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Effects of Different Dilution Water Types on the Acute Toxicity to Juvenile Pacific Salmonids and Rainbow Trout of Glyphosate and Its Formulated Products

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Glyphosate [N-(phosphonomethyl) glycine] is a broad spectrum herbicide manufactured by Monsanto Company. Its formulated product Roundup^R, also known as Vision^R in Canada, is a non-selective herbicide. Concerns have been raised about the applicability to natural water of the 96-h LC_{50} data for young salmon of Roundup^R assayed in reconstituted water (Servizi et al. 1987). This study compares the effects of reconstituted water and four natural sources of dilution water on the acute toxicity to juvenile Pacific salmonids and rainbow trout of glyphosate and its formulated products: Roundup^R, MON 8709 (a new formulation of glyphosate), and their surfactant MON 0818.

MATERIALS AND METHODS

The common names and concentration of test materials are summarized in Table 1. The test materials were supplied by Monsanto Company, U.S.A, Chesterfield, Missouri; and Monsanto Canada Incorporated, Delta, British Columbia.

A series of 96-h static acute toxicity tests was conducted using 5 species of juvenile salmonids (see footnote, Table 4) obtained in fresh water from British Columbia hatcheries. The tests were carried out from February to June 1988 in accordance with the protocols of Environment Canada (1980), Buchanan (1982), and the procedure outlined by Wan et al. (1988). Testing was undertaken at a temperature of 14 $\pm$ 1°C, and with a 16-h light:8-h dark photoperiod regime. The average loading density was 0.25 (0.10 - 0.4) g/L, and the test volume was 20 L. Ten fish were placed in each test vessel. The cumulative fish mortality was recorded and the LC_{50} values were calculated using the "Lethal" computer program developed by Stephan (1983).

Five different sources of water were used as dilution water: (1) city tap (Vancouver dechlorinated), (2) creek (Chehalis Creek, British Columbia), (3) reconstituted

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Table 1. List of test materials

Common name	Abbrev.	Concentration of active ingredient
Glyphosate (Technical grade)	Gly.	N-(phosphonomethyl) glycine; batch sample # 1 = 88.5 %; batch sample # 2 = 95.4 %
MON 0818	0818	75 % tallow amine surfactant
MON 8709	8709	41 % N-(phosphonomethyl) glycine as isopropyl-amine salt (30.5 % glyphosate equiv.), 10 % MON 0818, 49 % water and inerts; % by wt.
Roundup^{R} (Vision^{R})	Rdup.	41 % N-(phosphonomethyl) glycine as isopropyl-amine salt (30.5 % glyphosate equiv.), 15 % MON 0818, 44 % water and inerts; % by wt.

deionized city tap ($CaSO_4.2H_2O$, KCl, $MgSO_4$, and $NaHCO_3$ APHA 1985), (4) well (Cypress Well, British Columbia, Canada), and (5) lake (Cultus Lake, British Columbia). Each water was checked once for chlorinated pesticide residues and at regular intervals for its quality. Duplicate 50 mL water samples were collected at random from the mid concentration of each coho test solution to ascertain glyphosate concentration. Sampling was conducted after the addition of test chemicals and the 30-min preaeration process but before fish introduction.

The screening of water samples for extractable metals, nutrients, and chlorinated pesticides was conducted (using in-house methods) at Environment Canada Laboratory, West Vancouver, B.C. Glyphosate and amino methylphosphonic acid (ampa, the major metabolite of glyphosate) residue analyses in water were performed (using methods developed in-house) by the British Columbia Research Laboratory, Vancouver, B.C., Canada. The recovery rates for glyphosate and ampa in water were both 98.5 $\pm$ 1.0 %; n = 5, i.e., (mean $\pm$ S.E.; n = quality control samples). No attempt was made to recover the tallow amine residues of MON 0818, as a high resolution recovery method for this chemical is presently not available.

RESULTS AND DISCUSSION

Chemical quality of each source of water was relatively consistent throughout the study period from February to June 1988, (Table 2). Each water differed from the other significantly ($p < 0.05$) in terms of alkalinity, conductivity, hardness, pH, and some chemical elements/ions (those above the detection limits). The five dilution waters can be categorized into three main water types: (1) soft water (city, creek); (2) hard water (lake); and (3) intermediate between soft and hard water (reconstituted and well). Notably, the ratio of hardness for city, creek, reconstituted, well, and lake water was respectively: 1, 2, 8, 13, and 16 times that of soft (city) water. This ratio indicates the

Table 2. Quality* of dilution waters

Parameter analyzed	Type of dilution water				
	Soft (city)	Soft (creek)	Intermediate (reconstituted)	Intermediate (well)	Hard (lake)
Alkalinity	2.5 ± 0.2	6.7 ± 0.2	29.2 ± 0.4	27 ± 1	65 ± 0.7
Chemical elements/ions					
C (total)	4 ± 1	4.8 ± 0.7	7.2 ± 1	7.8 ± 1.8	17 ± 2.5
Ca	1.4 ± 0.1	3.3 ± 0.2	6.9 ± 0.1	18.5 ± 0.3	29.8 ± 0.3
K	0.1	0.1	1	2	0.1
Mg	0.2	0.3	5.5 ± 0.1	5.1 ± 0.1	2.7 ± 0.1
Na	0.7 ± 0.1	1.0 ± 0.1	13.6 ± 0.5	34.7 ± 0.2	3.3 ± 0.1
Si	1.4 ± 0.1	1.9 ± 0.1	ND	7.3 ± 0.1	2.8 ± 0.1
Cl	1.6 ± 0.9	0.8 ± 0.3	1.1 ± 0.4	63.6 ± 5	3.9 ± 1.1
SO_4	3.0 ± 0.1	4.5 ± 0.1	35 ± 0.5	15 ± 0.2	2 ± 0.4
Conductivity (umhos/cm)	17 ± 0.5	28 ± 3	148 ± 1	318 ± 26	180 ± 3
Hardness (total)	5.3 ± 0.2	10 ± 0.5	40 ± 0.5	68 ± 1.3	86 ± 1
pH (rel. U.)	6.3 ± 0.03	7.2 ± 0.05	7.8 ± 0.04	7.8 ± 0.1	8.2 ± 0.06

* - parameter measured in mg/L (mean + S.E., n = 8); ND - not detected; detection limits, < 0.001 - 0.01 mg/L

Table 3. Concentrations of test chemicals

Dilution water and test chemicals	Concentration of test chemicals (mg/L) at 0-h									Chemical Loss (%)			
	Theoretical				Measured		Calculated[c]						
	Gly.	0818	8709	Rdup.	Gly.	Ampa	0818	8709	Rdup.	Gly.	0818	8709	Rdup.
Soft (city)													
Glyphosate[a]	25	-	-	-	20.8	ND	-	-	-	16.8	-	-	-
MON 0818[b]	-	6.5	-	-	-	ND	4.9	-	-	-	24.7	-	-
MON 8709	15.3	-	50	-	13.7	ND	-	44.8	-	10.4	-	10.4	-
Roundup[R]	4.6	-	-	15	4.3	ND	-	-	14	6.5	-	-	6.6
Soft (creek)													
Glyphosate[a]	25	-	-	-	20	ND	-	-	-	16	-	-	-
MON 0818[b]	-	5.5	-	-	-	ND	4.1	-	-	-	25.4	-	-
MON 8709	15.3	-	50	-	13.4	ND	-	43.8	-	12.4	-	12.4	-
Roundup[R]	5.8	-	-	19	5.8	ND	-		19	0	-	-	0
Intermediate (reconstituted)													
Glyphosate[a]	75	-	-	-	71.8	ND	-	-	-	4.3	-	-	-
MON 0818[b]	-	3.7	-	-	-	ND	2.8	-	-	-	24.3	-	-
MON 8709	7.6	-	25	-	7.4	ND	-	24.3	-	2.6	-	2.8	-
Roundup[R]	3.8	-	-	12.5	3.6	ND	-	-	11.8	5.3	-	-	5.6
Intermediate (well)													
Glyphosate[a]	75	-	-	-	72.4	ND	-	-	-	3.5	-	-	-
MON 0818[b]	-	3.9	-	-	-	ND	2.9	-	-	-	25.6	-	-
MON 8709	7.6	-	25	-	7.3	ND	-	24	-	4	-	4	-
Roundup[R]	3.8	-	-	12.5	3.7	ND		-	12.2	2.6	-	-	2.4
Hard (lake)													
Glyphosate[a]	175	-	-	-	157	ND	-	-	-	10.3	-	-	-
MON 0818[b]	-	2.4	-	-	-	ND	1.8	-	-	-	25	-	-
MON 8709	5.8	-	19	-	5.4	ND	-	17.7	-	6.8	-	6.8	-
Roundup[R]	4.6	-	-	15	4.5	ND	-	-	14.7	2	-	-	2

a - technical grade glyphosate; b - technical grade 0818; c - back calculation based on a 30.5 % Gly. in Rdup. and MON 8709 (% by wt.), a 75 % tallow amine surfactant in MON 0818; ampa - amino methylphosphonic acid; ND - not detected, limit = < 1 mg/L

prevalence of Ca^{++} and Mg^{++} ions and the potential buffering capacity of each water in relation to the softest water (city) used in this test.

No detectable residues (limit of detection, 1 µg/L) were found in each water for the following chlorinated pesticides: DDT analogues, benzene hexachloride, cyclodiene and phenoxy compounds, PCB, PCP, and picloram. However, the measured concentration of glyphosate of each test material in different dilution water types in the coho test vessels before fish introduction was less than the corresponding theoretical concentration (Table 3). The data suggests that glyphosate loss occurred in the technical and the formulated materials. Losses for techni-

cal glyphosate and MON 8709 seemed to be greater in both soft and hard but not in intermediate water type. Several factors may have contributed to this loss, i.e., volatilization during the initial aeration process (Doudoroff et al. 1951); glass adsorption (Sharom and Solomon 1981); reduced solubility of test chemicals (Dow Chemical 1981); and possibly the varying purity of glyphosate in the technical material (Table 1).

The 24, 48, 72, and 96-h LC_{50} values for the test materials were adjusted to the measured concentration, except for MON 0818; its adjustment was based on the concentration of tallow amine surfactant provided by Monsanto Company (Table 1).

The results show that the LC_{50} values not only varied but also changed considerably during the 96-h of exposure for the different materials tested in different dilution water types (Table 4). Glyphosate appeared to have the least stability within the 96-h test period.

The 96-h toxicity data in Table 4 show that between fish species, glyphosate is most toxic to chum and rainbow trout in soft water (city, creek; hardness 5.3, 10 respectively), least toxic to chinook in hard water (lake, hardness 86) and equally toxic to the fish in intermediate water types (reconstituted, well; hardness 40, 68 respectively). MON 0818 is most toxic to pink and chum in hard water, and least toxic to coho in soft water. MON 8709 is most toxic to rainbow trout in hard water and least toxic to chinook in soft water types. This product is also similarly toxic to fish in intermediate water water type. Roundup^{R} is most toxic to chum in hard water, and least toxic to coho in intermediate water type, and to chinook, pink and rainbow trout in soft water.

The 96-h LC_{50} value appears to vary considerably for the same fish species tested under similar conditions. For example, the 96-h LC_{50} for rainbow trout (0.48 g) of Roundup^{R} in reconstituted water (pH 7.8, hardness 40 mg/L $CaCO_3$) of this test is 18 mg/L. Other studies using the same fish species under comparable rearing techniques and test conditions of fish size, pH, hardness, and water temperature in reconstituted water (APHA 1985) reported values varying from 1.6 mg/L (Folmar et al. 1979) to 22 mg/L (Mitchell et al. 1987). Different strains of rainbow trout tested under similar conditions may have varying 96-h LC_{50} values, but these values would be unlikely to vary by an order of magnitude.

Factors such as hardness and pH in water affect the toxicity to salmonids of heavy metals (Miller and Mackay 1980; Lauren and McDonald 1986). These factors, includ-

Table 4. Acute toxicities (adjusted for chemical loss) to juvenile Pacific salmonids and rainbow trout of glyphosate, MON 0818, MON 8709, and Roundup^{R} in different dilution water types

Dilution water types	Test Fish* Species	LC50 in mg/L** (95 % confidence limit)															
		Glyphosate				MON 0818				MON 8709				Roundup^{R}			
		24-h	48-h	72-h	96-h	24-h	48-h	72-h	96-h	24-h	48-h	72-h	96-h	24-h	48-h	72-h	96-h
Soft (city)																	
	coho	44	27	27	27	4.9	4.6	4.6	4.6	59	57	57	55	42	34	32	32
	chum	16	13	10	10	2.7	2.7	2.7	2.7	55	47	36	36	24	23	20	20
	chinook	24	22	22	19	2.9	2.8	2.8	2.8	84	79	73	67	35	33	33	33
	pink	26	14	14	14	4.5	4.5	4.5	4.5	88	54	48	48	35	33	33	33
	rainbow	21	11	11	10	2.9	2.4	2.3	2	88	62	48	48	33	33	33	33
Soft (creek)																	
	coho	55	37	36	36	4.1	3.5	3.4	3.2	54	51	51	51	27	27	27	27
	chum	26	25	24	22	2.6	2.4	2.4	2.4	62	58	58	58	31	27	25	19
	chinook	55	30	30	30	4.9	3	2.8	2.8	70	62	62	62	41	32	30	27
	pink	63	34	23	23	4.2	3.9	3	2.8	77	46	46	46	33	33	33	31
	rainbow	32	26	22	22	3.2	2.7	2.5	2.5	33	33	31	31	21	21	17	15
Intermediate (reconstituted)																	
	coho	153	122	117	112	2.8	2.8	2.8	2.8	45	34	34	34	52	38	33	33
	chum	112	101	101	99	2.7	2.7	2.7	2.6	34	34	34	34	18	18	17	15
	chinook	157	109	102	102	2.7	2.7	2.7	2.7	52	48	36	28	23	22	20	19
	pink	94	94	94	94	1.7	1.5	1.5	1.5	52	31	26	26	17	17	17	17
	rainbow	107	107	103	99	2.3	2.2	2	1.6	34	34	34	34	20	18	18	18
Intermediate (well)***																	
	coho	132	121	115	111	2.9	2.9	2.9	2.9	56	55	55	44	35	35	35	30
	chinook	128	108	108	108	2.6	2.6	2.6	2.6	51	47	45	45	28	28	27	22
	pink	102	102	102	102	5.3	3	2.6	2.6	68	52	34	34	35	30	20	19
	rainbow	115	108	108	93	2.6	2.6	2.6	2.6	42	42	35	29	20	19	19	18
Hard (lake)																	
	coho	210	205	182	174	1.8	1.8	1.8	1.8	25	25	25	25	14	13	13	13
	chum	202	178	157	148	1.5	1.4	1.4	1.4	25	25	23	23	17	12	11	11
	chinook	220	220	211	211	2	2	1.9	1.7	33	33	33	33	17	17	17	17
	pink	380	245	190	190	2.4	1.7	1.6	1.4	24	24	24	24	17	17	17	14
	rainbow	220	220	220	197	2	2	1.9	1.7	31	20	17	17	17	17	15	14

* - coho (Oncorhynchus kisutch), chum (O. keta), chinook (O. tshawytsha), pink (O. gorbuscha), rainbow trout (salmo gairdneri), sockeye (not available for testing); age = 2.6 ± 0.3 mo., 5 species (mean LC50 ± S.E., n); length = 4.3 ± 0.3 cm, 100 fish; weight = 0.5 ± 0.2 g, 100 fish

** - glyphosate & MON 0818 = mg active ingredient/L; MON 8709 & Roundup^{R} = mg product/L; *** - chum not tested

Table 5. Comparison of toxicity (mg/L) to salmonids in different dilution water types

Test chemicals and dilution water types		pH	Toxicity 96-h LC_{50} (Mean ± S.E.)	Statistical tests and comparison $N_1;N_2$[a]	Student "t" test	"t" Value	P < 0.05
Glyphosate							
1. Lake	(hard)	8.2	184 ± 12	5;5	3 vs. 1	6.71	S
2. Well	(intm.)[c]	7.8	104 ± 5	4;5[d]	3 vs. 2	0.51	NS
3. Recon[b]	(intm.)[c]	7.8	101 ± 3	5;5	3 vs. 4	17.44	S
4. Creek	(soft)	7.2	27 ± 3	5;5	3 vs. 5	17	S
5. City	(soft)	6.3	16 ± 3	5;5	1 vs. 5	13.44	S
MON 0818							
6. City	(soft)	6.3	3.3 ± 0.6	5;5	9 vs. 6	1.49	NS
7. Creek	(soft)	7.2	2.7 ± 0.2	5;5	9 vs. 7	1.11	NS
8. Well	(intm.)[c]	7.8	2.7 ± 0.1	4;5[d]	9 vs. 8	1.27	NS
9. Recon[b]	(intm.)[c]	7.8	2.3 ± 0.3	5;5	9 vs. 10	2.21	NS
10. Lake	(hard)	8.2	1.6 ± 0.1	5;5	6 vs. 10	2.8	S
MON 8709							
11. City	(soft)	6.3	51 ± 6	5;5	14 vs. 11	3.16	S
12. Creek	(soft)	7.2	50 ± 6	5;5	14 vs. 12	3	S
13. Well	(intm.)[c]	7.8	38 ± 5	4;5[d]	14 vs. 13	1.3	NS
14. Recon[b]	(intm.)[c]	7.8	31 ± 2	5;5	14 vs. 15	1.66	NS
15. Lake	(hard)	8.2	25 ± 3	5;5	11 vs. 15	3.88	S
Roundup[R]							
16. City	(soft)	6.3	30 ± 3	5;5	19 vs. 16	2	NS
17. Creek	(soft)	7.2	24 ± 3	5;5	19 vs. 17	0.8	NS
18. Well	(intm.)[c]	7.8	22 ± 3	4;5[d]	19 vs. 18	0.4	NS
19. Recon[b]	(intm.)[c]	7.8	20 ± 4	5;5	19 vs. 20	1.46	NS
20. Lake	(hard)	8.2	14 ± 1	5;5	16 vs. 20	5.06	S

a - sample size; b - reconstituted; c - intermediate; d -unequal sample size "t" analysis; S - significant; NS - not significant

ing alkalinity, conductivity and metal element/ionic content, may also affect, and possibly cause, the variation of acute toxicities to salmonids of glyphosate, MON 0818, MON 8709, and Roundup[R].

Table 5 compares the applicability of reconstituted water to natural sources of water in testing the acute toxicity to salmonids of glyphosate, MON 0818, MON 8709, and Roundup[R]. Glyphosate is significantly more toxic ($p < 0.05$) to juvenile salmonids in soft (city, creek) water than in reconstituted water. This chemical is also significantly more toxic ($p < 0.05$) to the fish in reconstituted water than in hard (lake) water. However, it is equally toxic to salmonids in similar (well) water type. MON 0818 toxicity to salmonids in reconstituted water is not significantly different ($p > 0.05$) to test results of the other water types.

Of the formulated products, MON 8709 is significantly more toxic ($p < 0.05$) to salmonids in reconstituted water than soft (city, creek) water but not in similar (well) water and hard (lake) water types. Roundup[R] toxicity to salmonids (96-h LC_{50} 20 mg Roundup[R]/L) in reconstituted water is not significantly different ($p < 0.05$) to test results of the other water types. This observation is contrary to the finding of Servizi et al. (1987) but confirms the study of Mitchell et al. (1987). Tests

of Roundup^R in a soft, slightly acidic water yield the upper range of the 96-h LC_{50} value (30 mg Roundup^R/L), while test results from a hard and slightly alkaline pH water produce the lower range (14 mg Roundup^R/L).

The surfactant MON 0818 is the most potent toxicant to salmonids when compared to glyphosate, MON 8709, and Roundup^R, irrespective of water types (Table 5). Glyphosate by itself is most toxic to salmonids in soft water and least toxic (12 times less) to the fish in hard water. MON 0818 is most toxic to salmonids in hard water. In the formulated products, both MON 8709 and Roundup^R are significantly more toxic ($p < 0.05$) to salmonids in hard water than they are in soft water, with Roundup^R almost twice as lethal as MON 8709. Roundup^R contains 50 % more MON 0818 surfactant than MON 8709. As well, MON 0818, MON 8709, and Roundup^R are significantly ($p < 0.05$) more toxic (twice as toxic) to salmonids in hard water than they are in soft water. This observation indicates that MON 0818 surfactant by itself and in the formulated products reduces the buffering capacity of hard water and thereby increases the toxicity of the materials to salmonids.

To sum up, this study indicates that a notable variation of 96-h LC_{50} values is attained for the same fish species when different water types are used in the bioassay of glyphosate, MON 0818, MON 8709, and Roundup^R. Variation of 96-h LC_{50} values for MON 0818, MON 8709, and Roundup^R is in the same order of magnitude, irrespective of water types. For glyphosate, the 96-h LC_{50} values for different water types can vary by an order of magnitude. Hardness of water and pH appear to be the key factors causing the variation of 96-h LC_{50} values. Roundup^R, MON 8709, and MON 0818 are more toxic to young salmonids in hard than they are in soft water, while the reverse holds for glyphosate. Salmonid 96-h LC_{50} data for Roundup^R and MON 0818, but not for glyphosate and MON 8709, obtained from reconstituted water (APHA 1985) are generally similar to 96-h LC_{50} values generated from natural sources of dilution water.

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