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Effects of Technical-Grade Active Ingredient vs. Commercial Formulation of Seven Pesticides in the Presence or Absence of UV Radiation on Survival of Green Frog Tadpoles

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Abstract Commercial formulations of pesticides contain both active and other ingredients. In some instances, the other ingredients have detrimental effects on nontarget species. Other factors such as UV radiation and predator cues have been shown to modify the toxicity of pesticides. In a laboratory study we compared the effects of technical-grade active ingredients to commercial formulations of seven common pesticides in the presence or absence of UV radiation on the survival of *Rana clamitans* (green frog) tadpoles over 96 h. We found a significant difference in the survival of tadpoles in technical-grade active ingredients versus commercial formulations in all of the pesticides tested. We also found that either the presence or the absence of UV radiation affected the survival of tadpoles in five of the seven pesticides tested. These results suggest that there is a need to test the effects of both active ingredients and commercial formulations of pesticides and, also, to include relevant abiotic factors like UV radiation treatments in the testing of pesticides because they can have a dramatic impact on the toxicity of some chemicals.

In 2001, Americans used an estimated 888 million lb of conventional pesticides for residential, agricultural, and industrial purposes (Kiely et al. 2004). Every year, new pesticides are introduced to the market. For a pesticide to be put on the market, it must be tested and approved by the Environmental Protection Agency (EPA) under guidelines set by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). This registration process includes toxicity tests on target and nontarget organisms. For outdoor-use products, nontarget animals include birds, fish, aquatic invertebrates, and honey bees. Amphibians are not included in this list, yet they are experiencing worldwide population declines that have been linked to pesticide exposure in some areas (Davidson et al. 2001, 2002; Davidson 2004).

The toxicity of a chemical can be affected by the formulation (Pereira et al. 2009) and, also, the presence of abiotic or biotic factors. The commercial formulation of a pesticide includes known technical-grade active ingredients as well as "other" ingredients, which are considered trade secrets and are not public knowledge. There are known cases where the "other" ingredients have made the commercial formulation more toxic than the technical-grade active ingredient (Mann and Bidwell 1999; Kitulagodage et al. 2008). Abiotic and biotic stressors, such as ultraviolet (UV) radiation (Zaga et al. 1998), pH (Edginton et al 2004), predator cues (Relyea 2003; Relyea and Mills 2001), and temperature (Boone and Bridges 1999), can interact with a pesticide to alter the toxicity. For example, a chemical may increase an organism's photosensitivity (Stacell and Huffman 1994), or UV may change the rate at which a chemical is broken down into a more toxic byproduct (Tilak et al. 1981). Data requirements for pesticide registration under FIFRA do not take into account any biotic or abiotic stressors other than the pesticide being tested, despite the dramatic effects they can have on toxicity.

In this study, we chose seven pesticides, and their technical-grade active ingredients, that could enter amphibian habitat through direct application (spray drift) or runoff to determine whether toxicity to amphibians was altered by pesticide formulation or UV exposure. *Rana clamitans* (green frog) tadpoles were exposed to a 50% dilution series of both the commercial formulation of the pesticide and the

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technical-grade active ingredient to expose tadpoles to estimated LC50 concentrations as well as an expected environmental concentration in the presence/absence of UV radiation. We hypothesized that commercial formulations would be more toxic to green frog tadpoles than technical-grade active ingredients because of the additional ingredients and that UV presence would interact with pesticides to alter toxicity.

Materials and Methods

Animal Collection

Green frog egg masses were collected from a forested pond in a mixed deciduous forest in Miami University's Natural Areas in Oxford, Butler County, Ohio, USA. A total of four clutches were collected on 25–26 May 2007. Clutches were combined in the laboratory, which was kept at 25°C on a 16:8-h light:dark cycle. These four clutches hatched on 29 May 2007. Free-swimming tadpoles (Gosner stage 25 [Gosner 1960]) from these clutches were used in the studies with carbaryl, permethrin, and malathion. Four other green frog clutches were collected from the same pond on 20 June 2007. Again, all four clutches were combined in the laboratory. These four clutches hatched on 25 June 2007, and free-swimming tadpoles (Gosner stage 25 [Gosner 1960]) from these clutches were used in the studies with glyphosate, β-cyfluthrin, bifenthrin, and imidacloprid. We used different clutches with these contaminants so that all tadpoles were tested at the same developmental stage (all tadpoles were Gosner stage 25 [Gosner 1960]) and at similar ages (between 1 and 4 weeks old posthatching).

All egg masses were kept in water collected from the pond masses they were collected in, and aged tap water ($\geq$3 days; pH 8.4; dissolved oxygen, 12.10 mg/L) was added daily until hatching to maintain oxygenated conditions and water level. After hatching, tadpoles were kept in aged tap water. The water was changed and the tadpoles were fed TetraMin (Blacksburg, VA, USA) fish food ad libitum daily.

General Experimental Design

Glass beakers with 500 ml of aged tap water were assigned to one of five pesticide treatments using a 50% dilution series (1×, 2×, 4×, or 8×) and one of two UV treatments (filtered or unfiltered). If the technical-grade chemical was dissolved in acetone during stock solution preparation, an acetone control (0.25 ml/500 ml) was used in each study. For a commercial formulation control we used aged tap water. The filtered UV treatments were used as a UV control. Each treatment was replicated five times in a randomized block design with one of each treatment on each of five shelves (i.e., blocks). For each study we randomly placed 10 tadpoles from the combined clutches in each beaker and spiked each beaker with the appropriate amount of stock solution (pesticide treatments), aged tap water (commercial controls), or acetone (technical controls) on the start date of each of the seven studies (Table 1). We put all of the lights on a timer so the tadpoles experienced a 16:8-h light:dark cycle. We did not feed the tadpoles during the studies, but they were fed prior to start of the study. Each study was terminated after 96 h.

Tadpole mortality was monitored daily and deceased tadpoles were promptly removed with a glass pipette. Tadpole mortality was determined with a tadpole's failure to move after gentle prodding with a glass pipette.

Pesticide Stock Solutions

We used seven common pesticides with different modes of actions: aceytlcholinesterase inhibitors (carbaryl and malathion), a neonicotinoid that acts on the nicotinic acetylcholine receptor (imidacloprid), sodium channel disruptors (β-cyfluthrin, bifenthrin, and permethrin), and an herbicide (glyphosate) to broaden the implications of this study. Concentrated stock solutions were made for all studies except the technical-grade imidacloprid (Table 2). To calculate the amount of commercial formulation or technical-grade active ingredient to add to make stock solutions, the following equation was used: $8 \times \text{concentration} \cdot \text{test volume} \cdot \text{stock solution volume} \cdot (8\times \text{ spike volume})^{-1} \cdot (\text{purity})^{-1}$. The commercial formulations were dissolved in

Table 1 Summary of active ingredients, start date of each 96-h test, and nominal test concentration (conc.) for both commercial and technical-grade treatments

Active ingredient	Start date	1× conc.	2× conc.	4× conc.	8× conc.
Carbaryl	4 June	2.75 mg/L	4.5 mg/L	11 mg/L	22 mg/L
Malathion	18 June	1 mg/L	2 mg/L	4 mg/L	8 mg/L
Imidacloprid	24 July	18.75 mg/L	37.5 mg/L	75 mg/L	150 mg/L
β-Cyfluthrin	9 July	7.5 µg/L	15 µg/L	30 µg/L	60 µg/L
Bifenthrin	16 July	0.125 µg/L	0.25 µg/L	0.5 µg/L	1 µg/L
Permethrin	11 June	2.5 µg/L	5 µg/L	10 µg/L	20 µg/L
Glyphosate	2 July	0.625 mg/L	1.25 µg/L	2.5 µg/L	5 mg/L

Table 2 Summary of pesticide stock solution production

Active ingredient/ CAS no.	Formulation	Amount added	Stock volume (ml)	(8×) spike volume (ml)	Purity (%)	(8×) nominal concen-tration	Test volume (ml)
Carbaryl 63-25-2	Commercial	1.956 g	10	0.25	0.225	22 mg/L	500
	Technical	442.0 mg	10	0.25	0.995	22 mg/L	500
Malathion 121-75-5	Commercial	480.0 mg	15	0.25	0.5	8 mg/L	500
	Technical	241.4 mg	15	0.25	0.994	8 mg/L	500
Imidacloprid 138261-41-3	Commercial	204.081 g	10	0.25	0.0147	150 mg/L	500
	Technical[a]			0.25			500
β-Cyfluthrin 68359-37-5	Commercial	48.0 mg	10	0.25	0.025	60 µg/L	500
	Technical	54.55 mg	450	0.25	0.99	60 µg/L	500
Bifenthrin 82657-04-3	Commercial	53.33 mg	800	0.25	0.03	1 µg/L	500
	Technical	2.02 mg	1000	0.25	0.99	1 µg/L	500
Permethrin 52645-53-1	Commercial	1.60 g	1000	0.25	0.025	20 µg/L	500
	Technical	40.0 mg	1000	0.25	0.99	20 µg/L	500
Glyphosate 38641-94-0	Commercial	5.0 g	10	0.25	0.02	5 mg/L	500
	Technical	105.3 mg	10	0.25	0.95	5 mg/L	500

Note: CAS numbers are provided to ensure that the same chemical was used in the commercial formulation and technical-grade active ingredient treatments

[a] See text for how technical-grade imidacloprid treatments were mixed

reversed-osmosis water, while the technical-grade active ingredients were dissolved in acetone for the concentrated stock solutions. To achieve test concentrations and a 50% dilution series (Table 1), we added 0.0131 ml (1×), 0.0625 ml (2×), 0.125 ml (4×), or 0.25 ml (8×) of either stock solution to 500 ml of aged tap water and 0.25 ml of aged tap water or acetone to commercial and technical controls, respectively. Therefore, the greatest amount of acetone added to any beaker was 0.25 ml. For the technical-grade imidacloprid treatments we dissolved 75.38, 37.69, 18.84, and 9.42 mg pure-grade imidacloprid (99.5% purity) in 500 ml of aged tap water to achieve concentrations of 150, 75, 37.5, and 18.75 mg/L, respectively. Although we did not confirm nominal concentrations in this study, we have confirmed them in previous studies using the same calculations described above, which resulted in approximately 109% of nominal (as in Boone and Bridges 1999).

We used LC50 values from the literature to set our nominal test concentrations and these reported LC50s generally fell between the 4× and the 8× concentrations in the dilution series. Reported LC50 values for tadpoles were used if available; if not, LC50s for fish were used. For the commercial formulation of carbaryl we used Sevin (GardenTech, Lexington, KY, USA), and concentrations were selected based on being near reported LC50s for tadpoles (Boone and Bridges 1999; Zaga et al. 1998; Bridges 1999). A commercial formulation of Malathion was used (Spectracide, St. Louis, MO, USA) and concentrations were selected based on being near reported LC50s for tadpoles (Relyea 2004; Bridges et al. 2002; Boone 2008). For the commercial formulation of imidacloprid we used 12 Month Tree & Shrub Insect Control (Bayer, Research Triangle Park, NC, USA), and concentrations were selected based on being near reported LC50s for fish (Feng et al. 2004). For β-cyfluthrin, the commercial formulation we used was Power Force Carpenter Ant & Termite Killer Plus (Bayer), and concentrations were selected based on reported LC50s for fish (Waller et al. 1993; Heath et al. 1994). The commercial formulation that we used for bifenthrin was Bug-B-Gon Max Lawn & Garden Insect Killer (The Ortho Group, Marysville, OH, USA) and concentrations were selected based on reported LC50s for fish (Drenner et al. 1993). For the commercial formulation of permethrin we used Cutter Bug Free Back Yard (Spectrum, St. Louis, MO, USA), and concentrations were selected based on being near reported LC50s for tadpoles and fish (Bridges et al. 2002; Boone 2008; Zaga et al. 1998). Finally, for glyphosate, the commercial formulation we used was Roundup (Monsanto, Marysville, OH, USA), and concentrations were selected based on being near reported LC50s for tadpoles (Relyea 2005; Wojtaszek et al. 2004; Edginton et al. 2004).

Ultraviolet Radiation

We exposed tadpoles to UV radiation by placing two 1.2-m shop lights above each shelf, with each shop light using two Vita-Lite fluorescent bulbs (Duro-Test Lighting, Inc., Philadelphia, PA, USA). There were two UV treatments: present (UV-A, 223.3–320.7 mW/m^2; UV-B, 14.64–31.97 mW/m^2; and visible light, 7.394–11.09 W/m^2) and a filtered control

(UV-A, 7.172–11.17 mW/m^2; UV-B, 0 mW/m^2; and visible light, 2.45–3.717 W/m^2). The UV ranges represent the highest and lowest UV measurements made on all shelves for each UV treatment because the beakers spanned an area on a shelf and did not experience the same UV levels. We also measured UV levels outside on 30 May 2007, a clear, sunny day (UV-A, 17.76 W/m^2; UV-B, 1.529 W/m^2; and visible light, 311.2 W/m^2). Therefore, the UV present treatments were exposed to ~1% of outside UV-A, 1%–2% of UV-B, and 2%–3.5% of visible light. These levels are well below the levels of UV-B that caused significant mortality for green frog larvae (8.5 W h/m^2 [Tietge et al. 2001]). For UV absent controls, we wrapped the outside of beakers with Makrolon polycarbonate sheets (Sheffield Plastics Inc., Sheffield, MA, USA) and then with fiberglass screening (charcoal; 1 × 2-mm openings); these materials were fastened to beakers with zip ties. For UV present treatments, we wrapped the outside of beakers with clear polyethylene GLAD Cling Wrap. UV was measured using a radiometer (Macam Photometrics Ltd., Livingston, Scotland). The UV present treatments were measured with the detector at the water line in the beakers and wrapped with the same clear plastic wrap used on the beakers. We measured the UV for the absent treatments by placing the detector at the height of the water line in the beakers and covering the detector with both polycarbonate and fiberglass screening.

Statistical Analyses

We used a repeated-measures ANOVA to examine how tadpole survival was affected by pesticide formulation, chemical concentration, UV treatment, chemical concentration × formulation, UV treatment × formulation, chemical concentration × UV treatment, chemical concentration × formulation × UV treatment, and block over time. Mortality (dead/total) was angularly transformed prior to analysis.

Results

Acteylcholinesterase Inhibitors

Carbaryl

The effects of carbaryl formulation, concentration, UV, the interaction of formulation × concentration, the interaction of concentration × UV, and the interaction of formulation × concentration × UV were significant over time (Table 3). Generally, the technical formulation caused slightly more mortality than the commercial formulation, although the differences were small and may not be biologically significant (Fig. 1a). The two highest concentrations of carbaryl increased mortality (Fig. 1b), and overall the presence of UV increased the toxicity of carbaryl (Fig. 1c). All three of these treatments interacted so that tadpoles at the highest concentration of carbaryl in either formulation and in the presence of UV experienced 100% mortality (Fig. 1d). Mortality was low at the two lowest concentrations despite the presence or absence of UV. Other treatments showed reduced toxicity in commercial formulations when UV was absent (Fig. 1d).

Malathion

The effects of malathion did not change over time because most mortality occurred within the first 24 h. However, between-subjects analyses indicate that tadpole mortality was significantly affected by malathion formulation, the concentration of malathion, and the interaction of formulation × concentration (Table 3). Increased concentrations in commercial formulation treatments significantly increased tadpole mortality (Fig. 2) but did not increase mortality in technical-grade treatments. Tadpole mortality was not significantly affected by the presence/absence of UV (Table 3).

Neonicotinoid

Imidacloprid

The effect of an imidacloprid formulation on tadpole mortality was significant but did not change over time and resulted in greater mortality in commercial treatments compared to technical treatments (Table 3). The effects of imidacloprid concentration, UV, and the interaction of formulation × concentration and of concentration × UV were significant over time (Table 2). At 4× and 8× concentrations, tadpoles in commercial treatments experienced greater mortality rates than tadpoles in technical treatments (Fig. 3a). Also, at lower concentrations, 1× and 2×, tadpoles in the presence of UV experienced relatively higher mortality rates than tadpoles in UV absent treatments, while at the higher concentrations, 4× and 8×, there was no difference in mortality between UV treatments (Fig. 3b).

Sodium Channel Disruptors

β-Cyfluthrin

The effects of β-cyfluthrin formulation, concentration, and the interaction of formulation × concentration on tadpole mortality were significant over time (Table 2). Generally,

Table 3 Results of repeated-measures ANOVA on the responses of tadpoles exposed to the commercial formulation or the technical-grade active ingredient (form) of seven pesticides (carbaryl, malathion, imidacloprid, β-cyfluthrin, bifenthrin, permethrin, and glyphosate) at five concentrations (conc.), 0, 1×, 2×, 4×, or 8×, in the presence or absence of UV radiation

Treatments	Carbaryl			Malathion			Imidacloprid			β-Cyfluthrin		
	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Between subjects												
Form	1	3.67	0.0588	1	217.4	<0.0001	1	109.67	<0.0001	1	185.01	<0.0001
Conc	4	85.39	<0.0001	4	110.55	<0.0001	4	61.94	<0.0001	4	216.68	<0.0001
Form × Conc	4	0.91	0.4626	4	110.55	<0.0001	4	40.38	<0.0001	4	18.86	<0.0001
UV	1	12.12	0.0008	1	0	0.9737	1	0.01	0.9413	1	3.39	0.0691
Form × UV	1	0.41	0.524	1	0	0.9737	1	0	0.9864	1	0.23	0.6357
Conc × UV	4	11.12	<0.0001	4	0.19	0.9423	4	0.32	0.8659	4	0.92	0.4538
Form × Conc × UV	4	0.35	0.8403	4	0.19	0.9423	4	0.35	0.8451	4	0.86	0.4889
Error	80			66			76			80		
Within subjects												
Time	3	165.41	<0.0001	3	1.82	0.1448	3	53.92	<0.0001	3	226.2	<0.0001
Time × Form	3	6.73	0.0002	3	1.82	0.1448	3	0.68	0.566	3	25.57	<0.0001
Time × Conc	12	53.26	<0.0001	12	0.97	0.4795	12	4.51	<0.0001	12	17.26	<0.0001
Time × Form × Conc	12	5.57	<0.0001	12	0.97	0.4795	12	3.09	0.0005	12	25.62	<0.0001
Time × UV	3	8	<0.0001	3	1.82	0.1448	3	4.34	0.0054	3	0.83	0.4773
Time × Form × UV	3	1.55	0.2029	3	1.82	0.1448	3	1.73	0.1608	3	0.93	0.4273
Time × Conc × UV	12	11.09	<0.0001	12	0.97	0.4795	12	1.86	0.0405	12	0.39	0.9679
Time × Form × Conc × UV	12	4.28	<0.0001	12	0.97	0.4795	12	0.66	0.7893	12	0.88	0.5658
Error (time)	240			198			240			240		

Treatments	Bifenthrin			Permethrin			Glyphosate		
	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Between subjects									
Form	1	9.29	0.0031	1	1.81	0.1818	1	70.29	<0.0001
Conc	4	176.47	<0.0001	4	7.19	<0.0001	4	77.96	<0.0001
Form × Conc	4	4.61	0.0021	4	4.25	0.0036	4	70.73	<0.0001
UV	1	1.68	0.1991	1	4.85	0.0306	1	0.05	0.8294
Form × UV	1	1.12	0.2929	1	0.92	0.3409	1	0.04	0.8405
Conc × UV	4	1.79	0.1388	4	2.18	0.0791	4	0.05	0.9957
Form × Conc × UV	4	0.95	0.4402	4	2.85	0.0292	4	0.04	0.9967
Error	80			80			80		
Within subjects									
Time	3	81.08	<0.0001	3	28.05	<0.0001	3	60.88	<0.0001
Time × Form	3	38.1	<0.0001	3	28.05	<0.0001	3	54.58	<0.0001
Time × Conc	12	22.27	<0.0001	12	16.95	<0.0001	12	61.26	<0.0001
Time × Form × Conc	12	19.01	<0.0001	12	16.95	<0.0001	12	54.92	<0.0001
Time × UV	3	0.29	0.836	3	18.15	<0.0001	3	1.3	0.2761
Time × Form × UV	3	1.09	0.3554	3	18.15	<0.0001	3	2.14	0.0958
Time × Conc × UV	12	1.55	0.1089	12	9.75	<0.0001	12	1.31	0.216
Time × Form × Conc × UV	12	1.66	0.0766	12	9.75	<0.0001	12	2.15	0.0146
Error (time)	240			240			240		

the technical formulation was more toxic (Fig. 4a) and all concentrations of exposure led to significant mortality (Fig. 4b). At 96 h, tadpoles in all technical-grade β-cyfluthrin treatments experienced near 100% mortality, while those at lower concentrations in commercial formulations experienced lower rates of mortality (Fig. 4c).

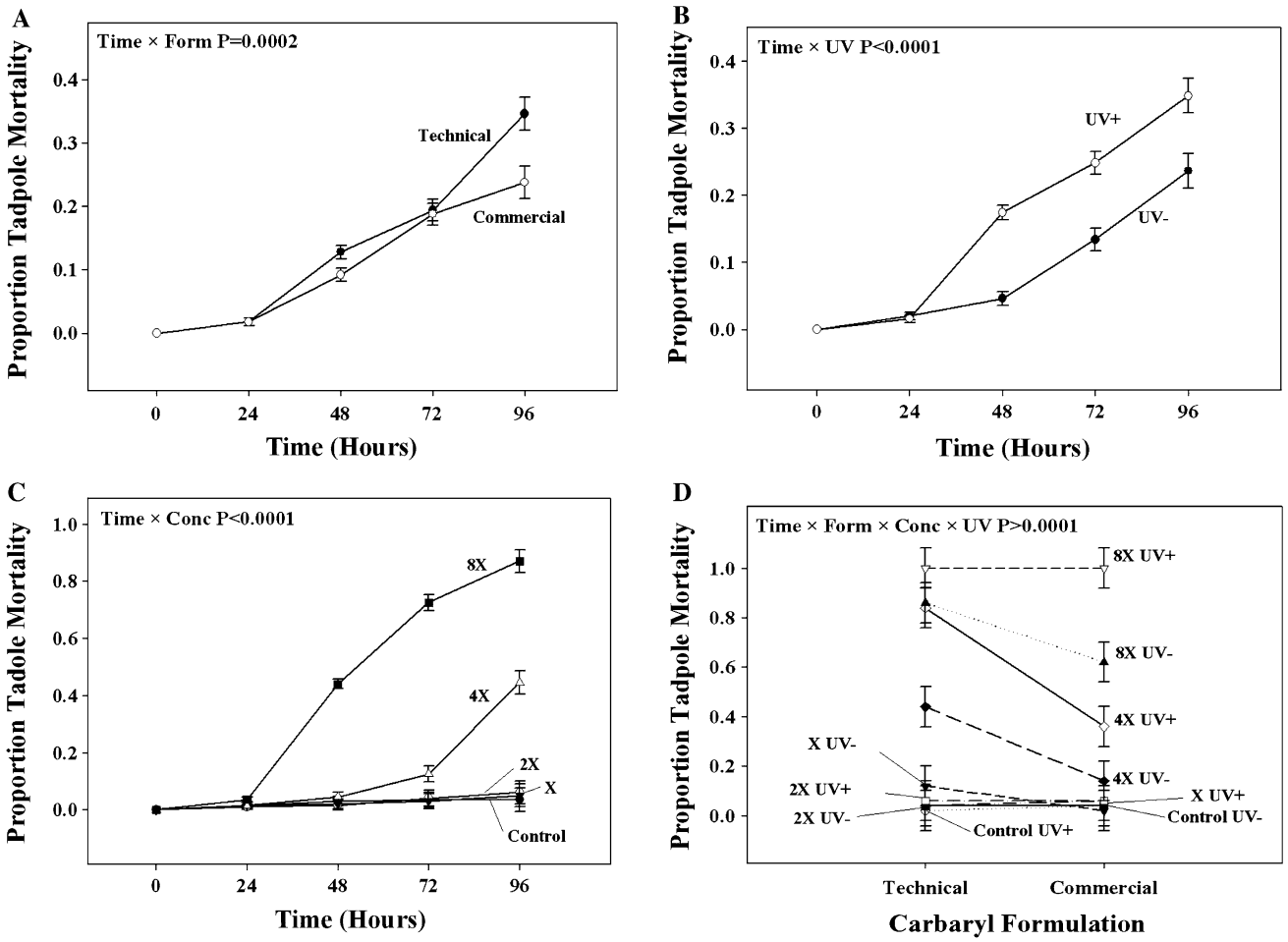

Fig. 1 Effect of **a** technical vs. commercial formulation of carbaryl, **b** carbaryl concentration, and **c** UV exposure over time on the mortality of green frog tadpoles. **d** Interactive effect of carbaryl formulation, concentration, and UV exposure on green frog tadpole mortality at 96 h. Error bars represent ±1 SE. Carbaryl exposures from control to high were 0, 2.25, 5.5, 11, or 22 mg/L and are labeled Control, 1×, 2×, 4×, and 8×, respectively

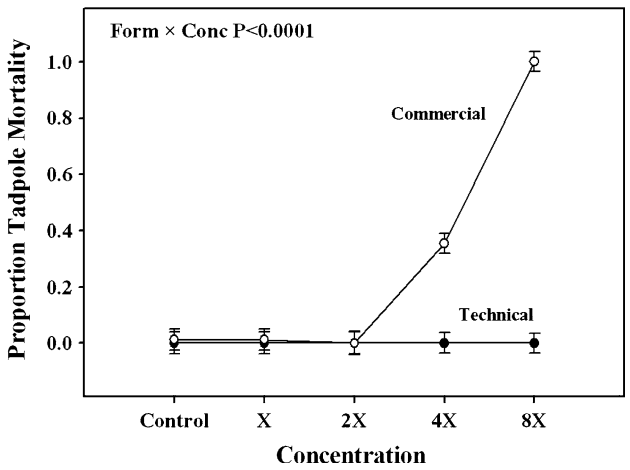

Fig. 2 Interactive effect of malathion formulation and concentration on green frog tadpole mortality at 96 h. Error bars represent ±1 SE. Malathion exposures from control to high were 0, 1, 2, 4, and 8 mg/L and are labeled Control, 1×, 2×, 4×, and 8×, respectively

Tadpole mortality was not significantly affected by the presence/absence of UV (Table 3).

Bifenthrin

The effects of bifenthrin formulation, concentration, and the interaction of formulation × concentration on tadpole mortality were significant over time (Table 3). Commercial formulations at the 4 × and 2 × concentrations were more toxic than the corresponding technical formulations initially, but by 96 h there were no significant differences in mortality between commercial and technical formulations at each concentration (Fig. 5).

Permethrin

The effects of permethrin formulation, concentration, UV, the interaction of formulation × concentration, the interaction of

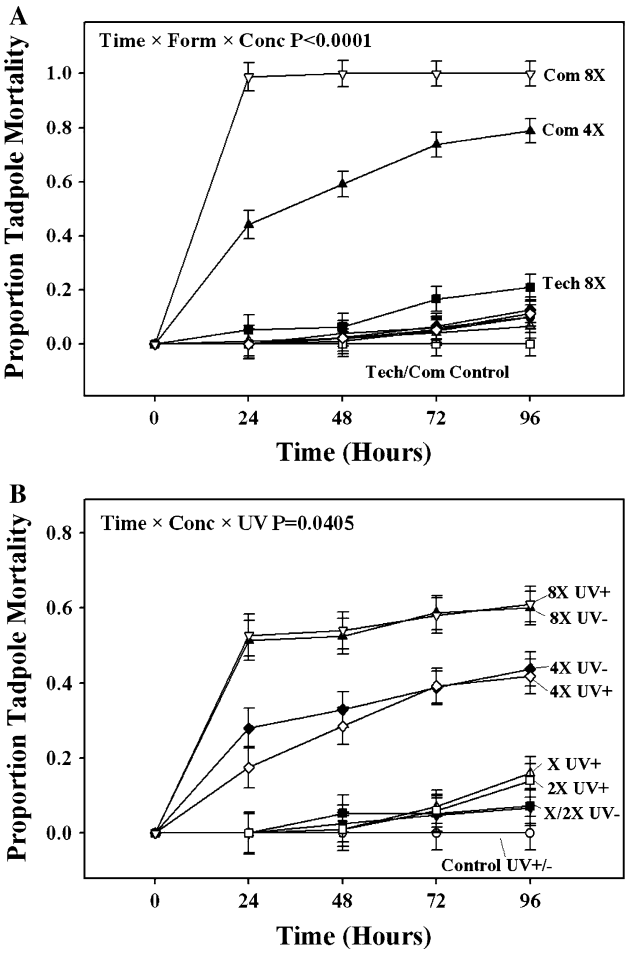

Fig. 3 Interactive effect of **a** imidacloprid formulation and concentration over time and **b** interactive effect of imidacloprid concentration and UV exposure over time on green frog tadpole mortality. Error bars represent ±1 SE. Imidacloprid exposures from control to high were 0, 18.75, 37.5, 75, and 150 mg/L and are labeled Control, 1×, 2×, 4×, and 8×, respectively

formulation × UV, the interaction of concentration × UV, and the interaction of formulation ×concentration × UV on tadpole mortality were significant over time (Table 3). Tadpoles experienced relatively low mortality in most treatments except with technical-grade permethrin at the highest concentration without UV (Fig. 6).

Herbicide

Glyphosate

The effects of glyphosate formulation, concentration, the interaction of formulation × concentration, and the interaction of formulation × concentration × UV on tadpole mortality were significant over time (Table 3). Mortality was low in all treatments except for high concentrations of the commercial formulation (Fig. 7).

Discussion

In this study we examined the effects of UV radiation and pesticide formulation on the survival of green frog tadpoles. We expected to find that commercial formulations of pesticides would be more toxic (Sayim 2008; Howe et al. 2004) and, also, that UV would interact with chemicals to enhance toxicity of pesticides (Zaga et al. 1998). We did find that both formulation type and UV presence/absence affected the survival of green frog tadpoles, which could alter the assessment of safe environmental levels, depending on which factors were included in the studies of pesticide toxicity. Commercial formulations differed in most cases from the toxicity of technical formulations, as also found by Pereira et al. (2009), and the presence of UV altered the toxicity of most of the pesticides tested.

The commercial formulation was more toxic than the technical-grade active ingredient in three of seven pesticides tested (malathion, glyphosate, and imidacloprid) and was toxic earlier than technical grade in one additional pesticide (bifenthrin). Differences in commercial formulation toxicity may be attributed to the addition of other ingredients. Commercial formulations that are more toxic than the technical formulation are a serious concern. With the exception of commercial formulations of glyphosate that include POEA, a surfactant known to be toxic to amphibians (Howe et al. 2004), the ingredients that make commercial formulations more toxic are unclear because manufacturers are not required to list their ingredients. Furthermore, the additional ingredients may make the tadpoles more vulnerable to the actual active ingredient. As has been found previously, malathion and glyphosate commercial formulations were more toxic to amphibians than the technical-grade active ingredient (Howe et al. 2004; Sayim 2008). In our study, imidacloprid also showed much greater toxicity in commercial formulations than the technical-grade chemical, while the commercial formulation of bifenthrin was more toxic initially at intermediate concentrations and eventually of similar toxicity to technical-grade bifenthrin at 96 h.

The additional ingredients added to the commercial formulation may make the pesticide more toxic to target organisms and less toxic to green frogs. For three of seven pesticides tested (carbaryl, permethrin, and β-cyflutrin), we found that technical-grade active ingredients were more toxic to green frog tadpoles than commercial formulations. While differences between formulations for carbaryl were statistically significant over time, they may not be biologically meaningful because differences were always <10%. In contrast, 8× (20 µg/L) concentrations of technical permethrin had a strong negative effect on tadpoles. This exposure is slightly more than double the reported expected environmental concentration of 9.4 µg/L (Pierce et al.

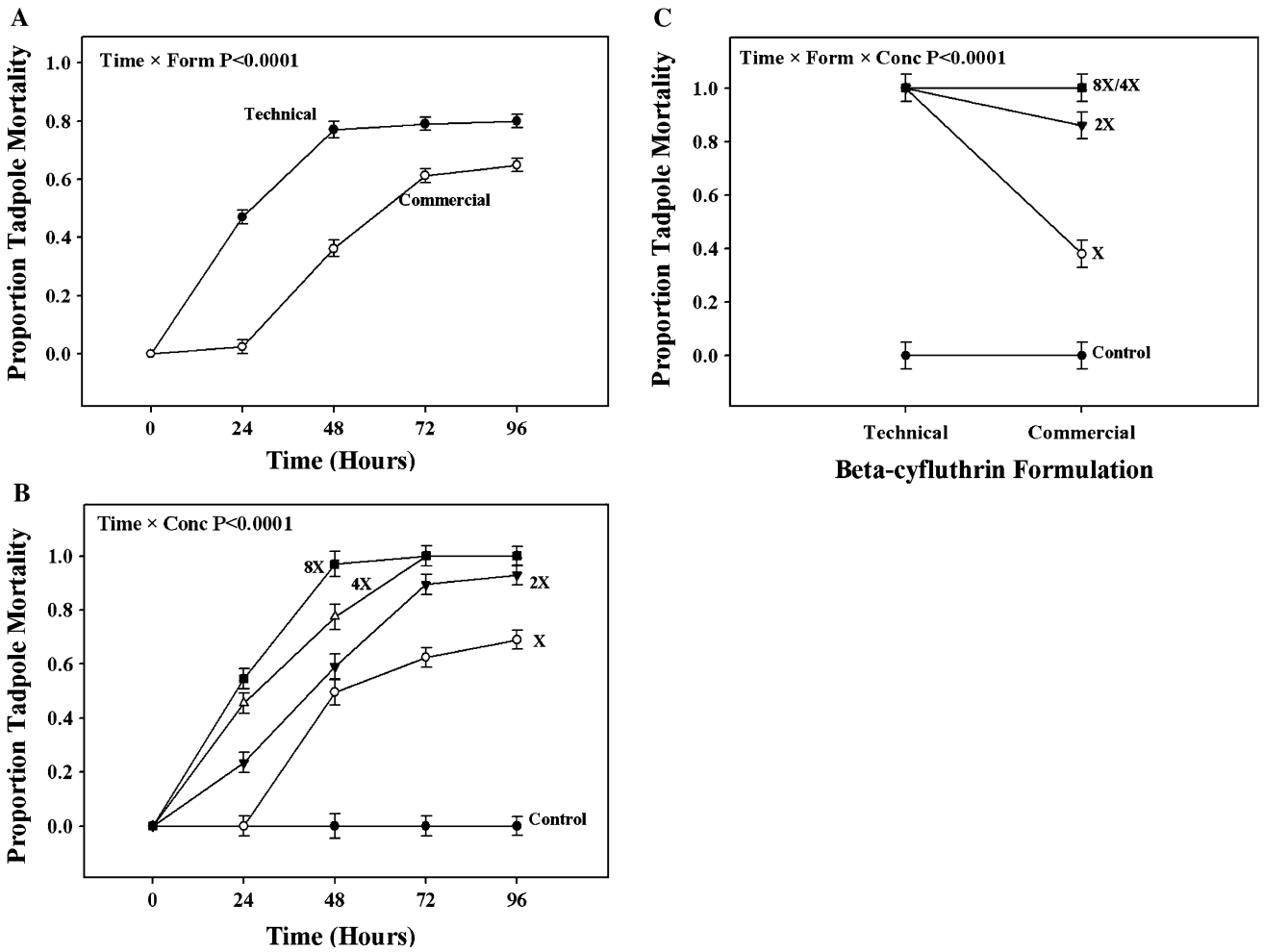

Fig. 4 Effect of **a** technical vs. commercial formulation of β-cyfluthrin and **b** β-cyfluthrin concentration over time on mortality of green frog tadpoles. **c** Interactive effect of β-cyfluthrin formulation and concentration on green frog tadpole mortality at 96 h. Error bars represent ±1 SE. β-Cyfluthrin exposures from control to high were 0, 7.5, 15, 30, and 60 µg/L and are labeled Control, 1×, 2×, 4×, and 8×, respectively

2005). This suggests that direct toxicity to permethrin may pose a smaller threat to natural populations. Technical β-cyfluthrin showed a relatively high mortality within the first 48 h; however, mortality was similar between technical and commercial at 96 h. This difference in formulation may be more important depending on the half-life; in alkaline systems it is short-lived, while in acidic or neutral systems it is more persistent (Gupta and Gajbhiye 2005). If technical formulations are slightly or greatly more toxic than commercial formulations in general, this may be less of a concern for nontarget wildlife that will more likely be exposed to commercial formulations. However, it suggests that assessing species with technical versus commercial formulations can potentially yield very different results and undermines our ability to predict the contaminants effect in more natural systems.

So while it is significant that commercial formulations were more toxic for many of the pesticides that we tested, it is also important to consider if these concentrations were likely to have direct effects on natural populations. The two highest concentrations of commercial malathion led to 35%–100% mortality. However, the 4× concentration (4 mg/L) is more than double the estimated postapplication rate for shallow water of 1.6 mg/L (Relyea 2004), which suggests that malathion may pose limited risk for amphibian populations. Animals exposed to the 8 × dose (5 mg/L) of commercial glyphosate also experienced a high mortality. Given that the expected environmental concentration is 1.43 mg/L (Wojtaszek et al. 2004), a concentrations at which we did not see any toxicity in our study, glyphosate may only pose a risk to amphibian populations if applied directly to surface waters to control aquatic plants. Tadpoles exposed to 4× (75 mg/L) and 8× (150 mg/L) concentrations of commercial imidacloprid experienced a very high mortality (~78%–100%). However, imidacloprid may only reach a concentration of about

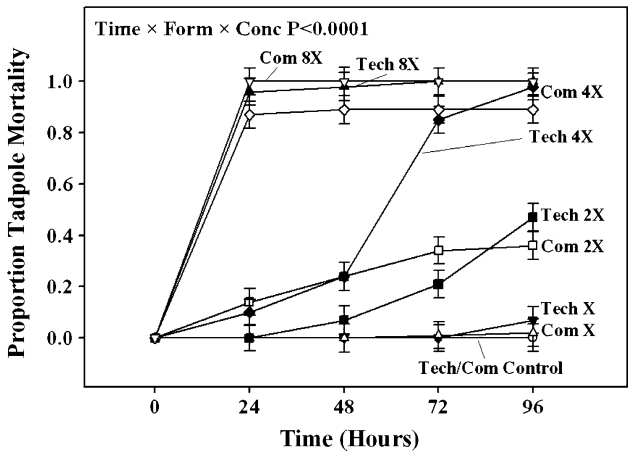

Fig. 5 Interactive effect of bifenthrin formulation and concentration over time on green frog tadpole mortality. Error bars represent ±1 SE. Bifenthrin exposures from control to high were 0, 0.125, 0.25, 0.5, and 1 μg/L and are labeled Control, 1×, 2×, 4×, and 8×, respectively

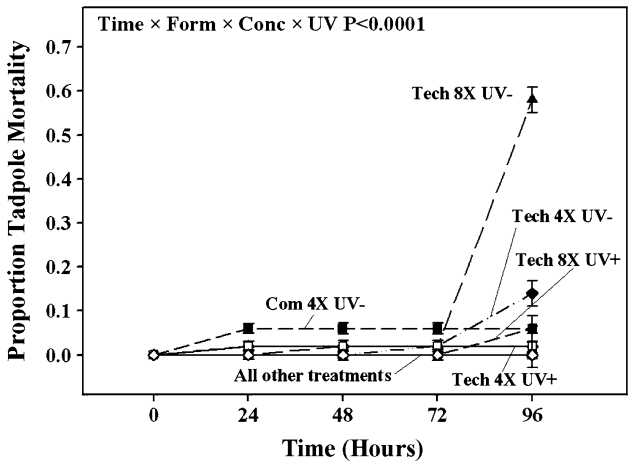

Fig. 6 Interactive effect of permethrin formulation, concentration, and UV exposure over time on green frog tadpole mortality. Error bars represent ±1 SE. Permethrin exposures from control to high were 0, 2.5, 5, 10, and 20 μg/L and are labeled Control, 1×, 2×, 4×, and 8×, respectively

22 μg/L in a 2-m-deep pond with direct application (SERA 2005), suggesting that imidacloprid may pose little risk to natural green frog populations. It is also worth noting that, in nature, organisms may experience adverse effects to these concentrations because they can be exposed for much longer than the 96 h we used in this study and may also experience repeated exposures (Relyea and Diecks 2008). Additionally, most expected environmental concentrations in the field are below the lethal effects measured in the laboratory, as we tested here, but sublethal concentrations can also have impacts on survival and fitness by altering the food web in terms of predator-prey interactions and abundance of food resources (Boone 2008; Relyea and

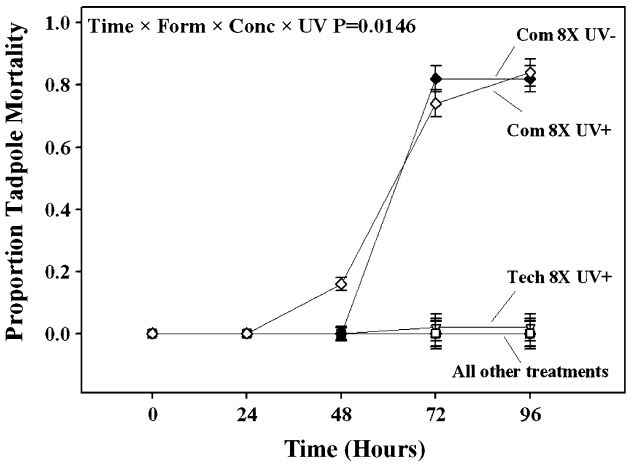

Fig. 7 Interactive effect of glyphosate formulation, concentration, and UV exposure over time on green frog tadpole mortality. Error bars represent ±1 SE. Glyphosate exposures from control to high were 0, 0.625, 1.25, 2.5, and 5 mg/L and are labeled Control, 1×, 2×, 4×, and 8×, respectively

Diecks 2008); therefore, although field concentrations may be sublethal, their effects could still have significant impacts on communities.

Natural factors, like UV radiation, have the potential to interact with contaminants and alter their toxicity but these factors are not often included in basic toxicity tests. We found that UV altered effects on mortality in four of seven pesticides (carbaryl, imidacloprid, glyphosate, and permethrin) over time. The levels of UV radiation used in this study were not high enough to be lethal to green frogs (Tietge et al. 2001) so there were no differences in mortality between pesticide control animals exposed to UV present and those who received filtered UV treatments. UV is known to interact with some chemicals to enhance toxicity, such as carbaryl (Zaga et al. 1998), because it increases the rate of breakdown into by-products that are more toxic than the original chemical (Tilak et al. 1981). We also found that carbaryl was more toxic in the presence of UV, particularly at 4× and 8× concentrations. Other studies have found glyphosate and imidacloprid to be photolytic (Lund-Høie and Friestad 1986; Wamhoff and Schneider 1999), so the breakdown products of these pesticides may also be more toxic. However, we did not see large differences in mortality between UV treatments for either pesticide. UV can also make contaminants less toxic by breaking chemicals down more rapidly into less toxic forms. We found that technical formulations of permethrin were less toxic in the presence of UV, suggesting that UV increased the breakdown of this insecticide into less toxic forms. Permethrin is moderately photolytic in water (Laskowski 2002). Increased rates of mortality in the filtered UV treatments of technical permethrin suggest that UV helps break it down into less toxic forms. Permethrin has a photolytic half-life in

pond water of 17.3–31.5 h (Rawn et al. 1982), suggesting that tadpoles with filtered UV would be exposed to greater levels of permethrin, which is supported by the higher mortality rates in filtered UV treatments. In natural systems with high dissolved organic carbon, however, the anticipated effects of UV may not be seen because high levels of dissolved organic carbon are known to attenuate UV-A and UV-B levels (Morris et al. 1995). For example, Bridges and Boone (2003) found than UV did not enhance the toxicity of carbaryl in pond mesocosms, as had been found in a laboratory study (Zaga et al. 1998), but instead both UV and carbaryl positively affected populations of green frogs. The authors suggest that carbaryl positively affected tadpoles because carbaryl eliminated zooplankton and thus increased the algal food resources (Bridges and Boone 2003). Therefore, taking into account the water system where exposure may occur is also important.

For all seven chemicals we tested, there was a significant difference in mortality between formulations. This argues for greater access to information regarding "other ingredients" used by manufacturers, so that independent research labs can test the toxicity of these ingredients. Additionally, this outcome suggests a need to test typical end use products on nontarget organisms, given that the effects can vary largely. In some cases, the presence or absence of UV altered the toxicity and identifies a need to incorporate natural stressors in the standard testing methods required for a pesticide to be registered by the EPA, which would provide a better understanding of the potential adverse effects of pesticides.

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