilii* collected from streams in locality of Sierra de La Ventana, Buenos Aires, Argentina.
Holding conditions prior to test	Individuals from each stream kept in separate containers with aerated dechlorinated tap water at 23 ± 1°C. After mating, females held individually for oviposting.
Life stage of the test species used	Embryos (blastula stage), larvae

Information on the test design	
Methodology used	Non-standard methodology but well described with schematic flow diagram. Exposure period to glyphosate, 96 and 48 h for embryos and larvae, respectively. After 96 h (~ 10% embryonic phase) embryos removed to clean medium and periodically checked until appearance of free-living larvae (FL). Once amount of FL >50% (between 28 and 38 days after test initiation) exposure to host (*Aedes aegyptii* larvae) for 72 h in clean water. Larvae bioassay, larvae exposed to glyphosate for 48 h, followed by immediate exposure to *A. aegyptii* larvae in control media for 72 h.
Form of the test substance	Glyphosate technical grade 95% w/v
Source of the test substance	Servicio Nacional de Sanidad y Calidad Agroalimentaria de Argentina (SENASA).
Type and source of the exposure medium	Reconstituted hard water (pH 7.6 – 8; hardness 160-180 mg $CaCO_3$ l^{-1})
Test concentrations used	Control, 0.1, 0.5, 1, 2, 4 and 8 mg a.e. l^{-1}.
Number of replicates per concentration	3
Number of organisms per replicate	Started with 2500-3000 eggs ml^{-1} for embryo test. No of larvae not stated.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static, in a rearing chamber at 23 ± 1°C, and 24 h dark.
Measurement of exposure concentrations	Verification of stock solution concentration – within 10% of nominal.
Measurement of water quality parameters	pH7.04 – 7.47 at test initiation. pH increased < 4% over exposure period
Test validity criteria satisfied	Embryo bioassays considered valid when the % non-viable embryos in the control group ≤ 10%. Larval bioassay considered valid when IIMA in control group > 2.
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comment	Endpoint larvae viability determined by evaluation of their infective capacity with the Infection Index Mean Abundance, IIMA, as

	total no. of *C. nobilii* larvae/by total no. *A. aegypti* larvae examined. No dose-response relationship between development time and glyphosate treatment in the embryo bioassay. However, IIMA was significantly reduced (< 75%) at all test concentrations compared to the control group but no significant difference between treatments. In the tests started with larvae there was significant dose-related decrease (~ 40- 80%) in viability in all test concentrations compared to controls with significant differences between treatments.

Reliability of study	**Reliable with restrictions** (some experimental detail not reported)
Relevance of study	**Not Relevant** (endpoint not direct toxicity but an indirect effect, no evidence that fewer infections leads to reductions at the population level)
Klimisch Code	**3**

Reference	Bowman, 1989 (Confidential data supplied by Monsanto Europe S.A.)

Information on the test species	
Test species used	*Oncorhynchus mykiss* (*Salmo gairdneri*) Rainbow trout
Source of the test organisms	Mt Lassen Trout Farm, Red Bluff, California
Holding conditions prior to test	Fish maintained in the dilution water for ~ 96 hrs prior to test initiation on a 16:8 light/dark photoperiod at test temperature. Fed hatched brine shrimp or commercial fish food daily.
Life stage of the test species used	Mean weight 1.3 (±0.37) g; mean length 49 (±4.5) mm.

Information on the test design	
Methodology used	OECD Guideline 204
Form of the test substance	Glyphosate technical (white powder), purity 97.67% [Lot XLI-203]
Source of the test substance	Monsanto Agricultural Company
Type and source of the exposure medium	Soft blended water (mixture of reverse osmosis and ABC well water) – pH 7.4-7.9; hardness 38-46 mg $CaCO_3$ l^{-1}; alkalinity 54-60 mg $CaCO_3$ l^{-1}; conductivity 104-120 µMhos cm^{-1}.
Test concentrations used	0, 6.5, 13, 25, 50 and 100 mg l^{-1} (nominal) 0, 5.8, 11, 22 , 52 and 98 mg l^{-1} (mean measured)
Number of replicates per concentration	1
Number of organisms per replicate	20
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through. 30 l test chambers. Diluter delivered 1 l test solution or control water to the test vessels at ~ 7 x hr^{-1}. Fed Rangens® salmon starter daily at 2% of initial body weight. Biomass loading for control group was 0.25 g l^{-1} d^{-1}.
Measurement of exposure concentrations	Samples taken days 0, 7, 14 and 21
Measurement of water quality parameters	Yes: Temp 14-15°C; pH 5.9 – 7.8; DO 7.6 – 8.4 mg l^{-1} (78-85% saturation)
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Yes
Comment	3 fish died in the 98 mg l-1 treatment group during days 0-7. Sub-lethal effects (loss of equilibrium, quiescence, light discolouration etc) were noted in one or two fish during the same period. Except for one fish having light discolouration on day 10 no other effects were seen at this test concentration. There were no treatment-related effects at any other test concentration. NOEC = 52 mg l^{-1}. Although not reported in the study, raw data for weight and length of individual fish at test termination were attached to the study report. No significant differences were apparent between treatments and control.

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Bringolf *et al*. 2007

Information on the test species	
Test species used	*Lampsilis siliquoidea*
Source of the test organisms	Brooding adult females were collected form Silver Fork of Perche Creek, Boone County, MO, USA. Roughly equal numbers of mature glochidia were obtained from the marsupial gills of at least 3 females by flushing using a syringe filled with well water. Juveniles were produced by transforming glochidia on young-of-year largemouth bass (*Micropterus salmoides*). After transformation and recovery from host fish, juvenile mussels were transferred to a culture system.
Holding conditions prior to test	Glochidia were used for toxicity tests only if initial viability ≥ 90%. Glochidia were acclimated to a 50:50 mixture of culture:dilution water for at least 2 h before initiation of tests. Juveniles fed ad libitum algal suspensions, water replaced weekly, temperature 22 - 23°C. Juvenile mussels acclimated to laboratory conditions by tempering into dilution water for 24 h prior to test initiation.
Life stage of the test species used	Juveniles (acute tests) 2 – 8 wks post-metamorphosis (mean shell length 732 ± 96 µm – 2196 ± 432 µm). Juveniles (chronic tests) 3 – 8 wks post-metamorphosis (mean shell length 1012 ± 118 µm – 2057 ± 416 µm).

Information on the test design	
Methodology used	ASTM 2006 – E2455-06 Standard guide for conducting laboratory tests with freshwater mussels.
Form of the test substance	Technical grade glyphosate IPA salt > 95% purity; technical grade glyphosate 98% purity
Source of the test substance	Chem Service, West Chester, PA, USA.
Type and source of the exposure medium	Reconstituted hard water per ASTM guideline
Test concentrations used	6 test concentrations glyphosate acid (0 – 200 mg l^{-1}). 5 test concentration glyphosate IPA salt (0, 0.8, 1.6, 3.1, 6.3 and 12.5 mg a.e. l^{-1}) chronic test.
Number of replicates per concentration	3
Number of organisms per replicate	~200 glochidia (subsample of 50 – 100 evaluated for survival) 7 – 10 juveniles
Nature of test system (static, semi-static or flow-through, duration, feeding)	48 h static (glochidia) 96 h semi-static (renewal at 48 h) juveniles 21 or 28 d semi-static (renewal at 48 or 72 h intervals) fed daily with solution of microalgae concentrates.
Measurement of exposure concentrations	Initial glyphosate exposure concentrations

	verified in each of 3 replicates from control, low, intermediate and high test concentrations. Measured glyphosate concentrations ranged from 82.2 – 104.4% of target concentrations (mean 94.2%, n=12). All results based on nominal concentrations.
Measurement of water quality parameters	Yes
Test validity criteria satisfied	> 90% control viability in all acute and chronic tests
Water quality criteria satisfied	Temp = 21.1 – 21.8°C; pH 8.22 – 8.76; conductivity 500 – 616 µS cm^{-1}; alkalinity 114 – 128 mg $CaCO_3$ l^{-1}; hardness 158 - 170 mg $CaCO_3$ l^{-1}; DO > 83% saturation.
Study conducted to GLP	No
Comment	48 hr EC50 (glochidia); 96 hr EC50 (juveniles) and 21-d EC50 (juveniles) > 200 mg a.e. l^{-1} technical grade glyphosate (< 63% affected at highest concentration). 48 hr EC50 (glochidia) 5.0 (95%CI 3.3 – 7.6) mg a.e. l^{-1} glyphosate IPA salt; 96 hr EC50 (juveniles) 7.2 (95%CI 5.5 – 9.6) mg a.e. l^{-1} glyphosate IPA salt and 28-d EC50 (juveniles) 4.8 (95%CI 3.0 - 7.6) mg a.e. l^{-1} technical grade glyphosate. Mean growth was calculated where ≥ 50% survival in the chronic test. Statistically significant reductions in growth were noted in the highest test concentration and 2 intermediate concentrations of technical grade glyphosate $NOEC_{growth}$ = 12.5 mg a.e. l^{-1}. For technical grade IPA salt there was a stimulatory effect on growth in concentrations where there was > 50% survival although this was not statistically significant.

Reliability of study	**Reliable with restrictions** (minor omissions in reporting)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Cedergreen and Streibig 2005

Information on the test species	
Test species used	1) *Lemna minor* L 2) *Pseudokirchneriella subcapita*
Source of the test organisms	1) *L. minor* in-house culture originally collected from local pond, Copenhagen, Demark in 1999. 2) *P.subcapita* in-house culture, originated from Institut for Vannforskning (NIVA), Norway
Holding conditions prior to test	1) Culture maintained in Ehrlenmeyer flasks in 'K'-medium, pH5 at 24°C and continuous lighting at 85-120 µmol m^{-2} s^{-1}. 2) Culture maintained at 5°C in dark.
Life stage of the test species used	Not specifically stated

Information on the test design	
Methodology used	1) 7-day growth test 2) 48 - 72 hr (ISO, 1989)
Form of the test substance	Technical grade glyphosate > 95% purity
Source of the test substance	Monsanto
Type and source of the exposure medium	For algal test medium buffered, where necessary, with sodium hydroxide or hydrochloric acid to achieve pH 8
Test concentrations used	8 dilutions, concentrations increasing by a factor of 2 between each step
Number of replicates per concentration	1) 6 (controls) 3 (treatments) 2) 6 (controls) 2 (treatments)
Number of organisms per replicate	1) 1 *L. minor* frond 2) 10,000 cells ml^{-1}
Nature of test system (static, semi-static or flow-through, duration, feeding)	1. Static, same environmental conditions as culture. 2. Static, placed on shaking table, continuous irradiance 80 µmol m^{-2} s^{-1}.
Measurement of exposure concentrations	No
Measurement of water quality parameters	pH confirmed in algal test where changes of > 1 pH unit were unacceptable.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	No
Comment	1) Relative growth rates were calculated from changes in frond surface area. 2) Relative growth rate was determined as the slope of the regression of the ln-transformed chlorophyll fluorescence as a function of time. Samples taken at 24, and 48 hrs, chlorophyll fluorescence of the samples measured 24 h after experiment termination i.e. 72 h.

Reliability of study	**Reliable with restrictions (**no analytical verification of exposure concentrations, some

	experimental detail not reported)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	E G & G Bionomics, 1975 (Confidential data supplied by Monsanto Europe S.A.)

Information on the test species	
Test species used	*Pimephales promelas*, Rafinesque
Source of the test organisms	Brood stock held at Aquatic Toxicology Laboratory of E G & G Bionomics.
Holding conditions prior to test	Not stated
Life stage of the test species used	Eggs

Information on the test design	
Methodology used	EPA, 1971. Recommended bioassay procedures for fathead minnow (*Pimephales promelas*, Rafinesque) chronic tests. By the Bioassay Committee, National Water Quality Laboratory, Duluth, Minnesota.
Form of the test substance	Glyphosate acid, 87.3% purity
Source of the test substance	Monsanto Agricultural Division, St Louis, Missouri, USA.
Type and source of the exposure medium	Aerated well water.
Test concentrations used	1.6, 3.2, 6.3, 12.5, 25.0 mg l^{-1} + dilution water control (nominal) 0.7 (± 0.2), 2.8 (± 0.5), 7.0 (± 0.8), 13.0 (± 1.4), 25.7 (± 1.0) mg l^{-1} + control [mean measured (± standard deviation)]
Number of replicates per concentration	2 duplicate aquaria per test concentration divided into 2.
Number of organisms per replicate	Two groups of 30 eggs incubated in each test aquaria (25°C). Dead eggs removed and counted daily until hatching complete (day 4). 40 fish divided into two groups of 20 were randomly selected and distributed to growth chambers in each aquarium. After 60 day measurements the number of fish released to each spawning chamber was impartially reduced to 15 after combining fish from the growth chambers. When secondary sexual characteristics were well developed (~ day 134) the number of fish in each tank was reduced initially to 4 males and 4 females and subsequently (day 179) to 2 males and 4 females. When spawning began (~day 112) eggs from each spawn were counted. 50 eggs from each of the first ten spawnings in each tank were incubated and hatching was monitored. 20 fry from the first 2 spawns in each tank in which at least 80% live hatch was observed were placed in their respective growth chambers. F_1 fish were sacrificed after all fry groups had completed 30 d exposure and spawning had nearly ceased in all aquaria for a period of 1 week (day 254).

Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through. 16:8 light/dark photoperiod. Fed 3 – 4 x daily with brine shrimp nauplii during first 45 days then fed 2 x daily frozen adult brine shrimp *ad libitum*.
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes: hatchability of eggs > 94% in all concentrations.
Water quality criteria satisfied	Yes. Hardness 37.8 (± 5.7) mg $CaCO_3$ l^{-1}; DO 8.1 (± 0.5) mg l^{-1}; pH 6.5 – 7.1 (25.7 mg l^{-1} glyphosate) and 6.8 – 7.6 (0 – 13.0 mg l^{-1} glyphosate); temp 25 ± 1°C.
Study conducted to GLP	Predates GLP guidelines
Comment	

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	E G & G Bionomics, 1975 (Confidential data supplied by Monsanto Europe S.A.)

Information on the test species	
Test species used	*Pimephales promelas*, Rafinesque
Source of the test organisms	Brood stock held at Aquatic Toxicology Laboratory of E G & G Bionomics.
Holding conditions prior to test	Not stated
Life stage of the test species used	1.5 g

Information on the test design	
Methodology used	EPA, 1975. EPA-660/3-75-009.
Form of the test substance	Glyphosate acid, 87.3% purity
Source of the test substance	Monsanto Agricultural Division, St Louis, Missouri, USA.
Type and source of the exposure medium	Aerated well water.
Test concentrations used	68, 75, 81, 87, 100, 120 and 140 mg l^{-1} + dilution water control (nominal)
Number of replicates per concentration	Not stated
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	pH ranged from 3.7 – 7.0; temp 19°C
Study conducted to GLP	Predates GLP guidelines
Comment	24 h LC50 84.9 mg l^{-1} (95% CI 72.7-99.3). 96 h LC50 could not be calculated as 100% mortality was observed in ≥ 87 mg l^{-1} while no mortality was observed among fish exposed to ≤ 81 mg l^{-1} glyphosate (nominal). Toxicity of glyphosate may be linked to pH alteration of the diluent water caused by the chemical. 100% mortality occurred where pH ≤ 4.5.

Reliability of study	**Reliable with restrictions** (no analytical verification of exposure concentrations; large variation in pH values between treatments and control)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	EG & B Bionomics 1978 (Confidential data supplied by Monsanto Europe S.A.)

Information on the test species	
Test species used	*Skeletonema costatum*
Source of the test organisms	In-house laboratory culture. Original culture from US EPA Research Laboratory, Narragansett, Rhode Island, USA.
Holding conditions prior to test	Cultures maintained at 20 ± 1°C; 2000 lux illumination; salinity 30 ‰.
Life stage of the test species used	No details given

Information on the test design	
Methodology used	EPA 1976: Bioassay procedures for the ocean disposal permit program. EPA-600/9-76-010. 96 p.
Form of the test substance	Glyphosate acid
Source of the test substance	Monsanto Company, St Louis, Missouri, USA
Type and source of the exposure medium	Appropriate amounts of glyphosate were dissolved in deionized water; the pH was adjusted to 8.0 with reagent grade NaOH, and then added to test containers.
Test concentrations used	0, 0.6 , 1.0, 1.8, 3.2 and 5.6 mg l^{-1} (nominal)
Number of replicates per concentration	2
Number of organisms per replicate	2 x 10^4 cell ml^{-1}
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Temperature and salinity
Test validity criteria satisfied	Yes
Water quality criteria satisfied	unknown: Temp 20±1°C; salinity 30‰; pH 8.3 - 8.4 (initial) and 8.2 – 8.5 (final)
Study conducted to GLP	Yes
Comments	A separate test was conducted using reference toxicant dodecyl sodium sulphate under the same test conditions. 96 h LC50 1.2 mg l^{-1} (95% CI: 0.6 – 2.3) in vivo chlorophyll *a* 96 h LC50 1.3 mg l^{-1} (95% CI: 0.7 – 2.5) cell number

Reliability of study	**Reliable with restrictions** (limited reporting, no analytical verification of exposure concentrations)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Elandalloussi *et al*. 2008

Information on the test species	
Test species used	*Ruditapes decussatus*
Source of the test organisms	Hatchery reared clams (25 mm) obtained from IPIMAR-INIAP, Tavira, Portugal
Holding conditions prior to test	Acclimated for ≥ 1 week at 22°C and 30‰ salinity in 5 l aquaria in filtered seawater.
Life stage of the test species used	Parasite *P. olensi* free

Information on the test design	
Methodology used	96 h acute toxicity test, no standard guideline method stated.
Form of the test substance	Glyphosate acid purity not stated
Source of the test substance	Sigma
Type and source of the exposure medium	Filtered seawater
Test concentrations used	10, 100, 1000 and 10000 µg l^{-1} + control
Number of replicates per concentration	2
Number of organisms per replicate	5
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static, 24 h renewal.
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not reported
Test validity criteria satisfied	Yes (no control mortality)
Water quality criteria satisfied	Values not reported
Study conducted to GLP	No
Comment	No mortality observed at any concentration tested.

Reliability of study	**Reliable with restrictions** (no analytical verification of exposure concentrations, some experimental detail not reported)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Fleming et al. 1991

Information on the test species	
Test species used	*Potamogeton pectinatus*
Source of the test organisms	*Potamogeton* turions were collected at 3 locations in Chesapeake Bay in 1986. Turions were sterilized, epidermal tissue removed, treated with antibiotics until axenic material was obtained.
Holding conditions prior to test	Turions sprouted in nutrient rich media (Murashige Shoot Multiplication Medium B, Carolina Biological Supply, and 10 g l-1 sucrose in deionized water). Vegetative material was propagated from rhizome tips. 3 clonal lines were established. Plants from each clonal line were distributed equally among treatment groups.
Life stage of the test species used	3-week old plants

Information on the test design	
Methodology used	3 separate 28 d trials – microcosm, heterotrophic and autotrophic systems, carried out for ASTM assessment.
Form of the test substance	Technical grade glyphosate
Source of the test substance	Not stated
Type and source of the exposure medium	Heterotrophic system sterilized culture medium (dependent on sucrose in the test medium). Autotrophic/microcosms – synthetic freshwater. Systems provided with substrate for rooting.
Test concentrations used	0, 1, 10, 100, 1000 and 10000 µg l^{-1}.
Number of replicates per concentration	Heterotrophic system /autotrophic system – 9. Microcosms - 5
Number of organisms per replicate	1 plant per Erlenmeyer flask (3 plants each from the 3 clonal lines) 3 plants per microcosm (one from each clonal line)
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static. All trials at 20 - 23°C, indoor microcosms under continuous fluorescent lighting ~ 70 µmol m^{-2} s^{-1}; hetero- and autotrophic trials 12:12 light :dark photoperiod. Water added to microcosm system to replace losses from evaporation, other 2 systems essentially closed.
Measurement of exposure concentrations	No – authors state that determinations in previous studies were within 15% of target concentrations.
Measurement of water quality parameters	Not reported
Test validity criteria satisfied	No validity criteria. Control growth increased by a factor ~ 5 - 6 over the 28 d test period.
Water quality criteria satisfied	pH autotrophic system 5.7
Study conducted to GLP	Not stated

Comment	Endpoint biomass production based on fresh weight at end compared to start weight expressed as % of the mean % biomass production of their respective controls. High intratrial variability in individual plant responses in both control and herbicide treatment groups noted in hetero- and autotrophic systems. Significant reduction in biomass production in highest test concentration in heterotrophic and microcosm systems (65 and 46%, respectively). In the microcosm system a stimulatory effect was observed at 1000 µg l^{-1} but not at 100 µg l^{-1}.

Reliability of study	**Reliable with restrictions** (no analytical verification of exposure concentrations, omissions in reporting experimental detail)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Folmar *et al.* 1979

Information on the test species	
Test species used	*1) Daphnia magna* *2) Gammarus pseudolimnaeus* *3) Chironomus plumosus* *4) Ephemerella walkeri* *5) Salmo gairdneri* *6) Pimpehales promelas* *7) Ictalurus punctatus* *8) Lepomis macrochirus*
Source of the test organisms	1-3) Lab culture – Columbia National Fisheries Research Laboratory 4) Collected from Clear Creek, nr Georgetown, Colorado 5- 8) Federal (USA) fish hatcheries
Holding conditions prior to test	5-8) held under lab conditions as described in Brauhn & Schoettger (1975) EPA report No: EPA-660/3-75-009
Life stage of the test species used	1) 1st instar 2) adults 3) early 4th instar 5 – 8) 0.5 – 2.2 g

Information on the test design	
Methodology used	Static toxicity testing methods as recommended by Committee on Methods for Toxicity Tests with Aquatic Organisms 1975
Form of the test substance	Technical grade glyphosate, glyphosate IPA salt (480.42 g l^{-1} a.i.), Roundup® formulation with surfactant (360 g l^{-1} a.i.), MON0818 (surfactant)
Source of the test substance	Monsanto Agricultural Products Company, St Louis, Missouri
Type and source of the exposure medium	Reconstituted water: pH 7.2; hardness 40 mg $CaCO_3$ l^{-1}; temp 22°C (3, 6 - 8); 12°C (5)
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	1) not stated 2) 10, except where average weight of fish exceeded 1.5 g when a second series of containers was used to maintain loadings < 1 g fish l^{-1} test solution
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes; test solutions adjusted daily to the initial pH
Study conducted to GLP	Not stated

Comments	Not all organisms were exposed to glyphosate acid or glyphosate IPA salt.

Reliability of study	**Reliable with restrictions** (no analytical verification of exposure concentrations, some experimental detail not reported)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Holdway and Dixon 1988

Information on the test species	
Test species used	*Jordanella Floridae*
Source of the test organisms	In-house brood stock 3 generations removed form original wild stock obtained from Florida.
Holding conditions prior to test	25.1°C, fed twice per day until start of test
Life stage of the test species used	2, 4 or 8-days old

Information on the test design	
Methodology used	Non-standard short 2 hour pulse exposure followed by 96 h observation period in clean water. Methodology well described. Comparison was carried out using fed and unfed juvenile fish of varying ages.
Form of the test substance	Glyphosate acid purity 95%
Source of the test substance	Cat No. PS 1051, Lot 10-10, Chem Service
Type and source of the exposure medium	Well water
Test concentrations used	0, 100, 1000, 10000 and 30000 µg l^{-1} (nominal)
Number of replicates per concentration	6
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	2 hour pulse exposure; fish where fed received brine shrimp nauplii twice daily
Measurement of exposure concentrations	Yes – mean assayed concentrations ranged from 103 – 111% of nominal. Results based on measured concentrations.
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes: T = 25.3°C; pH 7.96, DO 8.3 mg l^{-1}, hardness 372 mg $CaCO_3$ l^{-1}
Study conducted to GLP	Not stated
Comments	No mortality observed during bioassays with 2- and 4-d old fed and unfed juveniles. 8-d old fed juveniles were an order of magnitude less sensitive to glyphosate than unfed 8-d juveniles.

Reliability of study	**Reliable with restrictions** (non-standard test)
Relevance of study	**Relevant (**where fish were fed)
Klimisch Code	**2**

Reference	Howe *et al.* 2004

Information on the test species	
Test species used	*Rana clamitans (acute studies)* *Rana pipiens (chronic study)*
Source of the test organisms	Field collected egg masses from Otonabee River (44°21'N, 78°17'W) Ontario, Canada
Holding conditions prior to test	Standard conditions
Life stage of the test species used	Gosner stage 25

Information on the test design	
Methodology used	ASTM E729-96 (2000) Chronic study – exposure for 42 d, followed by rearing in clean water.
Form of the test substance	Glyphosate IPA salt, Roundup® Original, Roundup Biactive®, Touchdown®, Glyfos BIO®, Glyfos AU®, Roundup Transorb®, POEA
Source of the test substance	Roundup products – Monsanto (St Louis, MO, USA) Touchdown – Syngenta (Wilminton, DE, USA) Glyfos products – Cheminova (Wayne, NJ, USA)
Type and source of the exposure medium	Compliant with ASTM standard
Test concentrations used	0 – 18 mg a.e. l^{-1} (acute) : 0.6 and 1.8 mg a.e. l^{-1} (chronic)
Number of replicates per concentration	Acute – 3; chronic not specifically stated, evident ≥ 2
Number of organisms per replicate	20
Nature of test system (static, semi-static or flow-through, duration, feeding)	Acute - Static Chronic - Static renewal every 7 d (half change of water every 96 h to maintain acceptable ammonia levels). Transferred to contaminant free water on day 42. Experiments terminated on day 166 (≥80% surviving tadpoles in each control group reached metamorphic climax (Gosner stage 42).
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Acute – yes Chronic - no validity criteria - 38% mortality in controls over the course of experiment. EPA guideline OPPTS 850.1800 (Tadpole/sediment subchronic toxicity test) sets validity criteria of no more than 20% mortality over 30 days with the test species *R. catesbeiana*.
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	Some slow development attributed to either crowding in the test aquaria or social interaction between the animals. Despite the slight compromise to statistical power of the

	test overall this study was considered relevant to the assessment.

Reliability of study	**Reliable with restrictions** (some slow development possibly due to experimental conditions – relevant to chronic exposure only)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Hughes 1987a (Confidential data supplied by Monsanto Europe S.A.)

Information on the test species	
Test species used	*Lemna gibba*
Source of the test organisms	In-house culture originally obtained from Dr Cleland, Smithsonian Institution Radiation Biology Laboratory, Rockville, MD.
Holding conditions prior to test	Cultures maintained in synthetic 20-strength algal assay procedure nutrient medium (20X-AAP) under constant illumination and 25±2°C
Life stage of the test species used	7-day old stock culture

Information on the test design	
Methodology used	US EPA FIFRA Guideline 123-2 (Growth and reproduction of aquatic plants, Tier 2)
Form of the test substance	Glyphosate technical (white solid) purity 96.6% [lot no. NBP-3594465]
Source of the test substance	Monsanto Agricultural Company
Type and source of the exposure medium	As culture medium (base 800 ml distilled deionized water with 20 ml each macro- and micronutrient stock solutions. Volume brought to 1 l and pH adjusted to 7.5 ± 0.1).
Test concentrations used	0, 5, 9, 16, 28 and 50 mg l^{-1} (nominal). 0, 4.28, 9.02, 16.6, 29 and 49.4 mg l^{-1} (mean measured).
Number of replicates per concentration	3
Number of organisms per replicate	15 fronds [3, 4-frond colonies and 1, 3-frond colony aseptically added to each vessel].
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static. Flasks kept in Sherer Model RI-32LLTP Incubator at 25 ± 2°C, continuous illumination (4198-5813 lumens m^{-2}). Flasks randomly repositioned daily to minimize spatial differences in the incubator.
Measurement of exposure concentrations	Yes, samples retained for analysis of initial test concentration. At end of test contents of each flask filtered and replicate filtrates combined for analysis. Analysis by HPLC.
Measurement of water quality parameters	Temperature only
Test validity criteria satisfied	yes
Water quality criteria satisfied	N/A (FIFRA guidelines)
Study conducted to GLP	Yes
Comments	Mean frond count values at test termination for each test concentration were expressed as a % relative to that in the control. To determine EC25 and EC50 values log test concentration plotted against % inhibition expressed as probit. Inverse estimation least squares linear regression used to determine line of best fit, EC values and 95% CL. Parameters of the regression line determined using SAS software. The values for the 2 lowest test

	concentrations were stimulatory and therefore omitted from the regression analysis. 14-d EC25 = 18 mg l^{-1}; EC50 = 25.5 mg l^{-1}. 95% CL could not be determined.

Reliability of study	**Reliable**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Hughes 1987b (Confidential data supplied by Monsanto Europe S.A.)

Information on the test species	
Test species used	*Skeletonema costatum*
Source of the test organisms	In-house laboratory culture. Original culture from Culture Collection of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME.
Holding conditions prior to test	Cultures maintained in synthetic marine algal assay nutrient medium at 20 ± 2°C; photoperiod 14^{L}:10^{D} at 400 foot-candles; flasks manually shaken daily.
Life stage of the test species used	7 day old cultures

Information on the test design	
Methodology used	EPA 1974: EPA-600/9-78-101 and EPA 1982: EPA-540/9-82-020
Form of the test substance	Glyphosate acid, purity 96.6%
Source of the test substance	Monsanto Company, Chesterfield MO. USA (Lot No. 3594465)
Type and source of the exposure medium	Synthetic marine algal nutrient medium similar to culture medium. The test medium omits EDTA and the metal mix, minor salt mix and vitamins are added at lower concentrations (30 ‰ salinity)
Test concentrations used	0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg l^{-1} + medium control (nominal) 0.24, 0.28, 0.48, 0.94, 1.79, and 3.42 mg l^{-1} + control (< 0.05) (mean measured)*
Number of replicates per concentration	3
Number of organisms per replicate	10^{4} cell ml^{-1}
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, flasks manually shaken daily. 14^{L}:10^{D} photoperiod at 4306 ± 650 lumens m^{-2} provided by overhead cool-white fluorescent lights.
Measurement of exposure concentrations	Yes, on day 0 and at test termination.
Measurement of water quality parameters	Temperature and salinity
Test validity criteria satisfied	Current validity criteria of control growth ≥ 0.9 d^{-1} not met.
Water quality criteria satisfied	unknown:Temp 20±2°C; salinity 30‰; NO pH measurements
Study conducted to GLP	Yes
Comments	Cell counts (3 per replicate) were made using

Period (d)	Control growth rate (d^{-1})
0 to 3	0.43
0 to 4	0.51
0 to 7	0.51
2 to 4	0.78
2 to 7	0.62

	a Coulter Counter on test days 2, 3, 4 and 7. *For each nominal test concentration, the mean of the measured value on day 0 and the measured value on day 7 was calculated. The mean measured test concentrations were used as the basis for the data analysis. The mean measured values were ~ 107.8% of the nominal concentrations on day 0. The day 7 measured concentrations in the 2 lowest test concentrations were substantially > than day 0 values and a significant amount of glyphosate was detected in the control sample on day 7. These high recovery rates for day 7 are thought to be artefacts resulting from cross-contamination during the filtration process as no glyphosate was introduced at any point into the test system after day 0. Samples for analysis at test termination were not obtained from “blanks” as per protocol but were prepared from the filtrate of the test medium. Algae were removed from the triplicate flasks by filtration and the filtrates at each test concentration were combined into one sample which was then analyzed for glyphosate. Notwithstanding this the mean measured concentration was used to determine the results (with the exception of the control day 7 value). The report states that the cell count value for nominal test concentration 0.4 mg l^{-1} indicated stimulatory activity and was omitted from the statistical (regression) analysis. The tables show that it was nominal test concentration 0.2 mg l^{-1} that exhibited 13.6% growth stimulation. The results as presented in the study report were 7-day EC25 and EC50 values of 370 and 640 µg a.e. l^{-1}, respectively. A stimulatory effect of 13.6% was seen at test concentration 280 µg a.e. l^{-1} (measured) and was omitted from the statistical regression analysis. As the raw data were available an EC10 value was calculated (TRAP) using all the data and measured concentrations. This resulted in an EC10 of 348 (95% CI: 76 – 1590) and EC50 of 831 (95% CI: 246 – 2810) µg a.e. l^{-1} (data fit to model p = 0.999).

Reliability of study	**Unreliable**
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Ma *et al.* 2001

Information on the test species	
Test species used	*Chlorella pyrenoidosa*
Source of the test organisms	Institute of Wuhan Hydrobiology, Chinese Academic of Science
Holding conditions prior to test	Cells propagated photoautotrophically in liquid HB-4 medium at 25^{o}C under continuous cool-white fluorescent illumination (5000 lux cm^{-2})
Life stage of the test species used	-

Information on the test design	
Methodology used	Standard algal test methodology described
Form of the test substance	Technical grade glyphosate 95% purity
Source of the test substance	People's Republic of China
Type and source of the exposure medium	HB-4 medium
Test concentrations used	Range between 0 and 50 mg l^{-1} + control
Number of replicates per concentration	3
Number of organisms per replicate	6 x 10^{5} cells ml^{-1}
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static; same environmental conditions as culture, orbital shaker (100 rpm)
Measurement of exposure concentrations	No
Measurement of water quality parameters	-
Test validity criteria satisfied	Yes
Water quality criteria satisfied	No pH analysis
Study conducted to GLP	No
Comments	-

Reliability of study	**Reliable with restrictions** (no analytical verification of test concentrations; some experimental detail not reported, no pH analysis)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Ma *et al.* 2002

Information on the test species	
Test species used	*Chlorella vulgaris*
Source of the test organisms	Institute of Wuhan Hydrobiology, Chinese Academic of Science
Holding conditions prior to test	Cells propagated photoautotrophically in liquid HB-4 medium at 25^{o}C under continuous cool-white fluorescent illumination (5000 lux cm^{-2}). Kept on a rotator shaker at 100 rpm.
Life stage of the test species used	-

Information on the test design	
Methodology used	Standard algal test methodology described
Form of the test substance	Technical grade glyphosate 95% purity
Source of the test substance	People's Republic of China
Type and source of the exposure medium	HB-4 medium
Test concentrations used	Range between 0 and 150 mg l^{-1} + control
Number of replicates per concentration	3
Number of organisms per replicate	8 x 10^{5} cells ml^{-1}
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Static; same environmental conditions as culture, orbital shaker (100 rpm)
Measurement of exposure concentrations	No
Measurement of water quality parameters	-
Test validity criteria satisfied	Yes
Water quality criteria satisfied	No pH analysis
Study conducted to GLP	No
Comments	-

Reliability of study	**Reliable with restrictions** (no analytical verification of test concentrations; some experimental detail not reported, no pH analysis)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Mann and Bidwell 1999

Information on the test species	
Test species used	*Litoria moorei; Crinia insignifera; Heleioporus eyrie; Limnodynastes dorsalis*
Source of the test organisms	Field collected
Holding conditions prior to test	Eggs and tadpoles held at 20^{o}C in same water as used for tests. Holding periods ranged for 1 – 3 w. Tadpoles acclimatized to test conditions 48 h prior to commencement
Life stage of the test species used	Tadpole (Gosner stage 25)

Information on the test design	
Methodology used	ASTM Standard E729-88a^{e1} (ASTM 1993)
Form of the test substance	Roundup®; Roundup® Biactive; glyphosate IPA salt (60.5% in water); Touchdown® (glyphosate trimesium 48% a.i.); glyphosate acid
Source of the test substance	All Monsanto Australia Ltd except Retail outlet - Touchdown® Davison Industries – glyphosate acid
Type and source of the exposure medium	Filtered lake water; aged tap water; USEPA soft water
Test concentrations used	5 concentrations, following range finding
Number of replicates per concentration	4
Number of organisms per replicate	5 (3 for Touchdown)
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	Semi-static renewal at 24 h Animals not fed; 12^{L}:12^{D} photperiod
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes: Temp = 19 – 21.3^{o}C No single test varied by > 1.5^{o}C: *L. moorei* 23 – 25^{o}C; DO 70% < > 80%; pH (glyphosate acid) 2.9 - 7.7; pH (others) 5.1 – 8.0.
Test validity criteria satisfied	Yes – 100% survival in controls
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	Order of toxicity: Roundup > Touchdown > glyphosate acid > Roundup Biactive > IPA salt. Some toxicity of glyphosate acid attributed to low pH values of test water for high concentrations

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Maule and Wright 1984

Information on the test species	
Test species used	*Chlorococcum hypnosporum*
Source of the test organisms	Culture Centre of Algae and Protozoa, Cambridge, England
Holding conditions prior to test	Maintained axenically on slants of the appropriate agar medium, kept in daylight at room temperature.
Life stage of the test species used	Inocula were from cultures in exponential phase (2-days old) grwon under the same light and temperature conditions as the test regime.

Information on the test design	
Methodology used	Non standard method. 25 x 5 ml compartment Repli-dishes were used. Experimental conditions: 25°C under continuous illumination (4000 lux).
Form of the test substance	Glyphosate, purity 96.7%
Source of the test substance	Monsanto Technical Center, Louvain-la-Neuve, Belgium
Type and source of the exposure medium	Knops solution
Test concentrations used	10 concentrations + control
Number of replicates per concentration	2
Number of organisms per replicate	Not stated
Nature of test system (Static, Semi-static or, Flow- through, duration, feeding)	static
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	
Test validity criteria satisfied	Validity criteria not stated
Water quality criteria satisfied	Not applicable
Study conducted to GLP	Not stated
Comments	Growth after 4 days was measured turbidometrically in 1 cm glass cuvettes at 685 nm. Growth inhibition curves were plotted and EC50 values deterined.

Reliability of study	**Reliable with restrictions (**some experimental details missing)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	McAllister, 1982 (Confidential data supplied by Monsanto Europe S.A.)

Information on the test species	
Test species used	*Daphnia magna*
Source of the test organisms	In-house culture maintained by ABC for 4 years. Primary culture obtained from Columbia National Fisheries Research Laboratory (CNFRL), Missouri, USA.
Holding conditions prior to test	Temp = 20 ± 2°C; lighting 50 – 70 footcandles on 16:8 light/dark photoperiod. Fed food preparation PR11-30 supplemented with algae (*Selenastrum capricornutum*) every 3 days
Life stage of the test species used	< 24 h old

Information on the test design	
Methodology used	ASTM 1979 – Draft No. 5, E35.21 ASTM 1981 – Draft No. 3, E47.01 USEPA 1975 – EPA-660/3-75-009
Form of the test substance	Glyphosate acid, purity 99.7%
Source of the test substance	Monsanto Chemical Company (lot # NBP 1782610[1992049]
Type and source of the exposure medium	ABC well water; pH 8.2; total hardness 255 mg $CaCO_3$ l^{-1}; DO 9.3 mg l^{-1}; conductivity 50 µmhos cm^{-1}.
Test concentrations used	25, 50, 99, 199 and 397 mg l^{-1} + dilution water control (nominal) 26 (± 2.4), 50 (± 3.5), 96 (± 8.0), 186 (± 17.0) and 378 (± 28.0) mean measured (± standard deviation) + dilution water control
Number of replicates per concentration	4
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Flow-through; fed 3 x daily P11-30, supplemented with algae once per day
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes; T = 20°C; pH = 6.1 – 8.1 (pH of the test concentrations decreased with increasing glyphosate concentrations. Within each test concentration pH in replicates did not vary by > ± 0.1 units); DO = 6.4 - 9.0 mg l^{-1}
Study conducted to GLP	Yes
Comments	No significant decreases in survival (≥98%, control 100%) or length of adult Daphnia were observed in any concentration tested. Lengths of daphnids were statistically greater in the lowest and highest test concentrations (mean values: 3.7, 3.9, 3.7, 3.6, 3.7, 3.8 mm, control through to highest test concentration, respectively). Reproduction was significantly decreased at the 3 highest concentrations (mean values 4.9, 6.5, 5.1, 4.1, 3.8, 1.7

	young/adult/ reproductive day). Reproduction in the lowest test concentration increased compared to controls. This increase in reproduction in the low test concentration, as well as the reproduction in the control, was within the range of production obtained in the controls of nine previous *Daphnia magna* chronic studies conducted at ABC Laboratories and was therefore discounted as an effect of exposure to glyphosate. This historical range of number of young/adult/reproductive day in the controls was 3.3-12.5.

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Neskovic *et al.* 1996

Information on the test species	
Test species used	*Cyprinus carpio* L
Source of the test organisms	Ribokombinat, Belgrade
Holding conditions prior to test	Acclimatized to laboratory conditions for 10 d prior to tests
Life stage of the test species used	13.5 – 15.3 cm body length

Information on the test design	
Methodology used	Non standard but well described
Form of the test substance	Technical grade glyphosate, purity 62%
Source of the test substance	ICI England
Type and source of the exposure medium	Chlorine free tap water; pH 7-7.5; total hardness 141-223 mg $CaCO_3$ l^{-1}; DO 7.5 – 11.5 mg l^{-1}; T=20±1°C
Test concentrations used	2.5, 5.0 and 10 mg l^{-1} + control
Number of replicates per concentration	1
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static; renewal every 24 h; fed daily
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	A separate acute test was reported in this study: 96 h LC50 = 620 (607 – 638) mg l^{-1}. The sub-lethal studies were conducted over 14 d of exposure. At 10 mg l^{-1} abnormal histopathologic changes were noted in the gills and liver. At 5 mg l^{-1} abnormal histopathologic changes were only noted in the gills. These changes were accompanied by increased alkaline phosphatase activity

Reliability of study	**Reliable with restrictions** (no analytical verification of exposure concentrations, non-standard endpoints, no replicates)
Relevance of study	**Relevance unknown**
Klimisch Code	**2**

Reference	Nielsen and Dahllöf, 2007

Information on the test species	
Test species used	*Zostera marina*
Source of the test organisms	Field collected at Skuldelev Beach, Roskilde Fjord, Denmark, in a water depth of 1.5 m. To standardize plants, only plants with 8 rhizomes were used.
Holding conditions prior to test	24 h acclimation in filtered seawater (12-15‰ salinity) at 10°C under aeration.
Life stage of the test species used	

Information on the test design	
Methodology used	72 h toxicity test; non-standard methodology but well described
Form of the test substance	Glyphosate acid purity not stated
Source of the test substance	Not reported
Type and source of the exposure medium	Filtered seawater
Test concentrations used	0, 0.1, 1.0, 10 and 100 µM
Number of replicates per concentration	3
Number of organisms per replicate	3
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static; 2 l aquaria with 2 cm glass pellets functioning as sediment.
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not reported
Test validity criteria satisfied	No validity criteria stated
Water quality criteria satisfied	Values not reported
Study conducted to GLP	No
Comments	The relative growth rate, t^{-1}, was normalized total length before exposure. The growth zone was weighed and the relative growth weight was also normalized to total length before exposure. The youngest leaf from every plant was grouped from every aquarium and frozen for subsequent chlorophyll analysis. No significant effect was observed on relative growth rate and the chlorophyll *a*-*b* ratio but relative growth rate in weight was stimulated to 140% of control at 10µM (1690 µg l^{-1}). There were indications of reduced relative growth rate in length, and increased content of chlorophyll *a*, which the authors concluded might suggest the plants were stressed, producing more chlorophyll *a* to keep up energy production, but that this resulted in a grwoth in wight rather than length.

Reliability of study	**Reliable with restrictions** (no analytical verification of exposure concentrations; some

	experimental detail not reported)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Pereira *et al*. 2009

Information on the test species	
Test species used	*1) Pseudokirchneriella subcapitata* *2) Daphnia magna* (clone A, *sensu*)
Source of the test organisms	In-house cultures
Holding conditions prior to test	1) maintained in nonaxenic batch cultures with Woods Hole MBL medium at 20±2°C and 24 h illumination. 2) monoclonal bulk cultures maintained in synthetic ASTM hard water medium with a standard organic additive and vitamins. Cultures were renewed every other day and fed with *P. subcapitata*, at a ration of 3.0×10^5 cells ml^{-1}.
Life stage of the test species used	1) exponential growth phase 2) < 24 h

Information on the test design	
Methodology used	1) OECD Guideline 201 (2006) except 96 h instead of 72h 2) OECD Guideline 202 (2004)
Form of the test substance	Glyphosate acid, purity 95%
Source of the test substance	Sapec Agro®, Portugal
Type and source of the exposure medium	Exposure medium same as culture medium
Test concentrations used	1) 61.5, 65.5, 81.9, 102.0, 160.0, 200.0 mg l^{-1} and clean MBL medium as negative control. 2) geometric dilution series up to 2,000 mg l^{-1} and control
Number of replicates per concentration	1) 3 2) 4
Number of organisms per replicate	1) 10^4 cells ml^{-1} 2) 5
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static; 16:8 light/dark photoperiod;
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes (implied by following OECD guidelines) values not reported
Test validity criteria satisfied	Tests carried out to OECD guidelines but unable to verify criteria met from data reported
Water quality criteria satisfied	Tests carried out to OECD guidelines but unable to verify criteria met from data reported
Study conducted to GLP	Not stated
Comments	Stock solution exhibited low pH and was adjusted with NaOH to comply with guideline (201 and 202) requirements.
Reliability of study	**Reliable with restrictions** (no analytical verification of exposure concentrations, some experimental detail not reported)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Roshon 1997

Information on the test species	
Test species used	*Myriophyllum sibiricum*
Source of the test organisms	University of Guelph, Ontario, Canada
Holding conditions prior to test	Same as test conditions
Life stage of the test species used	3 cm apical segments, without roots; axenic culture taken from 10 to 12 day old stock plants.

Information on the test design	
Methodology used	ASTM 1998 Vol 11.05 E 1913-97 pp. 1428-1442. (Draft Guideline at time of experimentation). Plants incubated at 25°C for 16 h photoperiod with light fluorescence 100-150 µmol m^{-2} s^{-1} and at 20°C during 8 h dark period
Form of the test substance	Technical grade purity 97%
Source of the test substance	Monsanto Company
Type and source of the exposure medium	Modified Andrews' liquid nutrient medium supplemented with 3% sucrose (pH 5.8 ± 0.1). Each test tube also contained 3 g artificial rooting substrate (Turface®).
Test concentrations used	0, 4.1, 12.3, 36.9, 110.6, 331.9, 995.6 and 2987 µg l^{-1} (geometric dilution series based on expected environmental concentration based on maximum label rate of 4.48 kg ha^{-1} as the highest test concentration)
Number of replicates per concentration	5
Number of organisms per replicate	1
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	No
Measurement of water quality parameters	Methodology states pH and conductivity of the medium in each test tube is measured every 24 h but no measurements are given.
Test validity criteria satisfied	Yes – stated as at least 3 surviving replicates of the controls and treatments.
Water quality criteria satisfied	Not stated
Study conducted to GLP	No
Comments	No significant difference between control plants and those exposed to 331.9 µg l^{-1} for growth rate, increased shoot length, root length, chlorophyll *a* and carotenoid content.

Endpoint	**IC25 µg l^{-1} (± 95% CI)**	**IC50 µg l^{-1}(± 95% CI)**
Growth rate	1100 (654; 1851)	> 2987
Increase in shoot length	1283 (760; 2165)	> 2987
Root length	599 (361; 993)	844 (673; 1058)

	Root number	2325	2798
	Fresh weight	594 (387; 910)	1474 (1058; 2054)
	Chlorophyll *a* (apical dry weight)	559 (217; 1439)	2157 (1002; 4643)

Reliability of study	**Reliable with restrictions (**no details reported on pH of test medium after addition of glyphosate and at end of 14 d test period)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Ruan *et al.* 2009

Information on the test species	
Test species used	*Caenorhabditis elegans* (wild-type N_2)
Source of the test organisms	Originally obtained from Caenorhabditis Genetics Center.
Holding conditions prior to test	Worms cultivated on nematode growth medium with a flat of *E. coli* strain OP50. Age-synchronous populations of N_2 were obtained by collection and culturing of eggs laid by emergent Dauer larvae.
Life stage of the test species used	L4 larvae

Information on the test design	
Methodology used	No standard guideline. 24 and 72 hr exposures. Endpoints assessed: behaviour (head thrash and body bend frequency); propogation (brood size and generation time – time from F0 egg to first F1 egg); body size. Exposures were performed in 24-well sterile tissue culture plates.
Form of the test substance	Technical grade purity not stated
Source of the test substance	Not stated
Type and source of the exposure medium	K medium
Test concentrations used	7, 70, 700 and 7000 µg l^{-1} + control
Number of replicates per concentration	Variable depending on endpoint being assessed. 10 replicates for propagation assessment
Number of organisms per replicate	Behaviour ability and body size – 15 per treatment Propagation – single nematode per individual well. Every P0 animal transferred to new well every 1.5 d. Progeny counted the day following transfer.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static – 24 hr exposures, not fed; 72 h exposure carried out with addition of food. Culture plates placed in incubator at 20°C.
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not reported
Test validity criteria satisfied	No guidelines
Water quality criteria satisfied	-
Study conducted to GLP	No
Comments	Body bend frequency decreased in dose-dependent manner and was significant in highest test concentration at 24 h but no differences at 72 h (graph shows slight increase in activity). Head thrash frequency decreased in dose-dependent manner and was significant in highest test concentration at 24 and 72 h. Generation time extended quantitatively but did

	not reach significance. Brood size declined in a dose-dependent manner and was significant at the highest test concentration after 72 h (20% reduction). No effect on body size.

Reliability of study	**Reliable with restrictions** (no analytical verification of exposure concentrations; non-standard test)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Saenz *et al.* 1997

Information on the test species	
Test species used	1) *Scenedesmus quadricauda* 2) *Scenedesmus acutus* (SAG No. 276-3a)
Source of the test organisms	1) Luján River, Buenos Aires Province 2) axenic strain from Culture collection of Algae, University of Göttingen, Germany
Holding conditions prior to test	Maintained in autoclaved modified Detmer's nutrient medium ;pH 7.5; total hardness 80.1 mg $CaCO_3$ l^{-1}; DO = saturation; temp 22±1°C; continuous cool-white fluorescent light 3000 lux cm^{-2}; 100 rpm shaker.
Life stage of the test species used	Inoculum prepared from 5 d stock culture

Information on the test design	
Methodology used	96 h algal growth test (USEPA 1989); same environmental conditions as culture
Form of the test substance	Technical grade isopropylamine salt 99.5% purity, and Ron-do formulation (48% a.i. and 15% surfactant oxide-coco-amide-propyl dimethyl amine)
Source of the test substance	Not stated
Type and source of the exposure medium	Detmer's nutrient medium
Test concentrations used	1) 3.1, 6.2, 12.5, 25, 50 and 100 mg l^{-1} tech grade isopropylamine salt; 2.5, 5, 10, 20 and 40 mg a.i. l^{-1} (Ron-do) 2) 6, 8, 10, 12, 14, 16, 18 and 20 mg l^{-1} tech grade isopropylamine salt; 5.1, 6.4, 8, 10, 12.5 mg a.i. l^{-1} (Ron-do)
Number of replicates per concentration	2
Number of organisms per replicate	5 x 10^4 cell ml^{-1}
Nature of test system (static, semi-static or flow-through, duration, feeding)	static
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	yes
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	Inconsistency between the text and tables with respect to the results.

	S. acutus	*S. quadricauda*
	mg l^{-1}	mg l^{-1}
NOEC	2.0	0.77
LOEC	4.0	1.55
96 h EC50	10.2 (95%CI 10.4 – 11.2)	7.2 (95%CI 4.4 – 8.9)

According to the text Dunnett's procedure was

	used to calculate the NOEC. Both NOECs are considerably lower than the lowest test concentration used, and are unlikely to be EC10 values. For *S. acutus* 10, 76 and 89% growth inhibition was seen at 8, 12 and 16 mg l^{-1}, respectively and for *S. quadricauda* 15, 60, 75 and 80% inhibition was seen at 3.2, 12.5, 25 and 50 mg l^{-1}. No statistically significant effect on chlorophyll 'a' content except *S. quadricauda* at 50 mg l^{-1} glyphosate.

Reliability of study	**Reliable with restrictions** (inconsistency in reporting of results)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Smyth *et al.* 1995 (Confidential data supplied by Syngenta Limited)

Information on the test species	
Test species used	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum* Printz, Strain ATCC 22662))
Source of the test organisms	Laboratory cultures maintained under axenic conditions.
Holding conditions prior to test	Cultures grown in same medium and environmental conditions as described for the test.
Life stage of the test species used	3-day old culture in exponential growth phase

Information on the test design	
Methodology used	EPA FIFRA Subdivision J Guideline 123-2. Growth and reproduction of aquatic plants. OECD Guideline 201 (1984)
Form of the test substance	Glyphosate acid (white solid); 95.6% w/w purity
Source of the test substance	Zeneca Agrochemicals.
Type and source of the exposure medium	Hoagland's M-medium
Test concentrations used	5.6, 10, 18, 32, 56 and 100 mg l^{-1} + culture medium control (nominal) 5.6, 10, 20, 33, 58 and 100 mg l^{-1} + control < LoD (0.004 mg l^{-1})
Number of replicates per concentration	6 (controls) 3 (test concentration)
Number of organisms per replicate	0.300 x 10^4 cells ml^{-1}.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static; no aeration, with orbital shaking at 100 rpm, under continuous "cool-white" illumination (5030 lux), in a Gallenkamp type INR-401 orbital incubator.
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Temp = 24 ± 1°C; pH 3.5 – 7.5 fresh solutions and 3.6 – 8.9 old solutions
Test validity criteria satisfied	Yes
Water quality criteria satisfied	A maximum increase of 2 pH units was observed in one of the replicates of the nominal 5.6 mg l-1 test concentration over the test duration. This pH shift was considered to be a function of the very high growth factors observed.
Study conducted to GLP	Yes
Comment	Algal particle densities were determined using a Coulter counter model ZB. Measurements were taken at 24, 48, 72, 96 and 120 h. Samples for analytical verification of test concentrations were taken at start of test and at end of test (from the appropriate "blanks"). Mean measured concentrations ranged from 100 – 111% of nominal values, therefore nominal test concentration values were used for the calculation and reporting of all results

	(LoD = 0.004 mg l^{-1}). pH ≤ 5.9 day 0 at test concentrations ≥ 18 mg l^{-1}. NOEC = 10 mg l^{-1} (biomass and growth rate). $EC50_{biomass}$ = 17 (95% CI 13 -22) mg l^{-1}; $EC50_{growthrate}$ = 21 (95%CI 16 -28) mg l^{-1}.

Reliability of study	**Reliable with restrictions** (large variation in pH values across test concentrations)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Smyth *et al.* 1996a (Confidential data supplied by Syngenta Limited)

Information on the test species	
Test species used	*Lemna gibba* (Strain G3)
Source of the test organisms	Laboratory cultures maintained under axenic conditions. Cultures originally obtained from University of Waterloo, Canada.
Holding conditions prior to test	Cultures grown in same medium and environmental conditions as described for the test.
Life stage of the test species used	Actively growing duckweed from 14 d old cultures used as inoculum for the test.

Information on the test design	
Methodology used	EPA FIFRA Subdivision J Guideline 123-2. Growth and reproduction of aquatic plants.
Form of the test substance	Glyphosate acid; 95.6% w/w purity
Source of the test substance	Zeneca Agrochemicals.
Type and source of the exposure medium	Hoagland's M-medium
Test concentrations used	0.75, 1.5, 3.0, 6.0,12.0, 24.0, 48.0, 96.0 mg l^{-1} + culture medium control (nominal) 0.7, 1.4, 2.9, 5.6, 12.0, 23.0, 48.0, 96.0 mg l^{-1} + control < LoD (0.003 mg l^{-1})
Number of replicates per concentration	3
Number of organisms per replicate	3 plants each consisting of 4 fronds
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static, renewal of test solutions on days 5 and 9; no aeration; light intensity 5250 – 5210 lux.
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Temp = 24.6 – 25.7; pH 3.5 – 4.9 fresh solutions and 3.6 – 5.8 old solutions
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes; recommended pH when using M-Hoagland's medium 4.8 -5.2. According to FIFRA guidelines any changes due to test chemical should be recorded but not adjusted.
Study conducted to GLP	Yes
Comment	NOEC for frond increase 2.9 mg l^{-1}; NOEC for weight increase 5.6 mg l^{-1}. From day 2 plants in 23, 48 and 96 mg l^{-1} test concentrations exhibited progressive, dose related symptoms, which included pale frond colouration, the emergence of stunted new frond growths and reduced root growth. The plants also floated at unnatural attitudes on the solution surface. Plants in 12 mg l^{-1} test concentration displayed similar symptoms from day 5. Pale colouration, stunted new fond growths and reduced root growth became apparent from day 9 in 5.6 mg l^{-1}. On day 14, a

	small number of fronds (< 5%) were observed to be showing pale colouration in all three 2.9 mg l^{-1} test replicate solutions. Since visually observed effects were apparent at concentrations ≥ 2.9 mg l^{-1}, the overall NOEC = 1.4 mg l^{-1}.

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Smyth *et al.* 1996b (Confidential data supplied by Syngenta Limited)

Information on the test species	
Test species used	*Navicula pelliculosa* (Strain UTEX 667)
Source of the test organisms	Laboratory cultures maintained under axenic conditions.
Holding conditions prior to test	Cultures grown in same medium and environmental conditions as used for the test.
Life stage of the test species used	3 day old culture in exponential growth phase

Information on the test design	
Methodology used	EPA FIFRA Subdivision J Guideline 123-2. Growth and reproduction of aquatic plants – EPA 540/09-82-020 (1982)
Form of the test substance	Glyphosate acid; 95.6% w/w purity
Source of the test substance	Zeneca Agrochemicals.
Type and source of the exposure medium	As described in EPA-600/9-78-018 (1978)
Test concentrations used	1.8, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0 and 100 mg l^{-1} + culture medium control (nominal) 1.9, 3.4, 6.2, 11, 19, 35, 61 and 110 mg l^{-1} + culture medium control (<LoD) (mean measured)
Number of replicates per concentration	6 (control) 3 (per test concentration)
Number of organisms per replicate	0.3 x 10^4 cells ml^{-1}
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, no aeration, shaken (140 rpm), continuous “cool-white illumination (4560 lux)
Measurement of exposure concentrations	
Measurement of water quality parameters	T = 24 ± 1°C; pH range 3.7 – 8.3 at start of test and 3.7 – 8.7 at test termination.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Large variation in pH. EPA guidelines recommend pH of nutrient medium to be 7.5 (±0.1) and is not to be adjusted after addition of algae. However, if the test chemical is highly acidic and reduces pH of test solution < 5.0 at the first measurement, appropriate adjustments to pH should be considered, and test solution measured for pH on each day of the test.
Study conducted to GLP	Yes
Comment	Algal particle densities were determined using a Coulter counter model ZB. Measurements were taken at 24, 48, 72, 96 and 120 h. Samples for analytical verification of test concentrations were taken at start of test and at end of test (from the appropriate “blanks”). Mean measured concentrations ranged from 106 – 111% of nominal values, therefore nominal test concentration values were used for the calculation and reporting of all results in the report (LoD = 0.0021 mg l^{-1}).

	Effects observed in the lowest 4 test concentrations in the 0 - 4 and 0 - 5 day growth periods were due to growth enhancement. No inhibitory effects were observed < 32 mg l^{-1}. No effects were seen in the 18 mg l-1 at time period. For both areas under the growth curve and growth rates there was a large difference in dose response between nominal 18 and 32 mg l^{-1} test concentrations. Transformation to a probability scale led to a skewed data set and resulted in E_bC50 and E_rC50 values of 17 mg l^{-1} (< NOEC). (95% CI 12 – 24 mg l^{-1})

Reliability of study	**Reliable with restrictions** (large differences in pH value between control and test concentrations >18 mg a.e.l^{-1})
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Smyth *et al*. 1996c (Confidential data supplied by Syngenta Limited)

Information on the test species	
Test species used	*Skeletonema costatum* (Strain CCAP 1077/1C)
Source of the test organisms	Culture Centre of Algae and Protozoa, Dunstaffnage marine Laboratory, Argyll, UK
Holding conditions prior to test	Maintained under axenic conditions and grown in the medium and under the environmental conditions used in the test.
Life stage of the test species used	3 day old culture in growth phase

Information on the test design	
Methodology used	USA EPA 1982: EPA 540/09-82-020 OECD 1984 (OECD Guideline 201)
Form of the test substance	Glyphosate acid, purity 95.6% w/w
Source of the test substance	Zeneca Agrochemicals
Type and source of the exposure medium	Sterile culture medium
Test concentrations used	1.0, 1.8, 3.2, 5.6, 10.0, 18.0, 32.0, and 56.0 mg l^{-1} + culture medium control (nominal) 0.99, 1.8, 3.0, 5.6, 9.4, 19, 32 and 59 mg l^{-1} + culture medium control (<LoD) (mean measured)
Number of replicates per concentration	6 (control) 3 (per test concentration)
Number of organisms per replicate	10^4 cells ml^{-1} (nominal) 0.776 x 10^4 measured particle density in inoculum.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static; no aeration, shaken, 16^L:8^D photoperiod at 4340 lux.
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	T = 20 ± 1°C; pH range 7.1 – 8.1 at start of test and 8.1 – 8.8 at test termination; salinity 30.5‰.
Study conducted to GLP	Yes
Comments	Algal particle densities were determined using a Coulter counter model ZB. Measurements were taken at 24, 48, 72, 96 and 120 h. Samples for analytical verification of test concentrations were taken at start of test and at end of test (from the appropriate “blanks”). Mean measured concentrations ranged from 94 – 106% of nominal values, therefore nominal test concentration values were used for the calculation and reporting of all results in the report (LoD = 0.023 mg l^{-1}). $NOEC_{biomass}$ at 72, 96 and 120 h = 1.8 mg l^{-1}. $EC50_{biomass}$ at 120 h = 12 (95%CI 7.6 -19) mg l^{-1}. $NOEC_{growth\ rate}$ = 1.8 mg l^{-1} at 72 h and 10 mg l^{-1} at 96 and 120 h. $EC50_{growth\ rate}$ = 18 (95%CI 10 - >42) mg l^{-1} at 72h and 24 (95%CI 12 - >56) mg l^{-1} at 120h.

Reliability of study	**Reliable without restrictions**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Smyth *et al*. 1996d (Confidential data supplied by Syngenta Limited)

Information on the test species	
Test species used	*Anabaena flos-aquae* (Strain CCAP 1403/13A)
Source of the test organisms	In-house laboratory culture
Holding conditions prior to test	Same as test conditions
Life stage of the test species used	3-d old culture in exponential growth phase

Information on the test design	
Methodology used	USA EPA 1982: EPA 540/09-82-020 OECD 1984 (OECD Guideline 201)
Form of the test substance	Glyphosate acid (white solid), purity 95.6% w/w
Source of the test substance	Zeneca Agrochemicals
Type and source of the exposure medium	
Test concentrations used	0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96.0 mg l^{-1} + culture medium control (nominal) <0.0021 (LoD control), 0.75, 1.5, 3.1, 6.1, 13, 24, 47 and 110 mg l^{-1} (mean measured)
Number of replicates per concentration	6 (control) 3 (per test concentration)
Number of organisms per replicate	2.05 x 10^4 cells ml^{-1}
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static; no aeration, with orbital shaking at 100 rpm, under continuous “cool-white” illumination (3600 lux), in a Gallenakmp type INR-401 orbital incubator.
Measurement of exposure concentrations	Yes, at start of test samples taken using the excess stock solution. At the end of the test using blank vessel (without algal inoculum) set up for this purpose, one per test concentration.
Measurement of water quality parameters	Yes. Large variation in pH. EPA guidelines recommend pH of nutrient medium to be 7.5 (±0.1) and is not to be adjusted after addition of algae. However, if the test chemical is highly acidic and reduces pH of test solution < 5.0 at the first measurement, appropriate adjustments to pH should be considered, and test solution measured for pH on each day of the test.
Test validity criteria satisfied	Yes
Water quality criteria satisfied	T = 24 ± 1°C; pH range 3.5 – 7.2 at start of test and 3.6 – 8.2 at test termination.
Study conducted to GLP	Yes
Comments	Mean measured concentrations ranged from 98 – 110% of nominal values, therefore nominal test concentration values were used for the calculation and reporting of all results (LoD = 0.0021 mg l^{-1}).

Reliability of study	**Reliable with restrictions** (large variation in pH values across test concentrations)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Sobrero *et al*. 2007

Information on the test species	
Test species used	*Lemna gibba* L.
Source of the test organisms	Field collected in El Pescado stream, Buenos Aires Province, Argentina.
Holding conditions prior to test	Stock cultures maintained in standardized growth conditions using a sterile nutrient solution, pH 6.5 at 24 ± 2°C; 16:8 h light:dark photoperiod with 80 µM m^{-2} s^{-1}, cool-white fluorescent light. Plants acclimated for 1 month prior to testing.
Life stage of the test species used	-

Information on the test design	
Methodology used	Environment Canada 1999. Biological test method: test for measuring the inhibition of growth using the freshwater macrophyte *Lemna minor*. EPS 1/RM/37.
Form of the test substance	Glyphosate acid technical grade, 95%w/w
Source of the test substance	Not stated
Type and source of the exposure medium	Sterile nutrient solution
Test concentrations used	0.5, 1.0, (2.5?), 7.5, 15, 25, 60 and 80 mg l^{-1} + control
Number of replicates per concentration	4
Number of organisms per replicate	Six fronds
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static; to ensure exponential growth 1 ml fivefold nutrient solution was added every 2 or 3 d during exposure period. Environmental conditions same as for culture.
Measurement of exposure concentrations	Yes; samples taken throughout exposure period, results reported for days 0, 5 and 10 (test termination). Analysis was done by HPLC-UV detection (206 nm) and previous derivatization with 9-fluoroenylmethyl chloroformate chloride. Results given for nominal glyphosate concentrations of 1, 2.5, 15 and 25 mg l-1. Day 0 within 4%, day 5 ranged between 10 and 33% below nominal, day 10 ranged between 15 and 34% below nominal.
Measurement of water quality parameters	pH and temperature
Test validity criteria satisfied	Control growth rate not cited; exposure concentrations not maintained within 80% of nominal and results based on nominal concentrations.
Water quality criteria satisfied	Yes; pH increased from 6.5 to 7.8 over 10 days.
Study conducted to GLP	No
Comments	At 0.5 mg a.i. l^{-1} no changes on the growth rate were detected during the exposure time. At 1 mg a.i. l^{-1}, a significant effect was observed

	during the first days, turning to a recovery of the growth rate by test termination (24% and 4% inhibition, day 2 and day 10, respectively). 7-d IC10 < 1 mg a.i. l^{-1}; 10-d IC10 4.6 mg a.i. l^{-1} (95%CI 2.4 – 6.7). The IC25 and IC50 showed an increase in toxicity over the exposure period.

Reliability of study	**Reliable with restrictions** (some experimental detail not reported, exposure concentrations not maintained at 80% of nominal for duration of test)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	St-Laurant *et al*. 1992

Information on the test species	
Test species used	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
Source of the test organisms	In-house culture
Holding conditions prior to test	Grown in 0.625X AAP with 187.5 µg l^{-1} EDTA disodium salt; 24 ± 2°C; cool white fluorescent light, 73 ± 9 µE m^{-2} s^{-1}.
Life stage of the test species used	4 – 7 day old cells

Information on the test design	
Methodology used	Microplate algal assay and assay bottle test EPA/600/4-89/001
Form of the test substance	Technical grade glyphosate acid purity not stated
Source of the test substance	Monsanto, Canada Inc.
Type and source of the exposure medium	Same as culture medium
Test concentrations used	10 + control, no further details provided
Number of replicates per concentration	Microplate assay 10 ((control) 5 (test concentrations); bottle assay 6 (control) 3 (test concentrations)
Number of organisms per replicate	20,000 cells ml^{-1}
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static; flasks continuously shaken (100 rpm); same environmental conditions as culture
Measurement of exposure concentrations	No
Measurement of water quality parameters	No
Test validity criteria satisfied	Unknown
Water quality criteria satisfied	Unknown
Study conducted to GLP	No
Comments	Cell growth measured with a Coulter Counter. Linear regression analysis of % growth inhibition in relation to controls. EC50 = 13.5 (95% CI 11.1 – 16.6) mg l^{-1} (flask assay); EC50 = 7.8 (95% CI 3.0 – 12.7) mg l^{-1} (microplate assay)

Reliability of study	**Reliable with restrictions** (no analytical verification of exposure concentrations; some experimental detail not reported)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Tate *et al*. 1997

Information on the test species	
Test species used	*Pseudosuccinea columella*
Source of the test organisms	Laboratory culture
Holding conditions prior to test	Reared in 2-gallon glass aquariums containing artificial spring water. Water hardness maintained at 80 – 120 mg $CaCO_3$ l^{-1}; DO 6 – 8 mg l^{-1}; pH 6.5 - 8.5 and ammonia nitrate levels 2 mg l^{-1}.
Life stage of the test species used	Freshly laid egg masses

Information on the test design	
Methodology used	Non-standard methodology but well described. Experiment lasted through to 3rd generation. Egg masses monitored every 3 days until eggs hatched (~ within 12-13 days). Each embryo within masses measured to determine growth and abnormalities. 2nd and 3rd generation egg masses of same size, shape and no. of embryos were obtained from dishes containing 1st and 2nd generation snails that were continuously exposed to glyphosate.
Form of the test substance	Glyphosate 97% purity
Source of the test substance	Chem Service, West Chester, Pennsylvania
Type and source of the exposure medium	Artificial spring water (same composition as culture)
Test concentrations used	Control, 0.1, 1 and 10 mg l^{-1}
Number of replicates per concentration	2
Number of organisms per replicate	1 egg mass
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static, renewal every 24h; snails fed endive lettuce leaves *ad libitum* and aquaria were aerated.
Measurement of exposure concentrations	No
Measurement of water quality parameters	pH 6.8 – 7.2; Temp 25 ± 2°C
Test validity criteria satisfied	No validity criteria stated; control response would appear to be acceptable
Water quality criteria satisfied	Yes
Study conducted to GLP	No
Comments	

Reliability of study	**Reliable with restrictions** (no verification of exposure concentrations)
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Tsui and Chu 2003

Information on the test species	
Test species used	*1) Pseudokirchneriella subcapitata* *2) Ceriodaphnia dubia* *3) Tetrahymenaa pyriformis*
Source of the test organisms	1) Culture Collection of Algae, University of Texas at Austin, USA 2) Aquatic Research Organisms, Hampton, NH, USA 3) Culture Collection of Algae and Protozoa, Cumbria UK
Holding conditions prior to test	Same as test conditions
Life stage of the test species used	Not stated

Information on the test design	
Methodology used	1) ASTM 1994 2) USEPA 1993 3) Schultz 1997
Form of the test substance	a) Glyphosate acid ≥ 97% purity b) Isopropylamine salt 56.8% a.i. c) Roundup® (commercial grade; 41% a.i.)
Source of the test substance	a) Fluka, Buchs, Switzerland b) Monsanto Chemical Co, St Louis, MO, USA c) Monsanto Chemical Co, St Louis, MO, USA
Type and source of the exposure medium	1) ASTM in deionised water 2) Reconstituted water (69.5 mg $CaCO_3$ l^{-1}) 3) Tetratox medium
Test concentrations used	5 – 8 (dilution factor = 0.5) following range-finding tests + control
Number of replicates per concentration	1) 3 2) 4 3) 3
Number of organisms per replicate	1) 20,000 cells ml^{-1} 2) 5 3) 2500 cells ml^{-1}
Nature of test system (static, semi-static or flow-through, duration, feeding)	static
Measurement of exposure concentrations	Yes – 100% (± 1% SD) recovery
Measurement of water quality parameters	Yes: T = 25 ± 1°C, 27 ± 1°C (*T. pyriformis*); pH 7.5 (alga) 8.07 (*C. dubia*) 7.4 (*T. pyriformis*); DO = > 90% at commencement: > 70% at end of test period
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	An additional study with *C. dubia* was carried out to assess the effect of environmental factors on Roundup toxicity. Temp 20 - 30°C no sig. diff with organisms acclimatized prior to testing. Increasing toxicity of Roundup from pH 6 to pH 9 > 16 mg ae l^{-1} to 3.78 mg ae l^{-1} at 24 h: 4.47 mg ae l^{-1} to 2.9 mg ae l^{-1} at 48 h. The decrease in LC50 at pH 6 from 24 to 48 h due

	to toxic effect of acidity (control mortality 25%). The addition of suspended particles (kaolin clay) increased toxicity with increasing amounts of suspended particles but control mortality was high therefore it is not possible to separate toxicity due to herbicide.

Reliability of study	**Reliable without restrictions**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Tsui and Chu 2003

Information on the test species	
Test species used	*1) Skeletonema costatum* *2) Euplotes vannus* *3) Arcatia tonsa*
Source of the test organisms	1) Culture Collection of Algae, University of Texas at Austin, USA 2) Aquatic Research Organisms, Hampton, NH, USA
Holding conditions prior to test	Same as test conditions
Life stage of the test species used	1) Not stated 2) Not stated 3) Adults

Information on the test design	
Methodology used	1) ASTM 1994 2) Modified from Coppellotti 1998 3) ISO 1997
Form of the test substance	a) Glyphosate acid ≥ 97% purity b) Isopropylamine salt 56.8% a.i. c) Roundup® (commercial grade; 41% a.i.)
Source of the test substance	a) Fluka, Buchs, Switzerland b) Monsanto Chemical Co, St Louis, MO, USA c) Monsanto Chemical Co, St Louis, MO, USA
Type and source of the exposure medium	1) ASTM in artificial seawater (30‰) 2) 1% Bactotryptone and 0.5% yeast extract in artificial seawater (30‰) 3) artificial seawater (30‰)
Test concentrations used	5 – 8 (dilution factor = 0.5) following range-finding tests + control
Number of replicates per concentration	1) 3 2) 3 3) 4
Number of organisms per replicate	1) 20,000 cells ml^{-1} 2) 1,000 cells ml^{-1} 3) 5
Nature of test system (static, semi-static or flow-through, duration, feeding)	static
Measurement of exposure concentrations	Yes – 53.5% (± 6% SD) recovery – recovery-corrected concentrations reported.
Measurement of water quality parameters	Yes: T = 20 ± 1-2°C; pH 8: DO = > 90% at commencement: > 70% at end of test period
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	

Reliability of study	**Reliable without restrictions**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Vendrell *et al*. 2009

Information on the test species	
Test species used	***1)*** *Scenedesmus acutus* (Meyens) ***2)*** *Scenedesmus subspicatus* CCAP 276/22 ***3)*** *Chlorella vulgaris* Beijerinck ***4)*** *Chlorella saccharophila* (Krüger) Migula
Source of the test organisms	**1** and **4** - isolated from samples collected at Albufera lake in Valencia (Spain) **2** – Institute of Freshwater Ecology, Ambleside, UK. **3** – Area of Environmental Toxicology (CISA-INIA, Spain)
Holding conditions prior to test	Stock cultures were maintained in a liquid medium (ASTM 1997. E 1218-97a) at 22 ± 2°C on a 12:12 light/dark photoperiod at 1,100 lux.
Life stage of the test species used	Exponential growth phase

Information on the test design	
Methodology used	Microplate bioassays, using polystyrene microplates with 12 x 8 flat bottom well of 400 µl capacity. Growth of cultures was measured at 450 nm wavelength using a microplate reader at 0, 24, 48 and 72 h (readings taken twice). ANOVA and Student Newman-Keuls multiple range test used to determine if treatments significantly different ($p \leq 0.05$).EC10 and EC50 values with 95% confidence limits estimated by linear regression of probit of % growth on log dose of glyphosate (ASTM 1997. E 1218-97a).
Form of the test substance	Analytical standard glyphosate acid, purity 97.5%
Source of the test substance	"Dr Ehrenstorfer Quality", Augsburg, Alemania
Type and source of the exposure medium	Algal culture medium
Test concentrations used	**1)** control, 0.1, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25.0 and 50.0 mg l^{-1} (nominal) **2)** control, 0.1, 0.39, 1.56, 6.25, 12.5, 25.0 and 50.0 mg l^{-1} (nominal) **3)** control, 0.1, 0.20, 0.39, 0.78, 1.56, 6.3, 12.5, 25.0, 50.0 and 100 mg l^{-1} (nominal) **4)** control, 0.1, 0.39, 1.56, 6.3, 12.5, 25.0, 50.0 and 100 mg l^{-1} (nominal)
Number of replicates per concentration	8
Number of organisms per replicate	**1** and **2** – 5 x 10^5 cells ml-1 **3** and **4** – 2.5 x 10^6 cells ml-1
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static. T = 24 ± 2°C and 8,000 lux. Microplates were shaken at 100 rpm.
Measurement of exposure concentrations	No
Measurement of water quality parameters	Not applicable.

Test validity criteria satisfied	Yes
Water quality criteria satisfied	Not applicable
Study conducted to GLP	Not stated
Comments	Stimulated growth was observed at 0.1 mg l^{-1} for *C. saccharophilia*.

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Wan *et al.* 1989

Information on the test species	
Test species used	*Oncorhynchus kisutch, O. keta, O. tshawytsha, O. gorbuscha* and *O. mykiss (Salmo gairdneri)*
Source of the test organisms	British Columbia hatcheries
Holding conditions prior to test	Not stated
Life stage of the test species used	2.6 months; mean length 4.3 cm, weight 0.5 g

Information on the test design	
Methodology used	EPS 1-WP-80-1 (Environment Canada, 1980) with modifications.
Form of the test substance	Technical grade Glyphosate (88.5% batch 1; 95.4% batch 2); 2 Roundup formulations both (41% a.i. glyphosate IPA salt) with different amounts of POEA
Source of the test substance	Monsanto Company, USA, Chesterfield, Missouri and Monsanto Canada Incorporated, Delta, BC
Type and source of the exposure medium	5 different dilution waters to test the effect of water hardness on toxicity
Test concentrations used	Not stated
Number of replicates per concentration	Not stated
Number of organisms per replicate	10
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes – recovery rates 98.5 ± 1%
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Yes
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	LC50 values based on measured concentrations, losses to system over 96 h period.

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Wang *et al.* 1994

Information on the test species	
Test species used	*1) Cyprinus carpio* *2) Oreochromis mossambicus*
Source of the test organisms	Not stated
Holding conditions prior to test	Test fish were fed once a day during a 1 w acclimatization period, and were not fed for 2 d prior to being used in a test.
Life stage of the test species used	1) 3.5 – 4.0 cm 2) 3.0 – 3.5 cm

Information on the test design	
Methodology used	Not stated
Form of the test substance	Technical grade glyphosate 94% purity
Source of the test substance	Monsanto Co. St Louis, Mo, USA
Type and source of the exposure medium	Not stated
Test concentrations used	Not stated
Number of replicates per concentration	2
Number of organisms per replicate	Not stated
Nature of test system (static, semi-static or flow-through, duration, feeding)	Not stated; no aeration
Measurement of exposure concentrations	Not stated
Measurement of water quality parameters	T = 22 ± 1°C
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Not stated
Study conducted to GLP	Not stated
Comments	This paper was primarily concerned with assessing accumulation of glyphosate and details of test methods for determining the LC50 were not given

Reliability of study	**Not reliable**
Relevance of study	**Relevant**
Klimisch Code	**3**