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Differential relative effect potencies of some dioxin-like compounds in human peripheral blood lymphocytes and murine splenic cells

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HIGHLIGHTS

- Relative potencies (REPs) of dioxin-like compounds (DLCs) in lymphocytes (PBLs).
- REPs for some DLCs varied by species (human vs. mouse) and congener.
- REPs for PCB 126 in human PBLs significantly lower than WHO TEF.
- *In vitro*-derived REPs similar to systemic REPs from *in vivo* experiments.
- Human PBL-derived REPs contribute to improved human risk assessment for DLCs.

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ABSTRACT

Human risk assessment for dioxin-like compounds is typically based on the concentration measured in blood serum multiplied by their assigned toxic equivalency factor (TEF). Consequently, the actual value of the TEF is very important for accurate human risk assessment. In this study we investigated the effect potencies of three polychlorinated dibenzo-*p*-dioxins (PCDDs), six polychlorinated dibenzofurans (PCDFs) and 10 polychlorinated biphenyls (PCBs) relative to the reference congener 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) in *in vitro* exposed primary human peripheral blood lymphocytes (PBLs) and mouse splenic cells. REPs were determined based on cytochrome P450 (CYP) *1A1*, *1B1* and aryl hydrocarbon receptor repressor (*AhRR*) gene expression as well as CYP1A1 activity in human PBLs and *Cyp1a1* gene expression in murine splenic cells. Estimated median human REPs for 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (1234678-HpCDD), 2,3,4,7,8,-pentachlorodibenzofuran (23478-PeCDF), 1,2,3,4,7,8-hexachlorodibenzofuran (123478-HxCDF) and 1,2,3,4,7,8,9-heptachlorodibenzofuran (1234789-HpCDF) were with 0.1, 1.1, 1 and 0.09, respectively, significantly higher compared to those estimated for mouse with REPs of 0.05, 0.45, 0.09 and 0.04, respectively. Opposite to these results, the estimated median human REP of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), was with 0.001 30-fold lower compared to the mouse REP of 0.03. Furthermore, human REPs for 1234678-HpCDD, 23478-PeCDF, 123478-HxCDF, 1234789-HpCDF and PCB 126 were all outside the ± half log uncertainty range that is taken into account in the WHO-assigned TEFs. Together, these data show congener- and species-specific differences in REPs for some, but not all dioxin-like congeners tested. This suggests that, more emphasis should be placed on human-tissue derived REPs in the establishment of a TEF for human risk assessment.

Abbreviations: AhR, aryl hydrocarbon receptor; BMR, benchmark response; CYP, cytochrome P450; DLC, dioxin-like compound; EROD, ethoxyresorufin-*O*-deethylase; HepG2, human hepatoblastoma cell line; 1234678-HpCDD, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin; 123678-HxCDD, 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin; 1234678-HpCDF, 1,2,3,4,6,7,8-heptachlorodibenzofuran; 1234789-HpCDF, 1,2,3,4,7,8,9-heptachlorodibenzofuran; 123478-HxCDF, 1,2,3,4,7,8-hexachlorodibenzofuran; 234678-HxCDF, 2,3,4,6,7,8-hexachlorodibenzofuran; NDL, non-dioxin-like; PBL, peripheral blood lymphocytes; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyls; PCB 74, 2,4,4′,5-tetrachlorobiphenyl; PCB 77, 3,3′,4,4′-tetrachlorobiphenyl; PCB 105, 2,3,3′,4,4′-pentachlorobiphenyl; PCB 118, 2,3′,4,4′,5-pentachlorobiphenyl; PCB 126, 3,3′,4,4′,5-pentachlorobiphenyl; PCB 153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; PCB 156, 2,3,3′,4,4′,5-hexachlorobiphenyl; PCB 167, 2,3′,4,4′,5,5′-hexachlorobiphenyl; PCB 169, 3,3′,4,4′,5,5′-hexachlorobiphenyl; PCB 189, 2,3,3′,4,4′,5,5′-heptachlorobiphenyl; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofuran; 12378-PeCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin; 23478-PeCDF, 2,3,4,7,8,-pentachlorodibenzofuran; REP, relative effect potency; TCDD, 2,3,7,8-tetrachlorodibenzodioxin; 2378-TCDF, 2,3,7,8-tetrachlorodibenzofuran; TEF, toxic equivalency factor.

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1. Introduction

The estimation of human risk for polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) is typically based on the concentration measured in blood serum multiplied by their toxic equivalency factor (TEF) assigned by the World Health Organization (Van den Berg et al., 2006). Consequently, the actual value of the TEF is crucial for accurate human risk assessment. Each congener-specific TEF expresses the relative aryl hydrocarbon receptor (AhR)-mediated potency of a dioxin-like compound (DLC) compared to 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD), the most potent and well-studied congener. Although each TEF is derived from a large number of relative effect potencies (REPs), these REPs are primarily based on rodent *in vivo* and *in vitro* data (Haws et al., 2006). However, human *in vitro* models show that the potency of a number of congeners may differ from those derived from rodent studies. For example, 2,3,4,7,8-pentachlorodibenzofuran (23478-PeCDF, WHO-TEF 0.3), 1,2,3,4,7,8-hexachlorodibenzofuran (123478-HxCDF, WHO-TEF 0.1) and 1,2,3,6,7,8-hexachlorodibenzofuran (123678-HxCDF, WHO-TEF 0.1) were found to be as potent as TCDD in human lymphoblastoid cells reporting aryl hydrocarbon hydroxylase (AHH)-inducing potency or for induction of cytochrome P450 1A1 (*CYP1A1*) gene expression in human keratinocytes (Nagayama et al., 1985; Sutter et al., 2010). Also, the potency of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) for ethoxyresorufin-*O*-deethylase (EROD) activity or *CYP1A1* mRNA induction in primary human hepatocytes, keratinocytes, peripheral blood lymphocytes (PBLs) and human hepatoblastoma cells (HepG2) is generally up to 100-fold lower than expected based on its WHO-TEF of 0.1 (Silkworth et al., 2005; Sutter et al., 2010; Van Duursen et al., 2005; Zeiger et al., 2001). However, even though these species-specific differences in potency, in particular for PCB 126, were acknowledged by the expert panel during the WHO-TEF re-evaluation in 2005, it was concluded that more information regarding the difference between rodents and humans is needed (Van den Berg et al., 2006). Furthermore, in aforementioned studies only a few congeners assigned with a TEF-value have been analyzed, while these species-specific differences in potency might also concern other DLCs. Within this study, we determined species-specific differences in potency for CYP1A1 activity and *CYP1A1*, *CYP1B1* and aryl hydrocarbon receptor repressor (*AhRR*) gene expression for 20 selected congeners consisting of four PCDDs, six PCDFs, eight dioxin-like PCBs and two non-dioxin-like (NDL) PCBs in primary human PBLs and murine splenic cells. As human peripheral blood is easy to collect, it is an interesting matrix for monitoring human health. Changes in AhR-mediated gene expressions in PBLs are widely used as biomarkers of human exposure to DLCs and polycyclic aromatic hydrocarbons (PAHs), despite the uncertainties and interindividual variability in their responses (Guida et al., 2013; Hanaoka et al., 2002; Hu et al., 2006; McHale et al., 2007; Van Duursen et al., 2005). Furthermore, present concerns regarding responses in human populations upon DLC exposure are more and more focused on extra-hepatic responses, including (neuro)development, reproductive functions, immunotoxicity and extra-hepatic carcinogenic responses (ECSCF, 2001; IARC, 2012; JECFA, 2001; Lauby-Secretan et al., 2013; UKCOT, 2001; USEPA, 2012). Consequently, studies with respect to human responses that focus on extra-hepatic tissues might be of more interest from a human risk assessment point of view.

In this study, the 20 selected congeners were divided into two groups. Group one consisted of seven congeners, *i.e.* TCDD, 1,2,3,7,8-pentachlorodibenzodioxin (12378-PeCDD), 23478-PeCDF, PCB 126, 2,3′,4,4′,5-pentachlorodiphenyl (PCB 118), 2,3,3′,4,4′,5-hexachlorobiphenyl (PCB 156) and the NDL-PCB, 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) that represent approximately 90% of the dioxin-like activity in the human food chain (Liem et al., 2000). These congeners have also been studied in an *in vivo* mouse study, where congener-specific REPs in liver and PBLs were calculated based on administered dose as well as based on liver, adipose tissue and blood plasma concentrations (van Ede et al., 2013). This allows us to compare *in vitro* with *in vivo* derived REPs based on either the administered dose or systemic concentrations. A second group of 13 congeners consisted of two PCDDs, five PCDFs, five DL-PCBs and one NDL-PCB, which are commonly found in human tissues and the food chain, but are of lower toxicological meaning.

2. Materials and methods

2.1. Chemicals

TCDD, 12378-PeCDD, 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (123678-HxCDD), 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (1234678-HpCDD), 2,3,7,8-tetrachlorodibenzofuran (2378-TCDF), 23478-PeCDF, 123478-HxCDF, 2,3,4,6,7,8-hexachlorodibenzofuran (234678-HxCDF), 1,2,3,4,6,7,8-heptachlorodibenzofuran (1234678-HpCDF), 1,2,3,4,7,8,9-heptachlorodibenzofuran (1234789-HpCDF) and PCB 126 were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). PCB118, PCB156 and PCB153 were purchased from Cerilliant Corp. (Round Rock, TX, USA). 2,4,4′,5-tetrachlorobiphenyl (PCB 74), 3,3′,4,4′-tetrachlorobiphenyl (PCB 77), 2,3,3′,4,4′-pentachlorobiphenyl (PCB 105), 2,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 167), 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 169), 2,3,3′,4,4′,5,5′-heptachlorobiphenyl (PCB 189) were purchased from Larodan Fine Chemicals (Malmö, Sweden). All congeners had a purity >99% except for 1234678-HpCDD (98.7%). The congeners were dissolved and diluted in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Stockholm, Sweden).

2.2. Cell preparation, culture and exposure

Human buffy coat from 11 healthy volunteers consisting of six male (age 24-, 43-, 46-, 55-, 65-, 66-years old) and five female (age 25-, 25-, 38-, 46-, 57-years old) all living in The Netherlands were obtained from Sanquin Blood Supply (Sanquin Blood Supply, Amsterdam, The Netherlands). The study was evaluated and approved by the Sanquin Executive Board and a written informed consent was obtained from all donors. PBLs were isolated using Ficoll-Paque gradient according to the manufacturer's instructions (GE Healthcare Europe, Diegem, Belgium). Murine splenic cells were isolated from 10-week old female C57Bl/6 mice purchased from Harlan laboratories (Venray, The Netherlands). Animals were euthanized by CO_2/O_2 and spleens were removed. To obtain a single cell spleen suspension, spleens were pressed through a 70 μm cell strainer (BD Biosciences, Bedford, MA, USA) and red blood cells were lysed with lysis reagent (containing 155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.8). The animals were handled in a humane manner and the study was approved by the Animal Ethical Committee (DEC Utrecht, Utrecht, The Netherlands).

Human PBLs were suspended in culture medium consisting of phenol red-free RPMI 1640 supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Breda, the Netherlands), 100 U/mL penicillin, 100 μg/mL streptomycin (Invitrogen) and 1.5% phytohaemagglutin (PHA) (Life Technologies, Bleiswijk, the Netherlands). Murine splenic cells were suspended in culture medium consisting of phenol red-free RPMI 1640 supplemented with 10% fetal bovine serum (FBS) (Invitrogen), 100 U/mL penicillin, 100 μg/mL streptomycin (Invitrogen) and 5 μg/mL Concanavalin A (Con A) (Calbiochem, Merck Millipore, Darmstadt, Germany). Cell concentrations were determined using a Beckman Coulter