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# Acute Toxicity of Six Forest Insecticides to Three Aquatic Invertebrates and Four Fishes

by

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## Abstract

Technical grade and field formulations of six experimental forest insecticides—methomyl, carbaryl, aminocarb, trichlorfon, fenitrothion, and acephate—were tested for acute toxicity against three species of aquatic invertebrates (a daphnid, *Daphnia magna*; an amphipod, *Gammarus pseudolimnaeus*; and larvae of a midge, *Chironomus plumosus*), and four species of fish (bluegill, *Lepomis macrochirus*; rainbow trout, *Salmo gairdneri*; fathead minnow, *Pimephales promelas*; and channel catfish, *Ictalurus punctatus*). Five of the six insecticides tested were highly toxic (EC<sub>50</sub>, 100–1,000 µg/L) or extremely toxic (EC<sub>50</sub>, ≤100 µg/L) to the daphnid, amphipod, and midge larvae; the 48-h EC<sub>50</sub>'s ranged from 320 to 0.10 µg/L for daphnids and midge larvae, and the 96-h LC<sub>50</sub> from 2 to 920 µg/L for the amphipod. The sixth insecticide, acephate, was not toxic to invertebrates at concentrations up to 50 mg/L. Five of the insecticides ranged from highly toxic (methomyl, to channel catfish) to relatively non-toxic (trichlorfon, to fathead minnows); the sixth, acephate, was only slightly toxic to the fishes tested. In tests with bluegills and rainbow trout, the toxicity of methomyl and fenitrothion was little influenced by temperature, pH, or water hardness but that of carbaryl, aminocarb, and trichlorfon generally increased with increasing temperature or pH, or both.

Extensive damage to forests by defoliating insects has been a problem of national concern for many years. The spruce budworm (*Choristoneura fumiferana*), the gypsy moth (*Lymantria dispar*), and the Douglas-fir tussock moth (*Orgyia pseudotsugata*) have caused annual losses estimated to exceed 67 million cubic meters of lumber. Efforts to control these forest insect pests with chemicals have increased to a point where millions of hectares of forests are treated annually (Eidt 1975; Maranck 1976). This turn of events has led to a situation where foresters have had to become increasingly sophisticated pest managers (Newton and Knight 1981). Much of the area involved is Federal land managed by the U.S. Forest Service. Large-scale spraying projects always cause insecticide contamination in forest streams. The environmental impact of such contamination is of concern because many streams have productive fisheries and contain a diverse population of fish-food organisms.

After cancellation of the registration of DDT as a forest insecticide, the U.S. Forest Service needed to find alternative insecticides that have the efficacy of DDT but are less persistent and less toxic to aquatic organisms. The Columbia National Fisheries Research Laboratory cooperated exten-

sively with the Forest Service in evaluating the acute toxicity of several registered and experimental insecticides with potential for control of forest insect pests. The Forest Service provided sufficient information on the anticipated use of several experimental insecticides to enable the selection of six to be screened for toxicity: methomyl, carbaryl, aminocarb, trichlorfon, fenitrothion, and acephate. In view of the possible broad use of these insecticides in different geographical locations and forest habitats, it was necessary to determine how they affect a variety of non-target organisms in the aquatic environment. To make this determination, researchers at the Columbia Laboratory and its field stations at La Crosse, Wisconsin, and Jackson, Wyoming, screened the six experimental forest insecticides against a total of seven species of fish and six species of aquatic invertebrates. Tests were conducted with organisms found in both coldwater and warmwater habitats, and in water types characteristic of forest streams. Although some of the organisms may not be representative of those in waters likely to be contaminated by forest insecticides, the results provide useful basic information for the evaluation of the relative toxicity of the candidate insecticides.

The results on the toxicity of seven experimental forest

insecticides to fish and aquatic invertebrates were compiled in three reports, of which the present is the third. Schoettger and Mauck (1978) investigated the toxicological effects of six of these forest insecticides on brook trout (*Salvelinus fontinalis*), Atlantic salmon (*Salmo salar*), and naiads of stoneflies (*Pteronarcys californica*), and the possible interaction of combinations of insecticides. Woodward and Mauck (1980) reported on the toxicity of five of the insecticides to cutthroat trout (*Salmo clarki*), naiads of a stonefly (*Pteronarcella badia*), and a freshwater amphipod (*Gammarus pseudolimnaeus*). In the present work, we investigated the acute toxicity of technical grade and field formulations of five of the six candidate insecticides selected, and the technical grade of one (carbaryl), to four species of fish (bluegill, *Lepomis macrochirus*; rainbow trout, *Salmo gairdneri*; fathead minnow, *Pimephales promelas*; and channel catfish, *Ictalurus punctatus*) and to three aquatic invertebrates (a daphnid, *Daphnia magna*; the amphipod *Gammarus pseudolimnaeus*; and larvae of a midge, *Chironomus plumosus*). Additionally, we investigated the effects of three water quality variables—pH, temperature, and hardness—on toxicity.

## Materials and Methods

The aquatic invertebrates used were first instar daphnids, mature amphipods, and early fourth instar midge larvae. The rearing method described by Sanders and Cope (1966) was used in maintaining a continuous supply of daphnids, and amphipods were obtained from cultures maintained in the laboratory (Sanders 1972). Rearing techniques described by Biever (1965) and Ivleva (1973) were used to maintain a continuously reproducing population of midges. We obtained bluegills, rainbow trout, and channel catfish from U.S. Fish and Wildlife Service hatcheries and held them in the laboratory, using methods proposed by Brauhn and Schoettger (1975) for maintaining research fish. Fathead minnows were reared from eggs produced at our laboratory.

Technical grade and field formulations of six experimental forest insecticides were provided by the following chemical companies: methomyl, E.I. du Pont du Nemours Company; carbaryl (technical formulation only), Union Carbide Corporation; aminocarb and trichlorfon, Chemagro Agricultural Division of Mobay Chemical Corporation; fenitrothion, Sumitomo Chemical Company (Japan); and acephate, Ortho Division of Chevron Chemical Company. Stock solutions of technical grade and liquid chemicals were prepared in acetone, and the wettable powder solutions were prepared in water or added directly to test containers. Concentrations were based on active ingredients.

We conducted the acute toxicity tests at 7, 12, or 22°C with fish and 17°C with invertebrates, using standard methods for static toxicity tests (Committee on Methods for

Table 1. Acute toxicity (96-h LC50 or 48-h EC50 [ $\mu\text{g/L}$ ] and 95 % confidence intervals) of six candidate forest insecticides to three aquatic invertebrates.

Form and percent active ingredient <sup>a</sup>	Organism and measure of toxicity <sup>b</sup>		
	Daphnid 48-h EC50	Amphipod 96-h LC50	Midge larvae 48-h EC50
<b>Methomyl</b>			
T, 95–98 %	8.8	920	88
	4.1–19.0	660–1,300	47–160
L, 24 %	7.6	720	32
	4.8–12.0	555–940	30–50
<b>Carbaryl</b>			
T, 99.5 %	5.6	16	10
	2.7–12.0	12–19	7–16
<b>Aminocarb</b>			
T, 98 %	320	145	295
	260–380	96–220	240–365
L, 17 %	19	29	30
	13–27	19–44	22–41
<b>Trichlorfon</b>			
T, 99 %	0.12	43	0.10
	0.08–0.22	22–83	0.06–0.18
WP, 80 %	0.08	17	0.12
	0.05–0.14	7–42	0.08–0.21
<b>Fenitrothion</b>			
T, 95 %	11	6.1	2.6
	5–21	4.6–8.1	1.7–3.8
L, 87.7 %	24	2.0	4.0
	19–30	1.0–3.0	2.0–5.0
<b>Acephate</b>			
T, 94 %	> 50,000	> 50,000	> 50,000
WP, 95 %	> 50,000	> 50,000	> 50,000

<sup>a</sup>L = liquid; T = technical grade; WP = wettable powder.

<sup>b</sup>All tests conducted at 17°C.

Toxicity Tests with Aquatic Organisms 1975). Reconstituted water, with pH of 7.4 and a hardness of 40 mg/L (as  $\text{CaCO}_3$ ), was used. To determine the effect of changes in these water quality factors on toxicity, we prepared hard water by adjusting reconstituted deionized water to 320 mg/L total hardness (as  $\text{CaCO}_3$ ), and used appropriate buffers to establish pH's of 6.5, 7.5, and 8.5, plus 9.5 for the test of aminocarb (Marking and Dawson 1973). The pH test solutions were checked daily and adjusted as necessary.

The measure of acute toxicity for daphnids and midge larvae was the 48-h EC50—the median effective concentration that immobilized 50 % of the organisms in 48 h. The susceptibility of amphipods and fishes was measured in terms of the 96-h LC50—the calculated concentration of chemical in water that killed 50 % of the test organisms in 96 h. The method of Litchfield and Wilcoxon (1949) was used to estimate the EC50's and LC50's, and 95 % confidence intervals.

## Results

## Fish

*Invertebrates*

Among the three species of aquatic invertebrates studied, daphnids were usually more sensitive to the insecticides than were midge larvae and amphipods (Table 1). The toxicities varied greatly, from extremely toxic for trichlorfon against daphnids (48-h EC<sub>50</sub>, 0.08 µg/L), to relatively non-toxic for acephate, which had no effect on any of the three invertebrates at concentrations up to 50 mg/L. With the exception of acephate, the liquid or wettable powder formulations were usually more toxic than the technical grade material.

Acute toxicities (96-h LC<sub>50</sub>'s) of the six forest insecticides ranged from 0.08 mg/L (extremely toxic) to more than 100 mg/L (relatively non-toxic) for rainbow trout, bluegills, channel catfish, and fathead minnows (Table 2). Channel catfish were more sensitive than the other species to methomyl, but tended to be among the less sensitive to the other insecticides. Fathead minnows were by far the least sensitive to trichlorfon. With the exception of acephate and fenitrothion, the field formulations were nearly always more toxic than the technical grade material. In channel catfish and fathead minnows the field formulation of aminocarb (17% active ingredient) was about 100 times

Table 2. Acute toxicity (96-h LC 50 [mg/L] and 95% confidence intervals) of six candidate forest insecticides to four species of fish.

Form and percent active ingredient <sup>a</sup>	Rainbow trout <sup>b</sup>	Bluegill	Channel catfish	Fathead minnow
<b>Methomyl</b>				
T, 95-98%	1.6	1.2	0.50	2.8
	1.2-2.2	0.9-1.4	0.4-0.7	1.8-4.3
L, 24%	1.2	0.66	0.30	1.8
	1.1-1.4	0.48-0.91	0.2-0.4	1.2-2.7
L, 29%	1.2	1.2	0.32	1.5
	0.76-1.9	0.92-1.6	0.27-0.37	0.89-2.5
<b>Carbaryl</b>				
T, 99.5%	2.2	7.0	15.8	14.6
	1.7-2.8	5.1-9.8	13.9-18.0	11.7-19.8
<b>Aminocarb</b>				
T, 98%	13.5	6.0	10.0	8.5
	11.3-16.2	4.3-8.4	6.9-14.3	6.5-11.1
L, 17%	0.14	0.10	0.13	0.08
	0.12-0.17	0.07-0.15	0.11-0.16	0.06-0.09
<b>Trichlorfon</b>				
T, 99%	1.1	3.3	7.6	>100
	0.8-1.6	2.3-4.8	5.7-10.1	
WP, 80%	0.7	1.0	7.8	>100
	0.5-1.0	0.7-1.4	3.6-5.1	
<b>Fenitrothion</b>				
T, 95%	1.0	1.0	4.3	3.2
	0.7-1.4	0.7-1.4	8.6-5.1	2.4-4.2
L, 93%	1.9	2.5	7.6	4.3
	1.4-2.6	1.8-3.5	6.0-9.7	2.3-7.9
L, 87.7%	2.4	4.3	4.8	3.6
	2.0-2.9	3.6-5.1	3.5-6.7	2.5-5.2
WP, 40%	1.3	2.4		
	1.2-1.4	2.0-2.9		
<b>Acephate</b>				
T, 94%	>50	>50	>50	>50
WP, 75%	>50	>50	>50	>50

<sup>a</sup>L = liquid; T = technical grade; WP = wettable powder.

<sup>b</sup>Rainbow trout tested at 17°C and the three other species at 22°C.

Table 3. *Acute toxicity (96-h LC 50 [mg/L] and 95 % confidence intervals) of five candidate forest insecticides to two species of fish in water of different temperature, pH, and hardness (ND = no data).*

Water property	Insecticide, percent active ingredient, and fish species									
	Methomyl (95-98 %)		Carbaryl (99.5 %)		Aminocarb (98 %)		Trichlorfon (99 %)		Fenitrothion (95 %)	
	Rainbow trout	Bluegill	Rainbow trout	Bluegill	Rainbow trout	Bluegill	Rainbow trout	Bluegill	Rainbow trout	Bluegill
Temperature (°C)										
7	2.0	ND	2.8	ND	27	ND	2.4	ND	1.0	ND
	1.4-2.8		2.1-3.6		19-38		2.0-2.9		0.7-1.4	
12	1.0	2.0	2.2	16	18	4.2	1.1	15	1.0	1.0
	0.67-1.6	1.4-2.8	1.7-2.8	11-22	13-26	3.2-5.5	0.8-1.6	11-21	0.7-1.4	0.7-1.4
22	ND	0.86	ND	8.2	ND	3.5	ND	0.8	ND	0.8
		0.64-1.2		6.2-11		2.4-5.1		0.6-1.0		0.6-1.1
pH										
6.5	1.5	0.48	1.1	5.4	ND	14	2.6	1.6	2.3	2.7
	1.1-2.0	0.32-0.71	0.8-1.3	4.3-6.8		12-18	2.3-3.0	1.3-2.0	1.9-2.7	2.2-3.3
7.5	1.1	0.60	0.8	5.2	22	12	0.8	1.3	2.1	2.8
	0.76-1.6	0.42-0.86	0.6-0.9	4.1-6.6	19-27	10-16	0.6-1.0	0.9-1.9	1.7-2.5	2.2-3.3
8.5	1.2	0.62	1.5	1.8	17	5.2	0.4	1.5	2.7	3.2
	0.78-1.8	0.37-1.0	1.2-1.8	1.4-2.3	16-20	3.8-7.1	0.3-0.5	1.2-1.9	2.2-3.3	2.0-4.7
9.5	ND	ND	ND	ND	<5	0.5	ND	ND	ND	ND
						0.41-0.60				
Hardness (mg/L as CaCO <sub>3</sub> )										
40	1.7	1.2	0.9	2.2	25	3.3	0.9	2.2	2.0	4.1
	1.2-2.4	0.82-1.8	0.6-1.4	1.6-3.1	18-35	2.3-4.7	0.6-1.4	1.6-3.1	1.5-2.6	3.1-5.4
320	1.4	0.84	0.8	1.0	12	4.6	0.8	1.0	2.3	2.3
	0.95-2.0	0.53-1.3	0.6-1.3	0.7-1.5	9.4-15	3.4-6.3	0.6-1.3	0.7-1.5	1.8-3.0	1.8-3.0

more toxic than the technical formulation (98 % active ingredient). The toxicity of both formulations of acephate exceeded 50 mg/L for all four species tested. Although the toxicity of technical aminocarb to rainbow trout and bluegills was affected little by temperature and hardness, its toxicity to bluegills increased progressively with increases in pH, and was nearly 30 times greater at pH 9.5 than at pH 6.5 (Table 3).

Differences in temperature (7 or 12°C for rainbow trout; 12 or 22°C for bluegills), pH (6.5 to 8.5), or water hardness (40 or 320 mg/L) did not consistently alter the acute toxicity of methomyl or fenitrothion to rainbow trout or bluegills (Table 3). However, increases in temperature increased the toxicity of trichlorfon to both trout and bluegills. Trichlorfon was about 20 times more toxic to bluegills at 22°C than at 12°C. Water hardness did not affect the toxicity of trichlorfon to either species.

Toxicity of carbaryl to rainbow trout was not appreciably changed by differences in temperature, pH, or hardness. In contrast, toxicity to bluegills at 12°C was about half that at 22°C. The toxicity of carbaryl to bluegills increased threefold when the pH of the test solutions was increased from 6.5 to 8.5.

## Discussion

Laboratory acute toxicity studies by Sanders and Cope (1968) showed that carbaryl is extremely toxic to naiads of two species of stoneflies (*Pteronarcys californica* and *Pteronarcella badia*), the 96-h LC50's ranging from 1.7 to 4.8 µg/L. Our results also indicated that carbaryl is indeed extremely toxic to three other aquatic invertebrates—daphnids, amphipods, and midge larvae (Table 1).

Field studies of the impact of carbaryl on stream communities have yielded data that complement these laboratory studies. Several studies have shown that populations of certain groups of aquatic invertebrates may be reduced or the community structure perturbed when forest insecticides or other non-persistent pesticides are applied directly to streams (Coutant 1964; Gibbs 1976; Jacobi and Degan 1977; Courtemanch and Gibbs 1980). For example, Courtemanch and Gibbs (1980) reported both short- and long-term effects on stream invertebrate populations exposed to carbaryl (Sevin-4-oil) during spruce budworm suppression in Maine. They found the initial post-spray response to be a 170-fold increase in invertebrate drift and a significant reduction in benthic populations of stoneflies, mayflies, and

caddisflies. Burdick et al. (1960) also reported that carbaryl was toxic to these same groups of aquatic insects and that spraying resulted in a reduction of the standing crop of total stream invertebrates. Long-term effects in general included shifts in population numbers within and between various functional groups of invertebrates, changes in feeding behavior, and suppression of certain taxa that are more susceptible than others to the chemical (Jacobi and Degan 1977; Courtemanch and Gibbs 1980). It should also be noted that carbaryl can potentiate the toxicity of certain insecticides and herbicides to rainbow trout when the fish are coexposed to a nonlethal concentration of carbaryl (Statham and Lech 1975).

Field studies (Barker 1964; Burdick et al. 1960; Haines 1981) and laboratory studies (Schoettger and Mauck 1978; Woodward and Mauck 1980) have shown that aquatic invertebrates are much more sensitive than fish to the candidate forest insecticides. For example, Schoettger and Mauck (1978) concluded that, with the exception of acephate, invertebrates appear to be about 100 times more susceptible than trout to the forest insecticides examined here. Woodward and Mauck (1980) also reported that the insecticides were considerably more toxic to stonefly naiads (*Pteronarcella badia*) than to cutthroat trout. The results of our studies also generally support this conclusion, except for our finding of high acute toxicity of methomyl to channel catfish.

In general, acephate appears to be the least toxic of the insecticides tested on aquatic organisms, and therefore is likely to cause the fewest environmental problems in aquatic habitats. Use of this information, and especially the results of Schoettger and Mauck (1978), in conjunction with field studies (Rabeni et al. 1980), has resulted in the establishment of a general policy in the State of Maine: all lakes and rivers are to be protected by a buffer strip and a zone sprayed only with acephate, while much of the rest of the area is sprayed with carbaryl.

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