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Toxicity of Rotenone to Selected Aquatic Invertebrates and Frog Larvae

More than 60 species of plants containing rotenone have been used for centuries by natives of the tropics for stunning and killing fish. Rotenone is relatively harmless to plants and mammals at normal use pattern concentrations, but is extremely toxic to many insects and other invertebrates. It appears to act through respiratory enzyme inhibition, and kills by oxygen deprivation (Metcalf and Flint 1962). Binns (1967) reported that nearly all phyla of aquatic invertebrates present in the Green River, Wyoming, were adversely affected by an application of rotenone that ranged from 2.5 to 10.0 mg/L. Almquist (1959) stated that most zooplankters and many benthic animals were killed by exposure to the 0.5- to 0.6-mg/L concentration normally used to eradicate fish in water reclamation projects. Chironomid larvae were killed by this concentration after 23 h of exposure; 1.0 mg/L killed some species of trichopteran larvae in 5 h. Bottom-dwelling organisms and periphytic cladocerans were more resistant to rotenone, and higher concentrations and longer exposures were required to produce mortalities equivalent to those in other aquatic forms. Schnick (1974) reviewed the piscicidal uses of rotenone and included invertebrate toxicity data.

Although much information exists relating to the effects of rotenone exposure on aquatic invertebrates, many of the data used to support the original registration must be updated to conform to present labeling requirements (Lennon 1967). Marking (1975) proposed a guide or protocol for this type of registration research. The present study is part of a series of investigations designed to determine the toxicity of Noxfish to aquatic nontarget organisms in support of its continued registration as a piscicidal chemical.

Noxfish, an emulsifiable concentrate containing 5% rotenone, was used for all of the toxicity tests. Stock solutions were prepared in acetone daily as needed. The volume of stock needed to yield a particular concentration was added volumetrically to each of the test vessels.

Static tests were performed in a manner similar to that outlined by Lennon and Walker (1964) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Most of the test organisms were collected from the wild, but *Daphnia*, *Catenula*, *Physa*, *Helisoma*, and *Palaemonetes* came from laboratory or pond cultures. All organisms were held under laboratory conditions for periods of 5 to 14 days before being used for exposures. Ten organisms were added to each of a series of 3.7-L (1-gal) glass jars (test vessels) containing 3 L of limed water (20 mg/L total hardness; pH 6.6). Test organisms were acclimated to their new surroundings for 16 h. As the toxicant was added, the test solutions were stirred gently to ensure homogeneity. Two controls, one with acetone and another with untreated water, were included for each of the tests. Temperature of the test solutions was controlled at 16 ± 1°C by immersing the jars in a

chilled water bath. The absolute determination of death for mollusks was difficult. They were considered dead when a gentle probing of the foot or other appendage with a sharp instrument elicited no response. Dead organisms were removed daily and discarded.

The method of Litchfield and Wilcoxon (1949) was used to derive LC50's (the calculated concentration in water that produced 50% mortality of test organisms) and 95% confidence intervals.

Of the invertebrates in our study the most sensitive species was an ostracod (*Cypridopsis* sp.), with a 96-h LC50 of only 0.34 mg/L (Table 1). The other invertebrates were more tolerant of rotenone than most fish. The most resistant organisms exposed were a snail (*Helisoma* sp.) and the Asiatic clam (*Corbicula manilensis*), for which the respective 96-h LC50's were 7.95 and 7.50 mg/L. Marking and Bills (1976), who reported LC50 values for rotenone for more than 20 species of freshwater fish, showed that common carp (*Cyprinus carpio*) was one of the most sensitive, with a 96-h LC50 of only 0.05 mg/L; the black bullhead (*Ictalurus melas*) was much more resistant (LC50 = 0.39 mg/L). Values for channel catfish (*I. punctatus*), bluegill (*Lepomis macrochirus*), and largemouth bass (*Micropterus salmoides*) were intermediate (0.164, 0.141, and 0.142 mg/L, respectively).

The values for the Asiatic clam and the helisomid snail were more than 150 times that of the carp and about 50 times that of the other three fish species. The remaining invertebrates were 2 to 20 times as tolerant as fish. Larvae of the southern leopard frog (*Rana sphenocephala*) were 3 to 10 times more tolerant than the fish used by Marking and Bills (1976).

Table 1. Acute toxicity of Noxfish to aquatic invertebrates and frog larvae in limed water in static tests at 16 ± 1° C.

Organism	LC50 and 95% confidence interval (mg/L) at 1 h	3 h	6 h	24 h	96 h
Flatworm	—	8.95	6.40	5.10	1.72
Catenula sp.	— —	8.27–9.68	4.72–8.68	3.70–7.03	1.15–2.57
Daphnid	0.118	0.0960	0.0360	0.0275	—
Daphnia pulex	0.102–0.137	0.0807–0.114	0.0317–0.0409	0.0239–0.0316	— —
Ostracod	2.80	2.55	2.15	0.490	0.340
Cypridopsis sp.	2.35–3.34	2.11–3.08	1.80–2.56	0.299–0.803	0.280–0.557
Freshwater prawn	28.3	24.0	6.35	5.15	1.12
Palaemonetes kadiakensis	22.8–35.0	19.9–28.9	5.43–7.43	4.44–6.00	0.760–1.65
Dragonfly naiad	—	275	34.0	4.70	1.00
Macromia sp.	— —	230–329	19.6–58.9	1.45–15.2	0.730–1.59
Backswimmer	105	21.0	9.00	3.42	1.58
Notonecta sp.	86.5–128	17.7–25.0	6.79–11.9	2.27–5.15	0.727–3.44
Caddisfly larva	10.7	8.00	3.55	—	0.605
Hydropsyche sp.	7.98–14.5	6.69–9.56	2.88–4.38	— —	0.329–1.17
Whirligig beetle, adult	47.5	8.30	8.00	3.55	0.700
Gyrinus sp.	32.6–69.2	5.42–12.7	5.51–11.6	2.05–6.15	0.400–1.21
Snail	—	—	—	6.35	4.00
Physa pomilia	— —	— —	— —	5.61–7.19	3.45–4.63
Snail	—	—	—	—	1.75
Oxytrema catenaria	— —	— —	— —	— —	1.00–3.06
Snail	—	33.5	33.5	30.0	7.95
Helisoma sp.	— —	28.0–40.1	28.0–40.1	24.1–37.3	4.63–13.7
Buckley's filter clam	—	—	—	—	2.95
Elliptio buckleyi	— —	— —	— —	— —	2.23–3.90
Flattened filter clam	—	—	—	—	2.00
Elliptio complanata	— —	— —	— —	— —	1.53–2.61
Asiatic clam	—	—	—	—	7.50
Corbicula manilensis	— —	— —	— —	— —	5.74–9.81
Southern leopard frog larva	0.830	0.775	0.635	0.580	0.500
Rana sphenocephala	0.795–0.867	0.740–0.812	0.596–0.677	0.494–0.680	0.423–0.591

On the basis of these laboratory toxicity tests we conclude that most invertebrates should be safe during applications of rotenone. We suspect that the benthic forms are even more resistant when they reside in a natural substrate. However, fish and invertebrates are more sensitive to chemicals in laboratory tests than in the natural environment, and the ultimate safety should be evaluated under field conditions.

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Simple, Versatile Microscope Stage for the Identification of Pinned Adult Insects

Species determination of pinned adult aquatic insects often requires detailed examinations at high magnification. Many of the key morphological characters (such as palpal, antennal, or tarsal segments) are found on the ventral side and are often difficult to view. Specimens must be examined at various angles or oriented at peculiar angles to the source of illumination to provide suitable contrast for examination of these minute parts.

Several commercial models of insect microscope stages, based on the types depicted by Oldroyd (1958), are available for use in holding and orienting specimens on the stage plate. The simpler models often consist of corks, lumps of clay, or plasticine on small, angled supports. The more expensive models, which sometimes have cork mounting stages, may incorporate complex pivoting beams ("universal holders") or ball-and-socket components to provide flexibility. The simpler models necessitate frequent repinning and reorientation, which often result in specimen breakage. Although the pivoting beam mechanism is the most versatile of conventional holders, the beams and their supports often interrupt the light path and make it difficult to manipulate the specimen. Specimens mounted on stages are often elevated near the upper limit of the "working range" of the microscope, and some structures may be outside the focusing range of the microscope. The eye pieces may have to be raised