CROSS-VALIDATION STUDY OF THE DR-CALUX®-BIOASSAY COMPARISON TO MICRO-EROD BIOASSAY AND CHEMICAL ANALYSES

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Introduction

A cross-validation study was started in June 2000 analyzing 3 standards (PCB-126; 23478-PCDF; 23478-PBDF), 4 mixtures of PCBs/PCDD/Fs and 13 sample extracts of fly ashes (n=3), emission gas (n=1), PCB capacitor oils (n=2), mineral oil (n=1), sediments (n=3) and feed stuffs (n=3) at BioDetection Systems (BDS; Amsterdam, The Netherlands) and later at Kaneka Corporation (KC; Takasago, Japan). The aim was to proof the reliability of the DR-CALUX®-bioassay and to compare with the Micro-EROD-bioassay and chemical analyses.

Methods and Materials

a.) DR-CALUX®-bioassay (BDS): The validation samples were analyzed at BDS according to the guidelines from BDS (www.biodetectionsystems.com) and recently published studies[1,2].
b.) DR-CALUX®-bioassay (Kaneka Corporation, KC): The samples were also analysed at KC according to a.), but the luciferase activity was measured using LucLite™ (Packard) and the TopCount NXT® Microplate Scintillation & Luminescence Counter (Packard).
c.) Studies with Micro-EROD-bioassay (with H4IIE cells) were according to earlier publications[3,4].

Results and Dicussion

At first, the DR-CALUX® technology was validated with 2,3,7,8-TCDD, several dioxin-like compounds and mixtures in a cross-validation study with BioDetection Systems (BDS). The EC_{50} value [in pM and the coefficient of variation CV], correlation coefficient [R^2] value of the EC_{50} curve, minimal quantification limit [MQL] and minimal detection limit [MDL] of 2,3,7,8-TCDD analyzed by DR-CALUX®-bioassay in the cross-validation study with BDS [EC_{50}: 10 pM/ R^2: 0.99/ MQL: 1.0 pM/ MDL: 0.29 pM (n=10)]; reported by Bovee et al. (1998)[1] [EC_{50}: 7 pM, limit of detection: 0.27 pM); Murk et al. (1996)[5] [EC_{50}: 10 pM] and in the present study analyzed at KC [EC_{50}: 14.9 pM (n=88), CV:14% / R^2: 0.99; MQL: 1.4 pM; MDL: 0.38 pM; (n= 20, independent tests)] were comparable. Several PCB/PCDD/F congeners showed similar REP values analyzed in

these two laboratories by DR-CALUX® -bioassay and additional by Micro-EROD bioassay (see Graph 1; and Table 1). TEQ values of several mixtures of PCBs and PCDD/Fs measured by DR-CALUX® -bioassay in these two laboratories, were in the expected range. Additional, the values analyzed by Micro-EROD and calculated (based on the WHO-TEFs, 1997) were comparable.

In the second step, different matrices such as PCB oils, mineral oils, combustion gas, fly ashes, feedstuffs and environmental (sediments) samples were analyzed by DR-CALUX®-technology in both laboratories. Several samples were additional measured by Micro-EROD-bioassay and/or chemical analysis (see Graph 2, 3 and Table 1). Graph 4 compares the ratio between the obtained results from the DR-CALUX®-bioassay performenced at the laboratory of KC and at BDS, in a resulting R^2 value of 0.76 ($P < 0.01$).

Conclusion

The reliability of the DR-CALUX®-technology in a cross-validation study between two laboratories and in comparison to the Micro-EROD bioassay and chemical analysis could be demonstrated.

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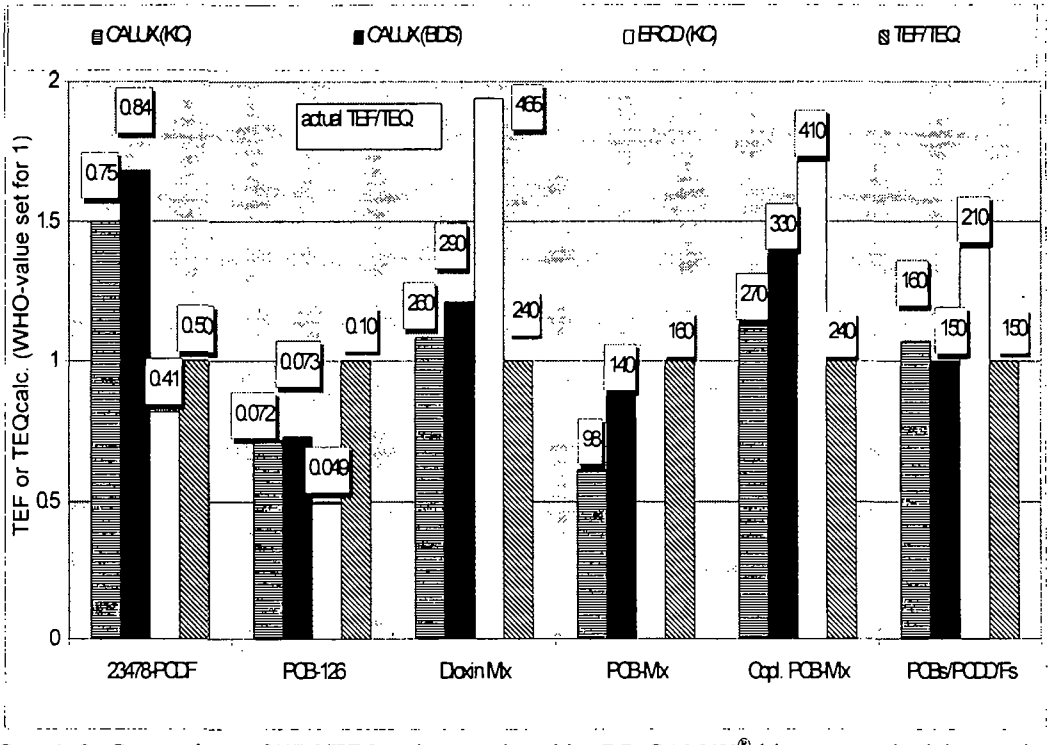
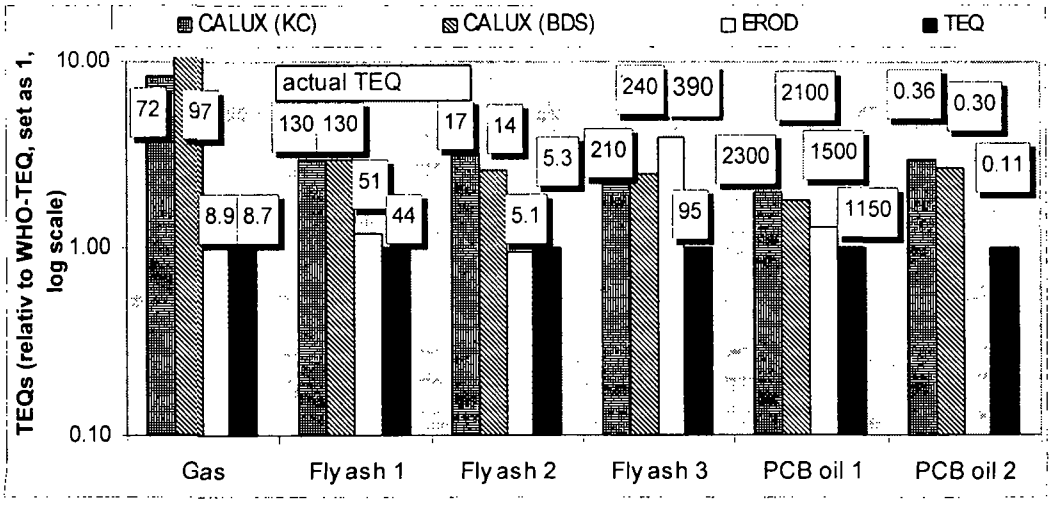
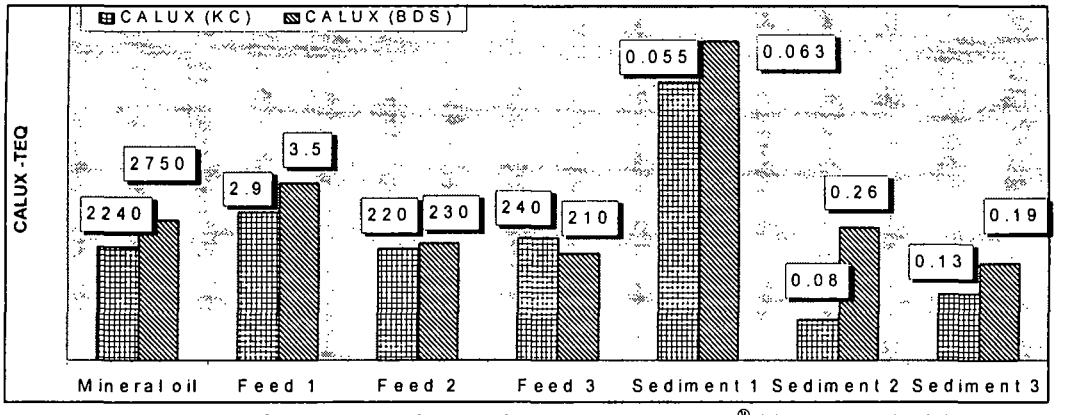
Graph 1: Comparison of TEF/TEQ-values analyzed by DR-CALUX®-bioassay at the laboratories of KC and BDS in comparison to the EROD-bioassay and chemical analysis (WHO-TEFs/TEQs)



Graph 2: Comparison of TEF/TEQ-values analyzed by DR-CALUX®-bioassay at the laboratories of KC and BDS in comparison to the EROD-bioassay and chemical analysis (WHO-TEFs/TEQs)



Graph 3: Comparison of TEQ-values for mineral oil, feed and sediment samples analyzed by DR-CALUX®-bioassay at the laboratories of KC and BDS (WHO-TEFs/TEQs)



Graph 4: Comparison of TEF/TEQ-values analyzed by DR-CALUX®-bioassay at the laboratories of KC and BDS for 3 standards (PCB-126; 23478-PCDF; 23478-PBDF), 4 mixtures of PCBs/ PCDD/Fs and 13 sample extracts tested in the present cross-validation study

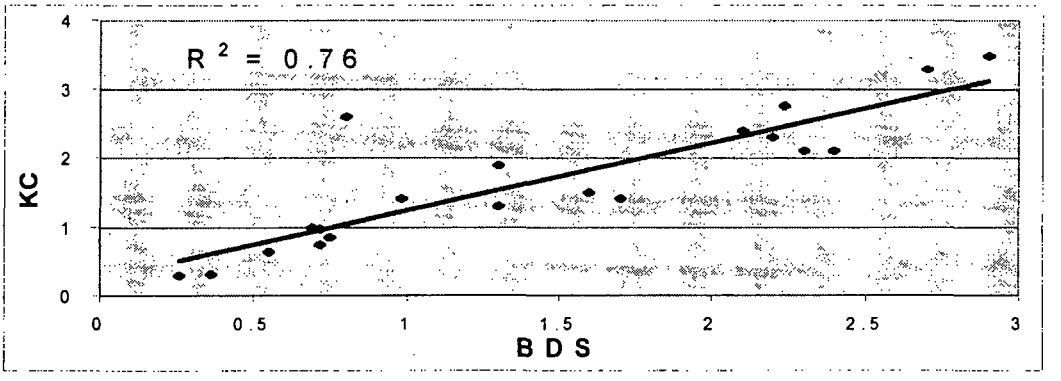


Table 1 : TEF and TEQ-values analyzed by DR-CALUX[c], Micro-EROD and chemical analysis for the in the present study tested cross-validation samples [CV, Coefficient of variation (%); [a]Not available; [b]TEF; [c]bio-TEQ; [d]TEQ-calculated (pg TEQ/ml); [e]TEQ-measured (PCBs + PCDD/Fs; ng-TEQ/ml, g or m^3); calculations of all samples concentration based]

	Kaneka Corporation (KC)	BDS	Ratio	KC	KC or BDS	Ratio
Samples	DR-CALUX [n; CV] (A)	DR-CALUX [n; CV] (B)	(A)/ (B)	Micro-EROD [n; CV]	WHO-TEF or TEQ (C)	(A)/ (C)
23478-PCDF	0.75 ± 0.06[b] [4; 8]	0.84 ± 0.16[b] [3; 23]	0.89	0.41 ± 0.17[b] [5; 42]	0.5[b]	1.5
23478-PBDF	0.069 ± 0.022[b] [5; 32]	0.099 ± 0.017[b] [2;-]	0.70	0.055 ± 0.021[b] [5; 38]	-	-
PCB-126	0.072 ± 0.014[b] [6; 19]	0.073 ± 0.016[b] [3; 22]	0.99	0.049 ± 0.018[b] [6; 36]	0.1[b]	0.72
Dioxin mixture (EPA-1613)	260 ± 39[c] [16, 15]	290[c] [2;-]	0.90	310 ± 94[c] [9; 30]	240[d]	1.1
PCB mixture	98 ± 21[c] [3; 21]	140[c] [2;-]	0.70	a	160[d]	0.61
Co-PCB mixture	270 ± 59[c] [11; 22]	330[c] [2;-]	0.82	260 ± 75[c] [13; 29]	240[d]	1.1
PCBs/PCDD/Fs	160 ± 32[c] [7; 20]	150[c] [1;-]	1.1	210 ± 51[c] [8; 24]	150[d]	1.1
2,2,4,4',5-PBDE	< 4.2 x 10^{-4}	< 4.2 x 10^{-4}	-	-	-	-
Combustion gas	72 ± 11[c] [6; 15]	97 ± 14[c] [3; 14]	0.74	8.9 ± 2.6[c] [5; 29]	8.7[e]	8.3
Fly ash 1	130 ± 13[c] [5; 10]	130 ± 10[c] [3; 8]	1.0	51 ± 6.7[c] [5/25]	44[e]	3.0
Fly ash 2	17 ± 3.0[c] [6; 18]	14 ± 1.4[c] [3; 10]	1.2	7.4 ± 1.4[c] [3/18]	5.3[e]	3.2
Fly ash 3	210 ± 36[c] [5; 17]	240 ± 26[c] [3; 11]	0.88	370 ± 150[c] [3; 42]	95[e]	2.2
PCB oil 1	2300 ± 370[c] [10; 16]	2100[c] [2;-]	1.1	1500 ± 180[c] [3; 12]	1150[e]	2.0
PCB oil 2	0.36 ± 0.05[c] [6; 15]	0.30[c] [2; -]	1.2	0.059[c] [4; 33]	a	3.6
Mineral oil	2240 ± 580[c] [6; 26]	2750[c] [2; -]	0.81	710 ± 310[c] [3; 44]	a	
Feed sample 1	2.9 ± 0.84[c] [9; 29]	3.5 ± 0.84[c] [6; 24]	0.83	2.7 ± 0.12[c] [3; 4]	a	
Feed sample 2	220 ± 49[c] [7; 22]	230 ± 55[c] [3; 24]	0.96	220 ± 55[c] [3; 25]	a	
Feed sample 3	240 ± 49[c] [7; 21]	210 ± 23[c] [3; 11]	1.1	230 ± 40[c] [3; 18]	a	
Sediment 1	0.055 ± 0.02[c] [8; 37]	0.063 ± 0.011[c] [3; 17]	0.87	0.027±5.5[c] [3; 20]	a	
Sediment 2	0.080 ± 0.021[c] [8; 26]	0.26 ± 0.096[c] (3; 37]	3.1	0.13 ± 0.015[c] [3; 11]	a	
Sediment 3	0.13 ± 0.02[c] [6; 17]	0.19[c] [2; -]	0.68	0.086 ± 0.022[c] [3; 26]	a	