ENANTIOSELECTIVE TRANSFORMATION OF CHIRAL PCBs AND THE INSECTICIDE FIPRONIL IN NATURAL ANOXIC SEDIMENTS

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Introduction

A significant number of industrial and agricultural chemicals in current use or of historical importance are chiral compounds; i.e., the two species (enantiomers) of the molecule are non-superimposible mirror images of each other. In most instances, the manufacture and application of chiral compounds occurs non-enantioselectively; thus, equal mixtures (racemates) of the enantiomers are likely introduced as contaminants of aquatic and terrestrial environments. Although the chemical and physical properties of the enantiomers of chiral compounds are identical, enantiomers generally have different biological and toxicological properties. With regard to fate processes in a biological system, enantioselectivity may occur in which one enantiomer is degraded faster than the other. To date, only limited data are available concerning the environmental fate of chiral compounds and their human and ecological effects.

Some PCBs and pesticides are considered important classes of chiral organic pollutants. Of the 209 possible PCB congeners, nineteen are chiral. Recent evidence from analysis of historically contaminated sediments indicates the occurrence of enantioselective transformation of PCBs *in situ*[1]. This result provides additional evidence that biological processes contribute to the transformation of pollutants in historically contaminated environments. Additional studies have presented evidence for the enantioselective transformation of other chiral environmental pollutants, including the pesticides *o,p'*-DDT, metalaxyl, and dichloroprop, to name a few[2,3]. For many chiral chemicals, however, few or no environmental biotransformation data are available.

The chiral pesticide fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1*H*-pyrazole-3-carbonitrile) is classified as a phenyl pyrazole insecticide. Fipronil provides long-term protection against specific soil and foliar pests that graze on a variety of crops, including corn, cotton, rice, and turf grass, and it is used to control fleas and ticks on pets as well as for cockroach and ant control[4,5]. Fipronil was registered as a pesticide in the US[6] in 1996 and the use of fipronil has increased worldwide during the past few years with an expected production of approximately 800 tons for the year 2000. The primary insecticidal action of fipronil involves blocking gamma-aminobutyric acid (GABA) regulated chloride channels, resulting in loss of control of neuron signaling and disruption of normal central nervous system function[4,7]. Importantly, the toxicity of fipronil is considered to be highly selective for arthropods, with inhibitory effects reported to be greater than 2000 times those of mammalian targets. Recently, there has been increased concern about the environmental and human health effects associated with fipronil use.

In this study, we examined the microbial transformation of two chiral PCB congeners and the insecticide fipronil in natural sediment microcosms. The specific goals of the study were to identify biotransformation pathways and determine if enantioselective microbial transformation of these chiral compounds occurred under natural environmental conditions.

Materials and Methods

Sediments. To assess microbial transformation of fipronil, anoxic sediments were collected from two distinct locations, representing a sulfate-reducing sediment (tidal wetland located at Aberdeen Proving Ground, Maryland) and a methanogenic sediment (freshwater pond located near Athens, GA). To assess PCB transformation, anoxic sediments were collected from the Twelve Mile Creek arm of Lake Hartwell, SC, an area of historical PCB contamination. Immediately following collection, all sediments were placed in glass jars, sealed, and stored at 4^{o}C prior to use.

Batch Kinetic Studies. Sediment slurries were prepared inside an anaerobic glove box containing an atmosphere of 99% N_2 and 1% H_2. The collected sediment was sieved (1-mm) and thoroughly mixed with either anoxic (N_2-sparged) site water or half-strength artificial seawater (Aberdeen wetland sediment) to achieve a sediment solids concentration of approximately 100 g/L slurry. Fipronil or PCB congeners were aseptically added at initial concentrations of approximately 4 or 10 ppm, respectively. Microcosms were incubated anaerobically at 25^{o}C on a rotary shaker and samples were collected over time to assess biotransformation. Sterile control microcosms were prepared by autoclaving sediment slurries at 120^{o}C for 30 minutes over three consecutive days.

Analytical Techniques. Gas chromatography (GC) techniques were used to quantify PCBs, fipronil, and transformation products. PCBs were extracted using acetone and fipronil was extracted with acetonitrile (followed by solvent exchange with hexane). PCBs, fipronil, and transformation products were analyzed by direct injection (1 uL) of the solvent extract using a 5890 Hewlett-Packard gas chromatograph equipped with an electron capture detector (ECD). Separation was achieved with a 30 m x 0.32 mm i.d., 0.25 mm film thickness, DB-5 capillary column (J&W Scientific, Folsom, CA).

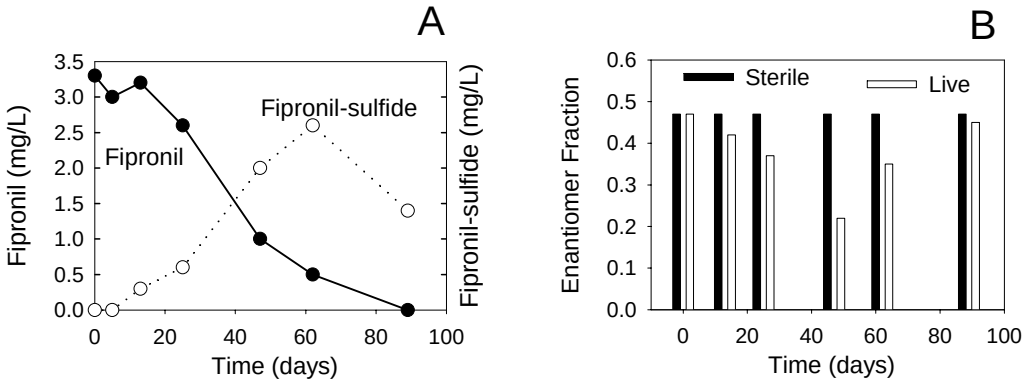
Enantiomer-specific chiral analysis was achieved using the same type GC and detector described above. Enantiomers of fipronil were separated using a 30-m x 0.25-mm x 0.2-um film thickness BGB-172 chiral column (BGB Analytic AG, Laufrainweg, Germany). The column temperature was as follows:150^{o}C, hold 2 min, 1^{o}C min^{-1} to 220^{o}C, hold for 20 min. PCB enantiomers were separated using a Chirasil-Dex column (25-m x 0.25-mm x 0.25-um, Chrompack). The column temperature was as follows: 100°C to 150°C at 10°C/min, 150°C to 200°C at 0.5°C/min. Helium (2 mL/min) was used as the carrier gas. The temperatures of injector and detector were maintained at 250°C and 325°C, respectively.

Results and Discussion

Separation of Fipronil Enantiomers. Fipronil enantiomers were separated by chiral GC using a commercially available modified 20% *t*-butyldimethylsilylated-*B*-cyclodextrin column. The enantiomeric fraction (EF = (+) enantiomer / [(+) enantiomer plus (-) enantiomer]) of the fipronil racemate used in all experimental studies was 0.47 (+/- 0.04).

Fipronil Transformation in Anoxic Sediments. Fipronil transformation in the microbially active, sulfidogenic Aberdeen wetland sediment slurries was associated with the production of fipronil sulfide, which subsequently decreased in concentration (Fig. 1A). Loss of fipronil sulfide coincided with formation of a previously unknown metabolite, fipronil sulfide amide. The half-life of fipronil in the sulfidogenic wetland sediment slurry was determined to be approximately 22 days. In autoclaved slurries, fipronil transformation products (i.e., fipronil sulfide) were observed only in trace amounts. In the microbially active (live) microcosms, transformation of fipronil was enantioselective. During incubation from day 13 to 62, the EF shifted to values significantly lower (EF of 0.18 to 0.40 ∀ 0.03) than observed for the sterile microcosms and parent chemical, confirming the faster degradation of the (+) enantiomer (Fig. 1B). These results are the first evidence of stereoselective degradation of fipronil. No evidence of enantioselective transformation of fipronil was detected in the microbially inhibited (sterilized) Aberdeen sediment microcosms (i.e., constant, racemic EF values of approximately 0.46 were observed for fipronil enantiomers throughout the 89-day incubation period).

Figure 1: Enantioselective Transformation of Fipronil in Anaerobic Aberdeen Sediment Slurries

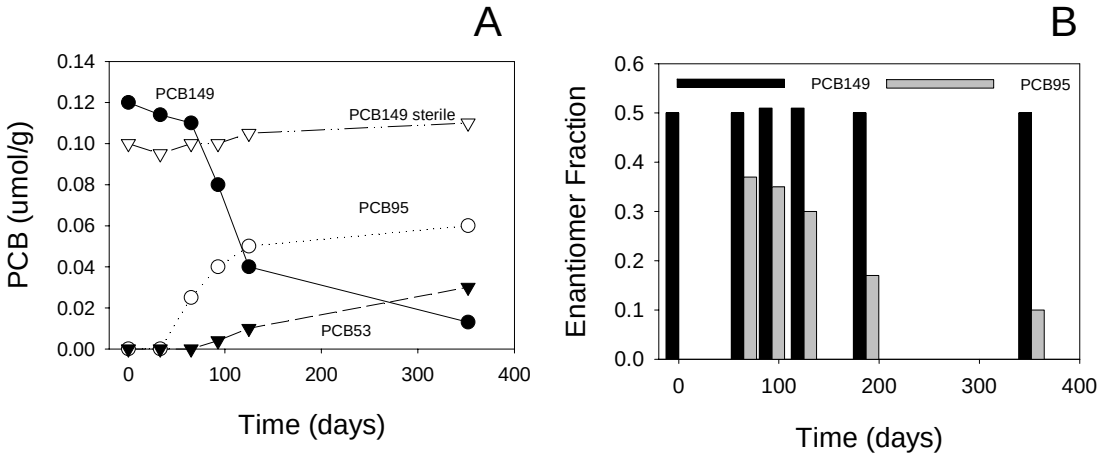


Fipronil transformation in the methanogenic sediments from the Athens site followed a trend similar to that observed for the Aberdeen sediment. In addition to differences in redox status, the Athens pond sediment was lower in pH (6.5 compared to 7.9) and almost double the %TOC than the Aberdeen sediment. The half-life of fipronil transformation in the Athens sediment slurry was approximately 25 days. Only minimal fipronil loss was observed in microbially inhibited (autoclaved) microcosms. As observed for Aberdeen sediments, transformation of fipronil in the pond slurries was also enantioselective, but the order of enantiomer transformation was reversed. During the rapid phase of fipronil transformation (days 46-88), the EF values increased from an initial value of 0.47 (+/- 0.05) to values ranging from 0.66 to 0.82. These results indicate that the rate of transformation of the (-) enantiomer was greater than the (+) enantiomer.

PCB Congener Biotransformation. Anoxic sediment slurries from Lake Hartwell SC reductively dechlorinated chiral PCB congeners 132 (234-236) and 149 (245-236) preferentially at the *meta*- and *para*-chlorine positions, respectively, after a 2-month lag period. Dehalogenation of PCB149 (Fig. 2A) resulted in the formation of PCB95 (236-25); the transformation was not enantioselective. EF values for PCB149 were constant (0.5) throughout the 350 day incubation

period. However, *meta*-dechlorination of the chiral product PCB95 to PCB 53 (25-26) was enantioselective (Fig. 2B). EF values for PCB95 decreased from 0.37 at day 60 (when initially detected) to a value of 0.10 at day 350. Therefore, enantioselective transformation of some chiral PCBs occurs and chiral PCBs may be useful chemical markers of dechlorination *in situ*.

Figure 2: Enantioselective Transformation of PCB95 in Anaerobic Lake Hartwell Sediment Slurries.



Conclusions

Enantioselective transformation of the chiral insecticide fipronil was observed in two distinct anaerobic sediment slurries; the enantiomer preference was opposite in the different sediments. Enantioselective dechlorination of chiral PCB congener 149 was not detected during its transformation, but dechlorination of the PCB149 dehalogenation product (PCB95) was enantioselective.

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