Bull. Environ. Contam. Toxicol. (1995) 55:870–877

Environmental Contamination and Toxicology

Lethal Effects of the Consumption of Field Levels of Paraquat-Contaminated Plants on Frog Tadpoles

C. A. Bauer Dial,* N. A. Dial

Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809, USA

Received: 30 September 1994/Accepted: 2 June 1995

The herbicide paraquat (1,1'-dimethyl-4,4'-bipyridinium dichoride) is used extensively in commercial agriculture. Paraquat is used as an aquatic herbicide in some countries but not in the United States. The rate of application for aquatic weed control usually ranges from about 0.1 to 2.0 mg/L, by weight in water (Newman 1970; Calderbank 1972). Relatively little is known regarding the effects of paraquat on aquatic animals. Dial and Bauer (1984) and Dial and Dial (1986) have reported lethal and teratogenic effects in young developing Rana pipiens tadpoles after treatment, beginning at early gastrula and at 15 d of age, of paraquat concentrations as low as 0.5 mg/L. A concentration of 0.1 mg/L paraquat did not produce a significant difference.

Paraquat is known to disappear from treated water quite rapidly due to its absorption and concentration by aquatic plants (Way et al. 1971; Calderbank 1972). The absorption of paraquat by aquatic plants can be considerable with residues of 2300 mg/kg and 1300 mg/kg reported in Chara sp. and Spirogyra sp., respectively (Ernest 1971).

Because plants can concentrate high levels of paraquat from water and frog tadpoles are known to be sensitive to paraquat exposure, it is important to learn whether consumption of paraquat contaminated plants by tadpoles is lethal.

The current study is an aquatic food chain study involving paraquat treated water, plants, and frog tadpoles. Only the water was treated with paraquat. Paraquat contamination of the plants was by absorption and concentration of paraquat from the water. Tadpoles became paraquat contaminated via feeding on contaminated plants.

Present address: 1025 10th Avenue, Helena, Montana 59601

Correspondence to: C. A. Bauer Dial

MATERIALS AND METHODS

Three concurrent runs were conducted, each of which consisted of 60 Rio Grande Leopard frog (Rana berlandieri) tadpoles which were exposed to treated and untreated Elodea canadensis and Myriophyllum sp. (species= elatinoides; verticillatum; scabratum) beginning when they were 21 d of age. Tadpoles were derived from eggs obtained from mature R. berlandieri females (Carolina Biological Supply Co., Burlington, North Carolina) which were induced to ovulate; eggs were artificially inseminated by the method of Rugh (1962). All tadpoles within a particular run were offspring of the same female; however, tadpoles from different females were used for the three runs. Tadpoles were reared in 21.6-cm culture bowls (30/bowl; 2 bowls/run). Only those developing normally were used.

The appropriate plant food was placed into the treatment bowls on a daily basis. The water was gently stirred to facilitate movement of the food to the bottom of the bowl. Sufficient food was available for feeding to be ad libitum. Dead tadpoles were removed three times daily to reduce the use of tadpoles as a food source.

Tadpoles were reared in 1000 mL of water. The water was a mixture of approximately 55:45 of 3 X distilled water and City of Terre Haute tap water, respectively. The water was mixed in 37.9 L aquariums and then aerated (activated charcoal) to remove chlorine. Aeration was discontinued after 2 d and the water was allowed to stand 2 additional days prior to it's use in order to have the dissolved oxygen similar to that in the culture bowls containing the tadpoles. The culture bowls and aquaria were glass covered to reduce evaporation. Alkalinity was readjusted immediately prior to water changes by adding an appropriate amount of distilled water to give the desired alkalinity of 88.0 to 89.0 mg/L as $CaCO_3$. All culture bowls received water from the same aquarium at the time the water was changed.

Water quality data were obtained on alternate days, at the time the water in the culture bowls was changed. Water in the aquaria was analyzed immediately prior to its use; and water from one bowl of each treatment per run was analyzed immediately prior to the water change. The water had the following characteristics: alkalinity 88.0-97.5 mg/L as $CaCO_3$, hardness 126.0-132.3 mg/L as $CaCO_3$, pH 7.5-7.8, temperature 23.4-25.3 C, dissolved oxygen 7.1-8.1 mg/L.

Elodea and Myriophyllum were exposed separately to 30.3 L of water in 37.9-L aquaria treated at 1.81 mg/kg paraquat. Each aquarium contained 40 g of Elodea or

Myriophyllum. Initially the plants remained at the surface of the water. At 8 d most of the Myriophyllum was located in the mid to lower 1/3 of the aquarium and had mushy leaves which were losing their integrity; it was at this time that the plants were removed from the paraquat treated water and allowed to dry. At 11 d most of the Elodea was located in the mid to lower 1/3 of the aquarium, some leaves were mushy and many leaves had abscissed from the main stem; it was at this time that the plants were removed from the paraquat treated water and allowed to dry. In both plant species some leaves and sprigs were lying on the bottom of the aquarium at the time of plant removal. The dried plant material was crushed and then used for tadpole food. Forty grams of Elodea or Myriophyllum were placed into 30.3 L of untreated water in 37.9-L aquaria for control tadpole food. The plants were removed from the untreated water at the time of removal of the Elodea and Myriophyllum as described above. The control plants were also dried immediately following their removal from the water.

The highest number of tadpoles feeding simultaneously during the first 25 sec of each minute was determined. The above data were collected for 15 min following the introduction of plant food. Food introduction stimulated feeding activity albeit food was available ad libitum. Feeding activity observations were made on days 0 and 1, and on alternate days thereafter. These data were collected for tadpoles exposed to control and treated Myriophyllum. Feeding activity was determined for 90 tadpoles (30/run) per treatment group.

All tadpoles were observed daily for determination of normal swimming behavior. Normal swimming behavior was set forth as the ability to swim, following probing of the tail, in a straight line, with no signs of abnormal swimming; this included normal "starts and stops".

Daily estimations of body size of tadpoles feeding on treated Elodea or Myriophyllum were made relative to the respective controls. Abnormal tails (short or bent) were also noted.

Three water samples were analyzed for paraquat content. The first water sample was collected after paraquat introduction into 30.3 L of water, in an aquarium, but before plant introduction. The second water sample was collected at the time of plant (Myriophyllum) removal from the paraquat treated water. Three hundred mL of water were collected from each aquarium and combined for each of the above water samples. The third water sample consisted of water from the culture bowls containing tadpoles fed treated Myriophyllum. One hundred mL were collected from each bowl at the time the water was

changed. The water was combined, then filtered 3 times to remove the Myriophyllum. The water samples were stored in a refrigerator.

Two plant samples were analyzed for paraquat content. The first plant sample involved the collection of Myriophyllum. One-half of the Myriophyllum in each aquarium was collected and combined for paraquat analysis at the time of plant removal from the paraquat treated water (8-d exposure period; the remaining Myriophyllum was immediately dried for tadpole food). The second plant sample involved the collection of Elodea. One-half of the Elodea in each aquarium was collected and combined for paraquat analysis at the time of plant removal from the paraquat treated water (11-d exposure period; the remaining Elodea was immediately dried for tadpole food). The plant material reserved for paraquat analysis was placed into a freezer at the time of collection.

The paraquat residue analyses were conducted by Hazleton Laboratories America, Inc. (Madison, Wisconsin). The method used is as described by Gill et al. (1983) and Worobey (1987). Plants were frozen, ground then extracted with acid using a reflux procedure. The extract was adjusted to pH 9-10 and cleaned by using a silica Sep-Pak. Aliquots of the final eluate were taken to dryness, disolved in the HPLC mobile phase and analyzed as their heptanesulfonate ion-pairs by HPLC with UV detection (254 nm). Four positive water controls were run at the 10 mg/L level; average recovery was 109%. The method has a published coefficient of variation (CV) of not greater than 8.27%.

Survivability, abnormal swimming behavior, abnormal tail data, and feeding activity data were compared using the Chi-square test.

It is important to note that during preliminary studies, tadpoles were observed feeding on plants suspended in the aquarium and on the bottom of the aquarium.

RESULTS AND DISCUSSION

Hazleton reported the following paraquat residues for the current study: water that plants were placed into--1.81 mg/L; Myriophyllum--1011.0 mg/kg; Elodea--72.6 mg/kg; water following Myriophyllum removal--1.61 mg/L. They did not detect paraquat at or above their limit of detection of 0.005 mg/L in the filtered water which had housed the tadpoles and plant material.

Survivability and observed gross effects are presented in Table 1. Significant mortality was observed on day 7 and thereafter for tadpoles fed paraquat treated Myriophy-

llum. Only 19.4% of the tadpoles lived to day 15 in contrast to 81.1% of controls living to day 15.

A significant number of tadpoles fed paraquat treated Myriophyllum were observed to have abnormal tails by day 3. Tail abnormalities were of two distinct types: flexed and short. Abnormal tails are of concern as tadpoles would likely not be as efficient in swimming which could affect predator avoidance behavior. In addition, the tail provides an energy source during metamorphosis.

Swimming behavior appeared normal in tadpoles fed treated Myriophyllum to day 4. However, a significant number of tadpoles exhibited abnormal swimming behavior on days 5-14, with the exception of day 9.

There were no differences in feeding between controls and tadpoles fed paraquat treated Myriophyllum on day 0. However, feeding activity was significantly affected in tadpoles fed treated Myriophyllum compared with tadpoles fed untreated Myriophyllum beginning on day 3. In the 0-5-min time interval, significant differences were found on days 1-13. In the 10-15-min time interval, significant differences were found on days 3-11, with the exception of day 5.

Body size of tadpoles fed treated Myriophyllum was affected; by day 15 treated tadpoles were approximately 85% the size of controls.

Significant differences were observed in swimming behavior, tail abnormalities, and feeding activity prior to the onset of significant differences in mortality.

No significant differences were found for survivability, feeding behavior, swimming behavior, tail abnormalities, or size between tadpoles fed treated and untreated Elodea.

Paraquat is known to disappear from treated water quite rapidly due to its absorption and concentration by aquatic plants (Way et al. 1971; Calderbank 1972). Yeo (1967) found that traces of paraquat could be detected in water at 12 d post treatment; but residues are usually below 0.01 mg/L paraquat within 4-7 d of application. The absorption of paraquat by aquatic plants can be considerable. Calderbank (1972) detected 112 mg/kg paraquat in Myriophyllum spicatum 2 d after application of 1 mg/L, and 36 mg/kg in Potamogeton pusillus 14 d after treating the water at 0.5 mg/L. Ernest (1971) reported paraquat residues of 2300 mg/kg in Chara sp. 8 d after treatment of 1.14 mg/L, and 1300 mg/kg in Spirogyra sp. 4 d after treatment. Rapid absorption by aquatic plants is the reason low concentrations of

paraquat (0.5-2.0 mg/L) are effective in controlling most aquatic weed species.

Way et al. (1971) examined paraquat residues in aquatic plants in two lakes for 32 d after treatment of 0.5 mg/L paraquat. In the first lake, an increase in weed paraquat residues occurred over the first 4 d to a maximum, after which there seemed to be a gradual decline, associated with the decay of the weed. Weed paraquat residues in the second lake peaked by 8 d after application. By day 16 the plants were rapidly disintegrating, and at 32 d it was difficult to collect enough plant matter for analysis.

Paraquat is not metabolized to any significant extent in plants (Funderburk and Lawrence 1964; Slade 1966; Calderbank et al. 1968). Calderbank et al. (1968) found that diquat residues in tubers persisted unchanged after several months of storage. The absorption, translocation, and distribution patterns for diquat and paraquat in plants are similar (Funderburk and Bozarth 1967). However, persistence studies indicate that paraquat is more persistent than diquat (Funderburk and Bozarth 1967). We did not have sufficient quantities of dried Elodea or Myriophyllum to perform paraquat residue analyses on the dried plant material; however based on the literature, it is unlikely that drying the Elodea and Myriophyllum in the current study affected the paraquat residues with regard to residues present in the non-dried plant material at the time of collection.

Drying the plant material immediately following it's removal from the paraquat treated water was the most feasible manner in which to handle the plants due to the neccessity of housing tadpoles in culture bowls to allow daily observations of mortality, teratology, swimming and feeding activity. In addition, the dried plant material likely remained constant in paraquat content. Elodea and Myriophyllum used for control tadpole food were handled in the same manner as the Elodea and Myriophyllum exposed to paraquat, with the exception of the water not containing paraquat. This resulted in healthy control plants at the time of their removal from the untreated water. This is in contrast to the condition of the Elodea and Myriophyllum exposed to 1.81 mg/L paraquat for paraquat treated tadpoles. We believe this does not present a problem as in preliminary studies 300 tadpoles in a 37.9-L aquarium were healthy and grew rapidly--to termination of the study at 6 wk--when fed Myriophyllum which had been placed in complete darkness for 3 wk to allow plant death.

In the current study significant differences were found in mortality, swimming behavior, tail abnormalities, and

Table 1. Effects of paraquat on survivability, swimming behavior, and tail abnormalities of R. berlandieri fed 0 paraquat plant (Myriophyllum) material (C) or 1011 mg/kg paraquat contaminated plant (Myriophyllum) material (T).

	Number Alive				Abnormal Swimming				Abnormal Tail			
	C		T		C		T		C		T	
Day a	#	%	#	%	#	%	#	%	#	%	#	%
1	180b	100.0	179b	99.4	0c	0.0d	0c	0.0d	0c	0.0d	0c	0.0d
2	180	100.0	177	98.3	0	0.0	0	0.0	0	0.0	0	0.0
3	180	100.0	177	98.3	1	0.6	0	0.0	1	0.6	21**	11.9
4	175	97.2	177	98.3	2	1.1	3	1.7	0	0.0	18**	10.2
5	174	96.7	174	96.7	1	0.6	7*	4.0	0	0.0	30**	17.2
6	174	96.7	167	92.8	1	0.6	16**	9.6	0	0.0	41**	24.6
7	172	95.6	147**	81.7	0	0.0	9**	6.1	0	0.0	29**	19.7
8	172	95.6	138**	76.7	1	0.6	8**	5.8	0	0.0	29**	21.0
9	165	91.7	127**	70.6	7	4.2	10	7.9	0	0.0	32**	25.2
10	161	89.4	110**	61.1	4	2.5	9*	8.2	0	0.0	42**	38.2
11	157	87.2	92**	51.1	2	1.3	11**	12.0	0	0.0	26**	28.3
12	156	86.7	75**	41.7	2	1.3	13**	17.3	0	0.0	29**	38.7
13	153	85.0	58**	32.2	7	4.6	12**	20.7	0	0.0	27**	46.6
14	149	82.8	41**	22.8	6	4.0	6*	14.6	0	0.0	17**	41.5
15	146	81.1	35**	19.4	3	2.1	2	5.7	0	0.0	12**	34.3

a Number of days after start of treatment.
b Number of surviving tadpoles; number at start=180.
c Number showing characteristic.
d Based on number of surviving tadpoles.
* Indicates a significant difference at the 5% level, Chi-square test.
** Indicates a significant difference at the 1% level, Chi-square test.

feeding activity of tadpoles fed 1011.0 mg/L Myriophyllum.

On day 15 many of the 35 surviving tadpoles fed paraquat treated Myriophyllum appeared normal except for their size and shorter tails. About 33.0% had tails that were about 2/3 control size.

No significant differences were observed in survivability, swimming behavior, body size, or tail abnormalities in tadpoles fed 72.6 mg/kg Elodea.

It appears that tadpole survivability under natural conditions would be related to the plant species upon which the tadpoles are feeding as plant species differ in their absorption and concentration of paraquat.

The current study indicates that significant tadpole mortality can result from tadpoles feeding on paraquat contaminated plant material. We are unaware of other

feeding studies of the nature reported here. Other aquatic herbicides should be investigated in a similar manner to ascertain whether they produce deleterious effects on tadpoles via this mode of contamination.

Acknowledgments. This research was supported in part by a grant from the University Research Committee, Indiana State University.

This paper is dedicated to N. A. Dial who died during the preparation of the manuscript.

REFERENCES

Calderbank A, McKenna RH, Stevens MA, Walley JK (1968) Grazing trials on paraquat-treated pasture. J Sci Fd Agric 19:246-250

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

Dial NA, Bauer CA (1984) Teratogenic and lethal effects of paraquat on developing frog embryos. Bull Environ Contam Toxicol 33:592-597

Dial NA, Dial CA (1986) Lethal effects of diquat and paraquat on developing frog embryos and 15-day old tadpoles (Rana pipiens). Bull Environ Contam Toxicol 38:1006-1011

Ernest RD (1971) The effect of paraquat on fish in a Colorado farm pond. Progressive Fish Culturist 33:27-31

Funderburk HH, Lawrence JM (1964) Mode of action and metabolism of diquat and paraquat. Weeds 12:259-264

Funderburk HH, Bozarth GA (1967) Review of the metabolism and decomposition of diquat and paraquat. J Agr Food Chem 15:563-567

Gill R., Qua SC, Moffat AC (1983) High-performace liquid chromatography of paraquat and diquat in vrine with rapid sample preparation involving ion-pair extraction on disposable cartridges of octadecyl-silica. Chrom 15:483-490

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

Rugh R (1962) Experimental embryology. Burgess, Mineapolis, Minnesota

Slade P (1966) The fate of paraquat applied to plants. Weed Res 6:158-167

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

Worobey (1987) Analytical Method for the Simultaneous Determination of diquat and paraquat residues in potatoes by high-pressure liquid chromatography. Pestic Sci 18:245-257

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