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Toxicity to *Daphnia magna, Hyalella azteca, Oncorhynchus kisutch, Oncorhynchus mykiss, Oncorhynchus tshawytscha,* and *Rana catesbeiana* of Atrazine, Metolachlor, Simazine, and Their Formulated Products

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Atrazine, metolachlor and simazine are herbicide active ingredients used for the selective control of broadleaf weeds and grasses in field, nursery, and tree fruit crops. A manufacturer of atrazine is the Drexel Chemical Company (Memphis, TN, USA); and likewise metolachlor and simazine are produced by Syngenta Crop Protection Canada, Inc. (Guelph, ON, Canada). The use of formulated products of these herbicides for weed control in coastal British Columbia, notably the Lower Fraser Valley, may result in the unintentional introduction of their active ingredients, carriers and transformation products into waterbodies inhabited by juvenile amphibian, salmon and their food organisms. Accordingly, the objectives of this study were: (1) to verify the acute toxicity of atrazine, metolachlor, simazine and their formulated products to a representative amphibian, aquatic invertebrates, and salmonids of the Pacific Northwest, and (2) to evaluate the sub-acute toxicity of atrazine, metolachlor and combinations of both to an indicator aquatic invertebrate that inhabits the streambed.

MATERIALS AND METHODS

The common and IUPAC technical name of herbicides and the composition of their formulated products are summarized in Table 1. Technical test materials were purchased from Chem Service (West Chester, PA, USA). The formulated products were bought locally from Evergro Canada Inc. (Delta, BC, Canada).

Standard stock solutions of atrazine, metolachlor and simazine were dissolved in acetone and kept under -4°C when not in use. Stock and test water concentrations were quantified by High Resolution Gas Chromatography (HRGC), using a Hewlett-Packard 5890 Capillary Gas Chromatograph (Hewlett Packard, Avondale, PA, USA) equipped with a Mass Spectrometric Detector (GC-MSD, Hewlett-Packard). All stock solutions were GC-MSD verified (limit of detection = 0.01 μg/L). Formulated products of these herbicides for bioassays were stored at 4°C and prepared as needed by weighing out the appropriate amounts for each test series. Herbicide residues in bioassay water and sediments were extracted via a liquid/liquid and solid/liquid extraction, respectively, using a solvent ratio of dichloromethane: methanol of 2:1. Fresh well water from the Pacific Environmental Science Centre (PESC), North Vancouver, BC, Canada, was the

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Table 1. Test materials

Common name	Chemical name, active ingredient, purity
Atrazine	6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-trianzine-2,4-diamine (98%)
Atrazine 500®	atrazine (48.5%) + related triazines (mostly pro-pazine (w/v) 1.5%) + proprietary carrier(s) (50%)
Metolachlor	2-chloro-6'-ethyl-N-(2-methoxy-1-methylethyl) aceto-*o*-toluidide (97.2%)
Primextra® II (w/w)	atrazine (28.7%) + S-metolachlor (35.8%) + Magnum® benoxacor (1.8%) + related triazines (propazine 0.07%) + ethylene glycol (4.5%) + proprietary surfactant (3 – 7%) + antifoamant (1 – 5%) + water (10 – 30%)
Princep® (w/v)	simazine (89%) + related triazines (mostly propazine 1%) + proprietary emulsifier (10%)
Propazine	6-chloro-N^2, N^4 di-isopropyl-1,3,5-triazine-2,4-diamine (99%)
Simazine	6-chloro-N^2, N^4 diethyl-1,3,5-triazine-2,4-diamine (99 %)

was the dilution water. Well water samples were taken monthly from January to June in 2004 and 2005 for water quality and pesticide residue analyses (Environment Canada 2003).

Test organisms and suppliers are listed in Table 2. Other test parameters, i.e., size, loading density, test conditions and volumes are summarized in Table 3. Acute toxicity bioassays were undertaken in accordance with the methods cited and/or outlined in Wan et al. (2005). The survival/growth test of *H. azteca* used Wheaton Styrene-Acrylonitrile Imhoff Settling Cones (Fisher Scientific, ON) described in the same study. Each test concentration series was conducted once for *R. Catesbeiana*, in duplicates for *D. magna*, and in triplicates for *H. azteca*, *O. kisutch, O. mykiss,* and *O. tshawytscha.* The test series for *R. catesbeiana* was not duplicated due to the high cost and supply shortage of that indicator organism.

Nominal test concentrations were used for the calculation of the lethal concentration of 50% of the population (LC50). Cumulative mortalities of test organisms were recorded at 24-h and 48-h for *D. magna*, only 96-h for *H. azteca*, and 24-h, 48-h, 72-h, and 96-h for salmonid fish and tadpoles. The LC50 values were calculated using the computer program developed by CETIS (2005). The acute toxicity tests were conducted from January to June in 2004 and 2005. Appropriate controls, viz., well water and acetone were included. Proprietary emulsifier/formulant tests were excluded however, as the manufacturers refused all requests for test samples.

To verify the starting (t = 0 h) test concentration, water samples (a composite total of 0.5 L from triplicate fish tanks) were taken soon after fish introduction from

Table 2. Test species, age and supplier

Test species	Date supplied	Age (mo.)	Source
Daphnia magna (daphnid)	Jan 2001	0.03	Aquatic Biosystems, Corvalis, OR
Hyalella azteca (amphipod)	June 1995	0.07 – 0.3	EPA, Corvalis, OR
Rana catesbeiana (American bullfrog tadpole)	Feb 2005	4-6	Ward's Natural Science, ON
Oncorhynchus kisutch (coho salmon)	May 2005	1	Chilliwack Hatchery, BC
O. mykiss (rainbow trout)	April 2004	1	Sun Valley Trout
	April 2005	1	Farm, Abbotsford, BC
O. tshawytscha (Chinook salmon)	Mar. 2005	4-5	Chilliwack Hatchery, BC

Table 3. Average test organism size and testing conditions†

Test organisms & (number/test)	Length (cm)	Wt. (g)	Den. (g/L)	Vol. (L)	pH	T°C	DO (mg/L)	Cond. (µS)
Daphnia (10)	neonate	NA*	NA	0.2	8.1	20	8.7	420
Hyalella								
Acute tests (10)	young	NA	NA	0.2	7.5	22	8.5	540
Survival tests (15)	young	NA	NA	0.15	7.6	23	9.0	430
Tadpole (10)	10	3.0	0.8	40	7.8	15	10	430
Chinook (10)	4.9	0.9	0.3	30	7.6	15	9.7	420
Coho (10)	4.2	0.7	0.2	30	7.7	15	9.9	450
Rainbow (10)	5.1	1.3	0.5	30	7.7	15	10.1	400

†water hardness = ~100 mg/L $CaCO_3$; photoperiod = 16:8-h light:dark regime; 15-min dawn/dusk. *NA = not available

one test concentration of the *O. mykiss* test series for quantification (including standards) by HRGC (Table 4). The acetone rinse (totaling 0.5 L) for the three empty vessels of that test also were pooled to determine the extent, if any, of glass adsorption of active ingredients. The *H. azteca* growth/survival rate with atrazine and metolachlor was recorded at day 28, when the dry wt. (24-h, 60°C) mass of surviving amphipods was determined. William's tests were used to ascertain whether the *H. azteca's* mass in each test sediment concentration was significantly different from those in the controls (CETIS, 2005). This test was designed to exclude the animal's reproductive phase.

Atrazine and metolachlor are both soluble in water at 33 mg/L (20°C) and 488 mg/L (25°C), respectively (Tomlin 2000). This study assumed that some transfer of residues occurred from contaminated sediments to the water phase via water solubility. It also assumed that contaminated sediments were the major cause of *Hyalella* impact, since the test organisms remained submerged below the surface of the bottom sediments about 90% of the time.

Table 4. Test concentrations in sediments (mg/kg), water (mg/L)

Test materials[a]	Nominal[b] concn.	Actual[c] concn.	Loss or gain (%)	Acetone rinse[d](96-h)
Residues in water of *O.mykiss* test series				
Control (acetone)[e]	ND[f]	ND	---	ND
Water	ND	ND	---	ND
Atrazine	4.0	2.7	- 32.5	0.220
Metolachlor	3.0	3.4	+ 11.7	0.650
Simazine[g]	113	42	- 62.3	54
Simulated[h] residues in sediments of *H. azteca* test series				
Atrazine	1.0	2.5	+ 247	---
Metolachlor	1.0	3.0	+ 300	---
(Atrazine+metolachlor)	2.0	5.3	+ 265	---

[a]Pool of 3 sub-samples from 3 test vessels of 1 treatment. [b]Nominal concentration. [c]Analytical concentration at 0.5 h equili-bration after fish introduction, i.e., $t = 0$ h. [d]Pool of 0.5L acetone rinse from 3 empty test (glass) vessels. [e]No mortality in highest test concentration: water (500 mg/L), sediment (2 mg/kg). [f]ND = not detected; detection limits: water = 0.01 µg/L, sediments = 20 µg/kg. [g]Princep® = 89% simazine. [h]Wan et al. (2005)

RESULTS AND DISCUSSION

Acute toxicity bioassays of simazine (99%), except with one of its formulated products, Princep®, were terminated after one test series. This herbicide was not very soluble in water (6.2 mg/L) or in organic solvents. Although more readily soluble in acetone than any other organic solvents, simazine solubility (1.47 g/L) in this solvent is about 21 times less than atrazine (Tomlin 2000).

Chemical quality of the diluent well water was generally similar throughout the study periods from January to June in 2004 and in 2005. Hardness ($CaCO_3$) ranged from 3.25 to 3.81 mg/L. Inorganic metal levels (mg/L) were: Pb, As, Cr, Cu, Mn, Mo, Sr (< 0.005); Cd, B, Be, Ti, Zn (< 0.002) and Ni, Sn (< 0.02). The means of other elements (mg/L) were: Al (0.12); Ca (1.2); Fe (0.07); K (0.13); Mg (0.10); Na (0.50), and Si (1.10). DO averaged 10.3 $\pm$ 0.8 mg/L, and The pH varied from 7.2 to 7.9. No chromatographic responses in well water were detected for organochlorine, organophosphorus and nitrogen type pesticides (detection limits, 0.05 - 0.10 µg/L). The analytical atrazine, metolachlor and simazine concentrations in the water of test vessels soon after fish introduction varied somewhat from the corresponding nominal concentrations (Table 4). The data suggest that there was a difference, possibly caused by various factors, e.g., volatilization during the initial aeration process, glass adsorption of test chemicals, and other unidentified causes discussed and cited in Wan et al. (2005). Atrazine-D5 (CDN Isotopes, QC, Canada) was the surrogate used to verify triazine recovery efficiency [av. = 80% (55% – 110%); n = 12].

Table 5. Acute toxicity of atrazine, metolachlor, simazine and their formulated products to indicator organisms

Test chemical & Species	LC50 (mg/L) 24-h	48-h	72-h	96-h
Atrazine (See[e] Table 4):				
D. magna	>250	72	-	-
H. azteca	-	-	-	13
R. catesbeiana	>16	>16	>16	>16
O. kisutch	14	14	12	12
O. mykiss	15	13	13	13
O. tshawytscha	22	20	20	19
Atrazine® 500:				
D. magna	>1,000	>1,000	-	-
H. azteca	-	-	-	33
R. catesbeiana	>480	>480	>480	>480
O. kisutch	50	48	47	43
O. mykiss	>250	48	40	38
O. tshawytscha	68	46	41	37
Metolachlor (See[e] Table 4):				
D. magna	80	13	-	-
H. azteca	-	-	-	6
R. catesbeiana	>25	17	16	14
O. kisutch	>20	15	9	9
O. mykiss	19	15	14	13
O. tshawytscha	44	15	13	13
Primextra® II Magnum:				
D. magna	135	44	-	-
H. azteca	-	-	-	9
R. catesbeiana	56	56	56	56
O. kisutch	14	13	13	13
O. mykiss	18	16	16	16
O. tshawytscha	16	16	15	15
Princep®:				
D. magna	>1,000	>1,000	-	-
H. azteca	-	-	-	270
R. catesbeiana	>2,000	>2,000	2,000	1,780
O. kisutch	390	330	330	330
O. mykiss	360	350	330	330
O. tshawytscha	1180	1020	930	910

Atrazine, metolachlor and their formulated products (Primextra®, Atazine® 500) border between moderately ($1 < 96\text{-h LC50} \leq 10$ mg/L) to slightly ($10 < 96\text{-h LC50} \leq 100$ mg/L) toxic to juvenile amphibian, crustaceans and salmonid fish (US EPA 1985), even if the toxicity data were adjusted for chemical loss or gain (Tables 4). This study suggests, however, that atrazine appears to be even less

Table 6. *Hyalella* 28-d survival: highest simulated concentrations

Treatment* (mg/kg)	Dry weight (mg ± SE)	Survival (% ± SE)
Control (n = 45/test)	0.56 ± 0.09	89 ± 4
Atrazine (high concn. 1.0)	0.70 ± 0.05	76 ± 16
Metolachlor (high concn. 1.0)	0.65 ± 0.03	91 ± 6
Atrazine + Metolachlor (high concn. 2.0)	0.71 ± 0.03	100 ± 0

*Cu reference toxicant tests = within acceptable chart limit

acutely toxic to freshwater organisms than the toxicity projected by Solomon et al. (1996). In contrast and based on similar acute toxicity ratings stated earlier, Princep® (89% granular simazine product) seems to be practically nontoxic (96-h LC50 = ≥100 mg/L) to freshwater amphibian, crustaceans and salmonid fish.

The order of increasing acute toxicity of test materials is the same for *D. magna* (48-h LC50), *H. azteca, R. catesbeiana, O. kisutch. O. mykiss and O. tshawytscha* (96-h LC50): Princep® ≤ Atrazine® 500 < atrazine < Primextra® < metolachlor (Table 5). Princep® is the least, and metolachlor the most, acutely toxic material. The highest simulated field concentrations of 1 to 2 mg/kg (actual 2.5 – 5.3 mg/kg; see Table 4) of atrazine, metolachlor or a combination of both ingredients in farm ditch sediments, however, had no significant negative impacts on the 28-d survival of *H. azteca* (Table 6).

Under the worst case Lower Fraser Valley (LFV) field scenario, a stream (15 cm depth, > 30 cm width, 0.002 m^3 sec^{-1}) unintentionally oversprayed once by aerial or ground-based operation with formulated products at the highest recommended application rate [e.g., 1.5 kg atrazine/ha, (1.6 kg/ha metolachlor + 1.3 atrazine kg/ha), or 4.5 simazine kg/ha] only has the potential to produce about 1.0 mg/L atrazine or metolachlor, and 3.0 mg/L simazine. Accordingly, the estimated concentrations vary from 6 to 19 times (atrazine, metolachlor or Primextra® II Magnum) and 11 to 590 times (Atrazine® 500 or Princep®) lower than the 48-h or 96-h LC50 values for freshwater juvenile amphibia, crustacea and salmonid fish (Table 5). Moreover, atrazine (and degradation product desethylatrazine), metolachlor and simazine residues found in farm ditches contiguous to fish streams in the LFV ranged from 0.30 to 0.60 µg/L in water and < 20 to 50 µg/kg in sediments *(unpublished data).* These concentrations varied from about 10^2 to 10^4 times lower than the 48-h or 96-h LC50 values of nontarget organisms (Table 5). When used under prescribed conditions, it is doubtful that acutely toxic levels of atrazine, metolachlor and simazine and their degradation products would occur in contaminated streams, except perhaps during accidental spills. To further protect habitat sensitive waterbodies, LFV farmers are encouraged to observe appropriate stream setbacks. Despite this evaluation, the verdict on sub-acute effects of triazine herbicide on nontarget organisms remains to be resolved. Recent studies indicate their potential negative effects on amphibian and salmon (Hayes et al, 2003; Sullivan and Spence, 2003; Waring and Moore, 2004).

In sum, atrazine, metolachlor and simazine are only slightly to moderately toxic to freshwater amphibia, salmonid fish and zooplankton of the Pacific Northwest. While their highest simulated streambed levels had no adverse impact on bottom dwellers, their potential sub-acute effects on nontarget organisms need further investigation.

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