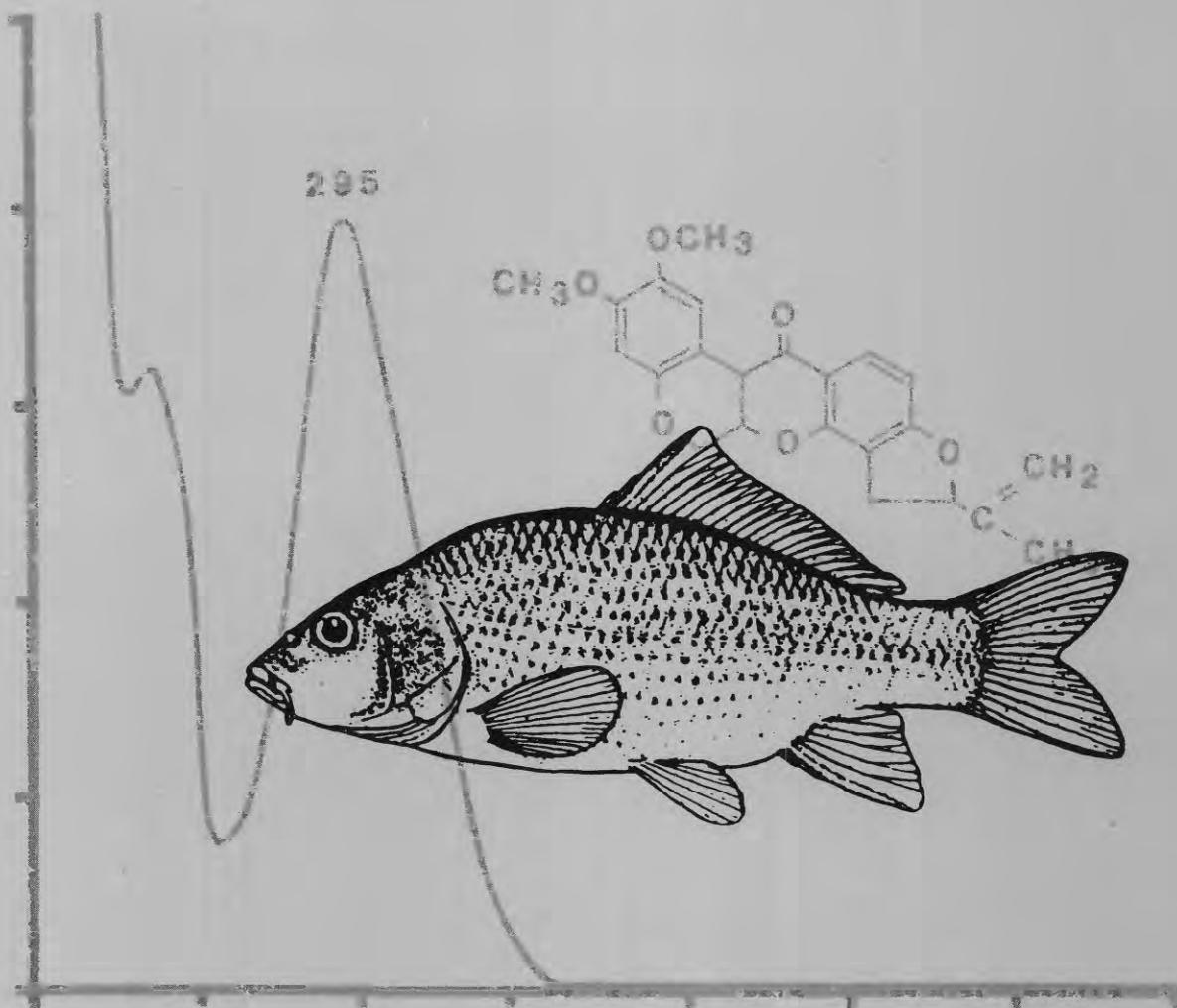


INVESTIGATIONS IN FISH CONTROL

92. Acute and Chronic Toxicity of Rotenone to *Daphnia magna*

93. Toxicity of Rotenone to Developing Rainbow Trout

94. Oral Toxicity of Rotenone to Mammals



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Washington, D.C. • 1988

Acute and Chronic Toxicity of Rotenone to *Daphnia magna*

by

J. J. Rach, T. D. Bills, and L. L. Marking

*National Fisheries Research Center
U.S. Fish and Wildlife Service
P.O. Box 818
La Crosse, Wisconsin 54602*

Abstract

The continued use of rotenone as a fish toxicant depends on the development of information requested by the U.S. Environmental Protection Agency for reregistration. To meet one of the requirements, we exposed *Daphnia magna* to rotenone in toxicity tests. In exposures of 0.5 to 10.0 $\mu\text{g/L}$ rotenone in a 48-h acute toxicity test, the EC50 was 3.7 $\mu\text{g/L}$; in exposures of 0.312 to 5.0 $\mu\text{g/L}$ in a 21-day chronic toxicity test, the EC50 was 2.1 $\mu\text{g/L}$. The no-observed-effect concentration was 1.25 $\mu\text{g/L}$.

Rotenone is widely used in fishery management for the removal of unwanted fish populations. Its usefulness stems from its high toxicity to fish, low toxicity to mammals, and rapid decomposition in the environment (Lennon et al. 1970; Haley 1978). Its use has been questioned, however, by environmental groups and others who are concerned about the use of chemicals in the environment.

The U.S. Environmental Protection Agency (EPA) has requested data needed for the reregistration of rotenone. During the last several years, studies of its toxicity, accumulation, and depuration in fish, mammals, invertebrates, and plants have been completed as part of the EPA requirements.

One of the studies required consisted of acute and chronic toxicity tests on *Daphnia magna*, the organism chosen because it is sensitive to toxic substances, is small, can be easily identified, is available from laboratories and commercial suppliers, and has been used extensively in toxicity testing (EPA 1984). The tests we conducted to meet this requirement are described here.

Materials

Analytical grade rotenone (96.47% pure) used for this study was obtained from the S. B. Penick Corporation, Lyndhurst, New Jersey. Purity was determined by high pressure liquid chromatography (HPLC) as described by Dawson et al. (1983).

Reconstituted water, prepared by adding selected salts to deionized water used for all exposures and culture waters, had the following characteristics: pH 7.6–8.0, hardness 160–180 mg/L as CaCO_3 , and alkalinity 110–120 mg/L as CaCO_3 (ASTM Committee E-35 on Pesticides 1980). Water samples were analyzed according to methods of the American Public Health Association et al. (1985).

Controls for the acute exposures included a blank water control, and a blank acetone control that contained acetone at a concentration equal to that in the highest exposure concentration. Controls for the chronic test were similar, except that algal food was added.

Exposure concentrations of rotenone used were 0.5, 2.5, 5.0, 7.5, and 10.0 $\mu\text{g/L}$ in the acute toxicity test and 0.312, 0.625, 1.25, 2.5, and 5.0 $\mu\text{g/L}$ in the chronic toxicity test. For both types of tests, rotenone stock solutions were prepared in 2-L volumetric flasks containing diluted water and rotenone; for the chronic toxicity tests, predetermined amounts of algal cells and food supplement were added. Rotenone concentrations in all tests were verified analytically by HPLC.

For the analysis of rotenone concentrations, we collected samples from the stock solutions before algal cells and food supplement were added. Rotenone was concentrated from the samples by the method of Dawson et al. (1983). The volume of water extracted increased with decreases in the theoretical rotenone concentration: 400 mL were extracted for the 5.0 $\mu\text{g/L}$ concentration and 800 mL for the 2.5- and 1.25- $\mu\text{g/L}$ concentrations. Water samples were filtered, buffered to pH 5.0, and passed through a disposable Baker C₁₈ column with the aid of a Baker-10 vacuum manifold. Extracted rotenone was then eluted from the columns with 2 mL of methanol.

For the direct analysis of eluted methanol samples, we used a Waters WISP710B autoinjector in conjunction with a Waters 510 HPLC pump, Waters 481 LC spectrophotometer, and a Micro-Pak (30 cm \times 4 mm) MCH-10 reverse-phase column. A mobile phase of methanol:water (78:22; volume:volume) was used at a flow rate of 1.0 mL/min. An ultraviolet spectrophotometer (wavelength 295 nm) enabled detection of rotenone in samples; attenuation was 0.01. Rotenone concentrations in the samples were calculated on the basis of peak area.

Cultures of *Daphnia magna* were maintained at $20 \pm 1^\circ\text{C}$ in a constant temperature water bath, at a photoperiod of 16 h light and 8 h darkness. We transferred adult brood stock daphnids to fresh water weekly in fire-polished pipettes with an inside diameter of 5–8 mm or at least 1.5 times the diameter of the daphnids. We introduced the organisms beneath the surface of the new medium to avoid trapping air under the carapace. Cultures were considered healthy and suitable for use when survival in each culture was $>90\%$ over a 2-week period, no air-locked daphnids were present, no ephippia were produced, and large numbers of young were present (EPA 1984).

Adult daphnids that were about to have their second to sixth broods were cultured under conditions similar to those described. Young daphnids produced from these adults provided brood stock for later testing. These young brood stock daphnids were reared in 2-L glass containers, each having 20 daphnids per 1,600 mL of water. The young produced by these brood daphnids were used for acute and chronic tests (EPA 1984).

The alga *Selenastrum capricornutum*, obtained from the EPA Laboratory, Duluth, Minnesota, was cultured at $20 \pm 2^\circ\text{C}$ in supplemented Woods Hole Marine Biological Laboratory medium (EPA 1984), as food for the daphnids. It was inoculated into three sterile culture vessels and allowed to bloom. The resulting cultures were used to maintain the *Daphnia magna* brood stock and to feed the experimental animals during the chronic toxicity study. The daphnids were provided algae at densities of 10^8 cells/L of test water, as recommended by EPA (1984). A liquefied solution of a commercial trout food, used to supplement the *Selenastrum* culture (EPA 1984), was added every other day to the exposure beakers and to *Daphnia* cultures to yield a concentration of 5 mg/L.

Procedures in Acute and Chronic Tests

A routine procedure was used to ensure that the young daphnids used in the tests were <24 h old. One day before the test, we removed 15 adult brood daphnids and transferred them into individual 100-mL beakers containing 80 mL of water and food. The next day we transferred the offspring to a 1-L beaker containing 800 mL of water. The young daphnids were then randomly pipetted into the exposure beakers used for the acute and chronic tests. Photoperiod in all tests was 16 h light and 8 h darkness.

In the acute test, daphnids were transferred into test vessels, which were 100-mL beakers containing 80 mL of test solution. Four beakers, each containing five daphnids, were required for each experimental treatment (control, acetone control, and each test concentration). The beakers were labeled, covered with watch glasses, and randomly placed in a water bath at $20 \pm 1^\circ\text{C}$. After 48 h, the beakers were removed and mortality was recorded. We determined EC₅₀ values (toxicant concentration resulting in immobilization or death of 50% of the exposed organisms) and estimated 95% confidence interval (C.I.) by the methods of Litchfield and Wilcoxon (1949).

In the chronic test, one daphnid was transferred into each of 10 100-mL beakers containing 80 mL of test solution with food supplement. As in the acute test, the beakers were labeled, covered with watch glasses, and randomly placed in a water bath at $20 \pm 1^\circ\text{C}$.

Daphnids for the chronic exposures were transferred to clean beakers with fresh test solutions and observations on mortalities and molting were made three times a week (Monday, Wednesday, Friday). Rotenone concentrations were verified by HPLC on days 0, 7, 14, and 19. Chemical characteristics of the reconstituted water used to prepare rotenone test solutions were checked on days 0, 7, and 19. The number of young produced and water

Table 1. Analysis of the reconstituted water for 21-day chronic toxicity test. (Temperature was 20°C throughout the test.)

Date (1986)	pH	Alkalinity (mg/L)	Hardness (mg/L)	Dissolved oxygen (mg/L)
16 April	8.34	112	166	9.0
23 April	8.18	108	164	^a
5 May	8.02	94	168	9.4

^aNo determination.

bath temperature were recorded daily. Surviving daphnids on day 21 were measured under a microscope equipped with an ocular micrometer. Lengths of the daphnids (top of the head to the base of the spine) in each concentration were statistically compared by analysis of variance and application of the Student-Newman-Keuls multiple range test (Sokal and Rohlf 1969).

Criteria for a valid *Daphnia* chronic toxicity test require that control animals produce an average of at least 40 young, and that survival in the control units be 80% or higher over the 3-week test (EPA 1984). The EC50 values and the 95% C.I. were determined by the method of Litchfield and Wilcoxon (1949).

Results

Chemical characteristics of the reconstituted water for the chronic study (Table 1) remained within acceptable limits established by the ASTM Committee E-35 on Pesticides (1980). Chemical characteristics of the water

in the rotenone exposures were analyzed at the start and end of batch replacements. Concentrations of components remained within the established acceptable ranges, except that alkalinity levels were slightly below normal (Table 2).

Rotenone concentrations of 0.312 and 0.625 µg/L were below minimum detection levels; only the solutions containing 1.25, 2.5, and 5.0 µg/L rotenone were analyzed (Table 3). Measured rotenone concentrations (mean ± SD) were 1.38 ± 0.078 µg/L for the 1.25-µg/L concentration, 2.63 ± 0.137 µg/L for the 2.5-µg/L concentration, and 5.27 µg/L for the 5.0-µg/L concentration.

In the acute toxicity test, all daphnids survived in the controls and at 0.5 µg/L rotenone, but only 5% survived at 10.0 µg/L (Table 4). The calculated 48-h EC50 value for rotenone was 3.7 µg/L (95% C.I., 2.5–5.5 µg/L).

In the 21-day chronic toxicity test, the survival rate in the acetone control was 80%, and the no-observed-effect concentration was 1.25 µg/L (Table 5). The calculated EC50 value for rotenone in the chronic 21-day test was 2.1 µg/L (95% C.I., 0.9–4.9 µg/L). Each surviving daphnid produced 40 or more young in the rotenone concentrations of 0.312–1.25 µg/L, but only 19 in the 2.5-µg/L concentration. The size (lengths) of daphnids in the control group and the rotenone-treated groups (Table 6) did not differ significantly.

Discussion

The standard toxicity tests described here were conducted to update the data base on rotenone toxicity to crustaceans. *Daphnia magna* was used as the test organism because of its importance in the aquatic environment and because it has been recommended by the EPA for

Table 2. Chemical characteristics of rotenone exposure water used for a batch replacement during the 21-day chronic toxicity test, freshly prepared and after 3 days of exposure. (Temperature was 20°C throughout the test.)

Test concentration (µg/L)	pH		Alkalinity (mg/L)		Hardness (mg/L)		Dissolved oxygen (mg/L)	
	Fresh	3 days	Fresh	3 days	Fresh	3 days	Fresh	3 days
0.0 (control)	8.02	8.16	94	106	168	166	9.4	8.7
0.312	8.29	8.44	94	97	164	164	9.4	8.8
0.625	8.30	8.41	105	100	168	166	9.2	8.7
1.25	8.31	8.43	104	98	166	164	9.4	8.6
2.5	8.26	8.45	111	103	164	166	9.4	8.8

Table 3. Rotenone concentrations ($\mu\text{g/L}$)^a determined by high pressure liquid chromatography during the 21-day chronic toxicity test.

Day	Date (1986)	Calculated rotenone concentrations ($\mu\text{g/L}$)		
		1.25	2.5	5.0 ^b
0	16 April	1.33	2.53	5.27
7	23 April	1.49	2.55	—
14	30 April	1.33	2.61	—
19	5 May	1.36	2.83	—

^aRotenone was not detectable at calculated concentrations of 0.312 or 0.625. Detection level was 1 $\mu\text{g/L}$.

^bAll organisms died within 5 days.

Table 4. Survival of daphnids ($n = 20$) exposed to rotenone in a 48-h acute toxicity test.

Concentration ($\mu\text{g/L}$)	Survival (%) ^a
0.0 (water control)	100
0.0 (acetone control)	100
0.5	100
2.5	80
5.0	25
7.5	15
10.0	5

^aEC50 = 3.7 $\mu\text{g/L}$; range, 2.5–5.5 $\mu\text{g/L}$.

Table 5. Survival of daphnids ($n = 10$) exposed to rotenone during a 21-day chronic toxicity test.

Concentration ($\mu\text{g/L}$)	Survival of test organisms (%) ^a	Number of young produced by test organisms	Average number of young produced per surviving adult
0.0 (water control)	90	483	53.7
0.0 (acetone control)	80	456	57.0
0.312	70	459	65.6
0.625	100	578	57.8
1.25	80	351	43.9
2.5	40	78	19.5
5.0	0	—	—

^aEC50 = 2.1 $\mu\text{g/L}$; range, 0.9–4.9 $\mu\text{g/L}$.

Table 6. Mean lengths (mm) of daphnids in chronic exposure groups.

Concentration ($\mu\text{g/L}$)	Length (mm)	
	Mean	SE
0.0 (water control)	3.82	0.17
0.0 (acetone control)	3.94	0.08
0.312	3.83	0.17
0.625	3.88	0.04
1.25	3.56	0.08
2.5	3.32	0.28

standard toxicity testing (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975).

The toxicity data indicated that daphnids were much more sensitive than fish to rotenone, as shown earlier by Kemp et al. (1971). The 48-h EC50 value for *Daphnia* was 3.7 $\mu\text{g/L}$, whereas Marking and Bills (1976) showed that, for fish, the LC50 ranged from 21.5 to 389 $\mu\text{g/L}$ of the formulated material. Even though the toxicity data for fish and daphnids were developed in a laboratory environment, it is likely that daphnid numbers would be reduced after rotenone was used to remove nuisance fish populations. However, the organisms are prolific and have

been shown to recover quickly from chemical treatments and adverse conditions (Jacobi and Degan 1977).

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Rach, J. J., T. D. Bills, and L. L. Marking. 1988. Acute and chronic toxicity of rotenone to *Daphnia magna*. U.S. Fish Wildl. Serv., Invest. Fish Control 92. 5 pp.

The continued use of rotenone as a fish toxicant depends on the development of information requested by the U.S. Environmental Protection Agency for re-registration. To meet one of the requirements, we exposed *Daphnia magna* to rotenone in acute and chronic toxicity tests. In exposures of 0.5 to 10.0 µg/L rotenone in a 48-h acute toxicity test, the EC50 was 3.7 µg/L; in exposures of 0.312 to 5.0 µg/L in a 21-day chronic toxicity test, the EC50 was 2.1 µg/L. The no-observed-effect concentration of rotenone to daphnids was 1.25 µg/L.

Key words: Rotenone, acute toxicity, chronic toxicity, *Daphnia magna*, no-observed-effect concentration.

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Toxicity of Rotenone to Developing Rainbow Trout

by

T. D. Bills, J. J. Rach, and L. L. Marking

*U.S. Fish and Wildlife Service
National Fisheries Research Center
P.O. Box 818
La Crosse, Wisconsin 54602*

Abstract

In a flow-through toxicity test, we exposed eyed eggs and larvae of rainbow trout (*Salmo gairdneri*) for 32 days to crystalline technical grade rotenone to determine its effects on survival, hatching, and growth. Rotenone concentrations of 1.0 to 10.0 $\mu\text{g/L}$ did not kill the eyed eggs, which hatched normally after 5 or 6 days of exposure. Mortality of young larvae increased as rotenone concentrations increased. After 32 days of exposure, and after all larvae had transformed to the swim-up stage, the LC50 was 2.08 $\mu\text{g/L}$. Hatching time was unaffected by exposures to rotenone, but growth was significantly decreased in fish that survived concentrations of 2.21 and 2.75 $\mu\text{g/L}$.

Rotenone has been used extensively as an insecticide, and has been used by fishery managers since the 1930's to remove unwanted fish populations from lakes and streams (Schnick 1974). The toxicity of rotenone to aquatic organisms is well documented (Marking and Bills 1976); however, owing to changes in regulations of the U.S. Environmental Protection Agency regarding the toxic effects of contaminants on aquatic organisms, the U.S. Fish and Wildlife Service undertook additional studies to determine whether rotenone can be safely used as a piscicide.

The present study (funded by the Division of Federal Aid of the U.S. Fish and Wildlife Service) was designed to evaluate the effects of rotenone on the eyed eggs and young of rainbow trout (*Salmo gairdneri*) under chronic exposure conditions. We observed the effects on hatching and on growth and mortality of larvae during a 32-day exposure. This period was chosen because embryos or larvae have been shown to be the life stages most sensitive to toxicants (Woltering 1984).

Materials and Methods

Crystalline rotenone (96.47% pure) for the study was supplied by S. B. Penick and Company. Tests were conducted with a proportional diluter (Mount and Brungs 1967) that provided about 12 turnovers of test solution daily. Test organisms were exposed to rotenone continuously for 28 days at the following concentrations (mean \pm SD): 0, 1.01 \pm 0.09, 2.21 \pm 0.266, 2.75 \pm 0.424, 4.37 \pm 0.092, 5.32 \pm 0.197, 7.52 \pm 0.577, and 10.0 \pm 0.436. We measured concentrations of rotenone in water by high pressure liquid chromatography (HPLC), using the method of Dawson et al. (1983). Samples for analysis were collected once every 5 days. Dissolved oxygen and temperature in test solutions were monitored daily.

Chemical characteristics of water used in the tests were determined in accordance with the methods outlined by the American Public Health Association et al. (1985): average pH was 8.0, total hardness was 138 mg/L as CaCO₃, and total alkalinity was 105 mg/L as CaCO₃.

Table 1. Cumulative mortality (%) in duplicated exposures (100 each) of eyed eggs and early larval stages of rainbow trout to rotenone in water at 12°C.^a

Day	Exposure concentration (µg/L)							
	0.00	1.01	2.21	2.75	4.37	5.32	7.52	10.04
1 ^b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5 ^c	0.0	0.5	0.0	0.0	0.0	0.0	0.0	6.0
10	3.5	2.0	6.0	17.0	97.5	99.0	100.0	100.0
15	4.5	2.0	33.0	91.0	100.0	100.0	100.0	100.0
20	6.0	2.5	47.5	94.0	100.0	100.0	100.0	100.0
25	6.5	3.0	53.0	96.5	100.0	100.0	100.0	100.0
32 ^d	7.0	4.0	58.0	96.5	100.0	100.0	100.0	100.0

^aLethal concentrations (µg/L) and 95% confidence at 32 days: LC50, 2.08 (1.98–2.18); and LC01, 1.00 (0.89–1.12).

^bAll eggs were eyed.

^cEggs began hatching at all concentrations.

^dAll surviving larvae reached swim-up stage and appeared to be searching for food.

Eyed rainbow trout eggs obtained from Trout Lodge, Inc., McMillan, Washington, were handled according to procedures outlined by Hunn et al. (1968). Mortality was determined daily and dead eggs or larvae were removed.

Testing was done in accordance with methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975), ASTM Committee E-35 on Pesticides (1980), and Horning and Weber (1985). Duplicate groups of 100 organisms were exposed to each test concentration. The LC01 and LC50 (concentrations producing 1 and 50% mortality) and 95% confidence intervals (C.I.) were calculated according to the method of Litchfield and Wilcoxon (1949). Data on growth in length after 32 days of exposure were analyzed by one-way analysis of variance, and the significance of differences in length among treatment means was determined by use of the Student-Newman-Keuls multiple range test.

Results and Discussion

Measured concentrations of rotenone in the exposure solutions ranged from 1.01 to 10.04 µg/L. Dissolved oxygen remained at 8.1 mg/L or higher and water temperature was held at 12.0°C throughout the tests.

No rainbow trout eggs died as a result of exposure to rotenone (Table 1). Hatching was not affected; all eggs hatched on the fifth or sixth day of exposure. All larvae exposed to concentrations of 4.37 µg/L or higher died within 5 days after hatching, and 90% of those exposed to 2.75 µg/L died within 15 days. The 32-day LC50 (and 95% C.I.) was 2.08 µg/L (1.98–2.18) and the 32-day LC01 was 1.00 µg/L (0.894–1.12).

Growth of fry that survived exposure to 2.21 or 2.75 µg/L rotenone was significantly less ($P < 0.05$) than that of the controls (Table 2). Studies of mammals chronically exposed to rotenone have also shown dose-related responses: In rats, pup weights and weight gains were both reduced after exposure to 37.5 and 75.0 mg/L diet concentrations of rotenone (however, at these high concentrations the reduced food intake may have caused the weight differences); rotenone exposure had no effect on the reproductive performance of either sex in two successive generations (MacKenzie and Kehoe 1983).

Noxfish, a 5% emulsifiable formulation of rotenone, was less toxic to eggs than to fingerlings in rainbow trout in static exposures (Marking and Bills 1976). The 96-h LC50 for newly fertilized eggs ranged from 2.5 mg/L in very hard water to 5.6 mg/L in very soft water; commensurate values for fingerlings were 53.0 µg/L in very hard water and 54.4 µg/L in very soft water. Accordingly, green eggs of rainbow trout were more resistant than

Table 2. Mean length of surviving rainbow trout after 32 days of exposure to rotenone at three concentrations.

Exposure concentration (µg/L)	Length (mm)	
	Mean	SD
0 (control)	26.7	1.27
1.01	26.8	1.65
2.21	21.7 ^a	1.35
2.75	23.6 ^a	0.53

^aSignificantly shorter ($P < 0.05$) than the controls or the fish exposed to 1.01 µg/L.

eyed eggs, and both egg stages were far more resistant than fingerlings.

There is little likelihood of chronic exposures of fish or fish eggs to rotenone because the approved use patterns and the label restrictions specify application periods of less than 12 h in streams and single applications in ponds or lakes. Rotenone dissipates rapidly and degrades in the natural environment (Schnick 1974); only in cold water does it remain toxic for more than 4 days. P. A. Gilderhus (personal communication) demonstrated that residues persisted for as long as 57 days in water when ice was present, but that treated waters were no longer toxic to fathead minnows (*Pimephales promelas*) after 30 days.

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Bills, T. D., J. J. Rach, and L. L. Marking. 1988. Toxicity of rotenone to developing rainbow trout. U.S. Fish Wildl. Serv., Invest. Fish Control 93. 3 pp.

Eyed eggs and resulting larvae of rainbow trout (*Salmo gairdneri*) were exposed for 32 days to technical grade rotenone to determine its effects on survival, hatching, and growth. Rotenone concentrations of 1.0 to 10.0 µg/L did not kill the eyed eggs, which hatched normally after 5 or 6 days of exposure. After 32 days of exposure, and after all larvae had transformed to the swim-up stage, the LC50 was 2.08 µg/L. Growth was significantly decreased in fish that survived concentrations of 2.21 and 2.75 µg/L.

Key words: Rotenone, embryo, larvae, swim-up, LC50, growth, hatching.

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Oral Toxicity of Rotenone to Mammals

by

Leif L. Marking

*U.S. Fish and Wildlife Service
National Fisheries Research Center
P.O. Box 818
La Crosse, Wisconsin 54602*

Abstract

Information required by the U.S. Environmental Protection Agency to support the continued use of rotenone as a fish toxicant includes data on toxicity to nontarget organisms. Information summarized here was developed in three mammalian safety studies of rotenone: chronic oral toxicity in rats, effects on reproduction in rats, and subchronic oral toxicity in dogs. The no-observed-effect level (NOEL) of rotenone, determined in rats in a 24-month exposure, was 7.5 mg per kilogram of diet. The only difference related to exposure was the lower weight of rats exposed to the higher concentrations (37.5 and 75.0 mg/kg)—probably because the animals ate less food as a result of taste avoidance. Rotenone administered continuously to two successive generations of rats at concentrations of 7.5, 37.5, and 75.0 mg/kg in the diet had no effect on the reproductive performance of either sex. The NOEL for toxicity again was 7.5 mg rotenone per kilogram of diet. In beagles that received daily doses in gelatin capsules, animals that received the highest daily dose rate of 10 mg/kg showed the most obvious effects: diarrhea; decreased feed consumption; weight loss during the first 2 months of exposure; mild anemia; and consistent decreases in blood glucose, total lipids, and cholesterol. A daily oral dose of 2 mg/kg produced only mild signs of these disorders, and the low dose of 0.4 mg/kg was considered the NOEL in dogs. Results of studies reported here and in the literature show that even unusually high treatment rates of rotenone do not cause tumors or reproductive problems in mammals.

In recent years, there has been increasing concern about environmental effects of pesticides, particularly their toxicity to nontarget organisms. The use of many pesticides has been curtailed or terminated because of suspected hazards of parent compounds or degradation products. Attention has been directed even at compounds that long were "generally regarded as safe."

Rotenone has been used as a fish toxicant for more than 50 years (Schnick 1974) and also has a long history of safe use as an insecticide. However, its continued registration for this use requires the development of information on its toxicity to nontarget organisms in accordance with

regulations of the U.S. Environmental Protection Agency (EPA).

Numerous formulations of rotenone have been used as fish toxicants (Schnick et al. 1986). The present registration carries two specific restrictions: Fish killed by rotenone cannot be used as food for humans or animals; and the toxicant can be applied only by, or after consultation with, State or Federal agencies. Rotenone is now the compound most widely used for the control or eradication of unwanted fish populations, and fish managers hope that the registration of rotenone for this purpose will be maintained. Current EPA regulatory guidelines indicate that

additional information on safety to nontarget aquatic organisms and mammals is needed.

Methods

Contract research on rats and dogs for determination of mammalian safety is summarized here. The studies were done in accordance with the Good Laboratory Practice Regulations as required in the Code of Federal Regulations [21 CFR 58.35(b)(6)(7)]. Authorized methods and standard procedures were used in each study and a Quality Assurance Unit performed inspections and verified the accuracy of the results. The data from each study have been submitted to the EPA for inclusion in the rotenone file.

Three studies are summarized:

1. Chronic toxicity study of rotenone in rats. Study 6115-100, Volumes 1-4, Hazleton Laboratories America, Inc., 3301 Kinsman Boulevard, Madison, Wisconsin. (U.S. Fish and Wildlife Service contract 14-16-009-81-043.)
2. Reproduction study for safety evaluation of rotenone using rats. Study 81007, Volumes 1-3, Hazleton Raltech, Inc., a subsidiary of Hazleton Laboratories America, Inc., 3301 Kinsman Boulevard, Madison, Wisconsin. (U.S. Fish and Wildlife Service contract 14-16-009-79-097.)
3. Subchronic oral dosing study for safety evaluation of rotenone using dogs. Midwest Research Institute Project 4853-B, Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri. (U.S. Fish and Wildlife Service contract 14-16-0009-79-115.)

Details of the studies and records of all the original data are in bound volumes on file at the National Fisheries Research Center, U.S. Fish and Wildlife Service, P.O. Box 818, La Crosse, Wisconsin 54602.

Chronic Toxicity of Rotenone in Rats

Chronic effects of rotenone in rats were determined by submitting the animals to continuous dietary exposure for 24 months. A total of 320 Fischer 344 rats, 6 weeks old, were assigned at random to groups (40 animals of each sex per group) and fed 0, 7.5, 37.5, or 75.0 mg of rotenone per kilogram of feed. Doses were based on the results of a 15-day subchronic study in which doses of 600 and 1,200 mg/kg caused death in rats of both sexes. The lower dose sequence for the present study was designed to establish a no-observed-effect level (NOEL).

The exposure that began when the rats were about 6 weeks old continued for 2 years. Body weights were recorded weekly for 12 weeks and every fourth week thereafter. Food consumption was recorded weekly through 12 weeks and then for weeks 26, 39, 52, 65, 78, 92, and 104. Urinalyses and hematology and blood chemistry tests were done at 3, 6, 12, 18, and 24 months of exposure. At terminal sacrifice, all animals were examined macroscopically, selected organs were weighed, and selected tissues were prepared for microscopic study.

The incidence of clinical signs did not differ significantly among groups. Males and females treated with 37.5 and 75.0 mg/kg had significantly lower mean body weights and cumulative dose-related body weight gains than did those of their respective control groups (Table 1). However, the feed consumption of females treated with 37.5 and 75.0 mg/kg was lower than that of control females, probably because of taste avoidance.

Lower total protein and albumin in high-dose-group females and higher serum urea nitrogen in females of the middle and high dose group seemed to be treatment related. However, no histopathology was noted that could be correlated with these changes.

Treatment-related findings were minor or lacking. The only macroscopic finding was the lower average body weight of animals treated at the high doses. In both sexes, terminal body weights and, correspondingly, organ weights and ratios of organ weight to body weight, were significantly lower in animals treated with 37.5 and 75.0 mg/kg of rotenone than in controls or animals treated at 7.5 mg/kg. No pathological evidence was observed in the hematology, blood chemistry, urinalysis, or histology of exposed individuals. The NOEL of rotenone for rats in a 24-month exposure was 7.5 mg/kg in the diet.

Effects of Rotenone on Reproduction in Rats

The present study was conducted to determine the effects of rotenone on reproductive function and fetal development in two successive generations of rats that had been exposed continuously through their diet. Information was collected from two litters (F_{1a} and F_{2a}) produced by two generations of rats (F_0 and F_{1a}) through the weaning of F_{2a} litters and terminal necropsy of F_{1a} adults. Immature Charles River CD(SD)BR rats (4 weeks old upon arrival) were later mated to provide F_0 generation animals.

Table 1. Summary of weights (g) of rats fed diets containing different concentrations of rotenone.

Sex and week of study	Dietary rotenone concentration (ppm)											
	0			7.5			37.5			75.0		
	Mean	SD	Number	Mean	SD	Number	Mean	SD	Number	Mean	SD	Number
Males												
0	118.3	6.40	40	117.8	5.57	40	115.0 ^a	5.24	40	115.9	5.81	40
20	348.5	24.03	40	343.3	21.30	40	342.1	21.07	40	342.7	16.03	40
40	390.4	26.68	39	385.5	26.25	40	380.4	24.46	40	380.0	18.81	40
60	412.0	32.04	39	410.7	28.73	37	401.6	27.54	39	402.0	20.21	39
80	401.2	28.59	36	399.0	32.46	36	383.0 ^a	24.59	37	365.1 ^a	24.16	39
104	384.2	26.46	25	382.8	33.43	24	354.1 ^a	21.30	34	327.8 ^a	17.71	31
Females												
0	90.1	3.61	40	90.5	3.06	40	89.1	3.87	40	89.8	2.67	40
20	209.0	9.95	40	210.8	8.42	40	198.0 ^a	8.63	40	175.1 ^a	8.02	40
40	233.8	12.24	40	231.4	9.59	40	211.1 ^a	9.50	38	180.9 ^a	8.14	39
60	271.7	17.35	40	266.9	19.41	40	235.2 ^a	11.95	37	190.5 ^a	8.22	38
80	292.8	22.01	39	284.6	21.21	39	229.0 ^a	12.48	35	185.0 ^a	7.98	38
104	306.5	31.94	29	309.1	15.95	32	239.2 ^a	19.29	27	187.7 ^a	10.58	35

^aGroup mean is significantly different from the mean of the control group at $P < 0.05$ (Dunnett's test).

The rotenone used in this study, which was 97–98% pure, was incorporated into the diet at rates of 0, 7.5, 37.5, and 75.0 mg/kg. For each treatment group, 15 males and 25 females were selected at random and continued on the rotenone-treated diet. The F₀ generation animals received selected concentrations of rotenone in the diet continuously from 6 weeks of age through weaning of F_{1a} litters and until they were necropsied during week 33 on test. The F_{1a} generation animals selected for the reproduction study were exposed to rotenone while in utero, through weaning, and in their diet continuously through termination of the study during week 32 of the test.

A dose-related decrease in the average body weight of parental male and female animals began in week 13 and continued throughout the study. Body weights of F₀ and F_{1a} generation male rats exposed to 37.5 and 75.0 mg/kg were significantly lower than those of control animals. However, reduced organ weights were detected only at the highest treatment level and seemed to be related to the reduced body weights.

Mean litter size at birth was smaller in the high treatment group than in the controls for the F_{1a} and F_{2a} litters (Table 2). Treatment-related reductions in pup weights and in weight gains were detected throughout lactation. There were no other significant differences in litter data, and no physical or behavioral abnormalities were apparent in any of the offspring.

It was concluded that rotenone administered to two successive generations of rats at concentrations as high as 75.0 mg/kg in the diet had no effect on reproductive performance of either sex or on fetal development. The NOEL for toxicity was determined to be 7.5 mg/kg of rotenone in the diet.

Subchronic Toxicity of Rotenone to Dogs

The subchronic toxicity of rotenone to dogs was determined by administering the compound in gelatin capsules daily at one of three treatment rates. Toxicity was ascertained by monitoring for toxic, pharmacologic, and behavioral changes; for feed consumption and body weight changes; for hematologic and blood chemistry changes; for gross necropsy and histopathologic examinations; and for the survival of treated animals. The intent was to define a toxic dose and a safe dose.

A total of 60 AKC-registerable beagles (30 of each sex), 4 to 5 months old, were used in the studies. In a preliminary 28-day study, the dogs were exposed to daily doses of 0.08 to 50 mg/kg to establish treatment levels to be used for the main study. Six males and six females were then given daily doses of 0.0, 0.4, 2.0, or 10 mg/kg of encapsulated rotenone for 26 weeks.

Table 2. Numbers and weights of rats in litters at day 21 of exposure to different concentrations of rotenone.

Generation and dietary rotenone concentration (ppm)	Number of litters	Litter size (number)		Survival		Pup weight (g)	
		Mean	SD	Mean	SD	Mean	SD
Generation F _{1a}							
0	23	9.7	0.56	98.7	3.44	47.5	4.56
7.5	18	9.6	0.77	98.4	5.01	45.9	3.53
37.5	22	9.1	1.57	96.9	8.17	37.7*	5.03
75.0	21	8.6	1.56	96.5	7.44	24.4*	4.08
Generation F _{2a}							
0	20	9.5	1.32	100	—	45.6	5.97
7.5	18	8.5	2.37	100	—	43.2	6.24
37.5	21	9.3	1.63	97.1	7.17	36.3*	6.58
75.0	21	8.0	2.65	95.1	11.12	24.6*	8.37

*Significantly different from control ($P < 0.01$).

The major effect caused by rotenone was on the gastrointestinal tract; diarrhea or soft stools occurred throughout the study and were more common in the males. The dogs became tolerant of the gastrointestinal effects and survived the entire test period, even at the highest dose rate. No treatment-related effects were seen in the urinalyses or in the histopathologic evaluations.

The high daily dose of 10 mg/kg adversely affected gastrointestinal functions: diarrhea, decreased feed consumption, and weight loss were observed during the first 2 months of exposure. In addition, dogs in this group showed a mild anemia and consistent decreases in blood glucose, total lipids, and cholesterol.

The daily dose of 2 mg/kg was less toxic and caused a milder form of the gastrointestinal effects. No decrease in body weight was evident until the later months of the study. The low daily dose of 0.4 mg/kg produced no observed effects and was considered the NOEL in dogs.

Discussion

Most studies on the toxicity of rotenone to mammals have been on the rat. Lehman (1952) found an increased incidence of nodules and tumors in the livers of rats fed rotenone; however, a later similar study on rats by Hansen et al. (1965) showed no tumors, and a mouse bioassay by Innes et al. (1969) revealed no tumorigenicity.

The toxicity of chemicals to mammals and fish is difficult to compare because the routes of exposure are

usually different. Rotenone is purported to be extremely toxic to fish and much less toxic to mammals; however, Erickson and Gingerich (1986) reported the intravenous LD₅₀ to be 305 µg/kg of rotenone in rainbow trout (*Salmo gairdneri*)—indicating that intravenous toxicity in fish is similar to that in mammals when rotenone is administered in defined doses by the same route.

In a study with dogs, Hansen et al. (1965) reported that groups of four beagles fed a diet containing fixed concentrations of rotenone for 28 months developed no unusual symptoms. The highest daily dose rate was 0.52 mg/kg of rotenone. In a simultaneous study with rats, Hansen et al. (1965) reported that 50 mg/kg had no effect, but that doses as high as 1,000 mg/kg caused reduction in weight gains. In another study, Khera et al. (1982) reported that exposure of rats to daily doses of 5 and 10 mg/kg of rotenone was associated with reductions in maternal body weight gain, fetal weight, and skeletal ossification.

Gosalvez and Merchan (1973) reported that 6 weeks or more of daily intraperitoneal dosing of rats with rotenone caused transplantable mammary tumors to develop some months later. This information placed suspicion on the safety of rotenone and led to the imposition by EPA of a temporary Rebuttable Presumption Against Registration. However, when other scientists were unable to reproduce the results of Gosalvez and Merchan, the restriction was removed. Results of the studies summarized here and in the literature show that even high doses of rotenone do not cause tumors or reproductive failure, nor adversely affect fetal development.

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Marking, L. L. 1988. **Oral toxicity of rotenone to mammals.** U.S. Fish Wildl. Serv., *Invest. Fish Control* 94. 5 pp.

Information summarized was developed from three mammalian safety studies of rotenone: chronic oral toxicity in rats, effects on reproduction in rats, and subchronic oral toxicity in dogs. The no-observed-effect level (NOEL) of rotenone, determined in rats in a 24-month exposure, was 7.5 mg per kilogram of diet. Rotenone administered continuously to two successive generations of rats at concentrations of 7.5 to 75.0 mg/kg in the diet had no effect on reproductive performance. The NOEL for rotenone in beagles that received daily doses in gelatin capsules was 0.4 mg/kg. Even these unusually high treatment rates of rotenone did not cause tumors or reproductive problems in mammals.

Key words: Rotenone, oral toxicity, no-observed-effect level, rats, beagles.

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(Reports 73 through 76 are in one cover.)

73. Formalin: Its Toxicity to Nontarget Aquatic Organisms, Persistence, and Counteraction, by T. D. Bills, L. L. Marking, and J. H. Chandler, Jr. 1977. 7 pp.
74. Chlorine: Its Toxicity to Fish and Detoxification of Antimycin, by L. L. Marking and T. D. Bills. 1977. 5 pp.
75. Malachite Green: Its Toxicity to Aquatic Organisms, Persistence, and Removal with Activated Carbon, by T. D. Bills, L. L. Marking, and J. H. Chandler, Jr. 1977. 6 pp.
76. Toxicity of Furanace to Fish, Aquatic Invertebrates, and Frog Eggs and Larvae, by L. L. Marking, T. D. Bills, and J. H. Chandler, Jr. 1977. 6 pp.

(Reports 77 through 79 are in one cover.)

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82. *Investigations in Fish Control*: Index to Numbers 1-72, 1964-1976, by R. A. Schnick and K. A. Graves. 1977. 19 pp.

(Reports 83 through 85 are in one cover.)

83. Survival of Two Species of Freshwater Clams, *Corbicula leana* and *Magnoniais boykiniana*, After Exposure to Antimycin, by L. L. Marking and J. H. Chandler, Jr. 1978. 5 pp.
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86. Registration of Thirty-three Fishery Chemicals: Status of Research and Estimated Costs of Required Contract Studies, by R. A. Schnick and F. P. Meyer. 1978. 19 pp.

(Reports 87 through 89 are in one cover.)

87. Ethyl-*p*-aminobenzoate (Benzocaine): Efficacy as an Anesthetic for Five Species of Freshwater Fish, by V. K. Dawson and P. A. Gilderhus. 1979. 5 pp.
88. Influences of Selected Environmental Factors on the Activity of a Prospective Fish Toxicant, 2-(Digeranyl-amino)-ethanol, in Laboratory Tests, by C. A. Launer and T. D. Bills. 1979. 4 pp.
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