The effects of selected agricultural chemicals on freshwater microalgae and cladocerans in laboratory studies, with particular emphasis on hormesis

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Liliana Zalizniak
B. Sc. (Physics), M.Sc. (Env. Manag.)

Biotechnology and Environmental Biology
School of Applied Sciences
Science, Engineering and Technology Portfolio
RMIT University
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DECLARATION

I declare that:

- Except where due acknowledgements have been made, the work is mine alone;
- The work has not been submitted previously, in whole or in part, to qualify for any other academic award;
- The content of the thesis is the result of work, which has been carried out since the official commencement date of the approved research program; and
- Any editorial work, paid or unpaid, carried out by a third party is acknowledged.

Liliana Zalizniak

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Chapters 4-7 are the journal articles, which include input from my supervisor for which I am grateful. These papers also benefited from the comments of anonymous referees.

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LIST OF ABBREVIATIONS

a.e.	acid equivalent
ai	active ingredient
ChV	chronic values
CPF	chlorpyrifos
EC_{50}	concentration of a substance causing a particular response (effect) in 50% of population
EEC	expected environmental concentration
GL	gigalitre
Gly	glyphosate
L	litre
LC_{50}	concentration of a substance causing death of 50% of a population
mc	measured concentration
mg	milligram
µg	microgram
nc	nominal concentration
pf	product formulation
RB	Roundup Biactive

LIST OF PUBLICATIONS AND CONFERENCES PRESENTATIONS

Papers published in peer reviewed journals

Zalizniak, L., Nugegoda, D., 2006. Effect of sublethal concentrations of chlorpyrifos on three successive generations of *Daphnia carinata. Ecotoxicology and Environmental Safety,* **64**(2), 207-214.

Zalizniak, L., Nugegoda, D., 2006. Roundup Biactive modifies cadmium toxicity to *Daphnia carinata. Bulletin of Environmental Contamination and Toxicology,* **77**(5), 748-754.

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Refereed abstracts

Zalizniak, L., Nugegoda, D., 2003. Effect of Sublethal Concentrations of Chlorpyrifos on Three Successive generations of *D. carinata.* In: Solutions to Pollutions: Programme Abstract Book, Christchurch, New Zealand, September-October 2003, p. 199. The Society of Environmental Toxicology and Chemistry Asia/Pacific- The Australasian Society of Ecotoxicology, Christchurch, New Zealand.

Nugegoda, D., Zalizniak, L., Heazelwood, P.J., Heffernan, J., 2003. Pesticides and Australian Freshwater Crustaceans. In: Solutions to Pollutions: Programme Abstract Book, Christchurch, New Zealand, September-October 2003, p. 48. The Society of Environmental Toxicology and Chemistry Asia/Pacific- The Australasian Society of Ecotoxicology, Christchurch, New Zealand.

Oral presentations at conferences

An oral presentation was given at the Biennial Conference of the Australasian Society for Ecotoxicology "From Reality to Regulation", 12-14 February 2001, Canberra. The title of the presentation "Effect of Sublethal Concentrations of Glyphosate on *Daphnia carinata*: Population characteristics of three successive generations".

Another presentation titled "Low concentrations of agrochemicals in the environment: are they safe? A case study of chlorpyrifos and glyphosate" was given at the 12th International Symposium on Toxicity Assessment, Skiathos, Greece, 12-17 June, 2005.

An oral presentation "Effects of low concentrations of glyphosate and chlorpyrifos on links in a freshwater trophic chain" was given at the Biennial Conference of the Australasian Society for Ecotoxicology, Melbourne, 26-29 September 2005.

Also several posters were presented at Conferences of Australasian Society for Ecotoxicology and Australian Society of Limnology.

Papers submitted for publication in peer reviewed journals, that are currently under review

Zalizniak, L., Nugegoda, D. Chlorpyrifos and glyphosate at low concentrations stimulate growth of freshwater algae *Chlorella pyrenoidosa* and *Pseudokirchneriella subcapitata.*

Zalizniak, L., Nugegoda, D. Effects of two formulations of a herbicide glyphosate on *Daphnia carinata* in multiple-generation toxicity tests.

CHAPTER 1

SUMMARY

This thesis examines the effects of 3 selected pesticides on a model freshwater food chain of a producer and consumer. Chapter 2 reviews the specific features of the Australian environment and why native Australian species should be used in evaluating the effects of toxicants to Australian biota, especially the effects of pesticides. Toxicity of the herbicide glyphosate (two formulations – technical grade and Roundup Biactive RB) and insecticide chlorpyrifos CPF to aquatic biota are examined. The importance of studying the toxicity of low (environmentally realistic) concentrations of pesticides to non-target organisms is introduced. Based on these the aims of the project are specified.

Chapters 3.1 and 3.2 are literature reviews on the toxicity of glyphosate and chlorpyrifos to aquatic organisms.

In Chapter 4 the requirements for the maintenance of algal and *Daphnia carinata* cultures are presented. Three species of algae were used in various experiments and for maintenance of *D. carinata* cultures: *Chlorella vulgaris, Chlorella pyrenoidosa* and *Pseudokirchneriella subcapitata*. Batch cultures were used for maintenance of the algae, grown in flasks on a light-table. Sub-culturing was conducted once a week or as required. Two media were used for maintenance of these cultures: Tamiya (Vasser 1989) and Keating (1985). The most widespread daphnid in Australia, *D. carinata* is considered to be one of the most suitable for toxicity testing of contaminants entering Australian freshwaters. Little data is available on the culture requirements of the

species, and this chapter evaluates the efficacy of different food types for culture of *D. carinata.* Different types of food were tested: *Chlorella vulgaris* cultured in two different media - Keating and Tamiya, *Chlorella pyrenoidosa* cultured in the same two media, and a suspension of trout pellets. Intrinsic rates of natural increase of individual cultures of *D. carinata* were determined from "life tables". The best food from among those tested in terms of providing adequate survival and fecundity of *D. carinata* were *C. pyrenoidosa* cultured in either Keating or Tamiya medium. Two different procedures of individual cultures are proposed for the maintenance of *D. carinata* for use in toxicity testing using different culture volumes.

In Chapter 5 the results of a series of 72-h toxicity tests with algae are presented. The effects of two formulations of the herbicide glyphosate (technical grade and Roundup Biactive®) and the insecticide chlorpyrifos on the growth of *Chlorella pyrenoidosa* and *Pseudokirchneriella subcapitata* were studied, and the EC_{50} values determined. With glyphosate and Roundup Biactive® the 72-h EC_{50} were: *C. pyrenoidosa* = 788 and 763 mg/L, and *P. subcapitata* = 429 and 397 mg/L, while hormesis was observed when *P. subcapitata* was exposed at concentrations equal to 7% and 4% of EC_{50} respectively. No such effect was noted for *C. pyrenoidosa,* although it is possible that this effect may be present at very low concentrations, which were not tested in this study. For chlorpyrifos the 72-h EC_{50} was well above environmentally realistic concentrations for both algae (3736 for *C. pyrenoidosa* and 2060 μg/L for *P. subcapitata*). However at concentrations 0.3-5 μg/L (with a maximum at 0.06% of EC_{50}) hormesis was observed for both species, where growth rate exceeded that of control by as much as 20% for *C. pyrenoidosa* and 40% for *P. subcapitata*. *P.*

subcapitata was more sensitive to all toxicants tested, and it was recommended as a test species for pesticides in preference to *C. pyrenoidosa.*

In Chapter 6 the effects of sublethal concentrations of chlorpyrifos (ranging from 0.005 µg/L (‘0.01 LC_{50}’) to 0.500 µg/L (‘1 LC_{50}’)) on population characteristics of individual culture of *Daphnia carinata* were investigated over 21 days with subsequent testing of the next two generations. The endpoints for the first and second generations observed were: survival, fecundity, time to first brood and number of offspring per female. The results were incorporated into the computation of the intrinsic rate of natural increase for daphnids in each of the treatments. Exposure to chlorpyrifos affected survival and fecundity of animals in the first generation. In the second generation the most affected endpoint was time to the first brood with an indication of hormesis. LC_{50} tests were then conducted using animals of the third generation from each of the exposures in individual tests. Despite the absence of a negative effect of chlorpyrifos in the second generation, results of testing the third generation showed a constant significant decline in LC_{50} in the order of control daphnids through to ‘0.1 LC_{50}’ pre-exposed daphnids (‘0.1 LC_{50}’, or 0.05 µg/L being the highest concentration in which animals survived exposure to the toxicant in the second generation).

In Chapter 7.1 the long-term toxicity of glyphosate (technical grade and formulation Roundup Biactive) to three successive generations of *D. carinata* was investigated. The experimental protocol was the same as for chlorpyrifos testing (Chapter 6). Glyphosate was tested in two different media: sea salt solution and M4 medium specially designed for daphnids, while Roundup Biactive was tested in M4 medium.

Results indicated that glyphosate and Roundup Biactive had relatively low toxicity to *Daphnia*. Hormesis was evident in sea salt medium exposures in the first and second generations of daphnids with technical grade glyphosate. When exposed to glyphosate and Roundup Biactive in M4 medium animals showed no indication of hormesis. It is hypothesized that glyphosate may have compensated for the lack of microelements in the sea salt medium, and possible mechanisms discussed.

In Chapter 7.2 the modifying effect of glyphosate on the toxicity of cadmium to *Daphnia carinata* was studied in long-term (21 days) exposures with two generations of cladoceran. It was found that low concentration of glyphosate (in the form of Roundup Biactive [RB]) reduces toxicity of Cd, and the performance of daphnia is enhanced in terms of animals' size, survival, fecundity, and consequently the intrinsic rate of natural increase in both generations of animals in the presence of glyphosate. However when the third generation was tested for their sensitivity to Cd in the 48-h LC_{50} experiments there was no difference between RB-free and RB-spiked treatments in pair wise comparison, indicating that no adaptation mechanisms were involved in the enhancement.

In Chapter 8 the overall discussion of the results with respect to observed hormesis is presented. The implications for the effects of the pesticides on environmental freshwater food chains are discussed and recommendations on modifying pesticide use are provided.

CHAPTER 2

INTRODUCTION

2.1 STATE OF THE AUSTRALIAN ENVIRONMENT

Among the inhabited continents Australia is the driest with over 80% of its land having an average rainfall of less than 600 mm/year. Large variations in climate and rainfall throughout Australia result in a great range of natural environments - from temperate south to tropical north with vast arid regions in the centre. Australia's inland aquatic ecosystems provide resources for multiple uses such as agriculture and industry and other human activities (potable water, fishing, recreation etc). To maintain the health of the aquatic environment it is essential to review the available knowledge on the current state of the environment, real and predicted impacts due to natural processes and human involvement, and based on such data to develop management tools to minimise the impact and its consequences.

The key findings of *Australia: State of the Environment 1996* (State of the Environment Advisory Council 1996) highlighted that Australia's inland waters are under increasing pressure from over-extraction, pollution, algal blooms, catchment modification, habitat destruction and flow regulation. Since 1996, the pressures on many inland waters have increased, with a substantial increase in water extraction, continued clearing of catchment and riparian vegetation, increases in the area of land affected by dryland salinity and increases in pesticide use (Australia: State of the Environment 2002).

According to *Australia: State of the Environment 2001 Report* ‘the total water use in Australia for 1996/97 was 24 100 GL (NLWRA 2001), an increase of 65% from 1985 (AWRC 1987). Seventy-nine per cent of water was extracted from surface waters (19 100 GL), while 21% was extracted from groundwater resources (5000 GL) (NLWRA 2001a). Seventy-five per cent of water extracted is used for irrigation, with irrigation water use increasing by 76% between 1985 and 1996/97 (NLWRA 2001a). Most of the growth in irrigation has occurred in New South Wales and Queensland, with the area of irrigated land doubling in these states over the last twenty years. Urban and industrial water use has also increased by 55% (NLWRA 2001a) between 1985 and 1996/97’. Increased water usage puts additional pressure on the remaining (depleted) water resourses in terms of maintaining healthy freshwater ecosystems, and making them increasingly sensitive to any pollution.

The Report recognises that ‘pesticides are possibly the most widespread pollutants, which are used extensively in agriculture with cotton, rice, sugar cane and horticultural crops. Since 1990, at least 20 fish kills in New South Wales rivers have been attributed to pesticides. Integrated pest management and best management practices for pesticide use are gradually being implemented and a new generation of more selective, less toxic pesticides is also being introduced. However, based on the experience of the past 20 years, pesticide use is likely to increase, potentially causing continuing pollution of inland waters’. In recognition of this, the current study was focused on the pesticides routinely used in large quantities in the Australian environment.

2.2 SPECIFIC FEATURES OF THE AUSTRALIAN AQUATIC ENVIRONMENT

Most ecotoxicological research and the subsequent setting of water quality criteria and related issues are based upon the data gathered in the Northern hemisphere and related to northern aquatic environments. However, the validity of application of these data to other geographical areas with distinctively different features is questionable (Hart 1982, Hobbs *et al.* 2004, Maltby *et al.* 2005).

Williams (1972) defined several distinctive features of Australian inland waters, which include:

1. The inapplicability of the concept of a standard composition for average fresh water;
2. The predominance of sodium and chloride ions in fresh waters;
3. The high proportion of saline/fresh standing water bodies;
4. The high concentration of phosphorus as phosphate in many lakes and reservoirs;
5. The absence of dimictic lakes, the presence of warm monomictic lakes (holomixis occur once, not twice as in the north, and takes place in winter at temperatures above 4^{o}C). The presence of unique thermal pattern in some highland lakes;
6. The pronounced seasonal and secular fluctuations in discharge values for rivers;
7. The high faunal endemicity

8. The absence of a well-defined seasonal terrestrial leaf-fall that affects the ecology of stream biota.

Because of the specific climatic regime (low and uncertain rainfall, absence of permanent snowfields where rivers can be replenished), topography (mostly flat planes with vast deserts in the centre of the continent), and high evapotranspiration rates, most of Australia lacks rivers or permanent standing waters. As a result of variable rainfall, Australian rivers have highly variable flows, which in turn have impact on the biota.

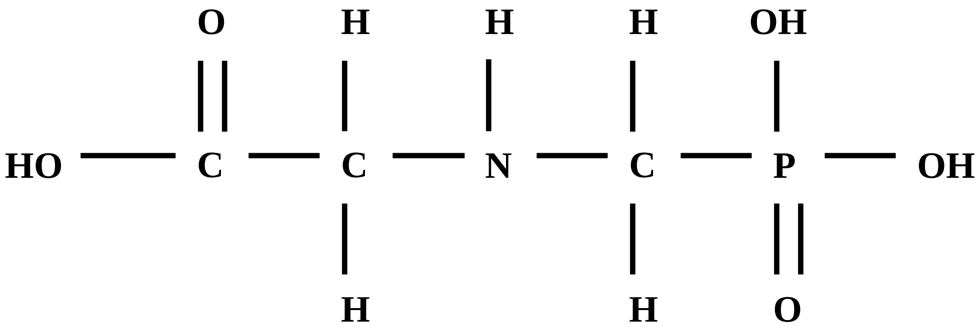
A large part of the aquatic Australian biota is endemic. Around 130 fish species that are endemic developed unique reproductive strategies adapted to variable flow and periods of drought. Many of the Australian freshwater invertebrates are also endemic, and their community compositions are different from those in the Northern hemisphere. Because of the seasonal nature of northern species, they have their population peaks at different times to avoid competition. This does not happen in Australia due to less pronounced seasons, consequently species interactions are different from those in the Northern hemisphere (Hart 1982).

All the above features make it necessary that toxicity values for *Australian* ecosystems be obtained using *Australian* native species, and related issues, such as the development of water quality criteria for Australia, be based on these data, and not on toxicity data derived from testing species from the Northern hemisphere. The reason why Australian and New Zealand Water Quality Guidelines rely predominantly on the Northern hemisphere data is lack of appropriate Australian data.

In the current study the use of a cosmopolitan cladoceran is proposed (*Daphnia carinata* King) in conjunction with cosmopolitan algal species, which are also native to Australia (unicellular green freshwater algae *Chlorella pyrenoidosa* and *Pseudokirchneriella subcapitata*), to investigate the effects of low concentrations of agricultural chemicals on non-target organisms in prolonged exposures since these species are found in Australian environmental trophic chains.

2.3 AGROCHEMICALS IN THE AUSTRALIAN ENVIRONMENT

The herbicide glyphosate (see Appendix 1 for properties and Fig. 2.1 for structure) was proposed for the study to investigate its effects on algae (potential target organisms) and a cladoceran (non-target organism), which feeds on these algae. Another agrochemical (that is also used in household applications in Australia) is the insecticide chlorpyrifos. Chlorpyrifos (see Appendix 1 for properties and Fig. 2.2 for structure) is highly toxic to crustaceans (of which daphnia is a representative), because they are closely related to insects. Though chlorpyrifos is not toxic to plants and algae, it is expected to influence their growth (though it is not known to what extent) due to its phosphorus content. Both chemicals are expected to influence the algae-cladoceran interactions due to their effects on at least one of the trophic links. Both agrochemicals are widely used in Australia and worldwide.



$$HO-C(=O)-CH_2-NH-CH_2-P(=O)(OH)-OH$$

Figure 2.1 Structure of glyphosate.

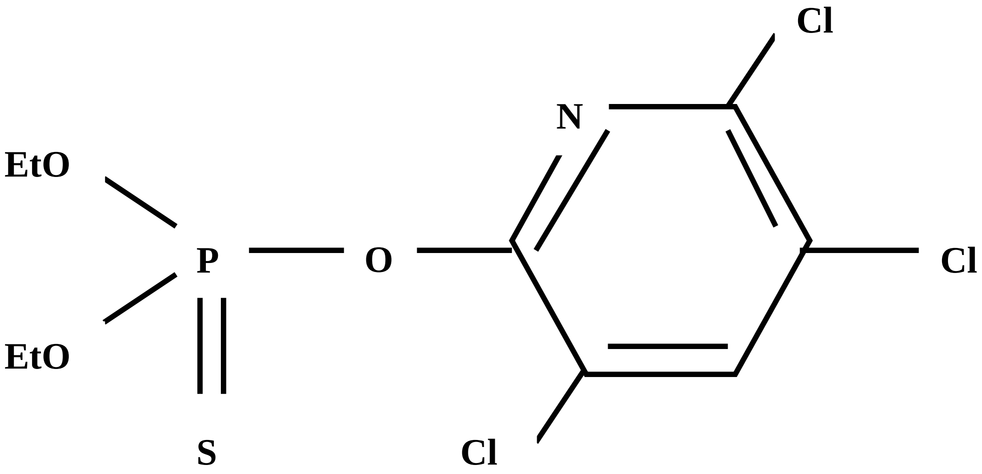


Figure 2.2 Structure of chlorpyrifos.

2.3.1 CHLORPYRIFOS IN THE AQUATIC ENVIRONMENT

CPF was introduced worldwide in 1965 to replace organochlorines and is one of the most widely used chemical organophosphate insecticides in the market today (Dow Agro Sciences www.dowagro.com). About 40 million kg of CPF is manufactured per year and it is an active ingredient in about 800 products in the USA (www.dowagro.com). There are 13 licensed producers of chlorpyrifos-based pesticides in Australia (NRA 2000). During the mid-1990s, 4-5.5 million kg were used annually in non-agricultural situations in over 17% of households in Australia. Agricultural usage estimates even more, with annual application of 4.5-10 million kg (NRA 2000). The National Water Quality Assessment Program (NAQWA) has been monitoring major watersheds in the US since 1991. The data reveals that concentrations of four organophosphorus pesticides (chlorpyrifos among them) exceed water quality criteria for aquatic life protection more often than other pesticides (de Vlaming *et al.* 2004).

Recently, the US EPA and the manufacturers of CPF agreed to eliminate nearly all household applications of the insecticide, but agricultural use continues worldwide, including Australia. For example, chlorpyrifos together with lindane, endosulfan and DDT was still the major concern in some parts of South Australia (Liston and Maher 1997). Chlorpyrifos was also detected in irrigation districts of New South Wales (Bowmer *et al.* 1998, Cooper 1996, Muschal 1998). It is estimated that in the sugar cane growing regions of Queensland, 74 500 kg of chlorpyrifos is used annually, which constitutes around 90% of all insecticide use by that industry (Hamilton and Haydon 1996). As a consequence of its widespread use, chlorpyrifos was detected in

Australian waterways at concentrations up to 0.525 mg/L (Humphrey and Klumpp 2000), while the recommended level of protection of 80 and 95% of species for fresh waters is 1.2 and 0.00004 µg/L respectively (ANZECC & ARMCANZ 2000).

Schulz (2001) studied a rainfall-induced runoff of pesticides from orchards into the Lourens River in South Africa, and found that as a result of such an event the pesticide contamination levels (including chlorpyrifos) were extremely high – they exceeded the national water quality standards and those established by the US EPA and may result in acute toxic effects on aquatic invertebrates and fish. He also conducted a probability analysis of 10-y rainfall data and found that such an event occurs approximately every 7 months. Considering that similar climatic conditions exist in Australia, a possibility of such events occurring here is very high (Muschal and Warne 2003).

In Australia not only agricultural but also household use of chlorpyrifos still continues. In March 1999 ecological and human health risk assessment of chemicals in sewage treatment plant discharges to the Hawkesbury-Nepean river system (NSW, Australia) found chlorpyrifos in the effluent of 2 inland sewage plants at levels that would constitute a risk to aquatic life (Sydney Water 2000). In addition this insecticide is commonly used in urban areas and appears in urban stormwater runoffs in the USA (Bailey *et al.* 1995), and the same pattern is expected to be present in Australia.

Organophosphorus pesticides such as chlorpyrifos are considered to be non-persistent in the environment; however, experimental research with ^{14}C-labelled chlorpyrifos has shown that this pesticide may persist for relatively long periods of time through sediment-water partitioning (Carvalho *et al.* 2002).

2.3.2 GLYPHOSATE IN THE AQUATIC ENVIRONMENT

Glyphosate was first reported as an herbicide in 1971. Three related products are now manufactured by Monsanto and Zeneca. In pure chemical terms glyphosate is an organophosphate because it contains carbon and phosphorous. However, it does not affect the nervous system in the same way as organophosphate insecticides, and is not a cholinesterase inhibitor, but rather it acts on various enzyme systems inhibiting amino acid metabolism in what is known as the shikimic acid pathway (Duke 1988). This pathway exists in higher plants and micro-organisms but not in animals.

Glyphosate product sales are currently worth approximately US$1,200 million annually worldwide and represent about 60% of global non-selective herbicides sales (Agrow 1995). The total world herbicide market was worth about US$14,285 million in 1995 (British Agrochemical Association 1996).

In UK arable agriculture, glyphosate was the 12th most extensively used pesticide active ingredient; the 5th most extensively used herbicide by weight with 251 tonnes being used; and 38th most widely applied herbicide, being applied over 334,529 ha annually in 1994 (MAFF 1995). In the US nearly 8,500 tonnes was being used on 5-8 million hectares annually in the years leading up to 1991(US EPA 1993).

The toxicity of glyphosate to mammals and birds is generally relatively low. Fish and aquatic invertebrates are more sensitive to glyphosate and its formulations. Some soil invertebrates including springtails, mites and isopods are also adversely affected by glyphosate (www.pesticideinfo.org). Of nine herbicides tested for their toxicity to soil micro-organisms, glyphosate was found to be the second most toxic to a range of bacteria, fungi, actinomycetes and yeasts (Carlisle and Trevors 1988).

However, while glyphosate alone has low toxicity, the formulation of glyphosate with the surfactant polyoxyethylene amine (POEA), which is widely used, is significantly more toxic (Wan *et al.* 1989; Servizi *et al.* 1987).

In Australia the recommended maximum level of glyphosate to ensure protection of 99 and 80% of aquatic freshwater life are 0.37 and 3.6 mg/L respectively (ANZECC & ARMCANZ 2000). Though glyphosate is considered non-persistent, it can last in the aquatic environment for a considerable time (up to ten weeks) and thus has the potential to affect non-target species with a short life cycle, such as cladocerans. Based on the scientific data available on glyphosate toxicity to aquatic animals, major organizations (USEPA 1993, WHO 1994) conclude that glyphosate and its formulation Roundup can be used with minimal risk to the environment. However it is possible that though glyphosate might not be toxic to animals, it still affects them at concentrations found in the environment.

In Australia most formulations of glyphosate have been banned from use in or near water because of their toxic effects on tadpoles and to a lesser extent on adult frogs. There is also concern about long-term sublethal effects of the herbicide on frogs

(Mann & Bidwell 1999). However, new formulations such as Roundup Biactive are excluded from the ban (Agrow 1996). Only few studies have been conducted on the toxicity of Roundup Biactive to aquatic biota (Mann and Bidwell 1999), and more data is required, especially on its long-term sublethal effects.

2.4 AIMS OF THE PROJECT

In order to determine the effects of low (environmentally realistic) concentrations of agricultural pesticides on non-target organisms, and considering all the issues presented above, the aims of the PhD project were as follows:

1. To investigate the effects of the herbicide glyphosate and its formulation Roundup Biactive on the growth of two Australian species of freshwater unicellular green algae *Chlorella pyrenoidosa* and *Pseudokirchneriella subcapitata* with special attention to the effects at environmentally realistic concentrations.
2. To study the effects of the insecticide chlorpyrifos on non-target organisms – algae *Chlorella pyrenoidosa* and *Pseudokirchneriella subcapitata* at low environmentally realistic concentrations especially with respect to hormesis and consequent potential algal blooms.
3. To study the effects of low concentrations of chlorpyrifos on population characteristics of *Daphnia carinata* in long-term multiple generation exposures.
4. To investigate the lethal and sublethal effects of low concentrations of glyphosate and its formulation Roundup Biactive on the growth and

reproduction of a native Australian cladoceran *Daphnia carinata* King in long-term exposures and using multiple-generation toxicity tests.

5. To establish the interdependence (if any) between the two trophic links when exposed to environmentally realistic concentrations of the above pesticides, and to evaluate the consequences of these exposures on an ecosystem.
6. To provide recommendations based on the results of the project on minimising detrimental effects of chronic exposure to environmentally realistic concentrations of the investigated pesticides on aquatic ecosystem health.

CHAPTER 3

EFFECT OF GLYPHOSATE AND CHLORPYRIFOS ON AQUATIC ORGANISMS - LITERATURE REVIEW

3.1 GLYPHOSATE TOXICITY TO DIFFERENT ORGANISMS

3.1.1 General issues

Various National environment agencies including Environment Canada (Peterson *et al.* 1994), USEPA, EU and the Australian National Registration Authority (NRA 1997) use an Expected Environmental Concentration (EEC) in evaluating the hazard of pesticides to non-target aquatic organisms. This concentration is calculated by assuming an overspray of a 15 cm deep water-body at the label application rate (Peterson *et al.* 1994). The EEC is then related to the EC_{50} for a given aquatic test organism.

In Canada, Vision® (containing 356 g/L of glyphosate as an active ingredient) is a major forest management herbicide, representing 81% of all herbicides sprayed on the forests. Because of the aerial method of application it can enter aquatic systems. Once in there, its half-life can vary from several days to ten weeks depending on the pH of the water (Trotter *et al.* 1990, cited in Morgan and Kiceniuk 1992). The Canadian Water Quality Guidelines recommend IMAC (Interim Maximum Accepted Concentration) for protection of aquatic life to be 65 μg/L. However, on occasion, the glyphosate levels were found to be up to 270 μg/L in some water bodies (Morgan and Kiceniuk 1992).

In Australia the recommended level of glyphosate to protect 99-80% of aquatic freshwater life is 0.37-3.6 mg/L respectively (ANZECC and ARMCANZ 2000).

Water quality parameters can affect the toxicity of glyphosate. Folmar *et al.* (1979) reported that increased temperature and pH both result in an increased toxicity of RB to rainbow trout. They also found that solutions of Roundup aged for up to 7 days in reconstituted water did not change in toxicity to midge larvae, rainbow trout, or bluegills. This indicates that the chemical can accumulate to dangerous levels in environmental waters if there are repeated applications within short time intervals.

There are few studies of effects of glyphosate, which may be of importance to human health. For example, Marc *et al.* (2004) demonstrated that various glyphosate-based herbicides induced cell cycle dysfunction.

3.1.2 Sediment-associated toxicity of glyphosate

Hartman and Martin (1984) demonstrated that the presence of suspended sediment in water significantly increased the acute toxicity of Roundup to *Daphnia pulex* (48-h EC_{50} for daphnia was 3.2 mg/L with suspended sediment and 7.9 mg/L without it) and decreased its toxicity to *Lemna minor*.

3.1.3 Effects of glyphosate on algae

Thomas *et al.* (1986) reported that water samples from arsenal waste sites were highly toxic to vascular plants, but were either stimulatory to or had no effect on *S. capricornutum* (now called *Pseudokirchneriella subcapitata*). Toxicants presented in the waste were suspected to be herbicides (including glyphosate) and their mixtures.

Anton et al. (1993) found that glyphosate was not toxic to the freshwater green alga *Chlorella pyrenoidosa* (see Table 3.1, entries 5-7). However Saenz et al. (1997) found in their study that much lower concentrations of glyphosate and its formulation Rondo caused inhibition of chlorophyll a synthesis in two green algae *Scenedesmus acutus* and *S. quadricauda* (Table 3.1, entries 9-12). Glyphosate inhibits the synthesis of the chlorophyll precursor 5-aminolevulinic acid (ALA) (Duke, 1988).

According to Shikha and Singh (2004) photosynthetic electron transport and O_2 evolution were initially stimulated by glyphosate at 50-200 mg/L, but were inhibited by higher concentrations 200-400 mg/L. Hernando *et al.* (1989) investigated chlorophyll and carotenoid content, greening process, photosynthetic and respiration rates and photosynthetic pigment content of *Chlorella pyrenoidosa* when grown in glyphosate concentrations raging from 0.1 mM to 1 mM (17 mg/L to 170 mg/L). The highest concentration inhibited growth completely; other concentrations reduced growth and photosynthetic pigment content. Glyphosate inhibited chlorophyll synthesis and reduced carotenoids. Oxygen evolution was also strongly inhibited. They concluded that glyphosate acts as an electron inhibitor, affecting both photosystems.

Peterson *et al.* (1994) estimated the Expected Environmental Concentrations for glyphosate formulations to be around 3 mg Gly/L. Authors found that among ten species of algae tested only diatoms and one cyanobacterium were sensitive to glyphosate. It appears that some algal species are more sensitive to glyphosate than others, and the variation is orders of magnitude. According to Saenz *et al.* (1997), the EEC for glyphosate is higher than the concentrations producing negative effects in algae in their study, and therefore the use of glyphosate formulation in aquatic environments may cause harmful effects on long-term development of *S. quadricauda* populations (as well as some others).

Christy *et al.* (1981) calculated EC_{50} (growth rate) of *Chlorella sorokiniana* to be 17.7 mg/L. Gardner *et al.* (1997) studied the effect of Rodeo$^{®}$ on growth of the freshwater green alga *Ankistrodesmus.* They found that the 96-h EC_{50} for this species was 74 mg/L (Table 3.1, entry 8). Maule and Write (1984) calculated the 96-h EC_{50} values of glyphosate for several algal species and found it to be non-toxic to microalgae. The most sensitive species tested was *Chlorococcum hypnosporum* with an EC_{50} of 68 mg/L, the least sensitive with an EC_{50} of 590 mg/L was *Chlorella pyrenoidosa.* Hess (1980) reported that a concentration of 1000 mg/L of glyphosate reduced the growth rate of *Chlamydomonas* to 30% of the control.

Hartman and Martin (1984) found that glyphosate did not produce any inhibitory effects on sprouting or early growth of sago pondweed *Potamogeton pectinatus* when treated with the concentrations up to 10.0 mg/L. However it stimulated plant growth at 1.0 mg/L. Schaffer and Sebetich (2004) found that low concentrations (0.125-12.5

mg/L) of Rodeo (a formulation of glyphosate) induced primary productivity of a phytoplankton community up to 168% of the control value.

3.1.4 Effect of glyphosate on freshwater fauna (single species data)

Most toxicity testing of glyphosate have been conducted using different species of fish (see Table 3.1, entries 91-142), e.g. goldfish *Carassius auratus* (Anton *et al.* 1994), rainbow trout *Oncorhyncus mykiss* (Anton *et al.* 1994, Morgan & Kiceniuk 1992), coho *Oncorhynchus kisutch* (Wan *et al.* 1989, Servizi *et al.* 1987, Mitchell *et al.* 1987), chum *Oncorhynchus keta* (Wan *et al.* 1989), chinook *Oncorhyncus tshawytsha* (Wan *et al.* 1989, Mitchell *et al.* 1987), pink salmon *Oncorhyncus gorbuscha* (Wan *et al.* 1989), rainbow trout *Salmo gairdneri* (Wan *et al.* 1989, Servizi *et al.* 1987, Mitchell *et al.* 1987, Folmar *et al.* 1979), carp *Cyprinus carpio* (Neskovic *et al.* 1996), sockeye salmon *Oncorhynchus nerka* (Servizi *et al.* 1987), mosquitofish *Gambusia yucatana* (Rendon-van Osten *et al.* 2005*).*

There has been an extensive study of the toxicity of different formulations of glyphosate to several species of Australian frogs (Mann & Bidwell 1999) (Table 3.1, entries 51-72) and other species of amphibians (Table 3.1, entries 73-90), and a few studies involving cladocerans (Table 3.1, entries 31, 36, 39-43, 45-47, 49) and other types of invertebrates and protozoans (Table 3.1, entries 21-30, 32-35, 37-39, 50). In general, not much attention was paid to the effects of glyphosate and its formulations on freshwater fauna. This is probably because glyphosate is considered to be non-toxic to animals, since they lack the metabolic pathway, along which the chemical reacts (the shickimate pathway is found only in plants). However some results suggest that though glyphosate might not be toxic to animals, it still affects them at concentrations found in the environment.

Morgan and Kiceniuk (1992) examined the effects of a two-months exposure to glyphosate, as Vision®, on the growth, behaviour, and gill and liver histopathology of rainbow trout. Concentrations tested were 6.25, 25 and 100 μg/L nominal concentration, the measured concentrations were 4.25, 8 and 45.75 μg/L respectively. There were no significant differences between control and treated animals in terms of all endpoints observed at all treatment concentrations, except one aspect of agonistic behaviour. At the highest tested concentration animals demonstrated higher frequency of aggressive behaviour – wigwags. It should be noted that this effect was observed at concentrations much lower than those found in some water bodies after spray application (Trotter *et al.* 1990).

Wan *et al.* (1989) found that the toxicity of glyphosate and its formulations depends on the type of dilution water used. Overall they found that variation of 96-h LC_{50} values for MON 0818, MON 8709 and Roundup® is in the same order of magnitude irrespective of water types. For glyphosate these values can vary by an order of magnitude depending on water type, with water hardness and pH being the most important contributing factors. Roundup®, MON 8709 and MON 0818 are more toxic to young salmonids in hard waters than they are in soft waters, while the reverse is true for glyphosate.

Edginton *et al.* (2004) compared toxicity of Vision® to several species of amphibians at different pHs and determined that it was more toxic to three species out of four at pH=7.5 than at pH=6, and the larval stage was more sensitive than the embryonic stage. Together with pH, other environmental factors such as availability of food can exacerbate chemical effects of Vision®, as was determined by Chen *et al.* (2004) in their experiments with *Simocephalus vetulus* and tadpoles of *Rana pipiens.* For both species, significant effects of the herbicide were measured at concentrations lower than the calculated worst-case value for EEC (1.4 mg/L ae), while high pH (7.5) increased the toxicity of herbicide to *S. vetulus.* Thompson *et al.* (2004) confirmed that amphibians are among the most sensitive organisms to glyphosate. However after conducting an *in situ* study they concluded that there was no risk to amphibians from glyphosate (as Vision®) application at recommended rates. Smith (2001) found that Kleeraway ® Grass was toxic to the tadpoles of two species of frogs: chorus frog *Pseudacris triseriata* and plains leopard frog *Rana blairi* at a concentration of 0.75 mg/L (as IPS equivalent) – about half of them died within 24 hours. However, further exposure of surviving animals to this concentration did not have any negative effect on their growth and development. Lajmanovich *et al.* (2003) found that glyphosate formulation Glyfos® induced death in 80% of tadpoles of *Scinax nasicus* at a concentration of 3.07 mg/L, with 75% malformed (craniofacial and mouth deformities, eye abnormalities and bent tails) in a 96-h exposure. Howe *et al.* (2004) observed that Roundup Original, Roundup Transorb and POEA (surfactant) significantly negatively affected growth and development of several species of amphibians in a chronic exposure to sublethal concentrations (0.6 mg/L of ae) of these compounds.

3.1.5 Effect of glyphosate on water communities.

Simenstad *et al.* (1996) conducted an intensive 119-day experiment in southern Willapa Bay, Washington, to evaluate the potential effects of a mixture of glyphosate (Rodeo®, 4.7 L/ha) and an associated surfactant, alkylarylpolyoxyethylene (AAPOE, X-77® Spreader, 1L/ha) on mudflat benthic communities. They concluded that there were no indications of either short- or long-term effects on the mudflat community after aerially applying this concentration of herbicide and surfactant. Though this study did not address either sublethal or indirect ecological effects of the herbicide application, there was an observed decrease in the exotic eelgrass *Zostera japonica*, that might be a longer-term, subtler response by the mudflat community. (Calculation of glyphosate concentration according to the Environment Canada procedure (Peterson *et al.,* 1994) gives us the concentration of active ingredient not more than 1.7 mg/L at the time of application, which in an estuarine environment will quickly decrease even further).

Perschbacher *et al.* (1997) studied the effect of sprayed herbicides (glyphosate among them) on the water communities (plankton productivity, zooplankton populations) and water quality. Though they did not provide all data in their paper, they stated that there were no significant differences between control and treated mesocosms, when sprayed with glyphosate at a rate of 0.43 kg/ha (see Table 3.1, entry 143). Similarly Kilbride and Paveglio (2001) conducted a 3-year study on effects of repeated applications of Rodeo to control smooth cordgrass *Spartina* sp. in Willapa Bay on aquatic biota. They concluded that under worst-case conditions short- and long-term detrimental effects of these applications would be highly unlikely.

In contradiction other researchers suggest that glyphosate could affect aquatic communities. For example, Bengtsson *et al.* (2004) measured the grazing rate of *Daphnia pulex* when pre-exposed to glyphosate via two vectors – water and food *Scenedesmus* spp. (exposure concentration in both cases was 50 mg/L), and found that the grazing rate was greatly reduced (40%) when exposed via the food route, suggesting greater toxicity of glyphosate to *Daphnia* than when exposed directly via water.

3.1.6 Bioconcentration of glyphosate

No bioaccumulation, biomagnification or persistence in a biologically available form is reported for glyphosate.

3.1.7 Toxicity of glyphosate formulations: active ingredient vs. surfactant

A number of researchers (Wan *et al.* 1989; Servizi *et al.* 1987) indicated that the surfactants in Roundup are more toxic to aquatic flora and fauna than the active ingredient glyphosate: MON8709 (Table 3.1, entries 120-124), MON 0818 (part of MON 8709, Table 3.1, entries 36, 105-107, 130-134). Mitchell *et al.* (1987) compared toxicity values for Rodeo herbicide alone and for Rodeo herbicide with X-77 surfactant as recommended for application by the manufacturer Monsanto. They found that the 96-h LC_{50} value of Rodeo/X-77 mixture was more than 4 times lower than Rodeo without the surfactant (130 mg/L of active ingredient and 580 mg/L respectively, Table 3.1, entries 112-114).

Tsui and Chu (2003) tested several formulations of glyphosate and Roundup surfactant using a number of marine and freshwater organisms (bacterium, algae, protozoans and cladocerans) to assess their relative toxicity. They found that polyoxyethylene amine (POEA) surfactant was the most toxic (normalized as acid equivalent) among four compounds tested, up to 6 times more toxic than Roundup and up to 360 times more than the isopropylamine salt of glyphosate (a usual active ingredient of glyphosate-based herbicides) (see Table 3.1, entries 1-4, 13-28 and 42-49 for comparison). Marc *et al.* (2005) also found POEA was highly toxic to the embryos of sea urchin *Sphaerechinus granularis* – irreversible damage and deaths occurred at concentrations >30 mg/L. Howe *et al.* (2004) confirmed that among several formulations of glyphosate and their surfactants, POEA was the most toxic, negatively affecting development of amphibians at a concentration 0.6 mg/L ae, and was lethal to 50% of *Rana clamitans* at a concentration 2.2 mg/L ae.

Several other authors have confirmed the toxicity of the surfactant to be higher than that of the active ingredient. These include Alberdi *et al.* (1996), who investigated the toxicity of RON-DO® in 48-h toxicity testing using *Daphnia magna* and *D. spinulata* (see Table 3.1, entries 40-41). RON-DO formulation contained 48% of glyphosate as isopropylamine salt and 15% of surfactant (oxide-coco-amide-propyl-dimethyl-amine). EC_{50} values were 66.18 mg/L for *D. spinulata* and 61.72 mg/L for *D. magna.* In comparison Henry *et al.* (1994) found 48-h LC_{50} value for *D. magna* to be 218 mg/L when exposed to Rodeo herbicide (Table 3.1, entry 39). Henry *et al.* (1994) also found that the surfactant X-77 used in some glyphosate formulations was about a 100 times more toxic to *D. magna* than Rodeo (48-h LC_{50} is 2 mg/L for X-77). In general X-77 Spreader® was 83-136 times more toxic than Rodeo when tested using different species of animals (Table 3.1, entries 37-39). Similarly Folmar *et al.* (1979) found that glyphosate contributed only a small percentage of the toxicity of Roundup® and that the surfactant in the formulation was the primary toxic agent (see also entries 33 and 95-98 of Table 3.1).

Mann and Bidwell (1999) determined the acute toxicity of technical grade glyphosate acid, glyphosate isopropylamine, and three glyphosate formulations to adults of one species and tadpoles of four species of southwestern Australian frogs in 48-h static/renewal tests (Table 3.1, entries 51-72). They found that among the tested formulations Roundup® Herbicide was the most toxic to the tadpoles (between 2.9 and 11.6 mg/L glyphosate acid equivalent [AE]). Touchdown® Herbicide was slightly less toxic (from 9.0 to 16.1 mg/L AE). All other formulations and technical grade glyphosate were practically non-toxic. These authors concluded that the surfactants in test formulations were the major contributing factor to their toxicity, and they should be studied further.

Everett and Dickerson (2003) studied the toxicity of technical grade glyphosate and formulation Roundup to ciliates *Tetrahymena thermophila* and *Ichthyophthirius multifiliis* and also concluded that Roundup was at least 100 times more toxic than technical grade glyphosate, confirming the toxicity of the products used in formulation.

Table 3.1 Glyphosate toxicity to different organisms (1979-2004). A.e. (acid equivalent), ai-active ingredient, pf-product formulation, ChV-chronic values (calculated as a geometrical mean between LOEC and NOEC), all mg/L

#	Glyphosate formulation 1	Species 2	Effect measured 3	Parameter		Reference 6
				Name 4	Value 5	
BACTERIA AND ALGAE						
1	Glyphosate acid	Marine bacterium *Vibrio fischeri*	Luminescence emission	15-min IC$_{50}$	17.5 (15.8-19.5) ae	Tsui and Chu, *2003*
2	Isopropylamine salt of glyphosate	Marine bacterium *Vibrio fischeri*	Luminescence emission	15-min IC$_{50}$	162 (150-177) ae	Tsui and Chu, *2003*
3	Polyoxyethylene amine (Surfactant in Rodeo®)	Marine bacterium *Vibrio fischeri*	Luminescence emission	15-min IC$_{50}$	10.2 (9.80-10.7) ae	Tsui and Chu, *2003*
4	Rodeo$^{®}$	Marine bacterium *Vibrio fischeri*	Luminescence emission	15-min IC$_{50}$	24.9 (23.9-26.0) ae	Tsui and Chu, *2003*
5	Glyphosate 36%	Alga *Chlorella pyrenoidosa*	Growth inhibition	96-h EC$_{50}$ NOEC 96-h	396-423 a.i. 108 a.i.	Anton *et al., 1993*
6	Glyphosate, technical grade, 38%	Alga *Chlorella pyrenoidosa*	Growth inhibition	96-h EC$_{50}$	380 a.i.	Anton *et al., 1993*
7	Glyphosate, technical grade, 54.9%	Alga *Chlorella pyrenoidosa*	Growth inhibition	96-h EC$_{50}$	1082 a.i.	Anton *et al., 1993*
8	Rodeo$^{®}$	Green alga *Ankistrodesmus*	Growth	96-hr EC$_{50}$	74 ± 47 a.i.	Gardner *et. al., 1997*
9	Ron-do	Alga *Scenedesmus acutus*	Chlorophyll *a* inhibition	NOEC LOEC ChV 96-h EC $_{50}$	3.2 Gly 4.08 Gly 3.61 Gly 9.08 Gly (8.4-9.7)	Saenz *et al, 1997*
10	Ron-do	Alga *Scenedesmus quadricauda*	Chlorophyll *a* inhibition	NOEC LOEC ChV 96-h EC $_{50}$	1.25 Gly 2.5 Gly 1.76 Gly 9.09 (8.06-10.2) Gly	Saenz *et al, 1997*

11	Glyphosate	Alga *Scenedesmus acutus*	Chlorophyll *a* inhibition	NOEC LOEC ChV 96-h EC_{50}	2 Gly 4 Gly 2.82 Gly 10.2(10.4-11.2) Gly	Saenz *et al, 1997*
12	Glyphosate	Alga *Scenedesmus quadricauda*	Chlorophyll *a* inhibition	NOEC LOEC ChV 96-h EC_{50}	0.77 Gly 1.55 Gly 1.09 Gly 7.2(4.4-8.9) Gly	Saenz *et al, 1997*
13	Glyphosate acid	Freshwater alga *Selenastrum capricornutum*	Absorbance at 680 nm	96-h IC_{50}	24.7 (22.8-16.7) ae	Tsui and Chu, *2003*
14	Isopropylamine salt of glyphosate	Freshwater alga *Selenastrum capricornutum*	Absorbance at 680 nm	96-h IC_{50}	41.0 (29.4-59.1) ae	Tsui and Chu, *2003*
15	Polyoxyethylene amine (Surfactant in Rodeo®)	Freshwater alga *Selenastrum capricornutum*	Absorbance at 680 nm	96-h IC_{50}	3.92 (1.57-9.58) ae	Tsui and Chu, *2003*
16	Rodeo$^{®}$	Freshwater alga *Selenastrum capricornutum*	Absorbance at 680 nm	96-h IC_{50}	5.81 (2.36-8.14) ae	Tsui and Chu, *2003*
17	Glyphosate acid	Marine alga *Skeletonema costatum*	Absorbance at 675 nm	96-h IC_{50}	2.27 (0.82-11.1) ae	Tsui and Chu, *2003*
18	Isopropylamine salt of glyphosate	Marine alga *Skeletonema costatum*	Absorbance at 675 nm	96-h IC_{50}	5.89 (3.14-10.4) ae	Tsui and Chu, *2003*
19	Polyoxyethylene amine (Surfactant in Rodeo®)	Marine alga *Skeletonema costatum*	Absorbance at 675 nm	96-h IC_{50}	3.35 (2.02-5.40) ae	Tsui and Chu, *2003*
20	Rodeo$^{®}$	Marine alga *Skeletonema costatum*	Absorbance at 675 nm	96-h IC_{50}	1.85 (0.33-10.49) ae	Tsui and Chu, *2003*

PROTOZOAN						
21	Glyphosate acid	Freshwater protozoan *Tetrahymena pyriformis*	Culture growth	40-h IC$_{50}$	648 (430-1280) ae	Tsui and Chu, *2003*
22	Isopropylamine salt of glyphosate	Freshwater protozoan *Tetrahymena pyriformis*	Culture growth	40-h IC$_{50}$	386 (95.2-2020) ae	Tsui and Chu, *2003*
23	Polyoxyethylene amine (Surfactant in Rodeo®)	Freshwater protozoan *Tetrahymena pyriformis*	Culture growth	40-h IC$_{50}$	4.96 (2.90-8.98) ae	Tsui and Chu, *2003*
24	Rodeo®	Freshwater protozoan *Tetrahymena pyriformis*	Culture growth	40-h IC$_{50}$	29.5 (11.3-66.0) ae	Tsui and Chu, *2003*
25	Glyphosate acid	Marine protozoan *Euplotes vannus*	Culture growth	48-h IC$_{50}$	10.1 (6.47-14.5) ae	Tsui and Chu, *2003*
26	Isopropylamine salt of glyphosate	Marine protozoan *Euplotes vannus*	Culture growth	48-h IC$_{50}$	64.09 (19.0-325) ae	Tsui and Chu, *2003*
27	Polyoxyethylene amine (Surfactant in Rodeo®)	Marine protozoan *Euplotes vannus*	Culture growth	48-h IC$_{50}$	5.00 (4.62-5.42) ae	Tsui and Chu, *2003*
28	Rodeo®	Marine protozoan *Euplotes vannus*	Culture growth	48-h IC$_{50}$	23.5 ae	Tsui and Chu, *2003*
INVERTEBRATES						
29	Glyphosate (commercial grade, 41%)	Rotifer *Brachionus calyciflorus*	Survival	24-h LC$_{50}$	28.0	Xi & Feng, *2004*
30	Rodeo® X-77	Leech *Nephelopsis obscura*	Survival	96-h LC$_{50}$	1177 (941-1415) 14.1 (10.7-19.0)	Henry *et. al., 1994*
31	Roundup® Herbicide (MON 2139 surfactant)	Water flea *Daphnia magna*	Survival	48-h LC$_{50}$	3.0 (2.6-3.4) a.i.	Folmar *et al., 1979*

32	Technical grade glyphosate (MON0573)	Midge larvae *Chironomous plumosus*	Survival	48-h LC$_{50}$	55 (31-97) a.i.	Folmar *et al., 1979*
33	Surfactant (MON0818)	Midge larvae *Chironomous plumosus*	Survival	48-h LC$_{50}$	13 (7.1-24) a.i.	Folmar *et al., 1979*
34	Roundup® Herbicide (MON 2139 surfactant)	Midge larvae *Chironomous plumosus*	Survival	48-h LC$_{50}$	18 (9.4-32) a.i.	Folmar *et al., 1979*
35	Roundup® Herbicide (MON 2139 surfactant)	Scud *Gammarus pseudoiemnaeus*	Survival	24-h LC$_{50}$ 48-h LC$_{50}$ 96-h LC$_{50}$	>100 a.i. 62 (40-98) a.i. 43 (28-66) a.i.	Folmar *et al., 1979*
36	Roundup (480 g/L of glyphosate as isopropylamine salt)	Cladoceran *Daphnia pulex*	Immobilization	96-h LC$_{50}$ (as Roundup) 96-h LC$_{50}$ (as glyphosate) 96-h LC$_{50}$ (as MONO818)	25.5 7.8 3.8	Servizi *et al., 1987*
37	Rodeo$^{®}$ X-77	Amphipod *Hyalella azteca*	Survival	96-h LC$_{50}$	720(399-1076) 5.3 (4.3-6.7)	Henry *et. al., 1994*
38	Rodeo$^{®}$ X-77	Midge *Chironomus riparius*	Survival	48-h LC$_{50}$	1216(996-1566) 10.0 (8.2-13.1)	Henry *et. al., 1994*
39	Rodeo$^{®}$ X-77	Cladoceran *Daphnia magna*	Survival	48-h LC$_{50}$	218(150-287) 2.0 (1.5-2.7)	Henry *et. al., 1994*
40	RON-DO (48% of glyphosate as isopropylamine salt)	Cladoceran *Daphnia spinulata*	Immobilization	24-h EC$_{50}$ 48-h EC$_{50}$	94.87(89.1-101) ai 66.18(61.1-71.8) ai	Alberdi *et. al., 1996*
41	RON-DO (48% of glyphosate as isopropylamine salt	Cladoceran *Daphnia magna*	Immobilization	24-h EC$_{50}$ 48-h EC$_{50}$	95.96(91.5-101.1) ai 61.72(58.8-64.2) ai	Alberdi *et. al., 1996*

42	Glyphosate acid	Freshwater crustacean *Ceriodaphnia dubia*	Survival	48-h LC$_{50}$	147 (141-153) ae	Tsui and Chu, *2003*
43	Isopropylamine salt of glyphosate	Freshwater crustacean *Ceriodaphnia dubia*	Survival	48-h LC$_{50}$	415 (339-508) ae	Tsui and Chu, *2003*
44	Polyoxyethylene amine (Surfactant in Rodeo®)	Freshwater crustacean *Ceriodaphnia dubia*	Survival	48-h LC$_{50}$	1.15 (1.04-1.27) ae	Tsui and Chu, *2003*
45	Rodeo®	Freshwater crustacean *Ceriodaphnia dubia*	Survival	48-h LC$_{50}$	5.39 (4.81-6.05) ae	Tsui and Chu, *2003*
46	Glyphosate acid	Marine crustacean *Acartia tonza*	Survival	48-h LC$_{50}$	35.3 (30.9-40.3) ae	Tsui and Chu, *2003*
47	Isopropylamine salt of glyphosate	Marine crustacean *Acartia tonza*	Survival	48-h LC$_{50}$	49.3 (38.4-63.1) ae	Tsui and Chu, *2003*
48	Polyoxyethylene amine (Surfactant in Rodeo®)	Marine crustacean *Acartia tonza*	Survival	48-h LC$_{50}$	0.57 (0.50-0.65) ae	Tsui and Chu, *2003*
49	Rodeo®	Marine crustacean *Acartia tonza*	Survival	48-h LC$_{50}$	1.77 (1.33-2.34) ae	Tsui and Chu, *2003*
50	Roundup	Freshwater mussel *Utterbackia imbecillis*	Survival	24-h LC$_{50}$	18.3 ± 12.9	Conners & Black, *2004*
AMPHIBIANS						
51	Technical grade glyphosate acid	Frog (tadpole) *Litoria moorei*	Survival	24-h LC$_{50}$ 48-h LC$_{50}$	127(90-180) 121(111-133)	Bidwell & Gorrie, *1995*
52	Technical grade glyphosate acid	Frog (adult) *Crinia insignifera*	Survival	24-h LC$_{50}$ 48-h LC$_{50}$	89.6(73.6-108.6) 83.6(67.4-103.6)	Bidwell & Gorrie, *1995*
53	Roundup® Herbicide (MON 2139 surfactant)	Frog (adult) *Crinia insignifera*	Survival	24-h LC$_{50}$ 48-h LC$_{50}$	52.6(39.3-70.5) ae 146(109-196) pf 49.4(40.5-60.2) ae 137(113-167) pf	Bidwell & Gorrie, *1995*

54	Roundup® Herbicide (MON 2139 surfactant)	Frog (tadpole) *Litoria moorei*	Survival	24-h LC_{50} 48-h LC_{50}	12.7(9.0-18.0) ae 35.3(25.0-50.0) pf 11.6(10.3-13.1) ae 32.2(28.6-36.4) pf	Bidwell & Gorrie, *1995*
55	Technical grade glyphosate acid	Frog (tadpole) *Litoria moorei*	Survival	24-h LC_{50} 48-h LC_{50}	88.6(79.8-98.3) 81.2(76.7-85.9)	Mann & Bidwell, *1999*
56	Glyphosate isopropylamine	Frog (tadpole) *Lymnodynastes dorsalis*	Survival	24-h LC_{50} 48-h LC_{50}	>400ae, >587pf >400ae, >587 pf	Mann & Bidwell, *1999*
57	Roundup® Herbicide (MON 2139 surfactant)	Frog (tadpole) *Lymnodynastes dorsalis*	Survival	24-h LC_{50} 48-h LC_{50}	4.6(4.1-5.2) ae 12.8(11.4-14.4) pf 3.0(2.8-3.2) ae 8.3(7.8-8.9) pf	Mann & Bidwell, *1999*
58	Touchdown® Herbicide (4 LC-E)	Frog (tadpole) *Lymnodynastes dorsalis*	Survival	24-h LC_{50} 48-h LC_{50}	14.7(14.0-15.4) ae 44.4(42.3-46.6) pf 12.0(11.4-12.6) ae 36.2(34.4-37.9) pf	Mann & Bidwell, *1999*
59	Roundup® Biactive (MON 77920)	Frog (tadpole) *Lymnodynastes dorsalis*	Survival	24-h LC_{50} 48-h LC_{50}	>400 ae >1111 pf >400 ae >1111 pf	Mann & Bidwell, *1999*
60	Glyphosate isopropylamine	Frog (tadpole) *Litoria moorei*	Survival	24-h LC_{50} 48-h LC_{50}	>343 ae >503 pf >343 ae >503 pf	Mann & Bidwell, *1999*
61	Roundup® Herbicide (MON 2139 surfactant)	Frog (tadpole) *Litoria moorei*	Survival	24-h LC_{50} 48-h LC_{50}	3.1(2.8-3.4) ae 8.6(7.8-9.4) pf 2.9(2.6-3.2) ae 8.1(7.2-8.9) pf	Mann & Bidwell, *1999*
62	Touchdown® Herbicide (4 LC-E)	Frog (tadpole) *Litoria moorei*	Survival	24-h LC_{50}	10.4(9.7-11.1) ae 31.4(29.4-33.6) pf	Mann & Bidwell, *1999*

63	Roudup® Biactive (MON 77920)	Frog (tadpole) *Litoria moorei*	Survival	24-h LC_{50}	333(305-363) ae 925(847-1008) pf	Mann & Bidwell, *1999*
				48-h LC_{50}	328(296-363) ae 911(822-1008) pf	
64	Glyphosate isopropylamine	Frog (tadpole) *Heleioporus eyrei*	Survival	24-h LC_{50}	>373 ae >548 pf	Mann & Bidwell, *1999*
				48-h LC_{50}	>373 ae >548 pf	
65	Roundup® Herbicide (MON 2139 surfactant)	Frog (tadpole) *Heleioporus eyrei*	Survival	24-h LC_{50}	8.6(7.8-9.5) ae 23.9(21.7-26.4) pf	Mann & Bidwell, *1999*
				48-h LC_{50}	6.3(5.6-7.1) ae 17.5(15.6-19.7) pf	
66	Touchdown® Herbicide (4 LC-E)	Frog (tadpole) *Heleioporus eyrei*	Survival	24-h LC_{50}	16.6(14.1-19.6) ae 50.2(42.5-59.3) pf	Mann & Bidwell, *1999*
				48-h LC_{50}	16.1(13.7-18.9) ae 48.7(41.5-57.1) pf	
67	Roudup® Biactive (MON 77920)	Frog (tadpole) *Heleioporus eyrei*	Survival	24-h LC_{50}	>427 ae >1186 pf	Mann & Bidwell, *1999*
				48-h LC_{50}	>427 ae >1186 pf	
68	Glyphosate isopropylamine	Frog (tadpole) *Crinia insignifera*	Survival	24-h LC_{50}	>466 ae >684 pf	Mann & Bidwell, *1999*
				48-h LC_{50}	>466 ae >684 pf	
69	Roundup® Herbicide (MON 2139 surfactant)	Frog (tadpole) *Crinia insignifera*	Survival	24-h LC_{50}	>5.1 ae >14.2 pf	Mann & Bidwell, *1999*
				48-h LC_{50}	3.6(3.3-4.1) ae 10(9.2-11.4) pf	
70	Touchdown® Herbicide (4 LC-E)	Frog (tadpole) *Crinia insignifera*	Survival	24-h LC_{50}	13.1(12.3-14.0) ae 39.6(37.2-42.2) pf	Mann & Bidwell, *1999*
				48-h LC_{50}	9.0(8.4-9.7) ae 27.3(25.5-29.3) pf	

71	Roudup® Biactive (MON 77920)	Frog (tadpole) *Crinia insignifera*	Survival	24-h LC_{50} 48-h LC_{50}	>494 ae >1372 pf >494 ae >1372 pf	Mann & Bidwell, *1999*
72	Roundup® Herbicide (MON 2139 surfactant)	Frog (metamorph) *Crinia insignifera*	Survival	24-h LC_{50} 48-h LC_{50}	88.7(68.6-114) ae 246(191-318) pf 51.8(42.1-63.8) ae 144(117-177) pf	Mann & Bidwell, *1999*
73	Glyfos®	Tadpole *Scinax nasicus*	Survival	24-h LC_{50} 48-h LC_{50} 72-h LC_{50} 96-h LC_{50}	4.78 (4.23-5.35) 3.62 (3.28-5.02) 3.23 (3.07-3.36) 2.64 (2.19-2.84)	Lajmanovich *et al., 2003*
74	Vision®	Amphibian larvae *Rana clamitans*	Survival	96-h LC_{10} 96-h LC_{50}	1.2-1.78 a.e. 2.70-4.34 a.e.	Wojtaszek *et al.*, *2004*
75	Vision®	Amphibian larvae *Rana pipiens*	Survival	96-h LC_{10} 96-h LC_{50}	3.26-7.31 a.e. 4.25-11.47 a.e.	Wojtaszek *et al.*, *2004*
76	Roundup Original®	Amphibian *Rana pipiens*	Survival	24-h LC_{50} 96-h LC_{50}	3.7 (3.5-3.9) ae 2.9 ae	Howe *et al., 2004*
77	Roundup Original®	Amphibian *Rana pipiens*	Survival	24-h LC_{50} 96-h LC_{50}	>8 ae 6.5 (6.1-6.8) ae	Howe *et al., 2004*
78	Roundup Original®	Amphibian *Rana sylvatica*	Survival	24-h LC_{50} 96-h LC_{50}	5.6 (5.2-6.1) ae 5.1 (4.9-5.4) ae	Howe *et al., 2004*
79	Roundup Original®	Amphibian *Rana sylvatica*	Survival	24-h LC_{50} 96-h LC_{50}	>8 ae >8 ae	Howe *et al., 2004*
80	Roundup Original®	Amphibian *Bufo americanus*	Survival	24-h LC_{50} 96-h LC_{50}	4.2 ae <4 ae	Howe *et al., 2004*
81	Roundup Original®	Amphibian *Bufo americanus*	Survival	24-h LC_{50} 96-h LC_{50}	>8 ae 8 ae	Howe *et al., 2004*
82	Roundup Original®	Amphibian *Rana clamitans*	Survival	24-h LC_{50} 96-h LC_{50}	2.0 (1.9-2.2) ae 2.0 (1.9-2.2) ae	Howe *et al., 2004*
83	Roundup Original®	Amphibian *Rana clamitans*	Survival	24-h LC_{50} 96-h LC_{50}	>8 ae 7.1 (6.6-7.6) ae	Howe *et al., 2004*

84	Glyphosate technical	Amphibian *Rana clamitans*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	>17.9 ae >17.9 ae	Howe *et al., 2004*
85	POEA	Amphibian *Rana clamitans*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	2.4 (2.2-2.5) ae 2.2 (2.1-2.4) ae	Howe *et al., 2004*
86	Roundup Biactive®	Amphibian *Rana clamitans*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	>17.9 ae >17.9 ae	Howe *et al., 2004*
87	Touchdown®	Amphibian *Rana clamitans*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	>17.9 ae >17.9 ae	Howe *et al., 2004*
88	Glyfos BIO®	Amphibian *Rana clamitans*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	>17.9 ae >17.9 ae	Howe *et al., 2004*
89	Glyfos AU®	Amphibian *Rana clamitans*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	9.0 (8.7-9.4) ae 8.9 (8.6-9.2) ae	Howe *et al., 2004*
90	Roundup Transorb®	Amphibian *Rana clamitans*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	2.3 (2.2-2.4) ae 2.2 (2.1-2.4) ae	Howe *et al., 2004*
FISH						
91	Technical grade glyphosate (MON0573)	Rainbow trout *Salmo gairdneri*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	140 (120-170) a.i. 140 (120-170) a.i.	Folmar *et al., 1979*
92	Technical grade glyphosate (MON0573)	Fathead minnow *Pimephales promelas*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	97 (79-120) a.i. 97 (79-120) a.i.	Folmar *et al., 1979*
93	Technical grade glyphosate (MON0573)	Channel catfish *Ictalurus punctatus*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	130 (110-160) a.i. 130 (110-160) a.i.	Folmar *et al., 1979*
94	Technical grade glyphosate (MON0573)	Bluegills *Lepomis macrochirus*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	150 (120-190) a.i. 140 (110-160) a.i.	Folmar *et al., 1979*
95	Surfactant (MON0818)	Rainbow trout *Salmo gairdneri*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	2.1 (1.6-2.7) a.i. 2.0 (1.5-2.7) a.i.	Folmar *et al., 1979*
96	Surfactant (MON0818)	Fathead minnow *Pimephales promelas*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	1.4 (1.2-1.7) a.i. 1.0 (1.2-1.7) a.i.	Folmar *et al., 1979*
97	Surfactant (MON0818)	Channel catfish *Ictalurus punctatus*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	18 (8.5-38) a.i. 13 (10-17) a.i.	Folmar *et al., 1979*
98	Surfactant (MON0818)	Bluegills *Lepomis macrochirus*	Survival	24-h LC$_{50}$ 96-h LC$_{50}$	3.0 (2.5-3.7) a.i. 3.0 (2.5-3.7) a.i.	Folmar *et al., 1979*

99	Roundup® Herbicide (MON 2139 surfactant)	Rainbow trout *Salmo gairdneri*	Survival	24-h LC_{50} 96-h LC_{50}	8.3 (7.0-9.9) a.i. 8.3 (7.0-9.9) a.i.	Folmar *et al., 1979*
100	Roundup® Herbicide (MON 2139 surfactant)	Fathead minnow *Pimephales promelas*	Survival	24-h LC_{50} 96-h LC_{50}	2.4 (2.0-2.9) a.i. 2.3 (1.9-2.8) a.i.	Folmar *et al., 1979*
101	Roundup® Herbicide (MON 2139 surfactant)	Channel catfish *Ictalurus punctatus*	Survival	24-h LC_{50} 96-h LC_{50}	13 (11-16) a.i. 13 (11-16) a.i.	Folmar *et al., 1979*
102	Roundup® Herbicide (MON 2139 surfactant)	Bluegills *Lepomis macrochirus*	Survival	24-h LC_{50} 96-h LC_{50}	6.4 (4.8-8.6) a.i. 5.0 (3.8-6.6) a.i.	Folmar *et al., 1979*
103	Roundup® Herbicide (MON 2139 surfactant)	Rainbow trout *Salmo gairdneri*	Survival:			Folmar *et al., 1979*
			Eyed eggs	24-h LC_{50}	46 (35-61) a.i.	
				96-h LC_{50}	16 (13-19) a.i.	
			Sac fry	24-h LC_{50}	11 (8.8-13) a.i.	
				96-h LC_{50}	3.4 (2.2-5.3) a.i.	
			Swim-up fry	24-h LC_{50}	2.4 (2.0-2.9) a.i.	
				96-h LC_{50}	2.4 (2.0-2.9) a.i.	
			Fingerling (1.0 g)	24-h LC_{50}	2.2 (0.93-5.2) a.i.	
				96-h LC_{50}	1.3 (1.1-1.6) a.i.	
			Fingerling (2.0 g)	24-h LC_{50}	8.3 (7.0-9.9) a.i.	
				96-h LC_{50}	8.3 (7.0-9.9) a.i.	
104	Roundup® Herbicide (MON 2139 surfactant)	Channel catfish *Ictalurus punctatus*	Survival:			Folmar *et al., 1979*
			Eyed eggs	24-h LC_{50}	43 (36-51) a.i.	
				96-h LC_{50}	nd	
			Sac fry	24-h LC_{50}	4.3 (3.6-5.1) a.i.	
				96-h LC_{50}	4.3 (3.6-5.1) a.i.	
			Swim-up fry	24-h LC_{50}	3.7 (3.4-4.1) a.i.	
				96-h LC_{50}	3.3 (2.8-3.9) a.i.	
			Fingerling (2.2 g)	24-h LC_{50}	13 (11-16) a.i.	
				96-h LC_{50}	13 (11-16) a.i.	

105	Roundup (480 g/L of glyphosate as isopropylamine salt)	Sockeye salmon *Oncorhynchus nerka* (fingerlings)	Survival	96-h LC$_{50}$ 96-h LC$_{50}$ (as glyphosate) 96-h LC$_{50}$ (as MONO818)	26.7-27.7 8.1-8.4 4.0-4.2	Servizi *et al.*, *1987*
106	Roundup (480 g/L of glyphosate as isopropylamine salt)	Sockeye salmon *Oncorhynchus nerka* (fry)	Survival	96-h LC$_{50}$ 96-h LC$_{50}$ (as glyphosate) 96-h LC$_{50}$ (as MONO818)	28.8 8.7 4.3	Servizi *et al.*, *1987*
107	Roundup (480 g/L of glyphosate as isopropylamine salt)	Rainbow trout (fry) *Salmo gairdneri*	Survival	96-h LC$_{50}$ 96-h LC$_{50}$ (as glyphosate) 96-h LC$_{50}$ (as MONO818)	25.5-28.0 7.8-8.5 3.8-4.2	Servizi *et al.*, *1987*
108	Roundup (480 g/L of glyphosate as isopropylamine salt)	Coho salmon (fry) *Oncorhynchus kisutch*	Survival	96-h LC$_{50}$ 96-h LC$_{50}$ (as glyphosate) 96-h LC$_{50}$ (as MONO818)	42.0 12.8 6.3	Servizi *et al.*, *1987*
109	Roundup (commercial formulation)	Rainbow trout *Salmo gairdneri*	Survival	96-h LC$_{50}$	12 (5.7-18) a.i.	Mitchell *et al.*, *1987*
110	Roundup (commercial formulation)	Chinook salmon *Oncorhynchus tshawytscha*	Survival	96-h LC$_{50}$	9.6 (7.9-13) a.i.	Mitchell *et al.*, *1987*
111	Roundup (commercial formulation)	Coho salmon *Oncorhynchus kisutch*	Survival	96-h LC$_{50}$	11 (5.7-18) a.i.	Mitchell *et al.*, *1987*
112	Rodeo/X-77	Rainbow trout *Salmo gairdneri*	Survival	96-h LC$_{50}$	130 (120-160) a.i.	Mitchell *et al.*, *1987*
113	Rodeo/X-77	Chinook salmon *Oncorhynchus tshawytscha*	Survival	96-h LC$_{50}$	140 (120-220) a.i.	Mitchell *et al.*, *1987*

114	Rodeo/X-77	Coho salmon *Oncorhynchus kisutch*	Survival	96-h LC_{50}	120 (68-220) a.i.	Mitchell *et al., 1987*
115	Glyphosate (technical grade)	Coho *Oncorhynchus kisutch*	Survival (range in different types of dilution water)	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	44-210 ai 27-205 ai 27-182 ai 27-174 ai	Wan *et al., 1989*
116	Glyphosate (technical grade)	Chum *Oncorhynchus keta*	Survival	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	16-202 ai 13-178 ai 10-157 ai 10-148 ai	Wan *et al., 1989*
117	Glyphosate (technical grade)	Chinook *Oncorhyncus tshawytsha*	Survival	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	24-220 ai 22-220 ai 22-211 ai 19-211 ai	Wan *et al., 1989*
118	Glyphosate (technical grade)	Pink salmon *Oncorhyncus gorbuscha*	Survival	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	26-380 ai 14-245 ai 14-190 ai 14-190 ai	Wan *et al., 1989*
119	Glyphosate (technical grade)	Rainbow trout *Salmo gairdneri*	Survival	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	21-220 ai 11-220 ai 11-220 ai 10-197 ai	Wan *et al., 1989*

120	MON 8709	Coho *Oncorhynchus kisutch*	Survival	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	25-59 product 25-57 product 25-57 product 25-55 product	Wan *et al., 1989*
121	MON 8709	Chum *Oncorhynchus keta*	Survival	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	25-62 product 25-58 product 23-58 product 23-58 product	Wan *et al., 1989*
122	MON 8709	Chinook *Oncorhyncus tshawytsha*	Survival	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	33-84 product 33-79 product 33-73 product 33-67 product	Wan *et al., 1989*
123	MON 8709	Pink salmon *Oncorhyncus gorbuscha*	Survival	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	24-88 product 24-54 product 24-48 product 24-48 product	Wan *et al., 1989*
124	MON 8709	Rainbow trout *Salmo gairdneri*	Survival	24 hr LC_{50} 48 hr LC_{50} 72 hr LC_{50} 96 hr LC_{50}	31-88 product 20-62 product 17-48 product 17-48 product	Wan *et al., 1989*

125	Roundup®	Coho *Oncorhynchus kisutch*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	14-52 product 13-38 product 13-35 product 13-33 product	Wan *et al., 1989*
126	Roundup®	Chum *Oncorhynchus keta*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	17-31 product 12-27 product 11-25 product 11-20 product	Wan *et al., 1989*
127	Roundup®	Chinook *Oncorhyncus tshawytsha*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	17-41 product 17-33 product 17-33 product 17-33 product	Wan *et al., 1989*
128	Roundup®	Pink salmon *Oncorhyncus gorbuscha*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	17-35 product 17-33 product 17-33 product 14-33 product	Wan *et al., 1989*
129	Roundup®	Rainbow trout *Salmo gairdneri*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	17-33 product 17-33 product 15-33 product 14-33 product	Wan *et al., 1989*

130	75% tallow amine surfactant MON 0818 (Part of MON 8709 – 10% w/w)	Coho *Oncorhynchus kisutch*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	1.8-4.9 ai 1.8-4.6 ai 1.8-4.6 ai 1.8-4.6 ai	Wan *et al., 1989*
131	75% tallow amine surfactant MON 0818 (Part of MON 8709 – 10% w/w)	Chum *Oncorhynchus keta*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	1.5-2.7 ai 1.4-2.7 ai 1.4-2.7 ai 1.4-2.7 ai	Wan *et al., 1989*
132	75% tallow amine surfactant MON 0818 (Part of MON 8709 – 10% w/w)	Chinook *Oncorhyncus tshawytsha*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	2.0-4.9 ai 2.0-3.0 ai 1.9-2.8 ai 1.7-2.8 ai	Wan *et al., 1989*
133	75% tallow amine surfactant MON 0818 (Part of MON 8709 – 10% w/w)	Pink salmon *Oncorhyncus gorbuscha*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	1.7-5.3 ai 1.5-4.5 ai 1.5-4.5 ai 1.4-4.5 ai	Wan *et al., 1989*
134	75% tallow amine surfactant MON 0818 (Part of MON 8709 – 10% w/w)	Rainbow trout *Salmo gairdneri*	Survival	24 hr LC$_{50}$ 48 hr LC$_{50}$ 72 hr LC$_{50}$ 96 hr LC$_{50}$	2.0-3.2 ai 2.0-2.7 ai 1.9-2.6 ai 1.6-2.6 ai	Wan *et al., 1989*
135	Vision (356 g/L of glyphosate as N-(phosphonometyl) glycine)	Rainbow trout *Oncorhyncus mykiss*	Survival	96-h LC$_{50}$	10.42(9.37-11.67) ai	Morgan & Kiceniuk, *1992*

136	Glyphosate commercial formulation 54.9% ai	Goldfish *Carassius auratus*	Survival	96-h NOEC 96-h LC_{50}	3431 ± 137 ai 4183.62 ± 83.5 ai	Anton *et al*, *1994*
137	Glyphosate commercial formulation 38% ai	Goldfish *Carassius auratus*	Survival	96-h NOEC 96-h LC_{50}	------ 9500-10000 ai	Anton *et al*, *1994*
138	Glyphosate commercial formulation 36 % ai	Goldfish *Carassius auratus*	Survival	96-h NOEC 96-h LC_{50}	2880 ai 9217 ai	Anton *et al*, *1994*
139	Glyphosate commercial formulation 54.9% ai	Rainbow trout *Oncorhyncus mykiss*	Survival	96 hr NOEC 96 hr LC_{50}	823.5 ai 4290.8 ai	Anton *et al*, *1994*
140	Technical grade 62%	Carp *Cyprinus carpio*	Survival	48 hr LC_{50} 96 hr LC_{50}	645 (632-655) ai 620 (607-638) ai	Neskovic *et al., 1996*
141	Technical grade 62%	Carp *Cyprinus carpio*	Biochemical &	Alkaline phosphatase (AP) activity in liver	Increased at 2.5, 5, and 10 a.i.	Neskovic *et. al., 1996*
				AP activity in heart	Increased at 10 a.i.	
				Glutamic-oxaloacetic transaminase (GOT) activity in liver and kidney	Increased at 2.5 and 5 a.i.	
				Glutamic-pyruvic transaminase (GPT) activity in kidney	Increased at 2.5 a.i.	
				GPT activity in serum	Increased at 5 and 10 a.i.	
			histopathological changes	Gills: epithelial hyperplasia and subepithelial edema	Found at 5 and 10 a.i.	
				Liver: congestion of sinusoid and signs of early fibrosis	10 a.i.	

142	Rival granular	Mosquitofish *Gambusia yucatana*	Survival	96-h LC_{10} 96-h LC_{50} 96-h LC_{90}	9.97 (3.53-13.91) 17.79 (12.19-25.36) 31.71 (22.95-84.71)	Rendon-von Osten *et al., 2005*
COMMUNITIES							
143	Roundup (application spray 0.43 kg a.i./ha)	Plankton communities with fish	DO, pH, T^{o}C, total ammonia and nitrite nitrogen, chlorophyll *a*		No adverse effect found	Perschbacher *et al., 1997*