OBSERVATIONS OF ENANTIOSELECTIVITY IN THE FATE, PERSISTENCE AND EFFECTS OF MODERN PESTICIDES

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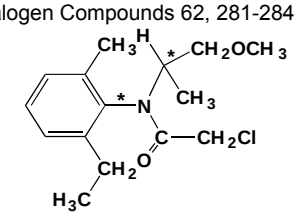
Introduction

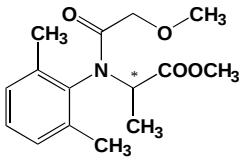
Chiral pollutants exist as 2 (or more) species, - *enantiomers* - that are non-superimposable mirror images of each other. Enantiomers have identical physical and chemical properties except when they interact with enzymes or other chiral molecules; then they usually react selectively. This *enantioselectivity* often results in different rates of microbial/biological transformation, differences in toxicity of the two enantiomers, and differences in activities toward biological species. Up to 25% of all pesticides are chiral molecules, and almost all are manufactured and applied as their *racemates*, mixtures of equal amounts of the enantiomers. Recently, however, the agrochemical industry and government regulators are beginning to take enantioselectivity into account [1,2]. For example, the (*R*)-(+)-enantiomer of dichlorprop (as well as the (*R*)-enantiomers of all the phenoxypropionic acid herbicides) is the herbicidally active species, while the (*S*)-(-)-enantiomer is inactive; so, to reduce the amount of herbicide used and avoid the possibility of the unnecessary enantiomer causing some adverse impact, several European countries have decreed that only the (*R*)-enantiomers will be used [3]. In addition, the two (*S*)-enantiomers of metolachlor **(Figure 1,** metolachlor has 2 chiral centers), one of the most widely used herbicides in the USA, are nine times more herbicidally active than the (*R*)-enantiomers , so its manufacturer successfully petitioned the EPA for registration of a formulation enriched to contain 88% of the (*S*)-enantiomers . This allows a 35% reduction in the amount of the herbicide applied, with the same effect. It seems obvious that the enantiomers of chiral pesticides should be treated as separate compounds and that accurate environmental and human risk assessments require an understanding of the relative persistence and effects of each enantiomer. This report emphasizes results of recent research on the fate and effects of enantiomers of specific chiral pesticides in use today; the so-called modern pesticides. **Figure 1** shows structures of the pesticides discussed here.

Methods and Materials

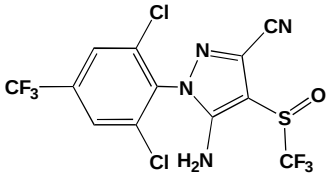
For the metolachlor field study, samples of soil selected from various depths of drilled cores and composited surface soils were extracted by shaking 16 hours with 80% methanol in water; this slurry was centrifuged and the supernatant extracted with methylene chloride, which was evaporated to appropriate volume for GC-MS analysis. Surface and run-off water samples of 4L volume were passed through 6mL SPE/LC-18 cartridges and eluted with methanol, which was evaporated for GC-MS analysis. GC-MS employed the SIM mode and an enantioselective column (BGB 172: 20% *tert*-butyldimethylsilylated beta-cylcodextrin dissolved in 15% phenyl/85% methylpolysiloxane) from BGB Analytik AG. For the metalaxyl degradation experiments, aliquots of the spiked soil slurries, taken with increasing time, were centrifuged and the supernatant analyzed directly by capillary electrophoresis in the MEKC mode. The electrolyte was a 30mM borate buffer of pH8.5 containing 100mM SDS, 15% acetonitrile, and 40mM gamma-cyclodextrin chiral selector; this resulted in baseline separation of metalaxyl enantiomers.



Metolachlor (* 2 chiral centers)



Metalaxyl



Fipronil

Figure 1. Structures of pesticides discussed in this report. * Marks the chiral center(s).

Results and Discussion

Because chiral pollutant residues exist in various environmental compartments, including biota and human tissue, it is important to analyze water, soil, sediment, biota and food samples expected to contain these pollutants to determine occurrences and ratios of the enantiomers. This will help establish the relative persistence of each enantiomer *in that matrix.* Such field occurrence data can be coupled with laboratory data on enantioselective transformation rates to allow a prediction of which enantiomer is more persistent in a particular environment.

There are many chiral pollutants with intermediate half-lives (days to months) that are expected to be transformed enantioselectively; that is, selective microbial transformation occurs before significant abiotic transformation. In earlier work[4], dichlorprop (2,4-dichlorphenoxy-2-propionic acid), an important herbicide, was shown to be transformed enantioselectively in surface soil after application to an experimental field. The (-)-enantiomer exhibited a half-life of about 4 days and the (+) about 8 days. This is a fortuitous situation, since the (+)-enantiomer is the active herbicide while the (-)-enantiomer is simply "ballast", and it seems prudent that the target-active enantiomer be the most persistent. As mentioned earlier, in several European countries recent regulations prescribe use of only the (+)-enantiomer[3].

Field studies were conducted to show the distribution of metolachlor and its enantiomers in an experimental U.S. Department of Agriculture watershed near Athens, GA, consisting of Cecil series soils (clayey, kaolinitic, thermic Typic Kanhapludult) developed in saprolite from Athens Gniess (grandioritic Gniess locally). The older, racemic formulation of the herbicide was applied to the watershed field in April 1999. Surface and subsurface soil samples and runoff water samples were obtained in early 2001 and extracted for the enantiomeric analysis of metolachlor by gas chromatography (GC). Although metolachlor has 2 chiral centers and contains 4 enantiomers, only 3 peaks can be separated by GC using the BGB 172 chiral phase. By comparing standards of the new *S*-enriched metolachlor with a racemic standard, it was shown that the first of the 3 separated peaks of racemic metolachlor is one of the *S* enantiomers and that the third peak is composed of one of the *R* enantiomers along with a smaller amount of the second *R* enantiomer. Thus, it is possible to calculate ratios of peak areas in the sample extracts, compare them with the ratios of the racemic standard, and so determine changes in relative concentrations of *R* and S enantiomers, which would indicate microbial degradation.

Table 1 shows preliminary results for samples from the experimental field site. The large differences in ratios between the two lower soils and the upper soil and the standard suggest considerable biotransformation, probably anaerobic in nature. The direction of the changes indicate loss of *R* enantiomers relative to *S* enantiomers; peak 1, the first eluting *S* enantiomer, increased relative to the other peaks, while peak 3, composed of *R* enantiomers, was not detected.

Table 1. Ratios of metolachlor GC peak areas (n=3)

	peak 1/peaks (2 + 3)	peak 3/peaks (1 + 2)
racemic standard	**0.30 ± 0.01**	**0.47 ± 0.03**
surface soil	**0.43 ± 0.04**	**0.52 ± 0.01**
5 m depth	**0.44 ± 0.02**	**0.43 ± 0.05**
6.8 m depth	**1.25 ± 0.04**	**0**
8 m depth	**1.13 ± 0.13**	**0**
run-off water	**0.41 ± 0.12**	**0.53 ± 0.09**

It is instructive to remember that the *S* enantiomers are the active components of metolachlor, while the *R* enantiomers are inactive and unnecessary. The ratios of peak areas for the run-off water and the surface soil were similar, as expected from dissolution of metolachlor from the surface soil by rain water.

It would be difficult to measure the kinetics of microbial transformation *in situ* at field sites. To collect significant amounts of data, it is necessary to select environmental matrices for laboratory microcosm studies, then spike the matrix, measure enantiomer concentration with time, and calculate rates of enantiomer transformation. Currently underway are experiments designed for determination of the microbial transformation in soil-water slurries of several modern pesticides of various classes: metalaxyl and metolachlor, a fungicide and herbicide, respectively, of the acetanilide class; dyfonate (fonofos), an organophosphorus insecticide; imazaquin, an imidazolinone herbicide; and fipronil, a phenypyrazole insecticide. These pesticides were spiked separately at 30 mg/L into several different soil-water slurries that were maintained in an aerobic state. Aliquots were removed periodically and analyzed for the residual pesticide by gas or liquid chromatography using chiral columns or capillary electrophoresis using chiral selectors. Enantiomeric ratios were measured to determine the degree of enantioselectivity, and kinetic plots allowed determination of degradation/transformation rate constants for each enantiomer.

The loss of metalaxyl, which was followed by capillary electrophoresis (CE), was relatively fast in one of the soils, with a high degree of enantioselectivity **(Figure 2).** Assuming first order kinetics, the disappearance rate constant (k) for the *R*-(+) enantiomer was calculated to be 0.063day $^{-1}$; the k value for the *S*-(-) enantiomer is 0.011day $^{-1}$, corresponding to half-lives of 11and 63 days. This same direction of enantioselectivity was also observed with the other 3 soils spiked with metalaxyl. Other investigators reported similar results[5], but later found reverse enantioselectivity in several soils[6]. Metalaxyl is one of the few pesticides marketed in a single-enantiomer formulation as well as a racemic formulation; the *R*-(+) is the active enantiomer. So, at least in these 4 soils, enantioselective degradation leads to longer persistence of the unnecessary enantiomer. Figure 2 also shows the appearance of the transformation product of the (+)-enantiomer, the corresponding acid. The enantioselective microbial degradation of fipronil will be reported in this meeting by Jones, et al.

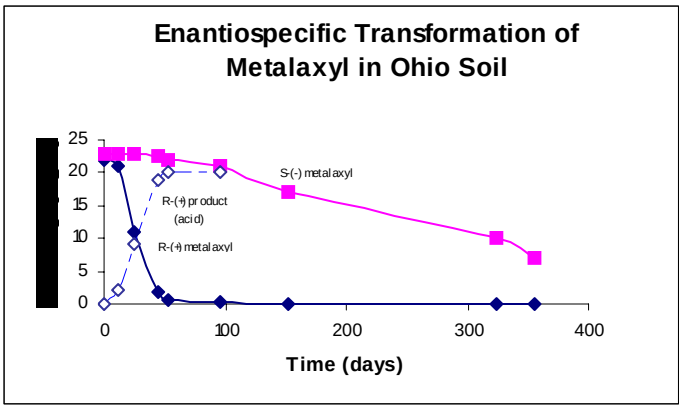


Figure 2. Microbial transformation of metalaxyl

In order to study the effects of enantiomers, it is necessary to separate them in enantiopure form. Enantiomers of 12 chiral pesticides have been separated in approximately 250mg quantities by preparative HPLC, and their optical rotations measured. Adequate quantities of each enantiomer of each pesticide have been submitted for endocrine disrupter (ED) screening tests. One remarkable result is that the (-)-enantiomer of *o,p*'-DDT was shown by these tests to be a much stronger endocrine disrupter than the (+)-enantiomer[7] . Preliminary results of ED screening of the separated enantiomers of fipronil show no estrogen activity, but enantioselective antiandrogen activity. Most of the androgen antagonist activity appears to be due to blocking of the androgen receptor. The (-)-enantiomer exhibited 95% blockage of DHT, whereas the (+)-enantiomer showed minimum (30-40%) antagonism.

In summary, non-racemic mixtures of the enantiomers of chiral pesticides are commonly found in environmental matrices such as soil and water because of enantioselective microbial transformation. In addition, there is other evidence that enantioselective occurrences may also be expected in dust and soil in the home environment and in food products. Because the persistence and toxicity of each enantiomer is likely to be different, each should be considered a separate pollutant. Coupling of environmental occurrence (exposure) and toxicity data for the enantiomers of pesticides and other pollutants will increase

the accuracy of risk assessment. In fact, determination that the non-target specific enantiomer of a chiral pesticide has an adverse effect on non-target species would justify the manufacture and application of only the target specific enantiomer – a pollution prevention measure.

Acknowledgments
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