# HUMAN KERATINOCYTES EXHIBIT SIGNIFICANTLY LOWER SENSITIVITY TO PCB 126 THAN RODENT MODELS PREDICT

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## Introduction

Human health risk assessment for dioxin-like compounds realistically should incorporate information derived from both human tissues and epidemiological studies. Recently, the U.S.A. National Academies of Science (NAS) review panel has deemed the toxic equivalency factor (TEF) methodology as a "reasonable" approach for the estimation of non-cancer human health risks of dioxin-like compounds[1]. Currently, TEFs are set by a World Health Organization (WHO) expert panel[2] using relative potency (REP) estimates in databases as most recently presented by Haws *et al.*[3] Since this panel chose to exclude all REPs derived from *in vitro* studies, which includes human data for several well-studied compounds, the current TEF approach impedes the incorporation of clearly relevant human data into the risk assessment process. One such "well-studied" compound is the polychlorinated biphenyl (PCB) congener PCB 126, which was assigned a TEF of 0.1 (i.e., $1/10^{th}$ as potent as 2,3,7,8-TCDD). The PCB 126 TEF value was derived from various *in vivo* rodent REPs and did not take into account several *in vitro* studies using human cells with REP estimates dramatically less than 0.1[4-7]. For example, Silkworth *et al.*[8] recently demonstrated that while the rodent derived PCB 126 TEF of 0.1 is reproducible in both rat hepatocytes or rat cell lines, the REP is about 0.002 when tested in primary human hepatocytes or human cell lines. This represents a 50-fold discrepancy in the estimated risk factors between these species. Furthermore, several *in vitro* interspecies comparisons have also consistently revealed that 2,3,7,8-TCDD is less potent in human cells compared those of rodents[7-14].

To further examine whether these findings of lower sensitivity of human cells as compared to rodent cells can be extended across congener classes (i.e., dioxins, furans, and PCBs) as well as to human tissues other than liver, we tested two congeners that have the same WHO-assigned TEF of 0.1 (i.e., PCB 126 and 2,3,7,8-TCDF) in comparison to 2,3,7,8-TCDD. Human skin was chosen because it is known to be a human tissue that is responsive to TCDD, typically resulting in chloracne following high exposures. This condition is mediated by the aryl hydrocarbon receptor (AHR) and associated with induction of cytochrome P450 1A1 (CYP1A1), a very sensitive biomarker for AHR activation[15,16]. Thus, the EC50s for CYP1A1 mRNA induction by TCDD, 2,3,7,8-TCDF, and PCB 126 were determined in primary cultures of normal human epidermal keratinocytes (NHEK) under defined chemical conditions. Relative potency values were calculated for TCDF and PCB 126 using these new human-derived EC50 values.

## Materials and Methods

### *Chemicals*

PCB 126 was obtained from Accustandard. TCDD and 2,3,7,8-TCDF were a gift from DOW Chemical (Midland, MI).

### *Keratinocytes*

NHEKs were purchased from Lonza (Walkersville, MD) and grown in chemically-defined keratinocyte-SFM (Invitrogen, Carlsbad, CA). Based on preliminary time course studies with TCDD, cells were exposed for 24h to TCDD ($10^{-12}$ nM to $10^{-7}$ nM), TCDF ($10^{-12}$ nM to $10^{-7}$ nM), or PCB 126 ($10^{-12}$ nM to $10^{-5}$ nM).

### *Real-time polymerase chain reaction (RT-PCR)*

The relative expression level of CYP1A1 mRNA was estimated using the RT-PCR procedure on an Icycler instrument as per the manufacturer's instructions (Bio-Rad, Hercules, CA). Total RNA was isolated using Stat-60 (Tel-Test, Friendswood, TX), reverse transcribed (Invitrogen), and CYP1A1 expression quantitated by RT-PCR using the

following primers, 5'-CATCCCCCACAGCACAACAAGAGA-3' and 5'-GCAGCAGGATAGCCAGGAAGAGAA-3'. For normalization, β-actin expression was also determined using the following primers, 5'-GCAGCAGGATAGCCAGGAAGAGAA-3' and 5'-CATCCCCCACAGCACAACAAGAGA-3'. The comparative Ct method, using the formula, 2 ΔΔCt (Applied Biosystems User Bulletin #2, Foster City, CA)[17] was employed to determine expression levels since the standard curves for CYP1A1 and β-actin primers resulted in comparable amplification efficiencies. For each set of dose response data, the highest CYP1A1 value was set to 100%. Dose-response curves were fitted by non-linear regression (GraphPad Prism) using the basic Hill equation.

**Results and Discussion**

The skin represents the only uniformly agreed upon target for dioxin toxicity in humans[1,18]. High exposure to dioxins can result in chloracne. Furthermore, primary skin cells show a robust inductive response to treatment with TCDD and can be passaged up to five times[19]. The current study investigates whether previous findings of lower PCB 126 potency in human liver cells[8] also extends to other cell types such as human keratinocytes, and to other dioxin-like chemical congeners of supposedly the same relative potency class such as TCDF. Figure 1 depicts the dose response induction of CYP1A1 mRNA for TCDD, TCDF and PCB 126 at 24 h in NHEKs. Although the current dose-response data does not refute basic aspects of the TEF concept such as the existence of parallel dose response curves of comparable efficacy for both PCB 126 and TCDF, these two compounds are clearly not equally potent AHR agonists in NHEKs. The relative potency of TCDF was calculated as 0.16 (Table 1), which is in line with the current $TEF_{TCDF}$ based on REPs from 9 *in vivo* rodent studies[3]. However, the human PCB 126 REP estimate for the current study was 0.002. This is considerably lower than the "order of magnitude" of uncertainty assigned to current TEFs[2]. An $REP_{PCB\ 126}$ of approximately 0.002 has also been previously demonstrated for EROD /CYP1A1 induction in primary human hepatocytes and the human-derived HepG2 cell line[8]. Figure 2 clearly shows that the approximately 2.5 orders of magnitude difference between the TCDD and PCB 126 potencies depicted in Figure 1 is also likely robust for time points between 3-48 hours.

Thus, data from the current study certainly helps fill in data gaps present in the interspecies and *in vitro-in vivo* extrapolation parallelogram as modified from that of Sobels *et al.*[20] and Sutter[21] (Figure 3) and questions the sole use of rodent-derived data for such interspecies extrapolations. The finding that the $TEF_{PCB\ 126}$ is much lower than 0.1 for NHEKs helps explain why chloracne is observed in those highly exposed to dioxin and/or furans, but not to in those exposed only to PCBs[22]. Furthermore, recent data on human sensitivity to this PCB congener reported here and elswhere[7-14] helps to explain the lack of excess hepatic tumors reported in worker populations most heavily exposed to PCBs[23].

This new information suggests that use of the current $TEF_{PCB\ 126}$ of 0.1 for human health risk assessment is scientifically unsupported for several reasons. Firstly, comparative studies employing a variety of *in vitro* systems have demonstrated a lower potency of TCDD in human cells as compared to rodent cells[7-14]. Secondly, the current study using NHEKs clearly demonstrated PCB 126 and TCDF (both TEF=0.1) are not equally potent in human cells. Finally, data from multiple *in vitro* studies[4-14], including the current study using NHEKs, indicate that the assumption that PCB 126 is $1/10^{th}$ as potent as TCDD in both rats and humans is incorrect. The use of human *in vitro* data in human health risk assessments of dioxin-like compounds (DLCs) would comply with the recommendations of NAS[1] that *"If significant differences in the REP of DLCs are found between humans and other species, then adjustments should be made in the TEFs…."* Since PCB 126 can contribute a significant portion of the total toxic equivalent (TEQ) load at many environmental sites, the use of rodent-derived TEFs for human health risk assessments, rather than a human derived value, will likely result in inappropriate estimates of potential health risks.

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**Table 1.** $EC_{50}$ and human REP values for TCDD, PCB126 and TCDF

| | TCDD | PCB126 | TCDF |
|---|---|---|---|
| $EC_{50}$ (nM)[a] | 0.28 | 129.9 | 1.72 |
| Human REP[b] | 1 | **0.002** | 0.16 |

**[a]$EC_{50}$ values were obtained from GraphPad Software using nonlinear regression of data presented in Figure 1.**
**[b]Human REP values were based on the $EC_{50}$ values, where TCDD value was set to one.**

**Figure 1.** Dose-response of induction of CYP1A1 RNA by TCDD, PCB126 and TCDF in normal human epidermal keratinocytes (NHEKs). Values plotted are an average (n=3) +/- standard deviation. Curves were fitted by nonlinear regression (GraphPad Prism).

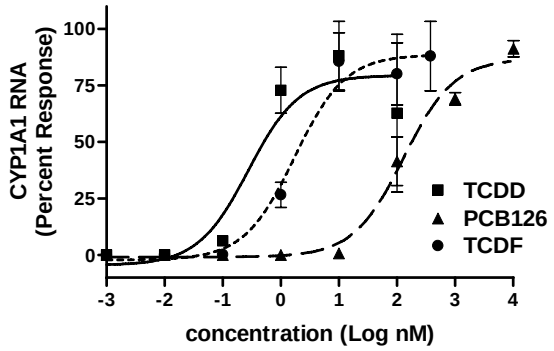


**Figure 2.** Time Course of CYP1A1 RNA response to TCDD (10 nM) and PCB126 (10,000 nM).

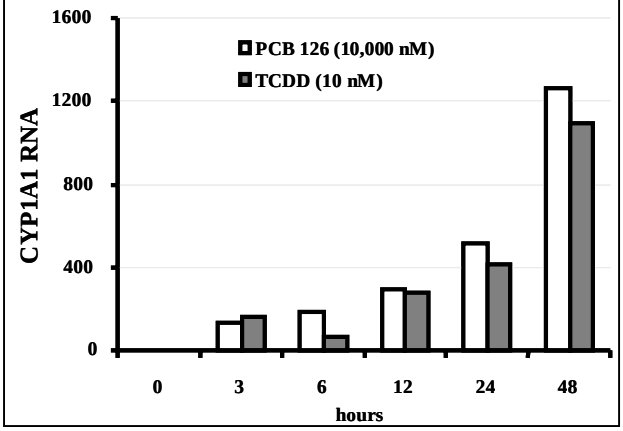


**Figure 3. Inter-species and *in vitro-in vivo* extrapolation parallelogram supplemented with data from current study**