

COMPARISON OF DR-CALUX TO HRGC/HRMS – TEQ MONITORING DURING KANECHLOR PCB DEGRADATION PROCESS USING METALLIC SODIUM DISPERSION

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Introduction

In view of increasing risk by waste PCB stockpiles, the Government of Japan is going to start regional treatments to destroy PCBs stored across the nation from this year onward ¹. For PCB containing oil treatment, which is of major concern, well-demonstrated chemical treatment technologies are adopted.

Oil treatment goal standard for PCB has been set as 0.5 ppm. Additionally, focus is also put on the control and reduction of dioxins, hydroxylated PCBs, *etc.* Basic concept for the judgment of oil treatment completion is the decreased level confirmation of those chemical concentrations. Those concentration levels should be checked during treatment at regular intervals using high resolution GC-MS (HRGC/HRMS). However, rapid and cost-effective methods are also necessary for everyday monitoring if the correlation between values of HRGC/HRMS and rapid methods is fully confirmed.

Monitoring of the fate of dioxin-like compounds during treatment is one of the important toxicological focuses. We have successively applied the DR-CALUX[®] bioassay to the monitoring during chemical dechlorination processes to meet the demands involving a risk assessment method and a screening method ²⁻⁴.

In this study, CALUX monitoring study was conducted during PCB Kanechlor (*i.e.*, KC-300, 400, 500) degradation by the sodium dispersion process, which will be actually adopted at one national treatment facility. CALUX-TEQs in the treated samples (cleaned-up as acid-stable fraction) were compared to the corresponding HRGC/HRMS-determined TEQs (WHO-TEQs) and a correlation between them was discussed. Further, those TEQ values were compared to those obtained for PCB oils treated by various chemical treatment methods in one common scale.

Materials and Methods

PCB treatment using the sodium dispersion process was carried out in a laboratory-scale reactor. KC-300, 400 and 500 (65 - 100 mg) were respectively added to *n*-hexadecane (500 - 800 mL) in the reactor. Then each mixture (approximately 100 mg PCB/g) was kept stirred at fixed

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temperature (lower temperature: 120 or 130°C, higher temperature: 160 °C) in the presence of nitrogen gas. Treatment reaction was initiated by injecting sodium dispersion (22-23% w/w, mean particle size 5-6 µm) at the sodium/PCB-chlorine (mol/mol) ratio of 130 into the reactor. 20 or 50 mL of treated oil was extracted from the reactor at appropriate reaction times. Reaction was stopped by adding water to the sampled oils to convert excess metal sodium to sodium hydroxide. Dioxin and PCB congener concentrations in samples were determined by HRGC/HRMS (HP6890/JMS-700; resolution 10,000; SP-2331, DB-5MS and HT-8 GC columns for 4-6Cl-PCDD/Fs, 7-8Cl-PCDD/Fs and PCBs, respectively).

Extraction from samples (aliquots of 1 - 7.5 g) was quantitatively made with DMSO (dimethylsulfoxide) to remove *n*-hexadecane. Then the DMSO fraction was diluted with water and followed by a re-extraction with *n*-hexane. The *n*-hexane fraction was evaporated and replaced with 50 or 100 µL of DMSO to yield labile (crude) fraction containing various hydrophobic compounds. Reflux method with silica gel-sulfuric acid was adopted for the fractionation of stable compounds. This method has been used through all the CALUX sample preparation for PCB treatment samples²⁻⁴. The *n*-hexane fraction (100 mL) after the DMSO/*n*-hexane extraction was refluxed with 50 g of silica gel-sulfuric acid (44%) at 70°C for 1 h. The refluxed fraction was evaporated and redissolved in 50 or 100 µL of DMSO as stable fraction. The procedural blank was also prepared through the same cleanup procedures to correct background of the obtained data.

The CALUX cell line (H4IIE-luc cell line) was obtained from Bio Detection Systems B.V. (Amsterdam, The Netherlands) and the CALUX was carried out as described by Asari *et al*⁵. The 2,3,7,8-TCDD standard dose-response curve was optimized using a cumulative fit function of 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

PCB, WHO-TEQ and CALUX-TEQ concentrations in the samples are shown in Table 1. The sodium dispersion process gave a remarkable reduction of PCBs to the level less than 500 ng/g (oil treatment goal) within five minutes after reaction initiation in the both cases of low and high temperature conditions. As shown for KC-400 treatment in Fig. 1, there showed little change in composition pattern of PCB homologues, which suggested that every PCB homologue was reduced uniformly, while dechlorination pathway by

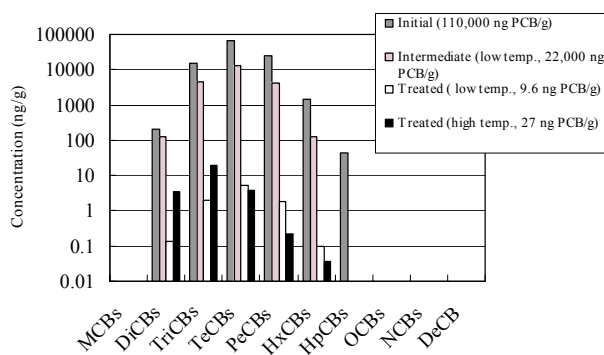


Fig. 1 Change in PCB homologue pattern of Kanechlor 400 samples during the sodium dispersion process.

sodium dispersion treatment appeared to be dependent on number and position of the substituted chlorine atom⁶. The trend of PCB homologue reduction pattern was similar for KC-300 and KC-500. With regard to WHO-TEQ, high values (240 - 2,000 pg/g) in the initial KC samples dropped remarkably to 0.0016 - 0.072 pg/g in the final treated samples.

For DR-CALUX, low limit of quantitation (LOQ) could be obtained by using 7.5 g of samples (LOQ: 0.3 – 0.5 pg-TEQ/g) and the coefficients of variation between triplicate assay results was almost below 30%.

CALUX-TEQ in the stable fraction (obtained after DMSO partition and silica gel/44% sulfuric acid reflux) could be a good correspondent to chemical TEQs since a good correlation between them have been observed in our CALUX monitoring study on PCB treatment²⁻⁴. In terms of results for stable fractions, CALUX-TEQs for the initial samples showed 740 – 1,300 pg/g. The samples showing WHO-TEQ values of more than 0.072 pg/g gave a quantitative response by the DR-CALUX and the final treated oils showed quite low CALUX-TEQ values ranged from ND – 1.7 pg/g corresponding to low PCB concentrations (1.7 - 45 ng/g) and low WHO-TEQs (0.0016 – 0.072 pg/g). The CALUX-TEQ (stable fraction)/WHO-TEQ ratio (B/C ratio) varied from 0.44 – 24 for the tested samples except for three treated samples which showed ND in the DR-CALUX. The difference in B/C ratio of treated samples among three Kanechlor treatment runs may be relatively consistent for the difference in Kanechlor compositions.

Theoretical CALUX-TEQs were calculated as below using our EC₅-based CALUX-TEFs for Co-PCBs⁷ and compared to the experimental CALUX-TEQs.

Theoretical CALUX-TEQ_{Co-PCBs} = $\sum (\text{CALUX-TEF}_{\text{Co-PCBs}} \times \text{HRGC/HRMS concentration})$

For all the samples except for the final KC-500 treated oil, experimental CALUX-TEQs were lower than the theoretical CALUX-TEQs (experimental/theoretical ratio = 0.03 -0.82). This could be due to weaker cell cross-reactivity of Co-PCBs compared to WHO-TEF and AhR-antagonistic effect of non-planar PCBs existing in the tested stable fractions.

The CALUX-TEQs of labile fractions were one to nine-fold higher than those of corresponding stable fractions in the initial and intermediate samples and showed small CALUX-TEQ values (5 - 13 pg-TEQ/g) in the final treated samples. Non-PCB substances contributive to this crude activity have not been characterized, though they are chemically labile.

Table 1. Results of chemical analysis and bioanalysis (DR-CALUX) for Kanechlor (treated) samples through the sodium dispersion process.

PCB	Degradation condition	React. time (min)	CALUX-TEQ (pg/g)					Chemical analysis		B/C ratio**	Remarks
			Crude fraction		Stable fraction		Theoretical value*	WHO-TEQ (pg/g)	Total-PCBs (ng/g)		
			Av.	%CV	Av.	%CV					
KC-400	low temp. □ 130 □ □	0	1600	14	1300	8.9	2900	2000	110000	0.65	
		1.5	16	8.5	2.0	41	67	4.1	22000	0.49	
		3	7.9	6.7	ND	-	ND	0.0052	9.6	-	Cleared treatment goal† < LOQ of CALUX
	high temp.□ 160 □ □	0.5	5.0	25	ND	-	ND	0.0033	27	-	Cleared treatment goal† < LOQ of CALUX
KC-300	low temp. □ 120 □ □	0	980	8.1	980	0.5	1200	240	91000	4.1	
		1.5	18	9.9	2.0	0	6.9	0.22	12000	9.1	Cleared treatment goal†
		5	13	14	ND	-	ND	0.0016	1.7	-	Cleared treatment goal† < LOQ of CALUX
KC-500	low temp. □ 120 □ □	0	990	4.6	740	11	1400	1700	95000	0.44	
		2	78	13	66	21	110	47	11000	1.4	
		5	8.9	11	1.7	0.8	0.28	0.072	45	24	Cleared treatment goal† < LOQ of CALUX

* Theoretical CALUX-TEQ = Sum (CALUX-TEF x analytical conc.)

** □ B/C ratio □ □ CALUX-TEQ in stable fraction, pg/g □ □ WHO-TEQ, pg/g □

† Japanese treatment goal for waste PCB oil is 500 ng PCB/g.

CALUX-TEQ and WHO-TEQ values of Kanechlor PCB treated samples during each of a photolysis method by ultraviolet irradiation (UV method), a catalytic hydrodechlorination method with palladium/carbon (Pd/C method), a UV-Pd/C combination method, a dechlorination method with potassium *t*-butoxide (t-BuOK method) and a sodium dispersion method reported in our studies²⁻⁴ were plotted in one common scale (Fig. 2). All the chemical PCB treatment methods reduced WHO-TEQ up to 10⁻¹ pg/g order or less in the final treated oil samples which cleared PCB treatment goal. The residual WHO-TEQ levels could be satisfactorily low even if compared to strict dioxin limit values such as those of food and feedstuffs in EU. The corresponding CALUX-TEQs for the samples showed 1 pg/g or less (CALUX LOQ: 10⁻¹ pg/g order) and consistent correlation between those two TEQs was observed.

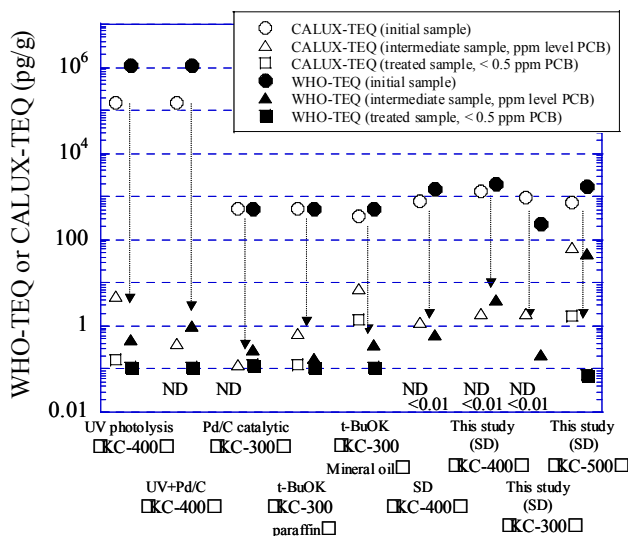


Fig. 2 Comparison of CALUX-TEQ and WHO-TEQ values of PCB (treated) samples during various chemical treatments.

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