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Effects of Different Dilution Water Types on the Acute Toxicity to Juvenile Pacific Salmon and Rainbow Trout of Fluroxypyr Formulated Product XRM-5084

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Methylheptyl ester (ME) of fluroxypyr (4-amino-3,5-dichloro-6-fluoro-2-pyridyl-oxyacetic acid) is a base chemical manufactured by DowElanco Company, Midland, Michigan, USA for the formulation of XRM-5084 (XRM). XRM is a selective and systemic herbicide used for the control of broadleaf vegetation. The possible registration in Canada and use of XRM for conifer release in forestry and industrial rights-of-way programs in coastal British Columbia may result in the accidental introduction of fluroxypyr into waterbodies inhabited by fish. The objective of this study was to evaluate the acute toxicity of XRM to juvenile Pacific salmonids and rainbow trout in different water types.

MATERIALS AND METHODS

The common name, abbreviation, and concentration of test material is respectively: XRM-5084, XRM, and 34.9 % methylheptyl ester of 4-amino-3,5-dichloro-6-fluro-2-pyridyloxyacetic acid + 65.1 % proprietary emulsifier. All test materials were supplied by DowElanco Company, Midland, Michigan.

A series of 96-hr static acute toxicity tests was conducted in fresh water using several species of juvenile salmonids [coho (*Onchorhynchus kisutch*), chinook (*O. tshawytscha*), chum (*O. keta*), pink (*O. gorbuscha*), rainbow trout (*O. mykiss*), sockeye (*O. nerka*)] obtained from British Columbia hatcheries. The tests were conducted from February to June 1991 in accordance with the procedure outlined by Environment Canada (1990) and Wan et al. (1989, 1991). Testing was carried out at a temperature of 14 ± 1°C, and with a 16-hr light:8-hr dark photoperiod regime. The average loading density was 0.25 (0.10 - 0.5) g/L, with a bio-assay test volume of 20 L. A 3-mm disposable plastic bag liner was used in each test vessel to hold the dilution water. Ten fish were used in each test vessel. The cumulative fish mortality was recorded and the LC50 values were calculated using the "Lethal" computer program developed by Stephan (1983).

Three different water types were used as dilution water: (1) **soft, acid pH** - city tap (Vancouver dechlorinated), (2) **hard, alkaline pH**

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- lake (Cultus Lake, British Columbia), and (3) **intermediate between (1) & (2)** - reconstituted deionized city tap ($CaSO_4 . H_2O$, KCl, $MgSO_4$, and $NaHCO_3$, APHA 1987). XRM-5084 contained an emulsifier which allowed the product to readily mix and disperse in water.

Triplicate 200-mL, 1-L, and 4.5-L water samples were collected from each water type. The 200-mL water samples were acidified with 10 drops of concentrated nitric acid and used for the determination of extractable metals, and chemical elements and ions content. The 1-L water samples were used for the determination of alkalinity, conductivity, hardness and pH, while the 4.5-L water samples were used for the analyses of organochlorine pesticide and other organic contaminant residues. The screening of water samples for metals, nutrients, and organic residues was conducted at Conservation & Protection Laboratory, West Vancouver, British Columbia, using methods developed by Environment Canada (1989).

Two 50-mL water samples were collected from the mid-concentration of each coho salmon test solution of each water type. Each water sample contained ten 5-mL composite sub-samples pooled to make up the 50-mL sample. The 0-hr samples were collected shortly after the addition and stir-mixing for about 1 min of the test material in the bioassay container. The 0.5-hr samples were taken after the 30-min aeration process but just before fish introduction, and subsequently samples were collected every 24-hr during the 4-d test.

Immediately after the 96-hr test run, the contents of the test vessel (where water samples were collected) were emptied and the plastic liner of the container was rinsed twice (2 x 100 mL) with acetone. This acetone solution was analysed for fluroxypyr residues. Two 5-mL XRM samples were submitted to the laboratory for fluroxypyr content validation. The calculation of the measured concentration of XRM in water samples was based on the fluroxypyr methylheptyl ester (F-ME) content analytically found in the product XRM sample (lot # GHD-1298-86), and not based on the F-ME content stated in the label of the sample bottle.

The analyses of fluroxypyr residues in water were conducted at the British Columbia Research Corporation, Vancouver, British Columbia. Fluroxypyr residues in the water samples were acidified, extracted with an organic solvent, and derivatized with diazomethane. The fluroxypyr free acid methyl ester and F-ME were analyzed in the same extract by using a Hewlett-Parkard GLC (Model 5890) equipped with an electron capture detector. A DB5 [30 m x 0.32 mm (i.d.), 0.25 um film thickness] fused-silica capillary column was used. The detector temperature was 350°C; injector temperature 240°C; column temperatures: initial 60°C for 0.5 min; ramped at 25°C/min to 150°C, 4°C to 220°C, then at 10°C to 260°C, and hold for 2 min. Standards for each compound were analysed along with the samples. The concentration of fluroxypyr free acid detected was converted to F-ME and a total F-ME result was reported. Method blanks and spiked samples were also analysed along with the samples and standards. The mean of blind quality control (QC) sample recovery rates of fluroxypyr was 96% $\pm$ 4 (S.E., n = 6).

RESULTS AND DISCUSSION

Chemical properties of each water type did not change throughout the study period from February to June 1991 (Table 1). Each dilution water differed from the other significantly ($p < 0.01$) in terms of alkalinity, conductivity, hardness, pH, and some chemical elements/ ions. Detectable residues (detection limit 1 ug/L) were not found in each water of the following compounds: total DDT, BHC, PCB, PCP, picloram, and simazine.

The measured concentrations of fluroxypyr product in different water types were much less than the nominal concentration (Table 2). The data suggest that chemical losses occurred, mainly due the adsorption of fluroxypyr onto the plastic liner, as indicated in the high recovery of herbicide residues [averaging 10 % (range, 9 - 11 %) for the three water types] in the acetone rinses of the empty plastic liner immediately after the 96-hr test run (Table 2). This observation confirms the findings of Sharom and Solomon (1981) who reported that pesticides adsorbed onto plastic or glass containers.

However, volatilization during the initial 30-min aeration process could also have contributed to the chemical losses (Doudoroff et al. 1951). Further losses of fluroxypyr in the test water beyond 0.5 hr were likely caused by the metabolic activities of the test fish, and the chemical and microbial degradation processes of the herbicide to the methoxypyridine and methoxypyridinol metabolites. No attempt was made to determine the residues of these metabolites in water, as the analytical standards of these chemicals were not available at the time of study.

The 96-hr LC50 values for XRM were adjusted, based on the measured concentration of this product in the water at 0.5 hr (water samples obtained just before fish introduction). Duplicate analytical results of XRM (lot # GHD-1298-86) test sample supplied by the manufacturer indicated that it contained 35 % methylheptyl ester equivalent of fluroxypyr. Adjustments based on QC spiked samples recovery rates were not made.

The data in Table 3 suggest that XRM is more toxic than Roundup^{R} to salmonids in soft and intermediate water (Wan et al. 1989). In hard water, it is as toxic as Roundup^{R} to the fish. However, XRM is less toxic to the fish than Garlon4^{TM} and Weedone CB irrespective of water types (Wan et al. 1987, 1990). As well, the data indicate that pink salmon is the most sensitive fish to XRM irrespective of water types. Amongst the six species of salmonids, rainbow trout is the least sensitive fish to this product in both intermediate and hard water, whereas in soft water, chum salmon is the most tolerant fish. One other notable observation is that almost all mortalities of fish occurred in the first 24-hr period for XRM in the three water types.

Table 4 compares the toxicities to juvenile salmonids of XRM in different water types. It shows that XRM is significantly ($p < 0.01$) more toxic to juvenile salmonids in soft and intermediate water. Alkalinity, conductivity, hardness, and pH of water did not appear to affect the acute toxicity of XRM. The order of increasing

Table 1. Characteristics* of dilution water.

Parameter analyzed	Abbrev.	Type of dilution water		
		Soft (city)	Intermediate (reconstituted)	Hard (lake)
Alkalinity	Alka.	1.2 ± 0.1	30.3 ± 0.5	63.1 ± 0.2
Chemical elements/ions	Chem.			
C (total)		1.7 ± 0.2	10.3 ± 0.1	16.3 ± 0.2
Ca		1.2 ± 0.1	7.2 ± 0.3	27.5 ± 0.2
K		< 2	< 2	< 2
Mg		0.2 ± 0.02	5.8 ± 0.1	2.5 ± 0.1
Na		0.6 ± 0.04	13.8 ± 0.5	2.8 ± 0.1
Si		1.9 ± 0.1	2.3 ± 0.2	3.8 ± 0.2
Cl		1.5 ± 0.1	1.1 ± 0.1	1.6 ± 0.2
SO_4		1.3 ± 0.2	43 ± 1	24.1 ± 0.3
total Chem.		8.4	83.5	77.6
Conductivity (umhos/cm)	Cond.	15 ± 0.6	162 ± 1	183 ± 2
Hardness (total)	Hard.	3.6 ± 0.2	42 ± 0.9	79.7 ± 1.1
pH (rel. U.)	pH	6.1 ± 0.1	7.6 ± 0.1	8.1 ± 0.1

* - parameter measured in mg/L (mean ± S.E., n = 3); detection limits, < 0.001 - 0.01 mg/L

Table 2. Concentrations of test chemical.

Test chemical & time	Concentration of test chemical (mg/L product)								
	soft water			intermediate water			hard water		
	nom.	mea.	rec. (%)	nom.	mea.	rec. (%)	nom.	mea.	rec. (%)
XRM									
0-h	30	19.7	66	30	19.1	64	30	21.4	71
0.5-h	-	16.9	56	-	11.4	38	-	15.1	51
24-h	-	7.1	24	-	7.4	25	-	9.1	31
48-h	-	6.6	22	-	7.7	26	-	8.6	29
72-h	-	4	13	-	6.3	21	-	6.6	22
96-h	-	3.4	11	-	3.1	11	-	2.3	8
acetone*	-	2.7	9	-	2.7	9	-	32.3	11

nom. = nominal; mea. = measured, product calculation based on analytical results of F-ME equivalent found in product sample; rec. = recovery, nearest whole number; * = acetone rinse of empty bioassay container plastic liner immediately after 96-hr test.

Table 3. Acute toxicities[1] to juvenile Pacific salmon and rainbow trout of fluroxypyr product XRM-5084, Roundup[R], Garlon4[TM], and Weedone CB in different dilution water types.

Dilution water & Fish[2] species	96-hr LC50 in mg/L[3]			
	XRM-5084	[a]Roundup[R]	[b]Garlon4[TM]	[c]Weedone CB
Soft (city)				
coho	17	32	2	10
chinook	13	33	3	-
chum	19	20	2	-
pink	12	33	1	2
rainbow trout	17	33	3	5
sockeye	15	-	1	-
Intermediate (reconstituted)				
coho	10	33	-	8
chinook	9	19	-	2
chum	10	15	-	2
pink	8	17	-	2
rainbow trout	12	18	-	4
sockeye	10	-	-	2
Hard (lake)				
coho	14	13	-	9
chinook	16	17	-	-
chum	14	11	-	-
pink	11	14	-	2
rainbow trout	17	14	-	6
sockeye	13	-	-	-

[1]. - adjusted for chemical loss (except Garlon4[TM]).
[2]. - age = 3.5 ± 0.5 mo.; length = 4.1 ± 0.1 cm, 30 fish; wt = 0.8 ± 0.1 g, 30 fish.
[3]. - mg product/L to the nearest whole number.
a, b, c - Wan et al. 1987, 1989, 1990.

toxicity of XRM is: soft < hard < intermediate water. As well, metals and chemical ions did not appear to influence the 96-hr LC50 of XRM.

The manufacturer's recommended experimental rates of XRM application for forestry and industrial brush control in Canada vary from a low of 0.56 kg acid equivalent (ae)/ha to a high of 1.12 kg ae/ha, or 0.5 to 1 lb ae/A (per. com. DowElanco, Saskatoon, 1991). These rates of treatment are equivalent to a respective low and high rates of 2.4 and 4.6 kg XRM/ha, or 2.1 and 4.1 lb XRM/A.

Under field conditions, the concentration of XRM in a water body unintentionally contaminated with this product at the lower rate of

Table 4. Acute toxicities[1] of XRM to salmonids in different dilution water.

Test chemical & dilution water types	Water quality					Toxicity 96-h LC50		Statistical tests[2]		
	Alk.	Chem.	Cond.	Hard.	pH	Mean (mg/L)	Mean $\log_e$	Means compared	t^3 Diff/S_D	Tukey's test ($p < 0.01$)
XRM-5084										
1. Lake (hard)	63.1	77.6	183	79.7	8.1	14.4	2.66	1 vs. 3	1.21	NS
2. Recon[a] (intm)	30.3	83.5	162	42	7.5	10	2.29	2 vs. 1	6.05	S
3. City (soft)	1.2	8.4	15	3.6	6.1	15.6	2.73	3 vs. 2	7.26	S

1 - adjusted for chemical loss; 2 - Steel & Torrie (1960); 3 - S_D = 0.061, df = 6, critical value for Tukey's test = 2.23 (5 % level), 3.17 (1 % level). a - reconstituted (intm = intermediate). S - significant; NS - not significant

application approximates 1.5 mg/L in 15 cm water, while the equivalent concentration under a similar condition at the high rate of treatment is 3 mg/L. During an accidental spill, these levels could be substantially higher. The concentration of XRM when used at both rates of treatment would not exceed the 96-h LC50 of salmonids and rainbow trout in the three water types, although little is known about the impact of sub-lethal concentrations of this herbicide to juvenile salmonids. Therefore, XRM could only generate acutely toxic conditions to young fish during accidental spills and misapplications.

To sum up, this bioassay study indicates that XRM is more toxic than Roundup[R] to juvenile Pacific salmonids and rainbow trout in soft and intermediate water. It is as toxic to salmonids as Roundup[R] in hard water. Changes in alkalinity, conductivity, hardness, and pH of water do not appear to affect the acute toxicity to young salmon of XRM. The potential to generate concentrations acutely toxic to salmonids of XRM at the recommended rates of application could only occur when streams are accidentally oversprayed or during spills. Pink salmon is the most sensitive fish to XRM irrespective of water types.

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