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Acute Toxicity of Garlon 4 and Roundup Herbicides to Salmon, *Daphnia*, and Trout

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The herbicides Garlon 4 (butoxyethyl ester of triclopyr; 3,5,6-trichloro-2-pyridinyloxyacetic acid) and Roundup (glyphosate; isopropylamine salt) have been used primarily in agriculture. The Canadian Forest Service (Forest Pest Management Institute, Sault St. Marie, Canada) and the British Columbia Ministry of Forests (Victoria, Canada) have been evaluating these herbicides for control of deciduous trees and bushes in areas to be reforested with marketable conifers. There was a shortage of published data concerning the acute lethality of Garlon 4 to aquatic life while published values for acute lethality of Roundup to rainbow trout (Salmo gairdneri) varied by a factor of about 30. To broaden the data base for these two herbicides to include species and water qualities typical of Pacific salmon (Oncorhynchus sp.), measurements of acute lethality were made at the Cultus Lake Salmon Research laboratory, Cultus Lake, B.C. using moderately buffered water, and at the North Vancouver laboratory of the Environmental Protection Service (EPS) using a weakly buffered water.

This report summarizes the acute lethality of Garlon 4, Roundup and a surfactant (MONO818) contained in the formulation of the latter to sockeye salmon (Oncorhynchus nerka), Daphnia pulex, rainbow trout (Salmo gairdneri) and coho salmon (Oncorhynchus kisutch).

MATERIALS AND METHODS

Although used for the same purpose, Garlon 4 and Roundup are chemically different. Garlon 4 is an oil soluble, water-emulsifiable butoxyethyl ester (BEE) formulation of triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) and was supplied by Dow Chemical Company, Midland, Michigan, U.S.A. According to a specimen label, Garlon 4 is 61.6% triclopyr BEE with an acid equivalent of 44.3%; the remaining 38.4% is inert ingredients.

Roundup, composed of glyphosate ($C_3H_8NO_5P$) and a surfactant (MONO818), is manufactured by Monsanto Canada, Ottawa.

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Glyphosate is present as isopropylamine salt (480 g/L) and the actual free acid glyphosate content is 30.5% of the Roundup formulation (pers. comm. Keith A. MacMillan, Monsanto Canada Ltd., 441 MacLaren Ave., Suite 350, Ottawa, Ont., K2P 2H3). The chemical formulation of MONO818 was not divulged but the concentration in Roundup is reported to be about 15% (pers. comm. Keith A. MacMillan, Ibid.). Samples of Roundup and MONO818 were supplied by Monsanto.

Water drawn from the 37-m depth of Cultus Lake was used for dilution of stock solutions in bioassays of Pitt Lake sockeye reared at the Cultus Lake Salmon Research laboratory, and Daphnia obtained from Deer Lake, Burnaby, B.C. but cultured in Cultus Lake water. This water has been tested regularly over 20 years and is stable with respect to hardness (84 mg/L), alkalinity (60 mg/L), pH (7.8), conductivity (168 umhos/cm) and turbidity (2 NTU).

Bioassays with sockeye fry and fingerlings were acute, lethal, static tests in glass aquariums containing 10, 20, or 50 L. Temperatures are reported in Results. Oil-free compressed air was supplied to maintain dissolved oxygen near saturation. Weight of fish in the test solution averaged 0.22 g/L for fry and 0.4 g/L for fingerlings. Ten fry or five fingerlings were used in each test concentration. The latter was used to obtain 0.5 g fish/L or less. Fish sizes are reported in Results. Mortalities were removed every 24 h and acute lethalities were quantified as 96-h LC50 values (Amer. Publ. Health Assoc. 1985).

Procedures for bioassays using rainbow trout and coho at the EPS laboratory were similar to those at Cultus Lake, except that 30 L aquariums and dechlorinated Vancouver city tap water (Capilano River; hardness 3 to 4 mg/L, alkalinity 2 to 3 mg/L, pH 6.3, conductivity 12 umhos/cm, turbidity near zero NTU) were used. The rainbow trout were obtained from Fraser Valley Trout Hatchery, Abbotsford, B.C. and coho salmon were from Dept. of Fisheries and Oceans, Capilano River Hatchery, Vancouver, B.C.

Bioassays using Daphnia pulex were carried out in 30-mL Griffin type glass beakers at room temperature (21°C). Five Daphnia were placed in each beaker and concentrations were tested in triplicate. Chlorella were introduced to dilution water as a food source. All Daphnia were <24 h old at the start of each bioassay. Supplemental aeration was not supplied but measurements indicated dissolved oxygen levels were greater than 50% of saturation at the beginning and end of bioassays. Absence of movement when the bioassay beaker was rotated was the criterion for effect and these observations were made and recorded each 24 h. Tests were carried out for 96 h to obtain 96-h EC50 values. The 96-h EC50 values were obtained from semi-logarithmic plots of effects versus concentration (Amer. Publ. Health Assoc. 1985).

Analyses of herbicide content were made at the British Columbia Environmental Laboratory, Vancouver, B.C. To check for hydrolysis of Garlon 4, multiple samples were collected from two concentrations at start of bioassays, stored at 4.5°C for three, four or 15 days, and acidified to halt hydrolysis. Water samples containing Garlon 4 were extracted with petroleum ether to recover triclopyr BEE and 2,3,5-trichloromethoxy pyridine. The sample was then extracted with dichloromethane under acid conditions to recover triclopyr acid and 3,5,6-tricloro-2-pyridinol. The extracts were cleaned up on florisil if required and analyzed by gas-liquid chromatography and electron capture. The latter procedures documented hydrolysis of triclopyr BEE to the acid form during bioassays. Samples of Roundup were collected from two concentrations at start of bioassays and at intervals of one to four days. Samples containing Roundup (glyphosate) were derivatized and cleaned up prior to ion suppression high performance liquid chromatography (HPLC). Analyses were performed on a Hewlett Packard 1084 B HPLC with an ultra sphere ODS five micron column. Recovery of Garlon 4 and Roundup from quality control samples averaged 90%, respectively.

RESULTS AND DISCUSSION

Analyses of selected Garlon 4 bioassay concentrations for triclopyr content (ester and acid) indicated that the percentage recovery of BEE plus acid averaged 103% of the nominal concentration of triclopyr acid equivalent (a.e.). The total amount recovered varied from 85 to 114% but was not influenced by hydrolysis time (Table 1). Thirty-three to 60% of triclopyr BEE hydrolyzed to triclopyr acid within three days. Hydrolysis reached 91 to 100% in 15 days. The half-life for hydrolysis of triclopyr BEE was about eight days in sterile buffered water at 25°C and pH 7, but in a natural water at unspecified temperature and pH the half-life was approximately one day (McCall 1985).

Table 1. Concentrations of triclopyr BEE and acid in selected bioassays of Garlon 4.

Nominal Garlon 4 (mg/L)	Nominal Triclopyr (a.e.)[1] (mg/L)	Total Hydrolysis Time (Day)	BEE[1] (a.e.)		Acid		Total BEE & Acid	
			mg/L	%	mg/L	%	mg/L	%
1.3	0.58	3	0.30	52	0.19	33	0.49	85
		3	0.28	48	0.29	50	0.57	98
		3	0.23	40	0.35	60	0.58	100
		3	0.33	57	0.29	50	0.62	107
		4	0.35	60	0.26	45	0.61	105
0.8	0.35	3	0.22	63	0.13	37	0.35	100
		4	0.22	63	0.18	51	0.40	114
0.8	0.35	15	0.064	18	0.32	91	0.38	109
		15	0.023	7	0.35	100	0.37	107

1 - a.e.; expressed as acid equivalent

The mean 96-h LC50 of Garlon 4 for sockeye was 1.3 mg/L at 4.5°C and pH 7.9, while the 96-h EC50 for Daphnia was 1.2 mg/L at 21°C (Table 2). The 96-h LC50's for coho and rainbow trout were 2.2 mg/L at 15°C and pH 6.2. The lower LC50 for sockeye than for coho and rainbow trout may be related to hydrolysis of triclopyr BEE as discussed below.

Due to hydrolysis, toxicity of Garlon 4 will depend on the relative toxicities of triclopyr BEE and acid. Furthermore, hydrolysis rate increases with temperature and pH (McCall 1985). There is reason to believe triclopyr acid is less toxic than the ester since studies with 2,4-D BEE demonstrated that the ester was at least 400 times more toxic than the acid was to sockeye (Martens et al. 1980). Rainbow trout fry and coho fingerlings were also less tolerant of various esters of 2,4-D than of the acid form (Meehan et al. 1974). The foregoing factors suggest that less triclopyr BEE would have hydrolyzed to acid at the temperature and pH of the sockeye bioassays compared to the coho and rainbow trout bioassays thereby causing a lower LC50 for sockeye.

It is anticipated that Canadian Forest Service and B.C. Ministry of Forests will continue testing Garlon 4 to determine suitability for silviculture. To assist evaluation of potential hazards of Garlon 4 to aquatic life, the toxicity of triclopyr acid should be measured. In addition, the influence of a range of pH and temperature on hydrolysis of triclopyr BEE should be defined. Photolysis is reported to breakdown triclopyr acid in a matter of hours under August sunlight in Canada (McCall 1985).

Table 2. Acute lethality of Garlon 4.

				Fish	
Test Organism	96-h LC50[1] (mg/L)	pH	Temp °C	Average Wet Wt (g)	Average Length (cm)
Sockeye:					
Fingerlings	1.4	7.90	4.5	4.50	7.1
Fry	1.2	7.90	4.5	0.22	2.9
Daphnia pulex	1.2[2]	7.50	21.0	--	--
Rainbow Fry[3]	2.2	6.20	15.0	0.33	3.4
Coho Fry[3]	2.2	6.20	15.0	0.29	3.4

1 - Nominal values
2 - 96-h EC50
3 - Data from Environmental Protection Service (M. Wan, Kapilano 100, Park Royal, West Vancouver, B.C., V7T 1A2)

Glyphosate and its primary metabolite, aminomethyl phosphonic acid (AMPA) (Folmar et al. 1979; Bardalaye et al. 1985) were measured in two concentrations of Roundup tested with sockeye fingerlings. Similar measurements were not made for all test solutions. The measured values of glyphosate equalled 102% and 96% of nominal values at the start of the two bioassays (Table 3). In the first test, after 24 h glyphosate decreased slightly to 97% of the nominal value and continued through 72 h. No change in glyphosate concentration was observed during the second bioassay. Little or no glyphosate was converted to AMPA during 96 h of bioassay. These data indicate that glyphosate was not transformed and little, if any, was taken up by test fish. This result is consistent with studies which showed that glyphosate is very slowly absorbed across the gastro-intestinal membranes of animals, including fish (Monsanto 1982a). No test method was readily available to measure MON0818.

Sockeye fingerlings and fry were equally susceptible to Roundup; the 96-h LC50 averaged 27.7 mg/L when tested at pH 7.7 to 8.0 (Table 4). For Daphnia the 96-h EC50 was 25.5 mg/L. Based on tests using Vancouver city water, the average 96-h LC50 for rainbow was 26.8 mg/L; for a single test of coho the 96-h LC50 was 42 mg/L. The pH's were not measured during bioassays using Vancouver city water but were estimated from tests of that water in our laboratory to decrease to 5.8 and 5.7 when 28 and 42 mg/L Roundup, respectively, were added. The pH of Cultus Lake water was not affected by doses of Roundup used in bioassays.

The 96-h LC50 of Roundup is listed as 11 and 5.3 mg/L for trout and Daphnia, respectively (Monsanto 1982a) but the species, water quality and temperature were not reported. In laboratory tests using dechlorinated Vancouver city water at 12°C, the 96-h LC50 of Roundup was 54.8 mg/L when rainbow trout fingerlings (1.4 g) were tested (Hildebrand et al. 1982). It was reported that pH ranged from 4.8 to 6.2 in test solutions but values for specific concentrations were not reported. The 96-h LC50 of Roundup was 48 mg/L when rainbow trout fingerlings were tested but water quality and temperature were not reported (Sacher 1978). The 96-h LC50

Table 3. Nominal and measured amounts of glyphosate in two bioassay concentrations.

Elapsed Time (h)	Roundup Nominal Conc (mg/L)	Glyphosate Calculated Nominal Conc (mg/L)	Glyphosate Measured Conc (mg/L)	Glyphosate Metabolite (AMPA) Measured Conc (mg/L)
0	32	9.8	10.0	<1.0
24			9.5	<1.0
48			9.5	<1.0
72			9.5	<1.0
0	24	7.3	7.0	<0.5
96			7.0	<0.5

Table 4. Acute lethality of Roundup.

	Roundup	Calculated Conc[2]				Fish	
Test Organism	LC50[1] 96-h (mg/L)	Glyphosate (mg/L)	MONO818 (mg/L)	pH	Temp °C	Ave Wet Wt (g)	Ave Length (cm)
Sockeye:							
Finger-	26.7	8.1	4.0	7.95	4.2	3.80	6.5
lings	27.7	8.4	4.2	8.00	4.2	3.70	6.5
Fry	28.8	8.7	4.3	7.70	4.5	0.25	3.0
Daphnia	25.5[3]	7.8	3.8	7.30	21.0	--	--
Rainbow	28.0	8.5	4.2	<6.3[5]	15.0	0.33	3.4
fry[4]	25.5	7.8	3.8	<6.3[5]	14.5	0.60	3.9
Coho fry[4]	42.0	12.8	6.3	<6.3[5]	15.0	0.30	3.5

1 - Nominal values
2 - Based on 30.5% glyphosate and 15% MONO818 in Roundup
3 - 96-h EC50
4 - Data from Environmental Protection Service (M. Wan, Kapilano 100, Park Royal, West Vancouver, B.C., V7T 1A2)
5 - See text

for Roundup equalled 1.3 and 8.3 mg/L in reconstituted dilution water at 12°C, pH 7.2 and hardness 40 mg/L in bioassays of one and two g rainbow trout, respectively (Folmar et al. 1979). These investigators also reported a 4.8 fold increase in LC50 as pH decreased from 7.5 to 6.5.

Since Roundup is a mixture of glyphosate and MONO818, it is informative to examine their acute toxicities. For glyphosate, 96-h LC50's were 106 and 962 mg/L for trout and Daphnia, respectively, based on tests using technical glyphosate (81% active, Monsanto 1982b). The species, temperature and pH were not specified for these tests. At pH 7.2 and 6.5 and 12°C the 96-h LC50 for glyphosate was 140 mg/L when rainbow trout were tested in reconstituted water (Folmar et al. 1979). For MONO818 the 96-h LC50 ranged from 2.0 to 3.5 mg/L for Daphnia, sockeye, coho and rainbow trout fry (Table 5). Using rainbow trout and reconstituted water, the 96-h LC50 for MONO818 was reported equal to 2.0 and 7.4 mg/L at pH 7.2 and 6.5, respectively, and 12°C (Folmar et al. 1979). It is evident from the foregoing that MONO818 is much more toxic than glyphosate.

The combined effect of glyphosate and MONO818 at concentrations occurring at the 96-h LC50 of Roundup can be examined using toxic units to assess the possibility of joint action or antagonism

Table 5. Acute lethality of surfactant (MONO818).

Test Organism	LC50[1] 96-h (mg/L)	pH	Temp °C	Fish: Average Wet Wt (g)	Fish: Average Length (cm)
Sockeye fry	2.60	7.90	5.2	0.35	3.3
Daphnia	2.00[2]	7.80	21.0	--	--
Rainbow fry[3]	3.20	6.20	14.0	0.30	3.4
Coho fry[3]	3.50	6.20	14.0	0.30	3.5

1 - Nominal values
2 - 96-h EC50
3 - Data from Environmental Protection Service (M. Wan, Kapilano 100 Park Royal, West Vancouver, B.C., V7V 1A2)

(Sprague 1970). For sockeye, coho and rainbow tested in moderately and well buffered waters, the toxic units of glyphosate at the LC50's of Roundup were estimated using the LC50 (106 mg/L) of glyphosate reported for trout (Monsanto 1982b). In each case, toxic units of glyphosate were 0.12 and less; a minor contribution to acute toxicity of Roundup (Table 6). Toxic units for MONO818 exceeded unity for tests in all but reconstituted water, where the toxic units were 0.63 and 0.15 depending on pH. The sum of toxic units for glyphosate and MONO818 ranged from 1.39 to 1.92 for the sockeye, Daphnia, rainbow and coho tested in moderately or weakly buffered waters. A sum of toxic units exceeding unity suggests antagonism, and in these waters glyphosate would appear to antagonize the toxicity of MONO818. In the case of rainbow fry tested in reconstituted water, the sum of toxic units was 0.17 and

Table 6. Toxic Units of glyphosate and MONO818 at the 96-h LC50 of Roundup[1].

Test Organism[2]	Glyphosate Conc mg/L	Glyphosate LC50[4] mg/L	Glyphosate T.U.	MONO818 Conc mg/L	MONO818 pH	MONO818 LC50[2] mg/L	MONO818 T.U.	Sum T.U.
Sockeye	8.7	(106)[5]	.08	4.30	7.9	2.6	1.65	1.73
Daphnia	7.8	962	.01	3.80	7.8	2.0	1.90	1.91
Rainbow	8.5	106	.08	4.20	6.2	3.2	1.31	1.39
Coho	12.8	(106)[5]	.12	6.30	6.2	3.5	1.80	1.92
Rainbow[3]	2.5	140[3]	.02	1.25	7.2	2.0[3]	0.63	0.65
Rainbow[3]	2.3	140[3]	.02	1.14	6.5	7.4[3]	0.15	0.17

1 - Toxic Units based on 30.5% glyphosate and 15% MONO818 in Roundup
2 - Table 4, except as noted
3 - Folmar et al. 1979
4 - Monsanto 1982b, except as noted
5 - Value reported for Rainbow trout (Monsanto 1982b)

0.65. These sums suggest that the combined effect of glyphosate and MONO818 were "more than additive" and contrary to the results obtained in the other four tests. This outcome raises doubt that the 96-h LC50's reported for Roundup in reconstituted water (Folmar et al. 1979) are applicable to natural waters.

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