

## DETERMINATION OF REP VALUES BY DR-CALUX® AND MICRO-EROD FOR SEVERAL PCDD/FS AND CO-PCBS, AND COMPARISON TO WHO TEF VALUES (1998)

K. Hosoe<sup>1</sup>, P. A. Behnisch<sup>2</sup>, H. Takigami<sup>3</sup> and S. Sakai<sup>3</sup>

<sup>1</sup>Life Science Research Laboratories, Kaneka Corporation, Takasago, Japan

<sup>2</sup>Institute for Environmental Protection Services, SGS Controll-Co.m.b. H, Wismar, Germany

<sup>3</sup>National Institute for Environmental Studies, Research Center for Material Cycles & Waste Management, Tsukuba, Japan

### Introduction

Cell based bioassays measuring AhR-dependent activity such as DR-CALUX® (www.biodetectionsystems.com) and Micro-EROD have been used to measure the effects of dioxin and dioxin-like compounds in the environment.<sup>1,2</sup> The dioxin-like potency of a single compound is expressed as relative potency (REP). EC<sub>50</sub> values, concentrations of compound eliciting 50 % of the maximum response by 2,3,7,8-TCDD, have widely used to determine REP values. On the other hand, it has been suggested that for chemicals that produce a dose-response curve not parallel and/or dose not exhibit the same maximal response as 2,3,7,8-TCDD, it may be more suitable to indicate activity of compounds as the "a REP range"<sup>3</sup>. In this study, as a part of the characterization of the responsiveness of the DR-CALUX®, REP values based on EC<sub>50</sub>, EC<sub>20</sub>, and EC<sub>5</sub> values were determined for several PCDD/F and dioxin-like PCB congeners in comparison with Micro-EROD.

### Methods and Materials

PCDD/F standards were obtained from Wellington Laboratories (1,2,3,7,8-PeCDD, 1,2,3,7,8,9-HxCDD, 2,3,4,7,8-PeCDF, 1,2,3,7,8,9-HxCDF and 2,3,4,6,7,8-HxCDF) or Cambridge Isotope Laboratories (rest of PCDD/F congeners). Co-PCBs were obtained from Cambridge Isotope Laboratories.

#### *DR-CALUX®*

Rat hepatoma H4IIE cells, stably transfected with a AhR-controlled luciferase gene construct, were seeded into 96-well plates. After 24 hours of growth, cells were exposed to 2,3,7,8-TCDD and test compounds. Following a 24 hour incubation time, luciferase activity was determined using the assay kit of LucLite (Packard) and TopCount NXT® Microplate Scintillation & Luminescence Counter (Packard)<sup>4,5,6</sup>.

#### *Micro-EROD*

Rat hepatoma H4IIEC3/T cells were seeded into 96-well plate. After 3 days of growth, cells were exposed to 2,3,7,8-TCDD and test compounds. After 72 hour incubation, ethoxyresorufin *O*-deethylase activity was measured fluorometrically using a multiwell fluorescence reader (Corona Electric)<sup>7,8</sup>.

#### *REP Calculation*

Dose-response curves for DR-CALUX® and Micro-EROD were fitted to a sigmoidal curve for which EC<sub>20</sub> and EC<sub>50</sub> values could be calculated (SlideWrite Plus Ver 5.0, Advanced Graphics

# BIOANALYSIS

Software). REP values based on  $EC_{20}$  ( $EC_{20}$ REP) and  $EC_{50}$  ( $EC_{50}$ REP) were calculated by dividing the  $EC_x$  for 2,3,7,8-TCDD by  $EC_x$  for test compound ( $x=20$  or  $50$ ). REP values based on  $EC_5$  ( $EC_5$ REP) were calculated by interpolation of the response induced by the test compound on the dose response curve for 2,3,7,8-TCDD. In this case, the diluted solution of the test compound that resulted in a response close to the  $EC_5$  of the 2,3,7,8-TCDD response was used<sup>9</sup>.

## Results and Discussion

Multiple endpoints of REP values based on  $EC_{50}$ ,  $EC_{20}$ , and  $EC_5$  values determined by DR-CALUX<sup>®</sup> and Micro-EROD were used to estimate activity of several PCDD/F and dioxin-like PCB congeners (Table 1).

### *Comparison of REP values determined by DR-CALUX<sup>®</sup> and WHO TEF values*

$EC_5$ REPs were similar to  $EC_{20}$ REPs and were approximately 1.2 - 3.3 times (mean: 1.7) higher than  $EC_{50}$ REPs for PCDD/Fs.  $EC_5$ REPs were 0.7 - 3.6 times (mean: 1.7) higher than  $EC_{50}$ REPs except PCB 157 and  $EC_{20}$ REPs were 1.0 - 1.5 times (mean: 1.3) higher than  $EC_{50}$ REPs for Co-PCBs. Among the REP values, the  $EC_{50}$ REPs were most close to WHO TEF values for 18 out of 22 compounds (PCDD/Fs and Co-PCBs). Of the 14 PCDD/Fs, none differed by more than 50% from the WHO TEF values, and the ratio between  $EC_{50}$ REPs and WHO TEF values were 0.4 - 4.6 (mean: 1.9). In the case of Co-PCBs, 7 compounds showed lower  $EC_{50}$ REPs than expected based on the WHO TEF values [the ratio between  $EC_{50}$ REPs and WHO TEF values were 0.01 - 0.7], while one Co-PCB (PCB 77) showed one order of magnitude higher than the WHO TEF value. Our  $EC_{50}$ REPs values for PCDD/Fs and Co-PCBs were in general in agreement with the results reported by Brown et al.<sup>10</sup> who obtained  $EC_{50}$ REPs values by CALUX<sup>®</sup> assay with mouse hepatoma H1L1 cells, stably transfected with a AhR-controlled luciferase gene construct indicating that there may be no difference of responsiveness between DR-CALUX<sup>®</sup>- and CALUX<sup>®</sup>-bioassays. Only in case of the 2,3,7,8-TCDF we measured a five times higher REP value in our study.

### *Comparison of REP values determined by Micro-EROD and WHO TEF value*

Although REP values for PCDD/Fs showed similar tendency observed in DR-CALUX<sup>®</sup>, difference among REPs were smaller than that in DR-CALUX<sup>®</sup>.  $EC_5$ REPs were similar to  $EC_{20}$ REPs and were 0.8 - 2.1 times (mean: 1.3) higher than  $EC_{50}$ REPs for PCDD/Fs. In contrast to the case of DR-CALUX<sup>®</sup>,  $EC_5$ REPs for Co-PCBs were equal or slightly lower (0.6 - 0.9 times) than  $EC_{50}$ REPs, and  $EC_{20}$ REPs were similar to  $EC_{50}$ REPs. For PCDD/Fs, difference between  $EC_{50}$ REPs and WHO TEF values was less than 50%. In the case of Co-PCBs, 7 compounds showed lower  $EC_{50}$ REPs than expected based on the WHO TEF values [the ratio between  $EC_{50}$ REPs and WHO TEF values were 0.01 - 0.5], while one Co-PCB (PCB 77) showed 5 times higher than the WHO TEF value. Our result shows an excellent agreement between REP values obtained by Micro EROD and DR-CALUX<sup>®</sup> indicating that these two bioassay may have similar responsiveness.

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Table 1. REP values determined by DR-CALUX<sup>®</sup> and Micro-EROD for several PCDD/Fs and Co-PCBs relative to 2,3,7,8-TCDD

Compound	WHO-TEF	DR-CALUX <sup>®(a)</sup>		Micro-EROD <sup>(b)</sup>		CALUX <sup>®(c)</sup>	
		EC <sub>5</sub> REP	EC <sub>20</sub> REP	EC <sub>5</sub> REP	EC <sub>20</sub> REP	EC <sub>50</sub> REP	EC <sub>50</sub> REP
TCDD	1	1	1	1	1	1	1
12378-PeCDD	1	0.75 (1.4) <sup>(d)</sup>	0.80 (1.5)	0.54 (1)	0.72 (1.2)	0.65 (1.1)	0.61 (1)
123478-HxCDD	0.1	0.43 (1.4)	0.42 (1.4)	0.30 (1)	0.18 (1.3)	0.21 (1.5)	0.14 (1)
123678-HxCDD	0.1	0.23 (1.6)	0.30 (2.1)	0.14 (1)	0.17 (1.1)	0.15 (1.0)	0.15 (1)
123789-HxCDD	0.1	0.14 (2.1)	0.10 (1.5)	0.066 (1)	0.059 (1.2)	0.090 (1.8)	0.049 (1)
1234678-HpCDD	0.01	0.15 (3.3)	0.14 (3.0)	0.046 (1)	0.071 (2.1)	0.074 (2.2)	0.034 (1)
2378-TCDF	0.1	0.49 (1.5)	0.51 (1.6)	0.32 (1)	0.35 (1.5)	0.29 (1.2)	0.24 (1)
12378-PeCDF	0.05	0.30 (1.9)	0.24 (1.5)	0.16 (1)	0.22 (1.0)	0.28 (1.3)	0.21 (1)
23478-PeCDF	0.5	0.93 (1.9)	0.90 (1.8)	0.50 (1)	0.58 (1.5)	0.60 (1.5)	0.39 (1)
123478-HxCDF	0.1	0.22 (1.7)	0.23 (1.8)	0.13 (1)	0.13 (0.9)	0.16 (1.1)	0.15 (1)
123678-HxCDF	0.1	0.059 (1.5)	0.055 (1.4)	0.039 (1)	0.082 (1.8)	0.065 (1.4)	0.046 (1)
123789-HxCDF	0.1	0.17 (1.5)	0.21 (1.9)	0.11 (1)	0.19 (1.6)	0.21 (1.8)	0.12 (1)
234678-HxCDF	0.1	0.33 (1.8)	0.34 (1.3)	0.18 (1)	0.092 (0.8)	0.12 (1.1)	0.11 (1)
1234678-HpCDF	0.01	0.040 (1.4)	0.039 (1.5)	0.029 (1)	0.019 (1.1)	0.020 (1.1)	0.018 (1)
1234789-HpCDF	0.01	0.048 (1.2)	0.060 (1.7)	0.041 (1)	0.032 (1.5)	0.032 (1.5)	0.021 (1)
PCB 77	0.0001	0.0015 (1.2)	0.0016 (1.2)	0.0013 (1)	0.0005 (1.0)	0.0005 (1.0)	0.0005 (1)
PCB 105	0.0001	2.5E-5 (2.1)	1.7E-5 (1.4)	1.2E-5 (1)	6.2E-6 (0.6)	1.1E-5 (1.1)	9.8E-6 (1)
PCB 114	0.0005	1.1E-4 (2.3)	6.8E-5 (1.4)	4.8E-5 (1)	2.8E-5 (0.9)	3.2E-5 (1.0)	3.2E-5 (1)
PCB 123	0.0001	8.6E-5 (3.6)	3.5E-5 (1.5)	2.4E-5 (1)	1.2E-5 (0.9)	2.0E-5 (1.4)	1.4E-5 (1)
PCB 126	0.1	0.079 (1.2)	0.071 (1.1)	0.065 (1)	0.044 (1)	0.051 (1.1)	0.046 (1)
PCB 156	0.0005	1.5E-4 (0.7)	2.5E-4 (1.2)	2.1E-4 (1)	7.7E-5 (0.8)	8.6E-5 (0.9)	9.9E-5 (1)
PCB 157	0.0005	7.7E-4 (9.6)	1.0E-4 (1.3)	8.0E-5 (1)	3.9E-5 (0.9)	4.7E-5 (1.1)	4.2E-5 (1)
PCB 169	0.01	0.0026 (0.8)	0.0034 (1.0)	0.0034 (1)	0.0016 (0.7)	0.0019 (0.9)	0.0022 (1)

<sup>a)</sup> REP was determined at least three independent experiments

<sup>b)</sup> REP was determined at least two independent experiments

<sup>c)</sup> Brown et al. (see reference 4)

<sup>d)</sup> Ratio to EC<sub>50</sub>REP