Acute Toxic Effects of Two Lampricides to Twenty-one Freshwater Invertebrates[1]

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ABSTRACT

We conducted laboratory static bioassays to determine acute toxicity of two lampricides—a 70% 2-aminoethanol salt of 5,2'dichloro-4'-nitrosalicylanilide (Bayer 73) and a mixture containing 98% 3-trifluoromethyl-4-nitrophenol (TFM) and 2% Bayer 73 (TFM-2B)—to 21 freshwater invertebrates. LC50 values were determined for 24-h exposure periods at 12.8 C. Organisms relatively sensitive to Bayer 73 were a turbellarian (*Dugesia tigrina*), aquatic earthworms (*Tubifex tubifex* and *Lumbriculus inconstans*), snails (*Physa* sp.) and (*Pleurocera* sp.), a clam (*Eliptio dilatatus*), blackflies (*Simulium* sp.), leeches (*Erpobdellidae*), and a daphnid (*Daphnia pulex*). The invertebrates most sensitive to TFM-2B were turbellarians, aquatic earthworms (*Tubifex*), snails (*Physa*), blackflies, leeches, and burrowing mayflies (*Hexagenia* sp.). Bayer 73 was generally much more toxic to the test organisms than TFM-2B. At lampricidal concentrations, TFM-2B was more highly selective than Bayer 73 against larval sea lampreys (*Petromyzon marinus*).

A program to control the sea lamprey (*Petromyzon marinus*) in the Great Lakes with the chemical 3-trifluoromethyl-4-nitrophenol (TFM) has been underway since 1958 (Applegate et al. 1961). The use of 5,2'-dichloro-4'-nitrosalicylanilide (Bayer 73 or Bayluscide) as a synergist reduces the amount of TFM needed for chemical treatment by about 50% (Howell et al. 1964). Reduction of the amount of TFM in turn reduces the cost of treatment considerably (Smith et al. 1974). A mixture that contained 98% TFM and 2% Bayer 73 by weight of active ingredient (TFM-2B), was considered to be the most desirable for field use. A heavy granular formulation of Bayer 73 developed for use on the bottom in estuaries and deltas was found to reduce sea lamprey larval populations in alluvial fans by about 89% (Manion 1969).

Data have been collected on the acute toxicity of TFM to several common aquatic invertebrates (Smith 1967), of Bayer 73 and TFM-2B to ostracods (Kawatski 1973), and of TFM and TFM-2B to stream invertebrates (Torblaa 1968); however, the agencies responsible for sea lamprey control needed additional data on toxicity to nontarget organisms to better evaluate the total hazard associated with the use of Bayer 73 and TFM-2B in the aquatic environment. The United States Environmental Protection Agency also needed this information to partly satisfy requirements for registration of the two materials. The purpose of the present study, therefore, was to determine by laboratory static bioassay the acute toxic effects of Bayer 73 and TFM-2B on several aquatic invertebrates.

METHODS

About 6,000 specimens, most identified to genus, were subjected to laboratory bioassays. The study included 21 invertebrates representing 4 phyla, 18 orders, and 21 families (Table 1). Most test specimens were collected in the Ocqueoc River, Presque Isle County, Michigan, and a few from other northern Michigan streams tributary to Lake Huron and Michigan and along the shore of Hammond Bay, Lake Huron. All test insects except water boatmen (Corixidae) were nymphs or larvae; all other invertebrates were adults. In making the collections, many invertebrates were manually dislodged or washed from vegetation or stones into containers; dip nets, fyke nets, and other devices were used as required; and aquatic earth-

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worms (*Lumbriculus inconstans*) were collected with an electric shocker. After collection, all specimens were held in the laboratory for at least 24 h before testing began. We considered this period to be adequate for the recovery of specimens possibly affected by handling. Sea lamprey larvae and fingerling rainbow trout (*Salmo gairdneri*) were included in the study for reference, since these two species are used as indicator organisms in pretreatment bioassays in sea lamprey control (Smith et al. 1974). The lampreys were collected with an electric shocker from the Rifle River, Ogemaw County, Michigan, and the trout were supplied by the Fish Division of the Michigan Department of Natural Resources.

Dilution water was taken directly from Lake Huron for each bioassay series. The physical and chemical characteristics of the water, which varied seasonally, were within the following ranges: temperature, 11 to 18 C; total alkalinity, 83.0 to 101.0 mg/liter as $CaCO_3$; phenolphthalein alkalinity, nil to trace; pH, 8.2 to 8.3; conductivity, 151.2 to 212.7 micromhos at 18 C; calcium hardness, 72.0 to 90.0 mg/liter; and total hardness, 100.0 to 136.0 mg/liter.

Most bioassays were conducted in 10-liter glass battery jars which contained 6.0 liters of test solution. The jars, covered with glass plates to limit evaporation, were placed in a controlled-temperature trough in which temperature was held at 12.8 C. The number of test specimens used in each bioassay varied with the availability of the organisms; usually 10 to 25 individuals of each species were exposed to each chemical at each test concentration. Four to six test concentrations of each chemical were usually employed for each species. The chemicals were introduced from fresh stock solutions made with Lake Huron water to give the desired test concentrations. Dissolved oxygen was maintained in each jar with compressed air delivered through a stone air breaker. Control groups of organisms were used in all bioassays. The condition of the organisms in the experimental and control groups was determined as precisely as possible, usually after 24 h, by microscopic examination and stimulation with a glass rod or dissecting needle. Facilities and techniques were modified in testing the snails, clams, and daphnids. The clams were exposed in 57-liter aquariums containing 40 liters of solution. After 24 h exposure, we placed snails and clams in fresh water for 96 h to enable us to differentiate between anesthesia and mortality. Daphnids were tested in 1.1-liter beakers containing 1.0 liter of solution.

Preliminary bioassays were conducted to establish effective concentrations. Maximum concentrations tested were 50 mg/liter for Bayer 73 and 100 mg/liter for TFM-2B. These concentrations are well above those lethal to sea lampreys. If these maximum concentrations were nontoxic to the invertebrates exposed, no additional tests were performed. If these preliminary concentrations were toxic, series of lower concentrations were tested that made it possible to produce regression lines that described the relation between log concentration in mg/liter and probit percent mortality. LC50 values (the concentration of toxicant killing 50% of the test organisms), their 95% confidence limits, and slope function values (antilogs of the reciprocals of the slope constant) for the regression lines were computed by the method of Litchfield and Wilcoxon (1949).

Mortality occurred in only one group of controls; 2 of 15 snails (*Physa* sp.) died of unknown causes. However, the toxicity data from bioassays in which the snails were exposed to the test concentrations were used because they met the criteria of Litchfield and Wilcoxon (1949) for a valid dose-effect response.

RESULTS AND DISCUSSION

Comparative Toxicity of Lampricides to Test Organisms

Bayer 73 was generally much more toxic than TFM-2B to the test organisms (Table 1). Exceptions occurred with water boatmen and burrowing mayflies: The LC50 for water boatmen was greater than 50.0 mg/liter with Bayer 73 and 40.0 mg/liter with TFM-2B, and for burrowing mayflies it was 6.9

TABLE 1.—*Toxicity of Bayer 73 and of TFM-2B to selected aquatic invertebrates, sea lamprey, and rainbow trout. (Invertebrate nomenclature after Stockard 1958.)*

Bioassay organism	LC50 and 95% confidence interval (mg/liter)		Slope-function value	
	Bayer 73	TFM-2B	Bayer 73	TFM-2B
Turbellarian				
Dugesia tigrina	0.048 (0.044–0.053)	1.5 (1.42–1.58)	1.20	1.10
Aquatic earthworms				
Tubifex tubifex	0.034 (0.031–0.037)	2.25 (1.98–2.55)	1.19	1.26
Lumbriculus inconstans	0.14 (0.12–0.18)	5.2 (4.8–5.6)	1.40	1.15
Leeches				
Erpobdellidae	0.42 (0.38–0.47)	4.0 (3.3–4.9)	1.13	1.25
Daphnid				
Daphnia pulex	0.8 (0.68–0.94)	7.35 (5.74–9.41)	1.50	2.23
Isopod				
Asellus militaris	23.0 (18.4–28.8)	31.0 (28.7–33.5)	2.69	1.38
Scuds				
Gammarus sp.	2.6 (2.34–2.88)	26.0 (24.4–27.7)	1.31	1.19
Crayfish				
Orconectes sp.	> 50.0	> 100.0		
Stoneflies				
Paragnetina sp.	1.07 (0.79–1.44)	15.4 (12.4–19.0)	1.56	1.25
Dragonflies				
Ophiogomphus sp.	> 50.0	> 100.0		
Water boatmen				
Corixidae	> 50.0	40.0 (32.8–48.8)		1.61
Non-burrowing mayflies				
Stenonema sp.	2.27 (1.68–3.08)	30.5 (26.1–35.7)	2.18	1.52
Burrowing mayflies				
Hexagenia sp.	6.9 (5.85–8.14)	4.0 (3.6–4.5)	1.39	1.14
Net-building caddisflies				
Hydropsyche sp.	2.45 (1.88–3.19)	30.0 (25.6–35.1)	2.45	1.66
Case-building caddisflies				
Helicopysche sp.	1.67 (1.18–2.37)	48.0 (38.1–60.5)	2.02	1.70
Blackflies				
Simulium sp.	0.255 (0.236–0.275)	3.45 (3.05–3.89)	1.28	1.73
Snipeflies				
Atherix sp.	> 50.0	> 100.0		
Dobsonflies				
Corydalus sp.	> 50.0	> 100.0		
Snails				
Physa sp.	0.106 (0.097–0.116)	1.93 (1.54–2.41)	1.32	1.92
Pleurocera sp.	0.355 (0.290–0.430)	9.4 (7.74–11.42)	1.73	1.73
Clam				
Eliptio dilatatus	0.382 (0.320–0.458)	4.7 (3.5–6.3)	1.53	1.62
Sea lamprey				
Petromyzon marinus	0.049 (0.043–0.056)	0.78 (0.684–0.889)	1.28	1.16
Rainbow trout				
Salmo gairdneri	0.14 (0.120–0.165)	3.85 (3.35–4.43)	1.30	1.17

mg/liter with Bayer 73 and 4.0 mg/liter with TFM-2B.

The LC50 values ranged from 0.034 mg/liter to more than 50.0 mg/liter for the invertebrates exposed to Bayer 73. LC50 values exceeded 50.0 mg/liter for crayfish, dragonflies, water boatmen, snipeflies, and dobsonflies. Isopods were also relatively resistant (LC50, 23.0 mg/liter). Among the most susceptible invertebrates were aquatic earthworms (*Tubifex*), turbellarians, and snails (*Physa*). Bayer 73 produced an LC50 of 0.049 mg/liter for sea lampreys—one of the lowest values in the series.

LC50 values for invertebrates exposed to TFM-2B ranged from 1.5 to more than 100.0 mg/liter. Crayfish, dragonflies, snipeflies, and dobsonflies were the most tolerant (LC50's exceeding 100.0 mg/liter). Water boatmen and casebuilding caddisflies were also relatively tolerant, with LC50 values of 40.0 and 48.0 mg/liter, respectively. The invertebrates most sensitive to TFM-2B were turbellarians, snails (*Physa*), and aquatic earthworms (*Tubifex*)—the same organisms that were most sensitive to Bayer 73. Sea lampreys were the most sensitive, with the lowest LC50 value (0.78 mg/liter) of all the organisms exposed to TFM-2B.

The slope function values of the regression lines in our study for all organisms with both chemicals were relatively low, indicating that the lowest concentration of the test substance that caused 100% mortality and the highest concentration that caused no mortality fell within a narrow range. These data indicate that care must be exercised in the use of these chemicals for sea lamprey control since small errors in application rates could result in high mortality of nontarget organisms or in low mortality of sea lamprey. In the Bayer 73 series, slope function values exceeded 2.0 for only four species and ranged from 1.13 to 2.69 for all organisms; in the TFM-2B series, the values ranged from 1.10 to 2.23, and exceeded 2.0 only in daphnids.

The toxicity of Bayer 73 and TFM-2B varied widely among the invertebrates tested, with the insects being generally tolerant. Two soft-bodied organisms, turbellarians and the aquatic earthworms (*Tubifex*), were especially susceptible to Bayer 73. The order of sensitivity among the invertebrates exposed was generally similar with both lampricides.

Implications of Lampricide Applications in the Field

Although TFM-2B displayed greater selective toxicity toward the sea lamprey, it was less toxic than Bayer 73 alone. The estimated MLC100 (minimum concentration that killed 100% of the lampreys) for TFM-2B in this study was 1.0 mg/liter. This concentration was nonfatal to all the other test organisms except snails (*Physa*). Judging by the mortality curve for *Physa*, 1.0 mg/liter of TFM-2B would produce a mortality of about 16%. We conclude that the judicious application of TFM-2B to control lamprey populations does not constitute a hazard to most freshwater invertebrates.

Bayer 73 has been used in a granular form to treat sea lamprey populations in estuarine and lacustrine habitats. The granules, which contain 5% active Bayer 73, sink rapidly and release most of the ingredient on the bottom. Information is lacking on the effects of this lampricide on invertebrate communities in treatment areas. The present study indicated that several invertebrates are vulnerable to Bayer 73 exposure. The estimated MLC100 for lampreys exposed to Bayer 73 was 0.1 mg/liter. Extrapolation from the mortality curves for other organisms indicated this concentration would kill nearly 100% of the turbellarians and aquatic earthworms (*Tubifex*), 42% of the snails (*Physa*), 16% of the aquatic earthworms (*Lumbriculus*), and 12% of the blackflies. Torblaa (1968) observed that invertebrate populations exposed to lampricides recovered substantially in 6 wk and fully in 1 yr, and suggested that reproduction by survivors from treatment areas and natural immigration from other areas restored the depleted populations. Invertebrate populations reduced by the use of granular Bayer 73 could be expected to similarly recover.

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