UPTAKE, ELIMINATION AND FISH BIOCONCENTRATION FACTORS FOR THE PERSISTENT HEXA- AND NONACHLOROBORNANES – M1 AND P-50

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Introduction

Knowledge of the uptake and elimination kinetics of chlorobornane (toxaphene) residue congeners by aquatic organisms is key to understanding their environmental fate. Most work to date has focused on a select few octa- and nonachlorinated congeners that are globally distributed and persistent.[1,2] However, recent evidence suggests that lower chlorinated toxaphene residue congeners, such as 2-exo, 5-endo, 6-exo, 8c, 9b, 10a-hexachlorobornane (aka Hx-Sed; B6-923 or M1)[3], are also detected in polar regions and thus may survive long range transport.[4] In this study, we followed the uptake and elimination of M1 by a model fish and compared rates with that for 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane (aka B9-1679 or P-50), a known persistent congener. From these experiments, we estimated the steady state bioconcentration factor (BCF) for M1 and compared our value with reported modeled and field-based values for toxaphene congeners with a similar degree of chlorination.

Materials and Methods

Adult female mummichogs (Fundulus sp.), a small minnow-like fish, were collected from an uncontaminated tidal creek near the Skidaway Institute of Oceanography in Savannah, GA (USA), and acclimated in 500 ml filtered seawater at 24oC for > 4 d (one fish per 800 ml glass jar). For days 0-3, M1 and P-50 in acetone were spiked to achieve 0.2 and 0.5 g/L, respectively, in microcosms with and without fish (controls). At day 4, toxaphene-exposed fish were transferred into clean water and fed a small amount of tropical fish food every third day. Fish, water from jars containing fish and water from no fish controls were collected after 1, 2, 3, 5, 7 and 10 d (M1) and after 1, 2, 4, 7, 14 and 31 d (P-50). Individual fish and water were analyzed in duplicate for each time point.

Whole fish (~5 g) were homogenized with Na2SO4, packed in a glass column, eluted with 100ml CH2Cl2 and fractionated using Florisil.5 Water samples (250ml) were extracted by shaking with 3

sequential 50 ml aliquots of hexane. Extracts were reduced and exchanged to hexane using a TurboVap II. Extracts were analyzed using a Varian 3400 GC with electron capture detection (GC-ECD), calibrated with 6 serial dilutions (2.2-111 ppb) of M1 and P-50 (Dr. Ehrenstorfer, Augsburg, Germany). The regression equation for calibration was highly linear for both analytes ($R2 > 0.99$). The recovery of DBOFB spiked into each sample prior to extraction as a surrogate was 78+36% for fish and 62+20% for water.

Results and Discussion

Preliminary experiments indicated that water concentrations of M1 or P-50 decreased measurably during the 4 day uptake period when microcosms were spiked with a single dose of toxicant on day 0. Modification of the protocol by spiking microcosms on a daily basis for the duration of the uptake period resulted in constant or slightly increasing water concentrations (data not shown), indicating that uptake was not limited by toxicant aqueous concentration.

M1 was taken up rapidly via the aqueous phase and appeared to reach steady state after day 2 (Fig. 1). Upon transfer to clean water, fish exposed to M1 eliminated the compound rapidly, with 42% on average remaining after 7 d, disregarding an anomously high concentration at day 7 (Fig. 1). This elimination rate was consistent with an earlier study ($t1/2 = 7$ d) utilizing naturally contaminated Fundulus from the Terry/Dupree Creek Superfund (toxaphene) site.6

P-50 was also taken up rapidly, reaching a concentration of ~300 ng/g wet wt in Fundulus by the end of microcosm spiking at day 4 (Fig. 2). Unlike M1, however, P50 did not reach an apparent steady-state after 3-4 days of exposure via the aqueous phase. For the elimination phase, P-50 tissue concentrations decreased at a much slower rate than M1, with 80% of the maximum concentration at day 4 remaining at the end of the experiment (day 31). This longer t1/2 for P-50 was also reported in experimentally2 and field-exposed7 fish.

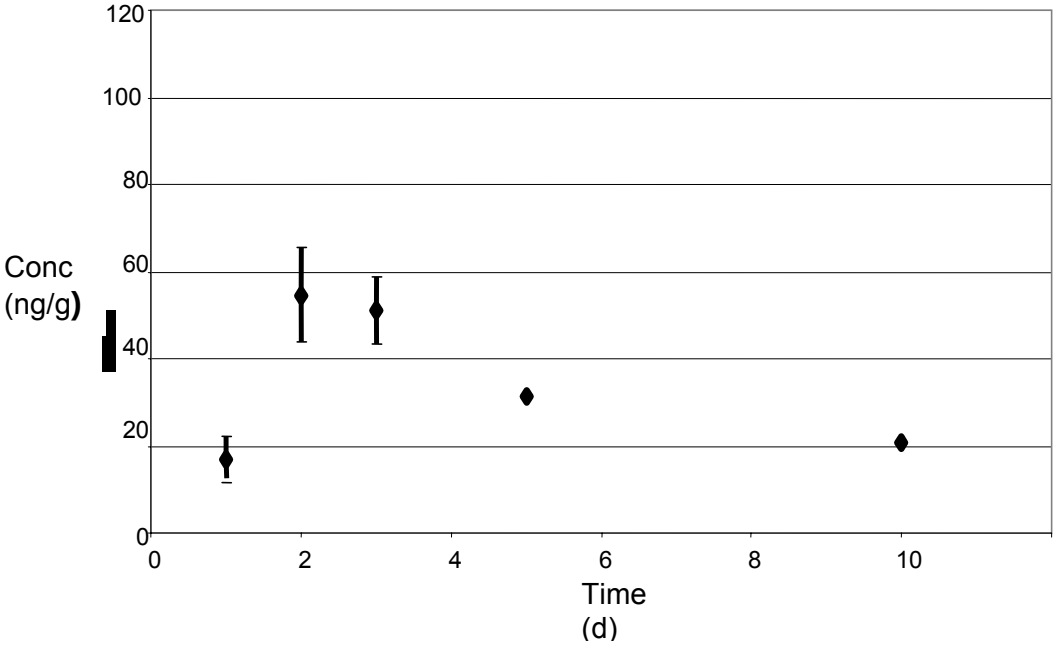

Fig. 1. Concentration of 2-exo,5-endo,6-exo,8,9,10-hexachlorobornane (M1) in fish during the uptake phase (days 0-3) and the elimination phase (days 4-10). Error bars represent 1□for duplicate microcosm determinations.

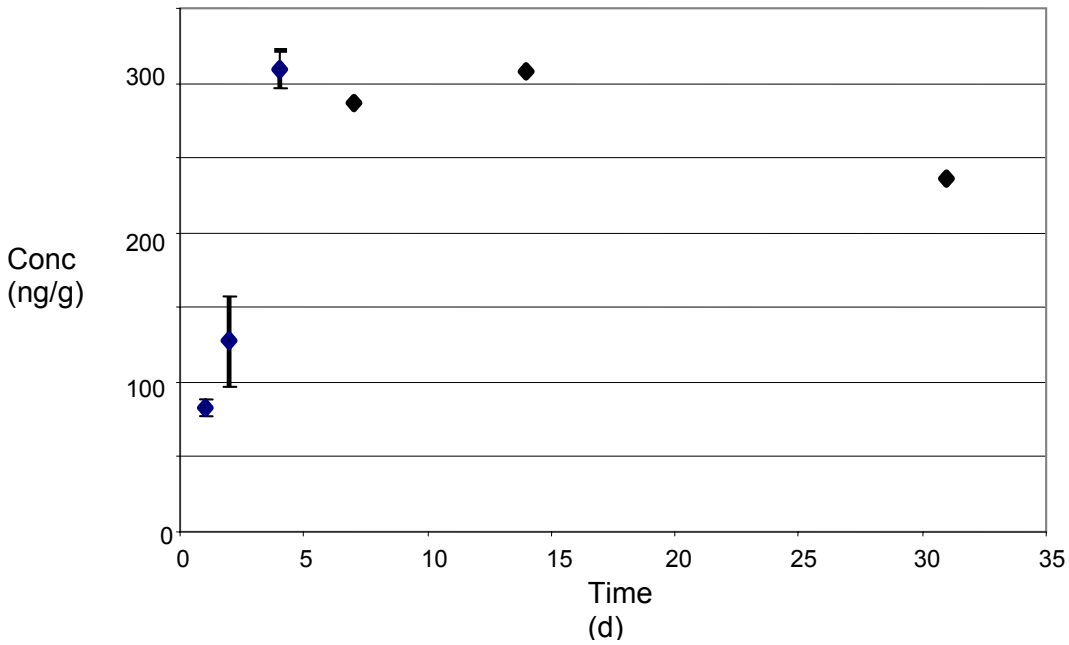

Fig. 2. Concentration of 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane (P-50) in fish during the uptake (days 0-4) and elimination phases (days 5-31). Error bars represent 1□ for duplicate microcosm determinations.

Because tissue concentrations did not increase after day 2 of exposure (Fig. 1), the bioconcentration factor (BCF), defined as the concentration in fish (ng/g wet) divided by the concentration in the aqueous phase (ng/ml), was estimated for M1 using day 3 data. The resulting BCF for M1 (3300) was roughly half (but of the same order of magnitude) of that reported for a hexachlorinated camphene of similar hydrophobicity (Table 1). In addition, our experimentally derived BCF for M1 follows the well-documented trend of increasing BCF with increasing hydrophobicity as measured by the octanol-water partition coefficient (Kow)10. These results are also consistent with the rapid elimination of Hx-Sed (i.e. M1) by the same fish species (Fundulus) in experiments with naturally contaminated specimens6.

Table 1. Bioconcentration factors (BCFs) for M1 and selected toxaphene congeners.

Compound	log Kow	BCF	BCF Ref
2-exo,5-endo,6-exo,8,9,10-hexachlorobornane (aka Hx-Sed, B6-923 and M1)	**~5.1[8]**	**3300**	**this study**
1,2,3,4,7,7-hexachloro-2,5-norbornadiene	**5.15-5.28[9]**	**6400**	**(9)**
1,2,3,4,5,7,7-heptachloro-2-norbornene	**5.28-5.55[9]**	**11200**	**(9)**
2-exo,3-endo,5-exo,9,9,10,10-heptachlorobornane (aka TOX7, B7-1457)	**5.80-5.93[9]**	**31500-43000**	**(9)**
2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlorobornane (aka P-26, B8-1413, T2)	**5.52[8]**	**133000-5660000**	**(9)**
2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane (aka P-50, B9-1679, Toxicant Ac and T12)	**5.84[8]**	**100000-680000**	**(9)**

8 Fisk et al. (1999)
9 Geyer et al. (1999)

Acknowledgements

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