MODIFICATION OF THE RHEOTROPIC RESPONSE OF RAINBOW TROUT (*SALMO GAIRDNERI*) BY SUBLETHAL DOSES OF THE AQUATIC HERBICIDES DIQUAT AND SIMAZINE

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ABSTRACT

The rheotaxis and swimming speeds of rainbow trout in response to a water current simulated by moving a striped background past the fish were observed following 24-h exposures to field application concentrations of the herbicides diquat, simazine and their commercial formulations. Fish tissues were subsequently analysed to assess the extent of uptake of the herbicides. Toxicological modification of rheotaxis and swimming speeds was observed in fish exposed to diquat and its formulation Reglone A® resulting in an increased incidence of downstream drift. Simazine residues in fish increased in proportion to treatment levels whereas diquat residues in fish levelled off with increasing treatment levels. The toxicological modification of rheotropism is presented as a sensitive bioassay to assess sublethal effects of biocides.

INTRODUCTION

The literature concerning the effect of pollutants in general on fish emphasises the relationship between acute mortality, concentration and exposure time (Kleerekoper, 1976). The physiological, developmental and behavioural modifications due to exposure to low, subacute concentrations of such compounds are of great ecological significance but have only recently been investigated. In particular, the toxicological modification of any aspect of a behavioural pattern may impair the animal's capabilities to respond adaptively to its environment. As many aquatic herbicides are used at application rates far below acute toxicities, the behavioural toxicology of such compounds must be investigated in assessing the total ecological damage inflicted by their use.

Rheotropism is a term used to cover all the reactions that a fish might make in

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response to a current of water, either directly as a response to water flowing over the body surface or indirectly as a response to the visual, tactile or inertial stimuli resulting from the displacement of fish in space (Harden-Jones, 1968; Arnold, 1974). The rheotropic response is composed of an orientational and a kinetic component; fish generally turn to head into a current and adjust their swimming speeds in response to the rate of the current. Several environmental factors have been observed to affect the orientational and kinetic components of rheotropism (Arnold, 1974) and evidence exists to suggest that such environmental regulation of rheotropism plays an important role in the migration of fish (Dodson & Young, 1977). Thus, this behaviour is of particular ecological significance. Furthermore, rheotropism, as any other behaviour pattern, represents the final integrated result of a diversity of biochemical and physiological processes (Warner *et al.*, 1965) and as such is more comprehensive and sensitive in detecting subtoxic effects than biochemical or physiological parameters. This paper reports the results of experiments designed to reveal modifications of the rheotropic behaviour of rainbow trout caused by short-term exposures to the aquatic herbicides diquat, simazine and their commercial formulations, and to relate any such modifications to the actual doses received as revealed by analysis of herbicide residues in fish tissue.

MATERIALS AND METHODS

The herbicides used in these tests were as follows: (a) Diquat (technical grade, 6,7-dihydrodipyrido [1,2-a: 2',1'-c] pyrazinediium ion; aqueous solution) and its commercial formulation Reglone A® (278 g/litre diquat dibromide) (Chipman Chemicals Ltd); (b) Simazine (technical grade, 2-chloro-4,6-*bis* (ethylamino)-S-triazine; white, crystalline, solubility in water = 5 mg/litre) and its commercial formulation Princep 80W® (80 % active ingredient by weight; wettable powder formulation) (Ciba-Geigy Canada Ltd).

One-year-old rainbow trout were obtained from a local fish hatchery and held under a 16-h daylength for the duration of the experiments. Groups of ten fish each were exposed for 24 h to concentrations of each compound equal to its lowest field application rate, its highest field application rate and five times the mean field application rate. Control groups of ten fish each were held for 24 h in clean water. All exposures were carried out in aerated local well water at 15 °C under static conditions in 790 litre holding tanks (average load = 1·9 g fish tissue/litre). A chemical analysis of the water is presented in Table 1. Fish were introduced into the holding tanks for a 24-h acclimation period prior to the 24-h exposure period. Water temperature, pH and oxygen concentrations were monitored throughout the study. Oxygen concentrations never fell below 75 % saturation and the pH remained fairly constant.

A simple and efficient way of observing the rheotropic response is to simulate the

TABLE 1
CHEMICAL COMPOSITION OF WELL WATER USED FOR BIOASSAY EXPERIMENTS

Constituents or properties	*Concentration (ppm)*
Ca^{2+}	96·5
Mg^{2+}	29·1
Na^{+}	22·7
K^{+}	1·62
NO_3^{2-}	0·50
Total inorganic carbon	48·0
Total organic carbon	not detectable
pH	7·2

visual stimuli produced by displacement in a water current by moving the background past the fish. Responses to displacement of visual images are specifically referred to as optomotor responses and all observations of rheotropism were made in the optomotor tank illustrated in Fig. 1 and described by Dodson & Young (1977). All fish were observed singly in the optomotor tank with the background

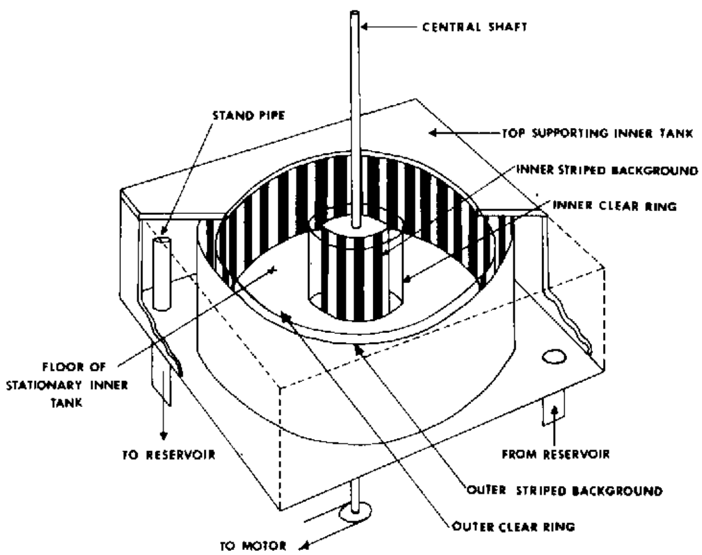

Fig. 1. The optomotor tank used to quantify the rheotropic response of rainbow trout. Part of the outer tank is cut away to reveal the outer revolving striped background. Outer diameter of stationary inner tank = 100 cm. Inner diameter of stationary inner tank = 20 cm.

revolving at a rate equivalent to a water current of 20 cm/sec at a water temperature of 15 °C. Each fish was allowed 10 min to acclimate to the optomotor tank. Any fish exhibiting excessively agitated behaviour or no response at all to the moving background, after the acclimation period, was not used in the experiment.

Responsive fish were observed and recorded for 15 min following the acclimation period.

The orientational component of the rheotropic response was quantified by recording the proportion of total observation time that fish spent swimming in the same direction as the moving background (frequency of positive rheotaxis), swimming in the opposite direction to the moving background (frequency of negative rheotaxis) and exhibiting no orientation to the moving background (frequency of no-response). In interpreting the results, it must be remembered that swimming in the same direction as the moving background is equivalent to swimming into a current. The kinetic component of the rheotropic response was quantified by recording the swimming speed of each fish during at least five circuits around the optomotor tank during periods of positive rheotaxis.

Preliminary analysis of the data indicated that, among the behavioural parameters recorded, frequency of positive rheotaxis, frequency of no-response and swimming speeds showed the greatest variation. These data were analysed using a standard one-way analysis of variance with missing observations. Individual *t*-tests were used to test the effects of specific treatment levels. Prior to detailed analysis, the residuals of each data set were checked for normality and constant variance. There was no evidence to suggest that these basic assumptions were not valid.

Diquat residues in fish tissue were analysed by randomly selecting one fish from each treatment level of each compound and grinding the tissue. Ten grams of ground tissue were blended with 20 ml of 0·5 M NH_4Cl and this was diluted with distilled water to a final NH_4Cl concentration of 2·5%. This mixture was centrifuged and suction-filtered. The resultant filtrate was run through an ion exchange column (Zerolit 225, BDH Chemicals) which consisted of a 25 ml burette with a glass wool plug near the stopcock. The column was prepared by passing 25 ml of saturated NaCl solution and 50 ml of distilled water through the column before use. The diquat was adsorbed on the column from the 2·5% NH_4Cl extraction solution and then eluted with saturated NH_4Cl. The eluate was collected and analysed according to the method of Calderbank & Yuen (1966). Fish tissue samples containing no diquat were 'spiked' with known amounts of the herbicide and used to measure the efficiency of extraction (recovery %). Standards of analytical grade diquat dibromide (Chipman Chemicals Ltd) were used to produce a standard curve. In order to ensure that the active ingredient was available to the fish during the 24-h exposure period, water samples were taken from the exposure tanks immediately after the addition of the highest treatment level of Reglone A® and that of technical grade diquat, and also 24 h later; the samples were analysed according to the method of Calderbank & Yuen (1966).

Gas chromatography was used to determine simazine residues in rainbow trout exposed to the technical grade material or to Princep 80W. Two hundred grams (wet weight) of chopped fish tissue were obtained from three randomly-selected fish at each treatment level and were extracted according to the method of Mattson *et al.* (1965). Water samples were taken from the exposure tanks immediately after the addition of the highest treatment dosage of both compounds and also 24 h later. The simazine concentration was measured in the same way. Gas chromatographic determinations of simazine levels in the extracted fish tissue and water samples were carried out on a 183 cm × 0·64 cm stainless steel column packed with 5 % Carbowax 20 M on 80/100 mesh Chromosorb W AW-DMCS. The flow rate of the nitrogen carrier gas was 50 ml/min, column temperature was 215 °C, injection port temperature was 225 °C and the elution time for simazine was 3·02 min. Columns were conditioned with simazine until constant recoveries were obtained. The flame ionisation detector on a Perkin-Elmer Model 900 gas chromatograph was used for all assays.

RESULTS

Fish were exposed to 0·5 and 1·5 ppm of diquat representing field application dosages ranging from 2·24 to 4·8 kg (AI)/ha, assuming a depth range of approximately 0·3 to 1 m. Fish were also exposed to 5 ppm of diquat representing a treatment level equal to five times the mean field application dosage. The observed variations in the recorded behavioural parameters and their analyses of variance are presented in Table 2. Fish exhibited no significant variation in the frequency of

TABLE 2

THE MEAN FREQUENCY OF POSITIVE RHEOTAXIS, THE MEAN FREQUENCY OF NO-RESPONSE AND MEAN SWIMMING SPEEDS OF RAINBOW TROUT EXPOSED FOR 24 H TO DIQUAT. n = TOTAL NUMBER OF FISH RECORDED; C = CONTROL TREATMENT; s^2 = EXPERIMENTAL ERROR; F = F-RATIO FOR TREATMENTS; p = LEVEL OF SIGNIFICANCE OF F

Parameters	*n*	*Concentration (ppm)* C	0·5	1·5	5·0	s^2	F	p
Positive rheotaxis (frequency)	38	0·77	0·75	0·80	0·65	0·032	1·23	>0·05
No response (frequency)	38	0·06	0·09	0·15	0·23	0·019	2·82	=0·05
Swimming speeds (cm/s)	38	22·6	14·1	17·5	16·5	40·21	3·09	<0·05

positive rheotaxis although the 5 ppm treatment level is significantly different when compared with the mean of all other treatments ($t = 1·81, p < 0·05$). Fish exhibited a significant increase in the frequency of no-response and a significant decrease in swimming speeds caused by short-term exposure to diquat. Rainbow trout exposed to 0·5, 1·5 and 5 ppm (AI) of Reglone A® exhibited similar behavioural modifications (Table 3).

TABLE 3

THE MEAN FREQUENCY OF POSITIVE RHEOTAXIS, THE MEAN FREQUENCY OF NO-RESPONSE AND MEAN SWIMMING SPEEDS OF RAINBOW TROUT EXPOSED FOR 24 H TO REGLONE A®. n = TOTAL NUMBER OF FISH RECORDED; C = CONTROL TREATMENT; s^2 = EXPERIMENTAL ERROR; F = F-RATIO FOR TREATMENTS; p = LEVEL OF SIGNIFICANCE OF F

Parameters	*n*	*Concentration (ppm)* C	0·5	1·5	5·0	s²	F	p
Positive rheotaxis (frequency)	39	0·82	0·74	0·77	0·64	0·049	1·25	>0·05
No response (frequency)	40	0·04	0·16	0·13	0·26	0·029	3·12	<0·05
Swimming speeds (cm/s)	38	17·9	18·7	15·4	12·2	24·88	3·35	<0·05

The analyses of water samples taken before and immediately after the 24-h 5 ppm exposures to diquat and Reglone A® indicated that diquat levels did not significantly decline over the exposure period (Table 4) and thus was available to the fish over the entire exposure period. Diquat residues in the tissue of fish exposed to diquat and Reglone A® were found in amounts below the concentrations in the water and appeared to level off between the 1·5 and 5 ppm treatment dosages (Table 5).

TABLE 4

RESULTS OF ANALYSES OF WATER SAMPLES TAKEN FROM EXPOSURE TANKS IMMEDIATELY AFTER (t_0) AND 24 H AFTER (t_{24}) THE ADDITION OF 5 PPM DIQUAT AND 5 PPM (AI) REGLONE A®. NUMBER OF SAMPLES IN EACH CASE = 3. SEM = STANDARD ERROR OF THE MEAN

Sample	*Measured concentration (ppm AI)* t_0 *Mean*	t_0 *SEM*	t_{24} *Mean*	t_{24} *SEM*
Water + diquat	4·7	0·50	4·6	0·35
Water + Reglone A®	4·5	0·24	4·6	0·32

TABLE 5

RESULTS OF ANALYSIS OF DIQUAT RESIDUES IN THE TISSUE OF FISH EXPOSED TO DIQUAT AND REGLONE A® FOR 24 H. DATA CONVERTED TO PPM AI, FISH TISSUE, DRY WEIGHT. NUMBER OF SAMPLES IN EACH CASE = 3. SEM = STANDARD ERROR OF THE MEAN

Sample	*Added (ppm AI)*	*Measured (ppm AI)* *Mean*	*SEM*
Diquat	0·5	0·25	0·07
Diquat	1·5	1·63	0·58
Diquat	5·0	1·88	0·53
Reglone A®	0·5	0·30	0·05
Reglone A®	1·5	1·88	0·52
Reglone A®	5·0	2·10	0·57

Rainbow trout were exposed to 1·0 and 4·0 ppm of simazine approximately equivalent to field application rates ranging from 0·71 kg/1000 m^3 of water to 4·3 kg/1000 m^3 of water. Fish were also exposed to 12·5 ppm of simazine representing a treatment level equal to five times the mean field application dosage. It was necessary to add 1 ml of Tween 80, a wetting agent, to each treatment level to create a homogeneous suspension of the technical grade material. Therefore, one group of fish was exposed to clean water plus 1 ml of Tween 80 in addition to the usual control group. Fish exposed to simazine plus Tween 80 exhibited a significant decrease in the frequency of positive rheotaxis, a significant increase in the frequency of no-response and a significant decrease in swimming speeds (Table 6). Rainbow

TABLE 6

THE MEAN FREQUENCY OF POSITIVE RHEOTAXIS, THE MEAN FREQUENCY OF NO-RESPONSE AND MEAN SWIMMING SPEEDS OF RAINBOW TROUT EXPOSED TO SIMAZINE FOR 24 H. n = TOTAL NUMBER OF FISH RECORDED; C = CONTROL TREATMENT; T_{80} = 1 ml OF TWEEN 80 TREATMENT; s^2 = EXPERIMENTAL ERROR; F = F-RATIO FOR TREATMENTS; p = LEVEL OF SIGNIFICANCE OF F

Parameter	*n*	*Concentration (ppm)* C	T_{80}	1·0	4·0	12·5	s^2	F	p
Positive rheotaxis (frequency)	48	0·88	0·73	0·68	0·66	0·50	0·064	2·77	<0·05
No response (frequency)	48	0·03	0·16	0·11	0·20	0·36	0·025	4·95	<0·01
Swimming speeds (cm/s)	41	19·8	16·8	19·7	13·4	7·5	25·94	8·37	<0·01

trout were exposed to 1·0, 4·0, 8·0 and 12·5 ppm (AI) of Princep 80W®. No Tween 80 was added as Princep 80W® is a wettable powder formulation. No significant variation occurred in the rheotropic behaviour of rainbow trout exposed to Princep 80W® (Table 7).

TABLE 7

THE MEAN FREQUENCY OF POSITIVE RHEOTAXIS, THE MEAN FREQUENCY OF NO-RESPONSE AND THE MEAN SWIMMING SPEEDS OF RAINBOW TROUT EXPOSED FOR 24 H TO PRINCEP 80W®. n = TOTAL NUMBER OF FISH RECORDED; C = CONTROL TREATMENT; s^2 = EXPERIMENTAL ERROR; F = F-RATIO FOR TREATMENTS; p = LEVEL OF SIGNIFICANCE OF F

Parameter	*n*	*Concentration (ppm)* C	1·0	4·0	8·0	12·5	s^2	F	p
Positive rheotaxis (frequency)	49	0·82	0·75	0·68	0·72	0·75	0·039	0·068	>0·05
No response (frequency)	49	0·04	0·11	0·12	0·17	0·11	0·023	0·97	>0·05
Swimming speeds (cm/s)	47	19·6	15·0	20·3	16·0	15·8	32·70	1·72	>0·05

Gas chromatographic analysis of water samples taken before and immediately after the 12·5 ppm exposures indicated that total simazine levels (dissolved and suspended) did not significantly decline over the exposure period (Table 8). Thus the simazine was in the water and available to fish over the entire 24-h exposure period. The results of the analysis of the tissue of fish exposed to simazine and Princep 80W® are presented in Table 9. Simazine residues were found in amounts approximately proportional to, but well below, the concentrations in the water.

TABLE 8
RESULTS OF ANALYSIS OF WATER SAMPLES TAKEN FROM EXPOSURE TANKS IMMEDIATELY AFTER (t_0) AND 24 H AFTER (t_{24}) THE ADDITION OF 12·5 PPM SIMAZINE AND 12·5 PPM (AI) PRINCEP 80W®. NUMBER OF SAMPLES IN EACH CASE = 3. SEM = STANDARD ERROR OF THE MEAN

Sample	Measured Concentration (ppm AI) t_0		t_{24}	
	Mean	*SEM*	*Mean*	*SEM*
Water + simazine	11·9	0·89	11·8	0·31
Water + Princep 80W®	12·9	0·23	11·6	0·68

DISCUSSION

This study has demonstrated that rainbow trout exposed to diquat and its formulation Reglone A® show significant variations in those behavioural parameters that in nature would lead to an increased incidence of downstream drift. Elevated frequencies of no-response will result in passive downstream movements as will slower swimming speeds during periods of positive rheotaxis. Thus, field application dosages of diquat getting into running water may lead to downstream displacement of trout populations. The ecological impact of such a behavioural modification would be greatest during periods of upstream spawning migration when a delay could impair the reproductive effort of the population.

TABLE 9
RESULTS OF ANALYSIS OF SIMAZINE RESIDUES IN THE TISSUE OF FISH EXPOSED TO SIMAZINE AND PRINCEP 80W® FOR 24 H. DATA CONVERTED TO PPM AI, FISH TISSUE, DRY WEIGHT. NUMBER OF SAMPLES IN EACH CASE = 3. SEM = STANDARD ERROR OF THE MEAN

Sample	*Added (ppm AI)*	*Measured (ppm AI)* *Mean*	*SEM*
Simazine	1	0·15	0·03
Simazine	4	1·03	0·18
Simazine	12·5	3·40	0·29
Princep 80W®	1	0·08	0·01
Princep 80W®	4	1·18	0·30
Princep 80W®	12·5	2·23	0·29

Diquat is a general aquatic herbicide used to control mixed submergents in reservoirs and lakes of recreational or commercial importance and is considered to be generally safe to wildlife and fish (WSSA, 1974). The acute toxicity of diquat to fishes has been studied in several investigations and 24-h LC_{50} values range from 12·3 to 315 ppm (AI) depending on fish species, the chemical makeup of the water and the formulation of diquat (Pimental, 1971). Alabaster (1969) established that the 24-h median lethal concentration to rainbow trout of diquat formulated as dibromide (as in Reglone A®) was 90 ppm (AI) in hard water. The most common field application dosage for diquat is approximately 1 ppm although in some situations local concentrations of up to 10 ppm have been found immediately after application (Bimber *et al.*, 1976). Thus, median lethal levels are well above normal field application dosages.

Little is known of the subtoxic effects of diquat on fish. Bimber *et al.* (1976) observed a significant level of respiratory stress, as indicated by the cough response, in two-year-old yellow perch exposed to subtoxic concentrations of diquat (1·0 to 5·0 ppm) over a period of 48 to 72 h. The reduced swimming speeds observed in the present study may, in part, be caused by respiratory complications although elevated cough responses were not observed. Folmar (1976) demonstrated that fingerling rainbow trout did not actively avoid field application dosages (up to 10 ppm) of diquat in a Y-maze over a period of one hour. The results of the present study indicate that fish exposed to diquat over longer periods of time may move passively downstream and, therefore, into decreasing concentrations of diquat. Thus, the modification of rheotropism in rainbow trout by subtoxic concentrations of diquat is, in effect, a passive avoidance mechanism.

The results of this study indicate that diquat is absorbed by rainbow trout but not in proportion to its concentration in the water. Furthermore, this study revealed higher levels of diquat residues in fish tissue than reported elsewhere. Gilderhus (1967) reported that residues of diquat in adult bluegills taken from ponds subjected to a single treatment of diquat at an application rate of 1·0 ppm increased from 0·14 ppm after 3 days to 0·49 ppm after 10 days, well below levels recorded in the present study after only 24 h. The levels reported by Gilderhus subsequently declined to 0·09 ppm after 3 weeks and to undetectable levels after 12 weeks. The differences in initial residue levels may be because diquat is rapidly adsorbed by organic materials and sediments (Grzenda *et al.*, 1966) which were not present in our exposure tanks. Thus, diquat was available to the fish in the present study at higher concentrations over the 24-h period than may be the case in a pond or reservoir treated with diquat. Hiltebran *et al.* (1972) exposed bluegills to diquat and demonstrated that as the length of exposure time increased, proportionally less diquat appeared to have been absorbed. They were unable to conclude whether this was due to the metabolism or elimination of diquat. A similar 'levelling off' of diquat residues in fish tissue was observed in the present study with increasing diquat concentrations rather than with increasing exposure time.

Rainbow trout exposed to simazine and Tween 80 show significant variations in those behavioural parameters that in nature would lead to increased downstream drift. However, no such variation was observed in fish exposed to Princep 80W®. Thus, it is concluded that the toxicological modification of rheotropism in the first instance is most probably due to an interaction between simazine and Tween 80. There is no indication that the rheotropic response of fish in nature would be adversely affected by field application concentrations of Princep 80W®.

Simazine is a widely used selective herbicide for the control of broadleaf and grass weeds in crops and in industrial areas. In recent years, it has been used as an aquatic herbicide to control filamentous algae and submerged and floating aquatic weeds. Toxicological investigations have shown simazine to have very low toxicity to fish and wildlife (WSSA, 1974). Published median lethal concentrations for simazine to fish vary widely depending on the species, water chemistry and the formulation of simazine. Alabaster (1969) reported the 24-h median lethal concentration for rainbow trout to a wettable powder formulation of simazine to be 95 ppm. In the present study, no mortality was observed after 24 h in a group of fish exposed to a 200 ppm (AI) solution of Princep 80W®. Thus, median lethal levels are well above normal field application dosages for Princep 80W®. We know of no other published studies concerned with the subtoxic effects of simazine on fish.

The results of this study indicate that rainbow trout exposed to simazine tend to absorb it in proportion to the concentration in the water. Similar results were obtained by Rodgers (1970) who demonstrated that green sunfish absorbed 0·95 and 2·29 ppm of simazine after 3 weeks exposure to 1 and 3 ppm respectively. Mauck *et al.* (1976) also reported that simazine residues in sub-adult bluegills were similar to, or less than, treatment levels. However, whereas Rodgers (1970) reported that no simazine residue was detected in fish after 7 days in fresh water, Mauck *et al.* (1976) reported simazine residues present in fish up to 1 year after the initial application, but observed no adverse effects on the fish. Mauck *et al.* (1976) suggested that the difference in persistence may have been related to the lack of vegetative material present at treatment times as simazine residues decrease more rapidly in ponds with a heavy growth of vegetation than in those with a light growth.

In conclusion, both diquat and simazine exhibit median lethal levels to fish that are well above suggested field application dosages. Both herbicides are absorbed by fish but neither appears to be concentrated nor to persist in either fish or water. Whereas simazine residues in fish increase in proportion to treatment levels, diquat residues in fish level off with increasing treatment levels. However, toxicological modification of rheotropism was observed only with diquat- and Reglone A®-treated fish. The increased incidence of downstream drift demonstrated by these fish in the laboratory warrants that careful attention be paid to fish movements in streams draining bodies of water treated with diquat. Finally, we feel that observing the toxicological modification of rheotropism in the laboratory not only permits predictions of possible ecological effects prior to the release of a particular toxicant into the environment, but also provides a sensitive and speedy means of assessing the relative hazards of large numbers of biocides, particularly those in which comparison of median lethal levels is ecologically meaningless because of their relatively low field application dosages.

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