Bull. Environ. Contam. Toxicol. (1991) 47:471–478

Environmental Contamination and Toxicology

Acute Toxicity to Juvenile Pacific Northwest Salmonids of Basacid Blue NB755 and Its Mixture with Formulated Products of 2,4-D, Glyphosate, and Triclopyr

M. T. Wan, R. G. Watts, and D. J. Moul

Environment Canada, Conservation and Protection, Environmental Protection, Pacific & Yukon Region, 224 West Esplanade, North Vancouver, British Columbia, V7M 3H7, Canada

Basacid Blue NB755 (BB) is a triphenylmethane dye manufactured by BASF Corporation, New Jersey, U.S.A. This product is used in British Columbia mainly as a marker by aerial and ground herbicide spray operators in forestry. Concerns were raised about the acute toxicity to young salmon of this dye and its mixture with several common herbicides. The objective of this study was to determine the acute toxicity to juvenile Pacific Northwest salmonids of BB and its mixture with formulated products of 2,4-D, glyphosate, and triclopyr in different sources of dilution water.

MATERIALS AND METHODS

The common names, abbreviations, and concentrations of test materials are summarized in Table 1. Test materials were obtained from local distributors: BB (Van Waters & Rogers), 2,4-D amine (Laters Chemicals Ltd.) and 2,4-D ester (United Agri Products), glyphosate (Monsanto Canada), except triclopyr, which was supplied by Dow-Elanco, Sarnia, Ontario.

A series of 96-h static acute toxicity tests was conducted in fresh water using several species of juvenile salmonids (*Onchorhychus kisutch*, *O. tshawytsha*, *O. keta*, *O. gorbuscha*, *O. mykiss*) obtained from British Columbia hatcheries. The tests were conducted from February to June 1990 in accordance with the procedure outlined by Wan et al. (1987; 1989). Testing was carried out at a temperature of $14 \pm 1^{o}C$, and with a 16-h light: 8-h 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** - 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). **BB** was water soluble while products of **2,4-D, glyphosate,** and **triclopyr** contained an emulsifier (see Table 1) which allowed the formulation to readily mix and

Send reprint requests to MT Wan at the above address

Table 1. Test materials

Common name	Abbrev.	Concentration of active ingredient (as stated in the labels)
Basacid Blue NB755	BB	ammonium salt of triphenylmethane (TPM) dye, 50 %; chromic sulfate, 0.5 %; oxalic acid, 0.5 %; proprietary surfactant & water, 49 %; % by vol.
2,4-D amine	2,4-DA	diethanolamine (DEA) salt of 2,4-dichlorophenoxyacetic, 69 %; water, 31 %; % by wt.
2,4-D ester	2,4-DE	isooctyl ester (IOE) of 2,4-dichlorophenoxyacetic acid, 60 %; petroleum solvent & proprietary emulsifier, 40 %; % by wt.
Garlon 4^{TM}	Gar4	butoxyethyl ester (BEE) of (3,5,6-trichloro-2-pyridinyl)oxy acetic acid (triclopyr), 61.6 %; kerosene & proprietary ingredients, 38.4 %; % by wt.
Roundup^{R} (Vision^{R})	Rdup	isopropylamine salt of N-(phosphonomethyl) glycine 41 % (30.5 % glyphosate equivalent); tallowamine surfactant, 15 %; water & inerts 44%; % by wt.

disperse in water.

The characteristics of each dilution water were determined according to the procedure outlined in Wan et al. (1990). Chemical properties of each water did not change throughout the study period from February to June 1990 (Table 2). Each dilution 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.

Two 50-mL water samples were collected from the mid-concentration of each coho salmon test solution. Each water sample contained ten 5-mL composite sub-samples 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. A 5-mL sample was collected from each test product for content validation of basacid blue, 2,4-D amine, 2,4-D ester, glyphosate, and triclopyr. The calculation of measured concentration of test chemicals was based on the analytical results of active ingredient content in each product and not based on the active ingredient content listed in the label (Table 1).

The analyses of 2,4-D, glyphosate, and triclopyr residues in water were conducted at British Columbia Research Corporation Vancouver, British Columbia. Water samples of 2,4-D and triclopyr were treated with a base to convert the amine or ester to the free acid. The free acids were extracted under acid conditions and converted to their methyl esters using diazomethane. The derivatives were analyzed by electron capture GLC. Glyphosate water samples were derivatized and analysed by HPLC (UV/VIS). BB was analyzed with a spectrophotometer, measuring adsorption at 630 nm. The mean ($\pm$ S.E.; $n = 4$) quality control (QC) sample recovery rates were: BB,

Table 2. Characteristics* of dilution water

Parameter analyzed	Tpye of dilution water		
	Soft (city)	Intermediate (reconstituted)	Hard (lake)
Alkalinity	1.4 ± 0.2	29.3 ± 0.5	66.1 ± 0.7
Chemical elements/ions			
C (total)	1.7 ± 0.3	7 ± 0.1	16.3 ± 0.2
Ca	1.3 ± 0.1	6.5 ± 0.3	28 ± 0.5
K	0.2 ± 0.01	1.1 ± 0.1	0.5 ± 0.06
Mg	0.1 ± 0.02	5.4 ± 0.3	2.4 ± 0.1
Na	0.6 ± 0.04	13.2 ± 0.7	2.8 ± 0.1
Si	1.6 ± 0.1	ND	3.5 ± 0.2
Cl	1.6 ± 0.1	0.9 ± 0.1	1.4 ± 0.2
SO_4	1.9 ± 0.2	38.7 ± 1	22.9 ± 0.4
Conductivity (umhos/cm)	14.9 ± 0.6	159 ± 3	186 ± 3
Hardness (total)	4.8 ± 0.2	38.9 ± 0.9	80.5 ± 1.1
pH (rel. U.)	6.3 ± 0.05	7.5 ± 0.06	8 ± 0.03

* - parameter measured in mg/L (mean ± S.E., n = 4); ND - not detected; detection limits, < 0.001 - 0.01 mg/L

100 ± 2 %; 2,4-D (acid equivalent), 97 ± 5 %; glyphosate and amino methylphosphonic acid (a major metabolite of glyphosate), both 95 ± 3 %; triclopyr, and 90 ± 4 %.

RESULTS AND DISCUSSION

The measured concentrations of 2,4-DE, Gar4, Rdup, and their mixture with BB in different water types were much less than the nominal concentration (Table 3). The data suggest that losses occurred, most likely due to (1) volatilization during the 30-min aeration process (Doudoroff et al. 1951), and (2) plastic adsorption (Sharom & Solomon 1981). In contrast, the measured concentrations of 2,4-DA and BB were greater than the nominal concentration in the three water types. The cause of this deviation is presently not known. In Rdup, higher chemical concentration was noted in soft and intermediate water but not in hard water, where the data suggest a chemical loss occurred.

The 24, 48, 72 and 96-h LC50 values for BB and the 96-h LC50 values for the rest of the test materials were calculated according to the measured concentration, based on the assumption that the bulk of the chemical loss occurred during the first 30-min pre-aeration process after chemical introduction and that the chemical concentration in the water stabilized after that time. Adjustments based on QC sample recovery rate were not made.

The data in Table 4 suggest that BB is quite toxic to salmonids. A 2-factor ANOVA (analysis of variance; Steel and Torrie 1960) was applied to the mean $\log_e$ (96-h LC50 + 1) data for this dye to test the effects of water type, species, and their interaction. For consistency, the ANOVA 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 soft water were

Table 3. Concentrations of test chemicals

Test chemicals	Concentration of test chemicals (mg/L product at 0-h)								
	soft water			intermediate water			hard water		
	nom.	mea.	rec. (%)	nom.	mea.	rec. (%)	nom.	mea.	rec. (%)
2,4-DA	405	496	123	400	415	104	405	463	114
2,4-DE	400	148	37	400	142	36	400	83.6	21
Gar4	0.8	0.7	88	3.6	2	56	4	2.2	55
Rdup[a]	15	14	93	12.5	11.8	94	15	14.7	98
BB	200	206	103	200	213	107	400	435	109
2,4-DA + BB	415	151	36	420	152	36	425	147	35
2,4-DE + BB	400	56	14	410	71	17	395	60.3	15
Gar4 + BB	3.6	1.4	39	7	3.1	44	4	1.5	38
Rdup + BB	22	25.7	117	32	32.5	102	30.5	24	79

nom. = nominal; mea. = measured & calculated based on analytical results of product active ingredient: BB, 50 % TPM; 2,4-DA, 64.3 % DEA; 2,4-DE, 61.8 % IOE; Gar4, 58.4 % BEE; Rdup, 31.8 % glyphosate; rec. = recovery to the nearest whole number; a - Wan et al. (1989).

included in Table 4 for information and visual comparison. The results of the 2-factor ANOVA suggest that water types and fish species have significant ($p < 0.01$) overall effects on the test results. Pink salmon is the most susceptible ($p < 0.01$) fish species to BB in intermediate and hard water, while sockeye salmon is the most sensitive fish in soft water.

Table 5 compares the toxicities to juvenile salmonids of 2,4-DA, 2,4-DE, Gar4, and Rdup to their mixture with 100 mg/L of BB. A 3-factor ANOVA (Steel and Torrie 1960) was carried out, the factors being herbicides, water types, and species. It indicates that the addition of this amount of dye to 2,4-DA, 2,4-DE, and Gar4 increases significantly ($p < 0.05$) the toxicity to young salmon of these products, irrespective of water types, except Gar4 in intermediate water. In contrast, the addition of 100 mg/L of the dye to Rdup did not significantly ($p > 0.05$) increase the toxicity to juvenile salmonids of the herbicide, irrespective of water types. However, the effects to the fish of the addition of BB greater than 100 mg/L to Rdup is presently not known.

Table 6 compares the toxicities to young salmonids of BB, 2,4-DA, 2,4-DE, Gar4, Rdup, and their respective mixtures with BB in different water types. Differences between water types were tested by Tukey's test for multiple comparison of means (Steel and Torrie 1960). It suggests that BB is significantly ($p < 0.05$) more toxic to young salmonids in soft water than in hard and intermediate water. The order of increasing toxicity of salmonids to BB is: intermediate < hard < soft water.

For 2,4-DA and (2,4-DA + BB), the order of increasing toxicity to fish is: hard < intermediate < soft water. In contrast, 2,4-DE and (2,4-DE + BB) are significantly ($p < 0.05$) more toxic to salmonids in hard than in soft and intermediate water. With Gar4 and (Gar4

Table 4. Acute toxicities* to salmonids of basacid blue

Dilution water	Fish** species	LC50 (mg product/L) 24-h	48-h	72-h	96-h
Soft (city)	coho	1154	332	332	332
	chinook	> 649	721	412	370
	chum	> 1130	801	452	427
	pink	> 680	> 680	420	398
	rainbow	> 1030	972	818	412
	sockeye	> 700	> 700	201	180
Intermediate (reconstituted)	coho	> 2038	1261	1144	1144
	pink	> 1126	> 1126	619	619
	rainbow	> 2114	1421	1370	1370
Hard (lake)	coho	569	392	377	377
	pink	674	265	252	239
	rainbow	3457	1525	1474	1474

* - adjusted for chemical recovery. ** - coho (Oncorhynchus kisutch), chinook (O. tshawytscha), chum (O. keta), pink (O. gorbuscha), rainbow trout (O. mykiss), sockeye (O. nerka); only 3 species tested in intermediate & hard water; mean LC50 ± S.E., n; age = 3.5 ± 0.5 mo.; length = 4.2 ± 0.2 cm, 65 fish; weight = 0.7 ± 0.1 g, 65 fish.

Table 5. Acute toxicities* to salmonids of 2,4-D amine, 2,4-D ester, Garlon 4, Roundup and their mixture with basacid blue in different water types

Dilution water	Fish** species	96-h LC50 (mg product/L) 2,4-DA	(2,4-DA + BB)	2,4-DE	(2,4-DE + BB)
Soft (city)	coho	472	115	156	76
	pink	291	100	30	16
	rainbow	409	116	167	56
Intermediate (reconstituted)	coho	493	191	158	82
	pink	363	122	70	26
	rainbow	511	187	164	84
Hard (lake)	coho	662	222	63	45
	pink	438	111	21	10
	rainbow	744	241	79	41

Dilution water	Fish** species	96-h LC50 (mg product/L) Gar4	(Gar4 + BB)	Rdup[1]	(Rdup +BB)
Soft (city)	coho	1.8	2.3	30	49
	pink	1.1	0.7	31	29
	rainbow	2.4	0.8	31	35
Intermediate (reconstituted)	coho	2.7	2.2	31	38
	pink	1.3	1.3	10	23
	rainbow	1.8	1.4	31	18
Hard (lake)	coho	2.4	1.5	13	17
	pink	0.8	0.2	14	10
	rainbow	1.5	1.4	14	17

* - adjusted for chemical recovery; ** - see Table 4 for fish species; BB = 100 mg/L basacid blue dye added per test. 1 - Wan et al. (1989).

Table 6. Toxicity of test chemicals to salmonids in different 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 ($p < 0.05$)
BB						
1. hard[b]	8	670	6.24	2 vs. 1	4.29	S
2. intm[b]	7.5	1039	6.9	2 vs. 3	8.01	S
3. soft	6.3	380	5.94	1 vs. 3	3.79	S
2,4-DA						
4. hard[b]	8	617	6.4	5 vs. 4	1.92	NS
5. intm[b]	7.5	455	6.11	5 vs. 6	1.03	NS
6. soft	6.3	392	5.95	4 vs. 6	2.94	S
2,4-DA + BB						
7. hard[b]	8	189	5.18	8 vs. 7	0.56	NS
8. intm[b]	7.5	167	5.1	8 vs. 9	2.55	NS
9. soft	6.3	111	4.71	7 vs. 9	3.11	S
2,4-DE						
10. hard[b]	8	54	3.88	11 vs. 10	6.07	S
11. intm[b]	7.5	129	4.79	11 vs. 12	1.64	NS
12. soft	6.3	118	4.55	10 vs. 12	4.43	S
2,4-DE + BB						
13. hard[b]	8	32	3.33	14 vs. 13	4.89	S
14. intm[b]	7.5	65	4.07	14 vs. 15	2.19	NS
15. soft	6.3	49	3.74	13 vs. 15	2.71	S
Gar4						
16. hard[b]	8	1.6	0.91	17 vs. 16	0.98	NS
17. intm[b]	7.5	1.9	1.06	17 vs. 18	0.39	NS
18. soft	6.3	1.8	1	16 vs. 18	0.59	NS
Gar4 + BB						
19. hard[b]	8	1	0.66	20 vs. 19	1.90	NS
20. intm[b]	7.5	1.9	0.95	20 vs. 21	1.96	NS
21. soft	6.3	0.9	0.65	19 vs. 21	0.05	NS
Rdup						
22. hard[b]	8	14	2.69	23 vs. 22	3.48	S
23. intm[b]	7.5	25	3.22	23 vs. 24	1.59	NS
24. soft	6.3	31	3.46	22 vs. 24	5.07	S
Rdup + BB						
25. hard[b]	8	15	2.73	26 vs. 25	3.53	S
26. intm[b]	7.5	26	3.26	26 vs. 27	2.44	NS
27. soft	6.3	38	3.63	25 vs. 27	5.97	S

a - (S_D = 0.1519, df = 16, critical value for Tukey's test = 2.58);
b - intermediate (reconstituted); S - significant; NS - non significant

+ BB), the toxicity is not significantly ($p > 0.05$) different between the three water types. Both Rdup and (Rdup + BB) are significantly ($p < 0.05$) more toxic to salmon in hard water with the order of increasing toxicity as follows: soft < intermediate < hard water. The influence of pH in water on the toxicity to salmonids of different test chemicals is presently not clear.

In British Columbia coastal forest areas, aerial and ground herbicide applicators commonly use an average concentration of 2200 mg/L BB (range, 1000 - 4400 mg/L) per 50L load/ha of spray mixture near salmon habitat (per. comm. Gladiuk and Scott 1990). This rate of application is equivalent to 0.1 kg BB/ha. An unintentional overspray of BB alone into streams with an average depth of 15 cm water has a potential to generate a concentration of about 0.07 mg/L BB. This concentration of BB is **lower than the 96-h LC50 of sockeye salmon** (the most sensitive fish) in **soft** water by an estimated multiple of 2600. A similar estimation for pink salmon, the most susceptible fish in **intermediate** and **hard** water, was respectively 8800 and 3400. Accordingly, the use of BB as an indicator (with no herbicides) at the above rates by foresters would not likely to generate acutely toxic conditions for young salmon via drift deposition into small fish streams.

However, this study shows that mixtures of BB at the rate of 100 mg/L with 2,4-DA, 2,4-DE, and Gar4 are 2 to 3 times more toxic to salmonids than 2,4-DA, 2,4-DE, irrespective of water types, and to Gar4 in soft and hard water. Accordingly, the use of BB as a dye indicator with these herbicides has to be treated with caution, as it has the potential to greatly increase the toxicity to salmonids of these products, particularly at rates higher than 100 mg BB/L.

This bioassay also indicates that BB at the rate of 100 mg/L mixture with Rdup does not significantly ($p > 0.05$) increase the toxicity to young salmonids of the herbicide. It is suggested that the use of this indicator for Rdup herbicide spray should not be greater than 100 mg/L (equivalent 8 mL BB/100L load), as the impact to young salmon of this coloring material at concentrations greater than the rate stated earlier have not been evaluated.

To sum up, this bioassay study indicates that basacid blue dye is quite toxic to salmonids, particularly in soft water. This dye (with no herbicides) is not likely to have an acute impact on salmonids at rates commonly used by foresters. However, the use of this dye as an indicator at the rate of 100 mg/L in spray mixtures increases the toxicity to young salmon of formulated products of 2,4-D amine, 2,4-D ester, Garlon 4 (except in intermediate water) but not Roundup, irrespective of water types. It is suggested that the use of basacid blue dye indicator for now for Roundup ground and aerial operations **should not exceed 100 mg/L (equivalent to 8 mL Basacid Blue NB755 per 100L spray mixture) per spray mixture load of 100L.**

Acknowledgments. We thank Messrs. R.H. Kussat, D.M. Wilson, D. A. Macleod - statistician (Environment Canada), S. Samis (Fisheries &

Oceans Canada), S. Szeto (Agriculture Canada) for their comments; Dow Elanco (Sarnia), Monsanto Canada (Vancouver), S. Yee, S. van der Geest, G. van Agglen for technical support; and Fisheries & Oceans Canada for partial funding of this study.

REFERENCES

American Public Health Association (1987) Standard methods for the examination of water and wastewater, 17th ed. American Public Health Association/American Water Works Association/Water Pollution Control Federation, Washington, DC 1268 p

Doudoroff P, Anderson BG, Burdick GE, Galtsoff P, Hart WB, Patrick R, Strong ER, Surber EW, VanHorn WM (1951) Bioassay methods for evaluation of acute toxicity of industrial wastes to fish. Sewage Ind Waste 23:1380-1397

Sharom MS, Solomon KR (1981) Adsorption and desorption of permethrin and other pesticides on glass and plastic materials used in bioassay procedures. Can J Fish Aquat Sci 38:199-204

Stephan CE (1983) Lethal program for computer analysis of LC50. Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota

Steel RGD, Torrie JH (1960) Principles and procedures of statistics with special reference to biological sciences. McGraw-Hill Book Company, Inc, New York

Wan MT, Moul DJ, Watts RG (1987) 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. Bull Environ Contam Toxicol 39:721-728

Wan MT, Watts RG, Moul DJ (1989) Effects of different dilution water types on the acute toxicity to juvenile Pacific salmonids and rainbow trout of glyphosate and its formulated products. Bull Environ Contam Toxicol 43:378-385

Wan MT, Watts RG, Moul DJ (1990) Acute toxicity to juvenile salmonids and rainbow trout of butoxyethyl esters of 2,4-D, 2,4-DP and their formulated product: Weedone CB and its carrier. Bull Environ Contam Toxicol 45:604-611

Received October 18, 1990; accepted March 12, 1991.