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Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors

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Abstract

Glyphosate-based herbicides (e.g. Roundup®) are extensively used in the aquatic environment, but there is a paucity of data on the toxicity of the formulated products and the influences by environmental factors. In this study, the acute toxicity of technical-grade glyphosate acid, isopropylamine (IPA) salt of glyphosate, Roundup® and its surfactant polyoxyethylene amine (POEA) to Microtox® bacterium (*Vibrio fischeri*), microalgae (*Selenastrum capricornutum* and *Skeletonema costatum*), protozoa (*Tetrahymena pyriformis* and *Euplotes vannus*) and crustaceans (*Ceriodaphnia dubia* and *Acartia tonsa*) was examined and the relative toxicity contributions of POEA to Roundup® were calculated. The effects of four environmental factors (temperature, pH, suspended sediment and algal food concentrations) on the acute toxicity of Roundup® to *C. dubia* were also examined. Generally, the toxicity order of the chemicals was: POEA > Roundup® > glyphosate acid > IPA salt of glyphosate, while the toxicity of glyphosate acid was mainly due to its high acidity. Microtox® bacterium and protozoa had similar sensitivities towards Roundup® toxicity (i.e. IC50 from 23.5 to 29.5 mg AE/l). In contrast, microalgae and crustaceans were 4–5 folds more sensitive to Roundup® toxicity than bacteria and protozoa. Except photosynthetic microalgae, POEA accounted for more than 86% of Roundup® toxicity and the toxicity contribution of POEA was shown to be species-dependent. Increase in pH (6–9) and increase of suspended sediment concentration (0–200 mg/l) significantly increased the toxicity of Roundup® to *C. dubia*, but there were no significant effects due to temperature change and food addition.

Keywords: Glyphosate; Surfactant; Acute toxicity; Bioassay battery; Environmental factors

1. Introduction

Glyphosate (*N*-Phosphonomethylglycine) is a non-selective and post-emergent herbicide. The major formulation is Roundup® in which glyphosate is formulated as isopropylamine (IPA) salt and a surfactant, polyoxyethylene amine (POEA), is added to enhance the efficacy of the herbicide. Another formulation, Rodeo®, contains the IPA salt of glyphosate without the surfactant and is primarily used for controlling aquatic weeds in some countries (e.g. United States). Owing to their high water solubility and extensive usage in the environment (especially in shallow water systems), the exposure of non-target aquatic organisms to these herbicides is a concern of ecotoxicologists.

Although there has been documentation on the physical, chemical and toxicological properties of glyphosate (Malik et al., 1989; WHO, 1994), the aquatic toxicity data for glyphosate-based formulations are relatively sparse with most previous work focusing on freshwater invertebrates and fishes (Folmar et al., 1979;

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Wan et al., 1989) and recently on frogs (Mann and Bidwell, 1999; Perkins et al., 2000). Work by Folmar et al. (1979) remained one of the most comprehensive, which compared the acute toxicity of technical-grade glyphosate acid, Roundup®, IPA salt of glyphosate and POEA to several freshwater invertebrates and fishes, and concluded that POEA was mainly responsible for the relatively high toxicity of Roundup®. However, there is no similar study to examine whether POEA also accounts for the toxicity of Roundup® to microorganisms such as bacteria, microalgae and protozoa which were very different from invertebrates and vertebrates (e.g. fishes and amphibians) in terms of morphology, cytogenetics and physiology. Furthermore, the lack of toxicity data of many kinds of pesticides on aquatic bacteria and protozoa was highlighted in a recent review (DeLorenzo et al., 2001), and this is also the case for glyphosate-based herbicides.

Several environmental factors such as pH and temperature have been identified to modify the toxicity of pesticides (Sprague, 1985; Fisher, 1990; Howe et al., 1994). For glyphosate-based formulations, Wan et al. (1989) examined the effects of different dilution waters on the toxicity of Roundup®, while Folmar et al. (1979) investigated the effects of pH and temperature on Roundup® toxicity. Nevertheless, these studies employed different organisms and test conditions and therefore could not easily generalize the individual effects of different factors on Roundup® (and glyphosate) toxicity.

This study was therefore carried out to differentiate the relative toxicity contribution of IPA salt of glyphosate and POEA to Roundup® using different groups of organisms with emphasis on microorganisms to provide new toxicity data of glyphosate on these species, and to evaluate the effects of various environmental factors on modifying the toxicity of Roundup® employing standard 48 h *Ceriodaphnia dubia* acute toxicity test (USEPA, 1993). Using a single and standard toxicity test allows better comparison between the influences of different environmental factors, rather than using different toxicity tests to address their influences.

2. Materials and methods

2.1. Test organisms

Seven organisms which represent four major taxonomic groups (viz. bacteria, algae, protozoa and crustaceans) were chosen, the selection of which was based on their frequent employment in toxicity tests, reported sensitivity to pollutants and wide occurrence in the environment. Except bacteria, all groups consisted of both a freshwater and a marine species. Freeze-dried bacterium (*Vibrio fischeri*) was purchased from Azur Environmental (Delaware, CA, USA). Algae, *Selenastrum capricornutum* (UTEX 1648) and *Skeletonema costatum* (UTEX LB2038) were obtained from the Culture Collection of Algae, the University of Texas at Austin, (Austin, TX, USA). Ciliates, *Tetrahymena pyriformis* (CCAP 1630/1F) and *Euplotes vannus* (CCAP 1624/13) were supplied by the Culture Collection of Algae and Protozoa (Cumbria, UK). Aquatic Research Organisms (Hampton, NH, USA) provided the cladoceran *C. dubia* and the Marine Biological Laboratory at Helsingør, (Helsingør, Denmark) supplied the copepod *Acartia tonsa*.

2.2. Test chemicals

Glyphosate acid (CAS: 1071-83-6; $\geqslant 97\%$ purity) and polyoxyethylene amine (POEA) (CAS: 61791-26-2; 100% a.i.) were purchased from Fluka (Buchs, Switzerland) and ChemService (West Chester, PA, USA), respectively. Roundup® (commercial grade; 41% a.i.) and isopropylamine (IPA) salt of glyphosate (CAS: 38641-94-0; 56.8% a.i.) were manufactured by Monsanto Chemical Co. (St. Louis, MO, USA). Glyphosate acid was included to allow comparison with previous studies (e.g. Wong, 2000).

2.3. Experiment I: comparison between different groups of organisms

A summary of test methods is given in Table 1. All experiments (except Microtox®) were conducted under the same growth conditions as the stock cultures. For the algal and protozoan tests, flasks were hand shaken twice daily to prevent settling of the cells. For each treatment, a range-finding test was conducted before the definitive test which consisted of 5–8 concentrations (dilution factor = 0.5). Because glyphosate is a weak acid and 1% aqueous solution of glyphosate has a pH of about 2 (Franz et al., 1997), the pH of the test media (except Microtox®) was measured before and after the test with a daily calibrated Orion pH-meter Model 920A (Boston, MA, USA). The dissolved oxygen content before and after the lethality tests of the cladoceran and copepod was measured with an YSI oxygen-meter Model 58 (Yellow Springs, OH, USA).

The calculation of the relative toxic contribution of IPA salt of glyphosate and POEA to Roundup® to each organism was adopted from the procedure of Henry et al. (1994), which is summarized below

$$TU_{IPA} = LC50_{IPA(Roundup)}/LC50_{IPA(alone)} \quad \text{and}$$
$$TU_{POEA} = LC50_{POEA(Roundup)}/LC50_{POEA(alone)}$$

where TU_{IPA} is toxic unit of IPA salt of glyphosate and TU_{POEA} is toxic unit of POEA, relative contribution of IPA salt of glyphosate:

Table 1
Summary of test procedures of the seven toxicity tests

	Test organisms						
	Vibrio fischeri	*Selenastrum capricornutum*	*Skeletonema costatum*	*Tetrahymena pyriformis*	*Euplotes vannus*	*Ceriodaphnia dubia*	*Acartia tonsa*
Freshwater/marine	Marine	Freshwater	Marine	Freshwater	Marine	Freshwater	Marine
Temperature (°C)	15	25 ± 1	20 ± 1	27 ± 1	20 ± 1	25±1	20 ± 2
Light intensity (lux)	–	4300 ± 430	4300 ± 430	0	400–800	500–1000	400–800
Photoperiod (light:-dark)	–	16:8	14:10	0:24	14:10	16:8	14:10
Test chambers	Glass cuvette	150 ml Erlenmeyer flask	150 ml Erlenmeyer flask	250 ml Erlenmeyer flask	150 ml Erlenmeyer flask	100 ml glass beaker	100 ml glass beaker
Volume of medium (ml)	2	30	30	50	30	50	50
Organisms stocked	–	20 000 cells/ml	20 000 cells/ml	2500 cells/ml	1000 cells/ml	5 individuals	5 individuals
Number of replicates	2	3	3	3	3	4	4
Control (without toxicant)	2	2	2	2	2	1	1
Media	Diluent + osmotic adjusting solution	ASTM in deionized water	ASTM in artificial seawater[a] (30‰)	Tetratox medium	1% Bactotryptone and 0.5% yeast extract in artificial seawater[a] (30‰)	Reconstituted moderately hard water (hardness 69.5 mg $CaCO_3$/l; alkalinity 50.9 mg $CaCO_3$/l)[b]	Artificial seawater[a] (30‰)
Sterilization of medium	No	0.22 μm filtration	0.22 μm filtration	Autoclaving	0.22 μm filtration	No	No
pH (average)	–	7.50	8.00	7.40	8.00	8.07	8.00
Test duration	15 min	96 h	96 h	40 h	48 h	48 h	48 h
Endpoint[c]	IC50	IC50	IC50	IC50	IC50	LC50	LC50
Biomass measurement	Luminescenceemission	Absorbance at 680 nm	Absorbance at 675 nm	Cell count by haemocytometer	Cell count by haemocytometer	Animal count	Animal count
Test guidelines/references	Microbics Corporation (1992)	ASTM (1994)	ASTM (1994)	Schultz (1997)	Modified from Coppellotti (1998)	USEPA (1993)	ISO (1997)

[a] Artificial seawater was prepared from Instant Ocean® sea salt in deionized water and filtered through Whatman® GF/A glass fiber filter; salinity was measured by conductivity meter of Orion Model 142 (Boston, MA, USA).

[b] Hardness was determined by measuring Ca and Mg concentrations separately by an atomic absorption spectrophotometer (Hitachi, Tokyo, Japan) and was calculated according to standard methods (APHA, 1995); total alkalinity was determined by an automatic ion analyzer (Skalar, Breda, Netherlands).

[c] IC50 = median growth inhibition concentration; LC50 = median acute lethal concentration.

$RC_{IPA} = TU_{IPA}/(TU_{IPA} + TU_{POEA})$

relative contribution of POEA:

$RC_{POEA} = TU_{POEA}/(TU_{IPA} + TU_{POEA})$

where $RC_{IPA} + RC_{POEA} = 1.0$.

RC_{IPA} and RC_{POEA} were then compared to determine which contributed more to the toxicity of Roundup®.

2.4. Experiment II: environmental factors in modifying Roundup® toxicity to C. dubia

The 48 h acute toxicity test with *C. dubia* was carried out as described in Experiment I, with the exception of varying four environmental factors—temperature, concentration of suspended particles, pH of water and algal food concentration.

Temperature. *C. dubia* was cultured at 20, 25 and 30 °C separately in different environmental chambers for one or more generations before the tests to produce <24 h old nenoates which were considered to be acclimated to that given temperature. The test water was stored in the given temperature for 2–3 h before the test.

Suspended particles. Kaolin (Acros, Fairlawn, NJ, USA) was added at 50, 100, 150 and 200 mg/l and the clay-Roundup® suspension was mixed for more than 2 h (Hartman and Martin, 1984) by a magnetic stirrer; kaolin was chosen because it is a typical component of many river sediments and the selected particle concentrations cover a wide variety of water types (i.e. from clean to turbid water). The clay suspension was agitated every 12 h to keep the particles suspended with the organisms removed during agitation.

pH. pH was adjusted to 6, 7, 8 or 9 by adding 1 ml of calibrating buffers (Riedel-de Haën, Seelze, Germany) to 50 ml of reconstituted moderately hard water and then either HCl or NaOH (Dave, 1984). Plastic vial with a tight lid was used as the test vessel to provide pH protection and the pH after 48 h deviated less than 0.3 unit in all treatments.

Food. Three algal (*Selenastrum capricornutum*) concentrations with cells in the log phase were concentrated by centrifugation and then added into synthetic water to result in 5×10^3, 5×10^4 and 5×10^5 cells/ml. This range of cell concentration that was adopted by Yu and Wang (2002) in a study of metal bioaccumulation using *Daphnia magna* should encompass a wide range of cell carbon contents in natural waters. The algae and Roundup® suspension was stirred overnight to allow sorption of the chemicals on algal surface.

2.5. Analysis of glyphosate concentration

Roundup® (i.e. IPA salt of glyphosate) was spiked onto two types of water—reconstituted moderately hard water and artificial seawater at 30‰ of salinity to determine the percentage recovery of the herbicide in different test media. The spiked concentrations of glyphosate ranged from 1.0 to 100 mg AE/l. Each treatment was replicated three times. Glyphosate was quantified using HPLC with pre-column derivatization (Miles et al., 1986). The measured concentration of glyphoaste was linear over 0.1–5.0 mg AE/l with $r^2 = 1.0$ (where AE = acid equivalent). The recovery for reconstituted moderately hard water was 100% (±1.0% S.D., $n = 3$) while that for artificial seawater was much lower, being 53.5% (±6.0% S.D., $n = 3$). Therefore, the nominal concentration of glyphosate (acid and IPA salts) was used for freshwater organism testing throughout the whole study while the recovery-corrected concentration was applied to marine organism testing (except Microtox®). In Experiment II, the decrease of glyphosate concentration in the water due to absorption/adsorption by particles was not quantified. POEA concentration in water was not determined as there is currently no validated method available for measuring POEA in water.

2.6. Validity of tests and data analysis

Except Microtox®, the growth inhibition tests were validated if the control attained double cell density or absorbance at the end of the test (ASTM, 1994); the acute lethal tests were validated if the control mortality did not exceed 10% (USEPA, 1993).

The IC50 (or median growth inhibition concentration) and 95% confidence interval were calculated by probit analysis for the growth inhibition test (Finney, 1971). The LC50 (median acute lethal concentration) and 95% confidence interval were calculated by the trimmed Spearman–Karber (TSK) analysis for lethal tests (Hamilton et al., 1977). All analyses were performed using statistical program issued by USEPA (1994). The criterion of "non-overlapping 95% confidence intervals" was used to determine significant difference ($p < 0.05$) between LC50s (APHA, 1995).

3. Results

3.1. Experiment I: comparison between different groups of organisms

Glyphosate acid caused the greatest decrease in pH, followed by IPA salt of glyphosate and Roundup® herbicide, while POEA slightly increased the pH of the test medium (Fig. 1). The dissolved oxygen contents of the water in the lethality tests were always greater than 90% of saturation at the beginning and above 70% at the end of the test.

The toxicity of glyphosate acid, Roundup®, IPA salt of glyphosate and POEA to the various test organisms are summarized in Table 2. Generally, Microtox® bac-

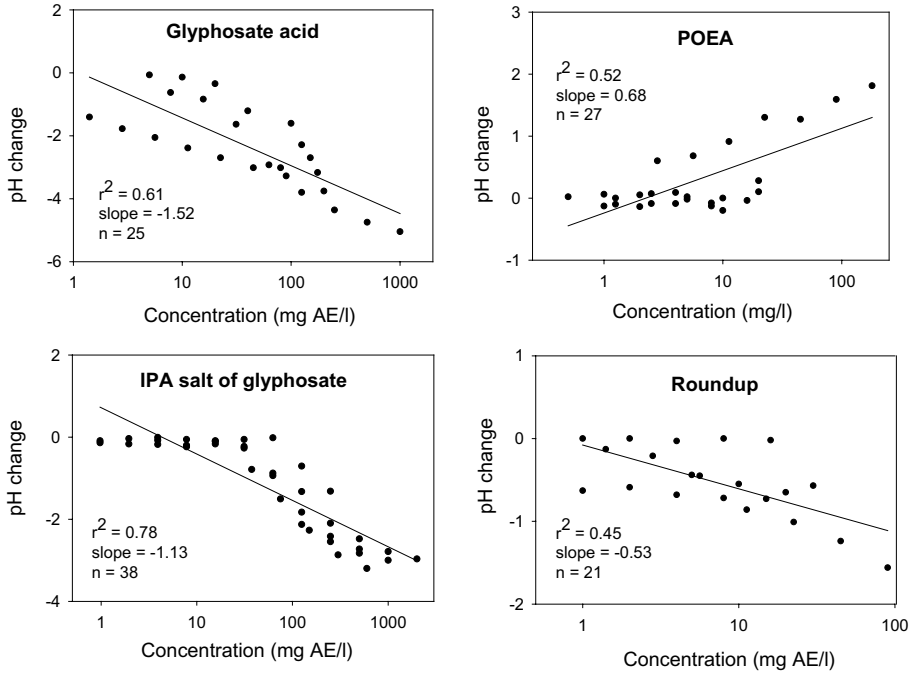

Fig. 1. pH changes with the four chemicals added to the different test media. Data from *Tetrahymena pyriformis* test were not included because buffer salts were added. AE = acid equivalent.

Table 2
Summary of results of the seven toxicity tests

Species	Endpoint[a]	Glyphosate acid (mg AE/l)[b]	IPA salt of glyphosate (mg AE/l)	POEA (mg AE/l)	Roundup® (mg AE/l)
Vibrio fischeri	15 min IC50	17.5 (15.8–19.5)[c]	162 (150–177)	10.2 (9.80–10.7)	24.9 (23.9–26.0)
Selenastrum capricornutum	96 h IC50	24.7 (22.8–26.7)	41.0 (29.4–59.1)	3.92 (1.57–9.58)	5.81 (2.36–8.14)
Skeletonema costatum	96 h IC50	2.27 (0.82–11.1)	5.89 (3.14–10.4)	3.35 (2.02–5.40)	1.85 (0.33–10.49)
Tetrahymena pyriformis	40 h IC50	648 (430–1280)	386 (95.2–2020)	4.96 (2.90–8.98)	29.5 (11.3–66.0)
Euplotes vannus	48 h IC50	10.1 (6.47–14.5)	64.09 (19.0–325)	5.00 (4.62–5.42)	23.5[d]
Ceriodaphnia dubia	48 h LC50	147 (141–153)	415 (339–508)	1.15 (1.04–1.27)	5.39 (4.81–6.05)
Acartia tonsa	48 h LC50	35.3 (30.9–40.3)	49.3 (38.4–63.1)	0.57 (0.50–0.65)	1.77 (1.33–2.34)

[a] IC50 = median growth inhibition concentration; LC50 = median lethal concentration.
[b] AE = acid equivalent.
[c] 95% confidence interval was in parentheses.
[d] 95% confidence interval cannot be calculated.

terium and protozoa (IC50s: 24.9–29.5 mg AE/l) were one order of magnitude less sensitive to the toxicity of Roundup® than algae and crustaceans (IC50s and LC50s: 1.77–5.81 mg AE/l). The toxicity for the other

three chemicals was in a decreasing order of POEA > glyphosate acid > IPA salt of glyphosate, except that IPA salt of glyphosate was twice as toxic as glyphosate acid to *T. pyriformis*. The ranges of IC50s or LC50s for glyphosate acid, IPA salt of glyphosate and POEA were 2.27–648, 5.89–386 mg AE/l and 0.57–10.2 mg/l, respectively. The contribution of IPA salt of glyphosate and POEA to Roundup® toxicity was shown to be species-dependent (Fig. 2). The relative contribution of POEA to Roundup toxicity was invertebrates > protozoa ~ Microtox® bacterium > algae, but the order was reversed for the IPA salt of glyphosate; POEA surfactant contributed more than 46% to Roundup® toxicity to all the test organisms.

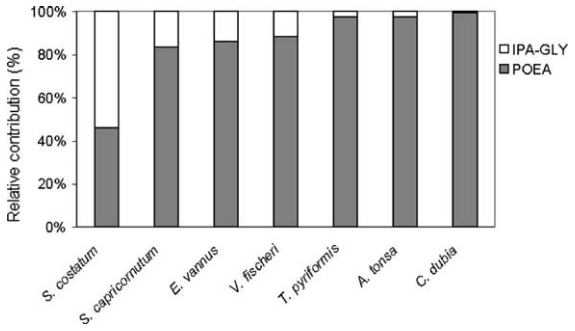

Fig. 2. Relative toxicity contribution of IPA salt of glyphosate (IPA–GLY) and POEA to Roundup® toxicity to different organisms.

3.2. Experiment II: environmental factors in modifying Roundup® toxicity to C. dubia

The effect of temperature on Roundup® toxicity was not significant, being slightly less toxic at 20 and 30 °C than at 25 °C (Table 3). The addition of kaolin clay at 150 and 200 mg/l was significantly ($p < 0.05$) more toxic than at 0–100 mg/l at 48 h. However, the addition of suspended particles alone (i.e. treatment without Roundup®) was more toxic at 150 and 200 mg/l as indicated by the relatively high mortality rates (30% and 60%, respectively). The pattern of increasing toxicity of Roundup® from pH 6 to 9 was more remarkable at 24 h than at 48 h. The decrease of LC50 at pH 6 from 24 to 48 h was mainly due to the toxic effect of acidity on *C. dubia*, as indicated by the high mortality at 48 h (25% in treatment without Roundup®); the difference between 48 h LC50s at different pHs diminished as compared with 24 h LC50s. However, increase of algal cell concentrations did not cause any observable linear relationship between food concentration and toxicity (both 24 and 48 h).

Table 3
Effects of environmental factors on Roundup® toxicity to *Ceriodaphnia dubia*

Treatments		24 h LC50s (mg AE/l)[a]		48 h LC50s (mg AE/l)	
Temperature (°C)	20	10.0 (7.37–13.6)[b]	b[c]	6.73 (5.72–7.91)	a
	25	6.43 (5.80–7.13)	a	5.39 (4.81–6.05)	a
	30	9.51 (8.32–10.9)	b	6.51 (5.75–7.37)	a
Suspended particles (mg/l)	0	6.43 (5.80–7.13)	a	5.39 (4.81–6.05)	c
	50	8.28 (6.74–10.2)	ab	4.76 (3.99–5.68)	c
	100	6.67 (4.81–9.26)	ab	3.61 (2.68–4.85)	bc
	150	8.00 (6.50–9.84)	ab	1.41 (0.66–3.02)[d]	ab
	200	11.6 (8.18–16.3)	ab	0.59 (0.40–0.88)[d]	ab
pH	6	>16.0[d]	c	4.47 (3.86–5.18)[e]	b
	7	14.3[f]	c	7.46 (6.17–9.02)	c
	8	8.57 (7.08–10.4)	b	7.13 (5.85–8.68)	c
	9	3.78 (3.18–4.50)	a	2.90 (2.47–3.41)	a
Food concentration (cells/ml)	0	6.43 (5.80–7.13)	a	5.39 (4.81–6.05)	a
	5000	9.51 (8.26–11.0)	b	8.28 (6.90–9.94)	b
	50 000	7.70 (6.15–9.63)	ab	7.19 (5.52–9.36)	ab
	500 000	9.19 (7.63–11.1)	b	6.50 (5.05–8.36)	ab

[a] AE = acid equivalent.
[b] 95% confidence interval was in parentheses.
[c] Values followed by the same letter were not significantly ($p > 0.05$) different from each other within the same column and the same treatment.
[d] LC50 was greater than the highest concentration tested.
[e] 48 h control mortality: 30% for 150 mg kaolin/l; 60% for 200 mg kaolin/l; 25% for pH 6 (i.e. greater than 10% control survival criterion).
[f] 95% confidence interval cannot be calculated.

4. Discussion

The sensitivities of aquatic microbes and crustaceans to glyphosate and its formulations were examined, and the effects of several important environmental parameters on the Roundup® toxicity were studied. In contrast to past studies (e.g. Folmar et al., 1979; Mann and Bidwell, 1999; Perkins et al., 2000), we found that the toxicity of Roundup® to aquatic organisms could be attributed to both the IPA salt of glyphosate and POEA, which depended on the group of organisms considered. Algae, which are photosynthetic, possess similar metabolic pathways to higher plants (e.g. aromatic amino acids synthesis), and were therefore more susceptible to the herbicidal effect of IPA salt of glyphosate than non-photosynthetic organisms.

When comparing the two algal species tested, *Skeletonema costatum* (diatom) was 7–10 times more sensitive than *Selenastrum capricornutum* (green alga) to glyphosate, either in acid or salt form, which was similar to the results of a previous study (Peterson et al., 1994) in which the inhibition of carbon uptake by Roundup® herbicide were 73–77% in diatoms (*Cycloetlla meneghiana* and *Nitzschia* sp.) but only 3–18% in green algae (*Selenastrum capricornutum* and *Scenedesmus quadricauda*). This may be attributed to the phylogenic variation between different algal groups (Wängberg and Blanck, 1988). Non-photosynthetic organisms such as bacteria, protozoa and crustaceans should be much more tolerant to the toxicity of IPA salt of glyphosate, and the same applied to fishes (Folmar et al., 1979) and frogs (Mann and Bidwell, 1999; Perkins et al., 2000), since these test organisms do not rely on the pathway of aromatic amino acids synthesis.

Glyphosate acid and IPA salt of glyphosate generally lowered the pH of the test media, the effect of which was stronger in the former. This may result in generally lower IC50s and LC50s for glyphosate acid than IPA salt of glyphosate. Gardner et al. (1997) reported a lower 96 h IC50 of glyphosate to green alga, *Ankistridesmus* sp., for pH un-adjusted than pH-adjusted test medium, while Mann and Bidwell (1999) reported a higher toxicity of glyphosate acid to tadpole of *Litoria moorei* which was attributed to acid intolerance of this organism. Although glyphosate acid has been studied in the past, it is neither included in the formulated products nor applied to the environment, and therefore studies with glyphosate acid may overestimate the toxicity of "glyphosate". It is therefore more appropriate to test the formulated products (e.g. Roundup® and Rodeo®) for their ecotoxicity. Buffered medium or pH-adjustment should be included in the toxicity tests for acidic toxicants to eliminate pH-associated toxicity because natural water has higher buffering capacity than test media in resisting dramatic pH change.

POEA was toxic to all the organisms tested, with IC50s and LC50s ranging from 0.57 to 10.2 mg/l. Microtox bacterium *V. fischeri* was the least sensitive species while the marine copepod, *A. tonsa*, was the most sensitive species to POEA toxicity (48 h LC50 = 0.57 mg/l). Our experiment used adults, and the LC50 of POEA could be much lower if napuliar stages were used, as the sensitivity of naupliar stages of *A. tonsa* to cypemethrin (a pesticide) was found to be 28 times higher than the adult stages after 96 h exposure (Medina et al., 2002). The toxicity of POEA or other frequently used surfactants should be fully evaluated with this highly sensitive species.

Change of temperature did not result in significant difference of Roundup® toxicity at 48 h. This contrasts with the results of Folmar et al. (1979) in which rainbow trout and bluegills showed greater susceptibility to Roundup® toxicity as temperature increased from 7 to 17 °C and 17 to 27 °C, respectively. Nevertheless, our study acclimatized *C. dubia* to the given temperature (more environmentally realistic), rather than increasing/decreasing the temperature before the toxicity tests as employed by Folmar et al. (1979); their procedures might subtly influence the biological responses to toxicants due to the temperature change. In addition, the temperature effect on modifying toxicity could not be easily generalized because it depended on the test chemicals and species (Cairns et al., 1975). The increase in pH of the test water from 6 to 9 significantly increased the acute toxicity of Roundup® to *C. dubia* (at 24 h), in which Roundup® was significantly more toxic at pH 9. This agrees with previous studies with fishes (Folmar et al., 1979; Wan et al., 1989), which demonstrated that glyphosate decreased but POEA increased the toxicity from acidic to alkaline pH, and thus POEA paralleled the trend of Roundup® in acute toxicity. The tallow amine surfactant is cationic at acidic and neutral pH but becomes non-ionic in alkaline pH (Cullum, 1994). Therefore, the non-ionic form of POEA should exert greater toxicity to the organisms, mostly through non-specific membrane disruption, (Maki, 1979; Schüürmann, 1990) and hence greater toxicity in alkaline medium.

The suspended particle used was kaolin which is typical of clay particles found in natural freshwater systems. The 48 h toxicity was shown to increase with clay concentration. The 48 h LC50s were higher for 150 and 200 mg kaolin/l which could probably be due to the combined effect of toxicity of suspended particle and active intake of sediment-Roundup® mixtures by the animals, because Roundup® was "concentrated" on the particle surface. Similarly, the addition of bentonite clay decreased the 48 h LC50 of Roundup® in *Daphnia pulex* (Hartman and Martin, 1984). These suggest that the POEA surfactant (and possibly glyphosate) could bind to the clay surface and be directly taken up by the filter-feeding cladocerans. However, we also need to consider

the absorption efficiency of glyphosate and POEA adsorbed on the particles by the organisms (Penry, 1998), which depends largely on test species, particle types and partition coefficient of the chemicals to the specific particles. The strong binding of IPA salt of glyphosate to kaolinite was demonstrated by Christy et al. (1981). Cells of *Selenastrum capricornutum* was the major food for *C. dubia* in this study, and the filter-feeding animals should take up the algal cells from the water column. However, a clear correlation between algal concentration and toxicity was not apparent from the data obtained and this may suggest POEA surfactant did not (extensively) bind to the algal surface or poorly absorbed by *C. dubia* from the clay particles.

In risk assessment of pesticides, both the toxicity of pesticides and the expected exposure to organisms should be considered. The maximum expected environmental concentration (EEC) of glyphosate in 15 cm water was 2.88 mg AE/l (Peterson et al., 1994; Perkins et al., 2000). This concentration would be hazardous to aquatic organisms if we consider Roundup® but not Rodeo® (i.e. because of greater margin of safety). Surfactant of lower toxicity could replace POEA in Roundup®. For example, a relatively new formulation (Roundup® Biactive) with an undisclosed surfactant manufactured by Monsanto Co., Australia is claimed to be safer to use than Roundup®. It has also been found that Roundup® Biactive was about 14 times less toxic than Roundup® to *C. dubia* with 48 h LC50 = 64.1 mg AE/l (Tsui and Chu, manuscript in preparation). Despite the rapid sorption of glyphosate and possibly the surfactant to sediment, the EEC of glyphosate may be much lower than 2.88 mg AE/l. However, environmental factors (e.g. pH and suspended sediment) may enhance the acute or chronic toxicity of glyphosate-based formulations.

Due to the removal of patent protection for Roundup® by Monsanto Co. and the development of genetically engineered glyphosate-resistant crops, the use of glyphosate-based formulations is expected to increase substantially. Therefore, further assessment of the environmental impact of glyphosate with different types and concentrations of surfactant is needed.

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