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The Toxicity of Glyphosate and Several Glyphosate Formulations to Four Species of Southwestern Australian Frogs

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Abstract. The acute toxicity of technical-grade glyphosate acid, glyphosate isopropylamine, and three glyphosate formulations was determined for adults of one species and tadpoles of four species of southwestern Australian frogs in 48-h static/renewal tests. The 48-h LC_{50} values for Roundup® Herbicide (MON 2139) tested against tadpoles of *Crinia insignifera*, *Heleioporus eyrei*, *Limnodynastes dorsalis*, and *Litoria moorei* ranged between 8.1 and 32.2 mg/L (2.9 and 11.6 mg/L glyphosate acid equivalent [AE]), while the 48-h LC_{50} values for Roundup® Herbicide tested against adult and newly metamorphosed *C. insignifera* ranged from 137–144 mg/L (49.4–51.8 mg/L AE). Touchdown® Herbicide (4 LC-E) tested against tadpoles of *C. insignifera*, *H. eyrei*, *L. dorsalis*, and *L. moorei* was slightly less toxic than Roundup® with 48-h LC_{50} values ranging between 27.3 and 48.7 mg/L (9.0 and 16.1 mg/L AE). Roundup® Biactive (MON 77920) was practically nontoxic to tadpoles of the same four species producing 48-h LC_{50} values of 911 mg/L (328 mg/L AE) for *L. moorei* and >1,000 mg/L (>360mg/L AE) for *C. insignifera*, *H. eyrei*, and *L. dorsalis*. Glyphosate isopropylamine was practically nontoxic, producing no mortality among tadpoles of any of the four species over 48 h, at concentrations between 503 and 684 mg/L (343 and 466 mg/L AE). The toxicity of technical-grade glyphosate acid (48-h LC_{50}, 81.2–121 mg/L) is likely to be due to acid intolerance. Slight differences in species sensitivity were evident, with *L. moorei* tadpoles showing greater sensitivity than tadpoles of the other four species. Adult and newly emergent metamorphs were less sensitive than tadpoles.

The widespread use of pesticides has been identified as a potential factor contributing to the global decline of amphibians (Barinaga 1990; Blaustein and Wake 1990). In Australia this prospect has gained some credibility through anecdotal reports of frog mortality and cessation of frog chorus following application of glyphosate based herbicides (NRA 1996; Tyler and Williams 1996).

Glyphosate (N–Phosphonomethyl glycine) is one of the most widely used herbicides in the world because of its efficacious weed control properties and negligible persistence in the environment. The physical, chemical, and toxicological properties of glyphosate have been well documented (Duke 1988; Malik *et al.* 1989; WHO 1994). Toxicological assessment of glyphosate-based formulations are, however, relatively sparse. A study by Folmar *et al.* (1979) remains one of the few comprehensive works on the toxicology of glyphosate-based herbicides to aquatic fauna. In that study, four materials—technical-grade glyphosate acid, the isopropylamine (IPA) salt of glyphosate, the commercial formulation Roundup® (MON 02139), and the Roundup® formulation surfactant (MON 0818)—were tested for acute toxicity against four species each of invertebrates and fish. Folmar *et al.* (1979) found that Roundup® Herbicide was more toxic than the active constituent glyphosate and that the surfactant, MON 0818 had a similar toxicity to Roundup®. Subsequent studies also concluded that the surfactant in Roundup® was responsible for its relatively high toxicity (Mitchell *et al.* 1987; Servizi *et al.* 1987; Wan *et al.* 1989).

Despite these findings, several authors concluded that under normal usage, Roundup® Herbicide did not present a hazard in the aquatic environment because both the glyphosate and surfactant would be diluted sufficiently in a large body of water or in a lotic aquatic environment, and therefore not constitute a toxic hazard (Sullivan *et al.* 1981; Hildebrand *et al.* 1982; Mitchell *et al.* 1987). However, in shallow, lentic, or ephemeral water bodies, at normal application rates, the concentration of surfactant may reach toxic levels, although this scenario has yet to be addressed.

The importance of ephemeral water bodies as breeding grounds for many of the world's amphibians cannot be overstated. In Australia, approximately half of the more than 200 species of frogs are dependent on seasonal bodies of water for completion of their reproductive cycles (Cogger 1992). Amphibian population viability within these systems can be compromised by changes in water chemistry or the introduction of pollutants (Hazelwood 1970; Freda 1986; Berger 1989).

This study examines the acute toxicity of several glyphosate formulations on tadpoles of four species and adults of one species of southwestern Australian frogs. Some of the preliminary data generated in this study have been detailed in a report to the Western Australian Department of Environmental Protection (Bidwell and Gorrie 1995). As a consequence of that

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report, the Australian National Registration Authority for Agricultural and Veterinary Chemicals (NRA) placed restrictions on the use of 84 glyphosate-based products in or over water (NRA 1996). The basis for the NRA restrictions was the toxicity of the surfactant component of those products. The surfactant is a polyoxyethylene amine derivative (POEA). Most of the products affected by the new restrictions are of similar composition and are typified by the widely used Roundup® Herbicide by Monsanto.

Materials and Methods

In general, toxicity test procedures employed in this study follow those outlined in the ASTM Standard E729-88a$^{\epsilon 1}$ (ASTM 1993).

Test Substances

Five test substances were used in this investigation. Roundup® Herbicide, Roundup® Biactive Herbicide, and the isopropylamine (IPA) salt of glyphosate (60.5% in water) were obtained from Monsanto Australia Ltd. in August 1996. Touchdown® Herbicide was purchased from a retail outlet, and glyphosate acid was provided by Davison Industries. Details on manufacturers and primary ingredients are provided in Table 1.

Test Organisms

Four species of frogs common to the southwest of Western Australia were selected for this study. The species were *Crinia insignifera, Heleioporus eyrei, Limnodynastes dorsalis,* and *Litoria moorei.* They serve as examples of the two major phylogenetic groups of frogs in Australia (Myobatrachidae and Hylidae) and are also representative of large and small frogs with varying habitat requirements (Cogger 1992; Tyler *et al.* 1994). *C. insignifera* is a small (14–29 mm s-v, snout-vent length) ground dwelling frog that inhabits areas temporarily inundated by water. *H. eyrei* is a medium sized (45–66 mm s-v) burrowing frog inhabiting sandy soils in areas prone to temporary inundation. *L. dorsalis* is a relatively large (60–73 mm s-v) ground frog that inhabits vegetation close to permanent water. *L. moorei* is also a relatively large (53–74 mm s-v) frog found in permanent waters where it inhabits emergent vegetation.

All animals were field-collected from areas with large populations. *C. insignifera* metamorphs and adults were collected from a single location in the Perth metropolitan area. *C. insignifera* eggs were harvested from the matings of adult animals collected in amplexus from the same location. *L. dorsalis* and *L. moorei* were collected as egg masses from a single location in the Mandurah district south of Perth. *H. eyrei* were collected as egg masses from two locations in the Perth metropolitan area.

The eggs and tadpoles were held in glass tanks fitted with air stones and held at approximately the same temperature (20°C) and in the same diluent water as was used during the tests. Holding periods ranged from 1 to 3 weeks prior to testing. The tadpoles were acclimatized to the test conditions for 48 h prior to the initiation of the tests. This involved transfer of the animals to a climate control room in which all tests were performed. During the holding and acclimation periods the animals showed no signs of disease or stress. Water quality was maintained by daily water changes. Ammonia levels were randomly monitored with a Merck Ammonium Aquaquant test kit. Daily water changes were adequate to maintain ammonium levels below 50 ppb. During the holding period the tadpoles were fed commercial fish food and pelletized rabbit chow. Adults and metamorphs were used within 2 days of capture to avoid stress associated with captivity.

Preparation of Test Concentrations

Prior to testing, a primary stock was prepared for each test substance as a nominal concentration of 1,000 mg/L glyphosate acid equivalent (AE). The diluent used for the stock solutions was either deionized water or US EPA Soft Water with a hardness of 40–48 mg/L $CaCO_3$ and a conductivity of approximately 210 µS/cm (ASTM 1993). Test concentrations were prepared just prior to the beginning of the tests using US EPA Soft Water, aged tapwater, or filtered (30 µm) lake water collected from Curtin Lake in Bentley, Perth (conductivity: 416 µS/cm). Those tests performed using lake water or aged tapwater were described by Bidwell and Gorrie (1995). These tests are specified below and denoted in Table 2 with a superscript † or ‡, respectively. Test solutions were renewed after 24 h with freshly made solutions.

Test Procedure

General: Prior to definitive tests, range finding tests were performed using various test concentrations between 1.0 mg/L and 1,000 mg/L (AE). If range-finding tests indicated no mortality at or above 400 mg/L (AE) (under normal field application rates, these chemicals are unlikely to reach or exceed concentrations of 400 mg/L AE), then the definitive test would be restricted to one 400 mg/L (AE) concentration. Where range finding tests indicated mortality below 400 mg/L (AE), the definitive test incorporated at least five concentrations, from which LC_{50} data were generated. Tests were run for 48 h rather than the standard 96 h since starvation was considered to be an important factor affecting the survival of young tadpoles. Animal condition was assessed and dead animals removed at 12-h intervals.

Tadpoles: Where possible, tadpoles from a single clutch were used for each test. All animals were at Gosner-stage 25 in their development (Gosner 1960). The average mass of at least 10 tadpoles (blotted dry) from the same clutch was used as an indication of tadpole weight. Biomass loading (defined as the total wet weight of tadpoles per liter of test water) was maintained below 0.6 g/L as recommended in ASTM guidelines (ASTM 1993). Either 400- or 600-ml acid-washed glass beakers with 200–500 ml of solution were used for all tests. The larger beaker was used for *H. eyrei* tadpoles. Four or five tadpoles were impartially allocated to each of the beakers until there was a total of 20 animals for each test concentration and a control group. In the case of the test using *H. eyrei* exposed to Touchdown® Herbicide, restricted numbers of animals necessitated the exposure of 12 tadpoles per concentration (three tadpoles per beaker). The beakers were indiscriminately arranged on a bench in a climate room. Animals were not fed for the 48-h duration of the tests. Glyphosate acid, glyphosate isopropylamine, Roundup® Herbicide, and Roundup® Biactive were tested simultaneously. Touchdown® was tested independently. Some of the preliminary tests using *L. moorei* (Bidwell and Gorrie 1995) were performed in solutions made up with filtered lake water. The tadpoles used in these preliminary tests are denoted *L. moorei*† in the Results section.

Adults and Metamorphs: Adult *C. insignifera* required specialized exposure chambers to ensure they remained in contact with the toxicants for the full exposure period. The chambers were constructed from short lengths of PVC pipe (50 mm diameter) which were covered at one end with nylon mesh. The other end was sealed with a 55-mm polystyrene petri dish. The frogs were placed inside, and the tube was placed into a beaker such that the cross-section was vertical. An air space at the top of the tube was large enough to allow frogs to cling to

Table 1. Commercial products used in acute toxicity tests

Product	Manufacturer's Code	Glyphosate Component	Surfactant Type
Roundup® [a] Herbicide	MON 2139 (Monsanto)	glyphosate (36%) isopropylamine	Polyoxyethylene amine (POEA)
Roundup® Biactive Herbicide	MON 77920 (Monsanto)	glyphosate (36%) isopropylamine	Undisclosed surfactants
Touchdown® [b] Herbicide	4 LC-E (ICI Crop Care)	glyphosate trimesium (48%)	alkylpolysaccharide & POEA

[a] Roundup® is a registered trademark of the Monsanto Chemical Company
[b] Touchdown® is a registered trademark of ICI Crop Care Australia

the mesh partially immersed, but small enough to prevent them from climbing out completely. Five frogs were exposed to each of five concentrations of Roundup® Herbicide or technical-grade glyphosate acid and a control.

Newly emergent *C. insignifera* metamorphs were too small (5–10 mm s-v) to be exposed in this manner. Instead they were exposed in glass tubes (one per tube), which were covered at one end with nylon mesh. Racks holding 10 tubes were then suspended in two replicate, 3-L beakers containing enough solution to immerse the tubes 5 mm deep. A froglet sitting upright in a tube was always partially covered with solution without having to swim. A swab of glass wool inserted in the top of the tube ensured that the frogs could not climb out of the solution. A total of 20 froglets were exposed in this manner to each of five concentrations of Roundup® Herbicide and a control.

All tests using postmetamorphic frogs were described by Bidwell and Gorrie (1995) and performed in solutions prepared with aged tapwater and are denoted *C. insignifera*‡ in the Results section.

Environmental Conditions

A Conviron C10 climate room was used to maintain a test temperature of 20 ± 1°C and a 12-h light and 12-h dark photoperiod. Temperature was monitored by an in-built continuous chart recorder. Temperature, dissolved oxygen (DO), and pH measurements were taken at the beginning of the test, and after 24 and 48 h. Temperature and DO were measured using a WTW OXI 320 oxygen meter, and pH was measured with a HANNA 8417 pH meter.

Analytical Chemistry

Water samples were taken at the beginning of the test and after 24 h (prior to test solution renewal). Selected samples were sent to the Australian Government Analytical Laboratories in Perth, Western Australia, for glyphosate determination by high-pressure liquid chromatography (HPLC) with post column derivitization and fluorescence detection. This procedure provided 93.9% recovery with a coefficient of variation of 2.2%. Only those concentrations which spanned the 0–100% mortality range were analyzed for glyphosate. Consequently, some of the lower concentrations were not analyzed. While individual controls (0 mg/L) were not analyzed, the deionized water supply was tested for background glyphosate levels and was found to be below the detection limit of 20 µg/L.

Data Analysis

Mortality data were used to generate LC_{50} values by the Spearman-Karber method (Hamilton *et al.* 1977, 1978) using the CT-TOX Multi-Method Program (CT-DEP 1990). Where available, initial measured glyphosate levels were used to generate LC_{50} values. Nominal data were used to generate LC_{50} values for *L. dorsalis, L. moorei*,† *C. insignifera*,‡ and *L. moorei* (Roundup®).

Results

Chemical Analysis for Glyphosate

Analysis for glyphosate at test initiation and after 24 h indicated no loss of glyphosate during this time period. Nearly all solutions sampled at 0 and 24 h exhibited increases in glyphosate of between 0.4 and 8.0% over the 24-h period. These increases may to be due to water evaporation because there was no humidity control employed. Alternatively, the discrepancies may in part be due to errors associated with volume measurement and instrumentation errors during analysis. Since initial glyphosate measurements have been used to generate LC_{50} data, reported toxicities in this study are likely to be slight overestimates.

Water Quality

For most of the tests, DO remained above 80%. DO dropped below 80% (but never below 70%) if the presence of dead animals allowed a bacterial build-up. The pH for all tests with technical-grade glyphosate acid ranged between 2.9 and 7.7. The pH for all tests with glyphosate IPA, Roundup® Herbicide, Roundup® Biactive, and Touchdown® Herbicide ranged between 5.1–8.0. The temperature for tests with *L. moorei*† ranged between 23.4 and 25.4°C. The temperature for all the other tests ranged between 19.0 and 21.3°C and for any single test, the temperature range was no greater than 1.5°C.

Acute Toxicity

Of the five compounds tested, Roundup® Herbicide was the most toxic, followed in decreasing order by Touchdown®, glyphosate acid, Roundup® Biactive, and glyphosate IPA (Table 2). For Roundup®, 48-h LC_{50} values ranged from 2.9 mg/L (AE) for *L. moorei* tadpoles, up to 11.6 mg/L (AE) for *L. moorei*† tadpoles, and up to 51.8 mg/L (AE) for *C. insignifera* metamorphs. For Touchdown®, 48-h LC_{50} values ranged from 9.0 mg/L (AE) for *C. insignifera* tadpoles to 16.1 mg/L (AE) for *H. eyrei* tadpoles. For Roundup® Biactive, all 48-h LC_{50} values were above 300 mg/L (AE). None of the control animals died in any test. The LC_{50} values for each chemical tested are presented in Table 2. Animal weights are presented in Table 3.

No mortality was observed for tadpoles of any species exposed to glyphosate IPA at approximately 400 mg/L (AE) (see Table 2 for exact concentrations). The 24-h and 48-h LC_{50} values generated for Roundup® Herbicide however, were all between 1.5 and two orders of magnitude lower than corre-

Table 2. Toxicity data for technical-grade glyphosate acid, glyphosate isopropylamine, and three glyphosate formulations for four species of western Australian frogs[a]

Species and Life Stage	LC_{50} (95% Confidence Limits, mg/L)									
	Technical-Grade Glyphosate Acid		Glyphosate Isopropylamine		Roundup® Herbicide		Touchdown® Herbicide		Roundup® Biactive	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Lymnodynastes dorsalis (tadpole)			**>400***	**>400***	**4.6 (4.1–5.2)***	**3.0 (2.8–3.2)***	**14.7 (14.0–15.4)***	**12.0 (11.4–12.6)***	**>400***	**>400***
			>587*	>587*	12.8 (11.4–14.4)*	8.3 (7.8–8.9)*	44.4 (42.3–46.6)*	36.2 (34.4–37.9)*	>1,111*	>1,111 *
Litoria moorei (tadpole)	**88.6 (79.8–98.3)**	**81.2 (76.7–85.9)**	**>343**	**>343**	**3.1 (2.8–3.4)***	**2.9 (2.6–3.2)***	**10.4 (9.7–11.1)**		**333 (305–363)**	**328 (296–363)**
			>503	>503	8.6 (7.8–9.4)*	8.1 (7.2–8.9)*	31.4 (29.4–33.6)		925 (847–1,008)	911 (822–1,008)
Litoria moorei† (tadpole)	**127 (90–180)***	**121 (111–133)***			**12.7 (9.0–18.0)***	**11.6 (10.3–13.1)***				
					35.3 (25.0–50.0)*	32.2 (28.6–36.4)*				
Heleioporus eyrei (tadpole)			**>373**	**>373**	**8.6 (7.8–9.5)**	**6.3 (5.6–7.1)**	**16.6 (14.1–19.6)**	**16.1 (13.7–18.9)**	**>427**	**>427**
			>548	>548	23.9 (21.7–26.4)	17.5 (15.6–19.7)	50.2 (42.5–59.3)	48.7 (41.5–57.1)	>1,186	>1,186
Crinia insignifera (tadpole)			**>466**	**>466**	**>**5.1§	**3.6 (3.3–4.1)**	**13.1 (12.3–14.0)**	**9.0 (8.4–9.7)**	**>494**	**>494**
			>684	>684	>14.2§	10 (9.2–11.4)	39.6 (37.2–42.2)	27.3 (25.5–29.3)	>1,372	>1,372
Crinia insignifera‡ (metamorph)					**88.7 (68.6–114)***	**51.8 (42.1–63.8)***				
					246 (191–318)*	144 (117–177)*				
Crinia insignifera‡ (adult)	**89.6 (73.6–108.6)***	**83.6 (67.4–103.6)***			**52.6 (39.3–70.5)***	**49.4 (40.5–60.2)***				
					146 (109–196)*	137 (113–167)*				

[a] LC_{50} values are expressed as mg/L glyphosate acid equivalent (bolded) or mg/L product formulation
† Data generated by Bidwell and Gorrie (1995) and for which lake water was the diluent
‡ Data generated by Bidwell and Gorrie (1995) and for which aged tapwater was the diluent
§ Data for which there were insufficient upper concentrations to record an LC_{50}
* Data for which nominal concentrations were used to calculate an LC_{50}

Table 3. Average weights of animals used in acute toxicity tests with standard deviations in parenthesis

Species and Life Stage	Average Animal Mass for Acute Toxicity Tests With: Glyphosate Acid, Glyphosate IPA, Roundup® Herbicide, Roundup® Biactive	Touchdown® Herbicide
L. dorsalis (tadpole)	21.2 mg (4.4)	8.8 mg (2.0)
L. moorei (tadpole)	17.2 mg (3.0)	28.8 mg (6.6)
L. moorei[a] (tadpole)	ND	
H. eyrei (tadpole)	57.6 mg (6.7)	82.7 mg (21.5)
C. insignifera (tadpole)	11.4 mg (2.1)	12.9 mg (2.4)
C. insignifera[a] (metamorph)	ND	
C. insignifera[a] (adult)	ND	

[a] Data generated by Bidwell and Gorrie (1995)

sponding glyphosate IPA test concentrations (Table 2). Touchdown® Herbicide produced LC_{50} values slightly higher than those produced for Roundup® (Table 2). Neither the trimesium salt nor the surfactant component used in this formulation were tested independantly.

Roundup® Biactive produced no observable toxic effects in *L. dorsalis*,*H. eyrei,* or *C. insignifera* tadpoles at 400, 427, and 495 mg/L (AE) respectively. However, this formulation was toxic to *L. moorei*, producing 24-h and 48-h LC_{50} values of 333 and 328 mg/L (AE), respectively (Table 2).

Glyphosate acid was far more toxic to *L. moorei* tadpoles (48-h LC_{50}, 81.2 mg/L AE) than glyphosate IPA salt (48-h LC_{50}, 343 mg/L AE) (Table 2).

There was no clear trend in species sensitivity, although tadpoles of larger species appear to be less sensitive. Comparing tests performed under similar conditions (*i.e.*, those tests not denoted with †), tadpoles of the largest species, *H. eyrei,* with an average mass of 57–83 mg (Table 3) were the least sensitive. The other three species did not show a clear size vs. sensitivity correlation. The tadpoles of *L. moorei* at an average mass of 17.2–28.8 mg (Table 3), were marginally more sensitive to both Roundup® and Touchdown® than the smaller *C. insignifera* tadpoles (11.4–12.9 mg). Furthermore, *L. moorei* was the only species that showed any mortality following exposure to Roundup® Biactive at concentrations lower than 400 mg/L (AE).

Adult and newly emergent metamorphs of the species *C. insignifera* were an order of magnitude less sensitive than tadpoles of the same species (Table 2); however, differing test conditions make this comparison tenuous. The difficulty in making comparisons between tests carried out under different conditions is well illustrated by the fourfold difference in 48-h LC_{50} values for tadpoles of *L. moorei*† (11.6 mg/L AE) and those for *L. moorei* (2.9 mg/L AE) exposed to Roundup® Herbicide (Table 2).

Discussion

Roundup® Herbicide was the most toxic of the formulations tested, followed by Touchdown®, and then Roundup® Biactive. The active ingredient in Roundup® Herbicide and Roundup® Biactive, glyphosate IPA, was found in this study to be nontoxic.

The POEA surfactant used in Roundup® was not tested independently, however, as in previous studies with fish and invertebrates (Folmar *et al.* 1979; Mitchell *et al.* 1987; Servizi *et al.* 1987; Wan *et al.* 1989), the surfactant component in Roundup® Herbicide appears to be primarily responsible for its toxicity. Reduction in the percentage of POEA surfactant has been shown to reduce the toxicity of glyphosate formulations (Wan *et al.* 1989). Furthermore, surfactants are known to interfere with gill morphology, causing lysis of gill epithelial cells and resulting in disruption of gill secondary lamellae (Partearroyo *et al.* 1991). Mortality is due either to asphyxiation or loss of osmotic stability (Able 1974).

The trimesium salt incorporated into Touchdown® has not been tested separately in this study. It is not possible therefore, to quantify the respective contributions of the surfactant and the active to the overall toxicity of the product. However, previous assessments of product toxicity to *Daphnia magna* indicate that the trimesium salt of glyphosate does contribute to the toxicity of Touchdown® Herbicide (NRA 1996).

Roundup® Biactive was 100 times less toxic than Roundup® in the most sensitive species, *L. moorei.* Furthermore, the LC_{50} values generated for Roundup® Biactive with *C. insignifera*, *H. eyrei*, and *L. dorsalis* are in agreement with the 96-h LC_{50} of >1,040 mg/L (product formulation) published for *Rana pipiens* (Monsanto 1996). The acute toxicity of Roundup® Biactive to *L. moorei* is presumably affected by the surfactant components, since no mortality was observed in equivalent concentrations of glyphosate IPA.

This study has included acute toxicity data for the isopropylamine salt of glyphosate and glyphosate acid. Glyphosate acid has been used previously in these kinds of tests as the comparative model to evaluate the toxicity of formulated products. Folmar *et al.* (1979) reported 96-h LC_{50} values for rainbow trout (*Oncorhyncus mykiss*), flathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), and bluegill sunfish (*Lepomis macrochirus*) as 140, 97, 130, and 140 mg/L, respectively, when exposed to glyphosate acid. No water quality data were reported for any of the above mentioned tests. The difficulty in interpreting such data is illustrated by the results obtained in this study. Our tests using *L. moorei* tadpoles exposed to glyphosate acid produced LC_{50} values similar to those reported by Folmar *et al.* (1979) for the four fish species; however, *L. moorei* tadpoles exposed to glyphosate IPA (the active constituent in both Roundup® and Roundup® Biactive) were unaffected at >340 mg/L. The discrepancy can be accounted for by the low pHs (<pH 3.0) encountered by tadpoles in the higher concentrations of glyphosate acid. It is well documented that amphibian larvae are intolerant to acid environments (Freda 1986).

There was a fourfold difference in sensitivity between *L. moorei* tadpoles and *L. moorei*† tadpoles exposed to Roundup® Herbicide. There are many reasons for variation in acute toxicity between tests carried out at different times. The age, size, and weight of *L. moorei*† tadpoles were not determined, and differences in these parameters are adequate to explain the observed differences in toxicity. Certainly tadpole size appears to be a mitigating factor as indicated by the greater tolerance of the larger *H. eyrei* tadpoles. Furthermore, the tests using *L.*

moorei[†] were conducted in dilution water collected from a local lake (Bidwell and Gorrie 1995). This water exhibited water chemistry parameters that differed from US EPA Soft Water (*i.e.*, higher conductivity). The toxicity of Roundup® has been shown to be affected by water chemistry (Servizi *et al.* 1987; Wan *et al.* 1989). Other reasons for variation between tests may be diet, differences in handling, and natural variations in sensitivity between different tadpole clutches.

Adult and new metamorph *C. insignifera* were less sensitive to Roundup® than tadpoles. This may be a reflection of their size, although metamorphs which are much smaller than adults expressed similar sensitivity to adults. It is more likely that the difference in sensitivity between terrestrial adults and aquatic tadpoles reflects their reduced reliance on exposed respiratory surfaces. While adult frogs do respire through their skin, they rely predominantly on pulmonary respiration. Studies in resting *Bufo marinus* indicate that 80% of the O_2 uptake is pulmonary with cutaneous respiration accounting for the remaining 20% (Bentley and Shield 1973). Cutaneous respiration may account for as little as 4% total respiration in active *B. marinus* (Withers and Hillman 1988).

The findings of this study indicate that there are minor differences in sensitivity between different species. While caution should be exercised in allocating any significance to the apparent sensitivity of *L. moorei* tadpoles, it is worth noting that this species is closely related to *Litoria aurea* from eastern Australia, which is currently listed as endangered (Tyler 1997). While there are no indications that *L. aurea* has become restricted in its distribution as a consequence of environmental contamination, the apparent sensitivity of a closely related species to the toxic effects of environmental contaminants may be worthy of further investigation.

The validity of extrapolating laboratory based acute toxicity data to the field situation is contentious. Data exist that suggest that environmental factors can either attenuate or exacerbate chemical toxicity (Kimball and Levin 1985). Natural amphibian habitat is usually chemically and physically complex, and acute toxicity tests may not replicate the chemical transformations and associated changes in toxicity that are likely to occur in complex ecosystems. The toxicological assessment of glyphosate-based formulations would therefore benefit from further studies using mesocosm or microcosm protocols that incorporate higher levels of biological organization.

The long-term sublethal effects of pesticide exposure are likewise not addressed by acute toxicity tests. A study by Tate *et al.* (1997) found anomalies in the development of third-generation *Pseudosuccinea columella* snails following three generations of continuous exposure to glyphosate. While natural populations of amphibians are unlikely to experience continuous glyphosate exposure, glyphosate application often coincides with the onset of the breeding season. It might be expected therefore, that succeeding generations of adult frogs (or tadpoles) may be exposed to an annual glyphosate pulse. Such regular exposures may have a cumulative effect that is only expressed after several generations. Generational effects of this kind can not be assessed by acute toxicity protocols as presented here.

Since the surfactant component of the tested formulations is the major contributor to their acute toxicity, it is important to evaluate the persistence of these chemicals. The POEA surfactant used in Roundup® is expected to be rapidly removed from the water column by a combination of sorption/binding to the sediment, microbial action and dilution, although its half-life in lentic systems may still extend to several days or weeks (NRA 1996). The narrow margin between no observable effect and 100% mortality associated with tadpoles exposed to surfactants (Presutti *et al.* 1994; Mann and Bidwell in preparation) and the ability of tadpoles to recover from short-term sublethal exposure to surfactants (Mann, unpublished observation) may indeed make lethality testing appropriate for the assessment of surfactant toxicity. In the absence of long-term pulsed or chronic exposure studies however, this interpretation remains speculative.

Notwithstanding the above considerations, a report to the Western Australian Department of Environmental Protection (Bidwell and Gorrie 1995) expressed concerns in regard to the application of glyphosate formulations over very shallow water bodies (<5 cm in depth), which constitute breeding habitat for frogs. Subsequent to that report, the Australian National Registration Authority for Agricultural and Veterinary Chemicals (NRA) stipulated that glyphosate formulations should exhibit no toxicity to aquatic organisms at concentrations of at least 100 mg/L (NRA 1996). Glyphosate formulations that contain 360 g/L glyphosate AE, when applied at the maximum rate of 10.6 kg/ha to a lentic water body of 5 cm in depth, would leave residues of approximately 21.1 mg/L (whole product). In such a situation, the regulations as they now stand in Australia would accommodate a fivefold safety margin (NRA 1996). Of the three formulations tested in this study, Roundup® Biactive is the only one that would comply with current regulations in Australia and is the only one of the three that is registered for use for aquatic weed control.

While acute toxicity tests have provided the necessary criteria for the NRA restrictions on the use of glyphosate-based herbicides in aquatic systems, similar criteria are not available for use of herbicides and other pesticides in terrestrial environments. Concern remains over the use of those products that are considered to be too toxic for use in an aquatic ecosystem but still registered for use in terrestrial habitats. In general, when applied in a terrestrial ecosystem, pesticides and associated surfactants will not be diluted to any substantial degree by the intrasoil water column. The toxic hazard to nontarget soil invertebrates and vertebrates (such as amphibians and reptiles), although somewhat localized, might be extreme. This argument remains speculative because toxicological assessments of terrestrial hazards are lacking and further research is needed.

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