BIOACCUMULATON AND ELIMINATION OF CHIRAL ORGANOCHLORINE COMPOUNDS IN LOW TROPHIC LEVEL ORGANISMS

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Introduction

Organochlorine compounds (OCs) such as polychlorinated biphenyls (PCBs), DDT, chlordanes, and hexachlorocyclohexane (HCH) were extensively used in the past. These chemicals remain ubiquitous environmental contaminants to this date, despite bans on production as long as 30 years ago. They remain a continued potential health threat today due to their continued persistence and recalcitrance. Thus, a key issue in assessing future trends of these pollutants, needed for rational risk assessment, is the need for accurate and reliable indicators of environmental degradation. Despite the wealth of knowledge concerning these chemicals and the major concentration decreases observed in recent years in most environmental media (e.g., air, water, sediment, biota, etc.), there are still difficulties in estimating the nature and extent of any future declines. These difficulties are due in part to a lack of a tracer for biological activity also unaffected by abiotic processes.

The environmental behavior of chiral compounds is a rapidly growing area of research providing enhanced insights into biological processes affecting these chemicals. In general, physical and chemical environmental processes are identical for stereoisomers of a chiral chemical. However, the enantiomers can have very different biological and toxicological effects due to differential interactions with other chiral molecules (e.g., enzymes in living organisms). As such, chiral analysis is a powerful tracer of biological processes in the environment, and is a promising means by which to understand biologically-mediated behavior of chiral pollutants and to estimate rates of environmental degradation of these chemicals.

The goal of this research is to assess the potential of low trophic level aquatic organisms to bioaccumulate and eliminate chiral OC pollutants stereoselectively. Previous research on chiral PCBs, for example (asymmetrically substituted congeners with hindered rotation about the C-C bond between the two phenyl rings due to the presence of 3-4 *ortho* chlorines) found significantly nonracemic compositions of many analytes in a wide assortment of environmental matrices, such as sediment[2] and biota[3]. Such residues could be caused by enantioselective *in vivo* biotransformation and/or uptake of nonracemic residues from prey. Laboratory-based bioaccumulation studies showed that rainbow trout can stereoselectively eliminate some chiral PCBs and OC pesticides enantioselectively[4], suggesting that piscivorous fish have greater metabolic activity towards xenobiotic chemicals than previously thought. However, it is not clear if this capability is also present in lower trophic level organisms (e.g., planktivorous forage fish),

despite suggestions of this potential based on chiral analysis of aquatic food webs[5]. In this study, we determine if fathead minnows (*Pimephales promelas*), a common low-trophic level species, can stereoselectively bioprocess chiral persistent pollutants in a controlled laboratory setting.

Methods and Materials

Fathead minnows were obtained from a baitfish farm in southern Ontario, and reared in 200 L fiberglass aquaria with flow-through, ultraviolet and carbon dechlorinated water (~9°C) with a 12 h light: 12 h dark photoperiod. Contaminated food was prepared by mixing commercial trout chow with racemic quantities of α-HCH, *cis*- and *trans*-chlordane, *o,p'*-DDT, and PCBs 45, 84, 91, 95, 132, 136, 149, 171, and 183 in hexane at various concentrations (Table 1), and slowly evaporating to dryness. Control food was prepared identically but without the addition of chiral congeners. Treatments consisting of fish fed control food, low concentration food, and high concentration food (ca. 200 adult individuals each) were fed this food for 40 days during the uptake phase of the exposure experiment, followed by a 121-day depuration phase during which all fish were fed control food. Three fish per treatment were sampled on days 0, 5, 10, 15, 20, 31, and 40 during the uptake period, and on days 45, 60, 66, 74, 102, 132, and 161 during the depuration period.

Sampled fish from each treatment at each time point were weighed and measured, and combined for chemical extraction. Fish carcasses (minus gastrointestinal tract) were similar to those of previous experiments[3, 6]. Briefly, tissues were mixed with Na_2SO_4 and solvent-extracted with a Polytron homogenizer. Extracts were centrifuged, concentrated, and passed through gel permeation chromatography (1:1 hexane:dichloromethane) to remove lipids. Extracts were solvent-exchanged to hexane, and interferences removed by silica column chromatography, followed by solvent exchange to isooctane and volume reduction to 1 mL. PCB 166 was added as a volume corrector, while PCBs 30 and 204 were used as surrogate recovery standards. Fish food was extracted in a similar manner.

Extracts were analyzed non-enantioselectively for PCBs and OC concentrations by gas chromatography with electron capture detection (GC/ECD) using chromatographic conditions described previously[7]. Enantioselective analysis was done by enantioselective GC/MS using a suite of chiral columns, as established in existing protocols[8]. Enantiomer compositions were expressed as enantiomer fractions (EFs)[9], defined as A/[A+B] where A and B are the (+) and (-) enantiomers, or the first-eluting/second-eluting enantiomers on Chirasil-Dex when elution order is unknown (e.g., PCBs 91 and 95).

Results and Discussion

Fish rapidly accumulated most analytes added to food (Figure 1). Concentrations reached a maximum of 125 ng/g for PCB 136 (sum of both enantiomers) in the high concentration food treatment. These values are substantially higher than the background concentrations of analytes in the fish, as illustrated by levels in fish from the control treatment (Figure 1). These levels are likely due to background accumulation of the target analytes by the fish before the experiment, as they were raised in an outdoor pond in rural Ontario that likely had trace inputs of PCBs and OC pesticides from regional and long-range atmospheric transport. Increases in analyte concentrations in control fish over the course of the experiment were due to the unavoidable presence of analytes in trace amounts in the food. However, these amounts are extremely low compared to the levels in treated food (Table 1) and accumulated by fish (Figure 1). In a similar vein, control fish had nonracemic amounts of PCB 136 (Figure 1) and PCBs 84 and 149 (data not shown), likely also due to contamination in the wild before the start of the experiment.

Figure 1 also clearly shows that fathead minnows maintained nonracemic EF throughout the experiment, despite the fact that the amount of analytes accumulated by fish in the high concentration treatment vastly exceeded their background concentrations. This maintenance was observed as well for PCBs 84 and 149, in which the (+) enantiomers were both preferentially depleted. PCBs 91, 95, and 174 were racemic in all treatments at all times. This result suggests that fathead minnows stereoselectively eliminated PCBs 84, 136, and 149 during the course of the experiment. This result is consistent with the observation of enantioselective elimination of PCB 136 by rainbow trout[4], suggesting that biochemical pathways controlling the processes responsible may be similar between the two species. The elimination of PCBs 84, 136, and 149 is consistent with structure-activity relations, as *ortho*-chlorinated congeners with *meta-para* vicinal hydrogen atoms are known to be substrates of CYP2B and CYP3A isozymes[10]. However, it is not clear why PCBs 91, 95, and 174 are not similarly attacked given similar structures present in those congeners. Maintenance of nonracemic EFs by fathead minnows may be due to a balance between competing sources (e.g., uptake vs. elimination) as observed for chiral toxaphene congeners in mummichogs[11]. Our study shows that the capability to eliminate chiral persistent pollutants is present within low-trophic level organisms, and that this activity, likely due to biotransformation, may affect the fate of such chemicals in the environment.

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Table 1. Concentrations (C_{food} ng/g wet wt) and enantiomer fractions (EFs) of chiral PCBs (mean ± SD) in treated food. Control food was racemic and < 3 ng/g for these congeners.

	Low treatment		High treatment	
PCB	**C food**	**EF**	**C food**	**EF**
84	60 ± 2	0.511±0.006	474± 120	0.507±0.010
91	74 ± 2	0.503±0.001	540 ± 82	0.502±0.003
95	82 ± 2	0.501±0.036	704± 290	0.501±0.001
136	109±6	0.504±0.002	781± 106	0.500±0.003
149	91 ± 2	0.499±0.004	686±142	0.503±0.002
174	73 ± 6	0.497±0.001	559 ± 62	0.505±0.005

Figure 1: Concentrations (ng/g wet weight) of (±)-PCB 136 and enantiomer fractions (EFs) in fathead minnows fed control food (top) and high concentration food (bottom).