THE DETERMINATION OF THE ACUTE TOXICITY OF ROTENONE AND BAYER 73 TO SELECTED AQUATIC ORGANISMS

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ABSTRACT

The acute toxicity of Bayer 73 to carp (Cyprinus carpio) and green sunfish (Lepomis cyanellus), the effects of sediment and ultraviolet radiation, and a standard curve for Bayer 73 were determined. The toxicity of Bayer 73 to carp increased with an increase in exposure time. The toxicity to carp and green sunfish decreased with an increase in hardness and pH. The presence of sediment particles does not appear to affect the efficacy of Bayer 73. Exposure of Bayer 73 to ultraviolet light for 96 hours did not affect its toxicity to carp. Fifty ug/l of Bayer 73 reduced the growth of Chlamydomonas sp. and Chlorella vulgaris by 50%. No growth of either species occurred when exposed to 100 ug/l of Bayer 73.

The toxicity of two formulations of rotenone, (Noxfish and Dri-Noxfish) were tested on frogs (Rana pipiens), crayfish (Orconectes immunis), scuds (Gammarus limnaeus), caddisflies (Hesperophylax sp.), and clams (Lampsilis sp.). Both formulations of rotenone are less toxic to the non-fish organisms than to fish. The effect of total hardness, pH, and exposure time varied with the organism tested. The toxicity of Dri-Noxfish was generally greater than Noxfish.

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INTRODUCTION

The use of fish toxicants reportedly came into existence in North America during the late 19th century (Lennon, Hunn, Schnick, and Burress, 1970). The first recorded use of a specific poison for eradication of fish was in 1913 when Titcomb (1914) applied copper sulfate to a lake in Vermont. In 1936, the Department of Fisheries of Canada used powdered rotenone to eradicate fish from a stream that supplied water to a fish hatchery (M'Gonigle and Smith, 1938). Application and use of various toxicants, such as antimycin, toxaphene, and rotenone, in water management programs have become widely used only since about 1950.

In 1895, Geoffroy isolated rotenone from a French Guiana plant called _Lonchocarpus nicou_. The powdered root of plants belonging to the Family Leguminosae had been used for centuries by natives of Borneo and Malay as a general fish poison (Ambrose and Haag, 1963). Rotenone, as a powdered derris root form, was first used in 1934 in Michigan. By the beginning of 1950, 34 states and Canada were using rotenone extensively (Lennon, Hunn, Schnick, and Burress, 1970).

There presently are 11 different formulations of rotenone commercially available. The two formulations used in this project were Noxfish[R1] and Dri-Noxfish[R2]. Noxfish contains 5% rotenone and

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[R1] Registered trade-mark of S. B. Penick Co., New York, N.Y.

[R2] Registered trade-mark of S. B. Penick Co., New York, N.Y.

and 10% other extracts from plant material (cubé) of the Family Leguminosae. Dri-Noxfish contains 20% rotenone and 40% other cubé extracts. Rotenone (Fig. 1), acts as an inhibitor of the mitochondrial respiratory chain. Its site of action may be between the NAD and flavoprotein or it may induce configural changes in the region of the $NADH_2$ dehydrogenase flavoprotein, and ubiquinone or cytochrome b (Burgos and Redfearn, 1965).

Bayer 73 (Bayluscide[R3]) was first synthesized by Gonnert and Schraufstatter (1958) as the 2-aminoethanol salt of 2', 5-dichloro-4'-nitrosalicylanilide (Fig. 2). Preliminary test results showed Bayer 73 was superior to the available molluscides copper sulfate, and sodium pentachlorophenate (Gonnert, 1961). The mechanism of action of Bayer 73 has not been isolated, although Gonnert (1962) reported that the active ingredient inhibited the enzymes in the respiratory and carbohydrate metabolism systems. The uptake of oxygen is inhibited at toxicant concentrations above 0.2 mg/l. Since its introduction, the use of Bayer 73 has continued to increase in removal of snails that are vectors of *Schistosoma sp.* and *Fasciola sp.*

Even though rotenone has been in wide use for 20 years, there is a lack of data as to the effect of rotenone on nontarget species. Although Bayer 73 has been in use for 12 years, definite results

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[R3] Registered trade-mark of Farbenfabriken Bayer AG, Leverkusen, Germany.

to the effect of certain environmental parameters on the efficacy of Bayer 73 have not been fully investigated. The following study was undertaken to determine the toxicity of rotenone to selected aquatic organisms and to determine the effect of ultraviolet radiation and sediment on the efficacy of Bayer 73.

LITERATURE REVIEW

The S. B. Penick Company (1962) listed the acute oral LD_{50} values of rotenone for chickens as 6.00 mg/kg of body weight, for rabbits as 1.70 mg/kg of body weight, and for rats as 0.02 mg/kg. Rotenone is nontoxic to lambs at 50 mg/l, and nontoxic to ducks at 25 mg/l. Pimentel (1971) listed the LD_{50} values for mammals (species not specified) as 132 mg/kg, mallards as 2000 mg/kg, and pheasants as 1414 mg/kg.

Brown and Ball (1943) demonstrated that the Tennessee sculpin (Cottus carolinae) was killed by rotenone in two hours with concentrations of 2 - 3 mg/l. Bridges and Cope (1965), using rotenone, conducted bioassays on rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), and bluegill (Lepomis macrochirus). Their results produced LC_{50} values for 24 hours of 31 ug/l, 33 ug/l, and 26 ug/l for rainbow trout, channel catifsh, and bluegill, respectively. In the same order, the 96 hour LC_{50} values were 27 ug/l, 28 ug/l, and 23 ug/l. Howland (1969) reported LC_{50} values for bluegill as 22 ug/l and for coho salmon (Oncorhynchus kisutch) as 150 ug/l. Marking (unpublished) listed LC_{50} values for both Noxfish and Dri-Noxfish. Noxfish values were: rainbow trout - 58 ug/l, carp (Cyprinus carpio) - 150 ug/l, green sunfish (Lepomis cyanellus) - 133 ug/l, and bluegill - 148 ug/l. For Dri-Noxfish, the LC_{50} values were: rainbow trout - 62 ug/l, carp - 326 ug/l, green sunfish - 156 ug/l, and bluegill - 150 ug/l.

The documentation of the effect of rotenone on invertebrate organisms is not as complete as it is for fish, with most reported data from field tests. Pimentel (1971) reported the 24 hour LC_{50} values of a 5% rotenone formulation on Pteronarcys californiea as 2.90 mg/l, Gammarus lacustris as 6.00 mg/l, and a 48 hour LC_{50} value for Daphnia pulex as 0.01 mg/l. Zischkale (1952) did not give LC_{50} values but listed concentrations required to produce a 25% or more kill in 48 hours for: Daphnia sp. (0.10 mg/l), Hyalella sp. (0.20 mg/l), Eurycypris sp. (0.10 mg/l), Palaemonetes sp. (4.00 mg/l), Culex aedes (2.00 mg/l), Chironomid larvae (0.10 mg/l), Physa sp. (4.50 mg/l), and Helisoma sp. (3.50 mg/l). Binns (1967), in a report on the effects of rotenone on the Green River in Wyoming, indicated that after treatment with concentrations of rotenone ranging from 2.50 - 9.40 mg/l, mayflies did not recover for 8 months and caddisflies recovered in about 12 months.

Almquist (1959) reported the exposure time and concentration of rotenone required to produce a 100% kill of Diaphanosoma sp. as 0.50 - 0.60 mg/l in 1 hour, Scapholeberis sp. as 0.50 - 0.60 mg/l in 2 hours, Chironomid larvae as 0.50 - 0.60 mg/l in 23 hours, Ceratium sp. as 1.00 mg/l in 4 hours, and ephemerid larvae as 2.00 mg/l in 7 hours.

Bayer 73 studies by Gonnert and Schraufstatter (1958) indicated that rats tolerated oral doses of 59 g/kg. Cows of 230 kg body weight received 2.30 grams/day orally for 93 days with no effect being noticed. Gonnert (1961), reporting on work by Hecht and Gloxhuber, reported that dosages to rats and rabbits of 5 g/kg, to cats and dogs of 1 g/kg, and to humans of 0.39 g/kg, produced no stress. Reports on laboratory and field tests of Bayer 73 on snails (intermediate hosts of schistosomiasis) have been reported by Gillet and Bruaux (1962), Gonnert (1961), Walker, Starkey, and Marking (1966), and Strufe (1962). Walker, Starkey, and Marking (1966) reported on the toxicity of Bayer 73 and related compounds to fish. Marking and Hogan (1967) completed a study of Bayer 73 and its relation to water temperature and water quality. Their tests on fish produced 24 hour LC_{50} values that ranged from 0.04 - 0.23 mg/l. Flathead catfish (Pylodictus olivaris), rainbow trout, and brown bullheads were the most sensitive species, with green sunfish and fathead minnows (Pimephales promelas) being the least sensitive (Marking and Hogan, 1967). The effects of temperature, organic sediment and ultraviolet light on the efficacy of Bayer 73 have been investigated by Gonnert and Schraufstatter (1958). Exposing Bayer 73 to mud at a temperature of 22 - 23 C at pH 7.70 - 7.80, they determined that approximately 66% of the active compound was present after 20 hours, 50% after 45 hours, and 38% after 70 hours. Gonnert (1961) tested the effect of temperature on the activity of Bayer 73, concluding that at concentrations of 0.30 mg/l, 50% of the snails tested died in 6 hours at a temperature of 11 C, and 80% died in 6 hours at 22 C. Strufe and Gonnert (1962) using a 40 watt Westinghouse sunlamp of 290 - 365 nM and assay water of 342 mg/l hardness, concluded that Bayer 73 was stable up to 240 minutes of exposure in weakly acid water. The action of mud on the toxicity of Bayer 73 in a control test without mud showed a 20% loss of activity in 120 hours. With the addition of river mud, a 70% loss of activity occurred in 120 hours. Gillet and Bruaux (1962) exposed two concentrations of Bayer 73 (0.5 and 0.25 mg/l) to a 30 watt Philips ultraviolet lamp at a distance of 104 cm. A 12 hour exposure demonstrated no effect, but a 24 hour exposure showed the activity at 0.50 mg/l reduced to 1/11 that of an unexposed control solution. The 0.25 mg/l solution resulted in an activity of 1/6 that of the unexposed control solution.

Strufe (1962) listed two methods for the determination of the concentration of Bayer 73 by colormetry. In the first method, Bayer 73 was extracted from the aqueous solution and then converted to a yellow color by the addition of alkali hydroxide. The second method involved the addition of Safranin TH to produce a red color. The complex was removed from the aqueous solution by organic solvents.

MATERIALS AND METHODS

Bioassay Procedures

The standard bioassay procedures used were those described by Lennon and Walker (1964). Two exceptions in the techniques for washing the 20 liter jars were used. After rinsing the jar, tap water instead of distilled water was used in conjunction with the activated charcoal. The second exception was the use of Deox[R4] and hydrochloric acid in washing jars.

All tests were conducted for 96 hours with mortalities recorded at 0.5, 1, 3, 6, 24, 48, 72, and 96 hours. All tests were maintained at 12 C in a flowing water bath. The maximum amount of solvent was added to each control vessel as was added to a test vessel. LC_{50} values, for all tests conducted, were calculated for each 24 and 96 hour period. One exception was the clam *Lampsilis sp.* for which a 96 hour LC_{50} value only was calculated. Reconstituted water was obtained by adding the appropriate salts to distilled water (Table 1).

Toxicant Preparations

Bayer 73 (control No. C910) was obtained from the Federal Fish Control Laboratory in La Crosse, Wisconsin. The formulation contained 99% 2-aminoethanol salt of 2',5-dichloro-4-nitrosalicylanilide.

R4 Registered trade-mark of National Chemsearch Corporation, Irving, Texas.

Noxfish (lot No. 1125-LMI-5) and Dri-Noxfish (lot No. 1625-LJM-11) were obtained from the S. B. Penick Company, New York, N. Y. Noxfish is a 5% rotenone, liquid-emulsifiable preparation, and Dri-Noxfish is a 20% dispersible powder.

Acetone was used as the solvent for Bayer 73. For Noxfish and Dri-Noxfish, glass double-distilled water was used. Stock solution for the three preparations was obtained by the general formula: 1.50 g (toxicant)/100 ml solvent.

Bayer 73 Tests

A standard curve for aqueous solutions of Bayer 73 at concentrations above 20 ug/l was established. The standard solutions were made from a common stock solution using glass-distilled water. The spectrum analysis was conducted on the Beckman DB spectrophotometer at 262 nM. Concentrations were plotted on the ordinate and absorbance was plotted on the abscissa.

The effects of water quality on the toxicity of Bayer 73 was determined for very soft (10 - 13 mg/l $CaCO_3$), soft (40 - 48 mg/l $CaCO_3$), hard (160 - 180 mg/l $CaCO_3$), and very hard (280 - 320 mg/l $CaCO_3$) water. For each water quality, 1 control and 11 concentrations of toxicant per test were used. The concentrations ranged from 50 - 400 ug/l. Each assay vessel contained 15 liters of water. The control vessel contained an additional volume of acetone and water equal to that used for the highest concentration. Each test was conducted for a 96 hour period. All test vessels were maintained at 12 C by a flowing water bath.

The effect of sediment on the toxicity of Bayer 73 was determined by adding sediment to each assay vessel. The sediment, consisting of organic muck (2% nitrogen by weight) was obtained from the Mississippi River near La Crosse, Wisconsin. The sediment was placed in a blender to thoroughly mix the material and to break apart large pieces of organic debris. The material was then autoclaved for two hours. After cooling and drying, the sediment was added to each assay vessel at the rate of 50 g/10 l of soft water. The material was allowed to settle for 24 hours. Ten of the appropriate species of fish were then added to each test vessel. After an additional 24 hours, various concentrations of toxicant were added. The tests were conducted for 96 hours.

The 10 milliliter aliquots (300 ug/l) of a Bayer 73 stock solution were added to glass petri plates. One-half of the plates were covered with Saran Wrap and exposed to ultraviolet light from a General Electric[R5] medium UV range sunlamp. The remaining petri plates were tightly sealed in aluminum foil. All plates were placed in a hood at a distance of one meter from the light source (flux density, 1,100 lux). Four plates were removed from the hood at 1, 2, 4, 8, 16, 24, 48, 72, and 96 hours. When the last plates were removed, the contents of each plate were added to a vessel containing 10 liters of soft water and 10 carp. The test procedures given before were then followed.

[R5] General Electric Lamp Bulb No. RS

Cultures of Chlamydomonas sp. and Chlorella vulgaris were grown on BBM (Bolds Basal Medium) for 10 days at 23 C in 12 hours of light and 12 hours of darkness. A one milliliter alloquot of the cultures were added to 200 ml of BBM in 500 ml Erlenmeyer flasks containing 1 - 500 ug/l of Bayer 73. After 8 days of growth the algae were centrifuged for 5 minutes at 5700 G., dried, and weighed.

Rotenone Tests

Both formulations of rotenone (Noxfish and Dri-Noxfish) were tested under identical conditions of temperature and water qualities (soft and hard water). Two tests were conducted for each water quality and for each organism tested. Crayfish (Orconectes immunis), frogs (Rana pipiens), and clams (Lampsilis sp.) were obtained from a biological supply house. Scuds (Gammarus limnaeus), and caddisflies (Hesperophylax sp.) were collected from a spring at Coon Valley, Wisconsin. All organisms, except the scuds, were acclimated to test conditions for 72 hours. The scuds were acclimatized for a 24 hour period. Crayfish, clams and frogs were tested in 15 liters of water. Caddisflies and scuds were tested in 1 gallon glass jars containing 1 liter of reconstituted water. All tests were conducted for 96 hours.

Statistical Procedures

The analytical procedures used were those described by Litchfield and Wilcoxon (1949). The concentrations (doses) were plotted on the abscissa of three phase log paper. The mortalities at each concentration were converted to a percent of the total number of organisms tested at that concentration. These percentages were converted to probits and plotted on the ordinate. The probits were used to convert the sigmoid line of dose effects to a straight line. The chi square was used to determine the goodness of fit of the data. The mathematics used established LC_{50} values (lethal concentration for 50% mortality) and 95% confidence intervals for each test conducted.

RESULTS AND DISCUSSION

Bayer 73

The effect of four different water qualities on the toxicity of Bayer 73 to carp and green sunfish was established to provide a point of reference for future tests. The results (Table 2) indicated that the toxicity of Bayer 73 to carp was directly proportional to exposure time. This trend did not appear to hold true for green sunfish except in hard water. The only difference in the toxicity of Bayer 73 to green sunfish, in relation to time, occurred in hard water, with a decrease of 47 ug/l noted between the 24 and 96 hour LC_{50} values. The second important result was that toxicity decreased with the increase in water hardness and pH. With carp, a decrease of 60 ug/l for very soft water and 30 ug/l for soft water was noted for 96 hour LC_{50} values as compared to the 24 hour LC_{50} values. This difference increased to 90 ug/l for hard water. The differences in toxicity between soft and hard water was 340 ug/l for 24 hours and 380 ug/l for 96 hours. The values established in this study are slightly lower, with the exception of hard water, than the values reported by Marking and Hogan (1967). Their study also showed Bayer 73 to have the highest toxicity at pH 7, the pH of soft water (Table 1).

The results of the effect of sediment on the toxicity of Bayer 73 to carp and green sunfish indicated a maximum 24 hour LC_{50}

difference of 20 ug/l to carp and 8 ug/l to green sunfish. The 96 hour LC_{50} value differences were 8 ug/l for carp and green sunfish (Table 3). The efficacy of Bayer 73 did not appear to be affected by sediment. These differences in values did not agree with a study by Strufe and Gonnert (1962) in which a 24 hour decrease in toxicant activity of 30% and a decrease of 50% in 96 hours was reported. However, the procedures utilized in the two studies varied considerably. Strufe and Gonnert used nonsterile river mud, mechanical stirring, carbon-filtered tap water and a temperature of 22 - 23 C. The only variable in the present study, in relation to the standard bioassay test, was the addition of the sediment itself. The differences in the results of the two studies might be accounted for by the differences in temperature and sterility of the sediments.

The differences between the toxicity of UV radiated Bayer 73 and nonradiated Bayer 73 on carp were small (Fig. 3). The slope of the two lines appeared to be due to the aging or self oxidation of the toxicant. Strufe and Gonnert (1962) made a similar test using a 70% wetable powder. Their results showed that after a 200 minute exposure to UV radiation, the activity of Bayer 73 was reduced by an appreciable amount. The difference in the formulations of Bayer 73 might be a possible reason for the differences between the study by Strufe and Gonnert and the study presented here.

Bayer 73 concentrations of 50 ug/l reduced the growth of both Chlamydomonas sp. and Chlorella vulgaris by approximately 50%. No growth occurred at concentrations of 100 ug/l (Fig. 4). Gonnert (1961), reporting on the field tests of Shilf and Garnett, indicated a decrease in Chara sp. and Spirogyra sp. at a concentration of 1 mg/l.

The standard curve established for Bayer 73 (Fig. 5) was applicable to the laboratory situation. Although Strufe (1962) presented two methods for quick estimates of toxicant concentrations for field work, accuracy was not established for concentrations below 100 ug/l. Although the results were linear, the analytical method presented here is not accurate for concentrations below 20 ug/l. This method measured the toxicant concentration and not a reaction product such as is used in colormetric determinations.

Noxfish

Tests were conducted with Noxfish utilizing frogs (Rana pipiens), crayfish (Orconectes immunis), Scuds (Gammarus limnaeus), caddisflies (Hesperophylax sp.), and clams (Lampsilis sp.). Its toxicity varied with species, with soft and hard water, and with length of exposure times (Table 4). The most resistant species appeared to be the crayfish. LC_{50} values for 24 hours, for soft and hard water, were 34.50 and 47.20 mg/l respectively. The 96 hour values, however, were lower than for any of the other organisms tested. The LC_{50} values were 1.02 and 1.18 mg/l for soft and hard water respectively. Noxfish was more toxic to scuds in hard water at 24 hours while the reverse was true for the other organisms tested.

All values obtained for the test organisms were higher than established LC_{50} values for rainbow trout (0.05 - 0.06 mg/l), for carp (0.07 - 0.15 mg/l), green sunfish (0.11 - 0.16 mg/l), and for bluegill (0.15 - 0.19 mg/l) (Marking, unpublished).

Almquist (1959) reported 100% kill of cladocera, midges, and protozoa at Noxfish concentrations of less than 1.50 mg/l in 24 hours or less. However, Pimental (1971) reported a 24 hour LC_{50} value of 6 mg/l for the scud (*Gammarus lacustris*).

Dri-Noxfish

The test results using Dri-Noxfish followed the same general pattern as that of Noxfish. The crayfish showed the highest 24 hour LC_{50} values of any organism tested, 10.75 mg/l for soft water and 9.62 mg/l for hard water (Table 5). The toxicity of Dri-Noxfish was generally greater than that of Noxfish. This would indicate that toxicity was directly related to the rotenone content. One exception was the reduced toxicity of Dri-Noxfish to the clam (*Lampsilis sp.*).

All values obtained in this study, for both the 24 and 96 hour tests, were higher than values obtained by Marking (unpublished) for fish. LC_{50} values for 24 hours, obtained by Marking ranged from 0.06 mg/l for rainbow trout to 0.35 mg/l for carp. Hard water values were 0.07 mg/l and 0.15 mg/l for rainbow trout and green sunfish, respectively. LC_{50} values for 96 hours were 0.04 mg/l for rainbow trout and 0.24 mg/l for carp. Hard water LC_{50} values were 0.07 and 0.08 mg/l for rainbow trout and green sunfish, respectively.

SUMMARY AND CONCLUSIONS

1. The acute toxicity of Bayer 73 to carp (Cyprinus carpio) and green sunfish (Lepomis cyanellus) was determined. The effects of sediment and ultraviolet radiation on the toxicity of Bayer 73 were also studied.

2. A standard curve established for Bayer 73 indicated a linear relationship between absorbance, at 262 nM and toxicant concentration. Accuracy was established for concentrations as low as 20 ug/l.

3. Bayer 73 toxicity to carp increased with an increase in exposure time. An increase in toxicity with time was not noted for green sunfish except in hard water.

4. Bayer 73 decreased in toxicity to carp and green sunfish with an increase in water hardness and pH.

5. The efficacy of Bayer 73 did not appear to be affected by sediment. The largest differences noted between tests with sediment and those without sediment was 20 ug/l for carp (in 24 hours) and 8 ug/l for green sunfish in 24 and 96 hours.

6. A 96 hour exposure of Bayer 73 to medium wave length ultraviolet light did not affect its toxicity to carp.

7. A 50 ug/l concentration of Bayer 73 reduced the growth of Chlamydomonas sp. and Chlorella vulgaris by 50% in 8 days. At a concentration of 100 ug/l, no growth occurred.

8. The toxicity of two formulations of rotenone, Noxfish and Dri-Noxfish, were tested on frogs (*Rana pipiens*), crayfish (*Orconectes immunis*), scuds (*Gammarus limnaeus*), caddisflies (*Hesperophylax sp.*), and clams (*Lampsilis sp.*).

9. Noxfish (5% rotenone) was more toxic to fish than to the organisms tested. The effect of water hardness, pH, and exposure time varied with the organism tested.

10. Tests conducted with Dri-Noxfish (20% rotenone) demonstrated higher values than values reported for fish. The toxicity of Dri-Noxfish was generally greater than Noxfish. This would indicate that toxicity is directly related to rotenone content.

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APPENDIX

TABLE 1. SALTS REQUIRED FOR RECONSTITUTED WATER

Water Type	Salts Added In mg/liter $NaHCO_3$	$CaSO_4$	$MgSO_4$	KCl	pH Range	Total Hardness ($mg/l\ CaCO_3$)
Very Soft	12	7.5	7.5	0.5	6.4-6.8	10-13
Soft*	48	30.0	30.0	2.0	7.2-7.6	40-48
Hard	192	120.0	120.0	8.0	7.6-8.0	160-180
Very Hard	384	240.0	240.0	16.0	8.0-8.4	280-320

* Standard bioassay water

TABLE 2. TOXICITY OF BAYER 73 TO CARP AND GREEN SUNFISH IN VERY SOFT, SOFT, HARD, AND VERY HARD WATER

Organism	Water Quality	LC_{50} Values and 95% Confidence Intervals (ug/l) at	
		24 Hours	96 Hours
Cyprinus carpio	Very Soft	170 (133-215)	110 (94-128)
	Soft	150 (56-399)	120 (103-139)
	Hard	490 (349-687)	400 (340-469)
	Very Hard	450 (385-525)	--
Lepomis cyanellus	Very Soft	86 (73-100)	86 (73-101)
	Soft	170 (152-189)	170 (152-189)
	Hard	260 (236-285)	213 (192-235)

TABLE 3. LC_{50} VALUES AND 95% CONFIDENCE INTERVALS (ug/l) FOR BAYER 73 TO FISH IN SOFT WATER, WITH AND WITHOUT SEDIMENT (50g/l)

Organism	With Sediment		Without Sediment	
	24 Hours	96 Hours	24 Hours	96 Hours
Cyprinus carpio	170 (140-206)	128 (109-146)	150 (56-399)	120 (130-139)
Lepomis cyanellus	139 (117-153)	102 (94-111)	131 (120-143)	94 (81-110)

TABLE 4. LC_{50} VALUES AND 95% CONFIDENCE INTERVALS (mg/l) FOR NOXFISH IN SOFT AND HARD WATER

	24 Hours		96 Hours	
Species	Soft Water	Hard Water	Soft Water	Hard Water
Rana pipiens	4.8 (3.7-6.3)	24.0 (21.1-27.2)	4.8 (3.7-6.3)	5.8 (3.6-9.8)
Orconectes immunis	34.5 (20.3-58.2)	47.2 (30.2-61.3)	1.0 (0.4-2.0)	1.2 (0.4-3.8)
Gammarus limnaeus	11.6 (6.2-13.6)	9.2 (5.4-14.5)	8.0 (5.7-8.8)	2.5 (1.3-4.7)
Hesperophylax sp.	10.4 (6.7-14.9)	15.0 (9.8-19.0)	3.4 (0.8-4.2)	2.5 (1.2-6.3)
Lampsilis sp.	--	--	2.7 (1.2-6.5)	2.3 (1.2-4.0)

TABLE 5. LC_{50} VALUES AND 95% CONFIDENCE INTERVALS (mg/l) FOR DRI-NOXFISH IN SOFT AND HARD WATER

	24 Hours		96 Hours	
Species	Soft Water	Hard Water	Soft Water	Hard Water
Rana pipiens	7.3 (6.3-10.3)	7.9 (6.2-9.8)	4.6 (4.3-4.9)	3.2 (2.3-4.3)
Orconectes immunis	10.8 (6.9-19.0)	9.6 (5.4-14.4)	0.7 (0.2-1.5)	0.4 (0.3-0.6)
Gammarus limnaeus	6.6 (3.7-10.0)	5.8 (3.8-9.0)	1.7 (0.7-4.0)	1.8 (0.9-3.6)
Hesperophylax sp.	5.1 (4.1-6.0)	5.1 (4.3-5.9)	3.2 (2.2-4.0)	3.6 (3.0-4.7)
Lampsilis sp.	--	--	12.9 (6.6-39.1)	11.3 (4.8-20.8)

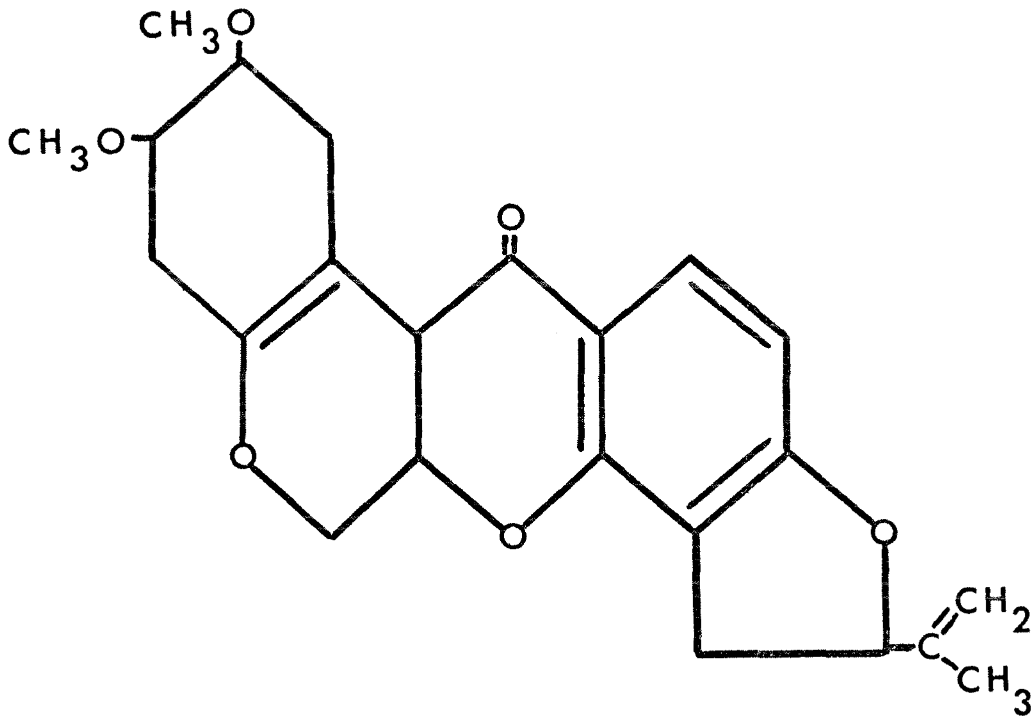

Figure 1. Structural formula of rotenone.

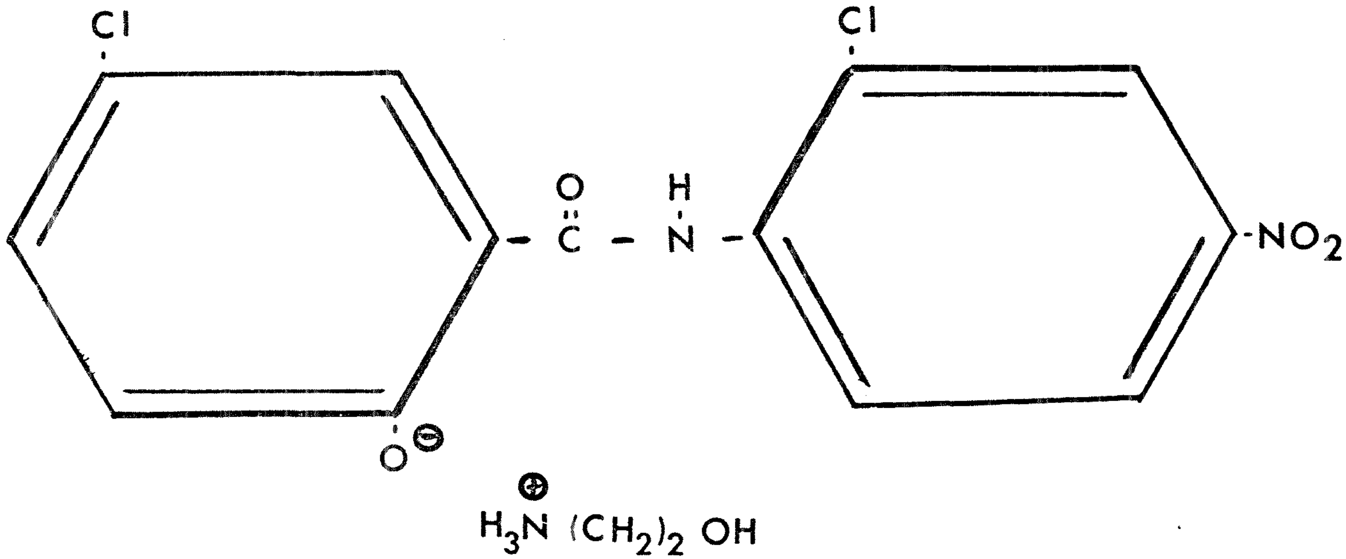

Figure 2. Structure of 2-aminoethanol salt of 2′, 5-dichloro-4′-nitrosalicylanilide.

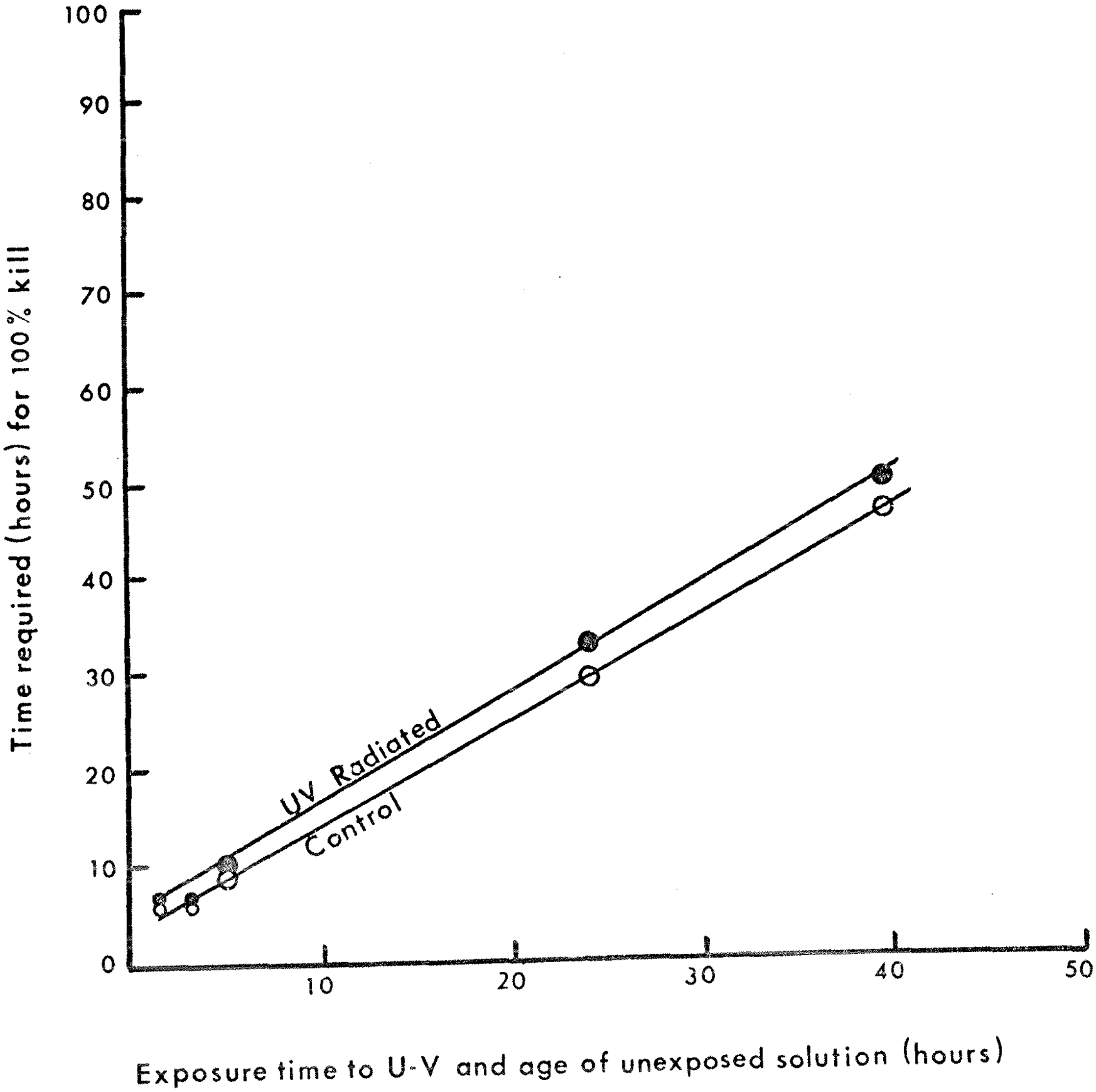

Figure 3. Time required for 100% kill of test organisms, with Bayer-73 radiated with U-V light (carp - 300 ug/L).

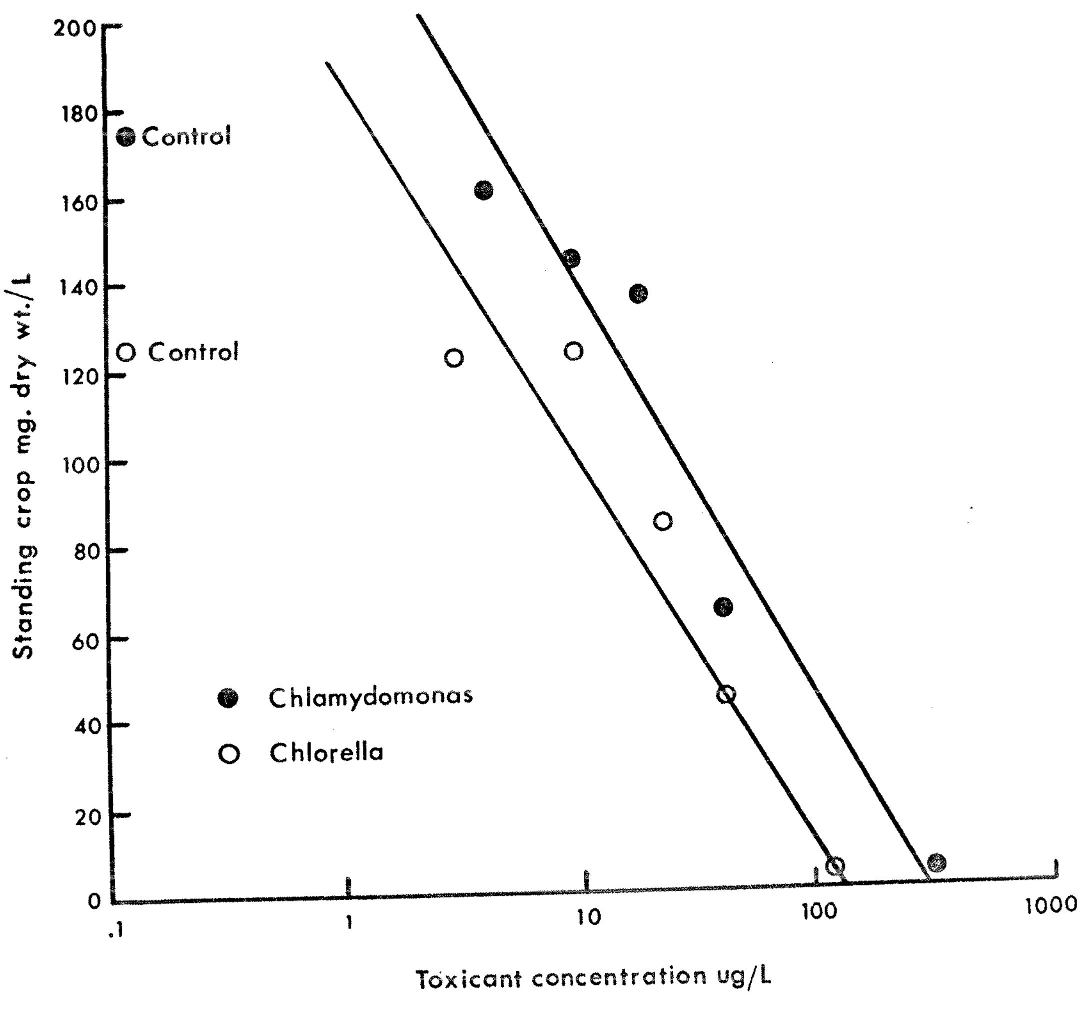

Figure 4. Reduction of standing crops of Chlamydomonas sp. and Chlorella vulgaris due to increased concentrations of Bayer-73.

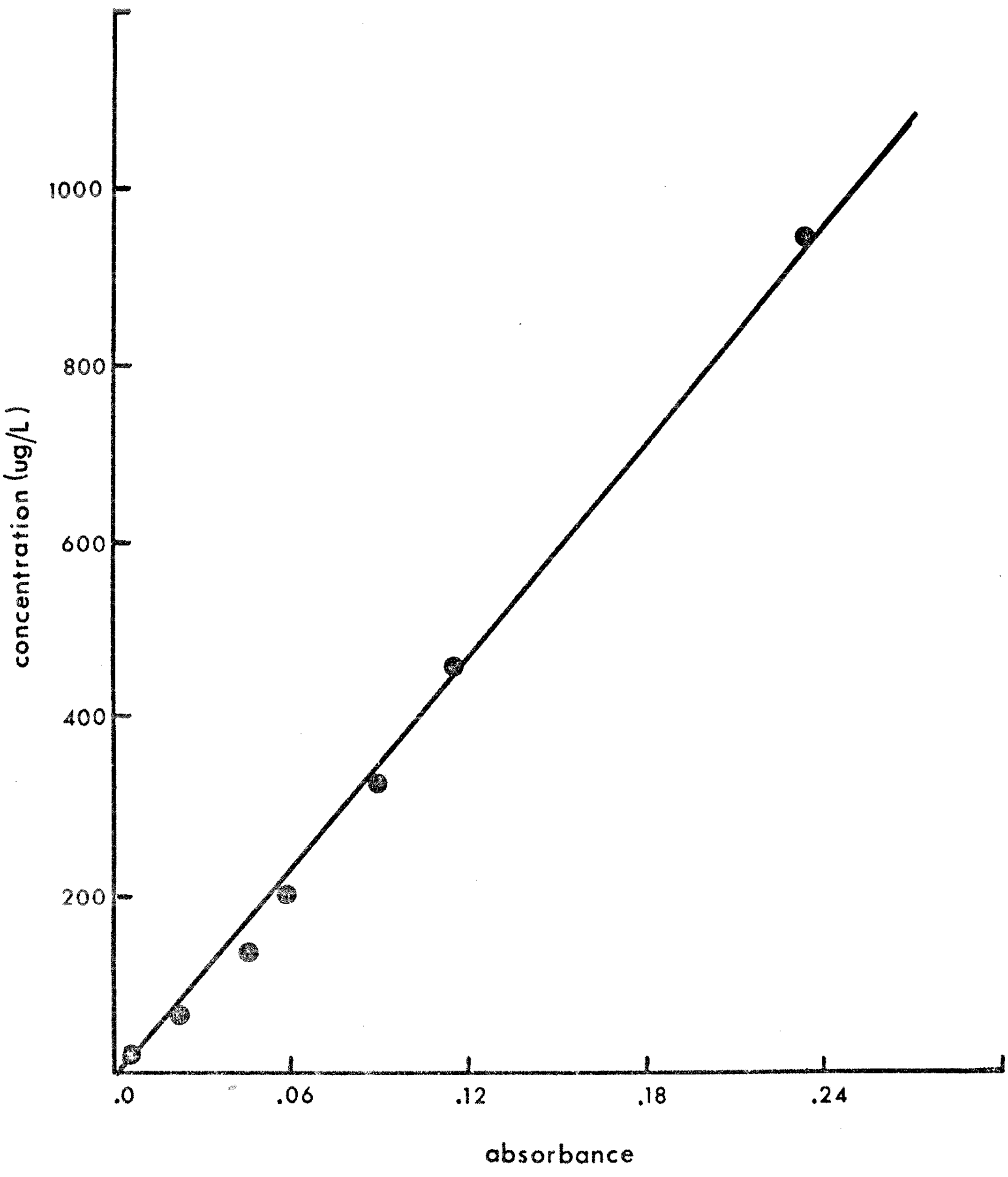

Figure 5. concentration-absorbance curve for Bayer 73 (262 nM).