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## Toxicity of rotenone to some species of coarse fish and invertebrates

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The toxicity of rotenone was determined for seven species of fish and three species of invertebrates. The results show that there is a marked variation in sensitivity of different species to rotenone. The survival times of roach *Rutilus rutilus* (L.) were reduced by a rise in temperature and also by a reduction in water hardness. Temperature affected the rate at which rotenone is degraded; 3 days after being prepared a solution containing 2 mg/l of 5% rotenone was non-toxic to roach over a 7-day period at 20° C, but it was still toxic for at least 11 days at 11.5° C. The data are discussed in relation to the use of rotenone in fisheries management.

### I. INTRODUCTION

Rotenone is derived from the roots of *Derris elliptica* Benth., a South-east Asian leguminous plant grown commercially for rotenone production. Derris root has been used for centuries for killing fish and an extract from the roots of closely related genera, which also contain rotenone, is still used by the inhabitants of tropical Africa and South America. In North America it has been used for over 30 years in fisheries management (Krumholz, 1948) but its use for this purpose has been illegal in England and Wales until the 1965 Act amending Section 9 of the Salmon and Freshwater Fisheries Act, 1923, enabled it to be used subject to certain consents.

The most common use of rotenone in fisheries management has been for the removal of coarse fish from enclosed waters prior to establishing a trout fishery. Only limited toxicity data for British coarse fish is available (Alabaster, 1970) and for this reason experiments have been carried out to determine the acute toxicity of rotenone to rudd *Scardinius erythrophthalmus* (L.), gudgeon *Gobio gobio* (L.), roach *Rutilus rutilus* (L.), mirror carp *Cyprinus carpio* L., Crucian carp *Carassius carassius* (L.) and perch *Perca fluviatilis* L. A single test was carried out using grass carp *Ctenopharyngodon idella* Val. and the toxicity of rotenone was determined for three species of invertebrates: *Asellus aquaticus* L., *Gammarus pulex* L. and *Cyclops* sp.

Rotenone is generally available as derris in powder form or as a liquid concentrate and commercial preparations normally contain 5% of the active ingredient. Liquid derris is convenient for use in fisheries work because emulsions are more easily dispersed in water than are powders and they also have a greater capacity for penetrating thermally stratified bodies of water (Almquist, 1959). The experiments described in this paper were made with liquid derris (5% rotenone)\*. The concentrations used in the experiments are expressed in terms of these formulations.

\*Murphy Chemical Company Limited, Wheathampstead, Hertfordshire, England, or Buggés Insecticides Limited, Sittingbourne, Kent, England.

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## II. METHODS AND RESULTS

## TOXICITY OF ROTENONE TO FISH

The tests were made in 600×300×300 mm aquaria each holding 40 l. From a stock solution containing 100 mg/l liquid derris (5% rotenone), dilutions were made with dechlorinated Metropolitan Water Board tap water which has a pH value of 7.9, a total hardness of 260 mg/l (as CaCO<sub>3</sub>) and 400 mg/l total dissolved solids. The dilutions were made within 1 h of the stock solution being prepared and the water in each aquarium was stirred to achieve a uniform distribution before the fish were introduced. No attempt was made to maintain the initial concentration of rotenone and no chemical analyses were made during the tests. Table I lists the species and concentrations used in the tests; 10 roach were used for each test but with other species the number was restricted to 5. A control was carried out for each test.

TABLE I. The species and concentration of liquid derris (5% rotenone) used for acute toxicity tests

Species	Concentration (mg/l)						Temperature (°C)	Supplier
	0.01	0.1	0.5	2.0	3.5	4.0		
Roach	—	x	x	x	x	—	—	10 (±0.5)
Roach	—	x	x	x	x	—	—	20 (±1.0)
Roach*	—	—	—	x	—	—	—	10 (±0.5)
Rudd	—	x	x	x	—	—	—	20 (±0.5)
Gudgeon	—	x	x	x	—	—	—	20 (±0.5)
Perch	x	x	—	x	—	—	—	10 (±0.5)
Perch	—	x	—	—	—	—	—	20 (±0.5)
Mirror Carp	—	x	x	x	x	—	—	11 (±0.5)
Crucian Carp	—	—	—	x	—	x	x	11 (±2.0)
Grass Carp	—	x	x	x	—	—	—	20 (±0.5)
Roach	—	—	—	x	—	—	—	10 (±0.5)
Gudgeon	—	—	—	x	—	—	—	20 (±0.5)

\*Additional test with a soft dilution water of total hardness (as CaCO<sub>3</sub>) 20 mg/l (see text).

All the fish used had been kept in the laboratory for at least 2 weeks prior to testing and were fed regularly on *Tubifex* sp. and *Daphnia* sp. Before being used in an experiment with soft water, roach were acclimated in a water with a total hardness of 140 mg/l (as CaCO<sub>3</sub>) for a week and were subsequently transferred to the test water, with a total hardness of 20 mg/l (as CaCO<sub>3</sub>), for a week immediately prior to the test.

Acclimation of fish to above-ambient test temperatures were made at 2° C intervals, with at least 3 days at each temperature.

During all experiments the tanks were aerated to maintain the dissolved oxygen concentration at, or near, the air saturation value and temperatures were taken at frequent intervals. The period of fish survival—taken as up to the cessation of movement—was recorded. Fish were weighed and measured immediately after tests were completed (Table II).

From the test data the median survival times and the values at which 5 or 95% of the fish would be expected to be killed were estimated graphically by plotting on logarithmic probability paper the cumulative percentage survival against the logarithm of concentration and fitting a line by eye. The results are shown in Figs 1 and 2.

There is a marked variation in resistance of the various species to rotenone. Crucian carp were the most resistant followed by mirror carp. Next, in resistance, were roach, grass carp, gudgeon and rudd. Perch were the least resistant species tested, being killed in about 400 min at an initial concentration of 0.1 mg/l.

TABLE II. The mean lengths and weights of fish used in the toxicity tests

Species	Length (mm)	Weight (g)
Roach	110 (±16)	16.9 (± 8.8)
Rudd	110 (±10)	25.2 (± 7.2)
Gudgeon	110 (±30)	14.9 (±11.2)
Perch	105 (±16)	16.3 (± 7.8)
Mirror carp	173 (±48)	126.6 (±49.6)
Grass carp	104 (±22)	24.2 (±12.4)
Crucian carp	200 (±26)	201.5 (±90.8)
Crucian carp*	65 (±12)	9.0 (± 4.1)

\*Test in 2 mg/l only.

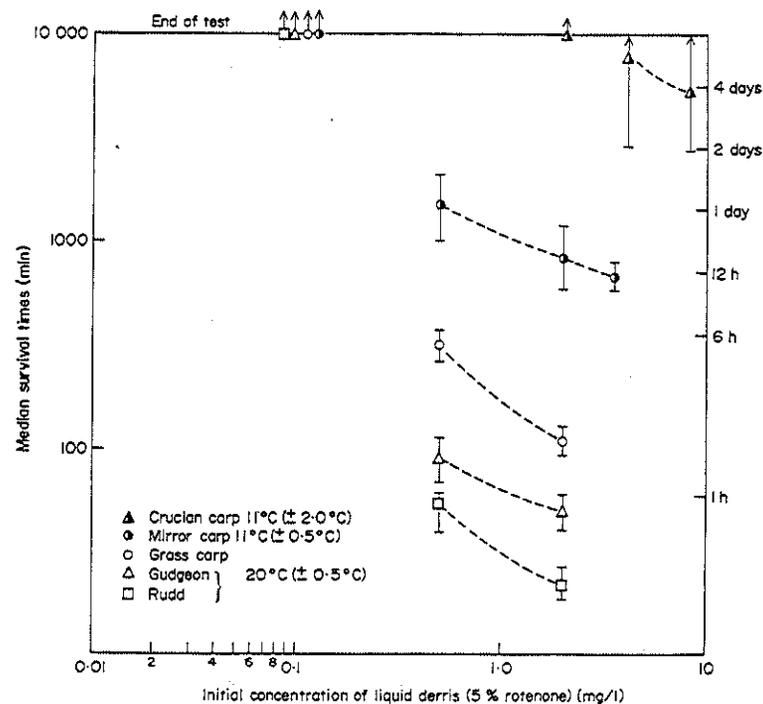


FIG. 1. Toxicity of liquid derris (5% rotenone) to five species of coarse fish showing median, 5 and 95% survival times.

An increase in temperatures markedly reduced the survival time of roach (Fig. 2) but it seems possible that the 48-h median lethal concentration (LC 50 value) at 10 and 20° C would be similar. Survival times of roach were shorter in soft water than in hard; a test with 2 mg/l derris in a dilution water with a total hardness of 20 mg/l gave a median survival time some 45 min less than the comparable test in hard water (Table III); this difference was significant at the level of  $P < 0.05$ .

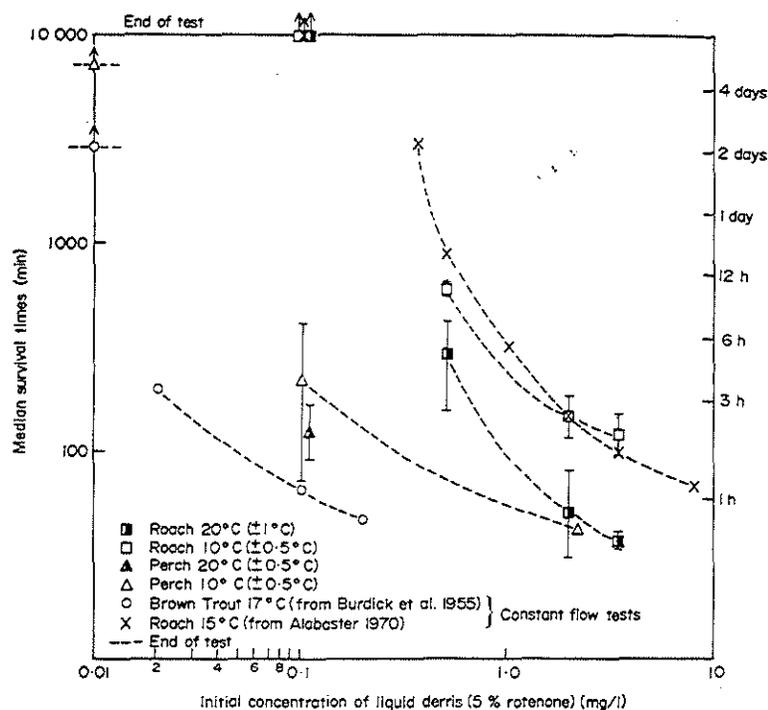


Fig. 2. Toxicity of liquid derris (5% rotenone) to roach, perch and brown trout showing median, 5 and 95% survival times.

TABLE III. Survival times of roach exposed to liquid derris (5% rotenone) in soft and hard dilution waters. Temperature 10° C ( $\pm 0.5^\circ$  C)

Hardness of dilution water (as mg/l CaCO <sub>3</sub> )	Initial derris concentration (mg/l)	Survival times (min)		
		95%	50%	5%
20	2.0	76	105	150
260	2.0	120	150	190

Two tests performed on roach and gudgeon in hard water to compare Buggés and Murphy's liquid derris formulations showed that their toxicities were almost identical at a concentration of 2 mg/l (Tables IV and V).

#### TOXICITY OF ROTENONE TO INVERTEBRATES

Tests were carried out on *A. aquaticus* L., *G. pulex* L., and *Cyclops* sp., using dilutions of rotenone prepared in the same way as for the fish toxicity tests. They had been kept in the laboratory and acclimated to the test temperature for at least 24 h prior to testing. Concentrations of 0.1, 0.5 and 2.0 mg/l were made up in 150 ml beakers immersed in a water-bath at 11° C ( $\pm 1^\circ$  C); the dissolved oxygen remained at or near saturation level during experiments. Temperatures were taken at frequent intervals and the individual periods of survival (up to the cessation of movement) were recorded. The results, shown in Table VI, indicate that *Cyclops* is extremely sensitive to rotenone, *Gammarus* is resistant and *Asellus* occupies an intermediate position.

TABLE IV. Survival times of roach in 2 mg/l of two commercial formulations of derris (5% rotenone). Temperature 10° C ( $\pm 0.5^\circ$  C)

	Survival times (min)	
	Buggés Insecticides Limited	Murphy Chemical Co. Limited
95%	135	120
50%	155	150
5%	185	190

TABLE V. Survival times of gudgeon in 2 mg/l of two commercial formulations of derris (5% rotenone). Temperature 20° C ( $\pm 0.5^\circ$  C)

	Survival times (min)	
	Buggés Insecticides Limited	Murphy Chemical Co. Limited
95%	38	42
50%	49	51
5%	62	62

TABLE VI. Percentage survival of *Asellus aquaticus* L., *Gammarus pulex* L. and *Cyclops* sp. exposed to liquid derris (5% rotenone) solutions

Species	No. of animals tested at each concentration	Initial concentration (mg/l)			Control	Duration of test (days)
		0.1	0.5	2.0		
<i>Cyclops</i> sp.	10	0%	0%	0%	100%	3
<i>Asellus aquaticus</i>	10	90%	70%	0%	100%	6
<i>Gammarus pulex</i>	10	100%	100%	90%	100%	6

#### DETOXIFICATION OF ROTENONE

An experiment was done on detoxification of rotenone in 40 l aquaria; five were maintained at 20° C ( $\pm 0.5^\circ$  C) and six at 11.5° C ( $\pm 1.0^\circ$  C) and all were aerated; batches of 10 and 5 roach being used at each temperature, respectively. All the fish were acclimated to the test temperature beforehand. The aquaria were filled with dechlorinated tap water to which sufficient liquid derris had been added to give a concentration of 2 mg/l. Immediately afterwards batches of fish were introduced into one aquarium at either temperature. At the higher temperature batches of fish were placed in aquaria on each of the following four days while at the lower temperature they were introduced 2, 3, 4, 11 and 16 days after the aquaria were first filled. Survival times were recorded and medians estimated as previously. The data obtained are shown in Fig. 3. Clearly temperature is very important in detoxification; 3 days after being prepared the derris was non-toxic to roach over a 7 day period at 20° C but at the lower temperature it was toxic for at least 11 days. In addition to the above tests

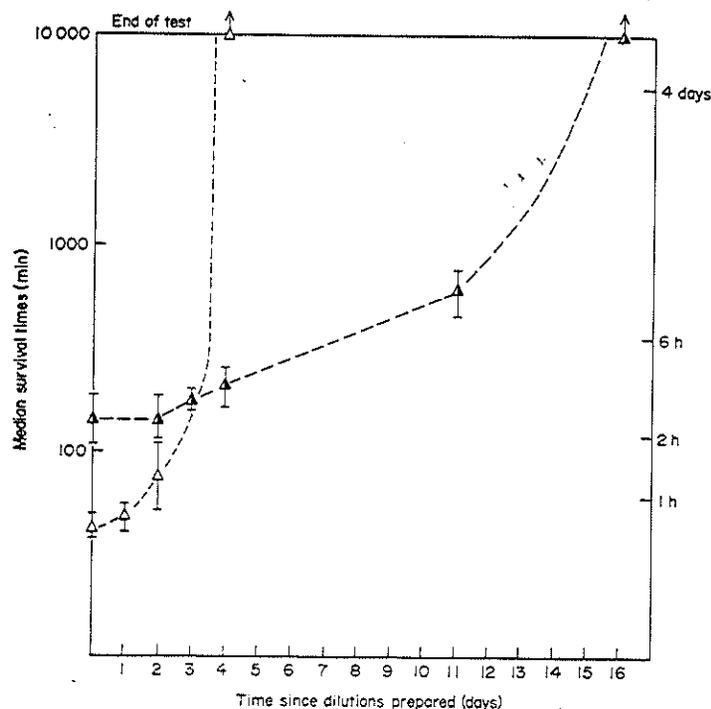


FIG. 3. Toxicity of 2 mg/l liquid derris (5% rotenone) to roach and the time required for detoxification. Vertical bars indicate 5 and 95% survival times.  $\Delta$ — $\Delta$ , 11.5°C ( $\pm 1.0^\circ$  C);  $\Delta$ — $\Delta$ , 20.0°C ( $\pm 0.5^\circ$  C).

carried out in a hard dilution water, a single 40 l aquarium was filled with a concentration of 2 mg/l derris made up in a soft dilution water with a hardness of 20 mg/l (as  $\text{CaCO}_3$ ). This aquarium was maintained at 11.5°C ( $\pm 1.0^\circ$  C) and a batch of roach acclimated at this temperature were introduced into the tank 16 days after the dilution had been prepared. These also survived 7 days.

### III. DISCUSSION

The toxicity data on fish suggested that an initial dosage of 2 mg/l of a formulation containing 5% rotenone would be adequate to kill roach, rudd, perch and gudgeon within a few hours in enclosed waters. If perch only were present then the dosage could be halved. Mirror carp were killed within 20 h at 2.0 mg/l at a temperature of 11°C, while Crucian carp, which appeared to be highly resistant, required a dosage of at least 8.0 mg/l if they were to be cleared from a pond. Field observations made during treatments using 2 mg/l for clearing small ponds suggested that bream *Abramis brama* (L.), pike *Esox lucius* L., 3-spined stickleback *Gasterosteus aculeatus* L. and 10-spined stickleback *Pygosteus pungitius* (L.) had a sensitivity somewhat similar to that of roach.

Alabaster (1970) tested Buggés liquid derris on roach in a constant flow apparatus at 15°C and his results are plotted in Fig. 2. The points he obtained do not all lie between the 10 and 20°C survival times for the static (non-replacement) tests, the

higher dilutions were less toxic than might be expected from the apparent effect of temperature. This may be due to the use of a constant flow apparatus for, paradoxically, dilutions of a proprietary fish toxin, based on rotenone, were found to be significantly more toxic to brown trout *Salmo trutta* L. and brook trout *Salvelinus fontinalis* Mitchell after being allowed to stand for between 2–24 h then they were on preparation (Loeb & Engstrom-Heg, 1970). The increase in toxicity was thought to be a reflection of a transition from the colloidal to the dissolved state.

Penick (1963) compared the toxicity of rotenone to six species of North American fish at 5 and 15°C and all species survived longer at the lower temperature in a given concentration except that the time concentration curves merged after three days. The same tendency can be seen in the roach data in Fig. 2. From the limited data available the dosage rates suggested above could apply to both hard and soft waters for although the shorter survival time of roach in soft water was significantly shorter it would probably not be of very great importance in practice. Of the invertebrates tested both *Cyclops* sp. and *A. aquaticus* would be killed by a dosage of 2 mg/l of 5% rotenone while *G. pulex* should survive. Almqvist (1959) and Lindgren (1960) found that there was a marked variation in sensitivity to dosages ranging from 0.1 to 10.0 mg/l of a proprietary fish toxin containing 2½% rotenone by different species of invertebrates and considered it unlikely that all animals would be killed during treatment. There is evidence to show that following rotenone application resistant species can increase rapidly, perhaps through lack of inter-specific competition. Smith (1941) observed large increases in the numbers of species of amphipods and molluscs which survived the treatment of a lake and Cusling & Olive (1957) found that the combined effect of rotenone and toxaphene killed off the chironomids but did not adversely affect the oligochaete population and, although the chironomids took nine months to re-populate the water, the standing crop of oligochaetes increased during the interim period. Anderson (1970) found that *Gammarus lacustris* Sars survived in a treated lake and subsequently stocked trout fed more frequently on this animal than had the original population. The use of rotenone does not appear to affect adversely the food supply available for fish which are subsequently introduced into the treated water provided that at least some of the resistant species are present to start with.

The work done on detoxification shows that rotenone is readily degraded in aquaria and a rise in temperature significantly increases the rate of detoxification. In the field exposure to light, suspended matter and adsorption by bottom deposits would also probable accelerate the process and usually restocking with fish should be feasible two weeks after treatment. If it is possible to choose the season when a water is treated the summer would be preferable since the faster degradation at higher temperatures would mean that not only would restocking be possible sooner but the time required to keep any outfalls to a river closed would be shortened. Although there is a colorimetric test for rotenone (Post, 1955), the easiest way to establish that the water is safe for restocking is to place brown trout, which are even more sensitive to rotenone than perch (Burdick *et al.*, 1955), in a perforated container in the water to see if they survive. Assuming an even distribution of rotenone the place where detoxification is most likely to be slowest is in the middle of the water column over the deepest part. Rainbow trout *Salmo gairdnerii* Richardson are not so suitable for this purpose since they are not very much more sensitive to rotenone than roach (Alabaster, 1970).

Although rotenone is an efficient fish poison, its successful use in the field will depend on achieving an even distribution of the required dosage to all parts of the water body. A number of techniques for aiding distribution have been tried. A common method is to pump diluted derris, from a holding tank in a boat, on to the surface of the water, or it can be poured into the propeller wash of an outboard motor (Penick, 1963). In the case of small ponds up to 0.5 ha in area an outboard motor can be fixed to the bank to create a circulation (Tate *et al.*, 1965). When treating large lakes an appreciable quantity of rotenone will have to be handled and Husley & Stevenson (1959) recommended using flat-bottomed boats each fitted with a rack to hold a 50-gallon drum of liquid derris; the rotenone was fed into the lake by gravity via a rubber hose from the drums. In waters deeper than 2.5 m weighted hoses should be used (Hooper, 1955).

Any deep holes or springs will need particular attention and Clemens (1952) described a technique that allows the concentration of rotenone being discharged to be varied, by connecting a dilution water intake pipe to the outlet from the rotenone tank and with a valve at the junction. Weedbeds also require careful attention; these may shelter fish fry and impede the penetration of rotenone, also the breakdown of rotenone can be accelerated by the oxygen given off by the photo-synthetic activity of submerged plants (Almquist, 1959). The successful eradication of fish by the use of rotenone will largely depend on the skill and thoroughness of the operators.

#### IV. SUMMARY

(1) Two commercial preparations of liquid derris, Murphy's and Buggés', each containing 5% rotenone had a similar toxicity to roach and gudgeon.

(2) The toxicity of Murphy's liquid derris to rudd, mirror carp, Crucian carp, grass carp and perch was also determined.

(3) There were marked variations in the sensitivity of the different species tested. Perch were the most sensitive and were killed by an initial concentration of 0.1 mg/l derris but for Crucian carp the minimum concentration lethal within 7 days was 4.0 mg/l. Other species were of intermediate sensitivity.

(4) A rise of 10° C reduced the survival time of roach in a concentration of 3.5 mg/l and 2.0 mg/l by a factor of three and in 0.5 mg/l by a factor of two.

(5) A reduction in water hardness from 260 mg/l (as CaCO<sub>3</sub>) to 20 mg/l (as CaCO<sub>3</sub>) reduced the survival time of roach in a concentration of 2.0 mg/l by approximately one-third but this is probably barely significant in practice.

(6) Three species of invertebrates *Asellus aquaticus* L., *Gammarus pulex* L. and *Cyclops* sp., were exposed to concentrations of 0.1, 0.5 and 2.0 mg/l liquid derris. *Cyclops* was killed at all concentrations within 3 days, *Asellus* at 2.0 mg/l within 6 days while *Gammarus* survived in this concentration for that period.

(7) Detoxification of a dilution containing 2 mg/l of liquid derris occurred within 3 days at a temperature of 20° C and between 11–16 days at 11.5° C.

(8) The results of the experiments are discussed in relation to the use of rotenone in fisheries management.

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