# Biotransformation rates of Ugilec141® (tetrachlorobenzyltoluenes) in rat and trout microsomes

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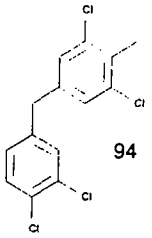
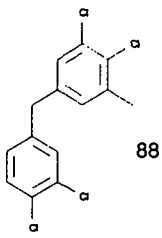
## Introduction

Ugilec 141®, an industrial mixture of 69 tetrachlorobenzyltoluene (TCBT) isomers, was introduced as a replacement of polychlorinated biphenyls (PCBs) on the European market in the early 1980s. The use of PCBs was prohibited because of their persistency in the environment and toxicological implications of these hydrophobic compounds. Due to structural similarities and similarities in physicochemical properties [1] with PCBs the use of Ugilec 141® was prohibited in 1994 by European Union. This likeness suggests that TCBTs may also bio-accumulate at all levels of the food chain [1]. However, only weak accumulation of Ugilec 141® has been demonstrated in fish and rat [2]. Similar biochemical changes in mice, like cytochrome P450 (CYP) induction, were reported after exposure to Ugilec 141® and the structurally related PCB#77. However the effect of Ugilec 141® was less pronounced [3]. It was hypothesized that biotransformation could explain the limited bioaccumulation of TCBTs as well as the limited induction of hepatic enzymes. In order to explain the limited bioaccumulation of TCBTs in various species we measured *in vitro* biotransformation rates of three TCBT isomers (fig. 1) in rat and trout hepatic microsomes. The theoretical affinity of all possible TCBT isomers for the Ah-receptor was estimated by computer simulation [1]. The isomers number 88 and 87 showed a high probability to fit into the Ah-receptor and may thus be expected to exhibit dioxin-like toxicity. Thus, for risk assessment purposes these isomers may be relevant.

## Material and Methods

Biotransformation rates of tetrachlorobenzyltoluenes (TCBT#87; (TCBT#88; TCBT#94) (synthesised by deLang [4] (purity >99%)) were determined *in vitro* by measuring the disappearance of the parent compound (PC) in time using untreated rat and rainbow trout hepatic microsomes. The maximum assay time was 2 hours for rat and 11 hours for trout. The substrate concentration ranged from 5 to 260 nM. The relative amount of PC was calculated by setting the amount of PC at t=0 at 100%.



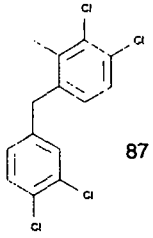




Figure 1 Chemical structure of 3 TCBT isomers.

The *in vitro* method was based on Boon et al. (1998)[5]. We used a NADPH regenerating system consisting of glucose-6-phosphate, NADP, and glucose-6-phosphate dehydrogenase. Control samples (n=3) without NADP were incubated for 1.5 (rat) or 11 hours (trout) to check the mass balance. No clean-up was performed after extracting PC with hexane. PCB#153 was used as internal standard (recovery >80%) because it is not metabolised in rat nor fish [6].. Samples were analysed by gaschromatography with a $^{63}$Ni-ECD detector. The individual data were plotted in Slidewrite and fitted according to first-order kinetics:

$$\frac{A_t}{A_0} = e^{-kt} \qquad (1)$$

where $A_t$ the amount at time t, $A_0$ is amount at t=0, $k$ is the biotransformation rate constant. To determine the major CYPs involved in the biotransformation of TCBT, we used microsomes of rats pretreated with phenobarbital (PB) or ß-naftoflavone (BNF). The isomers were incubated individually for 5 or 15 minutes. The relative amounts of PC from these incubation were compared with the results obtained from control microsomes. Microsomal enzyme activity was monitored during the incubation period by measuring CYP1A1 acivity using EROD assay [7]. This enzyme activity is used as indicator for total microsomal activity in time. A 25% decrease in EROD activity was measured in rat microsomes after 140 minutes while for trout microsomes the activity was reduced 50% after 11 hours.

**Results**

The amount of parent compound decreased in time for all three TCBT isomers in rat. After 60 minutes 65% of TCBT#87, 3 % of TCBT#88 and TCBT#94 was recovered. Trout microsomes showed no statistically significant decrease of all three TCBTs after 11 hours. The recoveries of PCB#153 as well as TCBT from the control sample were high (>80%), indicating that the mass balance conditions of the *in vitro* system were conserved. The non-enzymatic decrease of PC in time was 3 to 4 % in both species. This indicates that the decrease of PC is due to biotransformation. The velocity of the biotransformation reaction was linear to the substrate concentration (5-260 nM) for each TCBT isomer, indicating that saturation of metabolism did not occur. Thus the biotransformation rates were independent of the substrate concentrations. The biotransformation of TCBTs in rat microsomes are well described by equation 1. This indicates that TCBT biotransformation follows first-order kinetics. Fitting the experimental data resulted in estimates for $k$, the biotransformation rate constant from which half-lifes were

derived (table 1). Statistically significant differences were observed in biotransformation rate between the individual isomers in rat microsomes. TCBT#88 and #94 are metabolised 4-5 times faster than TCBT#87. Due to large variation in the trout biotransformation data no statistically significant difference in biotransformation rate between the isomers was observed. However, estimated half-lifes of trout and rat differ two to three orders of magnitude, indicating an inefficient biotransformation capacity of trout microsomes for TCBTs. Figure 2 shows the relative *in vitro* biotransformation of TCBT#87, #88, and #94 using either control, PB, or BNF induced microsomes. PB induced microsomes increased the percentage biotransformation of the 3 isomers 10 to 16 times relative to control microsomes. BNF induced microsomes increased the percentage biotransformation of TCBT#87 and #94 1.7 and 1.5 times, respectively, relative to control microsomes. Using induced microsomes, TCBT#88 and #87 were metabolised to a similar level while TCBT#94 was eliminated more rapidly. This is in contrast with our first experiment (table 1) where TCBT#88 and TCBT#94 are metabolised at similar rate while TCBT#87 is metabolised slower.

Table 1 Estimated first-order biotransformation rate constant $k$ ($min^{-1}.mg^{-1}$), confidence interval of $k$, and half-lifes for three TCBT isomers for rat and rainbow trout. For rat per time n=3 and for trout n=12

| TCBT | $k$ ($min^{-1}.mg^{-1}$) and 95% confidence interval | | half-life (min) | |
|---|---|---|---|---|
| | rat | trout | rat | trout |
| 87 | 0.016 (0.012-0.021) | 0.0037 (-0.006-0.014) | 43 | $5.2*10^3$ |
| 88 | 0.063 (0.051-0.074) | 0.0045 (-0.009-0.018) | 11 | $9.2*10^3$ |
| 94 | 0.076 (0.046-0.107) | 0.0081 (-0.001-0.017) | 9 | $1.1*10^4$ |

**Discussion**

In the present study, *in vitro* biotransformation rate constants of three TCBTs were obtained for rat and trout hepatic microsomes by measuring the disappearance of the parent compound. The disappearance of TCBT was shown to be a result of biotransformation by hepatic microsomal enzymes. So far it was unknown which enzymes are responsible for the metabolism of TCBT. In this study it was indicated that in the rat TCBTs are more rapidly metabolised by the PB inducible enzymes (e.g. CYP2B) then by BNF inducible enzymes (e.g. CYP1A). In biotransformation studies using PCBs it was determined that in rat CYP2B preferably catalyses the reaction of poly-ortho PCBs with non-planar conformation while CYP1A enzymes preferably catalyse the non-ortho PCBs which have planar conformation [8, 9]. Thus, based on these results it can be suggested that CYP2B enzymes play a significant role in the biotransformation of TCBTs, which could be explained by the non-planar conformation of TCBT molecules causing, in analogy with PCBs, a preferential biotransformation by enzymes of the 2B family.

In our study large differences in biotransformation rate constants between rat and trout microsomes were observed. Presently, it is unclear if these species differences in biotransformation are caused by the absence or low activity of certain CYPs (e.g. CYP2B) in fish or rats. Clearly, more studies are needed to elucidate the specific role of certain CYPs in

TCBT and PCB metabolism. Nevertheless, the preliminary results of our rat and trout experiments indicate that fish are less capable of metabolising TCBTs than mammals. Thus, some bioaccumulation of TCBTs in fish could be expected, but transport higher in the foodchain and possible biomagnification seems to be less likely.

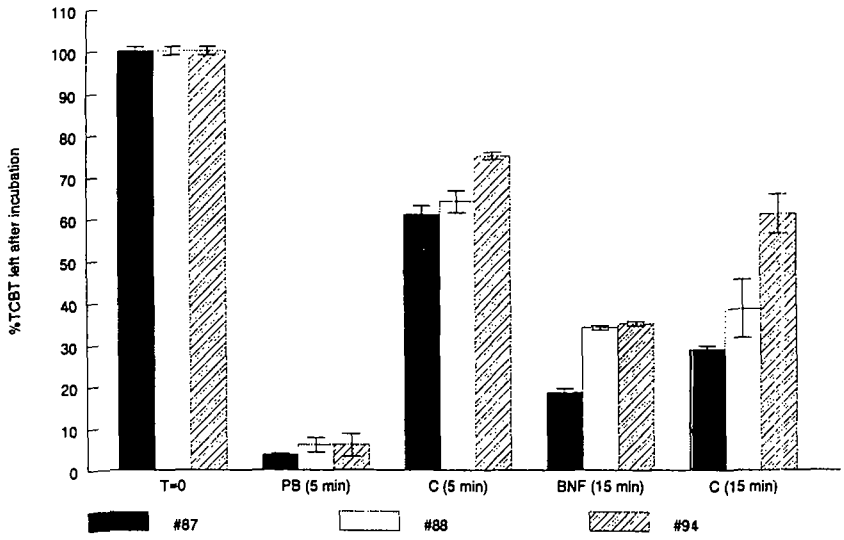



Figure 2 Percentage TCBT left after incubating TCBT with BNF and PB induced rat hepatic and C (control) microsomes for 0, 5, and 15 minutes at 37°C. (n=3)

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