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Acute Toxicity to Juvenile Pacific Salmonids and Rainbow Trout of Butoxyethyl Esters of 2,4-D, 2,4-DP and Their Formulated Product: Weedone CB and Its Carrier

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Butoxyethyl esters (BEE) of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2,4-dichlorophenoxy)propionic acid (di-chlorprop or 2,4-DP) are base chemicals manufactured by Rhone-Poulenc Agriculture, Mississauga, Ontario, for formulating Weedone CB. This product is a selective post-emergent herbicide used for the control of broad-leaf vegetation. The use of Weedone CB for forestry conifer release and industrial rights-of-way programs in coastal British Columbia may result in the accidental introduction of BEE of 2,4-D and 2,4-DP into waterbodies inhabited by anadromous fish. The aim of this study was to evaluate the acute toxicity of BEE of 2,4-D and 2,4-DP, Weedone CB and its carrier to juvenile salmonids of the Pacific Northwest.

MATERIALS AND METHODS

The common names, abbreviations, and concentrations of test materials are summarized in Table 1. All test materials were supplied by Rhone-Poulenc Agriculture, Mississauga, Ontario, except diesel oil, which was obtained locally. Diesel oil is often added by users to Weedone CB at the rate of 10 % oil: 90 % product v/v to enhance adhesion on vegetation.

A series of 96-h static acute toxicity tests was conducted in fresh water using several species of juvenile salmonids (see footnote, Table 4) obtained from British Columbia hatcheries. The tests were undertaken from February to July 1989 in accordance with the procedure outlined by Wan et al. (1987, 1988, 1989). Testing was carried out at a temperature of 14 ± 1°C, and with a 16-h light:8-h dark photoperiod regime. The average loading density was 0.25 (0.10 - 0.42) g/L, with a bio-assay test volume of 20 L. 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).

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Table 1. Test materials

Common name	Abbrev.	Concentration of active ingredient
2,4-D BEE (Technical)	2,4-D	2,4-dichlorophenoxyacetic acid (95 % butoxyethyl ester)
Dichlorprop BEE (Technical)	2,4-DP	2-(2,4-dichlorophenoxy)propionic acid (95 % butoxyethyl ester)
Weedone CB	Weedone	2,4-D butoxyethyl ester 11.9 %, 2-(2,4-DP) butoxyethyl ester 11.7 %, 76.4 % Car. A; wt/vol.
Weedone CB + diesel oil	Weedone+	2,4-D butoxyethyl ester 10.7 %, 2-(2,4-DP) butoxyethyl ester 10.5 %, Car. A 68.8 %, diesel oil 10.0 % (pour pt. -17.8°C); wt/vol.
Diesel oil	Diesel	100 % diesel oil (PetroCanada; pour pt. -17.8°C)
Carrier A	Car. A	100 % liquid Car. A (identity is proprietary information)

Three different water types were used as dilution water: (1) soft, acid pH - city tap (Vancouver dechlorinated), (2) hard, alkali pH - lake (Cultus Lake, British Columbia) (3) intermediate between (1) & (2) - reconstituted deionized city tap ($CaSO_4.2H_2O$, KCl, $MgSO_4$, and $NaHCO_3$, APHA 1987).

Each concentration of 2,4-D and 2,4-DP was dissolved in 10 mL acetone but not Weedone and Weedone+, as these two products contained an emulsifier which allowed the formulation to readily mix and disperse in water. An equal volume of acetone was added to the relevant controls.

The analysis of water samples for alkalinity, chemical element/ionic contents, conductivity, hardness, pH and chlorinated pesticides was carried out at Conservation & Protection Laboratory, Vancouver, B.C., using methods developed by Environment Canada (1989). Chemical characteristics of each water did not change throughout the study period from February to July 1989 (Table 2). Each water differed from the other significantly ($p < 0.01$) in terms of alkalinity, conductivity, hardness, pH, and some chemical elements/ ions. No detectable residues (detection limit = 1 ug/L) were found in each water for the following compounds: DDT analogues, BHC, cyclodiene and phenoxy compounds, PCB, PCP, and picloram.

The analyses of 2,4-D and 2,4-DP residues in water were conducted at British Columbia Research Laboratory, Vancouver, B.C., Canada. Water samples were treated with a base to convert the ester to the free acid. The free acids were extracted under acid conditions and converted to their methyl esters using diazomethane. The derivatives were analysed by electron capture GLC. The recov-

Table 2. Characteristics* of dilution water

Parameter analyzed	Type of dilution water: Soft (city)	Intermediate (reconstituted)	Hard (lake)
Alkalinity	1.6 ± 0.2	30.2 ± 0.5	63.9 ± 0.5
Chemical elements/ions			
C (total)	2.3 ± 0.4	1 ± 0.1	9.3 ± 1.4
Ca	1.2 ± 0.1	7.3 ± 0.3	29.8 ± 0.3
K	0.3 ± 0.07	1.2 ± 0.1	0.5 ± 0.1
Mg	0.2 ± 0.05	6.1 ± 0.2	2.7 ± 0.1
Na	0.6 ± 0.1	14 ± 0.4	2.9 ± 0.1
Si	1.2 ± 0.1	ND	2.6 ± 0.1
Cl	1.7 ± 0.5	0.9 ± 0.2	3.7 ± 1.3
SO_4	3.1 ± 0.2	41.3 ± 1	20 ± 0.3
Conductivity (umhos/cm)	14 ± 0.7	152 ± 2	175 ± 2.7
Hardness (total)	4.2 ± 0.1	43.4 ± 0.8	86 ± 1
pH (rel. U.)	6.1 ± 0.07	7.5 ± 0.09	8.1 ± 0.1

* - mg/L (mean ± S.E., n = 3), significantly different ($p < 0.01$); ND - not detected; detection limits, < 0.001 - 0.01 mg/L

ery rates for 2,4-D and 2,4-DP (acid equivalent) were 84 ± 7 % and 100 ± 2 % respectively, (mean ± S.E.; n = 4).

A 5-mL sample was collected from each test material (except car. A and diesel oil) for validation of 2,4-D and 2,4-DP content. The chemical assay results verified that the content of BEE of 2,4-D and 2,4-DP in each test material listed in Table 1 is according to the amount specified by the supplier. Two 50-mL water samples were collected at random from the mid-concentration of each coho salmon test solution (except diesel and car. A). Each water sample contained ten 5-mL composite subsamples pooled to make up the 50-mL sample. They were collected after the addition of test chemicals and the 30-min pre-aeration process but just before fish introduction.

RESULTS AND DISCUSSION

The measured concentrations of 2,4-D and 2,4-DP in different water types in the coho test vessels before fish introduction was much less than the corresponding theoretical concentration (Table 3). The data suggest that losses of 2,4-D and 2,4-DP occurred in both the technical and formulated products. Two main factors may have contributed to this loss: (1) volatilization during the initial aeration process (Doudoroff et al. 1951); and (2) glass adsorption (Sharom and Solomon 1981). Losses for 2,4-D and Weedone+ were apparently greater in soft, acidic water than in hard, alkaline water.

The 24, 48, 72 and 96-h LC_{50} values for 2,4-D, 2,4-DP, Weedone, and Weedone+ were calculated according to the measured concentration, but not for diesel oil and car. A, as their identities (proprietary information) were not determined by chemical assay.

Table 3. Concentrations of test chemicals

Dilution water and test chemicals	Concentration* of test chemicals (mg/L) at 0-h Theoretical	Measured	Chemical recovery (%)
Soft (city)			
2,4-D	2	0.99	50
2,4-DP	1.84	1.46	79
Weedone	3.69	2.28	62
Weedone+	1.65	0.43	26
Intermediate (reconstituted)			
2,4-D	2.18	1.43	66
2,4-DP	2.1	1.89	90
Weedone	2.94	1.14	39
Weedone+	1.68	0.77	46
Hard (lake)			
2,4-D	2.25	2.16	96
2,4-DP	2.06	1.64	80
Weedone	4.35	1.83	42
Weedone+	4	1.71	43

* - acid equivalent of BEE of 2,4-D, 2,4-DP, or (2,4-D + 2,4-DP in Weedone & Weedone+) via complete hydrolysis; a recovery correction made only for 2,4-D; mean of 2 samples

The data in Table 4 indicates clearly that 2,4-D, 2,4-DP, Weedone, and Weedone+ are highly toxic to salmonids. They are also more toxic to young salmon than car. A and diesel oil, irrespective of water types. As well, car. A is much more toxic to the fish than diesel oil.

A 3-way analysis of variance (Sokal and Rohlf 1969) was applied to the mean Log_e 96-h LC50 data (all values are within the same order of magnitude) for 2,4-D, 2,4-DP, Weedone, and Weedone+ to compare the interaction between water type, herbicide and fish species. For consistency, the analysis of variance was conducted using the 96-h LC50 data of only coho, pink, and rainbow trout for all water types. The 96-h LC50 data for chinook, chum and sockeye salmon in intermediate water were included in Table 4 for information and visual comparison. The results of the 3-way analysis of variance indicate that water type, herbicide, and fish species have significant ($p < 0.01$) overall effects on the test results.

Table 5 compares the toxicities to juvenile salmonids of 2,4-D, 2,4-DP, Weedone, and Weedone+ in different water types. It indicates that 2,4-D is significantly ($p < 0.05$) and increasingly more toxic to the fish as the pH of water decreases. The order of increasing toxicity is: lake < intermediate < soft water. The same result is true for Weedone+ but only when hard and intermediate water is compared to soft water. Accordingly, this study shows that water characteristics affect the acute toxicity to young salmon of 2,4-D and Weedone+ but not of 2,4-D and Weedone.

For soft water, the order of increasing toxicity to

Table 4. Acute toxicities[a] to juvenile Pacific salmonids of 2,4-D, 2,4-DP, Weedone, Weedone+, Car. A, and Diesel oil in different dilution water types

Dilution water	Fish[b] Species	LC50 in mg/L[c]											
		2,4-D				2,4-DP				Weedone			
		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	1.1	1.1	1.1	1.1	1.5	1.5	1.5	1.5	10.5	10	10	10
	pink	0.5	0.4	0.4	0.4	0.8	0.8	0.8	0.8	3.3	2.9	1.9	1.7
	rainbow	1	0.8	0.8	0.8	0.9	0.9	0.9	0.9	7.9	5.7	5	5
Intermediate	(reconstituted)												
	coho	1.5	1.5	1.5	1.5	2.2	2.2	2.2	2.2	8.3	8.3	8.3	8.3
	chinook	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	2.1	1.7	1.7	1.7
	chum	0.9	0.8	0.8	0.7	1.1	1.1	1.1	1.1	2.6	2.5	2.1	1.9
	pink	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8	1.9	1.9	1.9	1.9
	sockeye	1.4	1.4	1.4	1.4	0.7	0.7	0.7	0.7	2.3	2.3	2.3	2.3
	rainbow	1.3	1.3	1.3	1.3	1.4	1.4	1.4	1.4	4.1	3.7	3.7	3.7
Hard (lake)													
	coho	4.3	4.3	4.3	4.3	1.8	1.8	1.8	1.8	9	9	9	9
	pink	1.2	1.2	1.2	1.1	0.8	0.6	0.6	0.6	2.1	2.1	2.1	2.1
	rainbow	2.3	2.2	2.2	2.2	1.8	1.8	1.8	1.8	6	6	6	6
Dilution water	Fish[b] Species	LC50 in mg/L[c]											
		Weedone+				Car. A				Diesel			
		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	2.2	2.2	2.2	2.2	19.8	19.8	19.8	19.8	28972	28972	12345	10299
	pink	1.4	1.3	1.2	1.2	20.6	20.6	19.8	19.8	1972	276	84	74
	rainbow	3.4	2.1	2	2	14.8	14.8	14.8	14.8	>32000	5525	4600	3017
Intermediate	(reconstituted)												
	coho	6.6	6.6	6.6	6.6	20.7	20.7	20.7	20.7	>55560	>55560	33216	33216
	chinook	2.8	2.6	2.6	2.6	22.8	22	22	22	-	-	-	-
	chum	2.6	2.5	2.5	2.4	23.7	23.7	23.1	23.1	-	-	-	-
	pink	2.4	2.4	2.4	2.4	20.6	20.6	20.6	19.7	1829	376	133	123
	sockeye	2.9	2.9	2.9	2.9	26.1	26.1	26.1	26.1	-	-	-	-
	rainbow	5.8	5.8	5.8	5.8	20.1	20.1	20.1	20.1	168363	168363	28787	2186
Hard (lake)													
	coho	5.6	5.6	5.6	5.6	18.5	18.5	18.5	18.5	>26743	26743	4845	3333
	pink	2.4	2.4	2.2	2.2	17.7	17.7	17.7	17.7	1404	302	48	32
	rainbow	5.5	5.5	5.5	5.5	23.1	23.1	23.1	22.2	>23108	23108	5102	2447

[a]. adjusted for chemical loss (except Car. A & Diesel oil). [b]. coho (*O. kisutch*), chinook (*O. tshawytsha*), chum (*O. keta*), pink (*O. gorbuscha*), rainbow trout (*O. mykiss*), sockeye (*O. nerka*); only 3 species were selected for testing in soft & hard water; mean LC50 ± S.E., n; age = 3.5 ± 0.5 mo.; length = 4.2 ± 0.1 cm, 62 fish; weight = 0.7 ± 0.1 g, 62 fish. [c]. 2,4-D, 2,4-DP = mg active ingredient/L; Car. A, Diesel oil, Weedone, Weedone+ = mg product/L.

Table 5. Toxicity of test chemicals to salmonids in different dilution water

Test chemicals and dilution water type		pH	Toxicity 96-h LC50 Mean (mg/L)	Mean log	Statistical tests and comparison Means compared	t^a = Diff/S_D	Tukey's test ($p < 0.05$)
2,4-D							
1. Lake	(hard)	8.1	2.5	0.78	2 vs. 1	3.15	S
2. Recon[b]	(intm.)[b]	7.5	1.2	0.19	2 vs. 3	2.90	S
3. City	(soft)	6.1	0.8	-0.35	1 vs. 3	6.02	S
2,4-DP							
4. Lake	(hard)	8.1	1.4	0.27	5 vs. 4	0.17	NS
5. Recon[b]	(intm.)[b]	7.5	1.5	0.3	5 vs. 6	1.43	NS
6. City	(soft)	6.1	1.1	0.03	4 vs. 6	1.28	NS
Weedone							
7. Lake	(hard)	8.1	5.7	1.58	8 vs. 7	1.17	NS
8. Recon[b]	(intm.)[b]	7.5	4.6	1.36	8 vs. 9	0.75	NS
9. City	(soft)	6.1	5.7	1.5	7 vs. 9	0.43	NS
Weedone$^+$							
10. Lake	(hard)	8.1	4.5	1.4	11 vs. 10	0.59	NS
11. Recon[b]	(intm.)[b]	7.5	4.9	1.51	11 vs. 12	5.06	S
12. City	(soft)	6.1	1.8	0.56	10 vs. 12	4.48	S

a - (S_D = 0.1876, df = 12, critical value for Tukey's test = 2.97)
b - reconstituted (intermediate); S - significant; NS - not significant

juvenile salmonids is as follows: Weedone < Weedone$^+$ < 2,4-DP < 2,4-D (Table 6). However, in the three water types, 2,4-D and 2,4-DP toxicities to salmonids are not significantly ($p < 0.05$) different. The addition of 10 % diesel oil to Weedone increases significantly ($p < 0.05$) the toxicity of the product to salmonids only in soft water.

Under field conditions, the concentration of Weedone (sp gr 0.98) in a waterbody unintentionally oversprayed at the manufacturer's recommended rate of 50 L product/ha or 45.4 lb product/A (Rhone-Poulenc Agriculture 1988) approximates 34 mg/L in 15 cm water. During an accidental spill, this level could be higher. This concentration of Weedone is estimated to exceed the 96-h LC50 (in intermediate water) of juvenile salmonids when rapid dilution is not available by the following multiples: 4 (coho); 9 (rainbow trout); 15 (sockeye); 18 (chum); and 20 (chinook, pink). Therefore, when used at the labelled rate of application, Weedone could in the event of misapplication generate acutely toxic conditions for young salmon.

Several other notable observations could also be generalized by examining closely the results in Table 4. Firstly, during the 96-h exposure, the LC50 values remained almost unchanged after 24 h, except for Weedone in soft water and diesel oil in the three water types.

Secondly, between fish species, the data suggest that 2,4-D, 2,4-DP, Weedone, Weedone$^+$, and diesel oil are most toxic to pink salmon, irrespective of water types.

Table 6. Order of increasing toxicity to salmonids of test chemicals in different water type

Test water and chemicals	Toxicity 96-h LC50		Statistical tests and comparison		
	Mean (mg/L)	Mean log	Means compared	t^a = Diff/S_D	Tukey's test ($p < 0.05$)
Soft water					
1. 2,4-D	0.8	-0.35	1 vs. 2	2.03	NS
2. 2,4-DP	1.1	0.03	2 vs. 3, 4	2.83, 7.84	NS, S
3. Weedone+	1.8	0.56	3 vs. 1, 4	4.85, 5.01	S, S
4. Weedone	5.7	1.5	4 vs. 1	9.86	S
Reconstituted (intermediate) water					
5. 2,4-D	1.2	0.19	5 vs. 6	0.59	NS
6. 2,4-DP	1.5	0.3	6 vs. 7, 8	5.81, 6.45	S, S
7. Weedone	4.6	1.39	7 vs. 5, 8	6.40, 0.64	S, NS
8. Weedone+	4.9	1.51	8 vs. 5	7.04	S
Hard water					
9. 2,4-DP	1.4	0.27	9 vs. 10	2.72	NS
10. 2,4-D	2.5	0.78	10 vs. 11, 12	3.30, 4.26	S, S
11. Weedone+	4.5	1.4	11 vs. 9, 12	6.02, 0.96	S, NS
12. Weedone	5.7	1.58	12 vs. 9	6.98	S

a - (S_D = 0.1876, df = 12, critical value for Tukey's test = 2.97)
S - significant; NS - not significant

The exceptions are 2,4-DP and Weedone which are most toxic to chinook salmon in reconstituted water. As well, carrier A is most toxic to rainbow trout in soft water, while in intermediate and hard water it is most toxic to pink salmon. The order of increasing toxicity to other salmonids of car. A in intermediate water is as follows: sockeye < chum < chinook salmon.

Finally, diesel oil appeared to have the least stability within the 96-h test period. It yielded a wide range of LC50 values in the three different water types. Diesel oil toxicity to coho is lowest in intermediate water and highest to the fish in hard water. The 96-h LC50 values of diesel oil for salmonids (each test repeated at least twice) in different water types vary considerably (32 - 33216). The cause of this wide range of variation is presently not known. Diesel oil is most toxic to pink salmon when compared to coho salmon and rainbow trout irrespective of water type.

In summary, this bioassay study indicates that the butoxyethyl esters of 2,4-D, 2,4-DP, and Weedone, its carrier, and Weedone+ are highly toxic to juvenile salmonids of the Pacific Northwest. The acute toxicity to salmonids of 2,4-D and Weedone+ but not 2,4-DP and Weedone is affected by the characteristics of water. At the manufacturer's recommended rate of application (50 L product/ha), the potential to generate concentrations acutely toxic to salmonids of Weedone is high in streams unintentionally oversprayed. Pink salmon are much more sensitive to diesel oil carrier when compared to coho salmon and rainbow trout.

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