IN VITRO BIOTRANSFORMATION OF NON-DIOXINLIKE PCBs BY HUMAN AND RAT LIVER MICROSOMES – A STRUCTURE-ACTIVITY RELATIONSHIP STUDY

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Introduction

Different studies often report various half-life times of PCBs, not only for different species but also for the same species[1,2,3]. All studies agree, however, that biotransformation of PCBs highly depends on the substitution pattern of the PCBs. For lower-chlorinated biphenyls, biotransformation rates in general are inversely associated with the degree of chlorination, but other factors are more critical for higher chlorinated PCBs, including the number and position of vicinal H-atoms especially in the meta- and para-positions. In the current paper, in vitro biotransformation rates by human and rat liver microsomes were systematically determined for a test set consisting of ultrapure mainly non-dioxinlike (NDL) PCBs. The aim of the in vitro screening was to identify structural features steering biotransformation rates in different species. In addition, quantitative structure-activity relationships (QSARs) were determined to distinguish persistent from non-persistent PCB congeners and to predict in vitro biotransformation rates for untested congeners.

Materials and methods

A test set of twenty-two tri-to hepta-chlorinated NDL-PCBs was selected based on their environmental abundance and their wide variability in chemical and physical properties[4]. This test set consisted of PCB congeners 19, 28, 47, 51, 52, 53, 74, 80, 95, 100, 101, 104, 122, 125, 128, 136, 138, 153, 168, 170, 180, and 190. PCB congeners 126 and 118 were added to the test set as reference structures for DL-PCBs. Biotransformation rates were determined by incubation of mixtures consisting of 4-5 different PCB-congeners for four different incubation periods (t_0-t_3) with liver microsomes from livers of 33 (non-induced) donors with different background (age, gender, ethnics, history of drug use), representing an "average" human being. Incubations were performed at 37°C in 1 ml phosphate buffer containing 1 mg/ml microsomal protein and 1 mM NADPH. To avoid NADPH depletion, fresh NADPH was added every 15 min to the incubation mixture. Additional incubations were performed with microsomes prepared from livers from CYP2B/3A induced rats exposed to phenobarbital and from CYP1A induced rats exposed to 2,3,7,8-tetrachloro-p-dioxin (TCDD). Maximum incubation periods ranged from 3 min for induced rat microsomes to 490 min for non-induced human microsomes. After incubation, the incubation mixtures were extracted and the time-dependent decreases of parent compounds were analyzed in the extracts with gas-chromatography followed by electron capture detection (GC-ECD) on an Agilent 6890 gas chromatograph. Biotransformation rates were calculated by linear regression based on first order kinetics and expressed as half-life time ($t_{1/2}$).

Quantitative structure-activity relationships (QSARs) were determined by dividing the test set of 24 PCBs into a training set (16 PCBs) and a validation set (8 PCBs). The training set covered as much as possible of the chemical domain of the tri- to heptachlorinated PCBs, as was determined by running a principal component analysis (PCA) on the molecular descriptors of the congeners (e.g. retention times on various GC columns, semi-empirical based descriptors, ab initio based descriptors, UV absorption wavelengths, Log Sw, and log Kow). The relationship between $t_{1/2}$ and molecular descriptors were determined by partial least square (PLS) regression modeling. The predictive capability of the models was estimated by cross-validation and by predicting the $t_{1/2}$ for the compounds in the validation set. Models are evaluated using both internal (cross-validation) and external validation. The differences between the actual and the predicted $t_{1/2}$ values are calculated and expressed as root mean square error of predictions (RMSEP), i.e. the standard deviation of the estimated model error defined as the difference between the observed values and the values predicted by the model.

Results and discussion:
Congeners with at least two couples of vicinal H-atoms were very rapidly metabolized ($t_{1/2}$ <3 min) by liver microsomes from phenobarbital-exposed rat. Congeners with only one such couple were slower metabolized with $t_{1/2}$ ranging from 8 to 22 min. PCB congeners lacking a couple of vicinal H-atoms in meta-para position were only metabolized if hydroxylation at meta or para position could be achieved via arene oxide formation and a 1,2 chlorine shift. For this category of PCBs, $t_{1/2}$ estimates ranged from 27 to 68 min. For none of the other congeners any significant in vitro biotransformation was observed, except for PCB-128 which was the only congener in this subset with more than one couple of vicinal H-atoms in ortho-meta position.

In the in vivo situation phenobarbital induces CYP2B and CYP3A expression via activation of the constitutive androstane receptor (CAR) and the pregnane-X receptor (PXR). Because NDL-PCBs also induce CYP2B and CYP3A via CAR/PXR activation, in vitro biotransformation rates of NDL-PCBs by phenobarbital induced CYP2B/3A enriched microsomes are considered as highly relevant for the in vivo situation. In real life, however, PCB mixtures always consist of NDL-PCBs and dioxin-like (DL) PCBs. Although DL-PCB levels are generally much lower than NDL-PCB levels, their induction capacity to induce CYP1A activity via arylhydrocarbon receptor (AhR) is much higher. Therefore additional experiments were performed with CYP1A induced microsomes from rats exposed to TCDD. Congeners that were rapidly metabolized by CYP2B/CYP3A induced microsomes were also metabolized by CYP1A induced microsomes, but at much slower rates (e.g. PCB 101 in Fig. 1), except for PCB-122. Biotransformation of this specific congener by CYP2B/3A induced microsomes could also be inhibited by the CYP1A inhibitor alpha-naphthoflavone, suggesting that PCB-122 is metabolized by CYP1A rather than by CYP2B/3A. Some of the congeners that were not metabolized by CYP2B/CYP3A induced microsomes were also not metabolized by CYP1A induced microsomes (e.g. PCB-153 in Fig. 1), but others seemed to be metabolized very slowly (e.g. PCB-138 in Fig. 1).

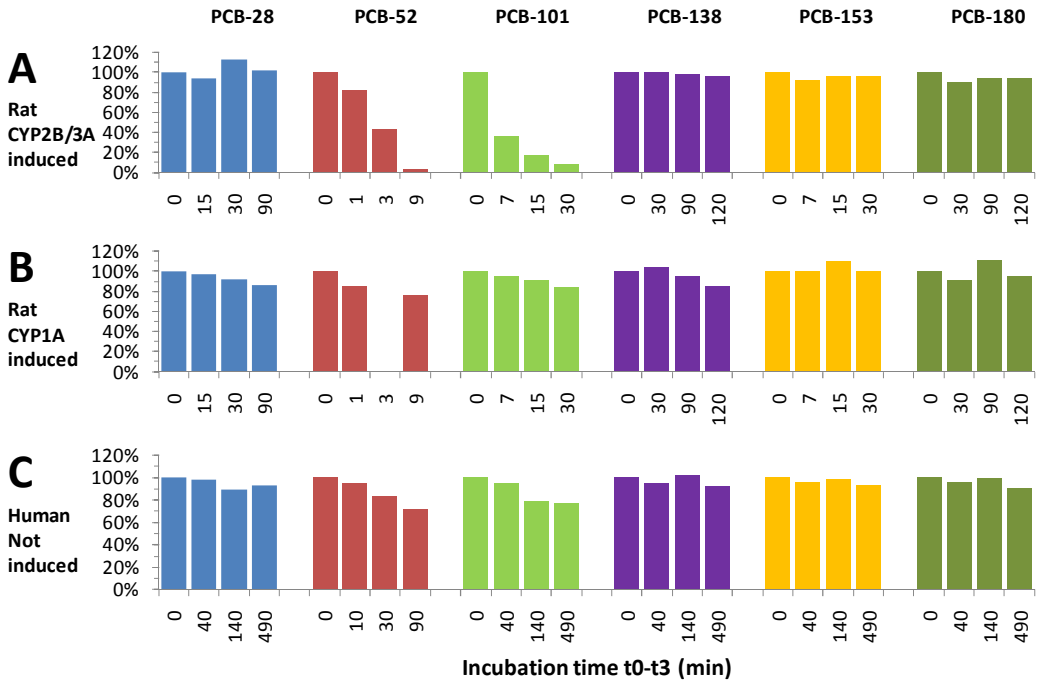

Figure 1: Biotransformation rates of six indicator PCBs by liver microsomes. **A**: CYP2B/3A induced rat liver microsomes; **B**: CYP1A induced rat liver microsomes; **C**: non-induced human liver microsomes.

Biotransformation rates in non-induced human liver microsomes were much slower than observed for CYP induced rat microsomes (Fig 1). Fastest biotransformation by human liver microsomes ($t_{1/2}$ ranging from 9 to 90 min) was observed for PCB congeners with vicinal H-atoms in meta-para position flanked by a third vicinal H-atom (i.e. meta-para-meta or ortho-meta-para). PCBs with only vicinal H-atoms in meta-para positions were metabolized at significantly lower rates ($t_{1/2}$ ranging from 277 to 456 min), while PCBs with only vicinal H-atoms in ortho-meta positions were not metabolized at all, except for PCB-100.

PLS modeling of the biotransformation data with human liver microsomes was done by a two step procedure. The first model was developed to enable separation of persistent PCB-congeners from non-persistent PCB congeners where all persistent congeners were assigned a $t_{1/2}$ of 1000 minutes. In total 41 tri- to hepta-PCBs were predicted to be persistent using a cut-off value of 800 minutes. The set of 11 PCB congeners that could be metabolized by human liver microsomes was again subdivided into a training set (n=7) and a validation set (n=4) to calculate a second QSAR model aimed for predicting $t_{1/2}$ values for non-persistent PCBs (Fig. 2). This model predicted $t_{1/2}$ values for human microsomes ranging from several minutes to 8 hours with an estimated prediction error of 50 minutes (RMSEP, $Q^2 = 0.54$). Further QSAR modeling on the biotransformation results with rat liver microsomes is currently in progress.

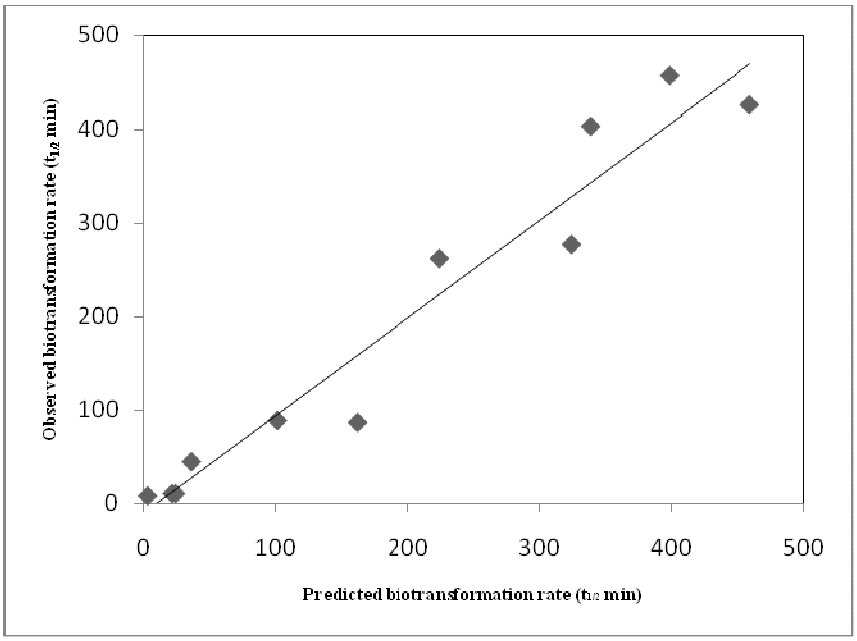

Figure 2: Observed versus predicted biotransformation rates ($t_{1/2}$) in human microsomes from the PLS model including only non-persistent PCBs.

The ultimate goal of this study is to make a better prediction of in vivo biotransformation rates of PCB congeners. In addition, a better understanding is obtained of the relative contribution of different CYP isozymes to PCB biotransformation and the differences between two species (i.e. rat and human) in structural requirements for biotransformation of PCBs.

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References:

1. Tanabe S, Nakagawa Y, Tatsukawa R. (1981); *Agric. Biol. Chem.* 45: 717-26
2. Öberg M, Sjödin A, Casabona H, Nordgren I, Klasson-Wehler E, Håkansson H. (2002); *Toxicol. Sci.* 70: 171-82.
3. Boon JP, Oostingh I, Van der Meer J, Hillebrand MTJ. (1994); *Eur J Pharmacol - Environ Toxicol Pharmacol Sect* 270: 237-51
4. Stenberg M, Andersson PL. (2008). *Chemosphere* 71: 1909-15.