Toxicity of Various Formulations of 2,4-D to Salmonids in Southeast Alaska[1]

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To determine acute toxicity to juvenile (1) pink, chum, coho, and sockeye salmon, (2) Dolly Varden char, and (3) rainbow trout, 2,4-D acid, butyl and isooctyl esters were tested in southeast Alaska. A comparable test was made in Oregon using coho salmon fingerlings. The mean percent mortality after 96 h and the highest concentration of herbicide that did not produce any mortality were determined for each formulation tested.

At less than 50 ppm 2,4-D acid produced no mortality except in pink salmon fry. The butyl ester was most toxic causing nearly complete mortality in all species at concentrations > 1.0 ppm and the isooctyl ester least toxic of the ester formulations. Alaskan and Oregon coho fingerlings were similar in their responses to 2,4-D acid, butyl and isooctyl esters. The toxicities of three different formulations of isooctyl ester, a PGBE ester, and butyl ester to Alaskan coho fingerlings were also determined. There were few or no differences in toxicity among isooctyl ester formulations. The butyl and PGBE esters were similar in toxicity.

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Nous avons fait des essais avec le 2,4-D acide et les esters de butyle et d'isooctyle dans le sud-est de l'Alaska en vue de déterminer leur toxicité aiguë sur les jeunes (1) des saumons rose, keta, coho et nerka, (2) de la Dolly Varden et (3) de la truite arc-en-ciel. Un essai semblable a été effectué dans l'Orégon sur des alevins de la grosseur du doigt de saumon coho. Le pourcentage moyen de mortalité après 96 h et la plus forte concentration d'herbicide qui n'entraîne pas de mortalités ont été déterminés pour chaque formulation essayée.

Le 2,4-D acide à moins de 50 ppm ne produit pas de mortalités sauf chez les alevins de saumon rose. L'ester de butyle est le plus toxique, causant des mortalités presque complètes chez toutes les espèces à des teneurs de > 0.1 ppm. L'ester d'isooctyle est la moins toxique de toutes les formulations essayée. Les alevins de la grosseur du doigt des saumons coho de l'Alaska et de l'Orégon réagissent de la même façon au 2,4-D acide et aux esters de butyle et d'isooctyle. Nous avons également déterminé la toxicité de trois formulations différentes d'ester d'isooctyle, d'un ester de PGBE et de l'ester de butyle sur des alevins de saumon coho de l'Alaska. Les différentes formulations d'ester d'isooctyle ne montrent que peu ou pas de différences. Les esters de butyle et de PGBE ont une toxicité semblable.

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[1]This publication reports research involving pesticides. It does not contain recommendations for their use nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

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MOST Alaskan fish populations have never been exposed to herbicides and they occupy waters with unique chemical and physical properties. There have been no previous tests of the toxicity of formulations of 2,4-D to Alaskan races of fish in their native waters and research has been inadequate to justify extrapolation of toxicity data from studies conducted in other parts of the United States to the Alaskan environment. Because the U.S. Forest Service is using 2,4-D for vegetation control in southeast Alaska, the present study was undertaken to test the toxicity of the chemical to salmonid fishes indigenous to the forested streams in this region (Sears and Meehan 1971).

In determinations of toxicity of pesticides to fish the phenoxy herbicides (2,4-D, 2,4,5-T, and 2,4,5-TP) have received considerable attention (Hughes and Davis 1963; Rawls 1965; Bond et al. 1960). These herbicides are available as esters, amines, or inorganic salts in a wide variety of commercial formulations. There are marked differences in toxicity to fish among formulations (Hughes and Davis 1963) and selection of formulations for use in the field should take into account the toxicity of specific formulations, as well as the toxicity of a general class of herbicide.

Material and methods — Chemicals — The herbicides used in these tests were: (1) purified 2,4-D acid; (2) Monsanto Crop Guard Weed Killer (*n*-butyl, isobutyl ester of 2,4-D[2]); (3) Monsanto 2,4-D Low Vol. Ester Weed Killer (isooctyl ester of 2,4-D); (4) Dow propylene glycol butyl ether (PGBE) ester of 2,4-D; (5) Diamond isooctyl ester of 2,4-D; and (6) Rhodia isooctyl ester of 2,4-D. Monsanto technical grade 2,4-D acid was recrystalized three times from benzene to obtain the purified 2,4-D acid. All other herbicides were used as received from the manufacturer and, we assume, are representative of these materials as available for field use.

Test animals — Resident fish of economically important species were chosen for testing. The species used in these experiments were: coho salmon, *Oncorhynchus kisutch* — fry and fingerlings; chum salmon, *O. keta* — fry; pink salmon, *O. gorbuscha* — fry; sockeye salmon, *O. nerka* — smolts; Dolly Varden, *Salvelinus malma* — several age-classes; and rainbow trout, *Salmo gairdneri* — several age-classes.

The fish were livetrapped in streams in the Juneau–Auke Bay vicinity. Rainbow trout were obtained from Baranof Island, about 120 miles south of Juneau. The fish were transported immediately after capture to the laboratory at Auke Bay.

All test animals were acclimated for 3–10 days in tanks. Fish that displayed abnormal behavior were not used as test animals.

Water chemistry — Water used during the course of the study was unfiltered and untreated, taken from a depth of 12.19 m (40 ft) in Auke Lake near the laboratory. Several indices of water chemistry were measured during March and September, 1970 and June 1971, using methods of Rainwater and Thatcher (1960). Samples of the water were also analyzed for background residues of chlorinated hydrocarbon insecticides and s-triazine and phenoxy- and picloram-type herbicides (Burchfield and Johnson 1965; Mattson et al. 1965; Anon. 1968).

Herbicide loss during testing — The influence of aeration and of presence of fish on persistence of 2,4-D during a 96-h exposure period was determined in bioassay jars each of which contained either 1 ppm acid equivalent 2,4-D acid, or butyl, or isooctyl ester The experiment was run in triplicate.

Water was drawn from the bioassay jars 24, 48, 72, and 96 h after introduction of herbicide. Quart samples were placed in glass jars containing 15 g of sodium hydroxide. Samples were then acidified to *p*H 1.0 with hydrochloric acid and extracted three times with 100-ml portions of benzene. The benzene extract was evaporated to dryness and methylated with diazomethane. Samples were diluted as needed with benzene and were assayed with a Microtech 2000 gas chromatograph. The 4-mm-ID × 1.2-m glass column was packed with 6% OV-1 coated, 60- to 80-mesh, Gas Chrom Q. The column was operated at 150 C with a flow of N_2 carrier gas of 90 ml/min at 40 psi. The herbicide was detected with the chlorine specific Infotronics T-300 microcoulometric detector. The precision of the analytical method is ±5%.

Bioassay methods — Modified bioassay methods of Doudoroff et al. (1951) and the Pesticides Regulation Division (1969) were used as guidelines in setting up the experiment. Wide-mouthed, 18.9-liter (5-gal) glass jars were used as containers and were arranged in rows of five in constant temperature water baths. All experiments were conducted in triplicate at 10 ± 1 C.

Each jar was fitted with a paraffin-impregnated plywood lid. We provided uniform aeration to all jars from an oilless compressor. Filtered air was pumped to each jar through glass tubing that was fitted with a disposable bubbler.

Fifteen liters of water were measured into each jar and allowed to reach 10 C before the chemicals were added. Aeration of the water was started at this time. Stock solutions of equal concentration of each herbicide formulation were prepared immediately before the test media were inoculated. Water was withdrawn from each jar to equal the volume of stock solution to be added. Because pure 2,4-D acid has a low solubility in water, dilutions were prepared by weighing the required amount of pure acid into each jar and adding

[2]Mixtures of *n*-butyl, isobutyl esters of 2,4-D are referred to as "butyl" ester. Use of trade names does not imply endorsement by the U.S. Department of Agriculture.

15 liters of water. Considerable stirring was required to dissolve the acid crystals.

Test solutions consisted of concentrations of 1, 5, 10, and 50 ppm of each chemical. Ten fish were placed in each jar. The mass of fish per jar was between 1.6 and 2.8 g of fish per liter. Fish were introduced into the test medium within 45 min after the introduction of the chemical. Observations of fish mortality were recorded 24, 48, 72, and 96 h after introduction of the fish. A fish was considered dead when all movements had ceased and no further responses could be obtained by touching or moving the fish. Fish that died during the tests were removed immediately.

After completion of each test, the jars and lids were rinsed with isopropyl alcohol, washed and rinsed twice in hot water and strong detergent, rinsed with acetone followed by hot water, and then rinsed twice with untreated lake water. The jars were then refilled with 15 liters of water and replaced in the water bath.

To evaluate applicability of bioassay work to widely different geographical locations, toxicity tests were done in Corvallis, Ore. using native coho salmon fingerlings, but using chemicals from the same containers used in Alaska. All test conditions were the same as those for the Alaskan tests.

Results and discussion — Chemical characteristics of the test water were generally similar throughout the study. Hardness as calcium and magnesium ranged from 10.0 to 33.64 ppm during the study period; such differences have no significant effect on toxicity of various organic herbicides, including 2,4-D (Ingles and Davis 1970).

Assay of test water for background pesticide residues showed no detectable quantities of s-triazine, phenoxy- or picloram-type herbicides, or the insecticides endrin, aldrin, dieldrin, hep-

TABLE 1. Mean percent mortality of fish after 96-h exposure to 2,4-D acid, butyl or isooctyl esters in static water toxicity tests.

Species	2,4-D	ppm 2,4-D a.e.[a] 1	5	10	50	N.E.[b] level (*ppm*)
Pink fry	Pure acid	10.0(0–20)[c]	13.3(10–20)	43.3(0–90)	100.0(0)	<1
	Butyl ester	100.0(0)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	40.0(0–70)	80.0(50–100)	100.0(0)	100.0(0)	<1
Chum fry	Pure acid	0.0(0)	0.0(0)	0.0(0)	66.7(0–100)	10
	Butyl ester	100.0(0)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	66.7(30–90)	60.0(20–100)	100.0(0)	1
Coho fry	Pure acid	0.0(0)	0.0(0)	0.0(0)	80.0(40–100)	10
	Butyl ester	100.0(0)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	6.67(0–10)	13.3(0–30)	100.0(0)	1
Sockeye smolts	Pure acid	0.0(0)	0.0(0)	0.0(0)	6.7(0–20)	10
	Butyl ester	70.0(10–100)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	6.7(0–20)	33.3(20–50)	63.3(20–100)	100.0(0)	<1
Alaska coho fingerlings	Pure acid	0.0(0)	0.0(0)	0.0(0)	0.0(0)	50
	Butyl ester	6.7(0–20)	93.3(80–100)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	0.0(0)	3.3(0–10)	40.0(10–60)	5
Dolly Varden fingerlings	Pure acid	0.0(0)	0.0(0)	0.0(0)	0.0(0)	50
	Butyl ester	16.7(10–30)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	0.0(0)	0.0(0)	66.7(30–100)	10
Rainbow fingerlings	Pure acid	0.0(0)	0.0(0)	0.0(0)	0.0(0)	50
	Butyl ester	53.3(30–70)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	0.0(0)	0.0(0)	63.3(40–80)	10
Oregon coho fingerlings	Pure acid	0.0(0)	0.0(0)	0.0(0)	73.3(50–100)	10
	Butyl ester	26.7(0–50)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	0.0(0)	0.0(0)	20.0(10–30)	10

[a]a.e. — acid equivalent.
[b]N.E. — the no acute effect level is the highest concentration tested that produced no mortality in test fish.
[c](0–20) — range in percent mortality in three replications.

tachlor, heptachlor epoxide, DDT[3], DDD, or DDE. The analytical method used is capable of quantitatively detecting 0.001 ppm of these pesticides. Therefore we feel that responses of our bioassay fish were only to the herbicides being tested.

Doudoroff et al. (1951) preferred not to aerate test solutions unless absolutely necessary, because they believed that chemical loss might result. In our tests, 45–50 mm coho (age 0+) died within 96 h when held in bioassay jars at 10 C without aeration. With aeration, 10 fish up to 150 mm in length could be held for 96 h in 15 liters of water with no apparent ill effects.

Measurements of persistence of 2,4-D pure acid, butyl ester, and isooctyl ester as influenced by aeration and fish showed no statistically significant difference among concentrations of herbicide subjected to different treatments after 96 h of exposure.[4] Some small "loss" of chemical occurred in the first 24-h period in all treatments, but may have been more apparent than real because these residues are as measured in the water and do not take into account herbicide that is reversibly adsorbed on various surfaces in the system. Some investigators suggest a maximum of 2 g of fish per liter of test solution to prevent depletion of test chemicals due to uptake or metabolism by the fish (Doudoroff et al. 1951). Because neither aeration nor presence of fish significantly decreased the concentration of any of the three herbicides tested, we feel the use of aerated solutions and up to 2.8 g of fish per liter of test solution was justified.

Because of the wide spread of concentrations tested in this study, much of the data shows either complete mortality or survival after 96- h exposure. These results precluded calculation of TL_m in the usual manner. Similarly, lack of variance where complete mortality or survival occurred also prevented statistical comparisons of percent mortality among treatments. However, the essential elements of our results are clear. Mean percent mortality (three replications) of test fish after 96-h exposure to various concentrations of chemical are shown in Table 1. There were some striking differences in toxicity among the chemicals tested. The butyl ester is much more toxic than the isooctyl ester or pure 2,4-D acid. The no-effect level in Table 1 is the highest concentration tested that caused no mortality in 96 h in any of the three replications. The no-effect level for butyl ester is considerably less than 1 ppm as relatively high mortality occurred at that level for nearly every species tested. The no-effect level for isooctyl ester ranged from 1 to 10 ppm for all fish except pink salmon fry which had 40% mortality at 1 ppm and sockeye smolts with 7% mortality at 1 ppm.

The 2,4-D is the herbicidally active component of each chemical tested; thus the chemical form of the herbicide and constituents of formulation have far

[3]Trace amounts of DDT were observed, but the level was less than 0.001 ppm.

[4]Data were analyzed by multivariate analysis of variance.

TABLE 2. Mean percent mortality of Alaskan coho fingerlings after 96-h exposure to 2,4-D acid, butyl, isooctyl, or PGBE[a] esters in static water toxicity tests.

2,4-D	ppm 2,4-D a.e.[b] 1	5	10	50	N.E.[c] level (*ppm*)
Pure acid	0.0(0)[d]	0.0(0)	0.0(0)	0.0(0)	50
Isooctyl ester (Monsanto)	3.3(0–10)[d]	0.0(0)	3.3(0–10)	83.3(60–100)	<1
Isooctyl ester (Diamond)	3.3(0–10)	0.0(0)	3.3(0–10)	96.7(90–100)	<1
Isooctyl ester (Rhodia)	0.0(0)	3.3(0–10)	16.7(10–30)	90.0(70–100)	1
PGBE ester (Dow)	26.7(0–80)	100.0(0)	100.0(0)	100.0(0)	<1
Butyl ester (Monsanto)	6.7(0–20)	93.3(80–100)	100.0(0)	100.0(0)	<1

[a]PGBE — propylene glycol butyl ether.
[b]a.e. — acid equivalent.
[c]N.E. — the no acute effect level is the highest concentration tested that produced no mortality in test fish.
[d](0); (0–10) — range in percent mortality in three replications.

more impact on fish than the 2,4-D alone. Toxicity data for specific formulations of herbicide should be consulted when selecting formulations for use in the field.

Various species of fish of similar size reacted generally the same to similar concentrations and formulations of 2,4-D. If pink, chum, and coho salmon fry are considered as one group and the older (and larger) rainbow, Dolly Varden, and coho fingerlings considered as another group, then the fry appear to be more sensitive to all three formulations. Sockeye smolts were intermediate between these two groups in sensitivity. Post and Schroeder (1971) found that various pesticides were more toxic to fish of lower body weight.

The tests on Oregon coho fingerlings showed that these fish behaved generally the same as the Alaskan cohos of similar size and age (Table 1).

Our test results for coho and rainbow trout are similar to results reported by other investigators (Bond et al. 1960; Kibby 1966) but this is the first report for other species and races of Alaskan fish. Alaskan land managers have felt that fish toxicity tests conducted in other parts of the United States might not apply to Alaskan fish in their natural environments. The similarities between our results and the results of others with coho salmon and rainbow trout and the direct comparison we made of the response of Oregon and Alaskan coho fingerlings make it clear that land managers can use a wide variety of fish toxicity test results in selecting chemicals for use in the coastal Alaskan environment.

Tests with aquatic insects yielded very erratic results and we found fish more reliable as indicators of toxicity of 2,4-D to stream organisms.

The relatively low toxicity of the isooctyl ester in comparison with the butyl ester prompted us to investigate the toxicity of several low volatile ester formulations. Isooctyl ester formulations from three manufacturers and the PGBE ester from one manufacturer were included to test for differences in toxicity among these products (Table 2).

There were no striking differences in toxicity among formulations of isooctyl ester, but the PGBE ester approached the butyl ester in toxicity (Table 2). Hughes and Davis (1963) found differences in toxicity of a given 2,4-D ester formulated by different manufacturers. They used warmwater fish and a narrower range of herbicide concentrations, and the differences in toxicity noted were not large in most instances. Our data do not provide a basis for choosing among formulations of isooctyl ester for field use, but clearly the butyl and PGBE ester formulations pose a more significant hazard to fish than the isooctyl esters if direct application of the chemical to surface water occurs.

It is doubtful that there are large degrees of difference in the effectiveness of various phenoxy herbicide esters in terms of brush control on forest lands (Hull 1967). Therefore, selection of specific phenoxy herbicide ester formulations should be made on the basis of the possible impact on aquatic organisms when the probability exists that the chemical will enter the water (Juntunen and Norris 1972).

Our study determined only relative "acute" toxicity among formulations of 2,4-D, and gave no information on "chronic" effects, such as reduced fecundity and growth potential, changes in body chemistry or genetic makeup, or alteration of behavioral characteristics.

The sex, size, life history (e.g. hatchery-reared vs. wild), condition, and acclimation of fish subjected to bioassay tests and various chemical properties of test water are factors that should receive more thorough consideration in testing of this type (Mount 1966; Hughes and Davis 1963; Sprague 1970; Cope 1971). Sprague (1969, 1970, 1971) reviewed in depth the methods for measuring toxicity of chemicals to fish. In addition, more research needs to be conducted on the various substances (e.g. surfactants and oil carriers) that are used operationally with chemicals like 2,4-D. In some cases, more than one chemical may be used in one area, and the synergistic effects of combinations of such chemicals deserve investigation.

The butyl ester and the PGBE ester of 2,4-D were considerably more toxic to fish than was the isooctyl ester in our tests. In general, the least toxic material that is effective should be recommended for use where there is any possibility that the chemical may enter the aquatic environment. No major differences were found in sensitivity among species of salmonids of comparable age or size exposed to a given chemical. Hence, bioassay work may be confined to the most readily available species. Results of the Oregon and Alaska tests were similar; therefore when circumstances make it impossible to undertake separate investigations, reasonable inferences may be made from other pesticide studies to the Alaskan freshwater environment.

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