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Acute and Chronic Toxicity of the Herbicide Metolachlor to the Water Flea *Daphnia magna* and the Soil Nematode *Panagrellus redivivus*

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Management Perspective

This study addresses one of the research needs identified in the derivation of the interim Canadian Water Quality Guideline for the protection of aquatic life, *i.e.,* the determination of the chronic toxicity of metolachlor to an invertebrate (the water flea, *Daphnia magna*). The results of this work, together with the work of others on metolachlor toxicity to aquatic organisms, and our earlier work on metolachlor persistence and fate in water, will be communicated to the Evaluation and Interpretation Branch for use in the possible setting of a full Canadian Water Quality Guideline for metolachlor for the protection of aquatic life.

Sommaire à l'intention de la direction

Cette étude porte sur un des besoins de recherche définis lors de la préparation de la recommandation provisoire pour la qualité des eaux du Canada pour la protection de la vie aquatique, c.-à-d. la détermination de la toxicité chronique du métolachlore pour un invertébré (la cladocère, *Daphnia magna*). On doit communiquer à la Direction de l'évaluation et de l'interprétation les résultats de ces travaux, aussi que ceux d'autres chercheurs portant sur la toxicité du métolachlore pour les organismes aquatiques, et les résultats de travaux antérieurs sur la persistance du métolachlore et ses transformations dans l'eau; ces résultats doivent être utilisés pour la mise en place éventuelle d'une recommandation définitive pour la qualité des eaux du Canada visant le métolachlore, pour assurer la protection de la vie aquatique.

Abstract

This study addresses one of the research needs identified in the derivation of the interim Canadian Water Quality Guideline of 8 μg/L for the protection of aquatic life, *i.e.*, the determination of the chronic toxicity of metolachlor to an invertebrate (the water flea, *Daphnia magna*). Also investigated were the acute toxicity of metolachlor to *D. magna*, and acute and genotoxic effects in the soil nematode, *Panagrellus redivivus*. The chronic and acute toxicity of metolachlor to *D. magna* decreased with increasing water hardness. In the chronic toxicity test, the calculated IC_{50} values were 1.4 mg/L for soft water and 11.4 mg/L for hard water. In the acute toxicity test, the 48-hour LC_{50} values for soft waters of different trophic status were in the range 4.2-7.9 mg/L; however, the 48-hour LC_{50} values for hard waters of different trophic status were in the range 15.7-16.5 mg/L. The soil nematode *P. redivivus* was much less sensitive to metolachlor than *D. magna*. The 96 hour *P. redivivus* bioassay showed no effects on survival, growth and maturation at concentrations of metolachlor less than 100 mg/L. The 96-hr LC_{50} value (survival) was about 400 mg/L of metolachlor, while EC_{50} values for growth and maturation of the survivors were > 600 mg/L and about 600 mg/L, respectively.

Résumé

Cette étude porte sur des besoins de recherche définis lors de la préparation de la recommandation provisoire pour la qualité des eaux du Canada de 8 µg/L pour la protection de la vie aquatique, c.-à-d. la détermination de la toxicité chronique du métolachlore pour un invertébré (la cladocère, *Daphnia magna*). On a également étudié la toxicité aiguë du métolachlore pour *D. magna*, ainsi que les effets de toxicité aiguë et génotoxiques chez le nématode du sol *Panagrellus redivivus*. La toxicité chronique et aiguë du métolachlore pour *D. magna* diminuait en raison inverse de la dureté de l'eau. Les valeurs calculées de CI_{50} obtenues avec l'essai de toxicité chronique étaient de 1,4 mg/L pour l'eau douce et 11,4 mg/L pour l'eau dure. Avec l'essai de toxicité aiguë, la plage des valeurs de CL_{50} (48 heures) pour de l'eau douce de divers états trophiques était de 4,2 à 7,9 mg/L; toutefois, celle des valeurs de CL_{50} (48 heures) pour de l'eau dure de divers états trophiques était de 15,7 à 16,5 mg/L. Le nématode du sol *P. redivivus* était beaucoup moins sensible au métolachlore que *D. magna*. Le bio-essai de 96 heures pour *P. redivivus* n'indiquait aucun effet sur la survie, la croissance ou la maturation à des concentrations de métolachlore inférieures à 100 mg/L. La CL_{50} (survie) du métolachlore était d'environ 400 mg/L, alors que les CE_{50} pour la croissance et la maturation des survivants étaient supérieures à 600 mg/L et d'environ 600 mg/L, respectivement.

Introduction

The herbicide metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide) (see Fig. 1) is a germination inhibitor used mainly for the weed control of grasses (Chesters *et al.,* 1989). It is the most heavily used agricultural pesticide in Ontario. An estimated 4.2×10^3 metric tons of agricultural pesticides (active ingredient) of all types were used in Ontario in 1993 (Hunter and McGee, 1994), down from 7.2×10^3 metric tons in 1988 (Moxley, 1989). Thirty-one percent of the 1993 total was metolachlor (1.3×10^3 metric tons, down from 1.7×10^3 metric tons in 1988), which was used mainly on crops such as soybeans, corn and beans.

In order that an assessment can be made of the hazards of metolachlor use to aquatic ecosystems, more information is required on its occurrence in water, its persistence and fate, and its toxicity to aquatic organisms.

In the development of an interim Canadian Water Quality Guideline for metolachlor for the protection of aquatic life, the Evaluation and Interpretation Branch of Environment Canada noted that there were few data on the acute and chronic toxicity of metolachlor to vertebrates and invertebrates, and on effects on phytoplankton and aquatic vascular plants (Kent *et al.*, 1991). The most sensitive fish species was rainbow trout (*Oncorhynchus mykiss*), with a 96-h LC_{50} of 2 mg/L (Weed Science Society of America, 1983). The 48-h LC_{50} for the water flea (*Daphnia magna*) was 25 mg/L (Mayer and Ellersieck, 1986). At that time the only chronic aquatic toxicity data were for fathead minnow (*Pimephales promelas*), for which a no-observed-effect-concentration (NOEC) for reproduction of 780 µg/L was reported (U.S. Environmental Protection Agency, 1987). Based on available toxicological data, the U.S. Environmental Protection Agency (1987) set an advisory acute concentration value of 355 µg/L, and an advisory chronic concentration value of 14.2 µg/L. The interim recommended Canadian Water Quality Guideline for the protection of aquatic life was set at 8 µg/L (Kent *et al.,* 1991). The

Evaluation and Interpretation Branch identified the need for studies on the chronic toxicity of metolachlor to vertebrates and invertebrates (Kent *et al.*, 1991). This report addresses one of these research needs, *i.e.*, the determination of the chronic toxicity of metolachlor to an invertebrate (the water flea, *Daphnia magna*). Also reported are the acute toxicity of metolachlor to *D. magna*, and acute and genotoxic effects in the soil nematode, *Panagrellus redivivus*.

Methods

Materials

Metolachlor was obtained from Ciba-Geigy Canada Ltd. (Mississauga, Ont.). Pesticide grade organic solvents were obtained from Caledon Laboratories (Georgetown, Ont.). The sodium sulfate used for drying organic extracts was heated to 500 °C for 24 h before use. All glassware was rinsed with pesticide grade solvents before use. Immunoassay kits for metolachlor were obtained from Quantix Systems, Cinnaminson, NJ, U.S.A. Water samples for the *D. magna* studies were collected from the following Ontario lakes: Blue Chalk Lake (oligotrophic, water hardness 8.5 mg/L); Gull Feather Lake (mesotrophic, water hardness 4 mg/L; and Moot Lake (eutrophic, water hardness 6.5 mg/L). These soft waters were also augmented with $NaHCO_3$,$CaSO_4$, $MgSO_4$ and KCl to produce a hardness of 170 mg/L. Chemical analyses of these lake waters before augmentation are given in Table 1. The *D. magna* were obtained from the Carolina Biological Supply Co., Burlington, NC, U.S.A., and the *P. redivivus* were obtained from Bioquest International Inc., Winnipeg, Man.

Analyses for metolachlor

The concentrations of metolachlor in all toxicity tests were determined by extraction of the test solutions with dichloromethane, concentration and solvent exchange into toluene, and gas chromatography (Liu *et al.*, 1995) using a Hewlett-Packard 5890 gas chromatograph with a single splitless injector - dual column - dual detector (nitrogen-phosphorus and flame ionization) technique. The injection volume was 2 µL. Both columns were DB-5 (Chromatographic Specialties, Brockville, Ont.). Column dimensions were 0.25 mm i.d. x 30 m in length, with 0.25 µm film thickness. Injector and detector temperatures were 200 and 300 °C, respectively. The initial column temperature was 80 °C (2 minute hold), and the program rate was 10 °/minute to 150 °C, then 4 °/minute to 280 °C, then 8 °/minute to 300 °C, followed by a 5 minute final hold. The helium carrier gas was maintained at 1 mL/minute with electronic pressure programming. A standard mixture of metolachlor was prepared and used to calibrate retention times and detector responses. The presence of metolachlor was taken to be confirmed if it eluted within the appropriate chromatographic "window" on both columns. Quantitation was done with the flame ionization detector.

In a separate part of this study a comparison was done between concentrations of metolachlor in test solutions determined by the two gas chromatographic detectors, and concentrations determined using the immunoassay kit. There was good agreement between all three methods (variation < 20%).

D. magna acute toxicity test

Acute toxicity (lethality) tests on *D. magna* were carried out according to generally accepted methods (*e.g.*, Eaton *et al.*, 1994). Typically three groups of ten neonates (first instar young, 12-24 hours old) were tested at metolachlor concentrations of 0.1, 1, 10 and 20 mg/L in hard or soft waters of oligotrophic, mesotrophic and eutrophic status. Toxicity testing was also done for control waters. Appropriate controls were used. The temperature was 21±1 °C, and the animals were not fed during the testing period. The number of dead animals in each vessel was recorded at 1, 2, 24 and 48 hours incubation time.

D. magna chronic toxicity test

This test was designed to measure chronic effects (survival and reproduction) on adult *D. magna* (Mount and Norberg, 1984). Neonates (< 24 hours old) were isolated and maintained at 25 °C, with daily feeding, for 10 days. On the eleventh day the adult *D. magna* were transferred to test or control waters, and incubated, with daily feeding at 25 °C for 7 days. The water was changed twice during the 7 day period, at days 3 and 5. The young were collected and counted after each hatching during this 7 day test period. A chronic reproductive effect was indicated if after the 7 day incubation period, all the original adults had survived and the mean brood size was more than one standard deviation less than the control mean brood size. The effects of metolachlor concentrations of 0.1, 1, 10 and 20 mg/L were tested only in mesotrophic soft water and hard water. Control waters were the mesotrophic soft water and dechlorinated Burlington tap water.

P. redivivus acute toxicity and genotoxicity assay

The soil nematode *P. redivivus* produces neonates designated as second stage juveniles (J2). Over a 96 hour period, the neonate J2s grow through two additional juvenile stages (J3 and J4) to the adult stage. Each stage of *P. redivivus* falls within a characteristic size range. Monitoring a population of J2 animals over a 96 hour period provides a method of measuring both lethal and sublethal effects of a tested sample. Lethal effects are determined by the reduction in the total number of animals in the population. The number of animals remaining at the J2 or J3 stages provides a measure of sublethal effects. Growth from J2 to J3, or from J3 to J4, requires very little gene activity, while growth from J4 to adult requires extensive gene activity. Many known mutagens will selectively inhibit the J4 to adult moult, and specific inhibition of growth of J4s to adults can be used as an indicator of potential mutagenicity in the test sample (Samoiloff, 1990).

In the toxicity test 100 J2 animals, in groups of 10, were exposed to various concentrations of metolachlor in culture media (not natural waters) for a 96-hour period at 21±1 °C, and monitored for survival, growth (J2 → J4) and maturation (J4 → adult stage). The range of metolachlor concentrations tested was up to 600 mg/L. Negative controls were used (M9Y growth medium).

Statistical analyses

Acute toxicity data for *D. magna* were analyzed with the U.S. Environmental Protection Agency's Probit Analysis Program (v. 1.5) for calculating LC/EC values. Chronic toxicity data for *D. magna* were analyzed by the method of Norberg-King (1993).

Results

Acute toxicity to *D. magna*

Table 2 summarizes the acute toxicity of metolachlor to *D. magna*. The 48-hour LC_{50} values for the three soft waters were similar, in the range 4.2-7.9 mg/L. Increasing the water hardness to 170 mg/L decreased the acute toxicity of metolachlor to *D. magna* by factors of 2-4. The 48-hour LC_{50} values for the three hard waters were also similar, in the range 15.7-16.5 mg/L. No differences were noted in the survival rates of *D. magna* in hard and soft waters that did not contain metolachlor.

The acute toxicity of metolachlor to *D. magna* determined in this study was similar to results that have been obtained before. For example, Mayer and Ellersieck (1986) determined a LC_{50} value of 23.5 mg/L (range 18.7-29.5 mg/L, temperature 17 °C, hardness 44 mg $CaCO_3$/L, pH 7.2, static test), and Vilkas (1976) (unpublished study reported in Kent *et al.*, 1991) determined a LC_{50} value of 25.1 mg/L (range 21.6-29.2 mg/L, no-observed-effects-level 5.6 mg/L, no other test details provided). These values are within the range of acute toxicity values in hard waters reported in this study.

Chronic toxicity to *D. magna*

Figure 2 shows the reproductive effects of metolachlor on *D. magna* in mesotrophic soft water and hard water (pH 5.8, temperature 25 °C). As was the case for acute toxicity, increasing water hardness decreased the chronic toxicity of metolachlor to *D. magna*. The 7-day IC_{50} (reproduction) values were 1.4 mg/L in soft water (hardness 4 mg $CaCO_3$/L) and 11.4 mg/L in hard water (hardness 170 mg $CaCO_3$/L). Significant mortality of the alga *Chlorella* (one of the food sources for the *D. magna*) at 0.5-1.0 mg/L was observed in both the soft water and the hard water. It should also be noted that there was an effect of water hardness on reproduction of *D. magna* in the control waters (no added metolachlor). There were slightly fewer young in the soft water controls (mean of three replicates 162, standard deviation 6.2) than in the hard water controls (mean 173, standard deviation 5.4). There was thus a significant difference in reproduction at the 10% level between soft and hard water controls.

Toxicity to *P. redivivus*

The 96 hour *P. redivivus* bioassay showed no effects on survival, growth and maturation at concentrations of metolachlor less than 100 mg/L (see Fig. 3). The 96-hr LC_{50} value (survival) was about 400 mg/L of metolachlor, while EC_{50} values for growth (J2 → J4) and maturation (J4 → adult stage) of the survivors were > 600 mg/L and about 600 mg/L, respectively. *P. redivivus* was much less sensitive to metolachlor than *D. magna*. Because there are no data in the literature on the toxicity of chloroacetamide herbicides to *P. redivivus*, these results are not discussed further in this report.

Discussion

This study has shown that the chronic toxicity of metolachlor to *D. magna* varies considerably with water hardness. The 7-day IC_{50} (reproduction) values were 1.4 mg/L in soft water (hardness 4 mg $CaCO_3$/L) and 11.4 mg/L in hard water (hardness 170 mg

$CaCO_3$/L). The chronic toxicity value in hard water is probably more relevant to conditions in southwestern Ontario, where metolachlor is applied heavily on crops such as corn, beans and soybeans. For example, the average water hardness of five sampling stations in Muddy Creek in the period November 1992 to November 1993 was 148 mg $CaCO_3$/L, and the average water hardness in one sampling station in Malden Creek over the same period was 212 mg $CaCO_3$/L (Bourgoin *et al.*, 1995). The IC_{50} (reproduction) value of 11.4 mg/L for *D. magna* is greater than the maximum acceptable toxicant concentration (MATC) of 780 µg/L for reproduction in fathead minnow (*P. promelas*) (Dionne, 1978, cited in Kent *et al.*, 1991) that was used in the derivation of the interim Canadian Water Quality Guideline for the protection of aquatic life. Some other studies of metolachlor toxicity to aquatic organisms that have been reported since the derivation of the interim guideline in 1991, and that may be relevant to the establishment of a full guideline, are the following: Heuer *et al.* (1991) found that for cucumber plants (*Cucumis sativus* L., "Delilah") (25 °C, light intensity 450 µE m^{-2} s^{-1}, photoperiod 13:11, Hoaglund nutrient solution) the 21-day EC_{50} for reduction in root growth (weight) was about 50 µg/L. However, after 4 weeks at this concentration, root weight was only about 10% less than that of controls. St-Laurent *et al.* (1992) determined a 96-h EC_{50} value of 50 µg/L for the alga *Selenastrum capricornutum*. Goncz and Sencic (1994) reported the following 28-day EC_{50} values (sunlight [14.3±1.5 hours], temperature 23±4.1 °C, culture medium) for the free-floating freshwater fern *Salvinia natans*: number of leaves (75 µg/L), amount of chlorophyll-a (80 µg/L), amount of chlorophyll-b (50 µg/L), wet weight (150 µg/L), and length of stems (50 µg/L).

There is relatively little information in the open literature on the occurrence of metolachlor in surface waters. Table 3 summarizes some readily-available information from the literature on the occurrence of metolachlor in aquatic ecosystems in Canada and elsewhere. Some of the data were included in the Canadian Water Quality Guideline development document (Kent *et al.*, 1991), but other data are more recent. It appears that although mean concentrations in water were generally less than the current interim guideline of 8 µg/L for the protection of aquatic life, maximum concentrations (usually after rainfall in May-July) sometimes exceeded the guideline. Bourgoin *et al.* (1995) estimated that more than 85% of the metolachlor in Muddy Creek water (southwestern Ontario) was transported in the "dissolved" phase (*i.e.*, centrifuged water) as opposed to suspended particulate material. They also noted that the concentrations of two other herbicides (atrazine and metribuzin) also exceeded their respective Canadian Water Quality Guidelines in Muddy Creek in June-July 1993. Since the summer months are a period of high productivity for macrophytes, plankton and other biota in Lake Erie's coastal marshes and nearshore zone, chronic exposures to herbicides, and cumulative effects of herbicides, may have deleterious effects on such organisms in those areas.

The Canadian Water Quality Guideline development document for metolachlor (Kent *et al.*, 1991) also identified data gaps on the aquatic fate and persistence of metolachlor. This lack of information was one reason that an application factor of 0.01 was used in the derivation of the interim guideline. [The usual practice in the derivation of Canadian Water Quality Guidelines for the protection of aquatic life is to multiply the most sensitive LOEC by a safety (application) factor of 0.1 to arrive at the guideline value. The factor of 0.1 was chosen to account for differences in sensitivity to a chemical due to differences in species, laboratory *vs.* field conditions, and test endpoints (Canadian Council of Ministers of the Environment, 1991).] Work at the National Water Research Institute indicates that metolachlor is at least moderately persistent in natural waters. Kochany and Maguire (1994) showed that metolachlor was fairly stable in lake water in the dark, with < 4% loss after 100 days. Sunlight photodegradation of metolachlor was faster than purely chemical degradation, but was still a relatively slow process, with estimated near-surface half-lives in lake water of 22 calendar days in summer and 205 calendar days in winter at 40° N latitude. In 5 mg/L solutions of dissolved organic matter, the estimated half-lives were 2-3 times longer, depending upon the season. Four dechlorinated photoproducts were identified in lake water, accounting, after 40 days of sunlight irradiation, for 18% of the metolachlor originally present. These products resulted from dechlorination, hydroxylation, dehydrochlorination with subsequent morpholine ring formation, and N-dealkylation. Liu *et al.* (1995) found no biodegradation or biotransformation of metolachlor in three test lake waters after an incubation period of 170 days. With a polynuclear aromatic hydrocarbon (PAH)-degrading bacterial culture as the test organism, metolachlor was estimated to have a biological degradation half-life much greater than that of medium molecular weight PAHs. The white rot fungus *Phanerochaete chrysosporium* was able to biotransform metolachlor. Based on the three identified metabolites, a tentative metabolic pathway of metolachlor biotransformation by *P. chrysosporium* was proposed, involving demethylation, hydroxylation, and hydrolytic dechlorination.

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Table 1. Chemistry of lake waters used in toxicity tests.*

Constituent	Blue Chalk L. (Oligotrophic)	Gull Feather L. (Mesotrophic)	Moot L. (Eutrophic)
Alkalinity (mg/L)	4.5	1.7	0.8
Al (μg/l)	10	72.3	106.7
Ca (mg/L)	2.6	2.5	2.1
Chlorophyll (μg/L)	1.4	3.8	6.9
Cl (mg/L)	0.4	0.4	0.4
Conductivity (μS/cm at 25 °C)	27.9	27.9	22.9
DIC (mg/L)	1.4	1.3	0.6
DOC (mg/L)	1.8	5.4	6.5
Fe (μg/L)	60.5	747.9	518.2
K (mg/L)	0.4	0.4	0.3
Mg (mg/L)	0.8	0.7	0.5
Mn (μg/L)	29.6	74.1	43.5
Na (mg/L	0.8	0.7	0.5
NH_4 (μg/L)	17.7	53.8	21.2
NO_3 (μg/L)	15.4	36.1	20.9
TKN (μg/L)	175.4	350.3	565.7
pH	6.7	5.8	5.6
Si (mg/L)	0.2	1.5	0.8
SO_4 (mg/L)	6.3	7.8	6.1
Total P (μg/L)	5.53	11.54	14.53

***Analyses performed by Natural Resources Canada (E.T. Cox).**

Table 2. Acute toxicity of metolachlor to *D. magna*.*

Water	pH	Hardness (mg $CaCO_3$/L)	48-hour LC_{50} (mg/L)	Range (mg/L)
oligotrophic	6.7	8.5	5.8	4.3 - 7.5
	6.7	170	16.3	11.7 - 28.6
mesotrophic	5.8	4	4.2	2.0 - 6.8
	5.8	170	16.5	11.2 - 33.2
eutrophic	5.6	6.5	7.9	5.6 - 10.8
	5.6	170	15.7	11.9 - 24.4

*Tests performed under static conditions at 21±1 °C; concentrations measured at the beginning of the test; the upper and lower limits of the range are at the 95% confidence interval.

Table 3. Metolachlor occurrence in aquatic ecosystems.*

Sample	Location	Concentration	Comments	Reference
Canada				
rain water	southern Ont.	max. 0.3 µg/L	May 1991	Hall *et al.* (1993)
		max. 0.2 µg/L	May 1992	
well water	southern Ont.	> 0.1 µg/L	25/91 wells in 1984	Frank *et al.* (1987)
well water	southern Ont.	> 0.1 µg/L	18/103 wells in 1986	Frank *et al.* (1990a)
		> 0.1 µg/L	34/76 wells in 1987	Frank *et al.* (1990a)
rural ponds	southern Ont.	5.7 ± 8.1 µg/L (range 0.6-15 µg/L)	mean in three ponds in 1981-1985	Frank *et al.* (1990b)
river water	Sydenham R. Dresden, Ont.	mean 5.7 µg/L	1984 (max. 28.0 µg/L)	Frank *et al.* (1990c)
		mean 2.0 µg/L	1985 (max. 5.1 µg/L)	
		mean 4.2 µg/L	1986 (max. 15.0 µg/L)	
		mean 4.4 µg/L	1987 (max. 14.0 µg/L)	
			(found in 19-27% of samples in 1984-1987)	
creek water	Kintore Cr., southwestern Ont.	n.d.- 9-10 µg/L	mid-May to mid-June, 1991	Hall *et al.* (1993)

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Table 3 cont'd

Sample	Location	Concentration	Comments	Reference
creek water	southern Ont.	mean 0.1-0.2 µg/L	Apr.-Dec. 1991 (detection frequencies 60-70%, "urban streams")	Struger *et al.* (1994)
creek water	southwestern Ont.	20-160 µg/kg d.w. in surficial sediments	1992-1993	Bourgoin *et al.* (1995)
		n.d.-520 µg/kg d.w. in suspended particulates	Muddy Creek, Apr.-Nov. 1992	
		0.02-80 µg/L	Muddy Creek, centrifuged water	
river water	Yamaska R. basin, Qué.	mean 1.7 µg/L (range n.d.-13.4 µg/L)	1986-1987, 24 locations	Maguire and Tkacz (1993)
river water	southwestern Ontario	≤ 4.1 µg/L	Grand, Saugeen and Thames Rivers, 1981-1985	Frank and Logan (1988)
<u>Outside Canada</u>				
rain water	Ohio	0.25-2.5 µg/L	max. in May-June 1985	Richards *et al.* (1987)
rain water	Iowa	mean 0.5 µg/L	67/325 samples in Oct. 1987 - Sept. 1990	Nations and Hallberg (1992)
ground water	Iowa	n.d.-4.5 µg/L	1985	Hallberg (1985), cited in Ritter (1990)

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Table 3 cont'd

Sample	Location	Concentration	Comments	Reference
river water	U.S.A.	> 1.0 µg/L	every sample in May-June 1991 from White R. (Indiana), Illinois R. (Illinois) and Platte R. (Nebraska)	Goolsby *et al.* (1991), cited in Wang and Squillace (1994)
river water	Mississippi R., U.S.A.	0.3-4.4 µg/L	1979-1985	Chesters *et al.* (1989)
	Sacramento R., CA, U.S.A.	0.8-1.8 µg/L	1979-1985	Chesters *et al.* (1985)
river water	Rhine R., Germany	mean 1.1 µg/L (range 0.1-3.2 µg/L)	34 samples, 1985-1986	Oehmichen and Haberer (1986)
lake water	U.S.A.	mean 0.9-1.2 µg/L	Upper Tuttle Creek Lake and Lower Tuttle Creek Lake, Kansas	Arruda *et al.* (1988)
river water	Ohio	0.2-1.8 µg/L (range n.d.-97 µg/L)	time-weighted means for 7 rivers in 1983-1991	Richards and Baker (1993)

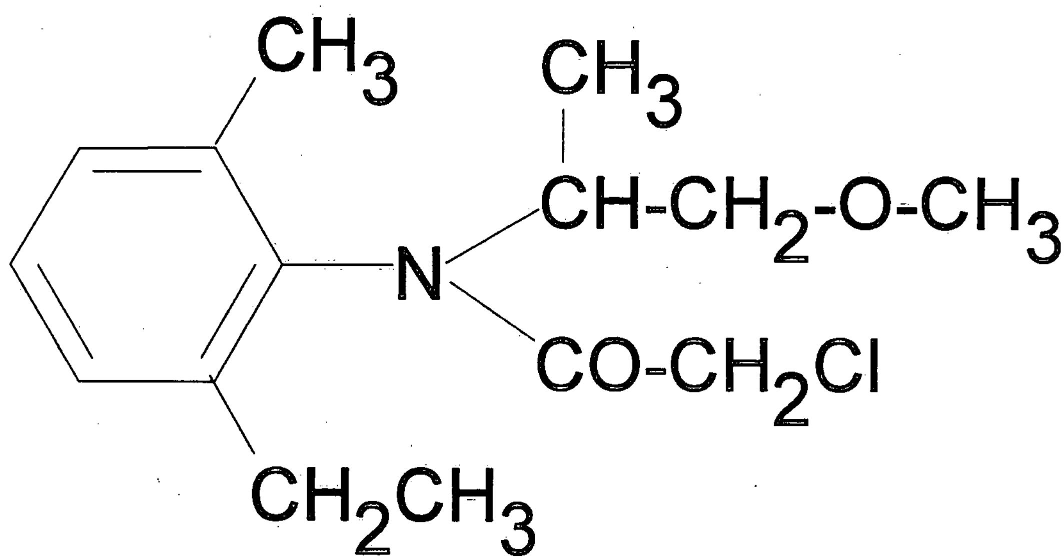
***Following are the recommended Canadian Water Quality Guidelines (Kent *et al.*, 1991): for the protection of aquatic life, 8 µg/L (interim); for raw water for drinking water supply, 50 µg/L; for livestock watering, 50 µg/L; for irrigation, 28 µg/L. "d.w." - dry weight; "n.d." - not detected.**

Figure Captions

Figure 1. Chemical structure of metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide).

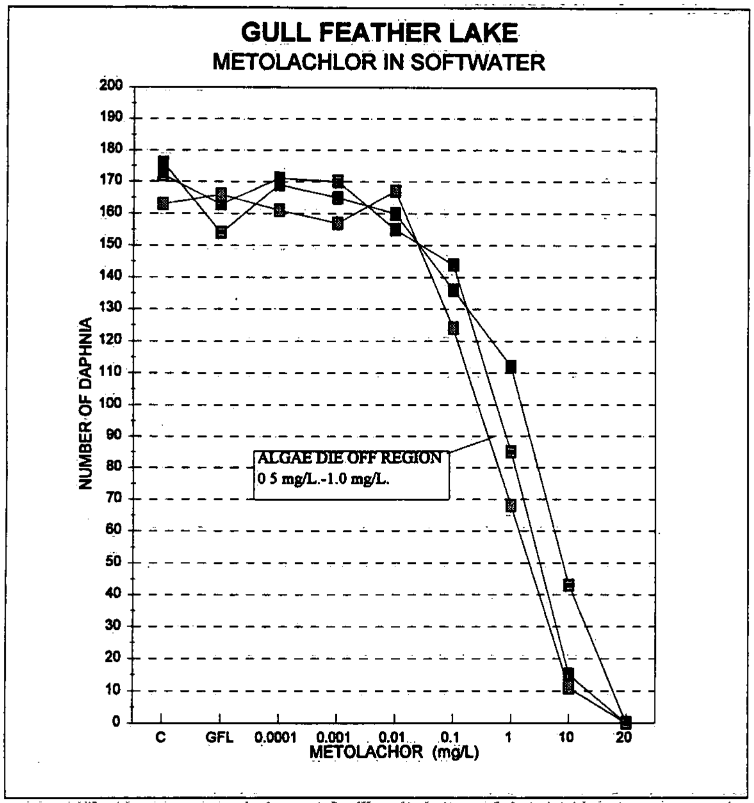
Figure 2. Reproductive effects of metolachlor on *D. magna* in mesotrophic soft water and hard water. The results of triplicate determinations are shown on the left hand side of the figure, while means are shown on the right hand side.

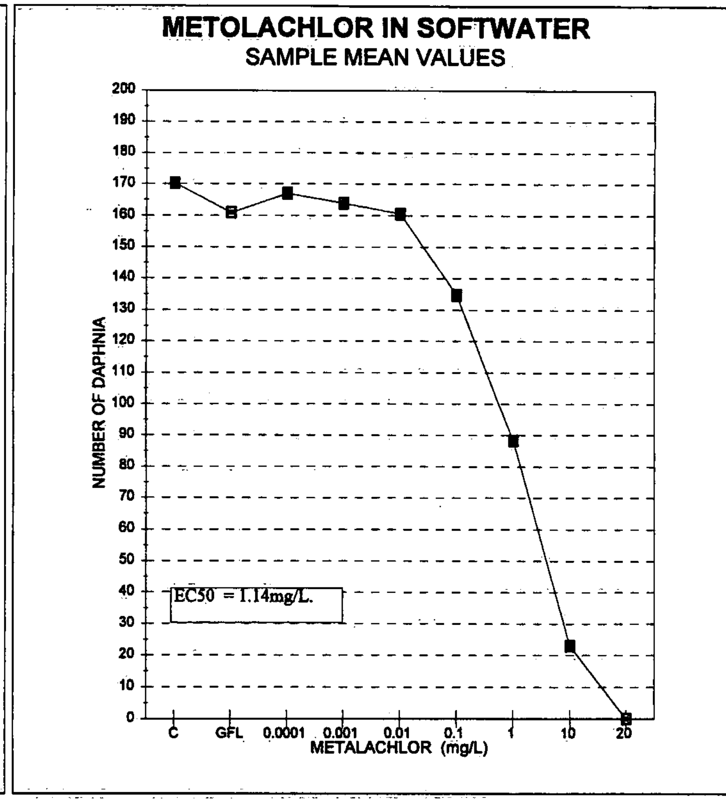
Figure 3. Effect of metolachlor on the survival, growth and maturation of *P. redivivus*. Fitness is calculated as the weighted mean of test survival, growth and maturation, relative to the control population (Samoiloff, 1990).

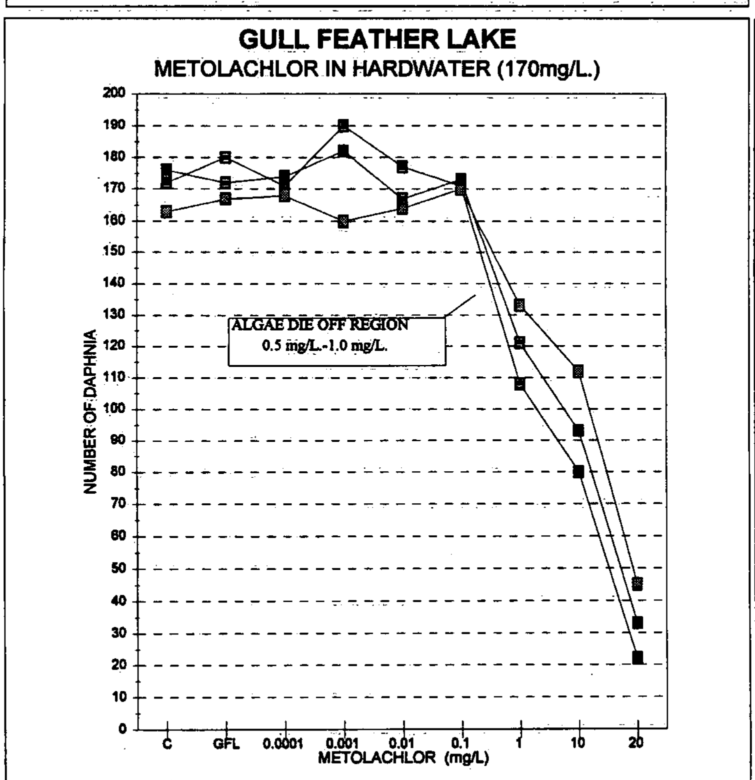


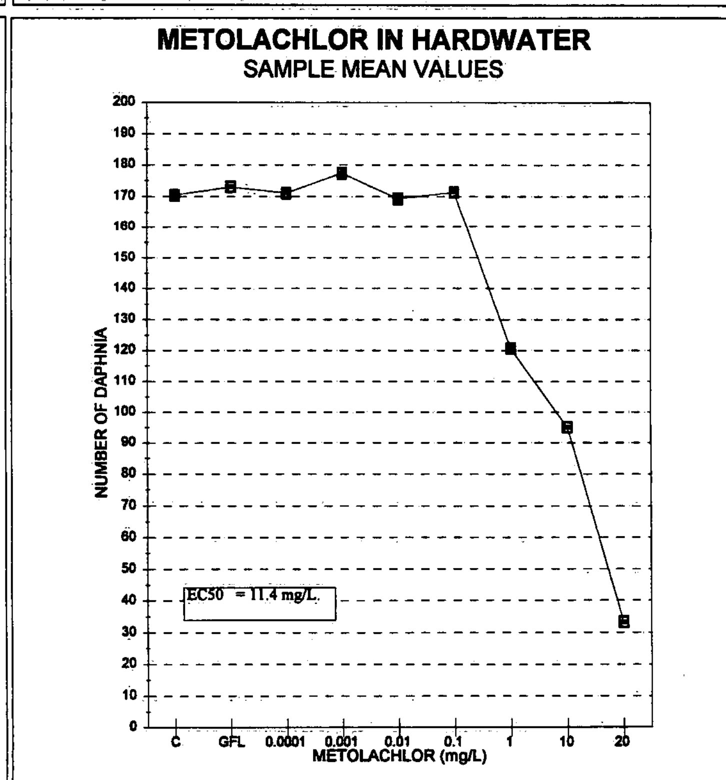
METOLACHLOR

EFFECT OF HARD AND SOFT MESOTROPHIC WATER ON CHRONIC TOXICITY OF D. MAGNA TO METOLACHLOR.



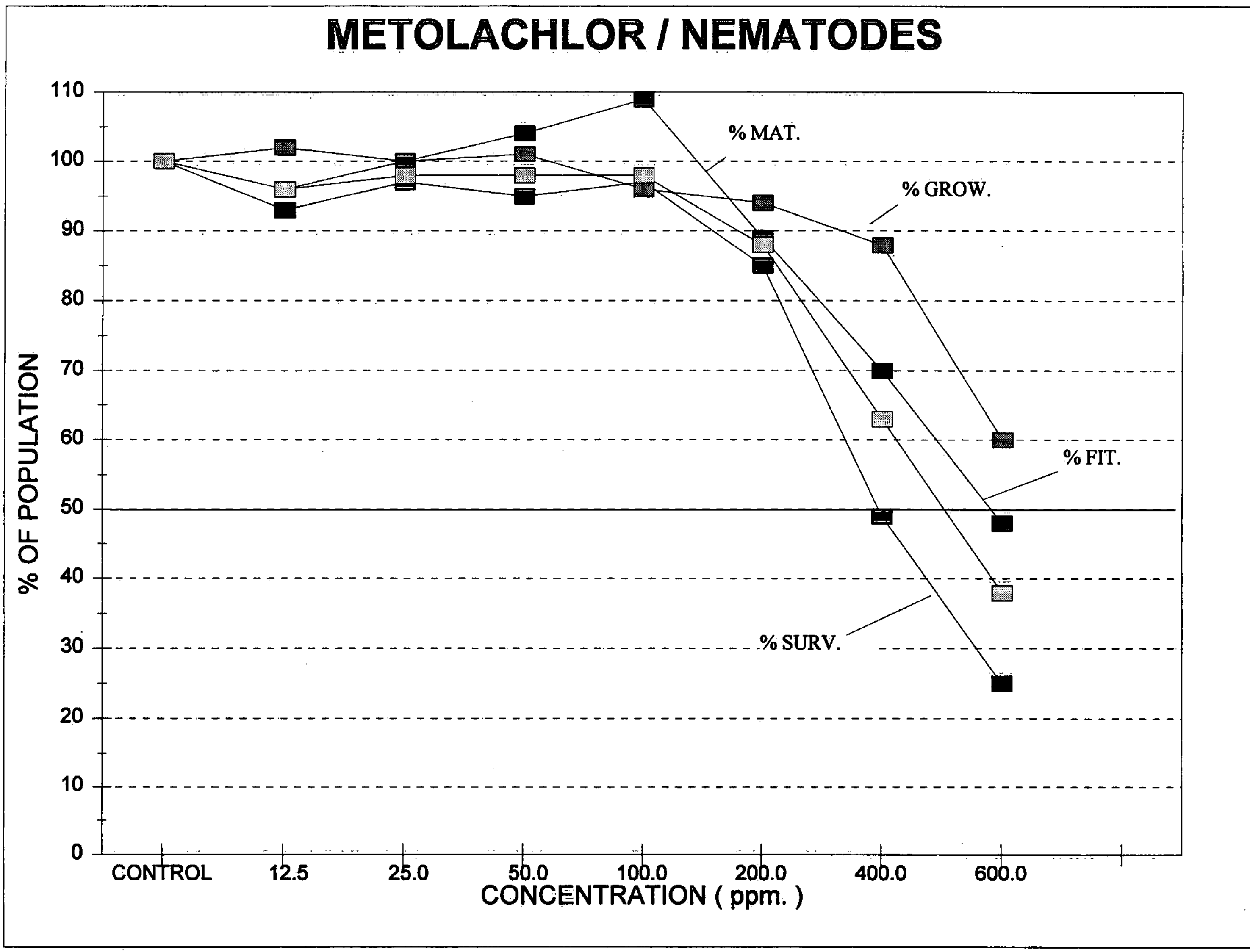






C = DECHLORINATED BURLINGTON TAP WATER.

GFL = GULF FEATHER LAKE WATER.



METOLACHLOR / NEMATODES
% OF POPULATION
110
100
90
80
70
60
50
40
30
20
10
0
% MAT.
% GROW.
% FIT.
% SURV.
CONTROL
12.5
25.0
50.0
100.0
200.0
400.0
600.0
CONCENTRATION (ppm.)

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Environment Canada Environnement Canada

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