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RESEARCH ARTICLE

Revised relative potency values for PCDDs, PCDFs, and non-ortho-substituted PCBs for the optimized H4IIE-luc in vitro bioassay

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Abstract While the World Health Organization 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalency factors are useful estimates of relative potencies of mixtures when conducting risk assessments, they are not useful when comparing the results of bioassays such as the H4IIE-*luc* to concentrations of TCDD equivalents calculated from instrumental analyses. Since there are thousands of dioxin-like compounds (DLCs), one use of screening assays is to determine if all of the aryl hydrocarbon receptor (AhR) active DLCs in a mixture have been accounted for in instrumental analyses. For this purpose, bioassay-specific relative potency (ReP) values are needed. RePs of 21 polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls that exhibit effects mediated through the AhR were determined by use of the H4IIE-*luc* assay. Different values of RePs are derived, depending on the statistical, curve-fitting methods used to derive them from the dose–response relationships. Here, we discuss the various methods for deriving RePs from in vitro data and their assumptions and effects on values of RePs. Full dose–response curves of 2,3,7,8-TCDD and other representative DLCs were used to estimate effective concentrations at multiple points (e.g., EC20-50-80), which were then used to estimate ReP of each DLC to 2,3,7,8-TCDD.

Keywords Dioxin-like compounds · AhR activity · In vitro bioassay · Toxic equivalency factor · Relative potency

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Table 1 Details of experimental design for QA/QC, summarized as for three parts of data set I–III (viz. depending on specific purposes given)

	Data set I	Data set II	Data set III
Specific purpose	Test general exposure conditions	Test reagents and reaction time	Test reproducibility
Condition details			
Exposure duration	24, 48, and 72 h	24, 48, and 72 h	48 h
Luciferase reagent-type	Steady glow, bright glow, and luc-lite	Steady glow and bright glow	Steady glow
Luciferase reagent-volume	50, 75, and 100 μL	100 μL	100 μL
Luciferase reagent-reaction time	10 min	Every 2 min for 2 h	10 min
Number of test sets	27	3	30
Number of test replicates	5	–	–
Number of total samples	135 (27 sets by 5 replicates)	192 (3 sets by 64 time measures)	30 (30 independent sets)
Data presented in	Fig. 1	Fig. 2	Fig. 3
Best conditions found	48 h (exposure duration) 100 μL (luciferase reagent volume)	48 h (exposure duration) steady glow (luciferase reagent type) 0–10 min (luciferase regent-reaction time)	stable response

Introduction

Among the persistent organic pollutants of concern worldwide, dioxin-like compounds (DLCs) including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) have the greatest potential to cause adverse effects on reproduction, immune function, or function as cancer promoters or endocrine disruptors (Reggiani 1981). Uses of some DLCs have been controlled under the Stockholm Convention; there are a number of DLCs released from human activities (USEPA 2006) that can be persistent and resistant to degradation and be biomagnified through the food chain (Tillitt et al. 1995; Van den Berg et al. 2006). Traditionally, DLCs in the environment have been identified and their concentrations quantified by use of gas chromatography–high resolution mass spectrometry (GC-HRMS). Such GC-HRMS techniques require expensive and specialized equipment together with highly trained personnel. These resources are often not available to agencies or research institutions, especially in developing countries. Even in the more developed countries, the analyses are time consuming and still expensive, which limits the number of samples that can be accommodated in such monitoring programs.

Based on studies that indicated the pivotal role of the aryl hydrocarbon receptor (AhR) in mediating most, if not all, of the toxic and biochemical effects induced by DLCs, such as PCDDs, PCDFs, and DL PCBs (Giesy and Kannan 1998, Giesy et al. 2002), the structure–receptor binding and structure–activity relationships, a toxic equivalency factor (TEF) approach was developed (Van den Berg et al. 1998). The sum of the products of concentrations of each DLC and corresponding TEF is defined as the 2,3,7,8-TCDD equivalent concentration. This system allows simplification of complex mixtures to a single estimator of toxic effects (Hilscherova et al. 2000). The TEF values developed by the World Health Organization (WHO) are meant for use in risk assessments and are specifically designed to be protective, not predictive. That is, TEF values are consensus values developed by a committee and based on a number of endpoints for several different species. In deriving the TEF values, to be protective, the assessment committee rounded upwards. While the WHO-TEF values are useful estimates of the relative potencies of mixtures, they are not useful when comparing the results of bioassays such as the H4IIE-*luc* to the concentrations of toxic equivalent (TEQ) calculated from instrumental analyses. Since there are thousands of DLCs, one use of screening assays is to determine if all of the DLCs in a mixture have been accounted for in instrumental analyses. For this purpose, bioassay-specific relative potency (ReP) values are needed.

Recombinant cell line bioassays, such as the H4IIE-*luc* applied in this study, have been used to assess AhR-mediated responses and determine dioxin-like activity in various environmental matrices such as water, soil, sediment, and food for last decade (Khim et al. 1999a,b; Hilscherova et al. 2000; Koh et al. 2004). More recently, a growing number of AhR-related bioassays including recombinant cell assays have been used as a supportive screening tool for the assessment of DLCs in environmental samples such as food and feed in EU, sediments in The Netherlands, and soil and fly ash of incinerators in Japan (Behnisch et al. 2001; JMOE 2006). However, comparison studies of the RePs of DLCs in H4IIE-*luc* are scarce (Villeneuve et al. 2000, 2002; Behnisch et al. 2003; Horii et al. 2009).

Table 2 Relative potency estimates for individual chemicals relative to the potency of 2,3,7,8-TCDD in the H4IIE-*luc* in vitro bioassay

Compounds	MW	Max concentration tested		%-TCDD-max.[a]		Condition		ReP20-50-80[b]		
		(ppb/well)	(fmol/well)	%-max.	At concentration (ppb/well)	Equal efficacy	Equal slope	ReP20	ReP50	ReP80
2,3,7,8-TCDD	322	3	2.3×10^{3}	100	3	–	–	1	1	1
1,2,3,7,8-PeCDD	356	1	7.0×10^{2}	131	0.3	yes	yes	2.9×10^{-1}	5.5×10^{-1}	1.0
1,2,3,4,7,8-HxCDD	391	10	6.4×10^{3}	117	3	yes	yes	6.4×10^{-2}	1.2×10^{-1}	2.4×10^{-1}
1,2,3,6,7,8-HxCDD	391	10	6.4×10^{3}	102	3	yes	yes	2.7×10^{-2}	4.7×10^{-2}	8.1×10^{-2}
1,2,3,7,8,9-HxCDD	391	30	1.9×10^{4}	119	10	yes	yes	3.1×10^{-2}	5.4×10^{-2}	9.4×10^{-2}
1,2,3,4,6,7,8-HpCDD	425	30	1.8×10^{4}	94	1	yes	yes	3.3×10^{-2}	5.6×10^{-2}	9.3×10^{-2}
2,3,7,8-TCDF	306	30	2.5×10^{4}	88	1	yes	yes	4.6×10^{-1}	2.7×10^{-1}	1.6×10^{-1}
1,2,3,7,8-PeCDF	340	10	7.3×10^{3}	141	10	yes	yes	1.2×10^{-2}	2.4×10^{-2}	4.7×10^{-2}
1,2,3,4,6,7,8-HpCDF	409	30	1.8×10^{4}	87	10	yes	yes	6.7×10^{-3}	1.1×10^{-2}	1.9×10^{-2}
PCB 77	292	1,000	8.6×10^{5}	74	1,000	yes	yes	8.0×10^{-5}	7.0×10^{-5}	NQ[d]
PCB 81	326	100	7.7×10^{4}	87	100	yes	yes	2.4×10^{-3}	3.4×10^{-3}	4.8×10^{-3}
PCB 126	326	30	2.3×10^{4}	83	10	yes	yes	6.2×10^{-1}	1.4×10^{-1}	3.3×10^{-2}
PCB 169	361	1,000	6.9×10^{5}	60	100	yes	yes	3.0×10^{-4}	3.3×10^{-4}	NQ
PCB 105	326	75,000	5.7×10^{7}	47	75,000	no	yes	4.3×10^{-7}	NQ	NQ
PCB 114	326	10,000	7.7×10^{6}	53	3,000	yes	yes	1.4×10^{-5}	4.9×10^{-6}	NQ
PCB 118	326	75,000	5.7×10^{7}	38	25,000	no	yes	3.3×10^{-7}	NQ	NQ
PCB 123	326	25,000	1.9×10^{7}	67	10,000	yes	yes	9.0×10^{-6}	8.2×10^{-6}	NQ
PCB 156	361	10,000	6.9×10^{6}	55	3,000	yes	yes	2.8×10^{-5}	1.6×10^{-5}	NQ
PCB 157	361	5,000	3.5×10^{6}	94	3,000	yes	yes	2.8×10^{-5}	4.1×10^{-5}	6.0×10^{-5}
PCB 167	361	10,000	6.9×10^{6}	19	10,000	no	no	NQ	NQ	NQ
PCB 189	395	25,000	1.6×10^{7}	25	25,000	no	no	9.5×10^{-8}	NQ	NQ

[a] Max-efficacy found to be over 50 %-TCDD-max (see %-max for full dose–response test; from six dilutions), appropriate to report ReP20-50 values for those compounds

[b] ReP20-50-80: RePs reported as the range of ReP estimates generated from multiple point estimates over a response range from 20 to 50 to 80 %-TCDD-max. (ReP-band)

[c] *NQ* not quantifiable for ReP calculation, dose–response relationship insufficient for estimate

The bioassay can be used in a screening mode to determine if more rigorous assessments are warranted; thus, the most appropriate use of bioassays is in a tiered screening mode. The results of the assay can be used to effectively eliminate samples from further consideration, but the results of the bioassay cannot be used to definitively determine the potential risks posed by mixtures of DLCs in various matrices. One effective use of the bioassays is to conduct potency-balance calculations so that when compared to the results of HRGC-MS, it can be determined if all of the DLC substances have been identified (Koh et al. 2004). However, to make these comparisons, bioassay-specific relative potency factors, i.e., RePs of individual target DLCs are necessary. Therefore, here, we have developed RePs for some of the major DLCs observed in samples from the Korean environment. In this study, dose–response relationships of 21 representative DLCs including 6 PCDDs, 3 PCDFs, and 12 DL PCBs are presented to estimate effective concentrations (e.g., EC50 or EC20-80), which were then used to estimate ReP of each DLC to 2,3,7,8-TCDD. As part of the study, the optimal conditions of the H4IIE-*luc* bioassay were revisited. These included exposure time, luciferase reagent type and volume, and when luminescence was measured. Finally, the revised RePs values of DLCs obtained from the present study were compared to existing TEFs and/or RePs, in terms of mass balance analysis, for various environmental samples reported.

Materials and methods

Experimental design

To optimize test conditions for the H4IIE-*luc* in vitro bioassay, three sets of QA/QC experiments were conducted: effect of

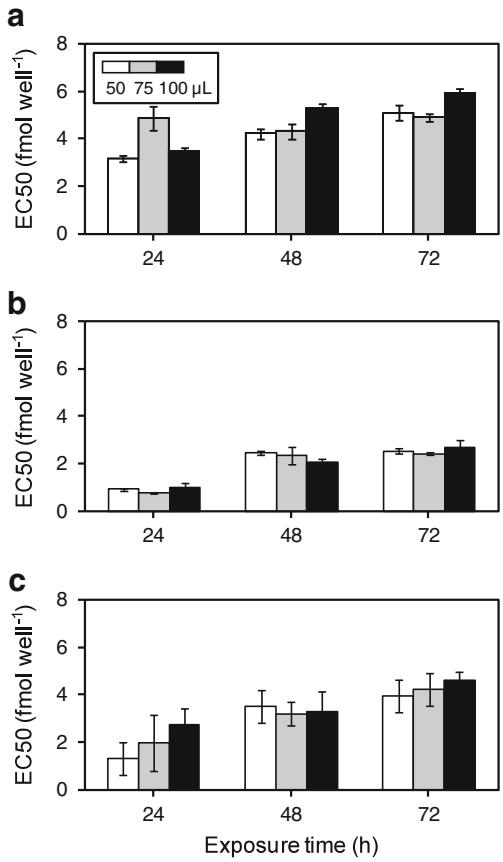

Fig. 1 Effects of exposure time (24, 48, and 72 h) and type (**a** steady glow, **b** bright glow, and **c** luc-lite) or volume (50, 75, and 100 μL) of luciferase reagent on the EC50 for induction of luciferase by 2,3,7,8-TCDD

duration of exposure, luciferase reagent (type, volume, and reaction time), and reproducibility from independent sets of 2,3,7,8-TCDD standard tests (Table 1). Next, full dose–response relationships were characterized for 21 target DCLs including 6 dioxins, 3 furans, and 12 DL-PCBs (Table 2) purchased from AccuStandard (New Haven, CT, USA). Chemicals were dissolved in base solvent (mostly hexane and some ethanol), and corresponding solvents were directly spiked into cell media as negative control for respective bioassays. Each standard solution was diluted, and a dilution series of 10 concentrations tested. Corresponding results of QA/QC and full dose–response characteristics for 21 DLCs are provided accordingly. Details of chemicals and test conditions are provided in Table 2.

Cell culture and bioassay

A standard operating procedure for cell culture and bioassay of the H4IIE-*luc* has been well documented previously (Khim et al. 1999a). In brief, H4IIE-*luc* cells were cultured in Dulbecco's modified Eagle's medium without phenol red, supplemented with 10 % fetal bovine serum, in 100-mm disposable Petri plates and incubated in a humidified 95:5 air/CO_2 atmosphere at 37 °C. Harvested cells were plated into the 60 interior wells of 96-well culture plates (250 μL per well) at a density of approximately 20,000 cells per well for the bioassay. The 36 exterior wells of each plate were filled with 250 μL of culture media. After 24 h of incubation, cells were exposed to 2.5 μL of prepared chemical solutions for certain periods of time to induce the reporter gene expression.

Luciferase assays were conducted after 24, 48, or 72 h of exposure. In brief, culture medium was removed by vacuum manifold, and the cells were rinsed with phosphate-buffered saline. Cells were lysed, and luciferase assay reagent (containing a luciferin substrate) was added to wells. Plates were scanned every 2 min for 2 h after the addition of luciferase reagent at 30 °C with a microplate luminometer GENios (Tecan, Mannedorf, Switzerland; see Table 1). All chemicals were determined to obtain full dose–response curves where dosed with different maximum concentrations (see Table 2) with threefold dilutions with the working range of chemical responses to be covered. Test and control wells were dosed with 2.5 μL of target solutions and appropriate carrier solvents. The final concentration of the carrier solvent was 1 %. Blank wells received no dose. A minimum of three control wells and three blank wells were tested on each plate.

Data analysis

Estimation of the ReP values from the dose–response relationship is done by fitting curves in which two assumptions were made. To assure accurate estimates of ReP, the assumptions of equal efficacy (maximum response achieved) and parallelism between the standard and unknown are required. In these studies, the efficacy of some of the test compounds was not the same as that of 2,3,7,8-TCDD. The second assumption that must be met is that the slopes of the log-transformed dose–response relationships are equivalent. That is to say that the slope of the dose–response relationship of the chemical for which a ReP value is being determined, must be parallel to that of the reference chemical (2,3,7,8-TCDD). If the slopes are equal then the values of the ReP determined by use of the EC (effect concentration) values between 20 (EC20) and 80 % (EC80) would all be the same (Villeneuve et al. 2000). The assumption of parallel slopes was tested by calculating RePs at multiple levels from 20 to 80 %-TCDD-max including 50 %-TCDD-max (EC50). ReP20 and ReP80 values are reported as an

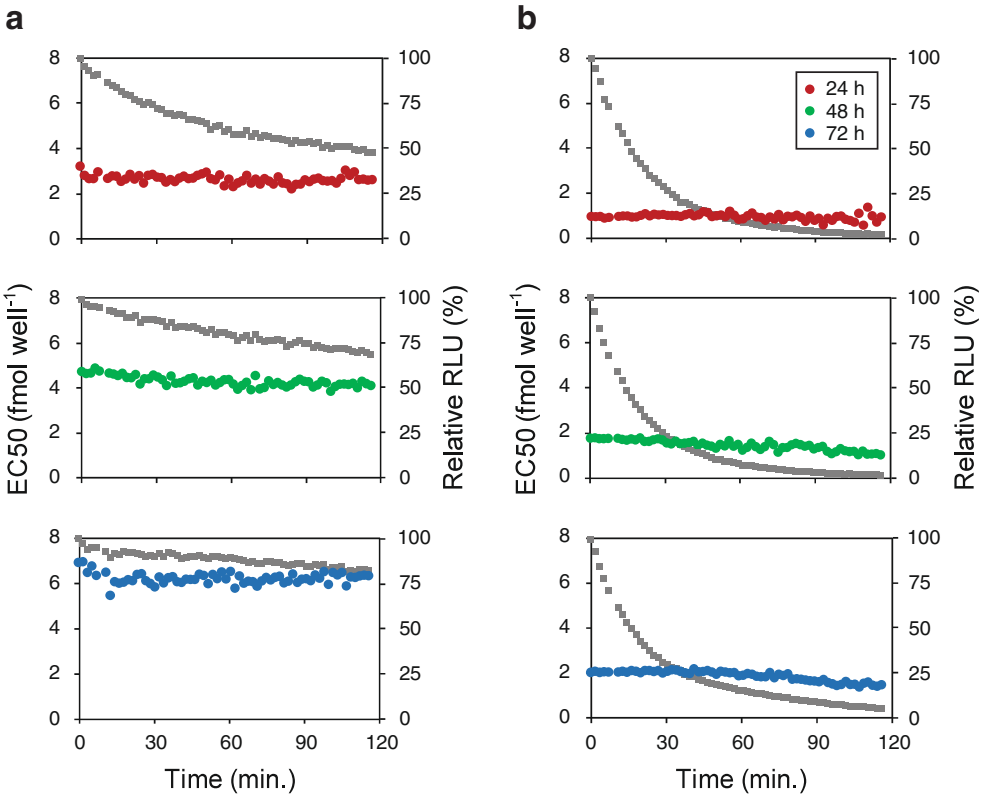

Fig. 2 Effects of luminescence reading time (0–120 min) after reagent addition on the EC50 of 2,3,7,8-TCDD (**a** steady glow and **b** bright glow), where *gray lines* indicate the luminescent signals over the time in each assay

estimate of the uncertainty in the relative potency estimate due to deviations from parallelism between the standard and sample curves (Villeneuve et al. 2002). The greater the variation among these estimates of RePs, the greater the violation of the assumption of parallelism. In cases where the observed maximum response for the sample was <50 %-TCDD-max, ReP20 was calculated in order to make the ReP20 comparable among samples.

Responses, expressed in mean relative luminance units (RLUs) for five replicate wells, were converted to percentage of maximum response observed for 2,3,7,8-TCDD (%-TCDD-max) standard curves generated on the same day. Potencies of samples relative to that of 2,3,7,8-TCDD were estimated. Responses were defined as significant at a threshold of three times the standard deviation (expressed in percent standard max) of the mean solvent control response (0 % standard max). The linear portion of each dose response (%-TCDD-max plotted as a function of log dose) was defined by eliminating points from the tails until an $r^2 \geq 0.95$ was obtained, and a linear regression model was fit to the remaining points. Linear regression equations for the target DLCs and corresponding TCDD standard were used to estimate the concentration associated with responses expressed as %-TCDD-max. Further, details regarding derivation of RePs values have been described previously (Khim et al. 1999a,b; Villeneuve et al. 2001, 2002; Horii et al. 2009).

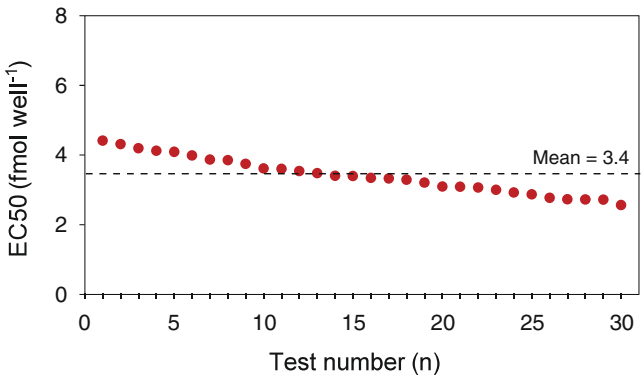

Fig. 3 Quality control chart of 2,3,7,8-TCDD standard response over independent assays (n=30, data given as mean with max to min values obtained), expressed as EC50. The TCDD-EC50 values were sorted in descending order for an easy reading

Results and discussion

QA/QC: optimization of assay condition

The H4IIE-*luc* assay was optimized for duration of time and volume of luciferase reagent. Differences in EC50 values for 2,3,7,8-TCDD standard were observed, depending on

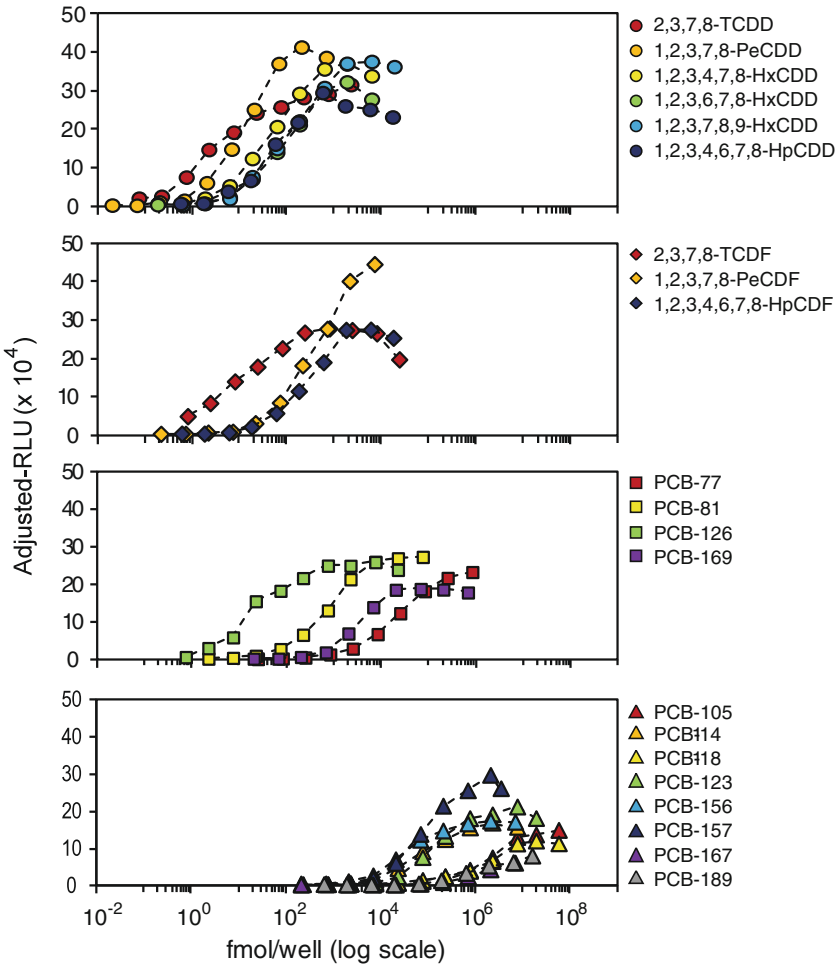

Fig. 4 Dose–response curves of PCDDs, PCDFs, and dioxin-like PCBs determined by H4IIE-*luc* assay. Assay condition given from the best conditions through QA/QC program shown in Table 1

duration of incubation with reagents (24, 48, or 72 h) and volume of reagent (50, 75, or 100 μL) during the course of conducting the H4IIE-*luc* bioassay (data set I in Table 1; Fig. 1). There was little effect of exposure duration on TCDD-EC50 values over the range studied, in general. The efficacy and potency of TCDD standard generally decreased with exposure duration, but maximum sensitivity with minimum variation (RSD) was observed with 48 h exposure among three cases. Results of the QA/QC experiments indicated that the steady glow and use of 50 μL reagent were most appropriate, respectively, considering the response range and statistical relevance (Fig. 1). Although exposure for 72 h (3 days) seemed to be conventional methods used for the H4IIE-*luc* assay in the earlier studies (Khim et al. 1999a; Villeneuve et al. 2002), results of the present study revealed that 48 h of incubation would be an acceptable alternative and appropriate for this type of screening bioassays.

Bright- and steady-glow reagents were investigated to determine the optimum time for stabilization of light emission and how long the reading time could be delayed (data set II in Table 1; Fig. 2) by determining TCDD-EC50 values at 2-min intervals over a period of ~2 h after adding either flash- or glow-type luciferase reagent. TCDD-EC50 values were only slightly different between the two types of luciferase reagent. While TCDD-EC50s were generally stable as a function of time both for steady and bright glow, luminescent signals, expressed as relative RLUs, were stronger over time with long half-life for the steady glow than bright glow. In addition, the fact that there was no significant change in TCDD-EC50 values over a period of 2 h indicated that there is no need to wait for stabilization of emission of light or that there is no need to correct for elapsed time for 2 h after the reagent added. In this study, steady glow was selected as the preferred reagent, and plate reading was conducted shortly after (~10 min) addition of the luciferase reagent for screening and testing target chemicals (Figs. 3 and 4).

Finally, a quality control chart was developed by conducting 2,3,7,8-TCDD standard tests with 30 independent measures (data set III in Table 1; Fig. 3). Compared to the results of other studies with CALUX®, the TCDD-EC50 values for the H4IIE-*luc* cell bioassay showed little variation (3.4±0.5 fmol per well) within an acceptable range (Khim et al. 1999b; Song et al. 2007). Results obtained in this study were similar to those observed for the mouse hepatoma cell bioassay (Windal et al. 2005).

Table 3 Comparison of TEFs (or RePs) for dioxin-like chemicals

Compounds	International TEFs		Assay-specific RePs		Existing TEFs (RePs) vs. H4IIE-*luc* Reps		
	WHO_{1998}[a]	WHO_{2005}[a]	CALUX[b]	H4IIE-*luc*[c]	WHO_{1998}/H4IIE-*luc*	WHO_{2005}/H4IIE-*luc*	CALUX/H4IIE-*luc*
2,3,7.8-TCDD	1	1	1	1	1.0	1.0	1.0
1,2,3,7,8-PeCDD	1	1	0.7	0.6	1.8	1.8	1.3
1,2,3,4,7,8-HxCDD	0.1	0.1	0.08	0.1	8.1×10^{-1}	8.1×10^{-1}	6.1×10^{-1}
1,2,3,6,7,8-HxCDD	0.1	0.1	0.1	0.05	2.1	2.1	2.1
1,2,3,7,8,9-HxCDD	0.1	0.1	0.06	0.05	1.9	1.9	1.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01	0.03	0.06	1.8×10^{-1}	1.8×10^{-1}	5.5×10^{-1}
OCDD	0.0001	0.0003	0.0003	NA	–	–	–
2,3,7,8-TCDF	0.1	0.1	0.07	0.3	3.7×10^{-1}	3.7×10^{-1}	2.5×10^{-1}
1,2,3,7,8-PeCDF	0.05	0.03	0.1	0.02	2.1	1.3	5.9
2,3,4,7,8-PeCDF	0.5	0.3	0.6	NA	–	–	–
1,2,3,4,7,8-HxCDF	0.1	0.1	0.1	NA	–	–	–
1,2,3,6,7,8-HxCDF	0.1	0.1	0.1	NA	–	–	–
1,2,3,7,8,9-HxCDF	0.1	0.1	0.1	NA	–	–	–
2,3,4,6,7,8-HxCDF	0.1	0.1	0.3	NA	–	–	–
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.02	0.01	8.9×10^{-1}	8.9×10^{-1}	2.1
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.04	NA	–	–	–
OCDF	0.0001	0.0003	0.002	NA	–	–	–
PCB 77	0.0001	0.0001	0.001	0.0001	1.4	1.4	2.0×10
PCB 81	0.0001	0.0003	0.005	0.003	3.0×10^{-2}	9.0×10^{-2}	1.3
PCB 126	0.1	0.1	0.04	0.1	7.0×10^{-1}	7.0×10^{-1}	2.7×10^{-1}
PCB 169	0.01	0.03	0.001	0.0003	3.0×10	9.0×10	3.3
PCB 105	0.0001	0.00003	0.000001	0.0000001	7.4×10^{2}	2.2×10^{2}	7.4
PCB 114	0.0005	0.00003	0.0001	0.000005	1.0×10^{2}	6.1	2.9×10
PCB 118	0.0001	0.00003	0.000001	0.0000001	1.8×10^{3}	5.4×10^{2}	1.8×10
PCB 123	0.0001	0.00003	0.0000003	0.00001	1.2×10	3.7	3.7×10^{-2}
PCB 156	0.0005	0.00003	0.0001	0.00002	3.2×10	1.9	9.0
PCB 157	0.0005	0.00003	0.000003	0.00004	1.2×10	7.3×10^{-1}	7.3×10^{-2}
PCB 167	0.00001	0.00003	0.0000003	0.000000001	1.2×10^{4}	3.6×10^{4}	3.6×10^{2}
PCB 189	0.0001	0.00003	0.0000002	0.000000001	1.5×10^{5}	4.6×10^{4}	3.1×10^{2}

NA not analyzed

[a] Van den Berg et al. (2006)

[b] USEPA (2008)

[c] This study

Dose–response characteristics: derivation of accurate RePs

Full dose–response curves for DLCs, including 6 PCDDs, 3 PCDFs, 4 coplanar-PCBs, and 8 mono-*ortho*-PCBs, were obtained by use of 10 concentrations in a serial dilution (Table 2; Fig. 4). Full dose–responses were obtained for all dioxins and furans, with their maximum responses >80 %-TCDD-max. However, some PCBs such as PCBs 105, 118, 167, and 189 did not reach a maximum efficacy comparable to 2,3,7,8-TCDD (Table 2). For 2,3,7,8-TCDD, the dose–response relationship was observed between 0.077 and 2,300 fmol/well and was found to be linear in the range of 0.23–77 fmol/well. The maximum response was observed at 233 fmol/well with an EC50 of 2.5–4.55 fmol 2,3,7,8-TCDD/well. For other PCDDs tested, full dose–response curves were observed with equal efficacy and equal slope compared to 2,3,7,8-TCDD. Slopes of dose–response curves were similar for 6 PCDDs, 3 PCDFs, and 4 PCB congeners;

Table 4 Comparison of TEQs calculated using different TEF or ReP values for assessment of potential AhR-mediated activity in environmental samples such as sediment in previously reported in the literatures

Country/region	Year	*n*	Concentration		TEQs			TCDD-EQ[b]	References
			PCDD/Fs Min–max Mean	DL-PCBs Min–max Mean	WHO_{1998} Min–max Mean	WHO_{2005} Min–max Mean	RePs[a] Min–max Mean	Min–max Mean	
China									
North Bohai Sea	2008	34	ND–42	–	ND–2.5	ND–2.5	ND–3.1	ND–28	Naile et al. 2011
			13		0.17	0.17	0.26	4.9	Hong et al. 2012
Perl River Delta	2000	3	1,700–3,800	–	1.4–6.5	1.7–6.7	5.1–16	–	Zhang et al. 2009
			2,500		3.3	3.5	9.0		
Shandong Peninsula	–	5	6.2–27	21–54	0.17–0.86	0.16–0.75	0.21–1.1	–	Pan et al. 2010
			16	36	0.58	0.53	0.70		
Wenyu River	2006	5	93–230	11,000–27,000	35–140	34–140	38–150	53–340	Luo et al. 2009
			150	22,000	100	100	110	200	
South Korea									
Lake Shihwa (Creeks)	2008	12	16–40,000	180–340,000	0.57–2,100	0.55–1,800	0.67–2,400	–	Moon et al. 2012
			6,300	180	280	240	320		
West coasts	2008	12	ND–140	–	ND–0.59	ND–0.58	ND–1.1	ND–11	Naile et al. 2011
			35		0.13	0.13	0.29	4.6	Hong et al. 2012
Hyeongsan River	2001	6	4.7–1,600	2.0–4,500	0.37–1,000	0.32–1,000	0.41–690	0.01–1,500	Koh et al. 2004
			470	1,600	220	210	160	320	
Japan									
Osaka (Urban area)	2003	8	24,000	97,000	790	770	1,000	–	Kishida et al. 2010
Portugal									
Mondego Estuary	2009	1	110	200	1.0	0.99	1.6	–	Nunes et al. 2011
Turkey									
Marmara Sea	2007	7	2.2–6.9	5.0–57,000	0.016–55	0.0084–18	0.042–18	–	Okay et al. 2009
			29	8,200	9.0	3.1	3.2		
Vietnam									
Can Gio	2003	10	240	250	5.1	4.9	6.1	–	Kishida et al. 2010

[a] TEQ values of DLCs were calculated using ReP values obtained from this study. RePs of some PCDD/Fs congeners not considered in this study were used the values from Behnisch et al. (2003)

[b] TCDD-EQ values were obtained using H4IIE-luc bioassays of sediment extracts

thus, the assumption of equal slopes among congeners was met for these chemicals. For those that the assumption of equal efficacies was not strictly met (for example, response lesser than 50 or 80 %-TCDD-max), such multiple estimates could not be made due to violation of the assumptions given.

RePs were determined by comparing the EC50 or EC20–80, if applicable, of each congener to that of 2,3,7,8-TCDD, which is the most potent agonist for the known AhR in the H4IIE-luc assay. For dioxins, ReP values for 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, and 1,2,3,4,6,7,8-HpCDD were determined to be 0.55, 0.12, 0.047, 0.054, and 0.056, respectively (Table 2). RePs for three PCDFs tested were slightly less than those of PCDDs, and ranged from 0.011 to 0.27 in the H4IIE-*luc* bioassay. RePs could be derived for all co-planar PCBs tested, with maximum of 0.14 for PCB 126 followed by PCB 81, 169, and 71 (Table 2). Relative potencies of all other PCBs were five to eight orders of magnitude lesser than that of 2,3,7,8-TCDD, indicating lack of dioxin-like activities in the H4IIE-*luc* assay.

Comparison to international TEF_{WHO}: application for environmental assessment

The RePs of 1,2,3,4,6,7,8-HpCDD was sixfold greater than $TEF_{WHO,}$ which is indicative of the need to have bioassay-specific RePs values when comparing TEQs derived from the bioassay with those calculated from concentrations and TEFs (Table 3). The RePs for the other PCDD congeners were all less than the corresponding TEF_{WHO}, which is consistent with the protective nature of TEFs and how they were derived. RePs of PCBs were found to be mostly less than the corresponding $TEF_{WHO.}$ However, PCB 81, which is known to be metabolized in vivo but not in the H4IIE-*luc* recombinant cells, exhibited greater potency in the H4IIE-*luc* bioassay (Birnbaum 1985). Thus, ReP values of certain compounds can be underestimated in the H4IIE-*luc* cell system due to lesser bioavailability or insufficient exposure duration for bioaccumulation.

Earlier studies reported a good predictive relationship between TEQ calculated by use of TEF_{WHO} and those determined in transactivation bioassasys (Birnbaum 1985; Chou et al. 2008), but this is case-specific. The RePs presented here for 21 DLCs in the H4IIE-*luc* cell bioassay were in general similar to the rank order of the TEF_{WHO}, but some of the ReP were much different than the respective TEF_{WHO}; thus, the use of assay-specific ReP should be applied in potency balance analysis (Table 3). There have been many reports published on environmental risk assessment that have used both chemical analysis and in vitro bioassay. They were generally conducted potency mass balances of AhR active compounds between instrumental and bioanalytical approaches in various environmental matrices including sediment, soil, and indoor dust. Here, the RePs values derived from the present study have been reapplied to calculate the TEQs for selected environmental samples reported previously (Table 4), targeting assessment of sediment DLCs. The TEQ values calculated by use of the RePs were found to be generally greater than those calculated by use of TEF_{WHO}, indicating underestimating that of sample potency by use of TEQ_{WHO}. Some of the recent studies used TEF values in developing potency balance comparisons due to lack of appropriate RePs values for PCDDs, PCDFs, and DL PCBs (Song et al. 2006; Nieuwoudt et al. 2009; Shen et al. 2009; Kang et al. 2011). Thus, it is suggested that more accurate potency balance comparisons can be achieved by use of established assay-specific RePs such as those reported in the present study. It is not appropriate to use TEFs because they are consensus values based on multiple endpoints and meant to be protective rather than predictive to calculate TEQ for use in potency balance calculations (Hong et al. 2012). Overall, this study will provide useful information on application of instrumental and bioanalytical approaches for the identification of contribution of known and unknown DLCs to the total dioxin-like toxicity in environmental samples.

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