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Evaluation of the Acute Toxicity to Juvenile Pacific Salmonids of Hexazinone and its Formulated Products: Pronone 10G, Velpar® L, and their Carriers

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Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl 1,3, 5-triazine-2,4(1H,3H)-dione] is a broad spectrum herbicide manufactured by E. I. du Pont de Nemours & Company. Its formulated products, Pronone 10G and Velpar^{R} L, are non-selective herbicides used for vegetation control. The use of these herbicides for brush control in coastal British Columbia forests may result in the accidental introduction of hexazinone into waterbodies inhabited by salmonid fish. The objective of this study was to assess the acute toxicity of hexazinone and its formulated products and their carriers to juvenile Pacific salmonids.

MATERIALS AND METHODS

The common name, abbreviation, and concentration of test materials are summarized in Table 1. All test materials were supplied by E. I. du Pont de Nemours & Company U.S.A., Incorporated, Wilmington, Delaware; and Du Pont Canada Incorporated, Guelph, Ontario.

A series of 96-h static acute toxicity tests was conducted from February to May 1987 in accordance with the protocols of Environment Canada (1980), Buchanan (1982), and the procedure outlined by Wan et al. (1987).

Juvenile Pacific salmonids were obtained in fresh water from British Columbia hatcheries and used as test fish, (Table 2). The average lengths of fish, weights, loading densities and test volumes were recorded, (Table 3).

Ten fish were placed in each test vessel. Duplicate tests were conducted for each test concentration. Cumulative fish mortality was recorded and the LC_{50} values were calculated using the "Lethal" computer program developed by Stephan (1983).

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Table 1. List of test materials

Common name	Abbrev.	Concentration of active Ingredient
Hexazinone	Hex.	95 % Hex.
Pronone 10G	Pro. 10G	10 % Hex./kg granular product
Velpar^{R} L	Vel. L	25 % Hex./L liquid product
Pronone 10G carrier	Car. P	100 % solid Car. P, (identity is proprietary information)
Velpar^{R} L carrier	Car. V	100 % liquid Car. V, (identity is proprietary information)

Table 2. Test fish species, ages, and suppliers

Fish Species	Date Obtained	Age (mo)	Hatchery
Chinook salmon (Oncorhynchus tshawytscha)	02/19/87	2.0	Capilano Hatchery Vancouver, B.C.
Coho salmon (O. kisutch)	04/24/87	1.5	Capilano Hatchery Vancouver, B.C.
Chum salmon (O. keta)	03/05/87	1.7	Inch Creek Hatchery, Dewdney, B. C.
Pink salmon (O. gorbuscha)	03/17/87	1.5	Quinsam Hatchery Campbell River, B.C.
Sockeye salmon (O. nerka)	04/09/87	1.5	Fisheries & Oceans Canada Sweltzer Creek Laboratory, Cultus Lake, B.C.
Rainbow trout (Salmo gairdneri)	01/21/87	1.7	Fraser Valley Trout Hatchery, Abbotsford, B.C.

A volume of either 20 or 30 L dechlorinated Vancouver City tap water was used in each test vessel, depending on the size of the fish. Loading densities ranged from 0.1 - 0.3 g/L, (Table 3).

Chemical quality of diluent water was relatively consistent throughout the study period from February to May 1987. Hardness (as Ca + Mg) ranged from 3.51 to 4.19 mg/L. Inorganic metals were less than the detection limits (mg/L) for most elements, i.e., < 0.005 for Co, Cr, Cu, Mo, V; < 0.002 for Cd, Ti, Zn; < 0.001 for B, Be; < 0.05 for As, P, Sb, Se; < 0.02 for Ni, Pb; and < 0.01 for Sn. The average concentration (mg/L) for the other elements was: K (0.18); Al (0.15); Ca (1.3); Fe (0.08); Mg (0.20); Na (0.90); and Si (1.15). Dissolved oxygen and temperature averaged 12 mg/L and 7.5^{o}C,

Table 3. Average fish lengths, weights, and test densities and volumes

Fish	Length		Weight		Loading Density	Test Volume
	cm	(range)	g	(range)	(g/L)	(L)
Chinook	4.4	(4.3-4.6)	0.7	(0.6-0.8)	0.3	30
Chum	4.1	(3.9-4.2)	0.4	(0.3-0.5)	0.1	20
Coho	3.8	(3.4-4.2)	0.5	(0.3-0.7)	0.2	20
Pink	3.3	(2.9-3.5)	0.2	(0.1-0.2)	0.1	20
Sockeye	4.3	(4.1-4.5)	0.7	(0.5-1.0)	0.3	30
Rainbow	4.9	(4.2-5.7)	0.9	(0.6-1.5)	0.3	30

respectively, and pH varied from 5.6 to 6.0 during the period of study.

Test concentrations were prepared in 100-mL beakers and then transferred to the test vessels. Acetone (15 mL per test sample) was used to dissolve the hexazinone. The diluent water of each test vessel was stirred for one minute with a glass rod immediately after the addition of the test chemical. An equal volume of acetone was added to the control vessel of the hexazinone test series. There were six nominal concentrations for each of the five chemicals tested, ranging from 220 mg/L to 20,000 mg/L, and a control for each test.

Duplicate 10 mL water samples were collected at random from two test concentrations of hexazinone and Velpar^{R} L but only one of Pronone 10G. Sampling was conducted after the 30-min aeration process but just before fish introduction, and at subsequent 24-h intervals. The water samples were analyzed to determine the actual amount of chemicals in the test vessels. Water samples were not collected from carriers P and V test vessels, as it was not possible to analyze these samples (proprietary information).

The water samples were analyzed for hexazinone by the British Columbia Ministry of Environment & Parks Laboratory, Vancouver. The samples were extracted with a solvent mix of 5 % acetone in dichloromethane. The extract was analyzed using a Hewlett Packard Model 5880 gas liquid chromatograph equipped with a nitrogen/phosphorus detector. The recovery rate of this method of analysis was close to 100 % (pers. comm. Masson GR, analytical chemist, British Columbia Ministry of Environment & Parks Laboratory, Vancouver, British Columbia, Canada, 1987).

RESULTS AND DISCUSSION

The analytical results indicate that the hexazinone

Table 4. Concentrations of hexazinone in water

Test Material	Concentration of Hexazinone (mg/L)						Chemical Loss (%)		
	Theoretical	Measured[a]							
	0 h	0 h	24 h	48 h	72 h	96 h	0 h	96 h	96-0 h[b]
Hex.	290	275	270	265	263	262	5	10	5
	370	258	258	249	218	226	30	39	9
							Av=18	25	7
Pro. 10G	360	160	160	130	140	140	56	61	5
	360	160	140	140	140	130	56	64	8
							Av=56	63	7
Vel. L	275	240	220	213	200	222	13	19	6
	290	247	245	211	200	206	15	29	14
							Av=14	24	10

a - mean of duplicate samples per concentration;
b - (96 h minus 0 h); Av = average, calculated to the nearest whole number

concentration of each candidate material in the water before fish introduction was less than the theoretical concentration (Table 4). The data suggest that some chemical loss occurred.

The average loss after the 30-min aeration process was approximately 18 % for technical hexazinone and 14 % for Velpar^{R} L. However, the chemical loss for Pronone 10G was 56 %. Despite the presence of fish, a further loss of chemical during the period from 0 h to 96 h in the test vessels containing hexazinone, Pronone 10G, and Velpar^{R}, respectively, only averaged 7 %, 7 %, and 10 % (Table 4).

A number of factors may have contributed to the initial chemical loss before fish introduction, viz., volatilization during the initial aeration process (Doudoroff et al. 1951); chemical degradation via demethylation and hydroxylation processes (Rhodes 1980); reduced solubility of test chemicals (Dow Chemical 1981); and glass adsorption of chemicals (Meehan et al. 1974).

Due to the high chemical loss in Pronone 10G test vessel, two 5-g samples of the granular product from a separate herbicide batch were sent to the analytical laboratory for hexazinone assay. The hexazinone content of these two samples averaged 6 %. This result suggests that the Pronone 10G batch sample used for the fish bioassay might contain 40 % less active ingredient. This disparity in hexazinone content could be a possible reason for the low recovery of the chemical in the Pronone 10G test series, as the theoretical test concentrations were calculated on the basis of a 10 %

active ingredient in the granular product.

Hexazinone degrades in water to at least eight transformation products, designated as A, B, C, D, E, F, G, and H (Rhodes 1980 and Holt 1981). Residues of degradation products A, B, and D were not detected in any of the water samples from the test vessels at 0, 24, 48, 72, and 96 h. The detection limit of each of these materials was 10 mg/L. No attempt was made to determine the residues of transformation products C, E, F, G, and H.

To compensate for the initial chemical loss, the calculation of the 24, 48, 72, and 96-h LC_{50} values for the test materials was adjusted to the measured concentration (Table 5). The results indicated that the LC_{50} values changed slightly after 96 h exposure. There was an average change of 14 %, 17 %, and 21 % for Velpar^{R} L, hexazinone, and Pronone 10G respectively (Table 5).

Table 5. Acute toxicities to juvenile Pacific salmonids of hexazinone, Pronone 10G, Velpar^{R} L, Pronone carrier P, and Velpar^{R} carrier V

Test Chemical	Test Fish Species	LC_{50} (mg/L) 24 h	48 h	72 h	96 h	LC_{50} Change 24-96 h (%)
Hexazinone	coho	290	282	265	246	15
	chum	321	288	288	285	11
	chinook	394	323	318	317	20
	pink	309	280	280	236	24
	rainbow	320	286	271	257	20
	sockeye	363	332	318	317	13
						Av=17 %
Pronone 10G	pink	1760	1621	1559	1408	20
	rainbow	2513	2084	2043	1964	22
						Av=21 %
Velpar^{R} L	coho	1192	1131	1041	923	23
	chum	934	934	934	934	0
	chinook	1096	1096	1096	1096	0
	pink	978	839	728	676	31
	rainbow	962	889	875	872	10
	sockeye	1167	974	927	925	20
						Av=14 %
Carrier P	rainbow	>20000	>20000	>20000	>20000	-
Carrier V	rainbow	4330	4330	4330	4330	0

The order of increasing toxicity to juvenile Pacific salmonids is as follows: carrier P, carrier V, Pronone 10G, Velpar^{R} L, and hexazinone (Table 6). Tests of carriers P and V for the other salmonid species were discontinued when the respective 96-h LC_{50} values were estimated to be greater than 4,000 and 20,000 mg/L for rainbow trout. The results suggest that carriers P and

Table 6. Order of increasing toxicity to Pacific salmonids of test chemicals

Test Chemicals	Toxicity 96-h LC_{50} (Mean ± S.E.)	N_1/N_2*	Student "t" test	"t" value	Level of Significance
1. Carrier P	> 20000	1[a]	-	-	-
2. Carrier V	4330	1[a]	-	-	-
3. Pronone 10G	1686 ± 393	2/6[b]	3 vs. 5	6.17	< 0.001
4. Velpar^R L	904 ± 61	2/6[b]	3 vs. 4	3.12	< 0.05
5. Hexazinone	276 ± 16	6/6	4 vs. 5	9.96	< 0.001

* - sample size; a - only rainbow tested; b - unequal sample size "t" analysis

V are of very low toxicity to juvenile salmonids.

Hexazinone was the most toxic test chemical. The 96-h LC_{50} for rainbow trout of this material was 257 mg/L. It was about 30 % more toxic than results reported by Kennedy Jr. (1984) for the same fish species. Within species, the order of increasing toxicity of hexazinone to salmonids was: chinook, sockeye, chum, rainbow, coho and pink. For Velpar^R L, toxicity increased by species as follows: chinook, chum, sockeye, coho salmon, rainbow trout, and pink salmon.

Pronone 10G and Velpar^R L are both significantly less ($p < 0.001$ for both) toxic to juvenile Pacific salmonids than hexazinone (Table 6). This result suggests that carriers P and V do not increase, but appear to decrease, the fish toxicity of hexazinone in the two formulated products. Velpar^R L is significantly ($p < 0.05$) more toxic to salmonids than Pronone 10G.

The toxic effect, if any, to juvenile Pacific salmonids of hexazinone transformation products designated as A, B, C, D, E, F, G, and H is presently not known. The likelihood of these chemicals occurring in the test vessels either singly or in combination was possible, since the limit of detection was set at 10 mg/L to measure the expected high concentrations of hexazinone in the water samples.

The highest recommended rate for non cropland use of hexazinone in the U.S.A. is 12 kg active ingredient/ha (Beste 1983). This rate of application is equivalent to 48 and 120 kg/ha of Velpar^R L and Pronone 10G respectively. At these rates of treatment, an unintentional overspray into a stream with a depth of 15 cm

water has a potential to generate a concentration of about 8, 33, and 78 mg/L for hexazinone, Velpar^{R} L, and Pronone 10G, respectively.

These levels are about 35, 27, and 22 times lower than the respective 96-h LC_{50} values for salmonids to hexazinone, Velpar^{R} L, and Pronone 10G. In Canada, the highest registered rate of application for hexazinone in forestry use is 4.3 kg per ha (DuPont 1986). This rate is 2.8 times lower than the highest rate recommended by Beste (1983) and provides an added measure of protection for the salmonids.

Although hexazinone is not as highly toxic as triclopyr ester herbicide to juvenile Pacific salmonids (96-h LC_{50} of 0.7 mg/L, Wan et al. 1987), it nonetheless may have an indirect effect on the fish. When applied at the rate of 4.0 kg per hectare, hexazinone persisted in the boreal environment for more than 500 days (Jensen and Kimball 1987).

Moreover, at the same rate of treatment, hexazinone has affected vegetation beyond the site of application (up to 100 meters off-site) because of its mobility in soils and surface runoff (Jensen and Kimball 1987; Feng 1987; Geisler and Newhouse 1987). Hence, this herbicide has the potential to affect stream side vegetation and possibly destroy important salmon habitat.

In summary, this bioassay study indicates that hexazinone is toxic to juvenile Pacific salmonids. This herbicide is more toxic to the same fish species than its formulated products: Pronone 10G and Velpar^{R} L. The carriers of both products are of low toxicity to the salmonids, and they appear to reduce the toxicity of hexazinone in the formulated materials. The toxic effect to young salmon of hexazinone transformation products is presently not known. However, due to its mobility and persistence, hexazinone has the potential to destroy riparian vegetation of salmon habitat.

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