CONSIDERATION OF *IN VITRO* HUMAN RELATIVE POTENCY DATA FOR PCB 126 IN DETERMINATION OF DIOXIN TOXIC EQUIVALENCY

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Introduction

Various regulatory agencies have endorsed the use of the dioxin toxic equivalency factor (TEF) [1] scheme to equate the human health risk of exposure to mixtures containing polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) (collectively, the so-called "dioxin-like" compounds [DLCs]) to that of exposure solely to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [2,3,4,5]. Van den Berg *et al.* [1] outline four criteria for inclusion in the TEF scheme: (1) the DLC must be structurally-related to PCDD/Fs; (2) the DLC must bind to the aryl hydrocarbon receptor (AHR); (3) the DLC must elicit AHR-mediated toxicity; and, (4) the DLC must be persistent and bioaccumulate. Current TEFs rely upon relative potency (REP) estimates obtained from orally-dosed rodent studies, where REPs can be calculated using TCDD/DLC EC50 ratios [6]. Thus, TEFs are expected to encompass both toxicokinetic and toxicodynamic aspects of congener potency. However, when relevant *in vivo* data were lacking for a DLC, the TEF panel considered *in vitro* REP evidence to set several TEFs (e.g., 1,2,3,6,7,8-HxCDD) [1]. Since quantitative information regarding human sensitivity to DLCs is likely to come primarily from *in vitro* assays measuring human AHR-mediated responses, we propose that human *in vitro* data be formally considered in future TEF updates using an interspecies extrapolation parallelogram approach similar to that described by Sobels [7]. Here, we outline evidence of interspecies differences for PCB 126 REP as a case study and discuss how this *in vitro* information might be incorporated into the current TEF scheme.

Results and Discussion

The most potent "dioxin-like" PCB congener in the TEF scheme is PCB 126, with a TEF value of 0.1. Several *in vitro* studies listed in the Haws *et al.* database [6] indicate that the REP of PCB 126 is significantly lower for human cells/cell lines compared to those derived from sensitive laboratory animals. This interspecies information, along with "in press" data from Silkworth *et al.* [8], was provided to the WHO expert panel during deliberations on the 2005 TEFs [1]. Although the WHO panel recognized possible species differences in REPs for PCB 126, the panel deemed, "…the present information too limited to make a decision other than to retain 0.1 as the WHO 2005 TEF." [1] Table 1 highlights *in vitro* REP data available for PCB 126 since the last update of the TEFs. Clearly, the human cell-based REP estimates are consistently lower than rodent and non-human primate estimates. This species difference has been demonstrated in cells from several critical tissues and replicated using multiple measurements of AHR activation, including microarray analysis. Furthermore, several studies have conducted side-by-side interspecies comparisons [8,9,10,11]. Overall, rodent-derived (mostly rat) PCB 126 REP estimates from *in vitro* and *in vivo* studies [6] are consistent with the current TEF of 0.1. Conversely, human *in vitro* REP estimates for PCB 126 are approximately 30-fold lower, i.e., 0.003.

The likelihood that any human *in vitro* REP measurement accurately reflects the *in vivo* REP can be examined by comparing laboratory animal-derived REPs obtained using *in vitro* assays versus *in vivo* studies. If the *in vitro* and *in vivo* REPs are in concordance for animals, then they are likely to be in concordance from humans. Figure 1 compares *in vivo* to *in vitro* laboratory animal-derived REPs for PCB 126 contained in the Haws *et al.* [6] database. The animal *in vitro* (n=21) and *in vivo* (n= 86) REPs for PCB 126 are in concordance, suggesting analogous pharmacokinetics for these congeners and differences in congener potency relate primarily to differential toxicodynamic characteristics. This finding is consistent with the observation of similar toxicokinetics for toxic equivalent (TEQ) doses of TCDD and PCB 126 in chronic rodent bioassays [12,13]. The metabolism of TCDD and PCB 126 is extremely slow and likely involves hepatic CYP1A enzymes. The tissue distribution patterns of both TCDD and PCB 126 rely upon hepatic sequestration where these congeners bind to basal and inducible levels CYP1A2 [14,15], an enzyme inducible by both congeners in human and rat primary

hepatocytes [8]. Furthermore, TCDD and PCB 126 bind to human and rat CYP1A2 with comparable affinities [16] and this enzyme is constitutively expressed at high levels in the liver of both species. Therefore, the weight-of-evidence suggests that TCDD and PCB 126 likely possess comparable toxicokinetics at TEQ-adjusted doses in both laboratory rodent strains and humans.

Table 1. *in vitro* REP data available for PCB 126 since 2005.

Species	Study	Cell Type	Tissue	Relative Potency [a]
Rat	Silkworth *et al.* (2005) [8]	Primary hepatocytes	Liver	0.12
Mouse	Peters *et al.* (2006) [17]	Hepa1c1c7 (altered)	Liver	0.04[b], 0.1[b], 0.02
Rat	Peters *et al.* (2006) [17]	H4IIE (altered)	Liver	0.03[b], 0.08[b], 0.1
Rat	Westerink *et al.* (2008) [10]	H4IIE	Liver	0.625 (??)[c], 1.35 (??)[c]
Rat	Carlson *et al.* (2009) [9]	Primary hepatocytes	Liver	0.06 (95% CI , 0.03-0.1)[d]
Rat	Sutter *et al.* (2010) [18]	H4IIE	Liver	0.082 (95% CI, 0.062-0.108)
Rat	Krckova *et al.* (2011) [19]	WB-F344 (liver epi.)	Liver	0.2
Rat	Krckova *et al.* (2011) [19]	RLE-6TN (lung epi.)	Lung	0.2
Rat	Neser *et al.* (2011) [11]	Primary hepatocytes	Liver	0.11[b], 0.058
Rat	Neser *et al.* (2011) [11]	H4IIE	Liver	0.12[b], 0.093
Rhesus monkey	Silkworth *et al.* (2005) [8]	Primary hepatocytes	Liver	0.13
Cyn. monkey	Peters *et al.* (2006) [20]	Primary hepatocytes	Liver	0.1± 0.04
Human	**Silkworth *et al.* (2005) [8]**	**HEPG2**	**Liver**	**0.002[b]**
Human	**Silkworth *et al.* (2005) [8]**	**Primary hepatocytes**	**Liver**	**0.003[b]**
Human	**vanDuursen *et al.*(2005) [21]**	**Per. lymphocytes**	**Blood**	**0.056[b], 0.006**
Human	**Westerink *et al.* (2008) [10]**	**HEPG2**	**Liver**	**0.02 (??)[b], 0.02 (??) [b,c]**
Human	**Carlson *et al.* (2009) [9]**	**Primary hepatocytes**	**Liver**	**0.002 (95% CI, 0.001-0.005)[e]**
Human	**vanDuursen *et al.*(2010) [22]**	**Per. lymphocytes**	**Blood**	**0.003**
Human	**Sutter *et al.* (2010) [18]**	**Keratinocytes**	**Skin**	**0.0022 (95%CI, 0.0019-0.0025)**
Human	**Neser *et al.* (2011) [11]**	**HEPG2**	**Liver**	**0.013[b], 0.0050**

[a] Multiple relative potency values from a single study are presented if more than one method to estimate relative potency was employed
[b] Denotes that apparent partial agonism of PCB 126 was not taken into account
[c] The EC50 value of TCDD is clearly incorrect comparing data in Figure 1A/B to EC50s listed in Table 2 of Westerink *et al* (2008), making these REP estimations unreliable (i.e., over-estimated)
[d] Geometric mean of REPs for 79 rat genes modeled for TCDD and PCB 126 dose response
[e] Geometric mean of REPs for 47 human genes modeled for TCDD and PCB 126 dose response

Using a parallelogram approach, the TEF for PCB 126 should be adjusted to 0.003. This adjustment is based upon (1) concordant REPs from *in vitro* and *in vivo* studies using laboratory animals and (2) interspecies differences in *in vitro*-derived REPs between humans and animals. Interspecies extrapolation is generally conducted with consideration of both toxicokinetic and toxicodynamic components [23]. For receptor-mediated

mechanisms, *in vitro* analyses of receptor activation in target tissues/cells are direct toxicodynamic measurements of bioavailable chemical. There is a scientific consensus that AHR activation is the initial key event in the mode of action for all "dioxin-like" toxicities. Quantification of *in vitro* activation of the AHR is likely the only mechanism to analyze relative differences in toxicodynamics among TCDD and DLCs in humans. Although improvements to the current TEF approach have been proposed, such as replacement of TEF point estimates with REP distributions and weighting frameworks [24, 25], none are designed to specifically address significant differences in congener REP between animal models and humans. Thus, evidence of species differences in sensitivity of responsive cells should be formally incorporated into the TEF scheme, as recommended by the National Academy of Sciences [26].

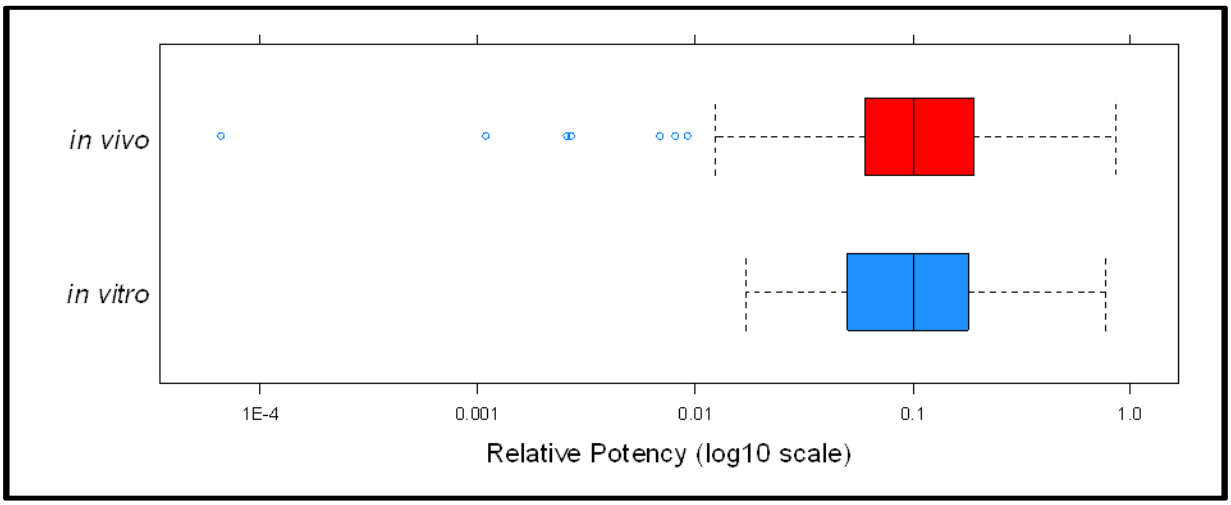

Figure 1. Standard Tukey boxplots comparing PCB 126 REP estimates derived from *in vitro* (n=21) versus *in vivo* (n=86) studies contained in the Haws *et al.* (2008) database. Since the objective was to compare laboratory animal responses, the eight REPs from *in vitro* studies using human cells were excluded. Center black line of each box represents the median value and hinges are the first and third quartiles. Whiskers extend to the most extreme data point no more than 1.5 times the interquartile range away from the box. Open blue circles represent the outliers.

Conclusion

Although humans have been exposed to high concentrations of PCBs in the past, there is no consistent evidence that the PCBs themselves, rather than non-PCB contaminants/co-exposures (e.g., PCDFs, chlorobenzenes), actually generated any "dioxin-like" health effects [27, 28]. This finding is rather disconcerting, particularly since: (1) the USEPA has suggested that TEFs can be used to project all cancer and non-cancer toxic endpoints attributed to TCDD to mixtures of DLCs [2]; (2) the reference dose for TCDD is set at a level below current US background intake extrapolated from a rare human exposure to extremely high levels of TCDD (i.e., the Seveso poisoning event)[3]; and, (3) TEFs rely upon rodent bioassays that do not account for established differences in species sensitivity to TCDD and DLCs. We propose that TEFs be adjusted for any interspecies differences in sensitivity to DLCs, using an approach similar to that described here, prior to use of the dioxin toxic equivalency method in human health risk assessment.

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