Bioaccumulation of Organochlorine Contaminants in Freshwater Invertebrates

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Introduction

Organochlorine (OC) compounds were extensively used in the past due to their favourable physicochemical properties. From electrical insulators (polychlorinated biphenyls (PCBs)) to insecticides (DDT, hexachlorocyclohexane, chlordanes, nonachlors), this class of compounds was used for a wide range of applications due to their chemical stability. Although these chemicals are present at low concentrations within the environment, their high affinity to lipids allows them to accumulate and magnify in organisms[1]. Although production of these chemicals has been banned, their inherent stability within the environment still raises concern.

OCs have been extensively studied in the literature, but the fact that some of these chemicals are chiral can help provide enhanced insight into biological processes affecting these chemicals within the environment. Chiral compounds exist as pairs of non-superimposable mirror images called enantiomers. Enantiomers have the same physical chemical properties, which determine their behaviour within the environment. However, their biological and toxicological properties may differ. Selective biotransformation/elimination of one enantiomer over the other will cause enantiomer enrichment within organisms. Thus, enantioselective analysis can act as a tracer of biological processes, particularly biotransformation, within organisms.

Enantiomer enrichment has been observed for chiral OCs within higher trophic level organisms (birds, seals, polar bears, whales) [2-5], little attention has been focused on lower trophic level organisms. Previous studies investigating lower trophic level organisms (zooplankton, invertebrates) in arctic marine food webs have found the proportion of enantiomers of chiral OCs to be similar to those observed in the water column, suggesting no, or limited biotransformation[2, 5]. However, enantiomer enrichment has been observed for chiral PCBs within the macrozooplankton *Mysis relicta* in Lake Superior [6]. This freshwater opossum shrimp is an opportunistic omnivore, which feeds on sediments during the day, and migrates vertically in the water column to consume phytoplankton and zooplankton at night [7]. *Mysis relicta* is an important part component of many aquatic food webs, comprising a major part of many fish species diets. Playing the role of both predator and prey, Mysids act as an important vector in the transport and recycling of contaminants through the aquatic food web [8]. It is unclear whether the enantiomer enrichment observed in *Mysis relicta* is due to enantiomer specific biotransformation/elimination or from uptake of enantiomer enriched signatures present in the sediment [9]. Thus, the goal of our research is to determine if *Mysis relicta*, under controlled environmental conditions, can enantioselectively biotransform chiral OC pollutants. This work will provide insight into biological processes that affect the fate of chiral OC pollutants at both lower and upper levels of the aquatic food web.

Methods and Materials

Mysids were collected from Kootenay Lake, British Columbia, Canada. Samples were obtained from vertical drops and horizontal tows using a zooplankton net and stored in coolers filled with lake water chilled to 5ºC. Mysids were transported back to the University of Alberta and transferred into holding tanks within an environmental chamber set at 5ºC.

Mysids were then transferred to individual control and experimental aquaria containing clean and spiked sediment respectively. Sediment in experimental aquaria was spiked with several chiral OCs. Target analytes included: PCBs (chiral congeners 45, 84, 91, 95, 136, 149, 174, 183, and achiral 153), *α*-HCH, *β*-HCH, *γ*-HCH, *trans*-chlordane, *o,p'* DDT, *trans*- and *cis*-nonachlor. After 10 days exposure to the spiked sediment, mysids were transferred to aquaria containing clean sediment to allow for a depuration phase. Control and experimental mysids collected on days 7, 10, 23, 35, 42, and 49, were placed into 4L beakers filled with clean water at 5º C to purge their guts for 24 hours. Mysids were then collected and placed in glass jars and frozen until needed for extraction of chiral OCs.

Mysids collected from each time point were weighed our and were prepared for chiral OC extraction. Briefly, samples were mixed with Na_2SO_4 using a mortar and pestle and OCs were extracted using dichloromethane via soxhlet extraction. Extracts were concentrated and passed through gel-permeation chromatography (GPC) (1:1 *n*-hexane: dichloromethane) to remove lipids. Extracts were solvent exchanged into hexane, concentrated and passed through alumina/silica chromatography as a final cleanup step. PCB 166 was added as a volume corrector while PCBs 30 and 204 served as recovery standards. Krill flakes and sediment were extracted in a similar manner. Sediment extracts required no GPC and activated copper was added to remove sulphur.

Extracts were analyzed by gas chromatography mass spectrometry using a suite of chiral columns under conditions previously reported [10]. Enantiomer composition was expressed as enantiomer fractions (EFs), which are defined as A/A+B, where A and B are the concentrations of the (+) and (-) or the first and second eluting enantiomers if the elution order is unknown [11].

Results and Discussion

A preliminary experiment was conducted to: Determine exposure time needed for mysids to achieve sufficient accumulation of chiral PCBs from the sediment, and to determine the biomass needed to produce a detectable signal. Mysids were exposed to spiked sediment for 7 days. Concentrations of target analytes for preliminary experiments in spiked sediments, krill flakes and mysids after 7-day exposure are given in Table 1. Using a conservative measure of EF precision of ± 0.032 (95% confidence interval) [6], enantiomer enrichment was observed in mysids for PCB 95 and to a lesser extent PCB 91 (Table 2) after a 7-day exposure to spiked sediment.

Results from preliminary studies suggest that *Mysis relicta* can stereoselectively uptake/eliminate PCB 95 and PCB 91 enantioselectively. These results may provide further insight into the biological processes that affect the fate of chiral OCs within the lower and upper trophic levels of food webs.

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Table 1.

Sample	Concentrations (ng/g)			
	PCB 91	PCB 95	PCB 136	PCB 149
Blanks (n = 3)	ND	ND	ND	ND
Control Sediment (n = 3)	ND	ND	ND	ND
Spiked Sediment (n = 3)	630 ± 32	846 ± 50	9170 ± 625	920 ± 60*
Kootenay Mysids	ND	ND	ND	ND
Krill Flakes (n = 2)	ND	ND	ND	ND
Spiked Mysids**	22.2	15.1	96	32.8

*n = 2

** After 7 day accumulation period.

Note: Concentrations expressed as wet weight.

Table 2.

Sample	Enantiomer Fractions (EFs)			
	PCB 91	PCB 95	PCB 136	PCB 149
Standard (n = 3)	0.501 ± 0.017	0.499 ± 0.008	0.512 ± 0.036	$0.503 \pm 6 \times 10^{-4}$
Spiked Sediment (n = 3)	0.502 ± 0.003	0.498 ± 0.001	$0.487 \pm 4 \times 10^{-4}$	$0.495 \pm 5 \times 10^{-4}$*
Spiked Mysids (~45 mysids)**	**0.466**	**0.318**	0.505	0.507

Boldface: significantly different from standard or spiked sediment.

** After 7 day accumulation period.