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PRACTICAL CALUX-MONITORING OF PCB WASTES DURING THEIR CHEMICAL DECHLORINATION TREATMENTS

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Introduction

In Japan, the law concerning special measures on the promotion of appropriate management of waste PCBs was approved and enacted in June, 2001. Upon the enforcement of this law, complete treatment of stockpile PCB wastes is scheduled by 2016. Non-incineration PCB treatment processes have been adopted and developed by the plant makers, PCB users and keepers. However, the environmentally sound treatment (destruction or irreversible conversion) of PCB wastes is a complex issue, because it should be strictly ensured that the technologies adopted for the treatment are acceptable both technically and socially (*i.e.*, health and economic impacts).

Monitoring for the presence of dioxin-like compounds in treated PCBs is one of the important toxicological focuses. It can be performed by strategies involving a risk assessment method in order to evaluate residual PCBs and undesirable treatment byproducts and a screening method in order to detect their level of interest rapidly. The use of the EROD (Ethoxy-Resorufin- *O*-Deethylase) and CALUX (Chemical Activated LUciferase eXpression) cell-based Ah receptor bioassays allows the identification and quantitation of dioxin-like contaminants in industrial effluents and environmental/biological samples[1-6] and they have been successively applied to the monitoring during some chemical dechlorination processes of waste PCBs as promising tools to meet the both strategic demands in our study[7, 8].

In this study, PCB treated samples during each of a photodechlorination method by ultraviolet irradiation (UV method), a catalytic hydrodechlorination method with palladium/carbon (Pd/C method), a UV-Pd/C combination method and a dechlorination method with potassium *t*-butyloxide (t-BuOK method) were prepared in a gradual way in terms of residual PCB concentrations. Extraction and clean-up from the samples were conducted to yield (acid-) stable fractions containing persistent organic compounds which were applied to the CALUX (DR-CALUX®). Treated PCB samples around 0.5 µg-PCB/g which has been set to as the treatment goal value for PCB oil in Japan were especially in focus and the detectability of CALUX for those samples were examined in combination with the comparison of CALUX-TEQs and GC/MS-determined TEQs (WHO-TEQs).

Materials and Methods

Treated PCB samples were obtained from five treatment runs using four methods described above. A detailed description of samples, type of treatment (treatment conditions), type of PCBs as the starting materials, type of solvent, sampling time, residual PCB concentrations is shown in Table 1 and 2. Determination of PCDD/Fs and Co-PCBs was performed using high-resolution GC/ high-resolution MS. PCB concentrations were measured by GC-ECD.

Reflux method with silica gel-sulfuric acid was adopted for the bioassay-directed fractionation of stable dioxin-like compounds in the samples. This method was effective for the removal of labile compounds (*e.g.*, polyaromatic hydrocarbons) in complicated sample matrix such as mineral oil [8] and waste wood samples[9]. Firstly, extraction from samples (0.005 - 25 g) was made with DMSO, which was followed by the re-extraction with n-hexane. The n-hexane fraction (100mL) was refluxed with 50 g of silica gel-sulfuric acid (44%) at 70°C for 1 h. Additional reflux was conducted with 25 g of silica gel-sulfuric acid (44%) until added silica gel remain colorless, if necessary). Then the treated fraction was processed to alumina column separation for removing residual oil. The final fractions were evaporated and replaced with DMSO and measured by the CALUX.

Table 1. Experimental scheme of PCB treatment using UV method and UV-Pd/C combination method.

Treatment		UV dechlorination				UV dechlorination + Pd/C method		
PCB	Kanechlor-400							
Solvent	2-propanol							
React. Time	Initial	38 min	114 min	300 min	30 h	During Pd/C method	During Pd/C method	After Pd/C method
PCB conc. (μg/g)	10,000	3,300	260	36	0.13	9.2	0.58	0.007
Sample No.	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8

UV method: test solution 8.7 L, NaOH 1%w/w, photochemical reactor 170 mm (diameter) X 1,500 mm (H) with a low pressure mercury lamp (λ_{max} = 254 nm), react. temp 50- 60 °C
Pd/C method: test solution 100 mL, Pd/C catalyst 0.2 g, react. temp 75 °C

Table 2. Experimental scheme of PCB treatment using Pd/C method and t-BuOK method.

Treatment	Pd/C method			t-BuOK method					
PCB	Kanechlor-300								
Solvent	Liquid paraffin						Mineral insulator oil		
React. Time	Initial	35 min	75 min	Initial	0.5 min	20 min	Initial	0.5 min	20 min
PCB conc. (μg/g)	52.9	2.2	< 0.1	52.9	1.89	< 0.1	53.9	2.05	0.16
Sample No.	K-1	K-2	K-3	K-1	K-4	K-5	K-6	K-7	K-8

Pd/C method: test solution 1.2 L, Pd/C catalyst 0.6 g, react. temp 260 °C, H_2 gas rate 0.2 L/min (0.5 min ventilation/ 5 min)
t-BuOK method: test solution 200 mL, t-BuOK 0.85 g, react. temp. 250 °C, N_2 gas rate 0.2 L/min

The CALUX cell line (recombinant rat H4IIE cell line) was obtained from Bio Detection Systems B. V. (Amsterdam, The Netherlands). The CALUX assay was carried out as described by Behnisch *et al*[6]. The standard dose-response curve was fitted using a cumulative fit function using Slide Write Plus Ver. 6.00 (Advanced Graphics Software). CALUX-TEQs for the tested samples were obtained from their dilutions so that their luciferase activities were in the reproducible lower part of the linear range corresponding to 1- 4 pM in TCDD.

Results and Discussion

The PCB concentrations, WHO-TEQ values, CALUX-TEQ values and CALUX-TEQ/WHO-TEQ ratio for the PCB treated samples were compared in Table 3.

The final treated oils obtained after conducting UV method, UV-Pd/C combination method, Pd/C method and t-BuOK method (liquid paraffin) showed quite low CALUX-TEQ values ranged from ND - 0.13 pg-TEQ/g corresponding to low PCB concentrations (ND - 0.13 μg/g) and low WHO-TEQs (0.11 - 0.13 pg-TEQ/g). The final sample (mineral oil) after t-BuOK treatment showed comparatively higher CALUX-TEQ value (1.4 pg-TEQ/g).

All samples showing WHO-TEQ values of more than 0.18 pg-TEQ/g gave a quantitative response in the CALUX. Very low limit of CALUX quantitation (LOQ) could be achieved by using 25 g of samples (LOQ: 0.047 – 0.18 pg-TEQ/g), which made it possible to quantify 10^{-1} pg-TEQ/g order of samples. In terms of residual PCB concentration, CALUX could detect the levels up to 0.1 μg-PCB/g for the tested samples.

Table 3. Results of chemical analysis and bioanalysis (CALUX) for various PCB (treated) samples.

Treatment	Sample	Chemical analysis		Theoretical CALUX-TEQ* (pg-TEQ/g)	Bioanalysis (CALUX)					B/C ratio (-)	Remarks
		PCB (ECD-GC) (μg/g)	WHO-TEQ(C) (pg-TEQ/g)		Sample (g)	LOD** (pg-TEQ/g)	LOQ*** (pg-TEQ/g)	CALUX-TEQ¶(B) (pg-TEQ/g)	CV ÷ (%)		
UV method (2-propanol)	T-1	10,000	**1,100,000**	1,300,000	0.005	320	770	**150,000**	13	0.14	Initial
	T-2	3,300	**NM†**	|	0.01	170	430	**590**	15	|	
	T-3	260	**NM†**	|	0.2	9.0	22	**28**	20	|	
	T-4	36	**0.47**	0.7	5	0.15	0.37	**4.8**	24	10	
	T-5	0.13	**0.11**	0.015	25	0.047	0.11	**0.17**	34	1.5	Cleared treatment goal §
UV + Pd/C method (2-propanol)	T-6	9.2	**NM†**	|	10	0.10	0.24	**1.6**	19	|	
	T-7	0.58	**1.0**	4.7	25	0.057	0.12	**0.40**	13	0.40	
	T-8	0.007	**0.11**	0.016	25	0.050	0.12	**ND**	|	|	Cleared treatment goal §
Pd/C method (liquid paraffin)	K-1	52.9	**520**	660	0.2	3.6	8.8	**530**	9	1.0	Initial
	K-2	2.2	**0.29**	0.98	10	0.11	0.28	**0.13**	23	0.45	
	K-3	<0.1	**0.13**	0.4	25	0.033	0.077	**ND**	|	|	Cleared treatment goal §
t-BuOK method (liquid paraffin)	K-1	52.9	**520**	660	Identical to K-1 before the Pd/C treatment			**530**	9	1.0	Initial
	K-4	1.89	**0.18**	0.43	10	0.080	0.19	**0.69**	13	3.8	
	K-5	<0.1	**0.11**	0.015	25	0.073	0.18	**0.13**	19	1.2	Cleared treatment goal §
t-BuOK method (mineral oil)	K-6	53.9	**520**	700	0.2	4.4	11	**340**	7	0.65	Initial
	K-7	2.05	**0.36**	0.69	2	0.78	1.9	**7.2**	7	20	
	K-8	0.16	**0.11**	0.015	25	0.017	0.047	**1.4**	12	13	Cleared treatment goal §

* Theoretical CALUX-TEQ $_{\text{Co-PCBs}}$= ∑ (CALUX-TEF $_{\text{Co-PCBs}}$ X analytical conc.)

** LOD: the limit of detection, *** LOQ: the limit of quantitation

¶ Analyzed at least three times, † NM: not measured

§ Japanese treatment goal for waste PCB oil is 0.5 μg-PCB/g.

CALUX-TEQ/WHO-TEQ ratio (B/C ratio) varied from 0.1 - 20 for the tested samples except for T-8 and K-3 which showed ND in the CALUX. The difference in B/C ratio of treated samples among the performed treatment runs was relatively consistent for the difference in treatment characteristics and sample matrix. For example, Pd/C-treated samples at the final stage (T-8 and K-3) gave no quantitative response in the CALUX, while they contained 0.11 – 0.13 pg-WHO-TEQ/g. For the UV-treated and t-BuOK treated (liquid paraffin) samples at the final stage (T-5 and K-5), CALUX-TEQs corresponded well to WHO-TEQs. The t-BuOK treated sample at the final stage (mineral oil, K-8) differed by a factor of 13.

Theoretical CALUX-TEQs were calculated by the following equation using our EC_5-based CALUX-TEF data for Co-PCBs[10] and compared to the experimental CALUX-TEQs.

Theoretical CALUX-TEQ $_{\text{Co-PCBs}} = \sum$ (CALUX-TEF $_{\text{Co-PCBs}}$ X GC/MS analytical concentration)

For the starting samples at every treatment (T-1, K-1 and K-6), the experimental CALUX-TEQs were lower than the theoretical CALUX-TEQs. This could be due to AhR-antagonistic effect of non-planar PCBs existing in the tested fractions. However, treated samples showed higher experimental CALUX-TEQs than the theoretical values during the UV and t-BuOK treatments, which suggests the occurrence of stable AhR agonists other than Co-PCBs. Meanwhile, lower CALUX-TEQs were obtained compared to the theoretical CALUX-TEQs during the Pd/C treatments. It was supposed that difference in treatment was reflected in above results.

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