Bull. Environ. Contam. Toxicol. (1990) 44:629–635

Bulletin of Environmental Contamination and Toxicology

Accumulation, Metabolism and Toxicity of Parathion in Tadpoles

Russell J. Hall

U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland 20708, USA

Earlier work exposing tadpoles to organophosphorus pesticides (Hall and Kolbe 1980) indicated the great resistance of tadpoles of the bullfrog (Rana catesbeiana) to these chemicals and their surprising ability to accumulate parathion and fenthion from water. These qualities seemed to make them an ideal model with which to test a hypothesis advanced by Burke and Ferguson (1969), who noted that parathion is more toxic to resistant mosquitofish in static water than in flowing water--a reversal of the pattern normally seen. They believed that the highly toxic metabolite paraoxon was produced by the fish and that its buildup in static systems resulted in the unexpected mortality. Amphibians have been shown to produce paraoxon (Potter and O'Brien 1964) and to accumulate the parent compound parathion to levels that are potentially hazardous to other organisms (Hall and Kolbe 1980). Significant accumulation of paraoxon might not only confirm Burke and Ferguson's hypothesis, but also could signal cause for concern about the environmental hazard this chemical might pose in aquatic food chains. In the course of examining paraoxon production by tadpoles, it would also be possible to learn more about their patterns of parathion uptake and elimination. Retention of residues is also a matter of concern, given the high levels observed in the earlier studies.

MATERIALS AND METHODS

Tadpoles were collected from the field at the Patuxent Wildlife Research Center. A first series of tests was conducted with R. catesbeiana tadpoles to examine accumulation and loss of residues, and a second examined toxicity of parathion in the same system with tadpoles of the green frog (R. clamitans). Six different exposure chambers, two static and four flow-through, were used in each test (Table 1). Containers for static systems each had 4 L of test water and differed only in that one had twice the number of tadpoles as the other. Well water was regulated in a pH range of 7.2 to 7.5 and averaged 100 mg/L hardness as $CaCO_3$; temperature was maintained at 20°C. For flow-through systems, technical grade parathion (o,o-diethyl-o-p-nitrophenyl phosphorothioate, 98.5%, Monsanto Chemical Company, St. Louis, Missouri) in acetone was injected into water by a single apparatus (Hall and Swineford 1980) and distributed to

test chambers through calibrated siphons at 0.5 or 5 mL/min. Each test chamber had a static volume of 4 L and each contained an equal number of tadpoles. Test chambers were arranged serially at each rate of flow so that a lower tank received water that had flowed through the tank above it. Thus, the water in each lower (2X) chamber was exposed to twice as many animals as that in the chamber (1X) above it, effectvely doubling the loading.

Table 1. Experimental Design.

Effective Loading[1]	Method of Exposure		
	Static	Low Flow	High Flow
1X	30 animals (51 g) in 4 L. Not renewed	30 animals (53 g) in 4 L renewed at 0.5 mL/min.	30 animals (47 g) in 4 L renewed at 5 mL/min
2X	60 animals (101 g) in 4 L. Not renewed	30 animals (57 g) in 4 L renewed at 0.5 mL/min by flow from 1X tank	30 animals (50 g) in 4 L renewed at 5 mL/min by flow from 1X tank

1 Biomass of test subjects to which each unit volume of water was exposed.

Water samples (100 mL) were collected after flow-through systems had been filled and flushed several times with dosed water and immediately before animals were introduced. In the first series of tests there were 30 R. catesbeiana tadpoles in each test chamber, 60 in the doubly loaded static system. Intended parathion concentration was 1 mg/L. Exposure continued for 96 hr and observation for another 18 d after parathion injection ceased, but water flow continued. Water was collected from each test chamber at 24, 48, 72, and 96 hr and saved for chemical analysis. Ten animals each were collected from the chambers at the end of exposure and at 4 and 18 d after cessation of parathion injection into water. In the second series of tests, R. clamitans in groups of 20 were used at rates of 10 or 20 animals per L of static volume. Exposure to parathion at 5 ppm continued for 24 hr, with observations for signs of toxicity continuing an additional 24 hr.

Water samples were refrigerated and within 1 wk of collection were extracted three times with methylene chloride and

concentrated to 10 mL on a rotary evaporator. A gas chromatograph with a flame photometric detector and a 5% OV-101 column at 200°C or a 1.5% OV-1711.95% QF-1 column at 195°C were used, depending on the quantity of residues present. These gave lower limits of reportable residues of 0.001 mg/L for parathion and paraoxon. Aminoparathion was identified on the basis of its mass spectrum, but not quantified. When large amounts of parathion were present, the lower limit of reportable residues for paraoxon was 1.0 mg/L. Additional compounds, probably metabolites of parathion, were detected by gas chromatography but not characterized. Average recoveries from spiked samples were 91% for parathion and 98% for paraoxon. Tadpole samples were extracted three times with hexane, concentrated on a rotary evaporator and analyzed with a gas chromatograph with a flame photometric detector and a 1.5% SP-2250/1.95% SP-2401 column at 200°C. Lower limits of reportable residues were 0.01 ppm for parathion and 0.5 ppm for paraoxon. Average recoveries were 93% for parathion and 96% for paraoxon. Residues in 10 water samples and one tissue sample were confirmed by gas chromatography/mass spectrometry.

RESULTS AND DISCUSSION

There was no mortality of tadpoles exposed to 1 mg/L parathion in the first series of tests. Parathion and related compounds in test water are shown in Table 2. A pattern of decreasing parathion with time can be seen in the unrenewed static systems. Paraoxon increased in the water with fewer animals as parathion decreased. Both parathion and paraoxon decreased after 48 hr in the chambers with greater loading. Presence of other metabolites increased with time and with parathion depletion. Parathion tended to increase in the flowing water systems, showing a greater overall tendency for increase in containers with lower loading and higher rate of renewal. At 96 hr it had attained as much as 2.2 times the intended 1 mg/L level and as much as 3.3 times the initial measured concentration. This increase appeared to be correlated with the observed increase in organic debris accumulating in the test water.

The failure of paraoxon to appear in measurable quantities in flowing water systems is not unexpected as its relatively great solubility would tend to remove it with the overflow water. Poor solubility of parathion in water probably explains its accumulation in the flow-through tests. It would have greater affinity for organic materials than for water and would adhere to animals, fecal material and molted skin in the test containers. As testing progressed, greater amounts of suspended debris and of adhering pesticide would be collected with water samples.

Residues of parathion in tadpoles after 96 hr of exposure and at 4 and 18 d after cessation of parathion injection are shown in Table 3. Concentrations in tadpoles were correlated with both loading and with the rate of flow, being lowest in static systems

Table 2. Parathion and related compounds in water in which tadpoles were exposed to a nominal 1 mg/L for 96 hr.

Exposure	Loading	Compounds Detected	Time (hr) 0	24	48	72	96
Static	1X	parathion	0.52	0.16	0.11	0.080	0.058
		paraoxon	-[1]	-	trace	0.042	0.060
		aminoparathion	-	-	-	-	-
		unidentified[2]	-	-	2	2	4
	2X	parathion	0.59	0.057	0.026	0.011	0.005
		paraoxon	-	0.042	0.062	0.024	0.016
		aminoparathion	-	+	-	-	-
		unidentified	-	-	-	2	-
0.5 mL/min	1X	parathion	0.70	0.48	0.57	1.2	1.8
		paraoxon[3]	-	-	trace	-	-
		aminoparathion	-	-	+	-	-
		unidentified	-	-	-	-	-
	2X	parathion	0.83	0.31	0.34	0.70	0.89
		paraoxon[3]	-	-	-	-	-
		aminoparathion	-	+	+	-	-
		unidentified	-	-	2	-	-
5 mL/min	1X	parathion	0.66	0.98	0.84	1.2	2.2
		paraoxon[3]	-	-	-	-	-
		aminoparathion	-	-	-	-	-
		unidentified	-	-	-	-	-
	2X	parathion	0.61	0.57	0.39	0.76	0.99
		paraoxon[3]	-	-	-	-	-
		aminoparathion	-	-	-	-	-
		unidentified	-	-	-	-	-

1 - = not detected; + = present, but not quantified
2 numbers shown are the number of compounds detected
3 the detection limit for paraoxon was 1 mg/L in these tests

and generally highest in chambers receiving the high rate of flow. Detectable residues of parathion (>0.01 ppm) had disappeared at 4 d in tadpoles in static systems, but surprisingly high residues remained in those from flowing water systems after 18 d. Bioconcentration factors at 96 hr varied from 33.6 to 75.9 and averaged 54.6; there was little evidence of a pattern in bioconcentration among the treatments. No metabolites were detected in tadpole samples.

Accumulation of parathion by tadpoles was similar to that reported earlier (Hall and Kolbe 1980). The unexpected persistence of residues is probably explainable by feeding

Table 3. Residues (ppm) of parathion in tadpoles at intervals after exposure to 1 mg/L parathion, analyzed as pools of 10 animals each.

Exposure	Loading	Time in Clean Water (d) 0	4	18
Static	1X	4.4	-	-
	2X	0.24	-	-
0.5 mL/min	1X	74	30	3.9
	2X	49	2.6	0.51
5 mL/min	1X	74	78	11
	2X	73	1.8	0.53

- = not detected.

habits of tadpoles and the adherance of parathion to organic material noted above. Tadpoles may ingest detritus or rasp algae or slime from surfaces (Wassersug 1980). Animals with the most persistent residues were in the chambers that first received inputs of parathion. Parathion probably accumulated in them by adhering to surfaces from which it was subsequently ingested by tadpoles. This phenomenon may be regarded an an artifact of the test systems, but similar effects can be envisioned in nature where flowing water and surfaces attractive to poorly soluble chemicals abound. Accounting for parathion adhering to surfaces and available for ingestion during this study, it seems likely that residues in tissues are essentially depurated in 4 d or less.

Mortality of tadpoles exposed for 24 hr to a nominal 5 mg/L parathion in the second series of tests is shown in Table 4. All mortality was restricted to treatments with the higher rate of flow or lower rates of loading. Total amounts of parathion to which tadpoles were exposed, calculated as the total amount of water used times measured concentrations of parathion, are 6.4 mg and 6.8 mg in the static systems, 10.9 mg in the 0.5 mL/min systems, and 38 mg in the 5 mL/min systems. In the static systems, each tadpole removed approximately 0.32 mg in 24 hr. The probable exposure in the 2X containers is therefore about 4.5 mg in the 0.5 mL/min systems and 32 mg in the 5 mL/min systems. The relationship between calculated exposure and mortality is shown in Fig.1.

Paraoxon may be 3 to 25 times as effective as parathion in inhibiting cholinesterase in mammalian tissue <u>in vitro</u> (Davies and Holub 1983). The mechanism of toxicity seems to be similar

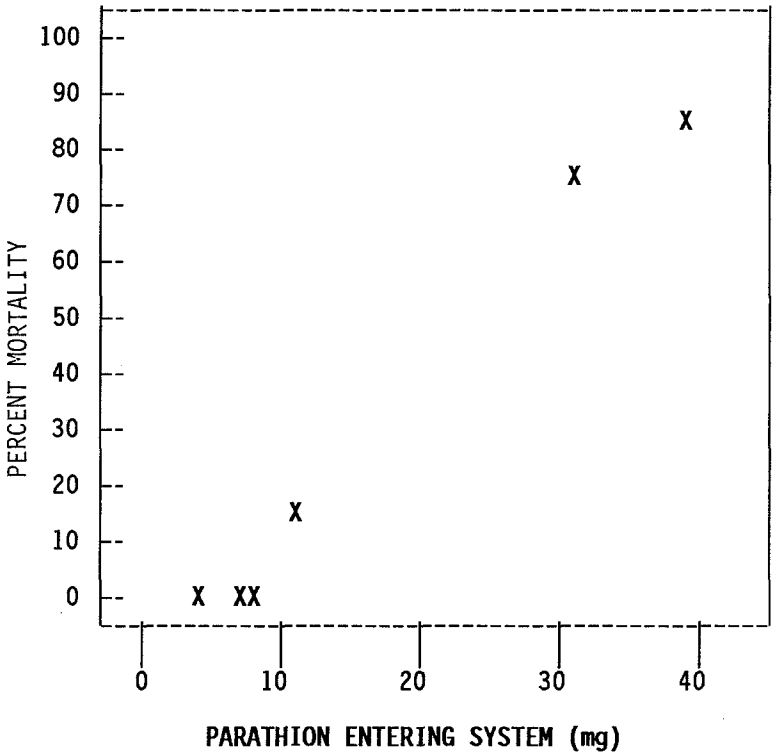

Figure 1. Relationship between the total amount of parathion calculated to have entered test chambers and percent mortality of tadpoles.

Table 4. Percent mortality of tadpoles exposed to 5 mg/L parathion for 24 hr.

Loading	Static	Exposure 0.5 mL/min	5 mL/min
1X	0	15	85
2X	0	0	75

in all vertebrates and thus it could be as much as 25 times as toxic to tadpoles as the parent compound, if one does not account for differences in distribution in aquatic systems due to its physical properties. The results of this study indicate that mortality of tadpoles does not begin until levels of parathion approach 5 mg/L; the maximum level of paraoxon observed in static systems, 0.062 mg/L would produce the equivalent toxicity of only 1.5 mg/L parathion even if its effects were multiplied the full 25 times. Therefore, one would not expect ambient paraoxon in

the relative amounts seen to contribute significantly to toxicity. Paraoxon levels reported earlier (Hall and Kolbe 1980) appear to have been in error, owing to significant interference from the metabolite aminoparathion.

Mortality in these experiments is explainable wholly on the basis of calculated exposure to parathion. No influence of ambient paraoxon is evident. Frog brain cholinesterase is remarkably resistant to cholinesterase inhibitors, however, being 100 times as resistant to paraoxon as chicken brain and 3 times as resistant as catfish brain (Wang and Murphy 1982). LC50 values for parathion in flow-through tests for aquatic invertebrates and for fish are 0.03 and 0.3 times the probable value for R. clamitans tadpoles (Mayer and Ellersieck 1986). Therefore it is likely that paraoxon released by tadpoles in aquatic systems could enhance the toxicity of parathion under certain circumstances.

Acknowledgments. Chemical analyses were performed by Elizabeth J. Kolbe and Thair Lamont, both research chemists in the Patuxent Wildlife Research Center Section of Environmental Chemistry. Critical reviews were provided by W. J. Fleming and J. B. Hunn.

REFERENCES

Burke WD, Ferguson DE (1969) Toxicities of four insecticides to resistent and susceptible mosquitofish in static and flowing solutions. Mosquito News 29:96-101

Davies DB, Holub BJ (1983) Comparative effects of organophosphorus insecticides on the activities of acetylcholinesterase, diacylglyceral kinase, and phosphatidylinositol phosphodiesterase in rat brain microsomes. Pestic Biochem Physiol 20:92-99

Hall RJ, Kolbe E (1980) Bioconcentration of organophosphorus pesticides to hazardous levels by amphibians. J Toxicol Environ Hlth 6:853-860

Hall RJ, Swineford D (1980) Toxic effects of endrin and toxaphene on the southern leopard frog Rana sphenocephala. Environ Pollut (Ser A) 23:53-65

Mayer FL Jr, Ellersieck MR (1986) Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. US Fish Wildl Ser Resour Publ 160

Potter JL, O'Brien RD (1964) Parathion activation by livers of aquatic and terrestrial vertebrates. Science 144:55-57

Wang C, Murphy SD (1982) Kinetic analysis of species difference in sensitivity to organophosphate insecticides. Toxicol Appl Pharmacol 66:409-419

Wassersug RJ (1980) Internal oral features of larvae from eight anuran families: functional, systematic, evolutionary and ecological considerations. Misc Publ Mus Nat Hist Univ Kansas 68:1-146

Received July 28, 1989; accepted October 14, 1989.