CHIRAL CURRENTLY USED PESTICIDES IN ONTARIO STREAMS IN 2003-2004

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Introduction

Many of the currently used pesticides (CUPs) in Canadian agriculture are chiral, including three herbicides that are applied in excess of 100 tonnes/y: metolachlor (554 t/y), mecoprop (252 t/y) and dichlorprop (112 t/y)[1]. Chiral pesticides may exist in two enantiomeric forms or several diasteromeric pairs of enantiomers and these enantiomers usually have different toxicological and pharmacokinetic properties. Liu et al. (2005)[2] reported large differences in toxicities of enantiomers for bifenthrin, permethrin, fonofos and profonofos in freshwater invertebrates *C. dubia* and *D. magna.* Microbial communities process the enantiomers of chiral chemicals at different rates[3], consequently non-racemic enantiomer ratios are detected in the environment even though many pesticides are applied as racemic mixtures. Garrison et al. (2006)[4] reported that the two enantiomers of metalaxyl have environmental half-lives in soil which differ by over ten fold, and different enantiomer degradation rates of bifenthrin and permethrin were found in sediment under field and laboratory conditions[2]. Moreover, single enantiomers of some pesticides can be subject to interconversion in the environment, as shown by Müller and Buser (1997)[5] in a study of mecoprop and dichlorprop enantiomerization in soil. The pesticide industry, in an effort to reduce chemical loadings while maintaining efficacy, is increasingly turning to products which contain only the active stereoisomer. Such "chiral switching" can decrease pesticide use, thereby reducing impacts on the environment[4]. There is a growing recognition to focus commercial formulations on the active enantiomers of chiral pesticides and consequences of this action need to be considered further at a regulatory level[4,6].

Surveys of CUPs, including the above three herbicides, have been conducted in Ontario streams[7], but this is the first enantioselective analysis to be done. This work was carried out to investigate the stereoisomer composition of residues in stream water samples taken during a time when both racemic, enriched or enantiopure products were in use. The intent was to establish a baseline of residue patterns in stream water which will be useful for assessing changes due to chiral switching; e.g. distinguishing freshly applied pesticides from carry-over from past usage.

Materials and Methods

Sample Collection, Storage and Extraction

Surface water samples were collected in the Ontario region in 2003 (n=164) and 2004 (n=229) generally between April and December. Water samples were collected in pre-cleaned 1 L amber glass bottles, stabilized with 100 mL dichloromethane (DCM) and transported to the laboratory for analysis. Samples for mecoprop and dichlorprop were acidified with H_2SO_4 to a pH of ~2.5-3.0 and refrigerated until analysis. Unfiltered acidified (mecoprop and dichlorprop) and non-acidified (metolachlor) samples were subjected to a three-step liquid/liquid extraction with a total volume of 200 mL DCM and the extracts were reduced to ~4-5 mL. The extract for acid herbicides was evaporated to dryness and the residue was redissolved in 4 mL acetone. The herbicides were derivatized to pentafluorobenzyl (PFB) esters[8] and cleaned up on silica gel (0.71 g, dried at 160 ℃, deactivated with 5% water) which was eluted with 10 mL 5% methanol in toluene. Samples for metolachlor analysis were cleaned up on a Florisil column (10 g, dried at 160 ℃, deactivated with 10% water), which was eluted with 200 mL 2% methanol in DCM and concentrated to ~1 mL in isooctane.

Quantitative/Enantioselective Analysis and Quality Control
Quantitative analysis was carried out on a 30 m HP5 column using an Agilent 6890 GC interfaced with a 5973 MSD operating in selected ion mode. Analytes were detected by electron capture negative ion (ECNI) mass spectrometry (mecoprop-PFB, m/z 213/215, and dichlorprop-PFB, m/z 233/235) or electron impact (EI) mass spectrometry (metolachlor, m/z 162/238). Enantioselective analysis of mecoprop-PFB and dichlorprop-PFB was done on the same instrument in ECNI and/or EI modes using 30 m Rtx βDEXcst (Restek, U.S.A) or 30 m Betadex-120 (Supelco, U.S.A) chiral stationary phase columns (both 0.25 mm i.d. , 0.25 µm film thickness). Monitored ions were the same as quantitative analysis. Separation of all four metolachlor stereoisomers was carried out by HPLC-MS/MS using a modular instrument consisting of an Agilent 1100 G1312A solvent delivery pump, 1100 LC binary pump and 1100 G1313A autosampler. The LC was connected to an API 2000 triple quadrupole mass spectrometer (Applied Biosystems, U.S.A.) operated in APPI (atmospheric pressure positive ionization) mode. The m/z ion transition 284.2 – 251.1 was monitored. Separations were done using a Daicel Chiralcel OD-H column, 150 x 4.6 mm, bonded phase of cellulose *tris*-(3,5-dimethylphenylcarbamate) coated on 5 µm silica particles (Chiral Technologies, U.S.A.) with a mobile phase composition of 99.8: 0.2 hexane:2-propanol [9]. Average recoveries of surrogates were: 2,3-dichlorophenoxy ethanoic acid (2,3-D) (for mecoprop and dichlorprop), 94.9±11.8% and trifluralin-d_{14} (for metolachlor), 72.3±23.7%. Recoveries of unlabeled mecoprop and dichlorprop (n=37) and metolachlor (n=12) were 86.3±8.9%, 83.8±9.0% and 82.2±7.9%, respectively. Results were not recovery corrected. Method Quantitation Limits (MQL), were 5 ng L^{-1} for mecoprop, 4.2 ng L^{-1} for dichlorprop, and 24 ng L^{-1} for metolachlor. Enantiomer fractions, EF = $R(+)/[R(+) + S(-)]$, which fell within the 95% confidence interval (C.I.) for standards (mecoprop 0.495±0.005, n=29; dichlorprop 0.489±0.009, n=26) were accepted to be racemic. Ratios of monitored ions were required to be within the ±95% C.I. for an acceptable analysis. Chiral data for metolachlor were expressed as S/R = (aS,C*S+aR,C*S)/(aS,C*R+aR,C*R), where aS and aR indicate axial chirality and C*S and C*R indicate carbon chirality. S/R expresses the ratio of herbicidally active/inactive stereoisomers[10]. This value was measured as 7.49±0.04 for a commercial S-metolachlor formulation and reported as ~9 by Buser et al. (2000)[10]. The measured S/R for a racemic metolachlor standard was 1.02±0.032.

Results and Discussion

Concentration Profiles
Ranges of concentrations and frequencies of occurrence ≥MQL for compounds of interest are given in Table 1. Metolachlor was the most frequently quantified chiral pesticide in 2003 and mecoprop in 2004. Dichlorprop frequencies were the lowest of the three in both years. Mecoprop and metolachlor concentrations in stream water samples were below Canadian Water Quality Guidelines (WQG)[11] in 2003; however, mecoprop concentrations in some sampling locations were above the guidelines in 2004 (1.2% of samples with conc. ≥ MQL).

Table 1. Concentration profiles of mecoprop, dichlorprop and metolachlor in Ontario streams in 2003-2004.

| Compound	Positive*/n	Frequency ≥MQL (%)	Concentration (ng L^{-1})					
			Min*	Max*	25th percentile*	Median*	75th percentile*	WQG[§]
2003								
Mecoprop	57/164	35.0	0.6	1900	3.0	5.80	130	4000
Dichlorprop	55/164	34.0	0.4	6.60	0.6	1.20	2.0	na
Metolachlor	71/164	43.0	24.0	1600	50.0	89.0	260.0	7800
2004								
Mecoprop	167/229	73.0	0.6	103000	3.30	28.0	121.0	4000
Dichlorprop	70/229	31.0	0.5	110.0	2.10	3.90	11.0	na
Metolachlor	130/229	57.0	24.0	5300	56.0	101.0	375.0	7800

* positive: ≥ MQL, [§]Canadian Water

A Student's t-test (2-tailed) showed that concentrations of metolachlor in samples from agricultural watersheds (average =501 ng L^{-1}, n=96) were significantly different from those in urban watersheds (average = 233 ng L^{-1}, n=49) (p =8.6x10^{-9}). Moreover, when agricultural row crops regions (n=48) were compared to fruitland (n=48) regions significantly lower metolachlor concentrations were found in stream water from row crop areas (p=2.6 x 10^{-3}). For mecoprop, there were no significant differences in concentrations between agricultural (n=39) and urban (n=23) watersheds (p=0.39) nor between agricultural row crop (n=11) and agricultural fruitland (n=28) areas (p=0.33).

Chiral Signatures of Herbicides in Stream Waters

Enantioselective analysis was carried out for samples in which concentrations of mecoprop (n=72), dichlorprop (n=10) and metolachlor (n=148) were sufficiently high. The distributions of EFs of mecoprop and dichlorprop and *S/R* of metolachlor are shown in Figure 1. *R*(+) mecoprop was preferentially enriched (EF > 0.5) in almost half of the samples, while *S*(-) mecoprop was enriched (EF >0.5) in 39% of the samples Figure 1A. Only 10 samples displayed high enough dichlorprop concentrations for chiral analysis in 2004 and 90% of samples showed preferential enrichment of *S*(-) dichlorprop, Figure 1B. Isomer distributions of metolachlor in Ontario streams in 2003-2004 were measured in 148 water samples (58 from 2003, 90 from 2004). The percentage of samples with *S/R* < 7.5, *S/R*=7.5-9.0 and *S/R*>9.0 are shown in Figures 1C (both seasons), 1D (2003) and 1E (2004), respectively. The majority of the samples displayed an *S/R* lower than 7.5, followed by *S/R*=7.5-9.0 and *13*>9.0 where overall *S/R* of metolachlor in both years was 6.73 ± 2.28.
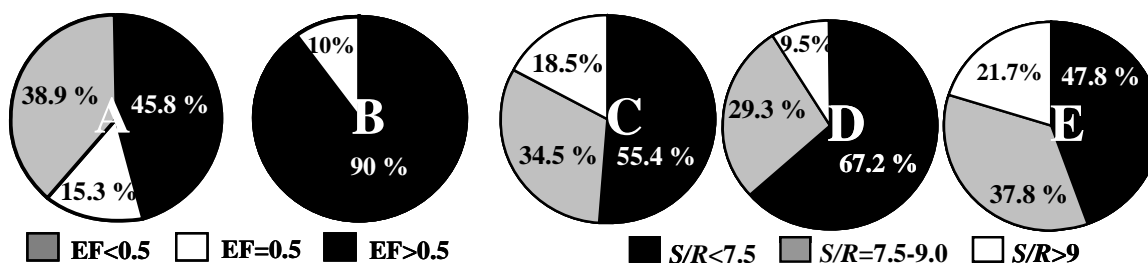
Figure 1. Left: Distribution of EFs (2003-2004 data together) of mecoprop (A) and dichlorprop (B). Right: Distribution of *S/R*s of metolachlor in 2003-2004 data together (C), in 2003 (D) and in 2004 (E)
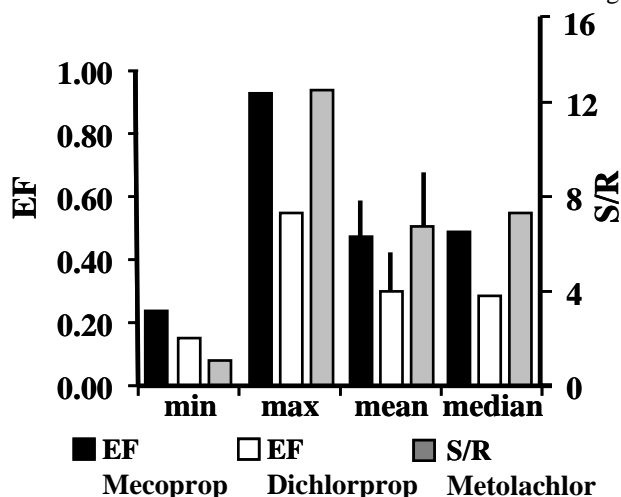
Figure 2. EFs (mecoprop & dichlorprop) and *S/R*s (metolachlor) in Ontario streams in 2003-2004.

Minimum, maximum, mean±SD and median values of EFs (mecoprop and dichlorprop) and *S/R*s (metolachlor) are shown in Figure 2. Minimum and maximum EFs for mecoprop were 0.236 and 0.928, and occurred in August and June 2004. For dichlorprop, the lowest and highest EFs were 0.152 and 0.549 in samples collected in June 2004. For metolachlor, 1.08 and 12.7 were the lowest and the highest *S/R* values in samples collected in June, 2004 and May, 2004.

The Canadian Pest Management Regulatory Agency states that last sales of racemic mecoprop products would be phased out between 2004-2005, and existing stockpiles could be used until the end of 2009[12].

If inputs of racemic mecoprop and mecoprop-P were the controlling factor of enantiomer compositions in the environment in these transition years, then we would expect racemic or *R*(+) enantiomer enriched compositions in stream samples.

But, results showed similar frequencies of $R(+)$ and $S(-)$ enantiomer enrichment for mecoprop, suggesting a more rapid degradation of the $R(+)$ enantiomer as well as possible interconversion of enantiomers, as found in soils [5,13,14]. Buerge et al. (2003)[15] reviewed reports of mecoprop and dichlorprop degradation and found that enantioselectivity was strongly influenced by soil pH.

Herbicide concentrations (C) in the watersheds ranged over several orders of magnitude (Table 1). Poor but significant correlation between EF vs log C (n=72) was found for mecoprop (r^2=0.11 and p=0.00436). Samples with higher concentrations showed EFs that were closer to racemic or enriched in $R(+)$, while the lower concentration samples showed more scattered EFs that varied on either side of racemic. If the higher concentrations were a result of recent applications, these results suggest that mainly racemic mecoprop was in use in these watersheds during 2003-2004. The correlation between S/R vs. log C of metolachlor was also weak but significant ($r^2 = 0.24$, p= 8.7×10^{-5}). Samples with higher metolachlor concentrations displayed S/R ratios similar to those in commercial S-metolachlor and samples with lower concentration showed more scatter. Use of both racemic and S-metolachlor in transition years could be a reason for this scatter since the chiral switch from racemic metolachlor to S-metolachlor began ~1998 in Canada[10], but in 2003 approximately equal usage of racemic metolachlor (261.8 tonnes) and S-metolachlor (293.7 tonnes) was reported[1].

EFs of mecoprop in agricultural watersheds (n=39) were not significantly different from those in urban watersheds (n=23) and no difference was found between agricultural row crop (n=11) and fruitland (n=28) watersheds (p>0.05 in both cases). However, S/Rs of metolachlor were higher in agricultural areas (n=96) (p=0.0003) than in urban watersheds (n=49) and within the agricultural grouping higher S/Rs were found in row crop areas (n=48) compared to fruitland areas (n=48) (p=0.036).

References
1. Brimble, S., Bacchus, P. and Caux P.-Y. *Pesticide Utilization in Canada: A Compilation of Current Sales and Use Data*, Environment Canada GoC, 2005, Cat. No. En4-56/2005E-PDF. ISBN 0-662-42085-3
2. Liu, W., Gan, J., Schlenk, D., Jury, W. *Proc. Nat. Acad. Sci*. 2005, 102: 701-706.
3. Müller, T.A., Kohler, H.-P. *Appl. Microbiol. Biotechnol*. 2004, 64: 300-316
4. Garrison A.W. *Environ. Sci. Technol.,* 2006, 40: 16-23
5. Müller, M.D., Buser, H-R., *Environ. Sci. Technol*. 1997, 31: 1953-1959
6. Magrans, J., Alonzo-Prados, J., Garcia-Baudin, J. *Chemosphere,* 2002, 49: 461-469
7. Struger, J., Fletcher, T. *J. Great Lakes Res*. 2007, 33: 887-905
8. Lee H.B., Peart T.E. Carron J.M., Tse H. *J. Assoc. Off. Anal. Chem.*, 1991, 74: 5, 835-842.
9. Polcaro, C.M., Berti, A., Mannina, L., Marra, C., Sinibaldi, M., Viel, S. *J. Liquid Chrom. Rel. Technol.*, 2004, 27: 1, 49-61
10. Buser, H.-R.; Poiger, T.; Muller, M. D. *Environ. Sci. Technol.*; 2000, 34, 13, 2690-2696
11. CCME. Appendix IX, *Canadian Water Quality Guidelines* 1991, Prepared by the Task Force on Water Quality Guidelines. [Updated and reprinted with minor revisions and editorial changes in Canadian environmental quality guidelines, Chapter 4, Canadian Council of Ministers of the Environment, 1999, Winnipeg.]
12. PMRA., *Reevaluation Decision Document: Mecoprop RRD2004-09,* 2004, Pest Management Regulatory Agency, Ottawa, May 2004
13. Garrison, A.W., Schmitt, Ph., Kettrup, A. *Environ. Sci. Technol.*, 1996, 30: 8, 2449-2455
14. Buser, H-R, Müller, M.D. *Environ. Sci. Technol.*, 1997, 31(7): 1960-1967
15. Buerge, I.J., Poiger, T., Muller, M.D., Buser, H.-R., *Environ. Sci. Technol.,* 2003, 37: 2668-2674