DETERMINING UPTAKE AND ELIMINATION RATES FOR THE TOXAPHENE COMPONENT B6-923 IN FISH (*FUNDULUS* SP.) USING ENANTIOSELECTIVE ANALYSIS

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Introduction

The multicomponent insecticide toxaphene contains several hundred penta- to dodecachlorobornanes[1]. However, only a few (mainly octa- and nonachlorobornanes) are not metabolized by higher organisms such as humans and marine mammals[2]. In anaerobic media, reductive dechlorination of chlorobornanes with geminal chlorine atoms yields several penta- to heptachlorobornanes[3], with B6-923 and B7-1001 being the principal metabolites[4]. As these metabolites are also present in the technical product but apparently do not biomagnify, one can assume that they are transformed/eliminated by higher organisms. In an elimination study with fish collected at a site highly contaminated with toxaphene[5], we test this hypothesis using enantioselective GC analysis.

Experimental

Samples

Mummichogs (*Fundulus* sp.) were collected in baited minnow traps from the cooling water discharge canal of a former toxaphene manufacturing facility near Brunswick, Georgia, USA. These fish were transported to the lab in coolers filled with aerated site water. Control fish with little or no toxaphene residues were collected from a reference site. Contaminated (2 tanks) and control fish (1 tank) were kept in flow-through fiberglass tanks that received filtered seawater from a brackish estuary with undetectable levels of toxaphene. Water temperature, pH and salinity were monitored daily. Fish were maintained for 60 days on a diet of commercial fish food (TetraMin). Two to three individual fish were collected from each tank 0, 3, 7, 14, 28 and 60 days into the elimination phase. Individual weight and length measurements were taken before they were composited and frozen at –20°C[6].

Sample clean-up

Samples were Soxhlet-extracted and cleaned up using Florisil column chromatography in accordance with Maruya and Lee[7]. Due to coelution of B6-923 with an unknown heptachloro CTT, the samples were fractionated on 8 g silica[8]. B6-923 was targeted in fraction 148-173 mL.

GC/MS analysis

GC/MS in the electron-capture negative ion mode was performed with a Hewlett-Packard HP5890 series II/5989 MS engine. A chiral stationary phase consisting of *tert.*-butyldimethylsilylated β-cyclodextrin (BGB Analytik, Adliswil, Switzerland) was installed in the GC oven. In the selected ion monitoring mode 2 m/z values were recorded for each degree

of chlorination (penta- to nonachlorobornanes) as described in detail elsewhere[8].

Results and Discussion

After 60 days, the toxaphene pattern in test fish was clearly modified relative to that at time 0, indicating that significant elimination had taken place within two months. The major components at the beginning, i.e. B6-923 and B7-1001, were below the detection limit by the end of the elimination phase (day 60). Due to expected variations in the toxaphene level of individual fish, our system was calibrated with B9-1679 (P-50), a component with a half-life in fish of > 1 year[9]. Accordingly, we assume that the level of B9-1679 (P-50) in mummichogs in our study would not have changed throughout the 60 day experiment. In support of this assumption, no shift in the ER of B9-1679 (P-50) was observed, which is also in agreement with findings of other studies on fish and marine mammals[10].
In contrast, a half-life of 7 days based on first order elimination kinetics was determined for B6-923. Furthermore, this elimination process occurred enantioselectively (**Table 1** and **Figure 1**).

Table 1: B6-923 level and ER during the elimination study. B6-923 levels of individual fish are normalized to the level of B9-1679 (P-50)

Day	0	3	7	14	28	60
Level	827	361	255	117	109	0
ER	1.3	1.6	2.65	6	inf.	-
Enantiomer-1	467	222	185	100	109	-
Enantiomer-2	360	139	70	17	-	-

Enantiomer-2 of B6-923 was excreted twice as fast as enantiomer-1, suggesting enantiomer specific half-lives of approx. 4 and 8 days for enantiomer-1 and enantiomer-2, respectively. Our finding that the B6-923 enantiomers were eliminated at a different rate, coupled with the observed constant ER (1.3) in fish at the start of our experiment indicates an equilibrium condition between uptake and elimination. Processes by which mummichogs take up toxaphene residues are ingestion of food (grass shrimp, plankton/detritus) and/or sediment, and absorption of water-borne contaminants via the gills. Since B6-923 was racemic in sediments and water from this site[3,8] and near racemic (1.1) in grass shrimp, the uptake of B6-923 in mummichogs can be expected to be largely racemic.
Knowledge of ERs at the start of the experiment and the experimentally determined enantiomer specific half-lives allows us to estimate the throughput of B6-923 by the mummichog, the most abundant teleost in the salt marsh environment. Assuming first-order kinetics and a constant ER of 1.3, 91% of the initial pool of B6-923 was taken up as the racemate and subsequently excreted within the half-life of the B6-923 enantiomers. The time 0 level of B6-923 was 990 ng/g. Assuming an average weight of 5 g per fish and steady state conditions, 128 ng/g fish of B6-923 are processed within 7 days. Expressed on an annual basis and assuming a constant total biomass of 100 kg mummichogs at the site, elimination of B6-923 by mummichogs would account for 4.7 g/year. Thus, elimination by mummichogs

is not likely to be rapid enough for bioremediation purposes, as supported by the presence of elevated toxaphene residue levels in sediments after several decades of natural attenuation. However, our results indicate that fish metabolize and subsequently eliminate reductive dechlorination metabolites such as B6-923, thus reducing their overall persistence in this environment. The results also confirm the hypothesis that anaerobic toxaphene degradation and toxaphene degradation in mammals is complementary.

Conclusions

Our results illustrate the utility of enantioselective analysis for characterizing the processing of chiral organic compounds under lab or field conditions. The shift in the enantioratio of B6-923 during our experiment clearly demonstrates enantiomer-specific elimination rates, suggesting that B6-923 (and other chiral compounds) should be considered as a two-component mixture formed by the two B6-923-enantiomers. Two-component mixtures are characterized by non-ideal first order kinetics (in contrast to single enantiomers). Our data indeed indicates a faster decrease during the first part compared with the second part of the elimination study. This was, however, not taken into account in our calculations. To do this, more data points, particularly at the beginning of the elimination study would be necessary to better characterize exact elimination kinetics. Nevertheless, our results obtained so far clearly demonstrate that any ER deviating from the source (usually the food) is an indication of an equilibrium and suggests active degradation of the compound. Under such conditions, enantioselective analysis is a useful tool for assessing persistence and biotransformation of chiral compounds in biota.

References

1. Saleh M.A. (1991) Rev. Environ. Cont. Toxicol. 118, 1
2. Vetter W. and Oehme M., Toxaphene. Analysis and Environmental Fate of Congeners. In: J. Paasivirta (Ed.), The Handbook of Environmental Chemistry, Volume 3, Part K, New Types of Persistent Halogenated Compounds. Springer-Verlag, Berlin Heidelberg, 1999, pp. 237-287
3. Vetter W., Bartha R., Stern G. and Tomy G. (1999) Environ. Toxicol. Chem. 18, 2775
4. Stern G.A., Loewen M.D., Miskimmin B.M., Muir D.C.G. and Westmore J.B. (1996) Environ. Sci. Technol. 30, 2251
5. Maruya K.A., Wakeham S.G., Vetter W. and Francendese L. (2000) Environ. Toxicol. Chem., in press.
6. Smalling K.L., Maruya K.A. and Vetter W. (2000) ACS Symposium Volumes, Extended Abstracts, Vol. 40, pp. 413-414.
7. Maruya K. and Lee R. (1998) Environ. Sci. Technol. 32, 1069
8. Vetter W. and Maruya K. A. (2000) Environ. Sci. Technol. 34, 1627
9. Delorme P.D., Muir D.C.G., Lockhart W.L., Mills K.H. and Ward F.J. (1993) Chemosphere 27, 1965
10. Vetter W. and Schurig V. (1997) *Review*, J. Chromatogr. A. 774, 143

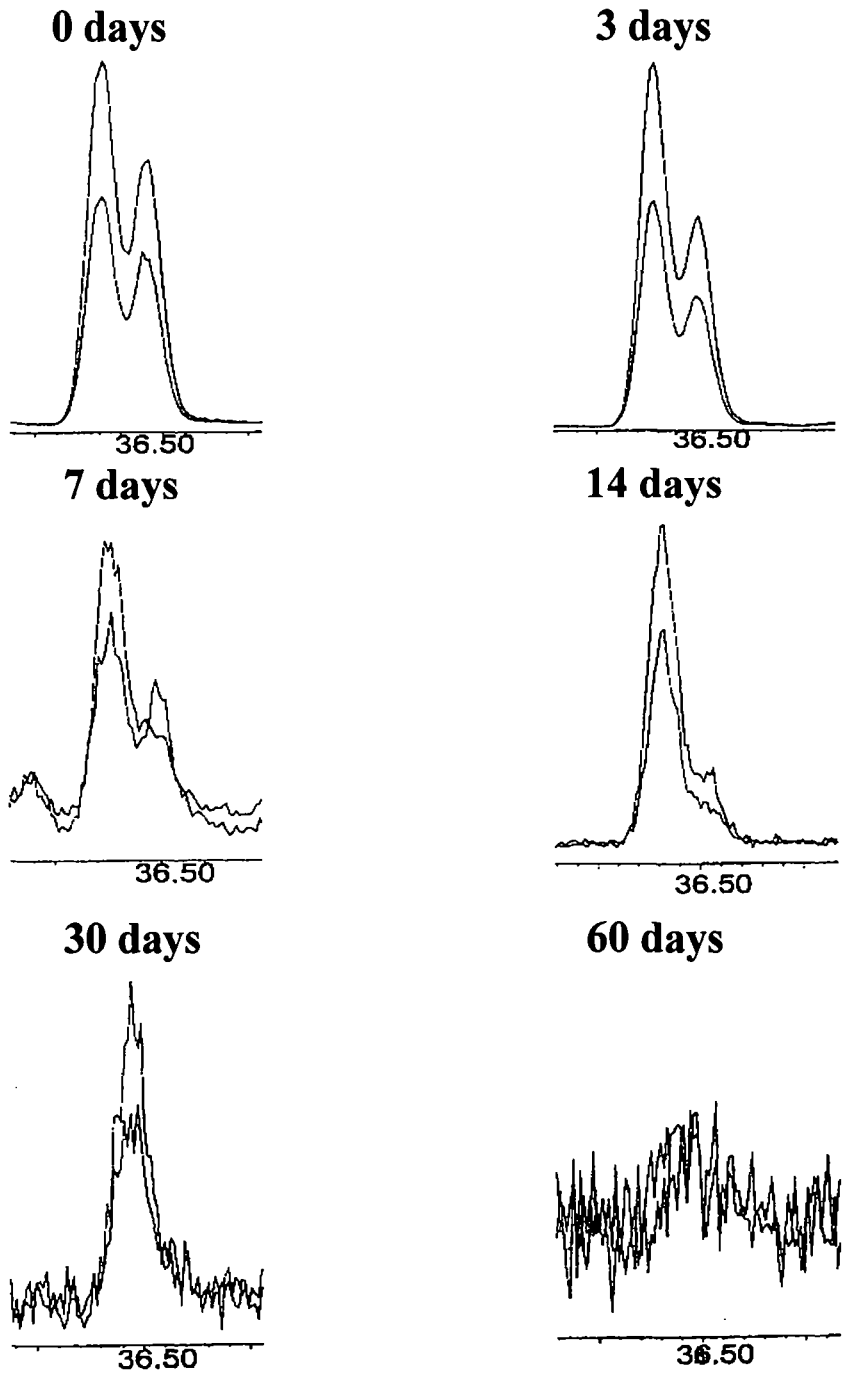

Figure 1: Partial GC/ECNI-MS chromatograms (m/z 307, m/z 309) showing the increase of the enantioratio of B6-923 during the elimination study with fish