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Lethal Effects of Diquat and Paraquat on Developing Frog Embryos and 15-Day-Old Tadpoles, *Rana pipiens*

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Diquat (6,7-dihydrodipyrido [1,2-a:2',1'-c] pyrazinediium) and paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) are widely used in agriculture and in the control of aquatic weeds. The rate of application for aquatic weed control is similar for both herbicides and usually ranges from about 0.1 to 2.0 parts per million (ppm), by weight in water (Newman 1970; Calderbank 1972). Bimber and Mitchel (1978) reported that application concentrations of diquat as high as 5.0 ppm are used for aquatic weed control and that local concentrations may significantly exceed recommended dosages. Relatively little is known of the effects of diquat and paraquat on early development in aquatic animals. Murray and Schreiweis (1977) reported paraquat induced teratogenic malformations in embryos of the teleost Oryzias latipes. Crawford and Guarino (1985) found that fry of the teleost Fundulus heteroclitus exposed throughout development to paraquat were slightly lethargic, and that fry exposed in the same manner to diquat were lethargic, arched, and exhibited convulsive tremors upon stimulation. Paulov (1977) has carried out developmental and body protein studies of the effects of paraquat in the amphibian Rana temporaria, and Dial and Bauer (1984) have reported lethal and teratogenic effects in young developing Rana pipiens tadpoles after treatment, beginning at early gastrula, of paraquat concentrations as low as 0.5 mg/L. Bimber and Mitchel (1978), to our knowledge, are the only investigators to examine the effects of diquat on developing frog embryos, and they used a treatment level of 100 ppm diquat--which is far in excess of recommended field application levels.

Based on the findings of Dial and Bauer (1984) and reports that paraquat is approximately twice as toxic as diquat in rats (Herbicide Handbook 1983) and five times as toxic in carp fingerlings (Singh and Yadav 1978) it was felt important to investigate the lethal effects of diquat on developing R. pipiens, using a range of concentrations, including concentrations at recommended aquatic application rates. In addition, 15-day old R. pipiens tadpoles were exposed to various concentrations of diquat and paraquat.

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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 eggs developing beyond the initial twenty-four hour period. Dechlorinated (aerated) City of Terre Haute water was used throughout the study. The water had 91 mg/L calcium, 32 mg/L magnesium, a hardness of 374 mg/L as $CaCO_3$, and an alkalinity of 295 mg/L as $CaCO_3$ (concentrations are means provided by the City of Terre Haute Waterworks Division for the months of study of January, February, and March, 1986). The water in the exposure bowls had a temperature of 20-21°C.

The onset of exposure occurred during 2 phases: early gastrula and when the tadpoles were 15 days of age. Eggs at the early gastrula stage (dorsal lip) were treated with nominal concentrations of 0, 2.0, 5.0, and 10.0 mg/L diquat (commercial source) and 0.5 and 2.0 mg/L paraquat (commercial source). Diquat exposure at the gastrula stage consisted of five 16-day runs, 3 using 40 embryos per dose group and 2 using 50 embryos per dose group (total 220 embryos per dose group). Paraquat exposure at the gastrula stage consisted of three 16-day runs, 2 using 25 embryos per dose group and 1 using 30 embryos per dose group (total 80 embryos per dose group). Previously unexposed tadpoles were treated with the above levels of paraquat and diquat, with the exception of 2.0 mg/L diquat, beginning when they were 15 days of age. Three 16-day runs were conducted using 20 tadpoles per dose group (total 60 tadpoles per group).

Static bioassay runs were made on separate occasions with eggs from different females. However, all eggs within a particular run, including each run in which the onset of exposure occurred at early gastrula and at 15 days of age, were from the same female. Embryos were reared in 11.4 cm finger bowls (maximum of 25/bowl) containing 100 ml of solution in all runs except runs dealing with exposure at 15 days of age, in which case 21.6 cm glass bowls containing 500 ml of solution were used. A glass plate was placed over each bowl to reduce evaporation. Solutions were changed in each bowl on alternate days until the run was terminated. Mortality was recorded daily. Comparisons between groups for survival were analyzed using chi-square.

RESULTS AND DISCUSSION

Survivability data are presented in Table 1, and discussed below. Eggs of R. pipiens were found to be resistant to diquat and paraquat as development proceeded normally to hatch (day 4) in all groups. No significant differences in survivability were detected

Table 1. Effects on survivability of diquat (N=220/group) and paraquat (N=80/group) exposure to R. pipiens at the gastrula stage and at 15 days of age (N=60/group)[a]

	Gastrula						15 Days of Age			
		Diquat (mg/L)			Paraquat (mg/L)		Diquat (mg/L)		Paraquat (mg/L)	
Day[b]	0	2	5	10	0.5	2	0	10	0.5	2
5	195[c]	196	191	199	71	69	59	57	60	46*
6	193	195	189	197	70	67	59	57	60	41**
7	193	193	188	194	70	60	58	57	60	38**
8	189	190	185	188	68	50**	58	56	60	25**
9	187	189	183	178	57*	29**	58	55	57	12**
10	187	187	176	135**	43**	5**	58	55	53	8**
11	186	184	171	128**	9**	0	57	54	48	6**
12	185	184	170	113**	6**	0	56	54	45*	6**
13	185	181	166*	102**	6**	0	56	51	42**	5**
14	182	180	161*	89**	5**	0	55	50	42**	5**
15	181	176	157**	82**	5**	0	54	48	41**	3**
16	180	175	156**	75**	5**	0	54	47	40**	3**
% live day 16	81.8	79.5	70.9	34.1	6.3	0.0	90.0	78.3	66.7	5.0

[a]Days 1-4 were eliminated from table as eggs were found to be resistant to diquat and paraquat; the first significant effect was on day 5 when treatment was at 15 days of age.

[b]Number of days after start of treatment; day 5 is day 1 post hatch when exposed at the gastrula stage.

[c]Number of surviving embryos.

* Indicates a significant difference at the 5% level, as compared with control of same age, chi-square test.

**Indicates a significant difference at the 1% level, as compared with control of same age, chi-square test.

between controls and 2 mg/L diquat, but treatment beginning at the early gastrula stage of 5 and 10 mg/L diquat and 0.5 and 2 mg/L paraquat resulted in significant (X^2, 1 df, $P < 0.05$) mortalities by days 13, 10, 9, and 8, respectively (days 9, 6, 5, and 4, respectively, post hatch). No tadpoles treated at early gastrula and subjected to 2 mg/L paraquat survived beyond 10 days after treatment (day 6 post hatch), and survivability was very poor in 0.5 mg/L paraquat and 10 mg/L diquat with only 6.3 and 34.1% respectively, living to day 16 (X^2, 1 df, $P < 0.01$). Control survival was good with 81.8% living to day 16. Survivability was statistically inseparable between controls and tadpoles treated at 15 days of age to 10 mg/L diquat. However, tadpoles treated at 15 days of age with 0.5 to 2.0 mg/L paraquat showed significant (X^2, 1 df, $P < 0.05$) mortalities by days 12 and 5, respectively. Only 5.0 and 66.7% of tadpoles treated at 2 and 0.5 mg/L paraquat, respectively, lived to day 16 of treatment (X^2, 1 df, $P < 0.01$) in contrast to 90.0% of the controls living to day 16.

There were no variations in mortality between runs for 0.5 and 2.0 mg/L paraquat or 2.0 mg/L diquat exposure at early gastrula nor in 2.0 mg/L paraquat exposure at 15 days of age; in all runs there were significant differences, with the exception of 2.0 mg/L diquat, in which no run was significant compared with controls. However, variations in mortality between runs was observed. When exposure was begun at early gastrula there were significant differences compared with controls in 2 of the 5 runs for 5 mg/L diquat, and in 4 of the 5 runs for 10 mg/L diquat. Significant differences compared with controls were found in only 1 of the 3 runs conducted when the tadpoles were 15 days of age and exposed to 10 mg/L diquat and 0.5 mg/L paraquat. As noted above, runs were made on separate occasions with eggs from different females and all eggs within a particular run were from the same female. The variation between runs may have been due to genetic variability of the frogs used, resulting in the offspring of the different egg masses having different tolerances to the pesticides used.

Diquat and paraquat exposure at 10.0 and 0.5 mg/L, respectively, resulted in higher mortalities when administered at early gastrula than at 15 days of age. However, 2.0 mg/L paraquat proved consistently lethal even when exposure was at 15 days of age.

Based on exposure levels necessary to produce similar lethal effects, paraquat is approximately twice as toxic as diquat in rats (Herbicide Handbook 1983) and 5 times as toxic in carp fingerlings (Singh and Yadav 1978). In the present study 0.5 mg/L paraquat was more toxic (6.3% alive day 16) than 10 mg/L diquat (34.1% alive day 16). Thus, in R. pipiens paraquat was more than 20 times as toxic as diquat. Diquat has been reported to be more teratogenic in mammals than paraquat (Selypes et al. 1980), but this was not found in R. pipiens in the present study. Paraquat has been reported to produce retardation of growth, significant levels of tail malformations, and poor head development (Dial and Bauer 1984);

these abnormalities were observed in the present study as well. Diquat, however, was not observed to produce any abnormalities.

Diquat and paraquat are known to disappear from treated water quite rapidly due to their absorption and concentration by aquatic plants (Way et al. 1971; Calderbank 1972). Way et al. (1971) found that paraquat lake water residues were not detectable by 16 days after application of 0.5 mg/L paraquat, and Yeo (1967) reported only traces of paraquat in water at the end of 12 days. The absorption of paraquat by aquatic plants can be considerable. Calderbank (1972) detected 112 ppm paraquat in Myriophyllum spicatum 2 days after application of 1 ppm, and 36 ppm in Potamogeton pusillus 14 days after treating the water at 0.5 ppm Ernest (1971) reported paraquat residues of 2300 ppm in Chara sp. 8 days after treatment of 1.14 ppm paraquat, and 1300 ppm in Spirogyra sp. 4 days after treatment. Available evidence shows that diquat absorption and concentration by aquatic plants is similar to paraquat (Calderbank 1972).

Because plants can concentrate astonishing levels of diquat and paraquat it would be important to learn whether consumption of diquat or paraquat contaminated plants or plant detritus by tadpoles is lethal.

After hatch, aquatic application levels of paraquat produced significant mortality for both treatment regimes, i.e., exposure beginning at the early gastrula stage of development and at 15 days of age. Common aquatic application levels of diquat did not produce significant mortality for either treatment regime.

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