ENANTIOSELECTIVE METABOLISM OF FOUR TOXAPHENE CONGENERS IN RAT AFTER INTRAVENOUS ADMINISTRATION

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Introduction

Toxaphene is a pesticide mainly consisting of hundreds of chlorobornanes (CHB). It is found world-wide in the environment. Due to differences in the persistency of congeners a much smaller number of toxaphene compounds is found in biota compared to the technical mixture. About 20 major congeners are found in fish, eight in marine mammals and only two major ones in humans, Parlar no.26 and 50[1]. Compared to other chlorinated insecticides such as DDT or Lindan, very limited knowledge about metabolic pathways and toxicokinetics is available for toxaphenes[1,2]. So far, the involvement of cytochrome P450 enzymes in the metabolism of toxaphene congeners has been reported[2,3].

Most toxaphene compounds are chiral. Bioaccumulation and metabolism in biota is usually enantioselective[1]. However, changes in the enantiomer ratios may occur throughout the food web and can often not be assigned to a specific organism. In addition, it has been reported that enantiomer ratios in technical products may deviate from racemic[1]. Therefore, detailed information about the metabolism of single toxaphene congeners can only be obtained by in vivo or in vitro studies in which species are exposed to selected congeners[4]. Moreover, the analysis has to be carried out enantioselectively.

The aim of this work was to study the enantioselective metabolism of the toxaphene congeners Parlar no. 26, 32, 50 and 62 in female Wistar rats by analysing brain, liver and adipose tissue at selected intervals after exposure.

Materials and Methods

Reference compounds and solvents. Stock solutions containing 5 ng/µl each of Parlar no. 26, 32, 50 and 62 (Promochem, Wesel, Germany) in iso-octane were diluted with n-hexane (J.T. Baker, Deventer, The Netherlands) to concentrations of 10 pg/µl, 20 pg/µl or 60 pg/µl. The solutions contained cis-chlordane (AccuStandard, New Haven, USA) as internal at a concentration of 12.71 pg/µl.

In vivo experiments. Thirty-two approximately 6 weeks old female Wistar rats (CPB:uWu, SPF) were obtained from the Central Animal Laboratory (Utrecht University, The Netherlands) with an average weight of 170.9 g. The toxaphene injection fluid was prepared by evaporating the solvent of 400 µl of a toxaphene stock solution, redissolving the residue in 200 µl of Tween 80 (Sigma, St. Louis, USA) and adding sterile saline[5]. The obtained 5% Tween 80 emulsion was injected into the tail vene at 1 µl/g body weight (doses: 49 µg/kg for

Parlar no. 26, 12µg/kg for Parlar no. 32, 71 µg/kg for Parlar no. 50 and 74 µg/kg bodyweight for Parlar no. 62). Three animals were sacrificed 4.0, 9.2, 24.5, 51.5, 74 and 131 h after exposure. Liver, brain and abdominal fat tissue were dissected and freeze-dried. Extraction and clean-up were performed as described in ref. 5. The organs were soxhlet extracted with dichloromethane/n-hexane (1:1 v/v) for 16 h, the extract concentrated and the solvent changed to n-hexane. Clean-up was carried out by column chromatography on 4 g Florisil (Merck, Darmstadt, Germany) deactivated with 5 % (w/w) of water. Toxaphene congeners were eluted with 48 ml of n-hexane.

Instrumentation. The isomerselective qunatification was performed using a Carlo Erba Mega 5360 gas chromatograph equipped with an electron capture detector and a 15 m x 0.2 mm i.d. separation column coated with 0.25 µm of DB5 (J&W Scientific, Folsom, USA). 1 µL was injected splitless (Carlo Erba A200S autosampler) and separated with the following temperature programme: 90 °C, isothermal for 2 min, then 20 °C/min to 210 °C, 2 °C/min to 237 °C, 20 °C/min to 260 °C.

For the enantioselective analyses a HP 5890II gas chromatograph/ HP 5989B mass spectrometer system was employed in the negative ion chemical ionisation (NICI) mode using methane as reagent gas. The $[M\text{-}Cl]^-$ fragments of the toxaphene congeners were detected in the single ion monitoring mode.

Determination of enantiomer ratios (ER). The two following capillaries were employed: Column A, 19 m length, 0.25 mm i.d., coated with 0.2 µm of 10 % heptakis(2,3,6-*O*-*tert*-butyldimethlylsilyl)-β-CD (TBDMS-CD) in OV 1701 (BGB 172, BGB-Analytik, Switzerland); column B, 12 m length, 0.25 mm i.d., coated with 0.15 µm of 25 % octakis(2,3,6-tri-*O*-ethyl)-γ-cyclodextrin (TEG-CD) in OV-1701 (home-made[6]). Sample volumes of 1 µl were injected splitless with a splitless time of 2 min and separated with the following temperature programme: Column A, 100 °C, isothermal for 2 min, then 20 °C/min to 170 °C, 2.5 °C to 230 °C, isothermal for 10 min; column B, 100 °C, isothermal for 2 min, 25 °C/min up to 150 °C, 2 °C up to 230 °C, isothermal for 10 min.

Results and Discussion

Enantioselective separation properties of the cyclodextrin capillaries. The TBDMS-CD capillary separated all four congeners into enantiomers. However, at temperature rates of 2.5 °C/min (total run time 38 min) the enantiomers of Parlar no. 26 and 32 were not well resolved (R_s 0.6 and 0.5, respectively). Only at a rate of 1 °C/min, a reasonable separation was achieved with a total run time of 68 min. In addition, Parlar no. 32 and 50 co-eluted. However, the selectivity of mass spectrometry allowed to determine the nonachloro congeners Parlar no. 50 free from interference from the heptachloro compound Parlar no. 32.

Alternatively, the co-eluting congeners Parlar no. 32 and 50 could be separated with the TEG-CD column, which was found very suitable for the enantioselective analysis of toxaphenes[6]. However, this capillary did not allow to split Parlar no. 26 into enantiomers, and the resolution for Parlar no. 62 was poorer than for TBDMS-CD. An example of the complementary enantioselectivity of TEG-CD and TBDMS-CD is given in Figure 1.

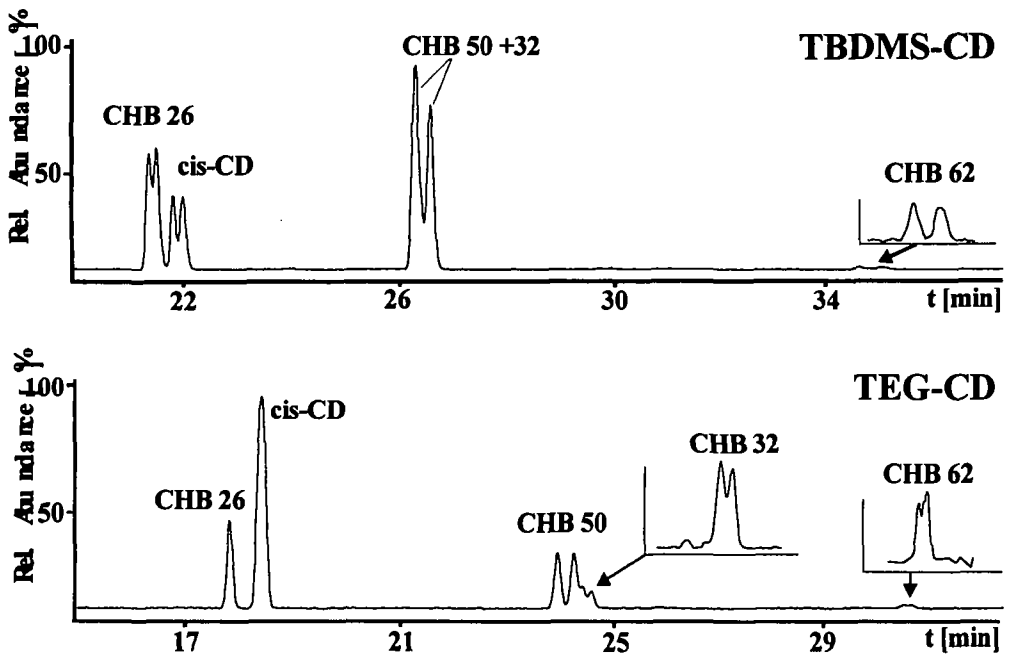

Figure 1: *Total ion chromatograms and mass chromatograms of a calibration solution* separated on TBDMS-CD and TEG-CD at a temperature rate of 2.5 °C/min.

Quality assurance. Retention times and enantiomer resolutions were well repeatable on both capillaries (≤0.8 % and ≤0.7 %, respectively, n=10). Enantiomer ratios (ERs) were determined as peak height ratios for resolutions between 0.5-1.0 resulting in reasonable repeatabilities of 6 % or less. Furthermore, the ERs for Parlar no. 50 and 62 were determined on both the TBDMS-CD and TEG-CD capillary. The average deviation for all samples was 0.04 for no. 50 and 0.11 for 62, respectively. The ER of the internal standard cis-chlordane was racemic for all samples (0.97 ± 0.05, n=20, only determined on TBDMS-CD) indicating no interferences due to matrix problems.

Most of the three samples taken at each time interval were analysed in duplicates. The ER deviation between duplicates was between 0.01-0.15 depending on congener and concentration. Deviations of 0.01-0.20 were measured between the three samples. Blank runs carried out after five separations did not contain detectable toxaphene amounts.

Observed enantiomer ratios. The administered congeners were all racemic within the precision of the determination (± 0.01-0.05). So far, most attention has been devoted to the determination of the ER of Parlar no. 26 and Parlar no. 50, which are the most stable congeners in biota including humans[1,4,7]. As can be seen from Figure 2, enantioselective degradation is observed for both congeners in rats. After 131 h the ER for Parlar no. 26 decreased to 0.67 ±0.04 in brain, to 0.72 ± 0.03 in fat tissue and to 0.58 ± 0.05 in liver. For Parlar no. 50 corresponding values confirmed on both enantioselective columns were 0.66 ± 0.03 (brain), 0.65 ± 0.05 (fat) and 0.62 ± 0.04 (liver). The ERs found are comparable with those in human milk and monkey adipose tissue as reported by Alder et al.[4]. Fish shows a more racemic distribution[1]. Boon et al.[2] as well as Drenth et al.[3] suggested that cytochrome

P450 enzymes of the CYP 2B subfamily are responsible for the degradation of toxaphenes. Fish has lower activities of these enzymes, which might explain the more racemic distribution in such biota[1].

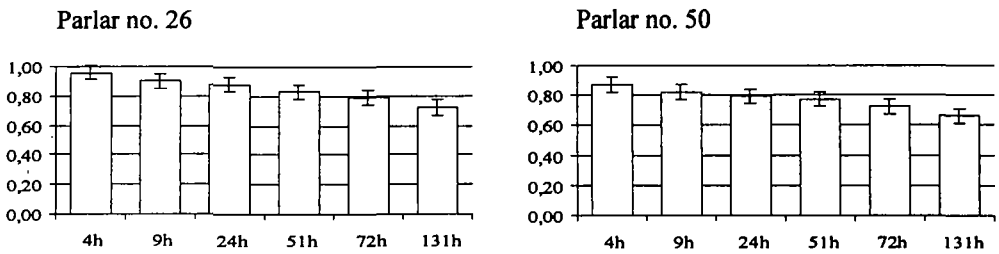

Figure 2: Time dependence of ERs for toxaphene Parlar no. 26 and 50 in rat adipose tissue.

Different CYP450 enzyme systems are involved in the metabolism of Parlar no. 32 and Parlar no. 62[2,3]. Further studies are still under investigation. The ERs for Parlar no. 32 were close to racemic. Average values were 0.93 ± 0.04 for adipose tissue and 0.97 ± 0.02 for liver. Levels in brain were close to or lower than the LOD. The ERs for Parlar no. 62 showed a significant alteration after 4 h but no significant increase or decrease for the rest of the experiment. Ratios were 1.24-1.54 for brain, 1.10-1.43 for adipose tissue and 1.12-1.65 for liver.

Toxaphene levels. Concentrations in the different rat tissues were determined on non-chiral stationary phases[3]. Levels in liver after 4 h were highest for Parlar no. 62 (139 ng/g wet weight (w.w.)), followed by Parlar no. 50 (106 ng/g w.w.), Parlar no. 26 (56 ng/g w.w.) and Parlar no. 32 (4 ng/g w.w.). The time to reach a level of ca. 1 ng/g was as follows: Parlar no. 32, 24h, Parlar no. 26, 131h, Parlar no. 50, 131h and Parlar no. 62, 131h.

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