Organohalogen Compounds, Volume 60, Pages 323-326 (2003)
1) Analysis g) Fast and/or Inexpensive Analyses

BIOASSAY MONITORING STUDY IN THE PCB DEGRADATION PROCESS USING METALLIC SODIUM DISPERSION

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Introduction

The PCB treatment technology presented in this paper uses chemical methods to dechlorinate PCB oil or PCB-contaminated oil by finely dispersed sodium as the active agent under inert atmosphere (nitrogen gas). PCB-sodium reaction (replacement of chlorine atom of PCB molecule with sodium atom) can be done at comparatively lower temperatures[1]. For PCB oil with low concentrations (greater than tens of ppm and less than 10,000ppm), the reaction is conducted at 50-60^{o}C and then followed by the addition of water to treated oil for the two-phase separation of the residual hydrophobic byproduct (e.g., biphenyl as major byproducts) and sodium chloride/hydroxide. On the other hand, high-temperature (160 ^{o}C) treatment is performed for the degradation of PCBs with high concentrations greater than 10,000ppm. In this case, polymerization of dechlorinated biphenyls occurs and the polybiphenyls can be separated from oil together with sodium chloride/hydroxide. Basically, PCB-sodium reaction occurs quickly and detailed time-course reaction mechanism could be hardly clarified. Furthermore, toxicological risk assessment for PCB treatment using this process has not been fully conducted, to the best of our knowledge.

In this study, Kanechlor 400, one of the Japanese commercial PCB products was applied to the sodium dispersion process on comparatively slow reaction conditions at low temperature (60^{o}C). Seven treated samples were taken with time and processed to the extraction and clean-ups for two *in vitro* bioassays: DR-CALUX® AhR reporter-gene bioassay (CALUX) and enzyme-linked immunosorbent assay adopting monoclonal antibody against 2,3', 4,4', 5-pentachlorobiphenyl (PCB #118-ELISA). Labile (crude) fraction containing hydrophobic byproducts and stable fraction containing persistent compounds such as PCBs resistant to acid treatment were prepared from the sampled oils. The two bioassays were applied according to their own assay characteristics. The CALUX was adopted to evaluate total biological TEQ in complex mixtures. The ELISA was used to simply and rapidly grasp the concentration of residual major PCB congeners (*e.g.*, #118).

Materials and Methods

PCB treatment using the sodium dispersion process was carried out in a pilot-scale reactor (500 mL volume) as shown in Figure 1. 60 mg of Kanechlor 400 was firstly added to 600 g of n-hexadecane in the reactor. Then the mixture was kept stirred at 60^{o}C in the presence of nitrogen gas. Treatment reaction was initiated by injecting 2 g of sodium dispersion (10% w/w, particle size < 10 μm) into the reactor. Each of 40 mL treated oil was extracted from the reactor at the reaction time of 0, 10, 15, 20, 25, 30 and 60 min. Reaction was stopped by adding small amount of water to the sampled oils to convert excess metal sodium to sodium hydroxide. PCB concentrations in samples were determined

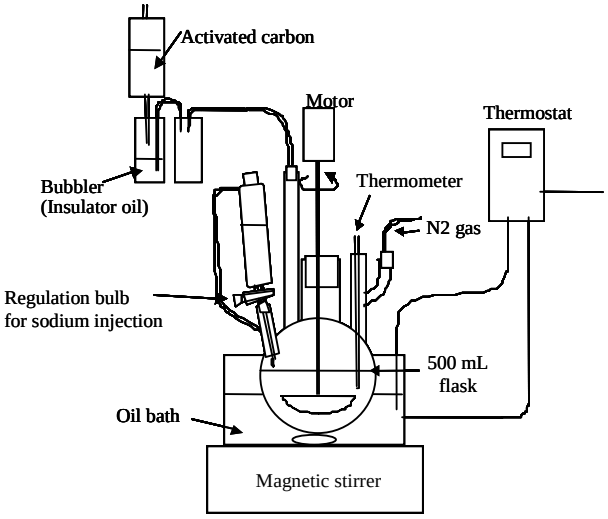

Figure 1. Laboratory-scale apparatus for the PCB treatment using sodium dispersion

by GC-ECD.

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 the re-extraction with n-hexane. The n-hexane fraction was evaporated and replaced with 50 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 was effective for the removal of labile compounds (*e.g.*, biphenyl and other polyaromatic hydrocarbons) in complicated sample matrix such as fly ash[2], mineral oil[3] and waste wood samples[4]. The n-hexane fraction (100mL) after the DMSO/n-hexane extraction was refluxed with 25 g of silica gel-sulfuric acid (44%) at 70°C for 1 h. The refluxed fraction was evaporated and redissolved in 50 µL of DMSO as stable fraction.

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 Behnisch *et al*[2]. The 2,3,7,8-TCDD 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.

PCB #118-ELISA was conducted using the ELISA kit manufactured by EnBioTec Laboratories (Tokyo, Japan). This kit adopts a monoclonal antibody against PCB #118 to bind PCB #118 in sample or a competitor-HRP conjugate in a competitive manner. The IC_{50} value of this ELISA for PCB #118 was 23.7 ng/mL with a detection limit of 6.5 ng/mL. The % cross reactivities based on IC_{50} in comparison with PCB #118 were observed for seven PCB congeners out of 27 predominant congeners contained in commercial PCBs as follows: PCB #28, 1.0; #31, 12.9; #33, 2.6; #66, 15.2; #70, 14.9, #105, 2.5, #110, 0.88. 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

PCB concentrations, CALUX-TEQ and ELISA results for labile and stable fractions, in the samples are shown in Figure 2 and Table 1.

Through the sodium dispersion process, PCB concentration decreased from 100ppm to 0.04ppm at 30 min and to the level below the quantitation limit at 1 h.

The CALUX-TEQs for the initial sample (PCB concentration 100ppm) showed 870 and 800 pg-TEQ/g in the labile and stable fractions, respectively (Figure 2). Then the CALUX-TEQ for stable fractions showed a remarkable drop to 2.5 pg-TEQ/g at 10 min and later remained stable (1-2 pg-TEQ/g) until the final sample showed 0.3 pg-TEQ/g. These values are similar to the CALUX-TEQs of the stable fractions for treated PCB oils (PCB concentration < 0.5ppm) by other PCB dechlorination technologies (ultraviolet irradiation, catalytic hydrodechlorination using palladium/carbon and degradation using potassium *t*-butyloxide)[5].

The CALUX-TEQs of labile fractions (at 20 and 60 min) were four times higher than those of corresponding stable fractions. This suggests the occurrence of labile AhR agonists, though the labile sample at the final stage showed a small CALUX-TEQ value (1.3 pg-TEQ/g). The major byproduct, biphenyl could be a candidate contributing to CALUX-TEQ of labile fraction. However, it possesses no agonistic activity up to 100 µM in the CALUX, which means it is at least eight orders of magnitude less potent than TCDD and cannot be detected even if it is contained to the extent of 10 µg/g in oil. It was confirmed that a large amount of AhR agonist was not formed as byproducts after the treatment run using Kanechlor 400.

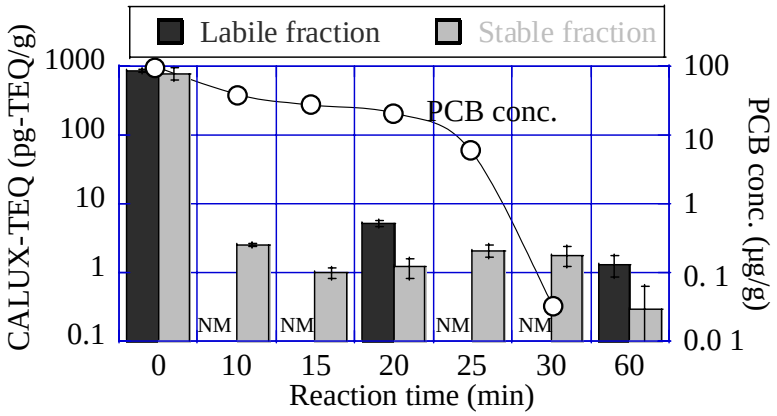

Figure 2. Time-course profile of CALUX-TEQ values obtained from labile and stable fractions and PCB concentrations in the Kanechlor 400 treated samples. Samples were assayed in triplicate. The vertical bar represents the standard deviation. NM: not measured.

The ELISA showed almost the same values (4.95 and 4.86 µg/g) for both fractions of the initial sample (Table 1). These results indicated the ELISA detected 5% of total PCB content, while Kanechlor 400 has been reported to contain 2-4 % of PCB #118[6]. This seems to be reasonable if taking cross reactivities of other PCBs into consideration.

During the treatment, samples were detectable to 6.4 µg-PCB/g at 25 min by the ELISA. The ELISA values decreased gradually and ELISA/GC-ECD value ratio also fell down in stable fractions. For example, ELISA value showed 0.7 µg/g which occupied 1.8% of total PCBs at 10 min, and likewise 0.0037 µg/g which was 0.058% of the PCB sum value at 25 min. During the dechlorination process, the ELISA might especially detect the occurrence of intermediate 3-4 chlorinated PCBs, though it requires reference congener analysis to confirm this.

For the measurement of both labile and stable fractions at 0 and 20 min, the ELISA values were in

good agreement between two fractions. So it could be considered that the antibody is not interfered with the sample matrix of labile fraction after removing n-hexadecane.
Further research using ELISA will focus on conducting a set of ELISA adopting some antibodies with different PCB recognition characteristics (*e.g.*, for each of lower chlorinated congeners and highly chlorinated congeners) and adding chemical congener analysis for comparison.

Table 1. ELISA results for labile and stable fractions and PCB concentrations in the Kanechlor 400 treated samples.

Samples	PCB conc. (µg/g)	Labile fraction		Stable fraction	
		ELISA (µg/g)	ELISA/GC rario	ELISA (µg/g)	ELISA/GC rario
0 min	100	4.95	0.05	4.86	0.05
10 min	39			0.7	0.018
15 min	33.8			0.44	0.013
20 min	20.9	0.15	0.0072	0.098	0.0047
25 min	6.38			0.0037	0.00058
30 min	0.039			ND	
60 min	ND	ND		ND	

The bioassay monitoring study allows screening of dioxin-like potency and toxicological risk assessment adopting the CALUX and screening and estimation of PCB content adopting the ELISA. It is necessary to obtain chemical and bioassay information varying the starting materials of PCBs and treatment conditions to make a comprehensive evaluation of the sodium dispersion process.

Acknowledgements
This research was funded by the Waste Management Research Grant from the Ministry of Environment of Japan. The authors wish to thank Prof. A. Brouwer of the BioDetection Systems B. V. and EnBioTec Laboratories Corporation.

References
1. Ariizumi, A., Otsuka, T., Kamiyama, M. and Hosomi, M. (1997) Journal of Environmental Chemistry 7 (4), 793
2. Behnisch, P. A., Hosoe, K., Shiozaki, K., Ozaki, H., Nakamura, K., Sakai, S. (2002) Environmental Science and Technology 36, 5211
3. Takigami, H., Hosoe, K., Behnisch, P. A., K., Shiozaki, K., Mizukami, H., Ohno, M. and Sakai, S. (2002) Organohalogen Compounds 58, 397
4. Asari, M., Takatsuki, H., Takigami, H. and Sakai, S. (2003) Organohalogen Compounds (in press)
5. Takigami, H., Ohno, M., Ohara, A., Shiozaki, K. and Sakai, S. (2003) Organohalogen Compounds (in press)
6. Takasuga, T., Inoue, T. and Ohi, E. (1995) Journal of Environmental Chemistry 5 (3), 647