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Relative effective potencies of dioxin-like compounds in rodent and human lung cell models

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ABSTRACT

Toxicity of dioxin-like compounds (DLCs), such as polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls, is largely mediated via aryl hydrocarbon receptor (AhR) activation. AhR-mediated gene expression can be tissue-specific; however, the inducibility of AhR in the lungs, a major target of DLCs, remains poorly characterized. In this study, we developed relative effective potencies (REPs) for a series of DLCs in both rodent (MLE-12, RLE-6TN) and human (A549, BEAS-2B) lung and bronchial epithelial cell models, using expression of both canonical (*CYP1A1*, *CYP1B1*) and less well characterized (*TIPARP*, *AHRR*, *ALDH3A1*) AhR target genes. The use of rat, murine and human cell lines allowed us to determine both species-specific differences in sensitivity of responses to DLCs in lung cellular models and deviations from established WHO toxic equivalency factor values (TEF) values. Finally, expression of selected AhR target genes was determined *in vivo*, using lung tissues of female rats exposed to a single oral dose of DLCs and compared with the obtained *in vitro* data. All cell models were highly sensitive to DLCs, with murine MLE-12 cells being the most sensitive and human A549 cells being the least sensitive. Interestingly, we observed that four AhR target genes were more sensitive than *CYP1A1* in lung cell models (*CYP1B1*, *AHRR*, *TIPARP* and/or *ALDH3A1*). We found some deviations, with strikingly low REPs for polychlorinated biphenyls PCBs 105, 167, 169 and 189 in rat RLE-6TN cells-derived REPs for a series of 20 DLCs evaluated in this study, as compared with WHO TEF values. For other DLCs, including PCBs 126, 118 and 156, REPs were generally in good accordance with WHO TEF values. This conclusion was supported by *in vivo* data obtained in rat lung tissue. However, we found that human lung REPs for 2,3,4,7,8-pentachlorodibenzofuran and PCB 126 were much lower than the respective rat lung REPs. Furthermore, PCBs 118 and 156 were almost inactive in these human cells. Our observations may have consequences for risk assessment. Given the differences observed between rat and human data sets, development of human-specific REP/TEFs, and the use of *CYP1B1*, *AHRR*, *TIPARP* and/or *ALDH3A1* mRNA inducibility as sensitive endpoints, are recommended for assessment of relative effective potencies of DLCs.

1. Introduction

The aryl hydrocarbon receptor (AhR) is a cytosolic ligand-activated transcription factor, a member of basic helix-loop-helix/Per Arnt Sim family of transcriptional regulators, which plays a key role in many physiological and pathophysiological processes, including toxicity of persistent polyhalogenated aromatic pollutants (White and Birnbaum, 2009; Murray et al., 2014; Denison and Farber, 2017; Kolluri et al., 2017). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent dioxin known and is often used as reference toxicant for estimation of "dioxin-like" toxicity. TCDD exhibits its adverse effects via a sustained, non-physiological AhR activation. Likewise, the toxicities of other dioxin-like compounds (DLCs) are quantified based on their ability to activate the AhR, as the capacity to activate the AhR is considered to be directly linked to their biological and toxic effects. Therefore, for risk assessment of DLCs, the toxic equivalency factor (TEF) approach has been established, based on their potencies to activate AhR-dependent endpoints, including expression of AhR target genes, such as cytochrome P450 (CYP) 1A1 and/or other drug metabolizing enzymes, relatively to TCDD. The current consensus WHO TEFs are based on

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multiple relative effect potencies (REPs) through combining available *in vitro* and *in vivo* studies (Haws et al., 2006; Van den Berg et al., 2006).

The differential sensitivity of various species to DLCs has been known for many years and can be linked to both species-specific binding affinities of DLCs to the AhR and to differential sets of target genes being regulated in various species (Denison et al., 2011; Murray et al., 2014). Also, toxicokinetic factors might play a role, especially when comparing *in vivo* REPs and *in vitro* REPs for DLCs that are rapidly metabolized. We have recently shown a large variation in bioassay sensitivity for AhR related readouts over several species, including rodents and humans (Ghorbanzadeh et al., 2014; Larsson et al., 2015). A huge variability has been also observed across cells of similar tissue origin, derived from mouse, rat and human hepatomas, with only a very limited set of genes being regulated similarly despite the same tissue origin (Dere et al., 2011). Similar to species specificity, tissue or cell specificity appears to be an important issue, when establishing REP values for DLCs. Although the lung is a significant target of TCDD (Walker et al., 2007; Yoshizawa et al., 2007), REP values for DLCs in lung tissue or lung cells are not well characterized. We identified only two subchronic *in vivo* studies focusing on estimation of REPs in murine lung (DeVito et al., 1997, 2000). No quantitative *in vitro* experimental data are yet available for development of REP values of DLCs in lung cells.

Therefore, the primary objective of the present study was to identify AhR target genes common for rodent and human lung, and to estimate REP values for a set of nineteen DLCs, based on the induction of canonical AhR target genes *CYP1A1* and *CYP1B1* in rat lung epithelial cells. In the second part of this study, REPs for individual genes and six selected DLCs were then compared across different species. We focused on estimation of species differences in sensitivity of lung cells for DLC-induced AhR target gene expression. In addition, we evaluated other frequently used AhR target genes, besides *CYP1A1*, which could be potentially more sensitive or inducible in these *in vitro* lung models. We addressed the following questions: i) are lung REPs in agreement with established WHO TEF values; ii) are there any significant differences among REPs developed in rat and human lung cells; iii) and, finally, are rat lung *in vitro* REP values comparable with *in vivo* rat data.

To answer these questions, we assessed gene expression changes of five AhR target genes (*CYP1A1*, *CYP1B1*, *TIPARP*, *AHRR* and *ALDH3A1*) in well-established rat, human and mouse models of lung or bronchial epithelial cell lines and compared full concentration scale-based responses. For rodent models, we used the following cell lines: rat lung epithelial RLE-6TN cell line, a model of alveolar type II epithelial cells (Driscoll et al., 1995), and non-tumorigenic murine lung epithelial cell line MLE-12 (Malkinson et al., 1997). For determination of human REPs, we used the human lung adenocarcinoma A549 cell line (representing again type II pneumocytes) and human non-transformed bronchial epithelial BEAS–2 B cells. Both human cell lines are frequently used in airway and lung toxicity studies (Bajaj et al., 2016).

2. Material and methods

2.1. Chemicals

A set of 4 PCDDs, 6 PCDFs, and 10 PCBs were selected (based on WHO-TEF values, number of chlorine atoms, substitution pattern, and environmental abundance). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (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 (TCDF), 2,3,4,7,8,-pentachlorodibenzofuran (23478-PeCDF), 1,2,3,4,7,8-hexachlorodibenzofuran (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 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). 2,3′,4,4′,5-Pentachlorobiphenyl (PCB118), 2,3,3′,4,4′,5-hexachlorobiphenyl (PCB156), and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB153) were purchased from Cerilliant Corp. (Round Rock, TX, USA). 2,4,4′,5-Tetrachlorobiphenyl (PCB74), 3,3′,4,4′-tetrachlorobiphenyl (PCB77), 2,3,3′,4,4′-pentachlorobiphenyl (PCB105), 2,3′,4,4′,5,5′-hexachlorobiphenyl (PCB167), 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB169), and 2,3,3′,4,4′,5,5′-heptachlorobiphenyl (PCB189) were purchased from Larodan Fine Chemicals (Malmö, Sweden). The mono-ortho substituted PCBs 118 and 156 as used for the *in vivo* experiments were purified using a charcoal column methodology as described in van Ede et al. (2014). All remaining 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). Pharmacological inhibition of AhR function was performed using CH223191 AhR antagonist at 10 μM concentration (Calbiochem, Darmstadt, Germany). DMSO and all other chemicals were supplied by Sigma-Aldrich (Prague, Czech Republic), if not stated otherwise.

2.2. Cells

The rat lung epithelial RLE-6TN cell line was obtained from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Hamś F12 medium (Invitrogene, Carlsbad, CA) supplemented with 2 mM L-glutamine, bovine pituitary extract (10 μg/ml), insulin (5 μg/ml), insulin-like growth factor (2,5 ng/ml), transferrin (1.25 μg/ml), epidermal growth factor (2.5 ng/ml) and 5% heat-inactivated fetal bovine serum (PAA, Pasching, Austria). The human lung epithelial A549 (ATCC) cells were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogene, Carlsbad, CA) supplemented with 25 mM sodium bicarbonate, 10 mM HEPES, and 10% heat-inactivated fetal bovine serum (PAA). Human bronchial epithelial BEAS–2 B (ATCC) cells were grown in a 1:1 mixture of DMEM with Hamś F12 (Invitrogene, Carlsbad, CA), supplemented with 25 mM sodium bicarbonate, 10 mM HEPES, and 5% heat-inactivated fetal bovine serum (PAA). The mouse lung epithelial MLE-12 (ATCC) cell line was cultured in HITES medium with 2.5% fetal bovine serum, as formulated by ATCC. All cells were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. The cells were routinely subcultured twice a week; passages between 15 and 25 were used. The A549 cells were seeded at density 85,000 cells/ml, the cell culture medium was changed after 72 h and the cells were exposed to a tested compound or vehicle (DMSO) for 24 h. Experimental design for other cell lines was as follows: BEAS–2 B cells were seeded at density of 95,000 cells/ml, and grown for 72 h growing before the change of the medium and exposure; MLE-12 cells were seeded at density 110,000 cells/ml, and grown for 72 h before the change of the medium and exposure; RLE-6TN cells were seeded at density 150,000 cells/ml, and grown for 120 h before the change of the medium and exposure. All tested cells were exposed to test DLCs or 0.1% *v/v* DMSO (vehicle control) for 24 h and total RNA was then isolated from cell lysates using NucleoSpin RNA II kit (Macherey-Nagel, GmbH, Duren, Germany) according to manufacturer's instructions.

2.3. In vivo samples

Rat lung tissue was obtained from the previously described *in vivo* study (van Ede et al., 2014), where DLC-induced biomarkers were examined in liver and peripheral blood lymphocytes. Briefly, eight-week-old female Sprague-Dawley rats (n = 6 per group) were treated with a single dose of the respective DLC by oral gavage and sacrificed after 3 days. The organs were then immediately removed, directly snap frozen in liquid nitrogen and stored at −80 °C. For the present study, total RNA was isolated from frozen lung tissue samples, using NucleoSpin RNA II kit (Macherey-Nagel). Thirty mg of each tissue sample was lysed in 1 ml of RA1 buffer with 10 μl β-mercaptoethanol and homogenized by 2.5-mm glass beads in a TissueLyser II (Retsch GmbH, Haan, Germany), using 30 Hz frequency for 40 s. The homogenate was then centrifuged (14,000 × *g*, 10 min) and 350 μl of supernatant was

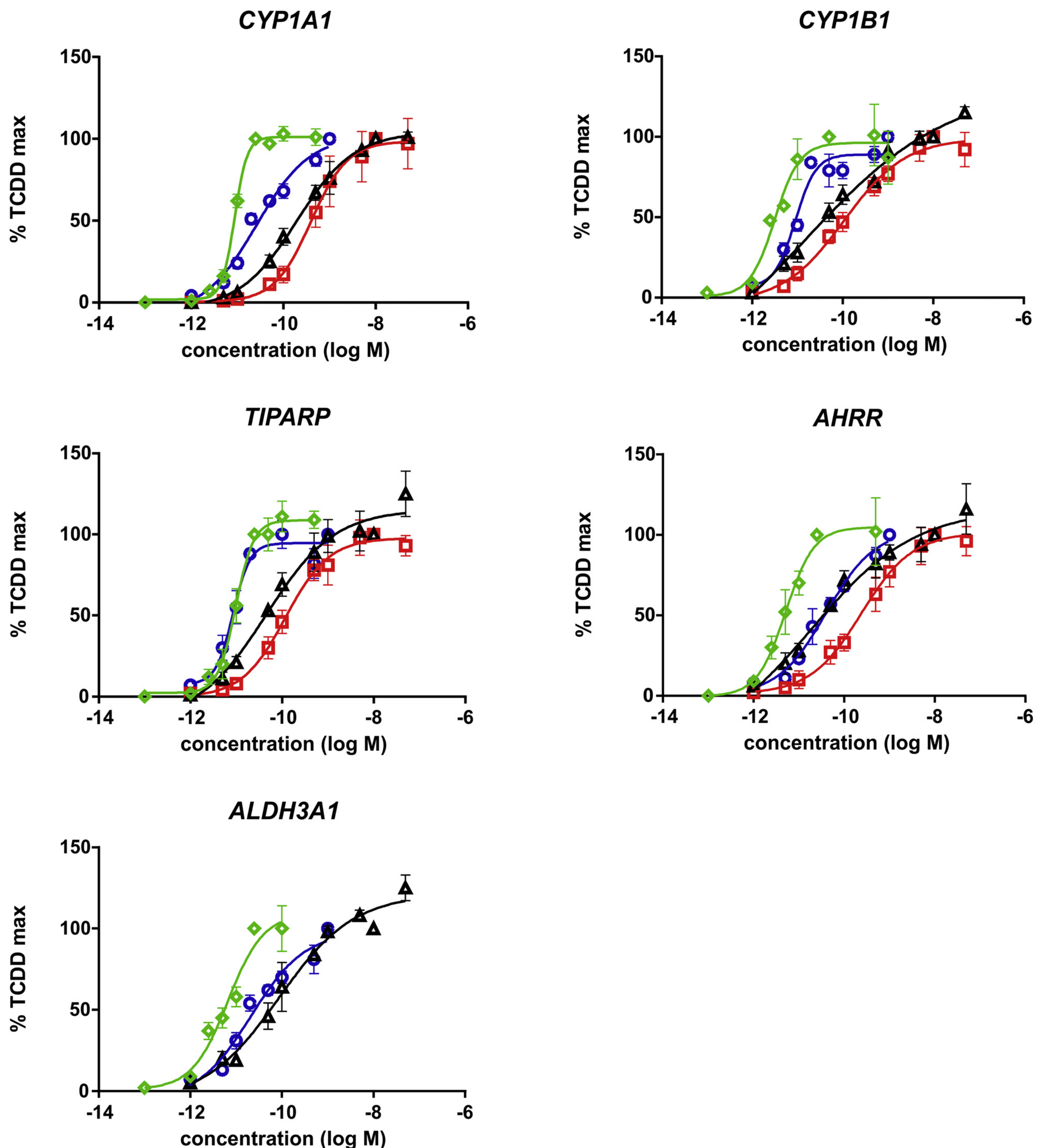

Fig. 1. Comparison of sensitivity of individual mRNA induction to TCDD in rat lung epithelial RLE-6TN cells (blue line), murine lung epithelial MLE-12 cells (green line), human lung adenocarcinoma A549 cells (red line) and human bronchial epithelial BEAS–2 B cells (black line) after 24-h exposure. Cells were treated with TCCD or vehicle (DMSO) and the levels of *CYP1A1*, *CYP1B1*, *AHRR*, *TIPARP* and *ALDH3A1* mRNA transcripts were determined by qRT-PCR. The results are expressed as% of TCDD maximal induction (means ± S.D. of three independent experiments conducted in triplicates).

transferred onto a NucleoSpin Filter unit placed in a collection tube and total RNA was isolated NucleoSpin RNA II kit was according to the manufacture's specifications.

2.4. Real time RT-PCR

The amplifications of the samples were carried out using QuantiTect Probe RT-PCR kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's specifications. The sequences of human, rat and murine primers and probes are presented in Supplementary Table S1. The amplifications were run on the LightCycler 480 II (Roche Diagnostics GmbH, Mannheim, Germany) using the following program: reverse transcription at 50 °C for 20 min and initial activation step at 95 °C for 15 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 60 s for TaqMan and UPL probes (Roche). For determination of rat *CYP1A1* and *ALDH3A1* the FRET probes were used with following program: reverse transcription at 50 °C for 20 min and initial activation step at 95 °C for 15 min, followed by 45 cycles at 95 °C for 15s, 58 °C for 30 s and 72 °C

for 30 s. Gene expression for each sample was expressed in terms of the threshold cycle (Ct), normalized to housekeeping gene porphobilinogen deaminase (ΔCt). ΔCt values were then compared between control samples (DMSO 0.1%) and samples treated with 2,3,7,8-TCDD and dioxin-like compounds to calculate ΔΔCt (ΔCt [control] – ΔCt [treatment]). No statistically significant impact of treatments on expression of reference gene was observed. The final comparison of transcript ratios between samples is given as $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). List of primers and probes, used in the study, is provided as Supplemental Table S1.

2.5. Data analysis and statistical analysis

Dose-response curves, effect concentrations and relative effect potency (REP) calculations were determined as described previously (van Ede et al., 2013, 2014), using a sigmoidal dose-response nonlinear regression curve fit with variable slope (GraphPad Prism 6.01, GraphPad Software Inc., San Diego, CA) and a benchmark response approach – the dose or concentration needed for a tested congener to reach 20% of the TCDD response, BMR_{20TCDD} (= EC_{20}). Additionally, when possible, EC_{50} values were also determined and used for REP estimation. All REP values were expressed relative to TCDD. When verifying the AhR-specific response with AhR antagonist, comparisons between treatments were made by one-way analysis of variance (ANOVA).

3. Results

3.1. Selection of AhR target genes, TCDD-dependent induction and verification of AhR target genes in lung cellular models

Based on previously published global gene expression data in human lung epithelial A549 cell line (Martinez et al., 2002) and our preliminary (unpublished) results as well as comparative studies in rat, mouse and human hepatoma cells (Dere et al., 2011), we selected two conventional (*CYP1A1*, *CYP1B1*), and three less frequently used AhR target genes (*TIPARP*, *AHRR*, *ALDH3A1*). Induction of *CYP1A1* and *CYP1B1* is a key AhR-dependent adaptive response to environmental chemical stress serving to detoxify both polycyclic aromatic hydrocarbons and halogenated aromatic compounds (Nebert and Dalton, 2006). Both *CYP1A1* and *CYP1B1* have been commonly used as biomarkers of exposure in numerous studies (Haws et al., 2006). The aryl hydrocarbon repressor (*AHRR*) and its expression has been shown to be AhR-dependent (Mimura et al., 1999). TCDD-inducible poly(ADP-ribose) polymerase (*TIPARP*) is known as a transcriptional repressor of the AhR, which represents a negative feedback loop in AhR signaling (MacPherson et al., 2013; Matthews, 2017). *ALDH3A1* is aldehyde dehydrogenase 3A1, which contributes to cell survival and cellular protection against lipid peroxidation (Black et al., 2012; Muzio et al., 2012).

We first compared potencies of TCDD to induce the expression of selected AhR target genes in rat, murine and human airway cell lines, namely in RLE-6TN, A549, BEAS–2 B and MLE-12 cell lines (Fig. 1). Full concentration-response experiments allowed us to quantify EC_{20} and EC_{50} values for all evaluated genes (Table 1). The only exception was gene expression of *ALDH3A1*, which was not induced by TCDD in A549 cells. Both murine and rat cells elicited similar induction responses, when EC_{20} values were compared. However, the murine MLE-12 cell line was slightly more sensitive. In contrast, the inducibility of all selected genes was significantly lower in human cell lines, with BEAS–2 B cells being slightly more sensitive towards AhR activation by TCDD than A549 cells. Interestingly, *CYP1B1* mRNA appeared to be the most sensitive target across all cell models. Gene expression of *TIPARP* and *AHRR* were also found to be more sensitive markers for AhR activation than *CYP1A1* in human cells (Table 1).

Next, the specificity of AhR-dependent response was verified in A549 and RLE-6TN cells, using co-treatment with pharmacological AhR inhibitor, CH223191, which specifically prevents TCDD-induced AhR activation (Kim et al., 2006). Indeed, the selected genes were regulated in an AhR-dependent manner in both cellular models, with *CYP1A1* mRNA induction being strictly AhR-dependent (Fig. 2).

Table 1
Comparison of EC20 and EC50 values in human, rat and mouse lung epithelial cell lines after 24-h exposure to 2,3,7,8-TCDD.

Gene	A549		BEAS-2B		RLE-6TN		MLE-12	
	EC_{20}	EC_{50}	EC_{20}	EC_{50}	EC_{20}	EC_{50}	EC_{20}	EC_{50}
CYP1A1	0.110	0.389	0.037	0.222	0.006[a]	0.029	0.005	0.009
CYP1B1	**0.016**	**0.123**	**0.006**	**0.086**	**0.003**[a]	**0.008**	**0.001**	**0.003**
TIPARP	**0.027**	**0.120**	**0.010**	**0.063**	0.004	**0.008**	0.005	0.010
AHRR	**0.037**	0.234	**0.005**	**0.049**	0.007	0.040	**0.002**	**0.004**
ALDH3A1	n.i.	n.i.	**0.010**	**0.099**	0.005	0.023	**0.002**	**0.004**

EC_{20} and EC_{50} values were expressed in nM; EC values in bold denote a higher sensitivity of the respective gene to TCDD induction, as compared with CYP1A1 (ratio of respective EC values for CYP1A1 and the analyzed gene ≥ 2); n.i., not induced.
[a] Data reported previously (Larsson et al., 2015).

3.2. Determination of EC and REP values for 20 DLCs in rat lung RLE-6TN cell line

Next, we performed full concentration-response experiments for a set of 20 highly purified PCDDs, PCDFs and PCBs in the rat RLE-6TN cell line, in order to determine EC_{20} (BMR_{20TCDD}) and EC50 values. Induction of *CYP1A1* and *CYP1B1* mRNA was used as a relevant endpoint (Table 2). Using this cell model, we observed a relatively high variability of REP values derived from inducibility of individual AhR target genes. Higher REPs, as compared with WHO-TEF values, were found for 2,3,7,8-tetraCDF, 2,3,4,6,7,8-hexaCDF, 1,2,3,4,7,8,9-heptaCDF, 1,2,3,4,6,7,8-heptaCDD and PCB 77. REP values for PCB 126 were calculated in rat lung epithelial cells in the range of 0.2-0.4, while

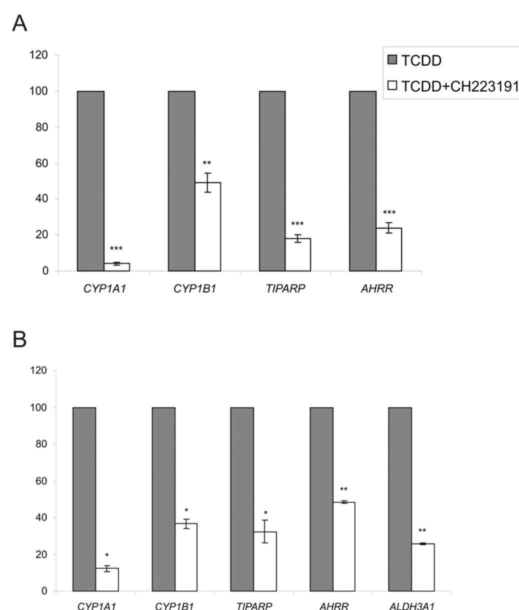

Fig. 2. Confirmation of AhR-dependent regulation of the respective genes in human A549 (**A**) and rat RLE-6TN (**B**) cell lines. Cells were pre-treated with AhR inhibitor CH223191 (10 μM) for 1 h and then exposed to TCDD (10 nM) or 0.1% DMSO (control) for 24 h. Levels of respective mRNAs were determined by qRT-PCR. All data represent results from three independent experiments performed in triplicates and are expressed as means ± S.D. Comparisons between individual treatments were made with ANOVA; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Table 2
REP values for 19 DLCs estimated based on induction of *CYP1A1* and *CYP1B1* mRNAs in RLE-6TN cells after 24-h exposure, compared with re-evaluated WHO-TEFs (Van den Berg et al., 2006).

compound	*CYP1A1*					*CYP1B1*					
	max. induction	BMR_{20TCDD} (EC_{20})[a]	REP (EC_{20})	EC_{50}	REP (EC_{50})	max. induction	BMR_{20TCDD} (EC_{20})[a]	REP (EC_{20})	EC_{50}	REP (EC_{50})	TEF (WHO 2005)
2,3,7,8-TCDD	100	0.006	1	0.029	1	100	0.003	1	0.008	1	1
1,2,3,7,8-PeCDD	93	0.012	0.5	0.049	0.6	115	0.005	0.6	0.016	0.5	1
2,3,4,7,8-PeCDF	93	0.135	0.05	0.676	0.04	118	0.058	0.06	0.257	**0.03**	0.3
PCB126	87	0.027	0.2	0.141	0.2	104	0.008	0.4	0.043	0.2	0.1
PCB118	60	562	0.00001	7413	0.000004	78	151	0.00002	562	0.00001	0.00003
PCB153	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	–
PCB156	65	46	0.0001	759	0.00004	108	7	**0.0005**	52	0.0002	0.00003
PCB74	45	1413	0.000004	n.a.	n.a.	71	263	0.000012	1380	0.00001	–
PCB77	67	1	**0.006**	14	**0.002**	103	0.282	**0.0115**	4	**0.002**	0.0001
PCB105	51	617	0.00001	n.a.	**n.a.**	84	89	0.00004	490	0.00002	0.00003
PCB167	26	5012	**0.000001**	n.a.	**n.a.**	55	447	0.000007	5623	**0.000001**	0.00003
PCB169	67	1	0.004	7	0.004	97	0.501	0.006	2	0.004	0.03
PCB189	60	871	0.000007	11482	**0.000003**	86	85	0.00004	550	0.00002	0.00003
2,3,7,8-tetraCDF	122	0.008	0.7	0.030	1.0	106	0.004	0.9	0.009	**1.0**	0.1
1,2,3,4,7,8-hexaCDF	108	0.025	0.3	0.110	0.3	113	0.006	0.6	0.032	0.26	0.1
2,3,4,6,7,8-hexaCDF	107	0.040	**0.2**	0.158	**0.2**	133	0.005	**0.69**	0.042	**0.20**	0.01
1,2,3,6,7,8-hexaCDD	101	0.107	0.06	0.513	0.06	88	0.023	0.14	0.182	0.05	0.1
1,2,3,4,7,8,9-heptaCDF	111	0.100	0.06	0.759	0.04	101	0.033	**0.10**	0.117	0.07	0.01
1,2,3,4,6,7,8-heptaCDF	91	0.468	0.01	1.445	0.02	94	0.102	0.03	0.347	0.02	0.01
1,2,3,4,6,7,8-heptaCDD	105	0.112	0.05	0.347	0.08	146	0.008	**0.43**	0.087	**0.10**	0.01

Estimation of REPs was performed based on either EC20 or EC50 values (expressed in nM), using a sigmoidal dose-response non-linear regression curve fit with variable slope; REP values in bold represent REP values deviating by more than 1 order of magnitude from the respective WHO-TEF value; n.a., not analyzed (maximum induction did not reach level corresponding to the respective EC value); –, not included in the WHO TEF list.

[a] Data reported previously (Larsson et al., 2015).

its WHO-TEF value is 0.1. Similarly, REP values of the most abundant mono-*ortho*-substituted PCBs, PCB 118 and 156, were comparable with WHO TEFs. In contrast, significantly lower REPs, as compared with established WHO-TEFs, were observed for other PCB congeners. Approximately one order of magnitude lower REPs were determined for PCBs 105, 167, 169 and 189, as well as for 2,3,4,7,8-PeCDF. For all dioxin-like PCBs, only a partial induction of *CYP1A1* and *CYP1B1* was achieved (Table 2). The complete set of concentration-response curves is presented in Supplemental Figs. 1 and 2.

3.3. Comparison of EC and REP values derived from inducibility of conventional and novel AhR target genes in rat RLE-6TN cells

We then determined the inducibility of *TIPARP*, *AHRR* and *ALDH3A1* mRNA after exposure to six selected DLCs, including TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, PCBs 126, 118 and 156, and non-dioxin-like PCB 153 (for complete concentration-response curves, see Supplemental Fig. 3), and calculated EC20, EC50 and respective REP values (Table 3). The largest deviation from the WHO-TEF value was surprisingly found for PCB 126 based on inducibility of *AHRR* mRNA. Also PCB 156 showed significantly higher REP values than expected based on the WHO TEF value. When compared with induction of *CYP1A1* and *CYP1B1* mRNAs, 2,3,4,7,8-PeCDF was found to exhibit significantly higher REP values based on *AHRR*, *TIPARP* or *ALDH3A1* induction. Here, the derived REPs were in a better accordance with the respective WHO-TEFs. Inducibility of other tested genes mostly were within the same order of magnitude.

3.4. Determination of EC and REP values in human lung epithelial A549 cells and their comparison with the values obtained in rat lung epithelial cells

In order to compare dioxin-like responses and respective REP values between rat and human lung epithelial cells, induction of *CYP1A1*, *CYP1B1*, *TIPARP* and *AHRR* mRNAs was determined in human lung epithelial A549 cells exposed to TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, PCB 126, 118, 153 and 156. Full concentration-responses curves are presented in Supplemental Fig. 4 . Based on these gene expression data, EC_{20}, EC_{50} and REP values were calculated for individual dose-responses. With the exception of 1,2,3,7,8-PeCDD, all congeners elicited significantly lower REP values for all AhR target genes assessed (Table 4). More than one order of magnitude lower human REPs were calculated for 2,3,4,7,8-PeCDF and, importantly, for all selected PCB congeners (PCB 126, 118 and 156), as compared with rat REPs (see Table 5, summarizing REP values).

3.5. Inducibility of AhR target genes in rat lung tissues after a single dose of TCDD or selected PCB congeners

Dose-responses of five AhR target genes were determined in lung tissue obtained from female Sprague-Dawley rats, three days after receiving a single oral dose of vehicle, TCDD, PCBs 126, 118, 153 or 156. Five different doses were administered in the range from 0.5 μg/kg b.w. (TCDD) up to 500 mg/kg b.w. (PCB 153), reflecting a similar range of administered TEQ doses based on the WHO-TEF values (van Ede et al., 2014). Inducibility of *CYP1A1*, *CYP1B1*, *TIPARP*, *AHRR* and *ALDH3A1* mRNA in lung tissue is shown in Supplemental Fig. 5 . The REP values were calculated using a benchmark response approach (BMR_{20TCDD}). Both intake (administered) and systemic doses, based on determination of concentrations of a congener in blood plasma (van Ede et al., 2014), were used for BMR calculations. REPs were calculated as a ratio of the concentration of BMR_{20} of TCDD and the BMR_{20TCDD} concentration of another tested congener (see Supplemental Table S2). For PCB 126, the highest inducibility with a REP value of 0.3 was calculated using *CYP1A1* mRNA. Other biomarkers (*CYP1B1*, *TIPARP*, *AHRR* and *ALDH3A1* mRNA) showed only a partial induction and lower REPs, compared with the PCB 126 WHO-TEF value. Both PCBs 118 and 156 elicited only a minimum induction of CYP1A1 mRNA. In contrast, a high potency was found for *TIPARP* and *ALDH3A1* after PCB 118 exposure and for *AHRR* and *ALDH3A1* after the PCB 156 exposure (Supplemental Fig. 5).

The *in vivo* REPs were then compared with *in vitro* REPs obtained in both rat and human lung cellular models in Table 5. Despite the large

Table 3
REP values of 6 DLCs based on induction of additional AhR target genes in rat lung RLE-6TN cells.

TIPARP						TEF (WHO 2005)
compound	max. induction	EC_{20}	REP (EC_{20})	EC_{50}	REP (EC_{50})	
2,3,7,8-TCDD	100	0.004	1	0.008	1	1
1,2,3,7,8-PeCDD	112	0.007	0.6	0.014	0.6	1
2,3,4,7,8-PeCDF	11	0.028	0.1	0.098	0.1	0.3
PCB126	123	0.012	0.3	0.030	0.3	0.1
PCB118	108	56.23	0.00007	138.0	0.00006	0.00003
PCB153	n.a.	n.a.	n.a.	n.a.	n.a.	0
PCB156	183	4.786	**0.0008**	18.62	**0.0004**	0.00003
AHRR						TEF (WHO 2005)
compound	max. induction	EC_{20}	REP (EC_{20})	EC_{50}	REP (EC_{50})	
2,3,7,8-TCDD	100	0.007	1	0.040	1	1
1,2,3,7,8-PeCDD	124	0.016	0.5	0.066	0.6	1
2,3,4,7,8-PeCDF	82	0.025	0.3	0.141	0.3	0.3
PCB126	141	0.006	**1.1**	0.018	**2.2**	0.1
PCB118	87	131.8	0.00005	398.1	0.0001	0.00003
PCB153	n.a.	n.a.	n.a.	n.a.	n.a.	0
PCB156	112	7.244	**0.001**	28.84	**0.001**	0.00003
ALDH3A1						TEF (WHO 2005)
compound	max. induction	EC_{20}	REP (EC_{20})	EC_{50}	REP (EC_{50})	
2,3,7,8-TCDD	100	0.005	1	0.023	1	1
1,2,3,7,8-PeCDD	93	0.007	0.6	0.036	0.6	1
2,3,4,7,8-PeCDF	102	0.065	0.1	0.309	0.1	0.3
PCB126	116	0.013	0.3	0.044	0.5	0.1
PCB118	46	323.6	0.00001	n.a.	n.a.	0.00003
PCB153	n.a.	n.a.	n.a.	n.a.	n.a.	0
PCB156	76	28.84	0.0002	173.8	0.0001	0.00003

EC_{20} and EC_{50} values were expressed in nM, for calculations see Table 2; REP values in bold represent REP values > 1 order of magnitude higher than estimated WHO-TEF values; n.a., not analyzed (maximal induction did not reach level corresponding to the respective EC value)

differences found among the REPs derived from individual gene expression and respective BMR_{20TCDD} values, the REPs calculated from administration (intake) doses were generally similar to the established WHO TEF values for selected dioxin-like PCB congeners. However, REPs derived from systemic doses were at least one order of magnitude lower for PCBs 118 and 156 (Table 5).

4. Discussion

The lungs are important targets for both TCDD and DLC toxicity in both rodents and humans (Walker et al., 2007; Yoshizawa et al., 2007). However, so far only two studies have focused on lung-specific dose-response relationships *in vivo* (DeVito et al., 1997; DeVito et al., 2000). These studies compared data from liver, lung and skin of female mice exposed subchronically to TCDD, TCDF, PCB 126 and several other persistent DLCs, using CYP1-dependent enzymatic activities as endpoint. Global gene expression data analysis of A549 cells exposed to TCDD suggested that the inducibility of some genes, such as *CYP1A1*, *CYP1B1* and *ALDH3A1*, could be used as potential biomarkers of exposure and toxicity of DLCs in lung epithelial cells (Martinez et al., 2002). More recently, induction of *CYP1A1*, *CYP1B1* and *TIPARP* mRNAs was shown to be a good marker of dioxin-like activity in A549 cells (Líbalová et al., 2014). Present study is the first to describe quantitative *in vitro* data for AhR activation in lung cellular models.

Many studies that attempted to quantify toxicities of DLCs based on their relative potencies to activate the AhR mostly use hepatic CYP1 enzymes as sensitive biomarkers of the AhR activation (Haws et al., 2006), although CYP1 induction *per se* is not a dioxin-like toxic effect. Selection of other AhR target genes to estimate relative potencies for AhR activation is not trivial, due to an extremely high variety and tissue/cell specificity of AhR-dependent gene expression responses. More than a decade ago, global gene expression analysis identified a large number of genes that respond to TCDD exposure in human hepatoma HepG2 cell line (Puga et al., 2000). This and other studies showed that multiple clusters of genes related to specific signal transduction pathways and various cellular events are affected by the persistently activated AhR, including regulation of cell cycle, cell proliferation, developmental and cancer-related processes (Barouki et al., 2007; Nault et al., 2013; Mulero-Navarro and Fernandez-Salguero, 2016). It was suggested that, both AhR-mediated changes in cell cycle progression and multiple crosstalks of AhR with other signaling pathways may indirectly affect AhR-mediated gene expression in a tissue/cell-specific manner (Puga et al., 2009; Mitchell and Elferink, 2009; Procházková et al., 2011). Comparison of gene expression responses in human HepG2, mouse Hepa1c1c7 and rat H4IIE hepatoma cells identified only a very limited set of commonly regulated AhR target genes (Dere et al., 2011). Similarly, divergent transcriptomic responses to AhR agonists were found in rat and human primary hepatocytes with only five orthologous genes being commonly regulated – *CYP1A1*, *CYP1B1*, *CYP1A2*, *NQO* and *HAL* (Carlson et al., 2009).

Therefore, in this study, we compared expression and inducibility of "core" (or canonical) AhR target genes, *CYP1A1* and *CYP1B1*, with other validated, but less frequently used AhR targets: *AHRR*, *TIPARP* and *ALDH3A1*. All selected genes were confirmed to be directly regulated by AhR activation in lung cell lines (Figs. 1 and 2). Therefore, they can be used as biomarkers of exposure to DLCs, as well as for testing species-specific responses. Through comparison of concentration-dependent induction of selected genes to TCDD in model rodent and human lung cells, we found that the lung cells responded to TCDD with a decreasing sensitivity in the following order: murine lung epithelial MLE-12 cells > rat lung epithelial RLE-6TN cells > human bronchial BEAS–2 B cells > human lung epithelial A549 cells (see Table 1). In

Table 4
REP values of 6 DLCs estimated in the human lung epithelial A549 cell line, compared with WHO-TEF values.

CYP1A1						TEF (WHO 2005)
compound	max. induction	EC_{20}	REP (EC_{20})	EC_{50}	REP (EC_{50})	
2,3,7,8-TCDD	100	0.110	1	0.389	1	1
1,2,3,7,8-PeCDD	88	0.078	1.4	0.331	1.2	1
2,3,4,7,8-PeCDF	78	3.715	**0.03**	16.22	**0.02**	0.3
PCB126	56	20.89	**0.01**	426.6	**0.001**	0.1
PCB118	n.a.	n.a.	**n.a.**	n.a.	**n.a.**	0.00003
PCB153	n.a.	n.a.	n.a.	n.a.	n.a.	0
PCB156	n.a.	n.a.	**n.a.**	n.a.	**n.a.**	0.00003
CYP1B						TEF (WHO 2005)
compound	max. induction	EC_{20}	REP (EC_{20})	EC_{50}	REP (EC_{50})	
2,3,7,8-TCDD	100	0.016	1	0.123	1	1
1,2,3,7,8-PeCDD	79	0.010	1.5	0.076	1.6	1
2,3,4,7,8-PeCDF	74	0.398	0.04	5.129	**0.02**	0.3
PCB126	80	1.413	**0.01**	25.70	**0.005**	0.1
PCB118	n.a.	n.a.	**n.a.**	n.a.	**n.a.**	0.00003
PCB153	n.a.	n.a.	n.a.	n.a.	n.a.	0
PCB156	36	2884	0.000005	n.a.	**n.a.**	0.00003
TIPARP						TEF (WHO 2005)
compound	max. induction	EC_{20}	REP (EC_{20})	EC_{50}	REP (EC_{50})	
2,3,7,8-TCDD	100	0.027	1	0.120	1	1
1,2,3,7,8-PeCDD	91	0.020	1.3	0.095	1.3	1
2,3,4,7,8-PeCDF	86	0.813	**0.03**	3.548	**0.03**	0.3
PCB126	73	3.890	**0.01**	22.91	**0.01**	0.1
PCB118	n.a.	n.a.	**n.a.**	n.a.	**n.a.**	0.00003
PCB153	n.a.	n.a.	n.a.	n.a.	n.a.	0
PCB156	18	n.a.	**n.a.**	n.a.	**n.a.**	0.00003
AHRR						TEF (WHO 2005)
compound	max. induction	EC_{20}	REP (EC_{20})	EC_{50}	REP (EC_{50})	
2,3,7,8-TCDD	100	0.037	1	0.234	1	1
1,2,3,7,8-PeCDD	88	0.017	2.2	0.132	1.8	1
2,3,4,7,8-PeCDF	68	1.148	**0.03**	21.38	**0.01**	0.3
PCB126	61	4.365	**0.01**	79.43	**0.003**	0.1
PCB118	n.a.	n.a.	**n.a.**	n.a.	**n.a.**	0.00003
PCB153	n.a.	n.a.	n.a.	n.a.	n.a.	0
PCB156	24	n.a.	**n.a.**	n.a.	**n.a.**	0.00003

EC_{20} and EC_{50} values are expressed in nM; REP values in bold represent REP values ≥ 1 order of magnitude lower than estimated WHO-TEF values; n.a., not analyzed (maximum induction did not reach level corresponding to the respective EC value)

our previous comparative study using 17 *in vitro* bioassays, the responses to TCDD in human cells were within the same order of magnitude, and they were generally 10–100 times weaker than those observed in rodent assays (Larsson et al., 2015). This could be due to distinct binding affinities of rodent and human AhR, or related with distinct species-specific sets of transcriptional co-regulators employed

Table 5
Comparison of REP values determined in A549 and RLE-6TN cells with WHO-TEF values.

compound	TEF (WHO 2005)	REPs			
		rat lung RLE-6TN cells[a]	human lung A549 cells[b]	rat lung (adm. dose)	rat lung (syst. dose)
2,3,7,8-TCDD	1	1	1	1	1
1,2,3,7,8-PeCDD	1	0.5–0.6	1.2–2.2	n.d.	n.d.
2,3,4,7,8-PeCDF	0.3	0.06–0.3	0.01–0.04	n.d.	n.d.
PCB126	0.1	0.2–0.5[c]	0.001–0.01	0.01–0.3	0.01–0.2
PCB118	0.00003	0.000004–0.0001	n.a.	0.00001–0.001	0.000006–0.0001
PCB156	0.00003	0.00004–0.001	**0.000005**[d]	0.00002–0.003	0.000007–0.00008

adm., administrated dose; n.a., not analyzed (maximum induction did not reach level corresponding to the respective EC value); n.d., not determined; syst., systemic concentration in plasma; REP values in bold represent REP values ≥ 1 order of magnitude lower than estimated WHO-TEF values.

[a] REP values based on induction of *CYP1A1*, *CYP1B1*, *TIPARP*, *AHRR* and *ALDH3A1* mRNAs.

[b] REP values based on induction of *CYP1A1*, *CYP1B1*, *TIPARP* and *AHRR* mRNAs.

[c] REP values do not include estimation of *AHRR* induction.

[d] REP values were based on estimation of EC20 value.

by the respective AhR transcriptional complex (Denison et al., 2011). Differences in inducibility of individual AhR target genes after TCDD exposure were also observed. Here, at least two-fold higher sensitivity of induction of *CYP1B1*, *TIPARP*, *AHRR* and *ALDH3A1* mRNA was found, as compared with *CYP1A1* mRNA inducibility, especially in human and murine cell models.

One of the major aims of this study was to determine REP values for all tested DLCs in rat lung model through performing concentration-response experiments after exposure to 20 DLCs (including PCB 153 as a negative control). For this purpose, RLE-6TN cell line was selected, using induction of *CYP1A1* and *CYP1B1* mRNA as an endpoint. We found some differences between established WHO TEF values and REPs derived from this experiment (Table 2). The BMR_{20TCDD} values from this particular set of EC_{20}, EC_{50} and respective REP values developed from full concentration-responses of *CYP1A1* and *CYP1B1* mRNA in RLE-6TN cells, were recently used for calculation of consensus toxicity factors (CTFs) for DLCs, where they have been combined with additional data from human and rodent bioassays (Larsson et al., 2015). Several PCB congeners, namely PCBs 105, 167, 169 and 189, showed significantly lower (more than one order of magnitude) REP values as compared with established WHO TEFs, while other DLCs (namely 2,3,7,8-tetraCDF, 2,3,4,6,7,8-hexaCDF, 1,2,3,4,7,8,9-heptaCDF, 1,2,3,4,6,7,8-heptaCDD and PCB 77) exhibited higher REPs compared to WHO TEFs. REPs of the other tested DLCs were generally in a good accordance with the respective WHO TEFs. Our data correspond with both the data obtained in rodent luciferase reporter assays (Ghorbanzadeh et al., 2014) and the overall CTFs derived based on principal component analysis of the data from rat *in vitro* bioassays, using primary hepatocytes, hepatoma cells, liver progenitor cells and primary murine splenic cells as model cells (Larsson et al., 2015).

The next set of experiments was performed in rat lung epithelial RLE-6TN cells, in order to compare the inducibility of novel AhR target genes *AHRR*, *TIPARP* and *ALDH3A1* by six DLCs and PCB 153. In general, similar EC and REP values were determined (within one order of magnitude range) for tested compounds. However, *CYP1A1* mRNA inducibility appeared to be a less sensitive endpoint than induction of other AhR target genes (Tables 2 and 3). Interestingly, using the inducibility of *AHRR* as an endpoint, PCB 126 exhibited an extremely high REP, and REP values of this PCB congener calculated from induction of other genes were also significantly higher. With exception of *CYP1A1* induction, also PCB 156 showed significantly higher REP values (for *CYP1B1*, *ALDH3A1*, *AHRR* and *TIPARP*) as compared with its WHO TEF value.

In order to compare the *in vitro* REPs derived in RLE-6TN cells with *in vivo* data, induction of AhR target genes was determined in the lung tissue of female rats exposed to single oral doses of TCDD and PCBs 126, 118 or 156. The obtained BMR_{20TCDD} and REP values are presented in Supplemental Table S2. So far, only two subchronic *in vivo* studies have described lung (and liver and skin) levels of enzymatic activities dependent on AhR activation in rodents exposed to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (DeVito et al., 1997), or selected PCB congeners (DeVito et al., 2000). In our study, despite a high variability of REP values derived from different *in vivo* endpoints (induction of *CYP1A1*, *CYP1B1*, *TIPARP*, *AHRR* and *ALDH3A1* mRNAs), we found that similar REPs can be derived both in rat lung epithelial cells and in lung tissue (for comparison, see Table 5). Consistently, lower REP values were derived when based on systemic doses, as compared with REPs based on administered doses. Interestingly, REPs derived from induction of *CYP1A1*, *CYP1B1* and *CYP1A2* mRNAs in livers of the same animals were 0.01-0.1 for PCB 126; PCBs 118 and 156 elicited only a weak induction below BMR_{20TCDD} (van Ede et al., 2014). Thus, the estimated BMR_{20TCDD} concentrations in lung tissue appeared to be higher than in the liver. Likely, toxicokinetics may explain these observed differences.

Distinct potencies of various ligands to bind to and to activate rodent and human AhR are well known (for review, see e.g. Denison et al., 2011). In order to investigate the differences in AhR-mediated responses between rat and human lung cellular models, we determined expression and inducibility of *CYP1A1*, *CYP1B1*, *TIPARP* and *AHRR* mRNA in human lung epithelial A549 cells exposed to six selected DLCs or PCB 153. These results provided us with two important observations. Firstly, all selected AhR target genes exhibited a higher sensitivity (i.e. lower EC20 values), than canonical *CYP1A1* mRNA biomarker, in human lung cells. Secondly, REPs of PCBs 126, 118 and 156 were much lower than their corresponding WHO TEFs or REPs derived from rodent cellular models, and often did not even reach the EC_{20} benchmark response (Table 4). This is in accordance with previously reported human CTFs derived from the data using human primary hepatocytes, human hepatoma cells, human keratinocytes and primary human lymphocytes: with exception of CTF for PCB 126 (0.003), it has not been possible to calculate REPs for any of other tested PCB congeners due to their inactivity in human bioassays (Larsson et al., 2015). Surprisingly, 2,3,4,7,8-PeCDF also elicited significantly lower REPs in lung cellular models, compared with both WHO-TEF (0.3) and rat and human CTFs (0.2 and 1.0, respectively). Although the explanation for this deviation is not clear, different human cell models used in this and the previous study may contribute to the observed REP differences.

In conclusion, we identified significant species differences in sensitivity toward AhR-dependent responses in lung cells, using REP values based on inducibility of a battery of AhR-dependent genes. Firstly, REPs for PCBs 126, 118 and 156 derived from rat lung cells and rat lung tissue were in a good accordance with WHO TEFs, whereas, in contrast, PCBs tested in human lung epithelial cells A549 cells elicited only low AhR-mediated activities (PCB 126 and 156) or no AhR-mediated activity (PCB 118). This strongly supports the recent suggestion to develop human-specific REP/TEFs based on toxicologically relevant endpoints (van Ede et al., 2016; van Duursen et al., 2017). Secondly, all selected AhR target genes were more sensitive biomarkers of AhR activation than *CYP1A1* mRNA in both rat and human lung epithelial cells. Determination of *CYP1B1*, *AHRR*, *TIPARP* and/or *ALDH3A1* mRNA inducibility could be, therefore, highly recommended as a complementary set of biomarkers for a more precise assessment of the AhR-mediated potencies of DLCs.

Conflict of interests

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tox.2018.05.004.

References

Bajaj, P., Harris, J.F., Huang, J.H., Nath, P., Iyer, R., 2016. Advances and challenges in recapitulating human pulmonary systems: at the cusp of biology and materials. ACS Biomater. Sci. Eng. 2, 473–488.

Barouki, R., Coumoul, X., Fernandez-Salguero, P.M., 2007. The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. FEBS Lett. 581, 3608–3615.

Black, W., Chen, Y., Matsumoto, A., Thompson, D.C., Lassen, N., Pappa, A., Vasiliou, V., 2012. Molecular mechanisms of ALDH3A1-mediated cellular protection against 4-hydroxy-2-nonenal. Free Radic. Biol. Med. 52, 1937–1944.

Carlson, E.A., McCulloch, C., Koganti, A., Goodwin, S.B., Sutter, T.R., Silkworth, J.B., 2009. Divergent transcriptomic responses to aryl hydrocarbon receptor agonists between rat and human primary hepatocytes. Toxicol. Sci. 112, 257–272.

DeVito, M.J., Diliberto, J.J., Ross, D.G., Menache, M.G., Birnbaum, L.S., 1997. Dose-

response relationships for polyhalogenated dioxins and dibenzofurans following subchronic treatment in mice. I. CYP1A1 and CYP1A2 enzyme activity in liver, lung, and skin. Toxicol. Appl. Pharmacol. 147, 267–280.

DeVito, M.J., Menache, M.G., Diliberto, J.J., Ross, D.G., Birnbaum, L.S., 2000. Dose-response relationships for induction of CYP1A1 and CYP1A2 enzyme activity in liver lung, and skin in female mice following subchronic exposure to polychlorinated biphenyls. Toxicol. Appl. Pharmacol. 167, 157–172.

Denison, M.S., Farber, S.C., 2017. And now for something completely different: diversity in ligand-dependent activation of Ah receptor responses. Curr. Opinion Toxicol. 2, 124–131.

Denison, M.S., Soshilov, A.A., He, G., DeGroot, D.E., Zhao, B., 2011. Exactly the same but different: promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. Toxicol. Sci. 124, 1–22.

Dere, E., Lee, A.W., Burgoon, L.D., Zacharewski, T.R., 2011. Differences in TCDD-elicited gene expression profiles in human HepG2, mouse Hepa1c1c7 and rat H4IIE hepatoma cells. BMC Genomics 12, 193.

Driscoll, K.E., Carter, J.M., Iype, P.T., Kumari, H.L., Crosby, L.L., Aardema, M.J., Isfort, R.J., Cody, D., Chestnut, M.H., Burns, J.L., et al., 1995. Establishment of immortalized alveolar type II epithelial cell lines from adult rats. In Vitro Cell Dev. Biol. Anim. 31, 516–527.

Ghorbanzadeh, M., van Ede, K.I., Larsson, M., van Duursen, M.B., Poellinger, L., Lucke-Johansson, S., Machala, M., Pěnčíková, K., Vondráček, J., van den Berg, M., Denison, M.S., Ringsted, T., Andersson, P.L., 2014. In vitro and in silico derived relative effect potencies of ah-receptor-mediated effects by PCDD/Fs and PCBs in rat mouse, and guinea pig CALUX cell lines. Chem. Res. Toxicol. 27, 1120–1132.

Haws, L.C., Su, S.H., Harris, M., Devito, M.J., Walker, N.J., Farland, W.H., Finley, B., Birnbaum, L.S., 2006. Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. Toxicol. Sci. 89, 4–30.

Kim, S.H., Henry, E.C., Kim, D.K., Kim, Y.H., Shin, K.J., Han, M.S., Lee, T.G., Kang, J.K., Gasiewicz, T.A., Ryu, S.H., Suh, P.G., 2006. Novel compound 2-methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o-tolylazo-phenyl)-amide (CH-223191) prevents 2,3,7,8-TCDD-induced toxicity by antagonizing the aryl hydrocarbon receptor. Mol. Pharmacol. 69, 1871–1878.

Kolluri, S.K., Jin, U.H., Safe, S., 2017. Role of the aryl hydrocarbon receptor in carcinogenesis and potential as an anti-cancer drug target. Arch. Toxicol. 91, 2497–2513.

Líbalová, H., Krčková, S., Uhlířová, K., Milcová, A., Schmuczerová, J., Ciganek, M., Kléma, J., Machala, M., Šrám, R.J., Topinka, J., 2014. Genotoxicity but not the AhR-mediated activity of PAHs is inhibited by other components of complex mixtures of ambient air pollutants. Toxicol. Lett. 225, 350–357.

Larsson, M., van den Berg, M., Brenerová, P., van Duursen, M.B., van Ede, K.I., Lohr, C., Luecke-Johansson, S., Machala, M., Neser, S., Pěnčíkova, K., Poellinger, L., Schrenk, D., Strapáčová, S., Vondráček, J., Andersson, P.L., 2015. Consensus toxicity factors for polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls combining in silico models and extensive in vitro screening of AhR-mediated effects in human and rodent cells. Chem. Res. Toxicol. 28, 641–650.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta $C_{(T)}$) method. Methods 25, 402–408.

MacPherson, L., Tamblyn, L., Rajendra, S., Bralha, F., McPherson, J.P., Matthews, J., 2013. 2,3,7,8-Tetrachlorodibenzo-p-dioxin poly(ADP-ribose) polymerase (TiPARP, ARTD14) is a mono-ADP-ribosyltransferase and repressor of aryl hydrocarbon receptor transactivation. Nucleic Acids Res. 41, 1604–1621.

Malkinson, A.M., Dwyer-Nield, L.D., Rice, P.L., Dinsdale, D., 1997. Mouse lung epithelial cell lines–tools for the study of differentiation and the neoplastic phenotype. Toxicology 123, 53–100.

Martinez, J.M., Afshari, C.A., Bushel, P.R., Masuda, A., Takahashi, T., Walker, N.J., 2002. Differential toxicogenomic responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin in malignant and nonmalignant human airway epithelial cells. Toxicol. Sci. 69, 409–423.

Matthews, J., 2017. AHR toxicity and signaling: role of TIPARP and ADP-ribosylation. Curr. Opinion Toxicol. 2, 50–57.

Mimura, J., Ema, M., Sogawa, K., Fujii-Kuriyama, Y., 1999. Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. Genes Dev. 13, 20–25.

Mitchell, K.A., Elferink, C.J., 2009. Timing is everything: consequences of transient and sustained AhR activity. Biochem. Pharmacol. 77, 947–956.

Mulero-Navarro, S., Fernandez-Salguero, P.M., 2016. New trends in aryl hydrocarbon receptor biology. Front. Cell Dev. Biol. 4, 45.

Murray, I.A., Patterson, A.D., Perdew, G.H., 2014. Aryl hydrocarbon receptor ligands in cancer: friend and foe. Nat. Rev. Cancer 14, 801–814.

Muzio, G., Maggiora, M., Paiuzzi, E., Oraldi, M., Canuto, R.A., 2012. Aldehyde dehydrogenases and cell proliferation. Free Radic. Biol. Med. 52, 735–746.

Nault, R., Forgacs, A.L., Dere, E., Zacharewski, T.R., 2013. Comparisons of differential gene expression elicited by TCDD, PCB126, betaNF, or ICZ in mouse hepatoma Hepa1c1c7 cells and C57BL/6 mouse liver. Toxicol. Lett. 223, 52–59.

Nebert, D.W., Dalton, T.P., 2006. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat. Rev. Cancer 6, 947–960.

Procházková, J., Kabátková, M., Bryja, V., Umannová, L., Bernatík, O., Kozubík, A., Machala, M., Vondráček, J., 2011. The interplay of the aryl hydrocarbon receptor and beta-catenin alters both AhR-dependent transcription and Wnt/beta-catenin signaling in liver progenitors. Toxicol. Sci. 122, 349–360.

Puga, A., Maier, A., Medvedovic, M., 2000. The transcriptional signature of dioxin in human hepatoma HepG2 cells. Biochem. Pharmacol. 60, 1129–1142.

Puga, A., Ma, C., Marlowe, J.L., 2009. The aryl hydrocarbon receptor cross-talks with multiple signal transduction pathways. Biochem. Pharmacol. 77, 713–722.

van Duursen, M.B.M., van Ede, K.I., van den Berg, M., 2017. One TEF concept does not fit all: the case for human risk assessment of polychlorinated biphenyls. Curr. Opinion Toxicol. 2, 103–108.

van Ede, K.I., Andersson, P.L., Gaisch, K.P., van den Berg, M., van Duursen, M.B., 2013. Comparison of intake and systemic relative effect potencies of dioxin-like compounds in female mice after a single oral dose. Environ. Health Perspect. 121, 847–853.

van Ede, K.I., Andersson, P.L., Gaisch, K.P., van den Berg, M., van Duursen, M.B., 2014. Comparison of intake and systemic relative effect potencies of dioxin-like compounds in female rats after a single oral dose. Arch. Toxicol. 88, 637–646.

van Ede, K.I., van Duursen, M.B., van den Berg, M., 2016. Evaluation of relative effect potencies (REPs) for dioxin-like compounds to derive systemic or human-specific TEFs to improve human risk assessment. Arch. Toxicol. 90, 1293–1305.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.

Walker, N.J., Yoshizawa, K., Miller, R.A., Brix, A.E., Sells, D.M., Jokinen, M.P., Wyde, M.E., Easterling, M., Nyska, A., 2007. Pulmonary lesions in female Harlan Sprague-Dawley rats following two-year oral treatment with dioxin-like compounds. Toxicol. Pathol. 35, 880–889.

White, S.S., Birnbaum, L.S., 2009. An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 27, 197–211.

Yoshizawa, K., Heatherly, A., Malarkey, D.E., Walker, N.J., Nyska, A., 2007. A critical comparison of murine pathology and epidemiological data of TCDD PCB126, and PeCDF. Toxicol. Pathol. 35, 865–879.