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Acute Toxicity of Selected Herbicides and Surfactants to Larvae of the Midge *Chironomus riparius*[1]

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Abstract. The acute toxicities of eight commercial herbicides and two surfactants to early fourth instar larvae of the midge *Chironomus riparius* were determined under static conditions. The formulated herbicides tested were Eradicane® (EPTC), Fargo® (triallate), Lasso® (alachlor), ME4 Brominal® (bromoxynil), Ramrod® (propachlor), Rodeo® (glyphosate), Sencor® (metribuzin), and Sutan (+)® (butylate); the two surfactants were Activator N.F.® and Ortho X-77®. In addition, technical grade alachlor, metribuzin, propachlor, and triallate were tested for comparison with the formulated herbicides. The relative toxicity of the commercial formulations, based on percent active ingredient, varied considerably. The EC50 values ranged from 1.23 mg/L for Fargo® to 5,600 mg/L for Rodeo®. Fargo®, ME4 Brominal®, and Ramrod® were moderately toxic to midge larvae; Lasso®, Sutan (+)®, and Eradicane® were slightly toxic; and Sencor® and Rodeo® were practically non-toxic. The 48-hr EC50 values of the two surfactants were nearly identical and were considered moderately toxic to midges. For two of the herbicides in which the technical grade material was tested, the inert ingredients in the formulations had a significant effect on the toxicity of the active ingredients. Fargo® was twice as toxic as technical grade triallate, whereas Sencor® was considerably less toxic than technical grade metribuzin. A comparison of the slope function values indicated that the toxic action of all the compounds occurred within a relatively narrow range. Published acute toxicity data on these compounds for other freshwater biota were tabulated and compared with our results. In general, the relative order of toxicity to *C. riparius* was similar to those for other freshwater invertebrates and fish. Maximum concentrations of each herbicide in bulk runoff during a projected "critical" runoff event were calculated as a percentage of the application rate lost in a given volume of runoff. A comparison between estimated maximum herbicide concentrations in runoff and results of acute tests indicated that Ramrod®, ME4 Brominal®, and Lasso® pose the greatest direct risk to midge larvae during a storm event.

A shift from conventional to conservation tillage and intensive production demands on a declining land base has led to greater use of herbicides in agriculture. These changes are particularly evident in the Northern Prairie Wetlands Region of North and South Dakota, where approximately one-half of the land surface is cropland and for most crops 80–90% of the acreage planted is treated with herbicides (Grue *et al.* 1986). This same region is characterized by the presence of numerous wetland potholes, poorly drained waterways, and slow moving streams. These prairie wetlands provide over 50% of the waterfowl production in North America from only 10% of the total continental production area (Smith *et al.* 1964).

Most herbicides are applied in spring and early summer, seasons that coincide with the period of maximum precipitation and prime waterfowl production. The diet of breeding ducks, particularly

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dabbling hens, consists primarily of aquatic invertebrates (Krapu 1974; Serie and Swanson 1976; Swanson *et al.* 1974), which provide a rich source of the highly digestible protein required for egg production and early growth of the young (Krapu and Swanson 1975). Invertebrates constitute 70 to 99% of the diet in laying hens (Swanson *et al.* 1979), 81% in flightless juveniles, and 57% in immature juveniles reaching flight stage (Krapu and Swanson 1977). Krapu and Swanson (1977) reported that 42% of the animal matter consumed by the pintail (*Anas acuta*) in North Dakota consisted of dipterans, of which 99% were larvae of the family Chironomidae.

Extensive use of herbicides has led to a growing concern about the movement of these chemicals from treated fields into wetland ecosystems. Little is known about the impacts of herbicides on waterfowl food organisms. Since most potholes are closed systems and herbicides are usually applied during the period of high runoff potential, indigenous biota may be periodically exposed to acutely lethal herbicide concentrations during storm events. The purpose of this study was to determine the acute toxicity of eight herbicides and two surfactants (substances that improve the efficacy of a herbicide) commonly used in the Northern Prairie Wetlands Region to larvae of the midge *Chironomus riparius*. For four of the herbicides, the technical grade material was also tested for comparison with commercial formulations. This study provides a starting point for evaluating potential impacts of these chemicals on wetland invertebrate communities.

Materials and Methods

Early fourth instar midge larvae were used in all toxicity tests. Larvae at this life stage were easily and accurately identified, minimizing handling stress, and are routinely used in acute toxicity tests conducted at the National Fisheries Contaminant Research Center, Columbia, Missouri. The larvae were obtained from a continuously reproducing laboratory culture (Biever 1965) maintained at the Yankton Field Research Station of the National Fisheries Contaminant Research Center in Yankton, South Dakota.

The eight formulated herbicides tested, including appropriate nomenclature and the supplier are given in Table 1.

The two nonionic surfactants, Activator N.F.® and Ortho X-77® Spreader were obtained locally and manufactured by Loveland Industries, Inc. and Chevron Chemical Company, respectively. In addition, technical grade alachlor, metribuzin, propachlor, and triallate were tested for comparison with their respective formulated herbicide.

Static acute toxicity tests were conducted at the Yankton Field Station according to standard toxicity testing protocol (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). Reconstituted water with pH 7.62–7.85, alkalinity 29.5–31.0 ppm as $CaCO_3$, and hardness of 42–44 ppm as $CaCO_3$ was prepared by adding reagent-grade salts to deionized water. The water was vigorously aerated and tempered to 22°C before testing. Midge larvae were tested at 22 ± 1.0°C in a temperature-controlled waterbath under natural light. Food was withheld during acclimation and testing.

Prior to testing, midge larvae were gradually acclimated from 100% holding (well) water to 100% dilution water over a 4 hr period. Holding water in the acclimation tank was diluted 50% with reconstituted water at 1 hr intervals during the acclimation period. Tests were conducted in 250-mL beakers containing 150-mL dilution water.

Each test consisted of exposing groups of 10 animals to a logarithmic series of six to eight concentrations of the test material based on the percent active ingredient, plus a solvent control (reagent-grade acetone) if required, and a control. The test materials were either added directly to the test vessel or pipetted from freshly prepared stock solutions. The amount of acetone added to the solvent control treatment was equal to the volume of stock solution added to the highest test concentration. Midge larvae were randomly distributed to the test chambers within 30 minutes after the toxicant was added. Determination of immobilization, defined as the lack of movement except for minor activity of appendages in response to gentle prodding, was made at 24 and 48 hr of exposure.

The measure of acute toxicity was the 48-hr EC50 (median effective concentration) based on immobilization. The Litchfield-Wilcoxon (1949) method was used to calculate EC50s, 95% confidence limits, and slope function values. Any two EC50 values were considered significantly different from each other if the 95% confidence limits did not overlap. Immobilization and mortality in the untreated control and solvent control did not exceed 10% in any of the tests.

Results and Discussion

The relative toxicities of the herbicides in the commercial formulations varied considerably (Table 2) ranging from moderately toxic for triallate, bromoxynil, and propachlor to practically non-toxic for metribuzin and glyphosate. Alachlor, butylate, and EPTC were slightly toxic to midge larvae. The qualitative rating of acute data was based on the toxicity rating scales of Christensen (1976) and the U.S. Environmental Protection Agency (unpublished). The range of EC50 values for each category were: 1–10 mg/L = moderately toxic, 10–100 mg/L = slightly toxic, and >100 mg/L = practically non-toxic. Excluding Rodeo® (48-hr EC50, 5600 mg/L), the range of EC50 values encompassed two orders of magnitude. All EC50 values were below the water solubility of their respective active ingredients. Fargo® (EC50, 1.23 mg/L triallate), the most toxic herbicide tested, was at 48 hr approximately 30 and 45 times more toxic than the other thiocarbamates, Sutan (+)® (EC50, 37 mg/L bu-

Table 1. Formulated herbicides tested

Trade name	Common name	Chemical name	Supplier
Eradicane®[a]	EPTC (plus R-25788)	*S*-ethyl dipropylthiocarbamate	Stauffer Chemical Co.
Fargo®	triallate	*S*-(2,3,3-trichloroallyl) diisopropylthiocarbamate	Monsanto Co.
Lasso®	alachlor	2-chloro-2′,6′-diethyl-*N*-(methoxymethyl) acetanilide	Monsanto Co.
ME4 Brominal®	bromoxynil	3,5-dibromo-4-hydroxybenzo-nitrile	Union Carbide
Ramrod®	propachlor	2-chloro-*N*-isopropyl-acetanilide	Monsanto Co.
Rodeo®	glyphosate	*N*-(phosphonomethyl)glycine	Monsanto Co.
Sencor®	metribuzin	4-amino-6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one	Mobay Chemical Corp.
Sutan (+)®	butylate (plus R-25788)	*S*-ethyl diisobutylthio-carbamate	Stauffer Chemical Co.

[a] Reference to trade names, commercial products, or manufacturers does not imply or constitute government endorsement or recommendation for use

tylate) and Eradicane® (EC50, 56 mg/L EPTC). Sutan (+)® and Eradicane® each contain Safener R-25788® in their formulation which may have modified the toxicity of the active ingredient. The herbicide antidote R-25788® is used in combination with thiocarbamate herbicides to increase the tolerance of corn and wheat to these herbicides. For the amides, propachlor was about 5.5 times more toxic than alachlor. From this limited data set, no consistent pattern of relative toxicities among the five classes of herbicides was observed.

Based on percent effective adjuvant, the EC50 values of the two surfactants were nearly identical and were moderately toxic to midge larvae. Watkins *et al.* (1985) reported similar findings for X-77® using bluegills (*Lepomis macrochirus*), for which both the 24-hr and 96-hr LC50 values were 5.5 mg/L.

The toxicities of technical grade propachlor, Ramrod®, technical grade alachlor, Eradicane®, and technical grade metribuzin increased significantly with exposure duration. This trend was not observed for technical grade triallate, Fargo®, ME4 Brominal®, Lasso®, Sutan (+)®, Sencor®, Rodeo® or the two surfactants, and may indicate a rapid loss in biological activity during the test period.

A comparison of toxicities between commercial formulations and their respective technical grade compounds revealed some interesting results. Based on percent active ingredient, Fargo® was approximately twice as toxic as technical grade triallate, whereas Sencor® and Rodeo® were significantly less toxic than technical grade metribuzin or glyphosate respectively (Table 3). Under standard test conditions, the "inert" ingredients had a pronounced effect on herbicide toxicity in these formulations. Folmar *et al.* (1979) reported that the presence of a surfactant in Round-Up® not only increased the biological activity of glyphosate to midges and rainbow trout (*Salmo gairdneri*) but was also the primary toxic component in the formulation. Using the freshwater copepod *Cyclops vernalis,* Robertson and Bunting (1976) found that commercial Amitrole-T® was about 127 and 48 times more toxic than reagent grade amitrole at 48 and 96 hr, respectively. We found no significant differences in toxicities between Ramrod® and technical grade propachlor or between Lasso® and technical grade alachlor. Mayer and Ellersieck (1986), using *Chironomus plumosus,* bluegills, channel catfish (*Ictalurus punctatus*), and rainbow trout reported similar results for propachlor and alachlor. However, technical alachlor was about three times more toxic than the 45% emulsifiable concentrate to *Daphnia magna*. These results suggested that "inert" ingredients formulated with technical grade chemicals may exhibit some biological activity and that commercial formulations

Table 2. Acute toxicity of selected herbicides and surfactants to fourth instar *Chironomus riparius* larvae under standard conditions

Formulation (% active ingredient)	EC50 (mg/L) and 95% confidence limits	
	24 hr	48 hr
Herbicides		
Triallate		
Technical material (96.3)	2.60 (1.52)[a]	2.30 (1.33)
	2.00–3.38	1.79–2.95
Fargo® (46.3)	1.80 (1.47)	1.23 (1.51)
	1.28–2.52	0.95–1.58
Bromoxynil		
ME4 Brominal® (39.4)	2.35 (1.33)	1.90 (1.23)
	1.83–3.02	1.58–2.28
Propachlor		
Technical material (94.4)	5.20 (1.24)	1.80 (1.46)
	4.31–6.27	1.30–2.50
Ramrod® (42.1)	6.30 (1.24)	2.20 (1.56)
	5.23–7.59	1.67–2.90
Alachlor		
Technical material (95.5)	27.5 (1.39)	10.0 (1.79)
	20.6–36.6	7.4–13.4
Lasso® (45.1)	13.1 (1.31)	12.5 (1.33)
	10.3–16.6	9.7–16.1
Butylate		
Sutan (+)® (85.1)	53.0 (1.23)	37.0 (1.37)
	44.2–63.5	28.0–48.9
EPTC		
Eradicane® (82.6)	>100	56.0 (1.24)
		46.4–67.5
Metribuzin		
Technical material (93.0)	175 (1.97)	43.5 (1.92)
	124–247	29.0–65.2
Sencor® (75)	190 (1.23)	130 (1.32)
	158–228	102–166
Glyphosate		
Rodeo® (53.5)	5900 (1.22)	5600 (1.22)
	4970–7000	4690–6690
Surfactants[b]		
X-77®	8.6 (1.41)	8.6 (1.41)
(Effective adjuvant, 90)	6.3–12.4	6.3–12.4
Activator N.F.®	10.1 (1.41)	8.9 (1.42)
(Effective adjuvant, 25.3)	6.6–13.3	5.9–12.7

[a] Slope function values given in parentheses
[b] Average EC50 and slope function for two tests, and minimum and maximum 95% confidence limits

should also be evaluated when the hazard potentials of herbicides are assessed.

The slope of the concentration-response curve is an index of the range of sensitivity within a group of organisms to a chemical (Rand and Petrocelli 1985). The narrow range in slope function (S) values (1.22 to 1.97) of the concentration-response curves (Table 2) suggested that the relative response of midge larvae to the herbicides and surfactants was similar, despite large numerical differences in EC50 values (potencies). The similarity in slopes may indicate that these chemicals have a similar mode of acute toxic action (Broderius and Kahl 1985). The small S values (steep slopes) indicated that concentrations required to immobilize midges were within a narrow range for each time interval, which suggests rapid uptake and onset of effects (Rand and Petrocelli 1985). Thurston *et al.* (1985) and Van Leeuwen *et al.* (1985) stated that highly toxic chemicals have a very specific mode of action, whereas chemicals with a low order of toxicity affect non-specific physiological processes, have non-selective toxicities, and probably have a similar mode of acute toxic action.

We found only limited published data on the toxicity of these agricultural chemicals to freshwater biota. A comparison of our results with acute toxicity values reported for fish and invertebrates is given in Table 3. Although no direct comparisons were made because the response measured and test conditions differed, the relative acute toxicity of these chemicals to different freshwater fauna generally followed the same order as for *C. riparius*. The one exception was the commercial formulation of butylate which was considerably more toxic to fish; however, only two fish species and probably a different formulation of the chemical were tested. For the compounds with comparative data, fourth instar *C. riparius* seem to be less sensitive than third instar *C. plumosus*. This apparent difference in species sensitivities may have been influenced by the life stage tested. Gauss *et al.* (1985) demonstrated that first instar *C. tentans* were significantly more sensitive than the fourth instar to copper. Overall, no single species was consistently the least or most sensitive to all the compounds tested. No toxicity data was found for ME4 Brominal® and Rodeo®.

To assess potential impacts of herbicides on prairie wetland invertebrate communities, toxicity data must be related to expected or measured concentrations in the water. Weber *et al.* (1980) stated that the extent of herbicide runoff from treated fields is determined primarily by rainfall intensity and the time that has elapsed between herbicide application and the rainfall event. The greatest risk of acute exposure to potentially toxic concentrations is from edge-of-field runoff during storm events occurring shortly after herbicide application. Based on rainfall simulation studies, a 10% loss of any pesticide is possible during a catastrophic event, although the probability of such an event is very low (Wauchope 1978). Weber *et al.* (1980) reported an

Table 3. Comparative acute toxicity (mg/L) of eight herbicides and one surfactant to freshwater invertebrates and fish

	Species						
	48-hr EC50			96-hr LC50			
Chemical and formulation[a]	*C. riparius* (this study)	*Chironomus plumosus*	*Daphnia magna*	Rainbow trout	Bluegills	Channel catfish	Reference[b]
Triallate							
T.M.	2.3	0.49[c]	0.08	0.62		1.7	1
			0.43[c]	1.2	1.3		2
E.C.	1.23		0.06[c]	1.0		1.1	1
				9.6	4.9		2
Bromoxynil							
T.M.			0.011[c]	0.05		0.023	2
Liq.	1.90						
Propachlor							
T.M.	1.80	0.79	6.9			0.23	1
			7.8	0.17	>1.4		2
Liq.	2.20	0.79	6.9			0.28	1
			13	0.42	1.6		2
Alachlor							
T.M.	10.0	3.2	21.0	2.4	4.3		1
			10.0	1.8	2.8		2
E.C.	12.5	2.5	7.7	1.4	3.2		1
			35	4.2	6.4		2
Butylate							
T.M.				2.1	0.21, 0.47		1
				4.2	6.9		2
E.C.	37			5.2	7.2		2
EPTC							
T.M.				19	27		2
E.C.	56			21	27		2
			0.95, 2.2	13	10, 24		3
Metribuzin							
T.M.	43.5		>100	42	92	>100	1
				>100	>100		2
W.P.	130						
Glyphosate							
T.M.			780	86	120		2
		55		140	140	130	4
Rodeo®	5600						
X-77®	8.6				5.5		5

[a] Abbreviations: T.M. = technical material; E.C. = emulsifiable concentrate; Liq. = liquid; and W.P. = wettable powder
[b] References: 1, Mayer and Ellersieck (1986); 2, Weed Science Society of America (1983); 3, J. Miller, Stauffer Chemical Company, personal communication; 4, Folmar *et al.* (1979); and 5, Watkins *et al.* (1985)
[c] Toxicity expressed as LC50

overall mean loss of 7% for various classes of pesticides under catastrophic conditions.

Due to the lack of chemical information on herbicide runoff from agricultural fields, herbicide concentrations in bulk runoff were estimated by multiplying the percent loss of herbicide applied by the depth of runoff. Wauchope (1978) defined a critical runoff event as one in which at least 1 cm of rainfall produces a runoff volume of 50% or more within 2 weeks after the application of a herbicide. To project a worst case scenario, we calculated the expected concentration of each herbicide (based on percent active ingredient) as 10% of the maximum recommended application rate in runoff water 1 cm deep (Table 4). For example, the concentration of Fargo® in runoff was calculated as follows: An application rate of 1.68 kg/ha equals 0.0168 mg/cm^2; a 10% loss would result in 0.00168 mg/cm^2 available for runoff; and the concentration in runoff 1 cm deep equals 0.00168 mg/cm^3 or 1.68 mg/L. Maximum concentrations reported in bulk runoff have been 1.7 ppm for propachlor and 0.18 ppm for

Table 4. Comparison of projected herbicide concentration in runoff with corresponding acute toxicity values

Herbicide	Application rate (kg/ha)[a]	Estimated concentration in runoff (mg/L)	Highest test concentration with no effect (mg/L)	48-hr EC50 (mg/L)	Ratio: 48-hr EC50 / concentration in runoff
Fargo®	1.12–1.68	1.68	0.56	1.23	0.73
ME4 Brominal®	0.56–1.12	1.12	1.00	1.90	1.70
Ramrod®	3.36–6.72	6.72	0.56	2.20	0.33
Lasso®	1.68–4.48	4.48	5.6	12.5	2.79
Sutan (+)®	3.4–6.7	6.7	32	37	5.52
Eradicane®	2.2–6.7	6.7	32	56	8.36
Sencor®	0.3–1.1	1.1	100	130	118
Rodeo®	0.34–4.48	4.5	3200	5600	1244

[a] Weed Science Society of America (1983)

alachlor (Wauchope 1978). During a critical runoff event, Fargo®, Ramrod®, ME4 Brominal®, and Lasso® pose the greatest direct risk to midges and other wetland biota with similar or greater sensitivities. Ramrod®, ME4 Brominal®, and Lasso® if applied to the soil surface pre- and early post-emergence would have a greater potential for runoff loss compared to Fargo® which is incorporated into the soil. Wauchope (1978) stated that a single-event loss of 10% is possible for any pesticide unless it is incorporated into the soil. The actual biologically available concentration in bulk runoff depends on partitioning between sediment and water, and other complex environmental factors. The other herbicides, if applied at the recommended rates, probably would not occur at concentrations acutely lethal to midge larvae. Additional acute testing, under laboratory and field conditions, on different life stages of other waterfowl food organisms are indicated for these four herbicides. Because these chemicals are phytotoxic, potential impacts on aquatic macrophyte and algal communities should be determined and appropriate herbicide combinations should also be tested. Further studies are needed to determine the fate and biological activity of these compounds in a wetland complex.

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