VALIDATION STUDY FOR PRACTICAL BIO-MONITORING OF WASTE PCB SAMPLES DURING THEIR DESTRUCTION TREATMENT USING DR-CALUX® ASSAY AND PCB IMMUNOASSAY

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Introduction

On the fate and behavior of PCBs during chemical destruction processes, bio/biochemical analyses can be promising tools to measure the reduction of PCBs and other undesirable by-products such as PCDD/DFs from a toxicology-directed viewpoint.

Mineral insulating oil and capacitor oil containing PCBs and their chemically treated samples are focused on as the test samples in this study. Clean-up procedures are designed and conducted for DR-CALUX® reporter-gene bioassay to measure dioxin-like compounds (AhR agonists) derived from PCB mixtures. Mineral insulating oil contains considerable amount (several %wt) of polyaromatic hydrocarbons (PAHs) originated from mineral oil, the AhR binding activity of which might be overwhelming to misevaluate the activity of PCBs. PCBs themselves are also the complex mixtures of AhR agonists and antagonists. 2-4 ortho PCBs have been reported to act as antagonists which mask the AhR binding activity of agonists of 0-1 ortho PCBs[1, 2, 3, 4]. Silica gel-H_2SO_4 (44%) reflux treatment was validated and used to remove PAHs in samples and then followed by a fractionation method using the activated carbon dispersed silica gel column to obtain the “agonistic” 0-1 ortho planar PCBs and PCDD/DFs fraction.

In addition to DR-CALUX® reporter gene assay for bio-TEQ measurement, Enzyme-linked immunosorbent assay (ELISA) adopting monoclonal antibody was also tried to be used to simply grasp the concentration of major PCB congeners (*i.e.*, #118) correlated with the whole PCB content. Those bioassay results were compared with those of chemical analysis {sum of PCBs, and (PCDD/DF+Co-PCB)-TEQ} to discuss applicability of these bio/biochemical analyses to monitoring of waste PCB treatment.

Materials and methods

Waste PCB samples

Actual PCB (treated) samples. PCB capacitor oil samples chemically treated by palladium/carbon (Pd/C) catalyst were available. Two samples of different treatment levels were selected. One PCB-containing insulating mineral oil sample (untreated) was also chosen as a test sample. Their chemical analysis on PCBs and PCDD/DFs had been conducted. Their PCB contents and I-TEQ values were described in Table 1.

BIOANALYSIS

Wide variety of range of PCB-containing mineral oil. PCB capacitor oil, composition of which was known by chemical analysis (*ca*. 96% PCB content; 24,000 ng-TEQ/g), was diluted to 1,000 mg-PCB/kg (24 ng-TEQ/g) with n-hexane. The solution was further diluted and adjusted as the various range of samples of 50, 5, 0.5 and 0.05 mg-PCB/kg with mineral oil for evaluating the sensitivity of bioassay.

Sample clean-up

Extraction from samples (0.5 - 1.5 g) was made with DMSO (75 mL X 4), which was followed by the re-extraction with n-hexane (225 mL X 3). The reduced volume (5 mL) of n-hexane fraction after evaporation was processed to the silica gel-H_2SO_4 (44 %) reflux treatment (150 g of silica gel-H_2SO_4 was mixed with 300 mL of diluted n-hexane fraction) at 70 °C for 1 h. Using this reflux method, we have experimentally confirmed > 99 % decomposition of 16 common PAHs (10 ìg each). Then the treated fraction was applied onto 4 g of activated carbon dispersed silica gel column (1.0 cm X 30 cm) that had been pre-conditioned with 200 mL of toluene and 200 mL of n-hexane in this order. Elution was conducted with n-hexane (240 mL) to afford a non-dioxin-like (2-4 ortho) PCB fraction, followed by 25 % dichloromethane/n-hexane (160 mL) to yield a dioxin-like mono-ortho PCB fraction and finally by toluene (800 mL) to obtain non-ortho PCBs and PCDD/DFs. The second and third fractions were combined, evaporated and then replaced with DMSO to yield the bioassay fraction.

Bio/biochemical analyses

DR-CALUX®: Rat hepatoma H4IIE cells, stably transfected with an AhR-regulated luciferase gene construct, were used. DR-CALUX® was carried out as described in the guidelines from BDS (www.biodetectionsystems.com) and recently published studies[5, 6]. Dose-response data for 2,3,7,8-TCDD (positive standard) were handled by an analysis software, Slide Write Plus Ver. 5.0 (Advanced Graphics Software) utilizing an 1-site ligand sigmoid curve fitting program. The concentration of "bio-TEQ" in the unknowns was determined by interpolation. Relative potency values based on EC_5 (EC_5 is approximately the limit of quantification) were adopted and the response data of sample close to the EC_5 value of 2,3,7,8-TCDD response curve was interpolated on the curve to calculate bio-TEQ concentration.

ELISA: The rapid and quantitative measurement of major congeners of PCBs was conducted using the ELISA kit manufactured by EnBioTec Laboratories. This kit applied a monoclonal antibody (EBT ACPM118A) to PCB #118 to bind PCB #118 in a target sample or competitor-HRP conjugate in a competitive manner. The IC_{50} value of this ELISA for PCB #118 was 114 ng/mL with a LDL of 34.6 ng/mL. The cross reactivities for a number of Co-PCBs and PAHs were determined at concentrations to 10 µg/mL. The % cross reactivities based on IC_{50} in comparison with PCB #118 were observed only on six Co-PCB congeners as follows: PCB #77, 70.1; #81, 23.1; #156, 14.3; #105, 10.4, #114, 6.7, #126, 3.3. The concentration of PCBs in unknowns was determined by interpolation on the logistic curve for the standard (PCB #118) handled by Microplate Manager Ver. 5.1 (Bio-Rad Lab.).

Results and Discussion

DR-CALUX® results for actual PCB (treated) samples

To ascertain the usefulness of the proposed clean-up method, fractions of the three actual PCB (treated) wastes were sampled at each level of clean-up procedures, replaced with DMSO and assayed using DR-CALUX® (Table 1). Overestimation (> 1,000 fold of interference) of bio-TEQ for Pd/C treated PCB capacitor oil-1 and PCB insulating oil samples (method A fractions) was remarkably improved by completing the proposed pre-treatment (method D). Finally, the ratio between bio-TEQ and I-TEQ values for Pd/C treated PCB capacitor oil-2 and PCB insulating oil became 2.8 and 3.3, respectively. Bio-TEQ value for Pd/C treated PCB capacitor oil-1 became under the determination limit.

Table 1 DR-CALUX® bio-TEQ values (µg/kg) and induction strength for actual waste PCB (treated) samples (results for each fraction of four clean-up methods).

(Treated) waste PCB samples	Pd/C treated PCB-1		Pd/C treated PCB-2		PCB insulation oil	
Pretreatment type	Bio-TEQ	Induction	Bio-TEQ	Induction	Bio-TEQ	Induction
Method A (DMSO/n-hexane extraction)	0.78	39	0.35	27	2,200	62
Method B (A+ silica gel-H_2SO_4 (22%) reflux)	0.36	14	0.45	25	3,100	60
Method C (A+ silica gel-H_2SO_4 (44%) reflux)	NM	NM	NM	NM	11	49
Method D (C+ activated carbon dispersed silica gel)	<0.016	36	0.31	42	1.0	77
PCB content (µg/kg)	<100		5,200		6,900	
I-TEQ µìg-TEQ/kg)	0.00072		0.11		0.30	
Bio-TEQ (method D)/I-TEQ	– –		2.8		3.3	

Key: NM, not measured

DR-CALUX® and ELISA results for a range of PCB- containing mineral oil

The extracts from insulating mineral oil had one of the strongest matrix effects in DR-CALUX® assay as mentioned above. Wide variety of range of PCB-containing mineral oil from 0.05 mg/kg to 50 mg/kg was prepared and fractions obtained by each of the method C and D were tested by DR-CALUX® and ELISA (Table 2).

DR-CALUX® data showed good agreement with I-TEQ values (Bio-TEQ/I-TEQ Ratio = 1.0 – 3.0) for 50 and 5 mg/kg PCB samples in both of method C and D fractions. DR-CALUX® can theoretically quantify the TEQ up to 0.1 ìg/kg level on this scale of sample clean-up, which was consistent with the assay results. Induction strength (response magnitude) became higher for method D fractions than for method C fractions (data not shown). However, difference between results with and without the use of activated carbon dispersed silica gel was not clearly observed in terms of Bio-TEQ for the prepared samples, which is of interest and still the focus of detailed investigation.

PCB "#118" contents measured by ELISA were well correlated with total PCB concentrations up to 0.5 mg/kg level with a correlation coefficient of 1.00 for both of method C and D fractions. The observed ELISA values were also highly correlated with the predicted values taking cross reactivities of Co-PCBs into consideration with a correlation coefficient of 1.00 for method C and D fractions.

For this ELISA, overestimation due to interference compounds (*e.g.*, non-coplanar PCBs) was not observed even for method C fractions. These data indicate that the PCB content can be estimated by the results of ELISA adopting antibody that binds to major PCB congeners.

Bio-monitoring methods detecting equal to or less than 0.5 mg-PCB/kg (treatment standard for waste PCB oil in Japan) are required to contribute to the routine monitoring in waste PCB treatment. Scale-up of sample preparation to enhance the determination level and application to various matrices of PCB untreated/treated samples are necessary for bio/biochemical analyses.

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Table 2 DR-CALUX® bio-TEQ values and ELISA values for a range of PCB-containing mineral oil (results for method C and D fractions).

		DR-CALUX®			ELISA		
Pre-treatment	PCB conc. (mg/kg)	Bio-TEQ (µg-TEQ/kg)	I-TEQ (µg-TEQ/kg)	Bio-TEQ /I-TEQ	Observed (µg/kg)	Predicted (µg/kg)	Ratio (Obs./Pred.)
Method C	50	1.6	1.2	1.3	2,200	2,000	1.1
	5	0.27	0.12	2.3	200	200	1.0
	0.5	0.23	0.012	19	19	20	0.95
	0.05	0.096	0.0012	80	ND	2.0	-
Method D	50	1.2	1.2	1.0	1,500	2,000	0.75
	5	0.36	0.12	3.0	210	200	1.1
	0.5	0.27	0.012	22	6.6	20	0.33
	0.05	1.0	0.0012	870	ND	2.0	-

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