Bull. Environ. Contam. Toxicol. (1984) 33:592–597

Bulletin of Environmental Contamination and Toxicology

Teratogenic and Lethal Effects of Paraquat on Developing Frog Embryos (*Rana pipiens*)

Norman A. Dial† and Cheryl A. Bauer

Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809; †Adjunct: Department of Anatomy, Indiana University School of Medicine, Terre Haute Center for Medical Education, Terre Haute, IN 47809

Paraquat (1,1'dimethyl-4,4'-bipyridinium dichloride) has been used for a number of years in the United States and other countries as a herbicide in the control of broadleaf weeds and grasses in crop fields, orchards, and berry patches. It is utilized as both a pre-emergence and post-emergence herbicide. Paraquat may also be used in aquatic weed control in some countries but not in the United States. The recommended field application rate is usually 0.25 to 1.0 lb. paraquat cation/acre. The rate of application for aquatic weed control ranges from about 0.1 to 2.0 parts per million (ppm), by weight in water (Newman 1970; Calderbank 1972).

Little is known of the effects of paraquat on embryonic development in aquatic animals. Murry and Schreiweis (1977) have shown teratogenic malformations in embryos of Oryzias latipes and Paulov (1977) has carried out developmental and body protein studies of the effects of paraquat in Rana temporaria. The present study was conducted to observe lethal and gross teratogenic effects of a range of concentrations of paraquat on Rana pipiens embryos, including concentrations at the recommended aquatic field application rates.

MATERIALS AND METHODS

Mature Rana pipiens females (Carolina Biological Supply Co.) were induced to ovulate and the eggs artificially inseminated by the method of Rugh (1962). Eggs were separated with scissors and allowed to develop to the 12-16 cell stage, at which time those developing normally were selected for stock embryos. This procedure resulted in a relatively high percentage of control and treated animals developing beyond the initial twenty-four hour period.

Eggs were reared in finger bowls containing 100 ml dechlorinated tap water and treated at early gastrula with 0, 0.1, 0.5, 2.0, and 10.0 parts per million (ppm). A glass plate was placed over each bowl to reduce evaporation. Three experimental runs were conducted, two using 30 embryos per bowl and one using 50 embryos (total 110 embryos per dose group). Runs were made on separate occasions with eggs from different females. However, all eggs within a particular run were from the same female. All embryos were maintained at 21±1°C. Solutions were changed in each bowl every other day until the experiment was terminated. Observations were made daily. Stage numbers referred to are those of Shumway (1940).

RESULTS AND DISCUSSION

Survivability and growth data are presented in table 1, and discussed below.

Eggs of R. pipiens were found to be very resistant to paraquat as development proceeded normally to hatch (day 4, stage 20) in all exposed groups. It was not until approximately 3 days post hatch that a significant (χ^2, 1 df, $p < 0.05$) increase in mortality was observed in all treated groups, with the exception of 0.1 ppm.

No tadpoles subjected to 2 and 10 ppm paraquat survived beyond 11 days after treatment (7 days post hatch), and survivability was very poor in 0.5 ppm with only 5.5% living to day 12 (χ^2, 1 df, $p < 0.01$). Considerable run variation was observed only in embryos treated at the 0.1 level. Survivability to day 12 at this level ranged from a low of 10% in one group, to a high of 86% in another (χ^2, combined, 1 df, $p > 0.05$). Control survivability was good with 75.5% living to day 12.

Growth rate was slowed in all treated groups, although only slightly so in embryos exposed to 0.1 ppm. A definite trend was observed between level of paraquat exposure and degree of retardation of growth. The maximum stage (Shumway 1940) of development attained by embryos declined as the dose level of paraquat increased. Some variation existed between runs in maximum stage reached by embryos in the respective groups. The stage given in table 1 represents the highest stage reached by the respective groups during the three runs. In one run, embryos exposed to 0.5, 2, and 10 ppm only reached stage 21, thus experiencing developmental arrest at day 5 (1 day post hatch).

A trend was also observed in maximum size attained by embryos exposed to the various dose levels of paraquat. Although development stage-wise was similar to controls in embryos treated with 0.1 ppm, embryos in stage 25 were only about 75% the size of controls. Surviving embryos treated with 0.5 ppm were only about 50% the size of controls at stage 25. No embryos treated 2 and 10 ppm reached stage 25, but maximum size of surviving embryos by day 10 (stage 23) was only about 40% that of controls.

A significant number of tadpoles exposed to levels of 0.5 ppm and above were observed to have abnormal tail development by day 5 (table 2; χ^2, 1 df, $p < 0.01$ for 0.5, 2, and 10 ppm). The most common tail abnormalities were, narrow margins, bent or drooped, and stunted growth (Fig. 1). Some tadpoles were observed to have poor head development (microcephaly), but this abnormality was not quantified.

Table 1. Effects of paraquat on survivability and growth of post-hatched *R. pipiens* embryos.[a]

Day[c]	Concentration (ppm)[b] 0		0.1		0.5		2		10	
5	101[d]	(21)[e]	103	(21)	99	(21)	102	(21)	103	(21)
6	100	(22)	103	(22)	96	(22)	92	(22)	100	(22)
7	99	(23)	89	(23)	85*	(23)	76**	(22)	87*	(22)
8	96	(24)	84	(23)	63**	(23)	36**	(23)	48**	(22)
9	94	(24)	83	(24)	36**	(23)	17**	(23)	14**	(23)
10	91	(25)	79	(24)	18**	(24)	8**	(23)	4**	(23)
11	85	(25)	76	(25)	11**	(24)	4**	(24)	0	-
12	83	(25)	76	(25)	6**	(25)	0	-	0	-
% live day 12	75.5		69.1		5.5		0		0	

[a] Days 1-4 were eliminated from table as eggs were found to be very resistant to paraquat.
[b] Number treated at each concentration = 110.
[c] Number of days after start of treatment (early gastrula); day 5 is day 1 post hatch.
[d] Number of surviving embryos.
[e] Growth stage number (Shumway, 1940; Stage 20 = hatch (day 4); Stage given is highest stage reached by the respective groups during the 3 runs.
* Indicates a significant difference at the 5% level, as compared to control of same age, chi-square test.
** Indicates a significant difference at the 1% level, as compared to control of same age, chi-square test.

Table 2. Some gross effects of paraquat on early frog development.

Level (ppm)	Abnormal tail day 5		Muscular response day 8		Muscular response day 12		Normal swimming behavior day 8		Normal swimming behavior day 12	
	N/S[a]	%	N/S	%	N/S	%	N/S	%	N/S	%
0	6/101	6	96/96	100	83/83	100	94/96	98	83/83	100
0.1	4/103	4	82/84	98	76/76	100	79/84	94	76/76	100
0.5	29/99*	29	30/63*	48	6/6	100	7/63*	11	6/6	100
2	48/102*	47	7/36*	19	0/0	-	0/36*	0	0/0	-
10	43/103*	42	14/48*	29	0/0	-	0/48*	0	0/0	-

[a]Number showing characteristic/number of surviving embryos.
*Indicates a significant difference at the 1% level, as compared to control, chi-square test.

Muscular response and swimming behavior appeared normal in nearly all embryos exposed to 0.1 ppm (table 2; χ^2, $p > 0.05$). Concentrations above this level, however, produced profound effects on motor ability. Only about 48% of surviving embryos exposed to 0.5 ppm showed any sign of muscular movement by day 8, and only 11% swam normally (χ^2, 1 df, $p < 0.01$, for both characteristics). No surviving embryos exposed to 2 or 10 ppm were observed to swim normally by day 8, but some exhibited limited muscular movement (table 2, χ^2, 1 df, $p < 0.01$ for muscular response at both levels).

Thus, after hatch (day 4), aquatic application levels of paraquat caused death, retardation of growth, significant levels of multiple tail malformations, poor head development, and produced profound effects on motor ability. Death and the aforementioned defects increased with time and increasing concentration levels.

It is well known that individual tolerances to pesticides exist and examples of this were observed, especially in embryos exposed to 0.5 ppm. Only 6 of the 110 treated at this level lived to day 12 (day 8, post hatch); all 6, surprisingly, appeared normal in all aspects, except size, including normal swimming behavior.

Variation between runs was observed in survivability of embryos treated at the 0.1 ppm level, and in maximum stage of development reached by embryos in the respective treated groups. As the three runs were conducted on separate occasions from different shipments of frogs, the observed variation between runs for the above probably reflects geographical tolerance differences to paraquat in R. pipiens.

Figure 1. Embryos treated for ten days. Left to right - control, 2 ppm, 0.5 ppm and 2 ppm. Figure demonstrates stunted growth and tail abnormalities observed in many treated embryos. Lower 1/3 of control tail not shown.

Paraquat is known to disappear from treated water quite rapidly due to its absorption and concentration by aquatic plants. Way et al. (1971) found that paraquat lake water residues were not detectable by 16 days after application of 0.5 ppm paraquat, and Yeo (1967) reported only traces of paraquat in water at the end of 12 days. Thus, the timing of paraquat application to aquatic ecosystems could be of utmost importance to R. pipiens, as well as other species.

Observations presented here indicate that natural populations of R. pipiens could be very seriously affected by aquatic application rates, as these rates usually range from 0.1 to 2.0 ppm (Newman 1970; Calderbank 1972). Survivability and stunted growth in Xenopus laevis embryos were observed to be similar to R. pipiens at concentrations reported here (Karen Buescher, pers comm). The authors plan to investigate the effects of diquat on R. pipiens early development. Diquat is chemically similar to paraquat and is currently in use in the United States for control of aquatic weeds.

Our studies and the considerable lack of information concerning paraquat effects on early development of other aquatic species suggest that the use of paraquat as an aquatic herbicide is suspect.

REFERENCES

Calderbank A (1972) Environmental considerations in the development of diquat and paraquat as aquatic herbicides. Outl Agr 7(2):51-54

Murray GJ, Schreiweis DO (1977) Teratogenic malformations in Oryzias latipes embryos treated with paraquat. J Ariz Acad Sci 12:41-42

Newman JF (1970) Ecological implications of the use of paraquat and diquat as aquatic herbicides. Chem Ind (London) 45:1424-1425

Paulov S (1977) Effect of the herbicide gramozone S paraquat dichloride on development and body proteins of amphibian, Rana temporaria. Biologia (Bratisl) 32(2):127-131

Rugh R (1962) Experimental embryology. Burgess, Minneapolis, 501 pp

Shumway W (1940) Stages in the normal development of Rana pipiens. Anat Rec 78:139-147

Way JM, Newman JF, Moore NW, Knaggs FW (1971) Some ecological effects of the use of paraquat for the control of weeds in small lakes. J Appl Ecol 8:509-532

Yeo RR (1967) Dissipation of diquat and paraquat, and effects on aquatic weeds and fish. Weeds 15(1):42-46

Received February 1, 1984; Accepted March 7, 1984