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

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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
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 CaCO₃; alkalinity, 34.6 mg/L as CaCO₃; conductivity, 103.2 μS; 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