

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

Malin Larsson,^{*,†} Martin van den Berg,[‡] Petra Brenerová,[#] Majorie B. M. van Duursen,[‡] Karin I. van Ede,[‡] Christiane Lohr,[⊥] Sandra Luecke-Johansson,[§] Miroslav Machala,[#] Sylke Naser,[⊥] Kateřina Pěňčíková,[#] Lorenz Poellinger,[§] Dieter Schrenk,[⊥] Simona Strapáčová,[#] Jan Vondráček,^{#,||} and Patrik L. Andersson[†]

[†]Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden

[‡]Endocrine Toxicology Group, Institute for Risk Assessment Sciences, Utrecht University, P.O. Box 80177, NL-3508 TD Utrecht, The Netherlands

[#]Department of Chemistry and Toxicology, Veterinary Research Institute, 621 32 Brno, Czech Republic

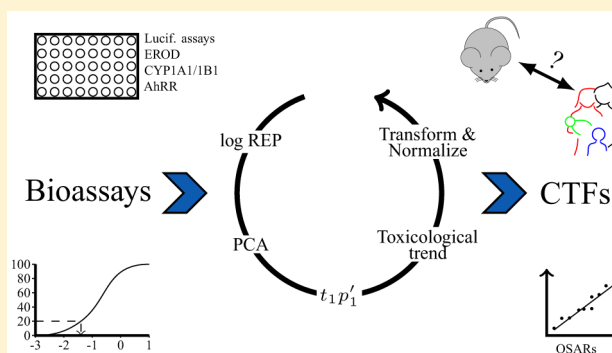
[⊥]Department of Food Chemistry and Environmental Toxicology, University of Kaiserslautern, Kaiserslautern 67663, Germany

[§]Department of Cell and Molecular Biology, Karolinska Institute, SE-171 77 Stockholm, Sweden

^{||}Department of Cytokinetics, Institute of Biophysics AS CR, 612 65 Brno, Czech Republic

Supporting Information

ABSTRACT: Consensus toxicity factors (CTFs) were developed as a novel approach to establish toxicity factors for risk assessment of dioxin-like compounds (DLCs). Eighteen polychlorinated dibenzo-*p*-dioxins, dibenzofurans (PCDD/Fs), and biphenyls (PCBs) with assigned World Health Organization toxic equivalency factors (WHO-TEFs) and two additional PCBs were screened in 17 human and rodent bioassays to assess their induction of aryl hydrocarbon receptor-related responses. For each bioassay and compound, relative effect potency values (REPs) compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were calculated and analyzed. The responses in the human and rodent cell bioassays generally differed. Most notably, the human cell models responded only weakly to PCBs, with 3,3',4,4',5-pentachlorobiphenyl (PCB126) being the only PCB that frequently evoked sufficiently strong responses in human cells to permit us to calculate REP values. Calculated REPs for PCB126 were more than 30 times lower than the WHO-TEF value for PCB126. CTFs were calculated using score and loading vectors from a principal component analysis to establish the ranking of the compounds and, by rescaling, also to provide numerical differences between the different congeners corresponding to the TEF scheme. The CTFs were based on rat and human bioassay data and indicated a significant deviation for PCBs but also for certain PCDD/Fs from the WHO-TEF values. The human CTFs for 2,3,4,7,8-pentachlorodibenzofuran, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin, and 1,2,3,4,7,8,9-heptachlorodibenzofuran were up to 10 times greater than their WHO-TEF values. Quantitative structure–activity relationship models were used to predict CTFs for untested WHO-TEF compounds, suggesting that the WHO-TEF value for 1,2,3,7,8-pentachlorodibenzofuran could be underestimated by an order of magnitude for both human and rodent models. Our results indicate that the CTF approach provides a powerful tool for condensing data from batteries of screening tests using compounds with similar mechanisms of action, which can be used to improve risk assessment of DLCs.



1. INTRODUCTION

Risk managers evaluate health risks associated with polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) by using toxic equivalency factors (TEFs). The TEF system provides a unique tool for assessing the effects of mixtures of compounds from these three classes on the aryl hydrocarbon receptor (AhR), based on the

assumption of additivity.^{1,2} Additivity is overall regarded as the most common and likely mechanism of action and can be modeled using, for instance, the concentration addition model if the target compounds act on, e.g., the same receptor (such as

Received: October 24, 2014

Published: February 5, 2015

the TEF system) or using the independent action model if the target compounds affect the same end point but through different mechanisms of action.^{3,4} In the TEF system, the toxicity of a mixture is evaluated by multiplying the concentration of each compound by its TEF factor and then summing the resulting products to obtain the mixture's toxic equivalency (TEQ) value. The TEQ value can then be used to assess and manage the risks associated with dioxin-like compounds (DLCs). The TEFs are based on expert judgments that take various sources of information into account. Primarily, *in vivo* results based on studies of AhR-mediated responses in rodents were used to establish the current TEF values by the World Health Organization (WHO-TEF).¹ However, also *in vitro* studies which provide important insights into toxins' mechanisms of action and possible differences in species sensitivity were used. In addition, *in silico* information was considered in the most recent TEF reevaluation.¹ By using information from *in vitro* and *in silico* studies to complement *in vivo* data, one can reduce reliance on animal experiments, save time and money, and predict the properties of untested compounds. Moreover, such approaches may yield more accurate predictions of human risk because they provide data based on human primary cells or cell lines; indeed, a growing number of studies have shown that AhR-mediated responses in rodents are more pronounced than in human cell models.^{5–8} Quantitative structure–activity relationships (QSARs) can be used to fill gaps in experimental data sets and to provide information on compounds' mechanisms of action.^{9,10} A QSAR is a statistical model that relates information on the structural and chemical properties of studied chemicals to their biological activity. QSARs have been established for DLCs using approaches based on linear regression such as partial least-squares to latent structures (PLS),^{11–15} multiple linear regression,^{16–18} and nonlinear methods including various approaches based on neural networks.^{19,20} Only a few published studies have considered all or most of the 29 compounds with WHO-TEF values. Most of these studies were performed *in vitro* using rat or mouse liver cell lines and focused on the induction of 7-ethoxyresorufin-O-deethylase (EROD) activity as measure of CYP1A1 activity or on the AhR-mediated induction of reporter gene luciferase activity.^{21–26} Behnisch et al. reported an extensive study based on the Micro-EROD system that included all of the WHO-TEF compounds and featured a relatively long exposure time of 72 h. Zeiger et al. compared the human hepatoblastoma cell line (HepG2) to rat hepatoma and primary cells in terms of the CYP1A activity induced on treatment with the most potent dioxin congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) and all WHO-TEF PCBs. They concluded that the relative effect potency values (REPs) were at least 10 times lower for 3,3',4,4'-tetrachlorobiphenyl (PCB77), 3,4,4',5-tetrachlorobiphenyl (PCB81), and 3,3',4,4',5-pentachlorobiphenyl (PCB126) in the human cells compared to the rodent cells. Silkworth et al. used the EROD assay and showed that the REP of PCB126 was 2 orders of magnitude lower in hepatocytes from human donors than from Sprague–Dawley rats. In summary, a number of studies suggest that humans are among the most dioxin-resistant species.^{7,8} The validity of directly extrapolating rodent-based TEFs in human risk assessment was therefore discussed during the most recent TEF reevaluation, which concluded that more research on human systems is needed.¹ Currently, primary human tissues such as lymphocytes

are being used to assess the risks to human health presented by dioxins and DLCs.^{27–29}

Here, we studied and compared human- and rodent-based bioassays using a set of DLCs that included 18 WHO-TEF compounds and two reference PCBs. The reference PCBs included 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153), which is an abundant non-dioxin-like PCB added as a negative control, and 2,4,4',5-tetrachlorobiphenyl (PCB74), which has previously been found to be active toward the AhR receptor and has been discussed for inclusion in the TEF scheme.^{1,30,31} The studied set represents environmentally relevant congeners from three chemical classes with a wide range of chemical properties, including different numbers of chlorines and substitution patterns. We recorded results from 17 bioassays in order to cover a wide spectrum of AhR-mediated responses and thereby enable the analysis of differences between species and individual tissues. The studied human cell types included primary keratinocytes, lymphocytes, and liver cells. We also performed bioassays based on rat (hepatocytes and hepatoma, liver epithelial, and lung epithelial cells), mouse/murine (hepatoma and primary splenic cells), and guinea pig intestinal adenocarcinoma cells. In the subsequent analyses, REPs were derived for each cell type and compared using multivariate statistics, and QSARs were developed to predict the effects of untested DLCs. A new approach for condensing multivariate data was developed, the consensus toxicity factors (CTFs), which were established for humans and rats using the large range of *in vitro* data gathered in this work.

2. MATERIALS AND METHODS

2.1. Chemicals. 2378-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 (2378-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 PCB126 were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). PCB74, PCB77, PCB81, 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 AccuStandard (New Haven, CT, USA), and 2,3',4,4',5-pentachlorobiphenyl (PCB118), 2,3,3',4,4',5-hexachlorobiphenyl (PCB156), and PCB153 were purchased from Cerilliant Corp. (Round Rock, TX, USA). All congeners were of >99% purity except for 1234678-HpCDD (98.7%). The congeners were dissolved and diluted in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Stockholm, Sweden). The molecular structures of the studied set are provided in the Supporting Information (Figure S1).

2.2. Biological Responses. The compounds were tested in 17 different *in vitro* assays (Table S1). In total, three types of bioassays (luciferase induction, EROD induction, and CYP1A1/CYP1B1/aryl hydrocarbon receptor regulator (AhRR) mRNA expression) in four species (rat, mouse, guinea pig, and human) and five target organs/tissues (liver, lung, spleen, lymphocytes, and keratinocytes) were studied. The Supporting Information (section S2) provides more detailed information on the bioassays.

2.3. Dose–Response Modeling. Dose–response modeling was conducted as described by Ghorbanzadeh et al. for all data except those relating to lymphocytes and splenic cells (Table S1), where separate curves were fitted for each human donor due to the high variation in the individual responses^{15,32} and the EROD studies (Lohr et al., unpublished results). For all bioassays, the concentration needed for a congener to reach a benchmark response (BMR) equal to 20% of

Table 1. BMR_{20TCDD} Concentrations in nM (or μ M for Mono- and Di-ortho PCBs)^a

compound ^b	rat liver epithelial cells		rat lung epithelial cells		rat liver		rat liver	mouse liver	guinea pig liver	human liver		human keratinocytes	
	Cyp1a1 mRNA ^c	Cyp1b1 mRNA ^c	Cyp1a1 mRNA ^f	Cyp1b1 mRNA ^f	EROD primary hepatocytes ^g	EROD H4IIE hepatoma cells ^g	Luc. ^h	Luc. ^h	Luc. ^h	EROD Primary hepatocytes ^g	Luc. HepG2-AZ-AhR cells	CYP1A1 mRNA	AhRR mRNA
Chlorinated Dibenzo- <i>p</i> -dioxins													
2378-TCDD	0.020	0.0019	0.0062	0.0032	0.0042	0.0038	0.0056	0.011	0.0015	0.11	0.19	0.12	0.10
12378-PeCDD	0.032	0.0050	0.012	0.0054	0.013	0.0060	0.012	0.0091	0.0018	0.058	0.069	0.064	0.039
123678-HxCDD	1.3	0.10	0.11	0.023	0.052	0.039	0.052	0.030	0.014	1.5	1.3	4.7	— ⁱ
1234678-HpCDD	0.17	0.0079	0.11	0.0076	0.14	0.080	0.20	0.095	0.019	0.70	0.59	0.42	0.15
Chlorinated Dibenzofurans													
2378-TCDF	0.018	0.0010	0.0083	0.0036	0.025	0.083	0.10	0.013	0.0056	0.89	1.8	0.73	1.5
23478-PeCDF	0.030	0.0059	0.13	0.058	0.045	0.0059	0.036	0.0096	0.0012	0.050	0.48	0.055	0.15
123478-HxCDF	0.11	0.0069	0.025	0.0056	0.13	0.042	0.075	0.025	0.0050	0.14	0.12	0.057	0.075
234678-HxCDF	0.091	0.00020	0.040	0.0047	0.14	0.073	0.096	0.018	0.0059	1.8	2.3	0.69	1.4
1234678-HpCDF	0.52	0.027	0.47	0.10	0.58	0.37	0.38	0.43	0.020	11	8.8	2.1	2.0
1234789-HpCDF	0.15	0.010	0.10	0.033	0.42	0.041	0.15	0.066	0.013	0.43	0.39	0.22	0.35
Non-ortho PCBs													
PCB77	2.4	0.13	1.1	0.28	9.28	67	40	6.0	0.71	— ^c	— ^c	— ^c	— ^c
PCB126	0.18	0.0030	0.027	0.0081	0.073	0.041	0.076	0.24	0.0081	26	58	50	— ^c
PCB169	2.6	0.058	1.38	0.50	5.8	4.7	2.6	21	0.11	— ^c	— ^c	— ^c	— ^c
Mono-ortho PCBs													
PCB74	14	0.17	1.4	0.26	— ^c	— ^c	4.2	4.5	1.1	— ^c	— ^c	— ^c	— ^c
PCB105	1.8	0.045	0.62	0.089	0.80	— ^c	1.3	6.1	0.012	— ^c	— ^c	— ^d	— ^d
PCB118	— ^c	— ^c	0.56	0.15	1.3	— ^c	1.6	2.9	0.13	— ^c	— ^c	— ^d	— ^d
PCB156	0.20	— ^c	0.046	0.0071	0.039	0.081	0.048	0.28	0.011	— ^c	— ^c	— ^d	— ^d
PCB167	— ^c	0.46	5.0	0.45	— ^c	— ^c	1.5	7.8	0.12	— ^c	— ^c	— ^d	— ^d
PCB189	5.5	0.072	0.87	0.085	— ^c	— ^c	— ^c	— ^c	0.12	— ^c	— ^c	— ^d	— ^d
Di-ortho PCB													
PCB153	— ^c	— ^c	— ^c	12	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^d	— ^d

^aData for primary human lymphocytes and primary murine splenic cells are provided in the Supporting Information (Table S3). ^bNames of compounds are abbreviated as described in section 2.1. ^cThe induced response was too weak to calculate a BMR_{20TCDD} value (see section 2.3). ^dCompound was tested at a concentration of 5 μ M after 3, 6, 12, and 24 h but achieved no induction and was therefore not tested further. ^eBrenerová et al. (unpublished results). ^fKrčková et al. (unpublished results). ^gLohr et al. (unpublished results). ^hGhorbanzadeh et al. (2014). ⁱThe coefficient of determination (R^2) was too low (see section 2.3).

the maximum 2378-TCDD response (BMR_{20TCDD}) was calculated to establish *in vitro* REPs. The benchmark response approach was chosen above calculating REPs based on 50% effect concentration (EC₅₀), as the Hill slope and efficacy between the individual congeners and 2378-TCDD were not for all congeners comparable. These differences could add a significant uncertainty in calculating EC₅₀ values. Several studies have shown that in these situations it might be more correct to use a LO(A)EL, benchmark response or multiple point estimates over the range of response from EC₂₀ to EC₈₀ to calculate REPs.^{1,33–38} For this study it was decided to use a benchmark response at 20% effect of 2378-TCDD as earlier described by Van Ede et al.^{36,37} The advance of a benchmark response at the lower part of the curve is that the agreement in curve shape is less essential. The 20% response of TCDD was chosen above 5 or 10% as these responses might be located in the lower bend or background noise of the curve. REPs for each congener were calculated as the ratio of the BMR_{20TCDD} for 2378-TCDD to that for the congener in question. The overall criteria for establishing BMR_{20TCDD} values were as follow:

- If defined, the maximum response for a given compound and assay (Y_{\max}) had to reach at least 25% of the maximum response to 2378-TCDD in the same assay ($Y_{\max\text{TCDD}}$).

- If a clear Y_{\max} value could not be defined, i.e., there was no well-defined top plateau in the response curve, Y_{\max} was set to the value of $Y_{\max\text{TCDD}}$.
- BMR_{20TCDD} values were only computed for a compound in a given assay if at least two points on its dose–response curve were above 10% of $Y_{\max\text{TCDD}}$.
- The coefficient of determination (R^2) value had to be above 0.80.

2.4. Statistical Analysis and Chemical Descriptors. The multivariate statistical analyses were performed using the SIMCA Version 13.0 software package. In principal component analysis (PCA), the variation in the data (X) is captured in a stepwise fashion, in the form of a series of principal components (PCs) that are defined by a set of score vectors t and loading vectors p (and model error E):

$$X = TP' + E = \sum_{a=1}^A t_a p'_a + E$$

The first score vector (t_1) is calculated to represent the largest variation in the data, the second score vector the second largest variation, and so on. The corresponding loading vectors p_1 and p_2

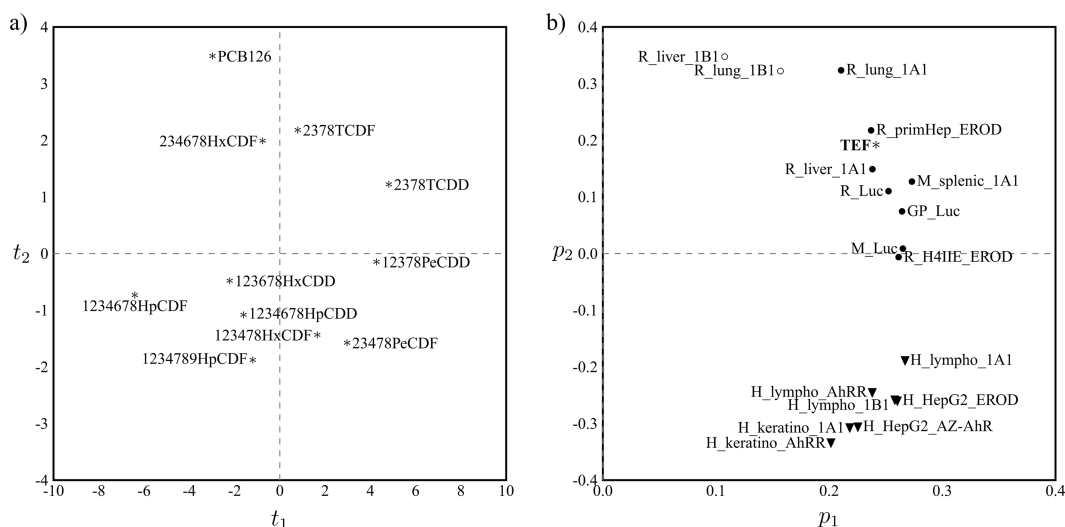


Figure 1. Principal component analysis (PCA) results based on the 17 *in vitro* responses from human and rodent bioassays and the World Health Organization toxic equivalency factors (WHO-TEFs). (a) Score plot for PC 1 (t_1) and 2 (t_2) explaining 64 and 17%, respectively, of the variation in the data. (b) Corresponding loading plot (p_1 versus p_2). In (b), the human responses are shown as triangles, the rodent responses as dots, and rodent CYP1B1 responses as open circles. More information about the compounds and bioassays is given in the Supporting Information (Figure S1, Table S1).

describe the impact of individual variables (in this case, the bioassay responses) have on the variation accounted for by t_1 and t_2 , respectively. Consensus toxicity factors were derived by using the t_1 and p_1 vectors from the rat and human PCA calculations to reflect the toxicological trends in the data. By multiplying t_1 and p_1 , we generated a condensed version of each bioassay's results. The resulting refined matrix was thereafter averaged row-wise (for each compound) to establish CTFs. Sections S5 and S7 of the Supporting Information provide more information on the computation of CTFs. The software SIMCA recommends excluding objects (vectors) with more than 50% missing values.³⁹ Suggested thresholds vary; for example, Chen recommends a limit of 20% missing values.⁴⁰ In present study 50% was used as cutoff, which most frequently resulted in exclusion of mono-*ortho* PCBs. This means that excluded variables were not able to distinguish between non- and mono-*ortho* PCBs, which is crucial information. For bioassays with less than 50% missing values, there were always active representatives among PCDD/Fs, non-*ortho* PCBs, and mono-*ortho* PCBs. For the rat PCA, missing data (which primarily related to certain mono-*ortho* PCBs) were replaced with approximations to account for inactivity, which otherwise would be lost information. The REP values for inactive compounds were assumed to be 1 order of magnitude lower than the lowest value determined in each of the affected bioassays. No approximations were used in the human PCA because the missing values pertained to compounds from different chemical classes to those for which data were available. Most inactive compounds in the human assays were PCBs, and to approximate an entire class of chemicals was considered irrelevant.

PLS is a modeling technique that searches for correlations between one or several Y variables (here, CTF values) and an X -matrix of properties (here, descriptors).^{41,42} The descriptors used for QSAR modeling are related to the chemicals' hydrophobicity, polarizability, reactivity, flexibility, size, and shape. In this work, the compounds' hydrophobicity was reflected using their octanol–water partition coefficients ($\log K_{ow}$). Reactivity is often described by molecular orbital energies, such as the energies of the highest occupied and the lowest unoccupied molecular orbitals (HOMO and LUMO) and the difference between them (GAP). Earlier studies have shown that GAP and HOMO are important variables for QSAR modeling of DLCs.^{13,14,43} In addition, atom-specific descriptors were included to describe reactivity and polarizability.¹⁴ A full list of the descriptors used in the PLS modeling is given in the Supporting Information (section S3), together with information on the statistics and validation of the QSAR models.

The variation in REPs versus WHO-TEFs was analyzed using box plots (see Figure 2, below). A one-sample t test was performed for each congener to detect potential outliers prior to median calculations of *in vitro* REP/TEFs. Any log REP/TEF ratios that were more than 1.5 times the interquartile range for each congener were marked as potential outliers, and an additional t test (one-sided, 95%) was performed to determine the significance of their deviation from the rest of the data.

2.5. QSAR Development and Validation Tools. All descriptors (Table S2) were used in the initial PLS modeling based on the training set and using the calculated CTFs as the response variable. The variable importance of the projection (VIP) statistic was used to indicate the influence of individual descriptors in the PLS models.^{39,42} Models using only the descriptors having a VIP value of 1 or higher were made, and if the model statistics improved, the refined model was selected as the final model. For the final QSAR models, the (final) VIP plots were used to locate the most important descriptors and to make a mechanistic interpretation of the models. For validating the developed QSAR models, the data set was divided into structurally representative training and validation sets using PCA (Table S7), as described in Ghorbanzadeh et al.¹⁵ For the modeling of human data, where only a subset of the 20 compounds (i.e., the 10 PCDD/Fs) was included, new training and validation sets were selected using the same principles (Figure S2). The reason for not using the training set of the rat model for the human model was that the Y-range of the training set from the rat model was smaller than achieved results of the human model. Using the same training set would force the model to extrapolate data, which should be avoided. The training set of the human model consisted of 12378-PeCDD, 23478-PeCDF, 123478-HxCDF, 1234678-HpCDD, 2378-TCDF, 123678-HxCDD, and 1234678-HpCDF, and the validation set included 2378-TCDD, 234678-HxCDF, and 1234789-HpCDF.

For the validation of QSAR models, the following concepts were used: cross-validation by dividing the training set into seven groups (Q^2) and by the "leave-one-out" method ($Q^2(\text{LOO})$). Model performance was expressed using root-mean-square error of estimation from the excluded items from the two methods of cross-validation (RMSEcv, RMSEE), and root-mean-square error of prediction (RMSEP) where the external validation set was used to calculate the model error. We applied the general rule stating that models with Q^2 values larger than 0.5 are predictive and that the difference between Q^2 and R^2 (the "goodness of fit") should not be too large.⁴⁴ The applicability domain of the QSAR models was defined by the

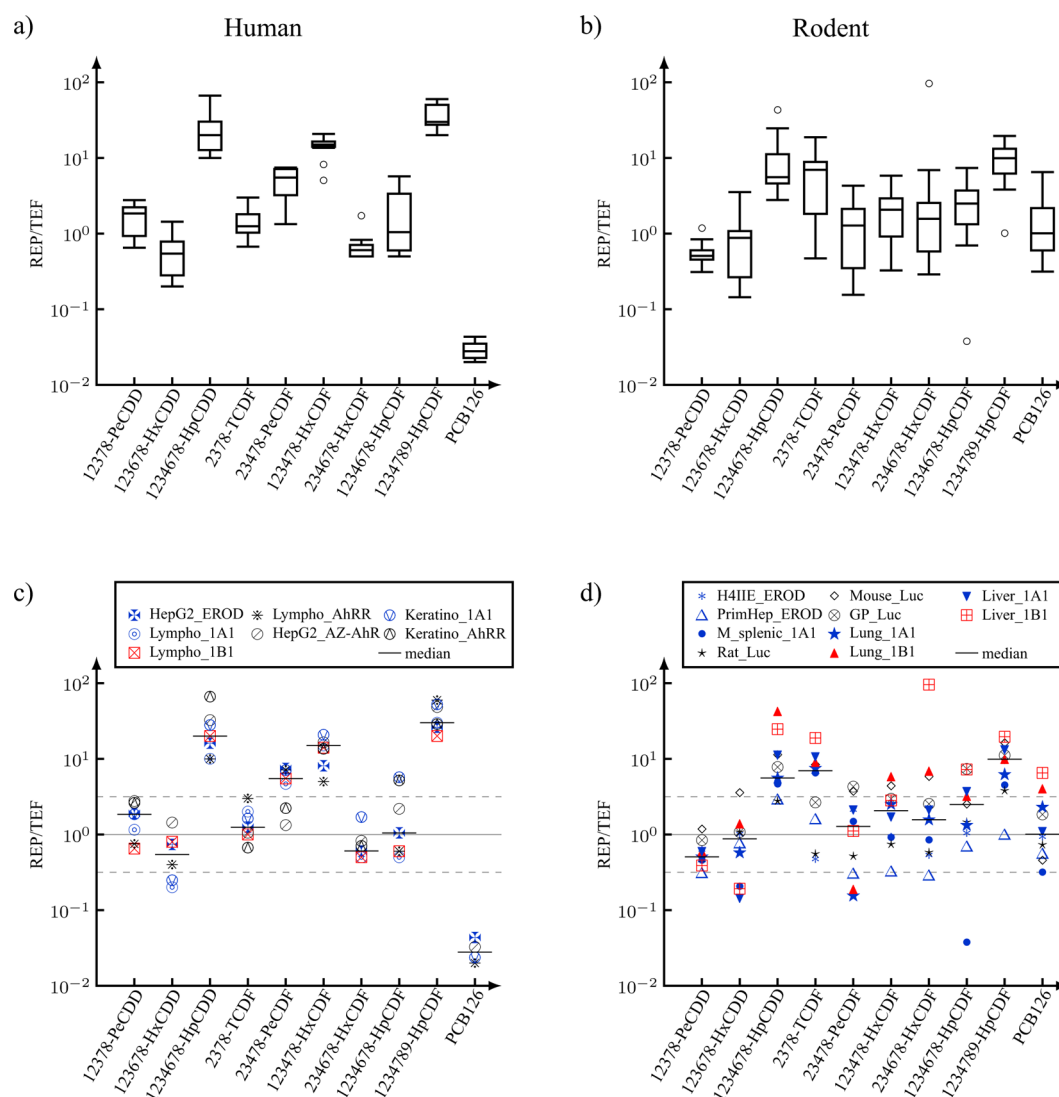


Figure 2. Ratios between relative effect potencies (REP) determined in the different bioassays and the corresponding World Health Organization toxic equivalency factors (WHO-TEFs). Ratios were determined for various PCDD/Fs and PCB126 in human (a) and rodent (b) cell models. Below are shown the individual REPs for the different human (c) and rodent (d) bioassays. CYP1A1-related responses are shown in blue and CYP1B1-related responses in red. Black lines show the median values for each congener, and gray lines indicate the half-log uncertainty range around the WHO-TEF. The outliers in panels (a) and (b) differed significantly from the other REP values determined for the congener in question ($p < 0.05$) and are therefore shown as unfilled circles. A further description of the compounds and each bioassay is given in the Supporting Information (Figure S1, Table S1), and details concerning the calculation of median values and outlier definitions can be found in section 2.4.

membership probability (PModXPS+) of each compound; a membership probability higher than 0.05 was considered to state that the compound was within the applicability domain. Predicted values on the 99% level in membership probability were also reported but indicated as values of lower significance.

3. RESULTS AND DISCUSSION

3.1. Raw Data Analysis. Table 1 presents the established BMR_{20TCDD} values from the 17 *in vitro* bioassays. PCB153 was inactive in all bioassays other than the rat lung *Cyp1b1* mRNA test, where it showed a weak induction. PCBs induced weak or negligible responses in all of the tested human systems: the only PCB for which a BMR_{20TCDD} value could be determined in the human cell models tested for all end points, was PCB126. Some of the rodent assays yielded relatively strong responses for most of the PCBs (e.g., the *Cyp1a1/1b1* mRNA assays using rat lung epithelial cells), but others (e.g., the EROD assays in primary rat hepatocytes and rat H4IIE hepatoma cells)

only exhibited strong responses for a few compounds. Interestingly, the BMR_{20TCDD} value of 2378-TCDD in rodent cells varied over an order of magnitude, from 0.0019 nM (in case of *Cyp1b1* mRNA detection in rat liver epithelial cell assay) to 0.011 nM (in the mouse luciferase assay). However, the responses to 2378-TCDD in human cells were all of the same order of magnitude, and they were 10–100 times weaker than those observed in the rodent assays. For example, the BMR_{20TCDD} value for 2378-TCDD in the human primary hepatocyte EROD assay (0.11 nM) was approximately 30 times greater than that in the rat primary hepatocyte EROD assay (0.0042 nM). The PCB126 responses observed in the EROD assays exhibited even greater differences between human ($BMR_{20TCDD} = 26$ nM) and rat ($BMR_{20TCDD} = 0.073$ nM) primary hepatocytes.

Data for compounds that yielded experimental REP values in most of the human and rodent bioassays (all of the PCDD/Fs and PCB126; see section 2.4) were analyzed by PCA

(Figure 1). Seventeen separate bioassays were considered in the PCA, which yielded three significant principal components (PCs) explaining 64%, 17%, and 8% of the overall variation in the data, respectively. The loading plot for the first two components clearly shows that the REP values from the AhR-related responses cluster around the WHO-TEFs (whose position is indicated by the asterisk in Figure 1b). Taking all three PCs into account, the REPs for the EROD responses in primary rat hepatocytes were most similar to the WHO-TEF values, at least for PCDD/Fs and PCB126 (Figure S4). In the first component, many AhR-related responses share similar loading values. This was expected since the bioassays all provide closely related end points, which reflect the activation of the AhR and/or transcriptional regulation of its direct gene target, the *CYP1A1* gene. The highest *t1* values were observed for 2378-TCDD and 12378-PeCDD. In agreement with their high WHO-TEF values, these were also the most potent compounds in a majority of the bioassays. The other PCDD/Fs have WHO-TEFs ranging from 0.1 to 0.01 and quite diverse *t1* values. The second PC clearly separated the human and rodent data (Figure 1b). This separation was partly due to the high human REPs of certain PCDD/Fs (i.e., the hepta-CDD/Fs, 123478-HxCDF, and 23478-PeCDF), but it was also strongly influenced by the human REPs for PCB126 being substantially lower than the corresponding rodent values. In addition, 2378-TCDF and 234678-HxCDF induced strong *Cyp1b1* mRNA responses in rodent cells but much weaker ones in human cells (Figure 1b). The human and rodent responses in the mRNA expression assays were also clearly separated with respect to the third PC, but the separation based on the luciferase or EROD responses was much less clear in this case (Figure S4). The PCA based on all of the rodent responses (Figure S5a) showed that the strengths of the responses induced by the PCBs were generally consistent with their WHO-TEF rankings, both within the non-*ortho* PCBs and when comparing non-*ortho* to mono-*ortho* PCBs. The most pronounced difference between the rankings based on WHO-TEF values and bioassay responses was observed for bioassays using primary murine splenic cells, in which mono-*ortho* PCBs exhibited relatively low activity (Table S4, Figure S5b). The PCA also clearly showed that PCB156 generally had higher REP values than the other mono-*ortho* PCBs. This is also reflected in the BMR values presented in Table 1, e.g., for the mouse luciferase assay.

3.2. Experimental REPs versus WHO-TEFs. The experimental REPs were compared to the WHO-TEFs for the most active compounds in all of the bioassays (Figure 2). In the human data set (Figure 2a), the results for 1234678-HpCDD, 123478-HxCDF, and 1234789-HpCDF differed strongly from their WHO-TEF values: their median REP values were around 10 times higher than their WHO-TEF values. Sutter et al. have previously shown that a human cell line can be as sensitive to 123678-HxCDF as to 2378-TCDD, indicating that human REPs of some congeners can differ from the WHO-TEFs.⁴⁵ Furthermore, the median rodent REPs for 1234678-HpCDD and 1234789-HpCDF, together with 2378-TCDF, were approximately 10 times greater than their corresponding WHO-TEFs (Figure 2b). Differences between the WHO-TEFs and rodent or human-based REPs could be related to species-, tissue-, and cell-specific variations in AhR sensitivity as well as ligand specificity of binding to the AhR.⁴⁶ The most pronounced deviation from the WHO-TEF rankings occurred for PCB126 in the human cell models: the average REP for this compound was 1.5 orders of magnitude lower than

its WHO-TEF. This is consistent with earlier findings suggesting that its REP values in human cells are 10 to 100 times lower than in rat cells.^{5,6} It is also interesting to note that the REP values for PCB126 were similar across different human cell models and bioassays (see Figure 2a). Most human REPs for 23478-PeCDF were just over half an order of magnitude greater than its WHO-TEF, whereas the REPs for the other PCDD/Fs were within half an order of magnitude of their WHO-TEFs. For human keratinocytes, there was no clear separation in gene expression (*CYP1A1* versus *AhRR*), but in general *CYP1A1* gene expression was closer to the median of all values, e.g., as seen for 1234678-HpCDD (Figure 2c). The same trend was seen for the primary human lymphocytes with respect to *CYP1A1* and *AhRR* gene expression (see, e.g., 1234678-HpCDF). The rodent REPs for all of the PCDD/Fs other than 1234678-HpCDD, 123478-HxCDF, and 1234789-HpCDF were close to their WHO-TEF values (Figure 2b). The rodent REP values (Figure 2b) varied over a much greater range than the human values (Figure 2a) because of the relatively high responses observed in the luciferase mouse and guinea pig assays, and in the rodent *Cyp1b1* mRNA expression assays in epithelial cells (Figure 2d). The latter one (*Cyp1b1*) also generated higher rodent REPs for the remaining PCBs (Figure S6a). For the human models, except for PCB126 and *CYP1B1* and *AhRR* gene expression in human lymphocytes, no REPs could be determined for the other PCBs tested (Figure S6b). The non-*ortho* PCBs 77 and 169, as well as the mono-*ortho* PCBs 167 and 189, dose-dependently induced gene expression of *CYP1B1* and *AhRR* in human lymphocytes for which REPs could be determined. Further analyses of tissue-specific responses and comparisons concerning PCBs are available in the Supporting Information (section S6).

3.3. CTFs for WHO-TEF Compounds. Ranking of environmental pollutants or predictions of environmentally important properties have been earlier performed by combining multivariate techniques, chemical descriptors, and crucial toxicological data.^{11,47–49} In particular, Andersson et al. used the score vectors from PCA to create a toxicological ranking of PCBs.¹¹ Here we have advanced these approaches by using QSARs in combination with PCA and vector multiplication and rescaling techniques to create the CTFs which were scaled as the REPs but also the WHO-TEFs, i.e., using 2378-TCDD as the reference compound. The CTFs use PCA to condense the data from the battery of tests for the studied compounds. Two PCA models were constructed based on data from the rat and human cell assays that summarized 97% and 91% of the total variation in the rat and human data sets, respectively, by one single principal component (Figures S9 and S10). The scores and loadings from the PCA were then used to calculate CTFs (Table 2). The highest human CTFs were observed for 23478-PeCDD, 23478-PeCDF, and 123478-HxCDF together with 2378-TCDD, which all had values close to 1 (Table 2). The human CTFs for 1234678-HpCDD and 1234789-HpCDF (0.2 and 0.3, respectively) were more than 1 order of magnitude higher than their WHO-TEFs (0.01). On the other side, the human CTF for PCB126 (0.003) was more than 1 order of magnitude lower compared to its WHO-TEF value (0.1). The rat CTFs were generally within half an order of magnitude around the WHO-TEF values. Two notable exceptions were PCB169, with a CTF 10 times lower than its WHO-TEF, and PCB156, with a CTF slightly greater than those of the other tested mono-*ortho* PCBs.

Table 2. Consensus Toxicity Factors for Compounds with World Health Organization Toxic Equivalency Factors

compound ^a	CTF		WHO-TEF
	rat	human	
Chlorinated Dibenzo- <i>p</i> -dioxins			
2378-TCDD ^b	1	1	1
12378-PeCDD ^b	0.5	1	1
123478-HxCDD ^c	0.2	0.03	0.1
123678-HxCDD ^b	0.06	0.06	0.1
123789-HxCDD ^c	0.3	0.002	0.1
1234678-HpCDD ^b	0.04	0.2	0.01
OCDD ^c	— ^e	0.005	0.0003
Chlorinated Dibenzofurans			
2378-TCDF ^b	0.2	0.1	0.1
12378-PeCDF ^c	0.2	0.6 ^{ex}	0.03
23478-PeCDF ^b	0.2	1	0.3
123478-HxCDF ^b	0.09	1	0.1
234678-HxCDF ^b	0.07	0.06	0.1
123678-HxCDF ^c	0.07	0.04 ^{ex}	0.1
123789-HxCDF ^c	0.3	0.02	0.1
1234678-HpCDF ^b	0.01	0.01	0.01
1234789-HpCDF ^b	0.05	0.3	0.01
OCDF ^c	0.007 ^{ex}	0.2 ^{ex}	0.0003
Non- <i>ortho</i> -substituted PCBs			
PCB77 ^b	0.0004	— ^d	0.0001
PCB81 ^c	0.0002	— ^d	0.0003
PCB126 ^b	0.09	0.003	0.1
PCB169 ^b	0.002	— ^d	0.03
Mono- <i>ortho</i> -substituted PCBs			
PCB74 ^b	0.000004	— ^d	—
PCB105 ^b	0.00001	— ^d	0.00003
PCB114 ^c	0.00006	— ^d	0.00003
PCB118 ^b	0.000009	— ^d	0.00003
PCB123 ^c	0.000009	— ^d	0.00003
PCB156 ^b	0.00008	— ^d	0.00003
PCB157 ^c	0.00003	— ^d	0.00003
PCB167 ^b	0.000007	— ^d	0.00003
PCB189 ^b	0.000007	— ^d	0.00003

^aNames of compounds are abbreviated as listed in the Materials and Methods. ^bBased on condensed information (PCA) from the experimental *in vitro* REPs. ^cBased on predictions from QSAR models.

^dNo value reported due to the inactivity of PCBs in the human bioassays. ^eNo value reported because the compound's membership probability value in the model was too low (below 99% confidence). Predictions were made at the 99% confidence level (marked "ex") and at 95% confidence level (unmarked). The membership probability values are located in the Supporting Information (Table S7).

The CTFs were used to establish QSAR models following the OECD principles for validation of QSAR models.⁵⁰ The rat ($Q^2 = 0.83$, $Q^2(\text{LOO}) = 0.86$) and human ($Q^2 = Q^2(\text{LOO}) = 0.71$) models were designed to predict the CTF values of compounds that have WHO-TEF values but were not tested in our assays (Figure 3). Notes on model statistics and descriptor dependence are presented in the Supporting Information (section S8). The applicability domain of the human model ranges the 2,3,7,8-chlorinated PCDD/Fs including all different substitution patterns for tetra- to heptachlorinated congeners. The rat model's applicability domain covers the whole WHO-TEF space, including tetra- to heptachlorinated PCBs with non- and mono-*ortho* substitution and the 2,3,7,8-chlorinated PCDD/Fs. No outliers were detected in the human model, whereas three were detected in the rat model: PCB169,

1234678-HpCDD, and OCDD (Table 2). The human model is more local, whereas the rat model covers three chemical classes, which may be the cause for the identified outliers. A low membership probability score was recorded for PCB169, which is lacking structurally similar representatives in the training set sharing the same potency (Table S7). The other hexachlorinated compounds have CTFs (and WHO-TEFs) 100 times lower (PCBs) or 100 times higher (PCDD/Fs) as compared with the CTF of PCB169. The three outliers are all highly chlorinated and thus on the range of the applicability domain, and we have earlier identified outliers among these compounds.¹⁵ Notably, OCDF showed very low membership score in both models but was defined as member on a 99% confidence level. The three compound classes share chemical characteristics of the chlorine atoms and aromatic rings but differ in flexibility where the PCBs are able to rotate over the biphenyl ring.⁵¹ QSAR models including PCBs for AhR mediated effects therefore adopt descriptor dependence related to the flexibility of PCBs, which is crucial for receptor interaction but may result in perturbations of model applicability and thus low membership probability scores of certain compounds, here the highly chlorinated congeners. The models were able to predict 10 (rat) and 7 (human) CTFs of the nontested WHO-TEF compounds. In the human model, two hexachlorinated congeners, 123789-HxCDD and 123789-HxCDF, were predicted between 1 and 2 orders of magnitude lower than their WHO-TEFs, whereas OCDD and OCDF had higher CTFs than WHO-TEFs (0.005 and 0.2, respectively). Furthermore, the prediction of 12378-PeCDF (0.6) was more than 1 order of magnitude higher than the corresponding WHO-TEF (0.03), but it should also be taken into account that the prediction's membership probability was low (Table S7). The remaining untested DLCs in the human model had values close to their corresponding WHO-TEFs, but always lower: 123478-HxCDD (0.03) and 123678-HxCDF (0.04). The predicted rat CTFs corresponded well with the WHO-TEFs, for instance all hexachlorinated congeners had CTFs close to their common WHO-TEF value (0.1) (Table 2). Among the untested PCBs (in the rat model) the CTFs ranged between 0.000009 and 0.00003, which is close to the common WHO-TEF value of all mono-*ortho*-chlorinated PCBs (0.00003). A notable difference from the WHO-TEFs were only encountered for 12378-PeCDF, whose rat CTF value (0.2) was an order of magnitude greater than its WHO-TEF (0.03) and identical to that of 23478-PeCDF.

4. CONCLUSIONS

The analysis of our *in vitro* data confirms earlier findings that human cells are less sensitive than rodent cells, as seen, e.g., for the PCB126-induced EROD activity in human cells (26 nM) as compared with rat primary hepatocytes (0.073 nM). The data also show the overall lower activity of PCBs in human cells. REPs were calculated from derived raw data for 20 compounds tested in 17 different bioassays, of which (to our knowledge) the human data provide previously not tested bioassays for such numbers of DLCs. All bioassays were analyzed individually and compared with the WHO-TEFs but also used as a basis for developing a novel TEF-comparable scale, the CTFs. The strength with the presented approach is that it focuses on finding the joint toxicological trend in the large data set of the *in vitro* screening campaign looking at all compounds at the same time, which is statistically more valid than studying each compound one by one. Another advantage with the approach is

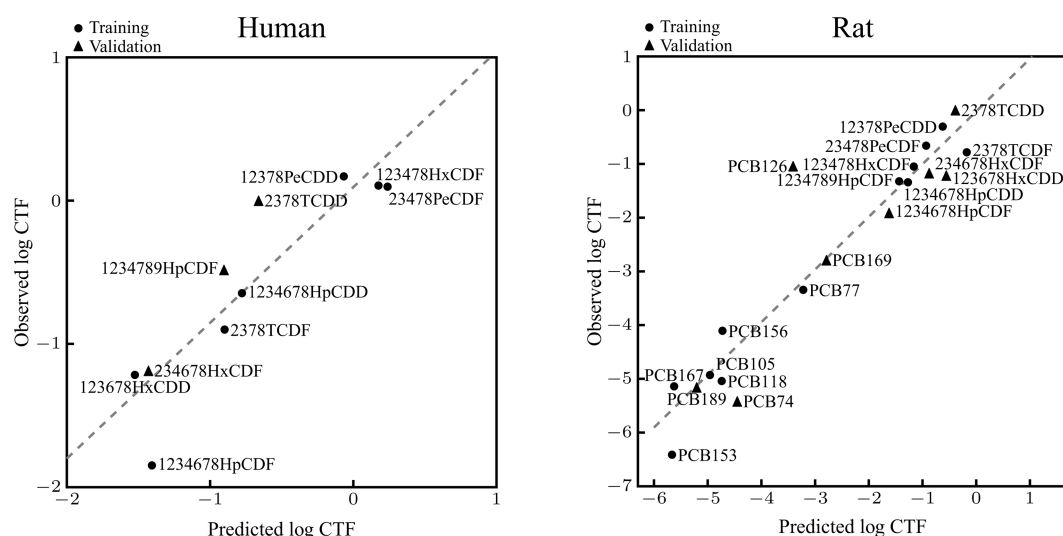


Figure 3. Observed versus predicted (log) consensus toxicity factors based on the human and rat CTF quantitative structure–activity relationship (QSAR) model, respectively. Molecular structures are given in the Supporting Information (Figure S1). The training set is marked with dots and the validation set with triangles in each QSAR model. More information on the QSAR development is given in section 2.5.

the use of PCA, which identifies the major variation and can handle noise from individual bioassays. The initial PCA of REPs clearly showed the similarity between the WHO-TEFs and the response patterns observed in the rodent AhR-based bioassays, but furthermore revealed that some of the human-derived REPs differed significantly from the WHO-TEFs. In general, the variation in the expression of different genes (i.e., *CYP1A1/1B1* and *AhRR*) was greater than the tissue-specific variation. The rat CTFs nicely resembled the WHO-TEFs, e.g., 2378-TCDD, 12378-PeCDD > all HxCDD/Fs, PCB126 > all HpCDD/Fs (and remaining PCBs), whereas the human CTFs showed less dependence on number of chlorine atoms: 23478-PeCDF = 123478-HxCDF = 2378-TCDD > 1234789-HpCDF, 1234678-HpCDD > 123678-HxCDD, 234678-HxCDF >> PCB126. Notably, the human CTF of PCB126 (0.003) was almost 30 times lower than both the WHO-TEF (0.1) and the rat CTF value (0.09). Calculated CTFs were complemented with predicted values using QSAR models developed according to the OECD principles for QSAR validation with a clearly stated end point, using a transparent modeling method and validation procedure, described applicability domain, and providing a mechanistic interpretation. Solid development of QSARs enabled us to fill data gaps and provide comprehensive ranking lists of studied DLCs. Although derived CTFs are based on specific *in vitro* responses, we suggest including them when the risks of the DLCs are being re-evaluated, together with findings from additional *in vitro* and *in vivo* studies covering complementary toxicological pathways, such as cancer and reproductive effects. The *in vitro* database presented here along with the novel CTFs provide further insights in congener-specific differences for a critical group of chemicals and differences between human and rodent cell responses. The present approach could be applied on other available *in vitro* as well as *in vivo* databases covering other mechanisms of action, e.g., mediated through the estrogen receptor. In the future, it would be interesting to apply the approach to related chemical classes, such as chlorinated naphthalenes, chlorinated and brominated diphenyl ethers, and brominated/mixed brominated and chlorinated analogues to the WHO-TEF compounds. Previously it has been suggested to include also

brominated analogues to the TEF scheme, assigning them TEF values in the same manner as for PCDD/Fs and dioxin-like PCBs.⁵² Hence, we strongly believe that the CTFs will be useful in refining the risk assessment of DLCs and as a means to condense multiple responses that share mechanism of action.

■ ASSOCIATED CONTENT

● Supporting Information

Molecular structures of the studied compounds, detailed information on the *in vitro* assays and chemical descriptors, analysis of the chemical diversity of the WHO-TEF compounds by PCA, BMR values of the lymphocytes and splenic cells, REPs of all 17 *in vitro* assays, plots of REPs versus WHO-TEFs for PCBs, description of the development of CTFs, QSAR statistics and validation, VIP plots, and membership probability values. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +46-90-786-5175. Fax: +46-90-786-7655. E-mail: malin.larsson@chem.umu.se.

Funding

This study was financially supported by the European Union through the project SYSTEQ (226694-FP7-ENV-2008-1).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Tine Ringsted (University of Copenhagen, Denmark) for her help with the dose–response modeling.

■ ABBREVIATIONS

1234678-HpCDD, 1,2,3,4,6,7,8-heptachlorodibenzo-*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; 123678-HxCDD, 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin; 12378-PeCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin; 234678-HxCDF, 2,3,4,6,7,8-hexachlorodibenzofuran; 23478-PeCDF,

2,3,4,7,8,-pentachlorodibenzofuran; 2378-TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; 2378-TCDF, 2,3,7,8-tetrachlorodibenzofuran; AhR, aryl hydrocarbon receptor; BMR, benchmark response; BMR_{20TCDD} , benchmark response equal to 20% of the maximum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin response; CTF, consensus toxicity factor; PC, principal component; PCA, principal component analysis; PCB, polychlorinated biphenyl; PCB105, 2,3,3',4,4'-pentachlorobiphenyl; PCB118, 2,3',4,4',5-pentachlorobiphenyl; PCB126, 3,3',4,4',5-pentachlorobiphenyl; PCB153, 2,2',4,4',5,5'-hexachlorobiphenyl; PCB156, 2,3,3',4,4',5-hexachlorobiphenyl; PCB167, 2,3',4,4',5,5'-hexachlorobiphenyl; PCB169, 3,3',4,4',5,5'-hexachlorobiphenyl; PCB189, 2,3,3',4,4',5,5'-heptachlorobiphenyl; PCB74, 2,4,4',5-tetrachlorobiphenyl; PCB77, 3,3',4,4'-tetrachlorobiphenyl; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; PLS, partial least-squares projection to latent structures; PModXPS+, membership probability value; Q^2 , cross-validated R^2 (using seven groups); $Q^2(LOO)$, cross-validated R^2 using the "leave-one-out" method; QSAR, quantitative structure–activity relationship; REP, relative effect potency; $RMSE_{CV}$, root-mean-square error for cross-validation; $RMSEE$, root-mean-square error of estimation; $RMSEP$, root-mean-square error of prediction; TEF, toxic equivalency factor; VIP, variable influence on projection

REFERENCES

- (1) 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., and 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.
- (2) Van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., Van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., and Zacharewski, T. (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* 106, 775–792.
- (3) Scholze, M., Silva, E., and Kortenkamp, A. (2014) Extending the Applicability of the Dose Addition Model to the Assessment of Chemical Mixtures of Partial Agonists by Using a Novel Toxic Unit Extrapolation Method. *PLoS One* 9, 2.
- (4) Backhaus, T., and Faust, M. (2012) Predictive Environmental Risk Assessment of Chemical Mixtures: A Conceptual Framework. *Environ. Sci. Technol.* 46, 2564–2573.
- (5) Zeiger, M., Haag, R., Hockel, J., Schrenk, D., and Schmitz, H. J. (2001) Inducing effects of dioxin-like polychlorinated biphenyls on CYP1A in the human hepatoblastoma cell line HepG2, the rat hepatoma cell line H4IIE, and rat primary hepatocytes: Comparison of relative potencies. *Toxicol. Sci.* 63, 65–73.
- (6) Silkworth, J. B., Koganti, A., Illouz, K., Possolo, A., Zhao, M., and Hamilton, S. B. (2005) Comparison of TCDD and PCB CYP1A induction sensitivities in fresh hepatocytes from human donors, Sprague-Dawley rats, and rhesus monkeys and HepG2 cells. *Toxicol. Sci.* 87, 508–519.
- (7) Okey, A. B., Boutros, P. C., and Harper, P. A. (2005) Polymorphisms of human nuclear receptors that control expression of drug-metabolizing enzymes. *Pharmacogenet. Genomics* 15, 371–379.
- (8) Harper, P. A., Wong, J. M. Y., Lam, M. S. M., and Okey, A. B. (2002) Polymorphisms in the human AH receptor. *Chem.-Biol. Interact.* 141, 161–187.
- (9) Benfenati, E. (2007) Predicting toxicity through computers: a changing world. *Chem. Cent. J.* 1, 1–32.
- (10) Cronin, M. T. D., Walker, J. D., Jaworska, J. S., Comber, M. H. I., Watts, C. D., and Worth, A. P. (2003) Use of QSARs in international decision-making frameworks to predict ecologic effects and environmental fate of chemical substances. *Environ. Health Perspect.* 111, 1376–1390.
- (11) Andersson, P. L., van der Burght, A., van den Berg, M., and Tysklind, M. (2000) Multivariate modeling of polychlorinated biphenyl-induced CYP1A activity in hepatocytes from three different species: Ranking scales and species differences. *Environ. Toxicol. Chem.* 19, 1454–1463.
- (12) Oprea, T. I., Kurunczi, L., Olah, M., and Simon, Z. (2001) MTD-PLS: A PLS-based variant of the MTD method. A 3D-QSAR analysis of receptor affinities for a series of halogenated dibenzoxin and biphenyl derivatives. *SAR QSAR Environ. Res.* 12, 75–92.
- (13) Diao, J. X., Li, Y., Shi, S. Q., and Sun, Y. (2010) QSAR Models for Predicting Toxicity of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans Using Quantum Chemical Descriptors. *Bull. Environ. Contam. Toxicol.* 85, 109–115.
- (14) Larsson, M., Kumar Mishra, B., Tysklind, M., Linusson, A., and Andersson, P. L. (2013) On the use of electronic descriptors for QSAR modelling of PCDDs, PCDFs and dioxin-like PCBs. *SAR QSAR Environ. Res.* 24, 461–479.
- (15) Ghorbanzadeh, M., van Ede, K. I., Larsson, M., van Duursen, M. B. M., Poellinger, L., Lucke-Johansson, S., Machala, M., Pencikova, K., Vondracek, J., van den Berg, M., Denison, M. S., Ringsted, T., and 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.
- (16) Romkes, M., Piskorska-Pleszczyńska, J., Keys, B., Safe, S., and Fujita, T. (1987) Quantitative Structure-Activity-Relationships—analysis of interactions of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin and 2-substituted analogs with rat, mouse, guinea-pig, and hamster cytosolic receptor. *Cancer Res.* 47, 5108–5111.
- (17) Lewis, D. F. V., Jacobs, M. N., Dickens, M., and Lake, B. G. (2002) Quantitative structure-activity relationships for inducers of cytochromes P450 and nuclear receptor ligands involved in P450 regulation within the CYP1, CYP2, CYP3 and CYP4 families. *Toxicology* 176, 51–57.
- (18) Gu, C., Jiang, X., Ju, X., Yu, G., and Bian, Y. (2007) QSARs for the toxicity of polychlorinated dibenzofurans through DFT-calculated descriptors of polarizabilities, hyperpolarizabilities and hyper-order electric moments. *Chemosphere* 67, 1325–1334.
- (19) Zheng, G., Xiao, M., and Lu, X. H. (2005) QSAR study on the Ah receptor-binding affinities of polyhalogenated dibenzo-*p*-dioxins using net atomic-charge descriptors and a radial basis neural network. *Anal. Bioanal. Chem.* 383, 810–816.
- (20) Luan, F., Ma, W. P., Zhang, X. Y., Zhang, H. X., Liu, M. C., Hu, Z. D., and Fan, B. T. (2006) QSAR study of polychlorinated dibenzodioxins, dibenzofurans, and biphenyls using the heuristic method and support vector machine. *QSAR Comb. Sci.* 25, 46–55.
- (21) Peters, A. K., Leonards, P. E., Zhao, B., Bergman, A., Denison, M. S., and Van den Berg, M. (2006) Determination of in vitro relative potency (REP) values for mono-ortho polychlorinated biphenyls after purification with active charcoal. *Toxicol. Lett.* 165, 230–241.
- (22) Behnisch, P. A., Hosoe, K., and Sakai, S. (2003) Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. *Environ. Int.* 29, 861–877.
- (23) Sanderson, J. T., Aarts, J., Brouwer, A., Froese, K. L., Denison, M. S., and Giesy, J. P. (1996) Comparison of Ah receptor-mediated luciferase and ethoxyresorufin-O-deethylase induction in H4IIE cells: Implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. *Toxicol. Appl. Pharmacol.* 137, 316–325.
- (24) Clemons, J. H., Lee, L. E. J., Myers, C. R., Dixon, D. G., and Bols, N. C. (1996) Cytochrome P4501A1 induction by polychlorinated biphenyls (PCBs) in liver cell lines from rat and trout and the

derivation of toxic equivalency factors. *Can. J. Fish. Aquat. Sci.* 53, 1177–1185.

(25) Mason, G., Farrell, K., Keys, B., Piskorskapliszczynska, J., Safe, L., and Safe, S. (1986) Polychlorinated dibenzo-para-dioxins—Quantitative invitro and invivo structure-activity-relationships. *Toxicology* 41, 21–31.

(26) Sawyer, T., and Safe, S. (1982) PCB isomers and congeners—induction of aryl-hydrocarbon hydroxylase and ethoxyresorufin o-deethylase enzyme-activities in rat hepatoma-cells. *Toxicol. Lett.* 13, 87–93.

(27) Hanaoka, T., Yamano, Y., Pan, G. W., Hara, K., Ichiba, M., Zhang, J. S., Zhang, S. J., Liu, T. F., Li, L. D., Takahashi, K., Kagawa, J., and Tsugane, S. (2002) Cytochrome P4501B1 mRNA levels in peripheral blood cells and exposure to polycyclic aromatic hydrocarbons in Chinese coke oven workers. *Sci. Total Environ.* 296, 27–33.

(28) Guida, M., Marra, M., Zullo, F., Guida, M., Trifuoggi, M., Biffali, E., Borra, M., De Mieri, G., D'Alessandro, R., and De Felice, B. (2013) Association between exposure to dioxin-like polychlorinated biphenyls and miR-191 expression in human peripheral blood mononuclear cells. *Mutat. Res., Genet. Toxicol. Environ. Mutagen* 753, 36–41.

(29) McHale, C. M., Zhang, L., Hubbard, A. E., Zhao, X., Baccarelli, A., Pesatori, A. C., Smith, M. T., and Landi, M. T. (2007) Microarray analysis of gene expression in peripheral blood mononuclear cells from dioxin-exposed human subjects. *Toxicology* 229, 101–113.

(30) Hamers, T., Kamstra, J. H., Cenijn, P. H., Pencikova, K., Palkova, L., Simeckova, P., Vondracek, J., Andersson, P. L., Stenberg, M., and Machala, M. (2011) In Vitro Toxicity Profiling of Ultrapure Non-Dioxin-like Polychlorinated Biphenyl Congeners and Their Relative Toxic Contribution to PCB Mixtures in Humans. *Toxicol. Sci.* 121, 88–100.

(31) Stenberg, M., Hamers, T., Machala, M., Fonnum, F., Stenius, U., Laay, A.-A., van Duursen, M. B. M., Westerink, R. H. S., Fernandes, E. C. A., and Andersson, P. L. (2011) Multivariate toxicity profiles and QSAR modeling of non-dioxin-like PCBs—An investigation of in vitro screening data from ultra-pure congeners. *Chemosphere* 85, 1423–1429.

(32) van Ede, K. I., Gaisch, K. P. J., van den Berg, M., and van Duursen, M. B. M. (2014) Differential relative effect potencies of some dioxin-like compounds in human peripheral blood lymphocytes and murine splenic cells. *Toxicol. Lett.* 226, 43–52.

(33) DeVito, M. J., Menache, M. G., Diliberto, J. J., Ross, D. G., and 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.

(34) Lee, K. T., Hong, S., Lee, J. S., Chung, K. H., Hilscherova, K., Giesy, J. P., and Khim, J. S. (2013) Revised relative potency values for PCDDs, PCDFs, and non-ortho-substituted PCBs for the optimized H4IIE-luc in vitro bioassay. *Environ. Sci. Pollut. Res.* 20, 8590–8599.

(35) Toyoshiba, H., Walker, N. J., Bailer, A. J., and Portier, C. J. (2004) Evaluation of toxic equivalency factors for induction of cytochromes P450 CYP1A1 and CYP1A2 enzyme activity by dioxin-like compounds. *Toxicol. Appl. Pharmacol.* 194, 156–168.

(36) van Ede, K. I., Andersson, P. L., Gaisch, K. P. J., van den Berg, M., and van Duursen, M. B. M. (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.

(37) van Ede, K. I., Andersson, P. L., Gaisch, K. P. J., van den Berg, M., and van Duursen, M. B. M. (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.

(38) Villeneuve, D. L., Blankenship, A. L., and Giesy, J. P. (2000) Derivation and application of relative potency estimates based on in vitro bioassay results. *Environ. Toxicol. Chem.* 19, 2835–2843.

(39) SIMCA 13.0; Umetrics AB: Umeå, Sweden, 2009.

(40) Chen, H. Principal Component Analysis With Missing Data and Outliers, Technical Report; Electrical and Computer Engineering

Department, Rutgers University: Piscataway, NJ, 2002; <http://venus.unive.it/romanaz/datamin/articoli/chen.pdf>.

(41) Jackson, J. E. *A User's Guide to Principal Components*; John Wiley: New York, 1991.

(42) Wold, S., Sjostrom, M., and Eriksson, L. (2001) PLS-regression: a basic tool of chemometrics. *Chemom. Intell. Lab. Syst.* 58, 109–130.

(43) Nevalainen, T., and Kolehmainen, E. (1994) New QSAR models for polyhalogenated aromatics. *Environ. Toxicol. Chem.* 13, 1699–1706.

(44) Golbraikh, A., and Tropsha, A. (2002) Beware of $q(2)!$ *J. Mol. Graph. Model.* 20, 269–276.

(45) Sutter, C. H., Bodreddigari, S., Sutter, T. R., Carlson, E. A., and Silkworth, J. B. (2010) Analysis of the CYP1A1 mRNA Dose-Response in Human Keratinocytes Indicates that Relative Potencies of Dioxins, Furans, and PCBs Are Species and Congener Specific. *Toxicol. Sci.* 118, 704–715.

(46) Denison, M. S., Soshilov, A. A., He, G., DeGroot, D. E., and 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.

(47) Servien, R., Mamy, L., Li, Z., Rossard, V., Latrille, E., Bessac, F., Patureau, D., and Benoit, P. (2014) TyPol—A new methodology for organic compounds clustering based on their molecular characteristics and environmental behavior. *Chemosphere* 111, 613–622.

(48) Papa, E., and Gramatica, P. (2010) QSPR as a support for the EU REACH regulation and rational design of environmentally safer chemicals: PBT identification from molecular structure. *Green Chem.* 12, 836–843.

(49) Eriksson, L., Andersson, P. L., Johansson, E., and Tysklind, M. (2002) Multivariate biological profiling and principal toxicity regions of compounds: the PCB case study. *J. Chemometr.* 16, 497–509.

(50) OECD Principles for the Validation, for Regulatory Purposes, of (Quantitative) Structure-Activity Relationship Models, 2004; <http://www.oecd.org/chemicalsafety/risk-assessment/37849783.pdf>.

(51) Andersson, P. L., Haglund, P., and Tysklind, M. (1997) The internal barriers of rotation for the 209 polychlorinated biphenyls. *Environ. Sci. Pollut. Res.* 4, 75–81.

(52) van den Berg, M.; Fiedler, H.; Tritscher, A. M.; Peterson, R. E. The use of toxic equivalency factors (TEFs) for polybrominated dibenzodioxins (PBDDs) and dibenzofurans (PBDFs) in risk assessment. Presented at the 51st Society of Toxicology Annual Meeting, San Francisco, CA, March 11–15, 2012; Abstract 2506.