IDENTIFICATION OF CYTOCHROME P450 ENZYMES INVOLVED IN THE BIOTRANSFORMATION OF PCB 77, PCB 136 AND UGILEC 141 ISOMERS USING HUMAN HEPATIC MICROSOMES.

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Introduction

Ugilec 141®, an industrial mixture of 69 tetrachlorobenzyltoluene (TCBT) isomers, was introduced on the European market in the early 1980s as a replacement of polychlorinated biphenyls (PCBs). Due to structural similarities in physicochemical properties [1] with PCBs the use of Ugilec 141® was prohibited in 1994 by the European Union.

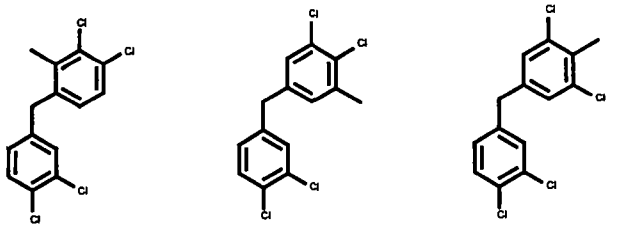


Figure 1 chemical structure of TCBT 87,88, 94 respectively

Based on the structural similarity with some PCBs, e.g. PCB 77, it has been suggested that some of these compounds could interact with the Ah-receptor. As a consequence dioxin-like toxicity might be expected [2]. The isomers that most likely have these characteristics are the isomers 87, 88 and 94 (see Fig.1). TCBT isomers appear to be less persistent in the environment than e.g. PCBs or PCDDs that could be caused by a more rapid biotransformation. As with other chlorinated aromatic hydrocarbons, the cytochrome P450 enzymes play a crucial role in the initial metabolic conversion to more polar compounds. In this study we used microsomal fractions from human livers to determine metabolic rates and detect the involvement of individual CYPs in the

biotransformation of Ugilec 141 isomers. As TCBTs are structurally related to PCBs, we also studied the microsomal biotransformation rates of two PCB congeners, 3,3',4,4'-tetrachlorobiphenyl (PCB 77) and 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136).

Material and Methods

Isolation of microsomes: Human liver tissue was obtained from redundant material after bipartitioning of livers procured from multi-organ donors. The human livers were handled as described before [3]. Consent from the legal authorities and from the families concerned was solicited for the explantation of organs for transplantation purposes. These liver samples (3-4 g) were used for the isolation of microsomes as described by Rutten et al. 1987[4]. The protein content was determined according to Bradford[4]. The calibration curve was made using bovine serum albumin as a standard.

Phenotyping of the microsomes:

Table 1. Enzyme assays performed to phenotype the human hepatic P450 activities

CYP	Substrate	Reference
1A2	Caffeine	[5,6]
2C9	Tolbutamide	[7,8]
2C19 /2B6	S-mephenytoine	[9,10]
2D6	Dextromethorphan	[11]
4A11	Lauric acid	[12]
3A4	Testosterone	[13]

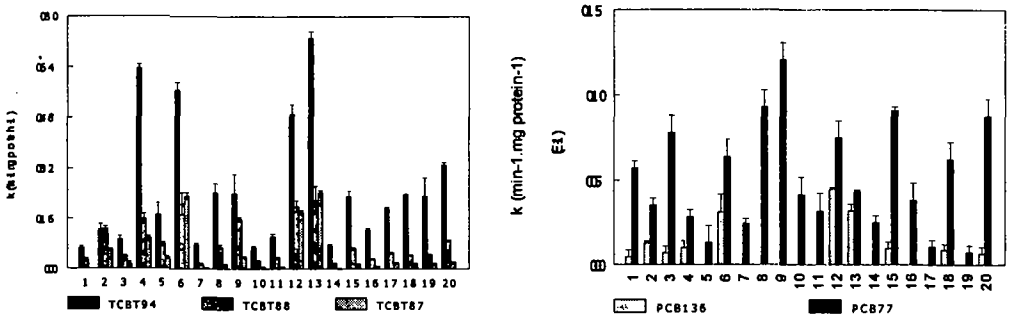
Biotransformation assay: Human hepatic microsomes were incubated (1 mg/ml) at 37°C with TCBT 87, 88, 94 (260 nM) in a total volume of 1 ml consisting of 0.05 M KH_2PO_4/Na_2HPO_4 phosphate buffer (pH=7.4), 1mM EDTA. Ugilec isomers and PCBs were dissolved in acetone (1 % final concentration). The biotransformation reaction was started by adding 50 µl of the NADPH-regenerating system (final concentration:1 mM NADP, 1 U Glucose-6-phosphate dehydrogenase, 3 mM $MgCl_2$, and 5 mM glucose-6-phosphate). The biotransformation assay was performed in triplicate. At four time points between 0 and 90 minutes 200 µl of the incubation mix was added to 1 ml ice cold methanol and 1 ml buffer solution to stop the biotransformation of the substrate. 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153) was used as an internal standard (260 nM) to determine the mass balance. PCB 153 is hardly metabolised in biota [7].

Extraction: The parent compound was extracted once by 4 ml n-hexane. Recovery of the extraction ranged between 90 and 100%. The organic phase was collected and evaporated at room temperature under air. The almost dry samples were then dissolved in 100 µl hexane.

GC-analysis: The samples were analysed on a Carlo Erba Mega 5360 GC-ECD equipped with a 15 m DB5 column (ID 0.32 mm, film thickness 0.25 µm) using split injection with a split ratio of 30 (T inj=250°C, Tcol=250°C). The area of TCBT relative to the area of PCB 153 was determined for each sample. The disappearance (biotransformation) of the parent compound (PCB or TCBT) was described by first order kinetics. The biotransformation rate was calculated by linear regression analysis after logarithmic transformation of the relative areas.

Correlation analysis: Spearman correlation test was performed between the microsomal enzyme activities and k using SPSS for Windows®. The statistically significant correlations indicated the CYPs that play a role in the metabolism of TCBT.

Results and conclusions



Figures 2 and 3. Microsomal biotransformation rates of TCBTs and PCBs in individual human livers.

In the figures 2 and 3 the biotransformation rate constants are presented from 20 individual donor livers. These results show that for TCBTs large differences are present among individuals as well as among the TCBTs. The biotransformation rate of TCBT 94 is about 2 to 10 times higher than for TCBT 88 and 94. The mean biotransformation rate of TCBT 94 is statistically significantly higher than TCBT 87 and 88 which are similar.

Table 1. Correlation coefficients (* = p<0.05,** = P<0.001) between human hepatic biotransformation rates and P450 activities for TCBTs or PCBs

P450 enzyme:	CYP2B6	CYP3A4	CYP1A2
Enzyme assay:	Nirvanol	6β-OH-T	Caffeine
TCBT 94	0.85**	0.56*	
TCBT 88	0.93**	0.60*	
TCBT 87	0.87**		
PCB 136	0.94**	0.79**	
PCB 77		0.63**	0.92 **

Table 1 shows the Spearman's correlation coefficients for those enzyme activities and biotransformation rates (*k*) that were statistically significant. From this table it can be concluded that CYP 2B6, 3A4 and 1A2 are the major P450 enzymes that are involved in the human biotransformation of these compounds. It should be noticed that CYP 2B6 and 3A4 are involved in the biotransformation of the *non planar* PCB 136 and TCBTs. In contrast, CYP1A2 seems to play a major role in the biotransformation of the *planar* PCB 77.

In general the biotransformation rate of TCBT 94 is at least two times greater than that of the TCBTs 88 and 87. The results that have been obtained for TCBTs could suggest that the presence of two non-adjacent chlorine atoms with a *para* methyl group in between facilitate the biotransformation significantly. For the PCBs 77 and 136 significant differences in the biotransformation rate could also be observed among humans. As with TCBTs individual biotransformation rates for both PCBs can vary almost one order of magnitude. The *non ortho* PCB 77 was metabolised more rapidly than the *tetra ortho* substituted PCB 136

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