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Comparative Toxicity of Guthion and Guthion 2S to *Xenopus laevis* and *Pseudacris regilla* Tadpoles

G. S. Schuytema, A. V. Nebeker, W. L. Griffis

U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, 200 SW 35th Street, Corvallis, Oregon 97333, USA

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The U.S. Environmental Protection Agency is developing water quality criteria for the protection of wildlife species (amphibians, reptiles, birds, mammals) to expand existing criteria currently based only on fish and other aquatic life. Criteria for only a few chemicals (DDT, PCBs, mercury, selenium) include wildlife data (Williams et al. 1989). Water quality criteria data based on the sensitivity of amphibians to potentially hazardous chemicals in the environment are needed (Schuytema et al. 1991; 1993). The development of a water quality data base for amphibians should also take the formulation of the pesticide into consideration.

Guthion (azinphosmethyl) is a widely-used organophosphate pesticide. Over 520,000 kg active ingredient (AI) were used in the United States on fruit crops and cotton in the major producing states in 1991 (USDA 1992a, 1992b). The large quantities of Guthion used in Louisiana sugar cane plantations can potentially enter surrounding wetlands and have adverse effects on commercially important crayfish populations (Sklar 1985). Similarly, direct application and associated run-off has the potential for adversely affecting non-target amphibian populations. There is little evidence to indicate Guthion would cause adverse effects through the food chain (USEPA 1986). Hall and Kolbe (1980) suggested, however, that based on their test results and the resistance of amphibians to cholinesterase inhibitors that a number of organophosphate pesticides may be concentrated to varying degrees and thus may represent a hazard to amphibian predators.

The purpose of this study was to evaluate mortality and growth in Xenopus laevis (African clawed frog) and Pseudacris regilla (Pacific Treefrog) tadpoles exposed to technical and formulation grades of Guthion, a representative organophosphate pesticide.

MATERIALS AND METHODS

Xenopus laevis (Daudin) tadpoles were raised from eggs obtained from a breeding colony at the Environmental Research Laboratory - Corvallis and were 2 wk old at the time of testing. They had been

Correspondence to: G. S. Schuytema

maintained at a temperature of 23 °C, a light-dark cycle of 16:8 hr, and had been fed dried ground Oregon Moist fish food pellets ad libitum. Pseudacris (= Hyla) regilla (Baird and Girard) tadpoles, 3 wk old at the time of testing, were raised from locally collected eggs and had been held in flowing 15 to 17 °C well water under the same light regime. They had been fed a slurry of microwaved lettuce, ground rabbit pellet, the alga Selenastrum and brine shrimp ad libitum. The P. regilla tadpoles were slowly raised to test temperature in the 24 hr prior to testing.

Test water was obtained from wells near the Willamette River at Corvallis, Oregon. Dissolved oxygen and pH were measured daily by electrode. Total hardness, alkalinity and conductivity were determined by USEPA Methods Nos. 130.2, 310.1 and 120.1, respectively, prior to the start of each test (USEPA 1979). Mean water quality parameters during testing were: dissolved oxygen, 7.5 mg/L; hardness, 37.2 mg/L as $CaCO_3$; alkalinity, 34.6 mg/L as $CaCO_3$; conductivity, 103.2 uS; median pH , 7.3. Water temperature was maintained at 23 ± 1 °C for the Guthion tests and at 24 ± 1 °C for the Guthion 2S tests.

X. laevis and P. regilla tadpoles were exposed to Guthion and Guthion 2S in 1,000-mL beakers containing 400, 500, or 1,000 mL of solution (Table 1). Initial tadpole biomass loading of the test vessels ranged between 0.41 to 0.48 g/L. All tests were static with daily renewal of exposure solutions; testing procedures followed standard procedures as guidelines (ASTM 1980). The tests were conducted in an environmental chamber and kept on a 16:8 light:dark cycle. The concentration of the dimethyl formamide carrier in all test solutions, including the carrier controls, was 100 ppm for Guthion Tests 1 and 2 and Guthion 2S Test 1, 50 ppm for Guthion 2S Test 2 and 37.5 ppm for Guthion 2S Tests 3 and 4. Each test was also run with a carrier-free control. Stocks of Guthion and Guthion 2S were prepared at the beginning of each test and were analyzed daily. No tadpoles were fed during the 4-d exposures, but P. regilla were fed 1% of their body weight/day of ground rabbit pellet during the last four days of the 8-d tests. Dead organisms were removed daily and mortality recorded. Survivors were euthanized with MS-222 (methane tricaine sulfonate, Sigma, St.Louis, Missouri).

The percent purity of the technical grade Guthion (O,O-Dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4-H)-yl)methyl]phosphorodithioate)was 99% (Chem Service Inc., West Chester, Pennsylvania). Guthion 2S was purchased from Mobay Corp., Kansas City, Missouri, and contained 22% active ingredient. Measured concentrations were analyzed from a 110-mL pooled water sample comprised of equal volumes taken from each stock bottle or replicate test beaker. The samples were extracted for Guthion with toluene via liquid-liquid extraction in a serum bottle on a reciprocating shaker (Henderson et al. 1977). Samples were analyzed on a Model 5890 Hewlett-Packard high resolution gas chromatograph (GC) with a 25 meter SE-54 column. The GC was operated in the split mode with a nitrogen-phosphorus flame ionization gas detector. Approximately 10% of the samples were run in duplicate. Several standards were analyzed at the beginning of

Table 1. Summary of static-renewal Guthion and Guthion 2S tests conducted with Xenopus laevis and Pseudacris regilla tadpoles

Test no.	Species	Age, wk	Days exposure	Test vol., mL	No. test conc.	Test[a] range mg/L	Rep./ conc.	Tadpoles/ rep.
Guthion								
1	Xenopus	2	4	400	5	0.02-8.70	2	12
2	Pseudacris	3	8	500	5	0.01-9.67	2	10
Guthion 2S								
1	Xenopus	2	4	500	7	0.02-11.40	1	10
2	Xenopus	2	4	500	5	0.25-1.41	3	12
3	Pseudacris	3	8	1,000	6	0.09-2.78	3	10
4	Pseudacris	3	4	1,000	5	0.09-1.44	4	6

[a] Based on measured active ingredient

an analytical run, with a single standard analyzed after every five samples. The detection limit for Guthion in water was 10 ug/L. The specific recovery of Guthion was 102.3% ± 0.8 (mean ± SE, n = 54). The mean coefficient of variation for paired duplicate samples was 4.8 (n = 56 pairs). Mean percent loss of Guthion in the test vessels over each 24-hr period was less than 10%.

All Guthion and Guthion 2S concentrations were expressed in terms of measured active ingredient. Data from replicates were pooled prior to calculating LC50s and 95% CI by the trimmed Spearman-Karber method (Hamilton et al. 1977). LOAEL (Lowest Observed Adverse Effect Level) and NOAEL (No Observed Adverse Effect Level) values based on mortality were determined by Dunnett's multiple comparison procedure (Computer Sciences Corporation 1988). Percentage mortality data were adjusted with an arc sine square root transformation prior to calculating LOAELs and NOAELs. Carrier controls were used in all comparisons with different exposure concentrations of Guthion. Guthion water exposure concentrations were calculated as the mean of the total number of daily exposure values (a daily exposure value was the average of the added measured stock solution at the start, and the measured test container sample at the end of each 24-hr period).

RESULTS AND DISCUSSION

X. laevis was slightly more sensitive than P. regilla to Guthion. The X. laevis 4-day LC50 was 2.94 mg/L in Guthion Test 1 as compared to 4.14 mg/L for P. regilla in Guthion Test 2 (Table 2). The Guthion 4-day NOAEL for X. laevis (0.34 mg/L) was approximately 5 times smaller than the 8-day NOAEL for P. regilla (1.78 mg/L), also indicating a higher sensitivity for X. laevis (Table 3). The 8-day LC50 for P. regilla (2.77 mg/L) was about the same as the 4-day X. laevis value. The mortality pattern at 4 d was very similar for X. laevis and P. regilla exposed to Guthion in tests 1 and 2, respectively. Both species had survived completely at 0.35 mg/L but

exhibited 100% mortality at 8.7 to 9.7 mg/L. The test with P. regilla was continued for an additional 4 d. Carrier-free and carrier control mortality in all of the X. laevis tests was never more than 5.5%; control mortality in all of the P. regilla tests was never more than 4.1%.

Table 2. LC50 values (active ingredient) for Xenopus laevis and Pseudacris regilla tadpoles exposed to Guthion and Guthion 2S

Test no.	Species	Days exposure	LC50 (95% CI), mg/L
Guthion 1	Xenopus laevis	4	2.94 (2.30-3.77)
Guthion 2	Pseudacris regilla	4	4.14 ([a])
		8	2.77 (1.82-4.22)
Guthion 2S 1	Xenopus laevis	4	0.59 (0.43-0.80)
Guthion 2S 2	Xenopus laevis	4	0.42 (0.38-0.46)
Guthion 2S 3	Pseudacris regilla	4	0.84 (0.72-0.97)
		8	0.76 (0.64-0.91)
Guthion 2S 4	Pseudacris regilla	4	0.46 (0.39-0.55)

[a] CI not calculable

The Guthion 4-day acute toxicity results obtained in the present study for X. laevis (LC50 of 2.94 mg/L) and P. regilla (LC50 of 4.14 mg/L) are very close to the 3.2 mg/L reported by Mayer and Ellersieck (1986) for Pseudacris triseriata. The 4-day LC50s of 0.109 mg/L (Sanders 1970) and 0.13 mg/L (Mayer and Ellersieck 1986) reported for Bufo woodhousii fowleri are 22-38 times lower than the values obtained for the frogs tested in the present study. Mulla et al. (1963) reported Guthion had no effect on Bufo boreas and Scaphiopus hammondi tadpoles at an application rate of 0.45 kg AI/ha. Meyer (1965) exposed Rana catesbeiana tadpoles to 1.0 mg AI/L Guthion for 48 hr with no effect. Guthion and Guthion 2S 4-day LC50 values for Xenopus laevis embryos (Schuytema et al. 1994) were 2 to 4 times higher than the present tadpole values, the reduced sensitivity perhaps due to a less well developed nervous system in embryos.

Based on mortality at 4 d, X. laevis and P. regilla exhibited similar sensitivities to Guthion 2S. There was 100% mortality for X. laevis at 1.3 and 0.8 mg/L, respectively, in Guthion 2S Tests 1 and 2; in Guthion 2S Tests 3 and 4 with P. regilla, 100% of the tadpoles were dead at 1.4 to 1.5 mg/L. The 4-d NOAEL values also indicated a fairly similar response by X. laevis (0.25 mg/L) and P. regilla (0.36-0.37 mg/L) to Guthion 2S (Table 3). The Guthion 2S 4-d LC50s for X. laevis ranged from 0.42 to 0.59 mg/L and from 0.46 to 0.84 mg/L for P. regilla (Table 2). The relative similarity of response of the two species to the two forms of Guthion suggest that in some cases X. laevis, a common laboratory animal, may be a suitable surrogate for wild populations such as P. regilla.

Table 3. NOAEL and LOAEL (active ingredient) values for Xenopus laevis and Pseudacris regilla tadpoles exposed to Guthion and Guthion 2S

Test no.		Days exposure	NOAEL[a] mg/L	LOAEL[b] mg/L
Guthion 1	Xenopus laevis	4	0.34	1.72
Guthion 2	Pseudacris regilla	8	1.78	9.67
Guthion 2S 2	Xenopus laevis	4	0.25	0.49
Guthion 2S 3	Pseudacris regilla	4	0.37	0.79
		8	0.37	0.79
Guthion 2S 4	Pseudacris regilla	4	0.36	0.70

[a] NOAEL = No observed adverse effects level (based on mortality)
[b] LOAEL = Lowest observed adverse effects level (based on mortality)

Both species were more sensitive to Guthion 2S than to Guthion. The X. laevis 4-d LC50 values were 5-7 times lower in frogs exposed to Guthion 2S (0.42-0.59 mg/L) than for those exposed to Guthion (2.94 mg/L) (Table 2). Similarly, 4-d LC50 values were 5-9 times lower in P. regilla tadpoles exposed to Guthion 2S (0.46-0.84 mg/L) than for those exposed to Guthion (4.14 mg/L) (Table 2). The NOAEL values (Table 3) indicated P. regilla to be about 5 times more sensitive to Guthion 2S (0.36-0.37 mg/L) than Guthion (1.78 mg/L). After 8 d exposure, 100% of the P. regilla tadpoles exposed to Guthion 2S (Guthion 2S Test 3) at 1.54 mg/L were dead, whereas in Guthion Test 2 at 8 d, 100% mortality did not occur until exposure to 9.7 mg/L Guthion. While the higher temperature used in the Guthion 2S tests (1 ^{o}C increase) may have caused some of the increased toxicity of Guthion 2S as compared to Guthion, it was very unlikely responsible for the entire increase.

The criterion value for Guthion of 0.01 ug/L (USEPA 1986) would appear to protect frogs since the lowest 4-d NOAELs for X. laevis and P. regilla based on Guthion mortality were 34,000 to 178,000 times greater.

The increased toxicity of Guthion 2S (based on active ingredient) as compared to the technical grade material illustrates the importance of including pesticide formulations in bioavailability and risk assessments, as "inert" ingredients can exert significant additional toxicity. Linder et al. (1990) noted a three-fold increase in acute toxicity to Rana pipiens for a commercial formulation of paraquat as compared to the technical grade, and emphasized the need to evaluate commonly available pesticide formulations since they may be the most frequently encountered forms in the environment. Mayer and Ellersieck (1986) studied the effects of formulation on the toxicity of water-borne chemicals and noted that the effects of "inert" ingredients may be as high as 2.5 orders of magnitude, and recommended further study of the toxicology of formulations.

Amphibians are widespread and important prey species for other wildlife such as birds, mammals and fish. They may also be sensitive indicators of environmental contamination because of exposure in both aquatic and terrestrial environments. The present study adds to the data base necessary for making informed decisions on the formulation of criteria protective of wildlife.

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