Bull. Environ. Contam. Toxicol. (1987) 39:721–728

Bulletin of Environmental Contamination and Toxicology

Acute Toxicity to Juvenile Pacific Salmonids of Garlon 3A™, Garlon 4™, Triclopyr, Triclopyr Ester, and Their Transformation Products: 3,5,6-Trichloro-2-pyridinol and 2-Methoxy-3,5,6-trichloropyridine

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Garlon 3A™ and Garlon 4™ are formulated triclopyr herbicides manufactured by The Dow Chemical Company. These products are selective post-emergent herbicides used for the control of broadleaf vegetation. The use of Garlon herbicides for conifer release in coastal British Columbia may result in the unintentional introduction of triclopyr and its degradation products into waterbodies inhabited by salmonids. The objective of this study was to evaluate the acute toxicity of formulated and technical triclopyr and its transformation products to juvenile salmonids of the Pacific Northwest.

MATERIALS AND METHODS

The common names of the test chemicals are summarized in Table 1. All test materials were supplied by Dow Chemical U.S.A., Midland, Michigan.

A series of 96-h static acute toxicity tests was conducted from March to June 1986 and March to April 1987 in accordance with the protocols of Environment Canada (1980) and Buchanan (1982). Under yearling juvenile Pacific salmonids were obtained as fry in fresh water from various British Columbia hatcheries and used as test fish, (Table 2).

Before testing, all fish were acclimated to laboratory conditions for a minimum period of 2 wk. They were reared in fiberglass tanks at an average ambient temperature of 6°C (range 5-8°C) and were fed a maintenance diet of Oregon moist pellets. Fish under test were not fed. The average lengths of fish, weights, loading densities and test volumes were recorded, (Table 3). The loading density and test volume were adjusted according to the size of the fish.

Ten fish per concentration were placed in each test vessel. Observations of fish mortality were recorded at 24

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Table 1. Nomenclature of test chemicals

Common name	Chemical name, active ingredient, purity
Garlon 3A™	triethylamine of triclopyr; 36% acid equivalent of triclopyr
Garlon 4™	butoxyethyl ester of triclopyr; 48% acid equivalent of triclopyr
Triclopyr ester	butoxyethyl ester of triclopyr; 99.7%
Triclopyr	triclopyr[((3,5,6-trichloro-2-pyridinyl) oxy)acetic acid]; 99.2%
Pyridinol	3,5,6-trichloro-2-pyridinol; 99.7%
Pyridine	2-methoxy-3,5,6-trichloropyridine; 99.7%

Table 2. Test fish species, ages, and suppliers

Fish Species	Date Obtained	Age (mo)	Hatchery
Coho salmon (*Oncorhynchus kisutch*)	05/15/86	1	Capilano Hatchery Vancouver, B.C.
Chinook salmon	05/07/86	4-5	Capilano Hatchery
(*O. tshawytscha*)	03/04/87	2-3	Vancouver, B.C.
Chum salmon (*O. keta*)	05/01/86	1	Little Qualicum Spawning Channel, Qualicum Beach, B.C.
Pink salmon	04/02/86	0.75	Quinsam Hatchery
(*O. gorbuscha*)	03/03/87	1.75	Campbell River, B.C.
Sockeye salmon	05/08/86	1	Fisheries/Ocean
(*O. nerka*)	05/12/86	2	Canada Research Laboratory, Cultus Lake, B.C
Rainbow trout (*Salmo gairdneri*)	25/02/86	1	Fraser Valley Trout Hatchery, Abbotsford, B.C.

48, 72, and 96 h after introduction. At each observation period, the temperature, pH, and dissolved oxygen of each test vessel were recorded. A fish was considered dead when movement ceased and no further responses could be obtained by prodding or moving the animal. Fish found dead during the test were removed at each observation. Cumulative fish mortality was recorded and the results used for the calculation of LC_{50} values based on the "Lethal" computer program developed by Stephan (1983).

The tests were conducted in 35-L glass aquaria. A maximum volume of either 20 or 30 L of dechlorinated Vancouver City tap water was used in each vessel, depending

Table 3. Average sizes of fish, weights, loading densities, and test volumes

Fish	Length		Weight		Loading Density	Test Volume
	cm	(range)	g	(range)	(g/L)	(L)
Coho	4.0	(3.5-4.5)	0.5	(0.3-0.9)	0.28	20
Chum	4.5	(3.9-5.0)	0.5	(0.3-0.8)	0.27	20
Chinook	6.8	(5.8-7.5)	2.7	(1.4-3.8)	1.00	30
Pink	3.5	(3.4-3.7)	0.2	(0.2-0.2)	0.10	20
Sockeye	3.9	(3.5-4.3)	0.5	(0.3-0.6)	0.24	20
Rainbow	4.1	(3.7-4.5)	0.7	(0.4-0.9)	0.33	20

on the size of the test fish. The test solutions were aerated to prevent possible premature fish mortality. Oil free compressed air was bubbled through the water column at the rate of 7.5 mL/min/L during the test.

The starting temperature of the test water was 8°C, and it stabilised at 14°C after 24 h in the test chamber. The test was designed to start at a lower temperature to minimize the shock to fish from the holding tank where the ambient water temperature ranged from 6 to 8°C.

Chemical quality of diluent water was generally similar throughout the study periods of March to June, 1986 and March to April, 1987. Hardness as $CaCO_3$ ranged from 3.25 to 3.81 mg/L during the study periods. Inorganic metals were less than the detection limits (mg/L) for most elements, i.e., (< 0.005) for Pb, As, Cr, Cu, Mn, Mo, Sr; (< 0.002) for Cd, B, Be, Ti, Zn; and (< 0.02) for Ni, Sn. The average concentration (mg/L) for the other elements was: K (0.13); Al (0.12); Ca (1.2); Fe (0.07); Mg (0.10); Na (0.50), and Si (1.10). Dissolved oxygen averaged 10.3 $\pm$ 0.8 mg/L, and pH varied from 5.6 to 6.0.

An appropriate amount of each test chemical was dissolved in 25-mL beakers with 10 mL of acetone to obtain the exposure concentrations, except for Garlon 3A™ and Garlon 4™ as both materials contained an emulsifier which readily mixed and dispersed in water. An equal volume of acetone was added to the appropriate controls. There were six nominal concentrations ranging from 0.30 mg/L to 1000 mg/L and a control for each test.

Duplicate 10 mL water samples were collected at random from one concentration of each test chemical after the 30-min aeration just before fish introduction. They were analyzed to determine the actual amount of chemicals in the test vessels.

The water samples were prepared and extracted according

to the method outlined by Lee et al. (1986). Ethyl acetate was used as the extraction solvent instead of dichloromethane. The ethylated extracts were analyzed with a Hewlett Packard Model 5890 gas chromatograph equipped with a Ni-63 electron capture detector. The residue recovery rates of this method of analysis according to Lee et al. (1986) were greater than 95 %.

RESULTS AND DISCUSSION

The analytical results show that the measured concentration of each candidate material in the water of the test vessels immediately prior to fish introduction was less than the corresponding nominal concentration (Table 4).

Table 4. Concentrations of Test Chemicals in Water

Test chemical	Concentration of Test chemical (mg/L)		Recovery (%)
	Nominal	Measured*	
Garlon 3A™	37.0	21.8	59
Garlon 4™	0.75	0.70	93
Triclopyr ester	1.17	0.98	84
Triclopyr	1.50	1.00	67
Pyridine	2.50	1.16	46
Pyridinol	1.56	1.03	66

* mean of duplicate samples

The data suggest that chemical loss occurred and the extent of loss appeared to vary for the different test chemicals. A number of factors may have contributed to this loss, viz., volatilization during the initial aeration process (Doudoroff et al. 1951); chemical degradation via photolysis and hydrolysis (McCall and Gavit 1986); reduced solubility of test chemicals (Dow Chemical 1981); and glass adsorption of test chemicals (Meehan et al. 1974).

Because of this chemical loss, the calculation of the 24, 48, 72, and 96-h LC_{50} values for the test chemicals was adjusted to the measured concentration (Table 5). The results show that the LC_{50} values changed only slightly after 96 h of exposure. The order of increasing toxicity to salmonids is as follows: Garlon 3A™, triclopyr, pyridine, pyridinol, Garlon 4™, and triclopyr ester, (Table 6). Triclopyr ester was the most toxic test chemical. Both Garlon 4™ and one of its transformation products, pyridinol were equally toxic to the salmonids tested.

Table 5. Acute toxicities to juvenile Pacific salmonids of Garlon 3A™, Garlon 4™, triclopyr acid, triclopyr ester, 3,5,6-triclopyr-2-pyridinol, and 2-methoxy-3,5,6-trichloropyridine

Test Chemical	Test Fish Species	LC50 (mg/L) 24 h	48 h	72 h	96 h
Garlon 3A™	coho	498	476	476	463
	chum	316	290	275	267
	sockeye	353	311	311	311
	rainbow	457	435	420	420
	chinook	472	312	283	275
Garlon 4™	coho	2.1	2.1	2.1	2.1
	chum	2.1	1.8	1.7	1.7
	sockeye	2.5	1.5	1.4	1.4
	rainbow	4.1	2.9	2.7	2.7
	chinook	4.2	2.7	2.7	2.7
	pink	1.9	1.3	1.2	1.2
Triclopyr ester	coho	1.0	1.0	1.0	1.0
	chum	0.4	0.3	0.3	0.3
	sockeye	0.5	0.4	0.4	0.4
	rainbow	1.1	1.1	1.1	1.1
	chinook	1.6	1.1	1.1	1.1
	pink	0.6	0.5	0.5	0.5
Triclopyr	coho	9.9	9.6	9.6	9.6
	chum	7.9	7.5	7.5	7.5
	sockeye	7.8	7.5	7.5	7.5
	rainbow	8.4	7.8	7.6	7.5
	chinook	9.7	9.7	9.7	9.7
	pink	13.3	8.8	6.1	5.3
Pyridine	coho	4.3	4.0	3.8	3.8
	chum	4.0	4.0	3.9	3.7
	sockeye	6.9	6.9	6.9	6.3
	rainbow	4.6	4.6	4.6	4.6
	chinook	3.2	2.9	2.9	2.9
	pink	1.3	1.2	1.2	1.1
Pyridinol	coho	1.8	1.8	1.8	1.8
	chum	1.8	1.8	1.8	1.8
	sockeye	2.5	2.5	2.5	2.5
	rainbow	1.5	1.5	1.5	1.5
	chinook	2.1	2.1	2.1	2.1
	pink	2.7	2.7	2.7	2.7

Table 6. Order of increasing toxicity to Pacific salmonids of test chemicals

Test Chemicals	Toxicity 96 h LC50 (Mean±S.E.)	n	Student "t" test	"t" value	Level of Significance
1. Garlon 3A™	347 + 44	5*	1 vs. 5	7.84	< 0.01
2. Triclopyr	7.9 ∓ 0.7	6	1 vs. 2	7.71	< 0.01
3. Pyridine	3.7 ∓ 0.8	6	5 vs. 6	4.60	< 0.01
4. Pyridinol	2.1 ∓ 0.2	6	2 vs. 6	9.89	< 0.001
5. Garlon 4™	2.0 ∓ 0.2	6			
6. Triclopyr ester	0.7 ± 0.2	6	(n = no. of fish species; * pink salmon not tested)		

Garlon 3A™, the amine product of triclopyr, is significantly less toxic ($p < 0.01$) to salmonids than Garlon 4™, ester formulation (Table 6). The results show that Garlon 3A™ was about 170 times less toxic to salmonids than Garlon 4™. Within the various salmonid species, Garlon 3A™ is more toxic to chum, chinook and sockeye salmon than to coho salmon and rainbow trout (Table 5). Garlon 4™ is slightly more toxic to pink and sockeye salmon than to chum, coho, chinook salmon, and rainbow trout.

Moreover, Garlon 3A™ and Garlon 4™ are significantly less toxic ($p < 0.01$) to salmonids than their respective active ingredients triclopyr and triclopyr ester (Table 6). This observation suggests that the emulsifiers used in formulating these products do not increase fish toxicity.

Triclopyr is significantly less toxic ($p < 0.001$) to salmonids than triclopyr ester, (Table 6). This acid is about 11 times less toxic to the fish than triclopyr ester. Within species, however, triclopyr is more toxic to pink, chum, sockeye salmon and rainbow trout than to coho and chinook.

Triclopyr ester is more toxic to juvenile chum, pink and sockeye salmon than to coho and chinook salmon and rainbow trout (Table 5). The 96-h LC_{50} of coho salmon for triclopyr ester is 1.0 mg/L, confirming a recent study by Mayes et al. (1986) who reported that this material to be highly toxic to both the juvenile and alevin stages of coho salmon. The respective 96-h LC_{50} values were 1.3 and 0.3 mg/L.

Test results of the two principal transformation products of triclopyr suggest that the pyridinol metabolite is somewhat more toxic to juvenile salmon than the pyridine (Table 6). The pyridinol degradation product is also as toxic to young salmon as the formulated tri-

clopyr ester. This transformation product is equally toxic to all species of juvenile salmon. The pyridine, however, is more toxic to pink salmon than the other five salmon species (coho, chum, chinook, sockeye and rainbow trout).

Under field conditions, the concentration of Garlon 3A™ in a stream unintentionally oversprayed during an aerial operation would not likely exceed a level greater than 10 mg/L in 15 cm water even at the highest recommended rate of application, (i.e., 10 kg active ingredient/ha), except perhaps during an accident. The potential of this product causing fish kills is therefore small when it is used under prescribed conditions. However, the use of the lower recommended rate of Garlon 4™, (i.e., 2.4 kg active ingredient/ha), has some potential to generate toxic concentrations (approximately 4 mg/L in 15 cm water) if the residues are not rapidly diluted and flushed out of the aquatic system.

It is doubtful that acutely toxic levels of both the pyridinol and pyridine degradation products would occur in contaminated streams, as their appearance requires the occurrence of certain chemical processes and, in the case of the pyridine, an incubation period involving microbial activity (Lee et al. 1986). However, there may be potential for sub-lethal effects of triclopyr and its transformation products in salmon waters contaminated by Garlon 3A™ and Garlon 4™ overspray. Studies have shown that low levels of these materials occurred in streams following aerial operations (Norris et al. 1976 and Wan 1986). The potential sub-lethal toxicity of triclopyr and its two transformation products to young salmon and other aquatic organisms is presently not known.

To sum up, this bioassay study indicates that Garlon 4™, the ester formulation of triclopyr, is highly toxic to salmonids of the Pacific Northwest. In contrast, Garlon 3A™, the amine formulation is considerably less toxic to fish. Of the two principal degradation products, the pyridinol metabolite is as toxic as Garlon 4™ to juvenile salmonids, while pyridine seems to be more toxic to young pink salmon than the other salmon species tested. Moreover, this study suggests indirectly that the emulsifiers used for the formulation of Garlon 3A™ and Garlon 4™ do not increase fish toxicity.

Acknowledgments. We wish to thank (1) Messr. D.M. Wilson, R.H. Kussat, G. Rawn (Environmental Protection), S. Szeto (Agriculture Canada), J. Servizi, R.W. Gordon, S. Samis (Fisheries & Oceans Canada) for their comments; and (2) Agriculture Canada Research Laboratory,

Environment Canada Laboratory Services, Vancouver, B.C., and Dow Chemical U.S.A., Midland, Michigan, for technical support.

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Received January 26, 1987; accepted May 22, 1987