INDUCTION OF CYP1A1 ACTIVITY IN FEMALE MOUSE AND RAT LIVER BY TCDD, 4-PeCDF AND PCB126: UPTAKE VERSUS SYSTEMIC DOSE LEVELS

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Introduction

Chlorinated dioxins and biphenyls (PCBs) commonly occur in the human food chain and can still be detected at levels that might cause long-term health effects. Exposure to dioxin-like compounds involves a complex mixture with a common mechanism of action involving endocrine, developmental, carcinogenic, immune and neurological effects. Risk assessment is performed with an additive model for mixture toxicity. Based on this the Toxic Equivalency (TEQ) concept was developed as a biomarker for exposure and risk. TEQs are the sum of congener-specific toxic equivalency factors (TEFs) multiplied by the concentration in a matrix, e.g., blood. TEF values are a composite quantitative value based on the relative effect potency (REP) of a compound to TCDD[1]. At present, human WHO-TEFs have been derived from a range of biomarkers that are congener- and endpoint-specific, mainly obtained from *in vivo* (semi)chronic animal studies with oral dosage as the principal route of exposure. Consequently, these are only applicable for exposure situations in which oral ingestion occurs. WHO-TEFs or 'intake' TEFs are commonly, but incorrectly used by regulatory authorities to calculate 'systemic' TEQs based on human blood and tissue levels and subsequently considered to be biomarkers for effect. However, at present there is no experimental validation that would either reject or accept the use of these 'uptake' TEFs and TEQs for human blood or tissues as biomarkers of effect. There are currently insufficient comparative studies, even for the toxicological most relevant congeners, for a balanced determination of 'systemic' TEFs and TEQs.

In this study, REPs of 4-PeCDF and PCB126 were determined in female mice and rats three days after receiving a single oral dose. REPs were calculated based on hepatic EROD activity, a marker for cytochrome P450 1A1 (CYP1A1) induction, using administered dose levels and liver tissue levels. The results presented here, are an initial step in the determination of 'systemic' REPs within the EU-funded project SYSTEQ (www.systeqproject.eu).

Materials and Methods

2,3,7,8-tetrachlorodibenzodioxin (TCDD), 2,3,4,7,8,-pentachlorodibenzofuran (4-PeCDF) and 3,3',4,4',5-pentachlorobiphenyl (PCB126) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada).

Female Sprague-Dawley rats and female C57BL/6 mice were purchased at 9 weeks of age from Harlan laboratories (Venray, The Netherlands) and allowed to acclimate for 1,5 week. The animals were housed in standard cages (46x35x19cm) and conditions (temperature 23 ± 2°C, 50% to 60% relative humidity, 12-h dark and light cycle) with free access to food and water. The animals were randomly assigned to 6 groups. The animals received a single dose of 0, 0.5, 2.5, 10, or 25µg TCDD/kg bw or 5, 25, 100, 250 or 1000 4-PnCF or PCB126/kg bw dissolved in corn oil by oral gavage at a dose volume of 10ml/kg bw (n=6/group). Animals were sacrificed at day 3 with CO2/O2. Blood was obtained from the abdominal aorta directly after decease. Plasma was immediately obtained from the blood sample by centrifugation and stored at -80°C. The liver was removed, weighed, snap frozen and stored until use at -80°C. All animal treatments were performed with permission of the Animal Ethical Committee and according to Dutch law on Animal Experiments.

Hepatic CYP1A1 activity was determined by means of ethoxyresorufin-O-deethylase (EROD) activity. Microsomal fractions of liver tissue were prepared using ultracentrifuation. 7-Ethoxyresorufin (5 µM) was added to the

microsomes and resorufin formation was measured by fluorescence at an excitation wavelength of 530 nm and emission wavelength of 590 nm every three minutes for at least 30 minutes.

Liver samples of three dose levels and control (corn oil) exposed animals of each compound were chosen for determining liver tissue levels. Compound analysis was performed in the lab of Dr. P. Andersson (Umeå University). Liver samples were cleaned using a combined solid phase extraction and clean-up column using Na2SO4. Lipid content was determined and the compounds were analysed by high-resolution GC/MS.

Curve fit was performed using sigmoidal dose-response association with variable slope (Hill equation) using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

Results and Discussion

A dose-dependent decrease in thymic weight and a dose-dependent increase in liver weight were observed in mouse and rat upon exposure to TCDD, 4-PeCDF or PCB126. No effects on body weight were observed by any of the compounds tested after three days (data not shown). In the liver samples, CYP1A1 activity was determined by measuring ethoxyresorufin-O-deethylase (EROD) activity in microsomal fractions of each individual liver sample (Figure 1). The relative effect potencies (REPs) of 4-PeCDF and PCB126 to induce hepatic EROD activity were calculated relative to the EC20 of TCDD for the mouse data. In the TEF methodology, the potency of a compound is usually calculated relative to TCDD using EC50 values, NOAELs or LOAELs. In this study, the EC20 value for TCDD was used as benchmark effect because the dose-response curves did not attain similar Ymax values or parallel slopes for different congeners. In rat liver, EROD activity was already maximally induced by TCDD at the lowest dose tested (0.5 µg TCDD/kg bw) and no dose-response curve could be obtained. Therefore, REPs for induction of CYP1A activity in rat liver were calculated relative to PnCDD. PnCDD has a WHO-TEF value of 1, comparable to TCDD. Using administered dose levels, REPs were 2 (4-PeCDF) and 10-fold (PCB126) lower than the WHO-TEF values in the mouse liver, but not in the rat liver (Table 1). Recalculating REPs using systemic dose levels (liver concentrations) showed that REPs decreased even further. Especially the REP for PCB126 in the murine liver showed a marked decrease from 0.01 (uptake) to 0.004 (systemic) in this study.

It is generally accepted that pharmacokinetic and pharmacodynamic parameters influence the REP of a dioxin-like compound. Especially 4-PeCDF accumulates in the liver to a higher degree than TCDD[2] and there are large variations in REPs decribed for 4-PeCDF[3]. Toyoshiba et al.[4] described a lack of consistency in administered dose-response induction by TCDD, 4-PeCDF and PCB126 of CYP1A1 and CYP1A2 in the rat liver and lung. This effect was attributed to the high sequestration of 4-PeCDF and therefore a stronger induction of hepatic CYP450 enzymes in the liver than the other compounds. From the same National Toxicology Program (NTP) study, it was concluded that the WHO-TEF value for 4-PeCDF appeared to somewhat overestimate the risk for developing neoplasms[5]. In our study, the 4-PeCDF REP for EROD induction in mouse liver was approximately 4-6-fold lower than the WHO-TEF, but no large effect on the REP was found between using administered dose versus liver concentration. Potentially, this is attributable to the short-term duration of this study compared to other studies. However, for PCB126 this effect was much clearer, especially in the mouse liver. The REP of PCB126 for EROD induction was 10- and 25-fold lower than the WHO-TEF value when calculated with administered dose or liver concentration, respectively. DeVito et al. also determined REPs for liver EROD induction based on administered dose and liver concentrations for PCB126 in female B6C3F1 mice exposed for 5 days/week for 13 weeks[6]. The REP of PCB126 was found to be similar when calculated based on administered dose or liver concentration and were 0.0053-0.011 and 0.0028-0.0095, respectively. However, the REP of PCB126 for hepatic CYP1A2 induction and skin CYP1A1 (EROD) were substantially lower and higher, respectively, when calculated with tissue concentrations instead of administered dose level. These results underline the general assumption that REPs are endpoint- and tissue (organ)-specific. Furthermore, pharmacokinetics and pharmacodynamics can greatly influence the REP. In practice this means that duration of the study and differences in compound distribution can play an important role. Using tissue concentrations instead of administered dose for REP calculations takes these differences to a certain extent into

account. Thus, tissue-specific REPs based on systemic dose levels might give a more accurate estimate of the potency of a single compound. In addition, in this way variation between species, including humans, due to pharmacokinetic differences is taken into account. Although the REPs described here substantially deviate from this WHO-TEF, it should be noted that our results have been obtained from a single endpoint using two rodent species. In view of this it should be realized that WHO-TEFs are based on a wide variety of endpoints and studies[1]. Further analysis of the tissues (e.g. lung, peripheral blood lymphocytes), systemic dose levels (fat, plasma) and effects by other dioxin-like compounds derived from the EU-SYSTEQ project should contribute to a better understanding of species- and tissue-specific REPs. Although the high dose levels in this study are not representative for human exposure (intake) levels, a better understanding of species-and tissue-differences in REPs will lead to a better human risk assessement for dioxin and dioxin-like compounds, when using human blood or tissue levels as the matrix of choice.

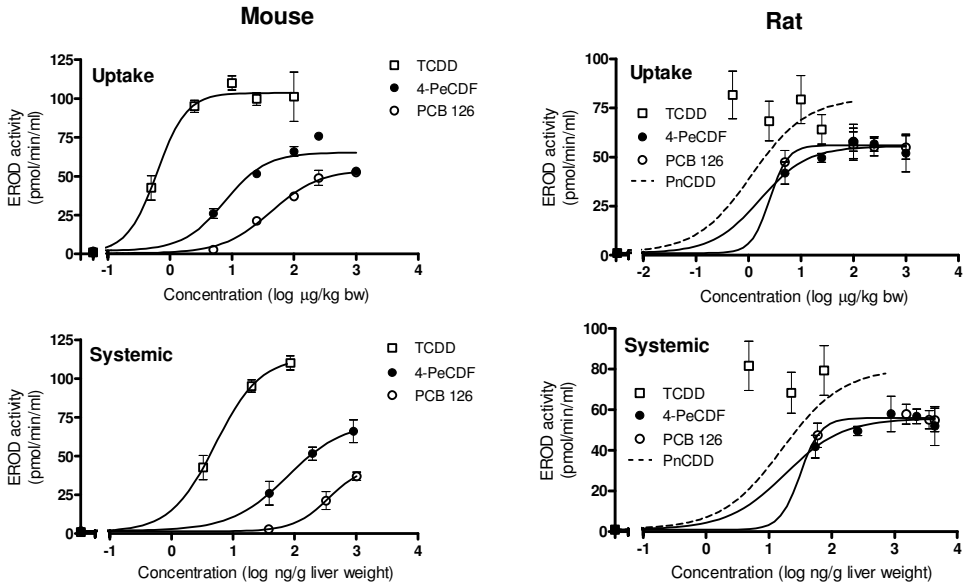

Figure 1. EROD induction in mouse (left panels) and rat (right panels) liver three days after a single oral dose of TCDD, 4-PeCDF or PCB126. Dose-response curves are calculated using administered dose (uptake, upper panels) or liver tissue levels (systemic, lower panels). Data are represented as mean ± SD (N=6).

Table 1. Relative Effect Potencies (REPs) for EROD induction in mouse and rat liver calculated using administered dose (uptake) and liver tissue levels (systemic). REPs were calculated using the EC20 of TCDD (mouse data) or EC20 of PnCDD (rat data).

	Uptake REP		**Systemic REP**		**WHO-TEF**[7]
	Mouse	Rat	Mouse	Rat	
4-PeCDF	0.07	0.3	0.05	0.5	0.3
PCB126	0.01	0.1	0.004	0.2	0.1

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