*Transactions of the American Fisheries Society* 108:632–640, 1979


# The Dynamics and Behavioral Toxicology of Aqua-Kleen® (2,4-D butoxyethanol ester) as Revealed by the Modification of Rheotropism in Rainbow Trout

JULIAN J. DODSON[1] AND COLIN I. MAYFIELD

*Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1*

**Abstract**

The rheotropic response of rainbow trout (*Salmo gairdneri*) to a water current, simulated by a striped background moving past the fish, was observed following 24-hour exposures to field application concentrations of the aquatic herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) butoxyethanol ester and its commercial formulation Aqua-Kleen®. Toxicological modification of rheotropism occurred that would in nature lead to an increased incidence of downstream movement. With increasing concentrations of Aqua-Kleen, the rheotropic response became more variable. As concentrations approached lethal levels, rainbow trout became extremely lethargic and could not avoid capture. Aqua-Kleen was lethal to rainbow trout at concentrations approximately twice the suggested field application level. The ester was taken up rapidly by fish and hydrolyzed to 2,4-D that was secreted back into the water.

The phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is widely used in canals, ponds, lakes, and waterways to kill floating weeds such as water hyacinth, submerged weeds such as pond weeds, and emergent and shoreline plants such as cattails and willows (CAST 1975). 2,4-D is available in a wide variety of commercial formulations as esters, amines, or inorganic salts. The reported toxicities to fish of these compounds are varied. The ester formulations are generally more toxic to fish than amine formulations and large differences in toxicity may occur within single formulations (Hughes and Davis 1963). Therefore, selection of a formulation for use in the field must take into account the toxicity of the specific formulation as well as that of the general class of herbicide.

The low-volatile butoxyethanol ester of 2,4-D is used extensively for aquatic vegetation control. It has been used for the control of water milfoil in partly salty areas of Chesapeake Bay and for the control of eelgrass in oyster growing areas of eastern Canada, and has been approved as part of a program to attack Eurasian milfoil in the Okanagan Lakes, British Columbia.

Most studies of pollution effects on fish emphasize the relationship between acute mortality, concentration, and exposure time. The physiological, developmental, and behavioral modifications due to exposure to sublethal concentrations of biocides are of great ecological significance but have only recently been investigated. In particular, the toxicological modification of behavior may impair the animal's ability to respond adaptively to its environment. As many aquatic herbicides are used at application rates below acute toxicities, the behavioral toxicology of such compounds must be investigated to assess the total ecological damage inflicted by their use.

Rheotropism is a term used to cover all the reactions that a fish might make in response to a current of water, either directly to water flowing over the body surface or indirectly to visual, tactile, or inertial stimuli resulting from the displacement of fish in space (Harden Jones 1968; Arnold 1974). The rheotropic response is composed of orientational and kinetic components; fish generally turn to head into a current and adjust their swimming speeds in response to the rate of the current. Several environmental factors affect these components of rheotropism and evidence exists to suggest that such environmental regulation of rheotropism plays an important role in the migration of fish (Dodson and Young 1977; Emanuel and Dodson 1979). Furthermore, these components of the rheotropic response are subject to alteration following exposure to sublethal doses of certain

[1] Present address: Département de Biologie, Université Laval, Québec, G1K 7P4. Send reprint request to this address.

TABLE 1.—*Chemical composition of well water used for bioassay experiments. Water samples were taken from March 20 to April 17, 1978.*

| Constituents or properties | N | Concentration, mg/liter | |
|---|---|---|---|
| | | Mean | SD |
| $Ca^{+2}$ | 5 | 116.25 | 3.88 |
| $Mg^{+2}$ | 5 | 33.12 | 2.09 |
| $Na^{+}$ | 5 | 28.20 | 2.08 |
| $K^{+}$ | 5 | 1.31 | 0.13 |
| $Fe^{-}$ | 10 | <0.1 | |
| $SO_4^{-2}$ | 5 | 56.66 | 2.88 |
| $Cl^{-}$ | 3 | 90.33 | 9.50 |
| $NO_3^{-}$ | 3 | 2.87 | 0.15 |
| Organic carbon | 3 | not detectable | |
| Inorganic carbon | 1 | 76.5 | |
| pH | 3 | 7.5 | 0.13 |
| Conductivity | 3 | 0.80 | 0.01 |

aquatic herbicides and form the basis of a sensitive bioassay to assess the relative sublethal properties of aquatic contaminants (Dodson and Mayfield 1979). This paper reports the results of experiments designed to establish lethal limits to yearling rainbow trout of 2,4-D butoxyethanol ester and its commercial formulation Aqua-Kleen®, to reveal modifications of rheotropism of rainbow trout caused by short-term exposures to sublethal concentrations of these compounds, and to relate any such modifications to the uptake of 2,4-D by fish.

## Methods

The compounds used in these tests were the butoxyethanol ester of 2,4-D (technical grade) and its commercial formulation Aqua-Kleen (29% active ingredient by weight; granular formulation; Amchem Products, Incorporated). Suggested field application rates for Aqua-Kleen range from 1.25 kg to 2.5 kg per 100 square meters representing concentrations of between 5 and 10 mg/liter in a depth of 2.4 meters and between 10 and 20 mg/liter in a depth of 1.2 m. These application rates represent active-ingredient concentrations of approximately 1.5 to 6.0 mg/liter.

Yearling rainbow trout were obtained from a local fish hatchery and held under a 16-hour day length for the duration of the experiments. The trout were 15 to 27 cm long with a mean fork length of 18.5 cm. Groups of 10 fish each were exposed for 24 hours to a range of field application concentrations of each compound. Control groups of 10 fish each were held for 24 hours in clean water. All exposures were carried out in aerated local well water at 15 C under static conditions in 790-liter insulated holding tanks (average load: 1.9 g fish tissue per liter). A chemical analysis of the water is presented in Table 1. Fish were introduced into the holding tanks for a 24-hour acclimation period prior to the 24-hour exposure period. Water temperature, pH, and oxygen concentrations were monitored throughout the study. Oxygen concentrations never fell below 75% saturation and pH and temperature remained fairly constant.

### *Rheotropism Bioassay and Lethal Concentrations*

A simple and efficient way to generate a rheotropic response is to simulate the visual stimuli produced by displacement of fish in a water current. We did this by moving a striped background past the fish. Responses to displacement of visual images are referred to as optomotor responses and all observations of rheotropism were made in the optomotor tank illustrated in Fig. 1 and described by Dodson and Young (1977). All fish were observed singly in the optomotor tank with the background revolving at a rate equivalent to a water current of 20 cm/second and at a water temperature of 15 C. Each fish was allowed 10 minutes to acclimate to the optomotor tank. Any fish exhibiting excessively agitated behavior 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 minutes following the acclimation period.

The orientational component of the rheotropic response was quantified by recording the proportions 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). 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.

These behavioral variables were analyzed by

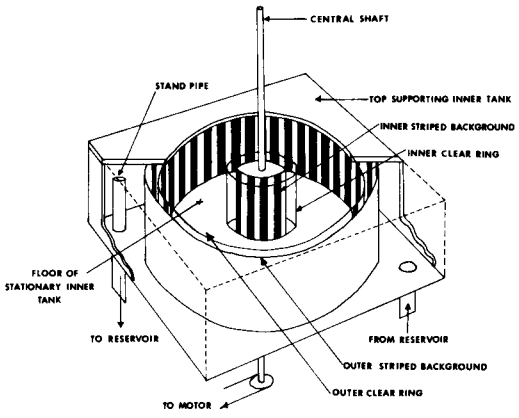


FIGURE 1.—*The optomotor tank used to quantify the rheotropic response of rainbow trout. Part of the outer tank is cut away to reveal the revolving striped background. Outer diameter of stationary inner tank = 100 cm. Inner diameter of stationary inner tank = 20 cm. Depth of water in stationary inner tank = 12 cm.*

standard one-way analyses of variance. Prior to detailed analysis, the residuals of each data set were checked graphically for normality and constant variance. There was no evidence to suggest that these basic assumptions were not valid. Because treatments (herbicide concentrations) were qualitatively similar and balanced ($N = 10$ for all treatments), the experimental error (variance, $s^2$) for each analysis of variance was used to generate the least significant difference (LSD; Sokal and Rohlf 1969) at the 5% probability level for each variable exhibiting significant variance. The means for each treatment were plotted with their appropriate LSD to illustrate the significant effects.

Preliminary tests revealed that fish became increasingly sluggish and insensitive as they were exposed to concentrations of these compounds approaching lethal concentrations. Fish in such a lethargic state showed no avoidance responses and were easily caught by hand. In the optomotor tank, such fish responded to the moving background in almost robot-like fashion, rarely altering speed or direction. Lethargic fish could be induced to exhibit nearly 100% positive rheotaxis or 100% negative rheotaxis by aligning the fish either with or against the moving background. Standard procedure for the bioassay was to place fish into the tank aligned with the moving background. In the case of normally responsive fish, the procedure did not effect the results of the test as such fish initially swam in all directions before exhibiting any consistent pattern of rheotropism and would alter speed and direction on several occasions over the 15-minute observation period. However, as this procedure influenced the outcome of the bioassay in the case of lethargic fish, any treatment group containing fish whose responsiveness was obviously impaired was not observed in the optomotor tank. Rather, concentrations in the exposure tanks were increased and treatment groups observed for mortality to determine 24-hour lethal concentrations.

Aqua-Kleen is a granular compound in which the active ingredient has been formulated on attaclay particles that sink to the bottom and slowly release the active ingredient. Aqua-Kleen was used in two ways in this experiment. Firstly, optomotor tank observations were made and lethal concentrations established using Aqua-Kleen ground to a fine powder, reducing particle size to a minimum. This was done to establish a homogenous solution or suspension of the active ingredient in the hope that the fish's exposure to the active ingredient was equivalent throughout the tank. Another series of treatment groups was exposed to whole Aqua-Kleen and observed only for lethargic behavior and mortality to assess any differences due to the physical state of Aqua-Kleen.

### *Herbicide Residue Analysis*

The 2,4-D butoxyethanol ester (2,4-DBEE) residues in the fish samples and in the water were analyzed according to the methods detailed in Anonymous (1974). Water samples (500 ml) were acidified to pH 2.0 with concentrated sulfuric acid, and 50 ml of chloroform was added and mixed with the water samples. After phase separation, the chloroform layer was removed and the chloroform treatment repeated. These chloroform extracts were washed with 100 ml distilled water and the aqueous layer was removed. After the chloroform extracts were dried with acidifed sodium sulfate, the residues were concentrated on a rotary evaporator to 5 ml, treated with 10 ml methanol, and again evaporated to 5 ml. This procedure was repeated until all traces of chloroform had been removed, after which the methanol was evaporated to 1 ml with nitrogen gas. The extract was methylated with 0.5 ml of $BF_3$-methanol complex at 50 C for 30 minutes. Five ml of

aqueous (5%) sodium sulfate was added to the tube and the methyl esters extracted with two 2-ml quantities of hexane which were then concentrated to 1 ml with nitrogen gas. This concentrate was passed through 2 cm of florisil and the esters were eluted with 10 ml of benzene. The benzene extract was concentrated to 0.5 ml with nitrogen gas and made up to exactly 1 ml. Water samples taken after 1, 5, 10, and 24 hours from two treatment concentrations of each compound were analyzed in this manner.

Residue analysis of fish tissue was performed by grinding three fish from each treatment and then blending the tissue (whole body). Fifteen-gram samples (wet weight) of blended tissue were blended with 400 ml of distilled water, acidified to pH 2.0, and extracted with chloroform. Any emulsion formation was destroyed by adding more chloroform. All other procedures were identical to those used for water analysis. Spiked water and fish tissue samples were also prepared and analyzed, together with analytical standard solutions of 2,4-D and 2,4-DBEE in benzene.

Gas liquid chromatography (GLC) was used to analyze all samples. The GLC procedure employed a 1.8-m × 0.64-cm glass column packed with 4% OV-101, 6% OV-210 on Chromosorb W.A.W., HDMS-treated, 80–100 mesh size at a column temperature of 195 C, injector temperature of 250 C, and an electron capture detector ($^{63}$Ni) at a temperature of 275 C. Nitrogen flowing at 60 ml min$^{-1}$ was the carrier gas and the retention times of 2,4-D and 2,4-DBEE were compared to aldrin in each GLC operation.

## Results

### *Rheotropism Bioassay and Lethal Concentrations*

Seven groups of 10 fish each were observed in the optomotor tank following 24-hour exposures to initial concentrations of 0.0 (control), 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 mg/liter (technical grade 2,4-DBEE). Fish exposed to higher concentrations of the technical grade material were not observed in the optomotor tank. At 8.0 mg/liter, fish began to exhibit lethargic behavior. At 9.0 mg/liter, 20% of the exposed fish died within 24 hours; at 10.0 mg/liter, 50%; and at 10.5 mg/liter, 90%. Surviving fish were all lethargic, being easily caught by hand. In general, the smallest fish appeared to be the least affected while the largest fish were the first to die.

Yearling rainbow trout observed in the optomotor tank exhibited significant variation in the frequencies of positive rheotaxis ($F = 4.25$; $P < 0.05$; $s^2 = 0.04$; $N = 70$) and no-response ($F = 2.38$; $P < 0.05$; $s^2 = 0.04$; $N = 70$) because of short-term exposure to technical grade 2,4-DBEE. The frequency of positive rheotaxis decreased with increasing concentrations of herbicide (Fig. 2A) and the frequency of no-response increased with increasing concentrations (Fig. 2B). No significant variation occurred in the frequency of negative rheotaxis ($F = 1.88$; $P > 0.05$; $N = 70$) or in swimming speeds ($F = 1.35$; $P > 0.05$; $N = 70$).

Seven groups of 10 fish each were observed in the optomotor tank following 24-hour exposures to initial concentrations of 0.0 (control), 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 mg/liter (active ingredient) of Aqua-Kleen ground to a powder. Fish exposed to higher concentrations were not observed in the optomotor tank. At 7.5 mg/liter, fish began to exhibit lethargic behavior. Twenty-four-hour mortalities were 30% at 8.5 mg/liter, 60% at 9.0 mg/liter, and 100% at 9.5 mg/liter. Surviving fish were very lethargic and the largest fish were the first to succumb to the effects of the compound.

Rainbow trout observed in the optomotor tank exhibited significant variation in the frequencies of positive rheotaxis ($F = 2.33$; $P < 0.05$; $s^2 = 0.03$; $N = 70$) and negative rheotaxis ($F = 3.36$; $P < 0.01$; $s^2 = 0.03$; $N = 70$) because of short-term exposure to Aqua-Kleen. No clear trend existed between the frequency of each behavioral variable and the concentration of the herbicide (Fig. 3). No significant variation occurred in the frequency of no-response ($F = 1.76$; $P > 0.05$; $N = 70$) or in swimming speeds ($F = 1.81$; $P > 0.05$; $N = 70$).

Yearling rainbow trout were also exposed to whole Aqua-Kleen but observed only for mortality after 24 hours of exposure. Lethargy was first observed at 10.5 mg/liter (active ingredient) but no mortality occurred until concentrations reached 14.0 mg/liter, at which concentration 70% of the fish died within 24 hours. This mortality rate remained constant at 15.0 mg/liter and 16.0 mg/liter, where testing was ended.

### *Herbicide Residues in Water*

The concentrations of 2,4-DBEE and 2,4-D in the water at various times over the 24-hour exposure period were determined from water

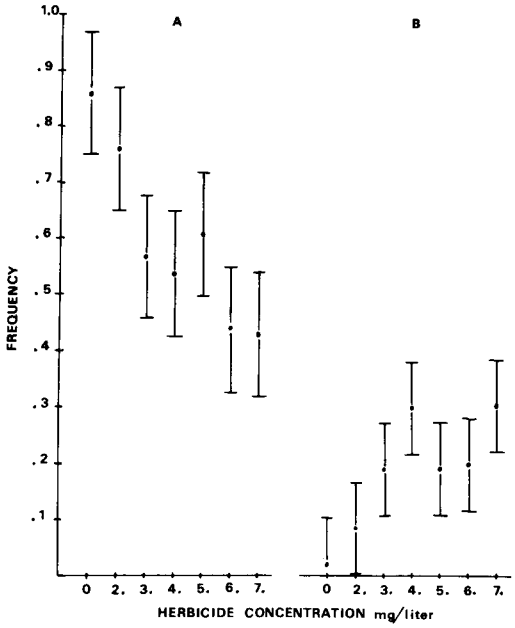


FIGURE 2.—*The effect of 24-hour exposures to the technical grade butoxyethanol ester of 2,4-dichlorophenoxyacetic acid on the frequency of positive rheotaxis (A) and the frequency of no response (B) of yearling rainbow trout. Each point on the graph represents the mean of 10 fish. Vertical bars indicate the least significant differences (LSD) as calculated from the analysis of variance of each behavioral variable. Overlapping bars indicate no significant difference between means at the 5% probability level.*

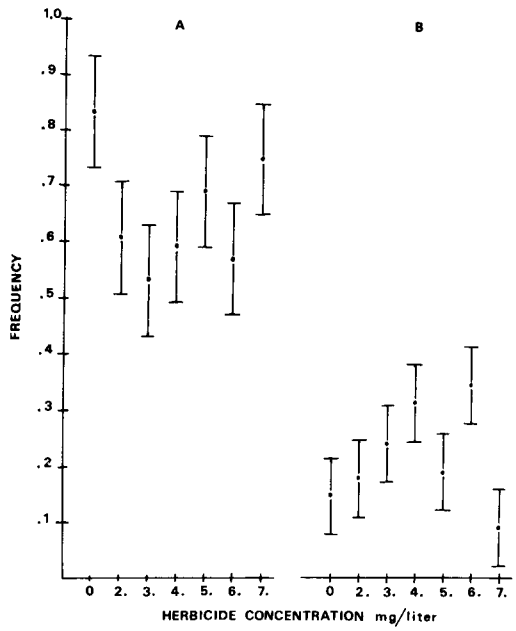


FIGURE 3.—*The effect of 24-hour exposures to ground Aqua-Kleen on the frequency of positive rheotaxis (A) and the frequency of negative rheotaxis (B) of yearling rainbow trout. Each point on the graph represents the mean of 10 fish. Vertical bars indicate the least significant differences (LSD) as calculated from the analysis of variance of each behavioral variable. Overlapping bars indicate no significant difference between means at the 5% probability level.*

samples taken from the tanks containing fish exposed to 6.0 mg/liter and 7.0 mg/liter technical grade 2,4-DBEE (Fig. 4), 8 mg/liter (active ingredient) and 9 mg/liter ground Aqua-Kleen (Fig. 5), and 12.5 mg/liter (active ingredient) and 13 mg/liter whole Aqua-Kleen (Fig. 6). In the case of all three forms of 2,4-DBEE, the concentration of 2,4-DBEE in the water declined rapidly over the 24-hour exposure period, whereas the concentration of 2,4-D increased over the first 5 to 10 hours of the exposure period. The concentration of 2,4-D remained relatively constant until the end of the exposure period in the case of the technical grade material, whereas it declined to relatively low levels after 24 hours in the case of the ground and whole Aqua-Kleen.

The concentrations of 2,4-DBEE and 2,4-D in water were also determined after 24 hours with no fish present in the water. When 1 mg/liter and 10 mg/liter of technical grade 2,4-DBEE were added to water, more than 80% of the 2,4-DBEE was present and only trace amounts of 2,4-D were detected after 24 hours.

### *Herbicide Residues in Fish Tissue*

The concentration of 2,4-DBEE in the tissue of rainbow trout exposed for 24 hours to the technical grade and formulated herbicide (ground) gradually increased as initial concentrations increased from 2 to 7 mg/liter, and then levelled off at tissue concentrations of approximately 0.3 μg/gram at initial concentrations ranging from 7 to 10 mg/liter (Fig. 7A and 7B). As initial concentrations of whole Aqua-Kleen increased from 7 to 18 mg/liter, the concentration of 2,4-DBEE in the tissue remained relatively constant at 0.5 μg/gram (Fig. 7C).

The concentration of 2,4-D in the tissue of rainbow trout exposed to the technical grade material and the ground Aqua-Kleen increased exponentially as initial concentrations increased from 2 to 10 mg/liter (Fig. 7A and 7B). The concentration of 2,4-D in the tissue of trout exposed to whole Aqua-Kleen continued to rise,

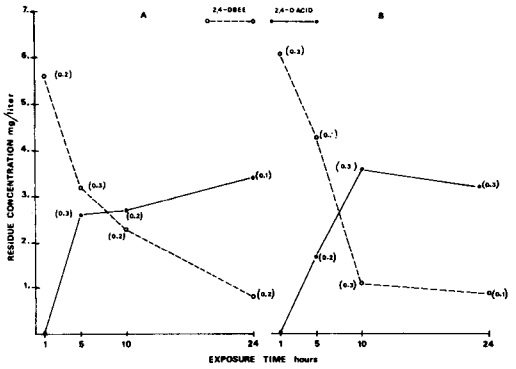


FIGURE 4.—*The concentration over 24 hours of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4-D butoxyethanol ester (2,4-DBEE) in water containing fish exposed to technical grade 2,4-DBEE. Each point on the graph represents the mean of three samples; figures in brackets represent the standard error of the mean. A: initial herbicide concentration, 6 mg/liter. B: initial herbicide concentration, 7 mg/liter.*

but less steeply than in the previous case, as initial concentrations increased from 7 to 18 mg/liter.

## Discussion

Rainbow trout exposed for 24 hours to field application concentrations of 2,4-D butoxyethanol ester and Aqua-Kleen exhibited several

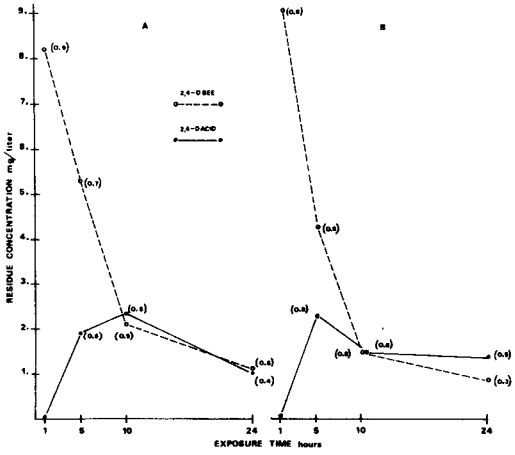


FIGURE 5.—*The concentration over 24 hours of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4-D butoxyethanol ester (2,4-DBEE) in water containing fish exposed to ground Aqua-Kleen. Each point on the graph represents the mean of three samples; figures in brackets represent the standard error of the mean. A: initial herbicide concentration, 8 mg/liter (active ingredient). B: initial herbicide concentration, 9 mg/liter (active ingredient).*

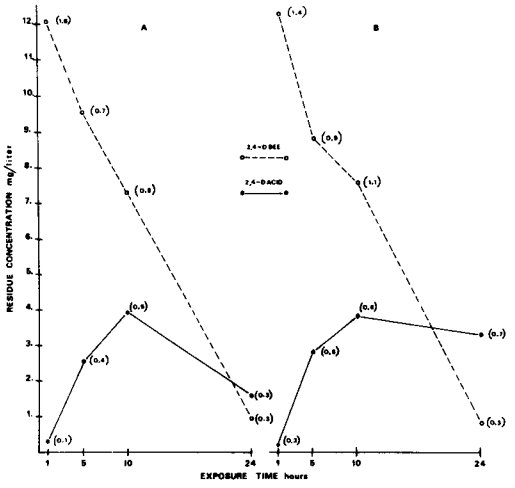


FIGURE 6.—*The concentration over 24 hours of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4-D butoxyethanol ester (2,4-DBEE) in water containing fish exposed to whole Aqua-Kleen. Each point on the graph represents the mean of three samples; figures in brackets represent the standard error of the mean. A: initial herbicide concentration, 12.5 mg/liter (active ingredient) B: initial herbicide concentration, 13 mg/liter (active ingredient).*

patterns of behavioral modification, the most evident of which was the lethargic behavior exhibited by fish exposed to concentrations approaching lethal levels. This behavior was characterized by a general appearance of sluggishness, little or no attempt to avoid capture, and an abnormally consistent pattern of rheotropism. The onset of lethargic behavior and the subsequent mortality of fish exposed to higher concentrations occurred at lower levels of the technical grade material and ground Aqua-Kleen than of whole Aqua-Kleen, suggesting that the intact granular formulation effectively decreased the toxicity of the herbicide. However, the levels of 2,4-DBEE and 2,4-D in the tissue of fish exposed to the same concentrations of all three forms of the herbicide were similar. Thus the apparent difference in toxicity between the intact and ground granular formulation cannot be explained solely by the extent of herbicide uptake after 24 hours. Rainbow trout exposed to technical grade 2,4-DBEE exhibited a significant decrease in the frequency of positive rheotaxis and a significant increase in the frequency of no-response. If the experimental variable of positive rheotaxis is interpreted as a measure of upstream orientation and the variable of no-response as a mea-

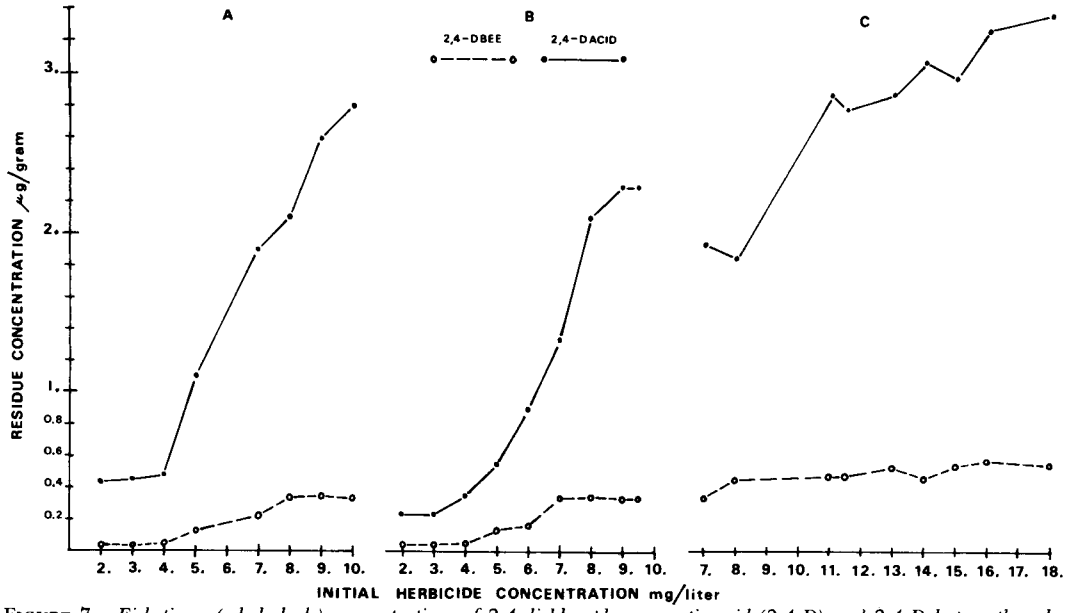


FIGURE 7.—*Fish tissue (whole body) concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4-D butoxyethanol ester (2,4-DBEE) following 24 hours of exposure to various concentrations of (A) technical grade 2,4-DBEE; (B) ground Aqua-Kleen; (C) whole Aqua-Kleen. Each point on the graph represents the value of one sample expressed as micrograms per gram of fish tissue (wet weight). The average moisture content of the fish tissue was 76.1%.*

sure of the absence of rheotropism, the observed toxicological modification of these variables would in nature lead to an increased incidence of downstream movement.

Rainbow trout exposed to ground Aqua-Kleen exhibited a different pattern of toxicological modification of rheotropism, although in nature such modification would again lead to an increased incidence of downstream movement. The frequency of positive rheotaxis initially decreased with increasing concentrations of herbicide but became variable as active-ingredient concentrations exceeded 4 mg/liter. The frequency of negative rheotaxis (interpreted as a measure of downstream orientation) initally increased with increasing concentrations of herbicide but became variable as concentrations exceeded 4 mg/liter. The levels of 2,4-DBEE and 2,4-D in the tissue of fish exposed to the technical grade material and ground Aqua-Kleen were similar. Thus, the difference in the toxicological modification of rheotropism between the two compounds cannot be explained as a function of the 24-hour uptake of the ester or the acid. It is possible that some unknown component of the formulated product was responsible for the observed differences.

The published median lethal concentrations (LC50) of 2,4-DBEE to fishes vary depending on the species of the fish and the formulation. The 96-hour LC50 for fathead minnows (*Pimephales promelas*) to a 90% active-ingredient sample of 2,4-DBEE was 5.6 mg/liter (Mount and Stephan 1967). Hughes and Davis (1963) established the 24-hour LC50 of bluegills (*Lepomis macrochirus*) to 2,4-DBEE to be 2.1 mg/liter, and Sanders (1970) established the 48-hour LC50 for the same species to be 1.1 mg/liter. The 24-hour LC50 of 2,4-DBEE to mosquitofish (*Gambusia affinis*) was 7.0 mg/liter (Hansen et al. 1972) and the 24-hour LC50 for harlequin fish (*Rasbora heteromorpha*) to 2,4-D butoxyethyl ester was 1 mg/liter (Alabaster 1969). In the present study, the lowest active-ingredient concentration of whole Aqua-Kleen tested that produced mortality in rainbow trout was 14 mg/liter, that of ground Aqua-Kleen was 8.5 mg/liter, and that of the technical grade material was 9.0 mg/liter. As field application rates of Aqua-Kleen represent active-ingredient concentrations of approximately 1.5 to 6.0 mg/liter, we may conclude that field application concentrations of 2,4-DBEE are fairly close to or overlap those concentrations lethal to several species of fish.

Little is known of the sublethal effects of 2,4-DBEE on fish. Rodgers and Stalling (1972) observed that channel catfish (*Ictalurus punctatus*) and bluegills exposed to 2,4-DBEE were very calm or almost tranquilized during the period when tissue residues were at maximum concentration. This is similar to the lethargy exhibited by our rainbow trout exposed to the herbicide. Hanson et al. (1972) demonstrated that mosquitofish avoided water containing 10 mg/liter and 1 mg/liter of 2,4-DBEE in preference for water free of herbicide. In the present study fish exposed to 2,4-DBEE exhibited behavior that in nature would lead to an increased incidence of downstream movement. Such behavior would cause fish to move into areas of decreased concentration of herbicide. Thus, the toxicological modification of rheotropism observed in rainbow trout is, in effect, a passive avoidance mechanism.

There are few studies reported in the literature dealing with the effects of toxicants in general on the orientational and locomotor behavior of fish. Davy et al. (1972) reported that chronic exposure to a subacute concentration of DDT (dichlorodiphenyltrichloroethane) impaired the retention mechanism involved in a highly significant time-dependent correlation between consecutive turns in the locomotor pattern of goldfish (*Carassius auratus*). Rand et al. (1975) reported changes in the behavioral response of goldfish to food odour and water flow following 24-hour exposures to a subacute concentration of the organophosphate parathion. It is impossible to evaluate whether these modifications of the locomotor orientation of fish are functionally related to those observed in the present study without clarifying the physiological basis of the impairments.

The dynamics of 2,4-DBEE in water is influenced by the presence of fish. In water without fish, the hydrolysis of 2,4-DBEE was slow, whereas in the presence of fish, over 80% of the ester was hydrolyzed after 24 hours. This rate appeared even greater in the case of ground and whole Aqua-Kleen. Rodgers and Stalling (1972) observed a similar situation and determined that fish rapidly absorb 2,4-DBEE, and after several hours of exposure begin to excrete 2,4-D back into the water. The appearance of increasing concentrations of 2,4-D in our water samples confirms this observation. Rodgers and Stalling (1972) concluded that the rapid uptake of 2,4-DBEE by fish may result from increased solubility and partitioning of the 2,4-D ester across the gill membrane and that hydrolysis of the ester to the acid apparently occurs after the ester passes through the gill membrane, as detectable quantities of 2,4-D acid were not concentrated from the water. The eventual decline in the concentration of 2,4-D in the water may have been due to photodecomposition and (or) microbial degradation (Schulz 1973). Adsorption of 2,4-D onto the attaclay particles may have been responsible for its more rapid decline in the tanks treated with Aqua-Kleen.

The fairly constant fish tissue concentrations of 2,4-DBEE after 24 hours of exposure to active-ingredient concentrations of 7 mg/liter and greater of the technical grade and formulated material suggest that the rate of hydrolysis of the ester to the acid was not constant but increased with increasing initial concentrations. In contrast, the increasing fish tissue concentrations of 2,4-D after 24 hours of exposure to increasing concentrations of the technical grade and formulated material suggest that the excretion rate of the acid was fairly constant and independent of initial concentrations.

Rodgers and Stalling (1972) established that the uptake of 2,4-DBEE and the elimination of 2,4-D varied according to species of fish and dietary intake. In general, peak 2,4-D concentrations occurred in the tissues after exposures of 2 to 6 hours to 2,4-DBEE. Exposures of up to 24 hours resulted in a striking decrease in total body residues. Therefore, the tissue concentrations observed in the present study after 24 hours of exposure represent minimal residue levels and the behavioral modifications observed may have resulted from damage caused by higher residue levels present earlier in the exposure period. The variable rate of ester hydrolysis and the excretion of the 2,4-D over the 24-hour exposure period may have contributed to masking the relationship between residue levels, toxicity, and sublethal effects. Further research involving shorter-term exposures will be required to localize the onset of behavioral impairment and to identify the concurrent residue levels.

In conclusion, this study has demonstrated that the butoxyethanol ester of 2,4-D and its commercial formulation Aqua-Kleen are lethal to rainbow trout at concentrations approaching

suggested field application concentrations. The attaclay particle formulation apparently decreases the toxicity of 2,4-DBEE but this cannot be explained in terms of the herbicide dynamics revealed in this study. More research is required to describe how the dynamics and toxicity of this and other herbicides are influenced by granular formulations. The impairment of positive rheotaxis at active-ingredient concentrations of 2 to 4 mg/liter may lead to the downstream displacement of trout populations. Such a behavioral modification could be advantageous as an avoidance mechanism, but could also be deleterious if rainbow trout were exposed to these concentrations during periods of upstream spawning migrations. The increasing variability of the rheotropic response and the subsequent lethargy of trout exposed to higher concentrations of Aqua-Kleen could be expected to impair migration, predator-avoidance, and a variety of other behavioral mechanisms. The results of this study warrant the implementation of controlled field experiments to document the behavior of fish and the dynamics of 2,4-DBEE and 2,4-D in water and fish during and after application of Aqua-Kleen to a variety of aquatic habitats.

## Acknowledgments

This study was supported by a grant from the Ontario Pesticides Advisory Committee to Julian J. Dodson and Colin I. Mayfield for which we express our thanks. We thank Amchem Products, Incorporated for providing the technical grade and formulated herbicide. We also wish to thank A. Deschene for valuable technical assistance during the investigations.

## References

ALABASTER, J. S. 1969. Survival of fish in 164 herbicides, insecticides, fungicides, wetting agents and miscellaneous substances. International Pest Control 11:29–35.

ANONYMOUS. 1974. Analytical methods manual. Inland Waters Directorate, Water Quality Branch, Ottawa, Canada.

ARNOLD, G. P. 1974. Rheotropism in fishes. Biological Reviews of the Cambridge Philosophical Society 49:515–576.

CAST (COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY). 1975. The phenoxy herbicides. Weed Science 23:253–263.

DAVY, F. B., H. KLEEREKOPER, AND P. GENSLER. 1972. Effects of exposure to sublethal DDT on the locomotor behavior of the goldfish (*Carassius auratus*). Journal of the Fisheries Research Board of Canada 29:1333–1336.

DODSON, J. J., AND C. I. MAYFIELD. 1979. Modification of the rheotropic response of rainbow trout (*Salmo gairdneri*) by sublethal doses of the aquatic herbicides diquat and simazine. Environmental Pollution 18:147–157.

DODSON, J. J., AND J. C. YOUNG. 1977. Temperature and photoperiod regulation of rheotropic behavior in prespawning common shiners, *Notropis cornutus*. Journal of the Fisheries Research Board of Canada 34:341–346.

EMANUEL, M. E., AND J. J. DODSON. 1979. Modification of the rheotropic behavior of male rainbow trout (*Salmo gairdneri*) by ovarian fluid. Journal of the Fisheries Research Board of Canada 36:63–68.

HANSEN, D. J., E. MATTHEW, S. L. NALL, AND D. P. DUMAS. 1972. Avoidance of pesticides by untrained mosquitofish, *Gambusia affinis*. Bulletin of Environmental Contamination and Toxicology 8:45–51.

HARDEN JONES, F. R. 1968. Fish migration. St. Martin's Press, New York, New York, USA.

HUGHES, J. S., AND J. R. DAVIS. 1963. Variations in toxicity to bluegill sunfish of phenoxy herbicides. Weeds 11:50–53.

MOUNT, D. I., AND C. E. STEPHAN. 1967. A method for establishing acceptable toxicant limits for fish—malathion and the butoxyethanol ester of 2,4-D. Transactions of the American Fisheries Society 96:185–193.

RAND, G., H. KLEEREKOPER, AND J. MATIS. 1975. Interaction of odour and flow perception and the effects of parathion in the locomotor orientation of the goldfish *Carassius auratus* L. Journal of Fish Biology 7:497–504.

RODGERS, C. A., AND D. L. STALLING. 1972. Dynamics of an ester of 2,4-D in organs of three species of fish. Weed Science 20:101–105.

SANDERS, H. O. 1970. Toxicities of some herbicides to six species of freshwater crustaceans. Journal of the Water Pollution Control Federation 42:1544–1550.

SCHULZ, D. P. 1973. Dynamics of a salt of (2,4-dichlorophenoxy)acetic acid in fish, water and hydrosol. Journal of Agricultural and Food Chemistry 21:186–192.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. H. Freeman and Company, San Francisco, California, USA.