# RAPID SCREENING SYSTEM OF PCBs IN TRANFORMER OIL USING IMMUNOCHROMATOGRAPHIC ASSAY

Akira Okuyama, Hiroshi Takenaka, Haruki Mizukami

EnBioTec Laboratories Co., Ltd., Chiyoda Parion Bldg., 6th Floor 2-3-16, Kanda Suda-cho Chiyoda-Ku, Tokyo 101-0041, Japan

**Abstract**

We evaluated our newly developed clean-up and immunochromatographic assay procedures for determination of PCBs in transformer oil. The results of a recovery test and reproducibility test using fresh and used transformer oil samples found the clean-up method to have sufficient ability to eliminate the matrix effects of oil samples on the immunochromatographic assay and showed good reproducibility. The detection limit and assay range were 0.033 and 0.046-0.97 mg-PCB/kg-oil respectively, suggesting the assay could sufficiently cover the domestic regulatory PCB concentration of 0.5 mg/kg in transformer oil. By using used transformer oil samples, we found the measurements of our immunochromatographic assay were highly correlated to HRGC/HRMS and LRGC/LRMS data. We concluded that the developed assay possesses the ability to screen PCB contamination in transformer oil.

## Introduction

Polychlorinated biphenyls (PCBs) are a major concern in environmental contaminants because of toxicity to humans and wildlife, and its low rate of degradation in the environment. Currently there is considerable concern regarding the occurrence of low levels of PCB contamination in insulating and transformer oils in Japan. Simple and cost effective analytical techniques to rapidly confirm the existence of PCB contamination in suspected oils is highly desired.

One possible alternative to conventional instrumental analysis is immunochemical techniques based on poly- or monoclonal antibodies. An immunochromatographic method is very simple, low-cost and requires no special equipments or skills. The objective of this study is to develop a useful screening method for the PCB-contaminated oils using immunochromatographic techniques. In this paper, we report performance of developed clean-up method and the immunochromatographic test to determine PCBs in transformer oils.

## Materials and Methods

All chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). Fresh PCB-free transformer oil was purchased from Matsumura Oil (Osaka, Japan). PCB-contaminated and PCB-free, used transformer oil samples with HRGC/HRMS and/or LRGC/LRMS measurements and anti-PCB monoclonal antibody were supplied by Dr. Ohmura of Central Research Institute of Electric Power Industry (Ibaraki, Japan). Plastic centrifuge tubes and glass vials were purchased from Iwaki (Tokyo, Japan) and Maruemu (Osaka, Japan). Commercial mixtures of PCBs, Kanechlor or KC-300, 400, 500 and 600 were purchased from GL science (Tokyo, Japan). KC-MIX was prepared by equally mixing KC-300, KC-400, KC-500 and KC-600.

### *Sample clean-up*

Oil sample (2.5 g) was added to 2.5 mL of dimethyl sulphoxide (DMSO) in a centrifuge tube and vigorously agitated by hand for more than 10 seconds. After centrifugation at 2,000 × g for 1 min,

2.0 mL out of 2.5 mL of the lower DMSO phase was added to a mixture of distilled water (1 mL) and *n*-hexane (2.5 mL) in another centrifuge tube. After vigorous shaking and centrifugation (2,000 × g, 1 min), the upper *n*-hexane phase was passed through a silica gel column filled with 6 g of 10% nitrate-impregnated silica gel. The eluate obtained with *n*-hexane was dried using a nitrogen stream and the residue was re-dissolved into 0.5 mL of DMSO.

***Immunochromatographic test***

The assay principle and construction of the immunochromatographic method were described previously[1, 2]. Briefly, a gold colloid labeled anti-PCB antibody which was dried in a glass vial was suspended with 100 µL of tris buffered saline (TBS), followed by an addition of 10 µL of pretreated oil samples. A 75 µL of gently agitated mixture was applied on an immunochromatographic test device and moved forward on a nitrocellulose membrane by a capillary action. After keeping for 20 minutes, a color intensity of a line on the membrane was measured by an immunoreader (Otsuka electronics Co., Ltd., Osaka, Japan). The line, designated as a trap line, was immobilized by a hapten-BSA conjugate. When no PCBs exist in samples, nearly all the anti-PCB antibody were trapped by the hapten-BSA conjugate, which resulted in an appearance of a red color on the trap line. When samples included PCBs, a complex of PCBs and the anti-PCB antibody passed through the trap line. A color intensity of the trap line decreased in proportion to the concentration of PCBs in the samples. The immunoreader was programmed to display the color intensity of the trap line.

**Results and Discussion**

***Validation of clean-up method***

The effects of an oil matrix on the immunochromatographic assay were examined. The fresh and used, PCB-free transformer oil were treated with the clean-up procedure described above and then spiked with known amounts of KC-MIX. As a result of immunochromatographic test of the spiked samples, good recoveries (80.8-119.8) were observed for both fresh and used oil (Table 1). This result suggests that the clean-up method had enough ability to eliminate the matrix effects of oil sample on the immunochromatographic assay.

Reproducibility and a recovery rate of the clean-up procedures were determined with PCB-free fresh oil spiked with 0.5 mg/kg of KC-MIX. The coefficient of variation (CV) for 5 replicates of clean-up procedures was 4.9 %, suggesting good reproducibility (Table 2). The recovery rate of the

**Table 1.** Recovery of KC-MIX from pretreated mineral oil

| Sample | Spiked level (ppb)[a] | 12.5 | 25 | 50 | 100 |
|---|---|---|---|---|---|
| Fresh oil | Observed level (ppb)[b] | 14.9 | 21.9 | 43.7 | 106.6 |
| | Recovery (%) | 119.1 | 87.7 | 87.4 | 106.6 |
| Used oil | Observed level (ppb) | 14.5 | 23.1 | 40.4 | 99.1 |
| | Recovery (%) | 116.0 | 92.5 | 80.8 | 99.1 |

a. Clean-up extracts from oil samples were spiked with known concentrations of KC-MIX. The mixing ratio of the extracts and KC-MIX were 9:1.
b. Immunochromatographic test was carried out in duplicate.

clean-up procedure was calculated from expected and actual measurements of immunochromatographic test. The expected measurement of immunochromatographic test for the oil spiked with 0.5 mg/kg of KC-MIX was 2.0 mg/kg provided 100% recovery (that is, the concentration factor was 4.0). By dividing expected measurement by actual immunochromatographic measurements in Table 2, the average recovery rate was calculated to be 40.9%.

***Response of the immunochromatographic test to Kanechlor mixtures***

Fresh oil was spiked with known concentrations of Kanechlor, treated with the clean-up method and then assayed by the immunochromatographic test. Figure 1 shows a response of the immunochromatographic assay to each of the Kanechlor. Each plot represents the mean signal from two separate clean-up procedures and subsequent immunochromatographic test. The IC50 value was 0.35, 0.19, 0.16 and 0.69 mg/kg-oil for KC-300, KC-400, KC-500 and KC-600 respectively.

***Assay performance of immunochromatographic test***

In order to evaluate the reproducibility of the total procedures from the clean-up to the immunochromatographic assay, the clean-up and assay procedures were repeated 5 times for transformer fresh oil spiked with various level of KC-MIX. Figure 2 shows a precision profile representing coefficient of variation (% CV) for each concentration of KC-MIX spiked oil. According to a validation method in the manual of bioassay for dioxins described by Ministry of the Environment of Japan[3], detection limit was calculated as a lower side of PCB concentration corresponding to 30% of CV. Similarly, an assay range was defined as a range of PCB concentrations corresponded to CVs less than 20%. The detection limit and the assay range assay were 0.033 and 0.046-0.97 mg/kg-oil respectively. The assay range was found to be sufficient to

**Table 2.** Reproducibility of the clean-up procedure

| Replicate[a] | 1 | 2 | 3 | 4 | 5 | Mean | % CV |
|---|---|---|---|---|---|---|---|
| Measurement (mg/kg)[b] | 0.83 | 0.77 | 0.79 | 0.83 | 0.87 | 0.82 | 4.9 |

a. KC-MIX (0.5 mg/kg) spiked oil sample was pretreated by clean-up procedure in 5 replicates.
b. Each measurement were mean value of immunochromatographic assay in duplicate

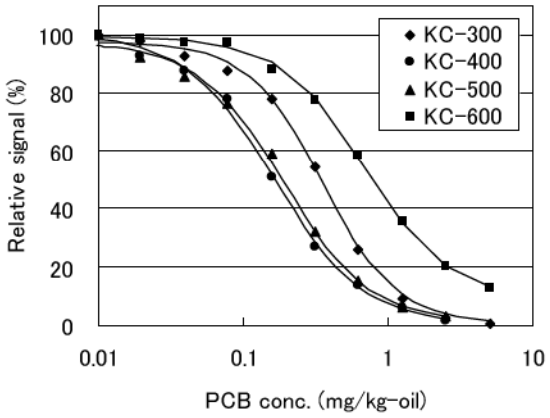


**Figure 1.** Standard curve for various Kanechlor (KC) mixtures.

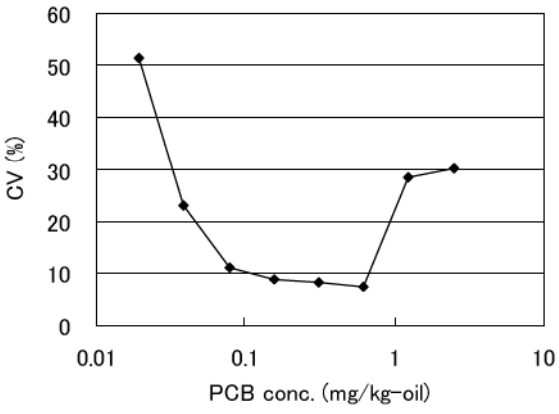


**Figure 2.** Precision profile using transformer fresh oil spiked with various level of KC-MIX.

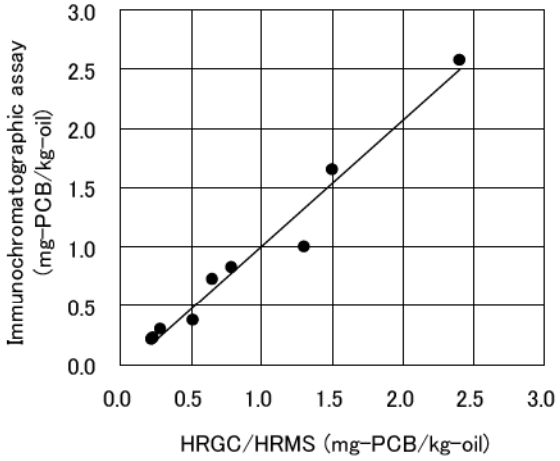


**Figure 3.** Comparison of measurements from the immunochromatographic assay and HRGC/HRMS analysis in used transformer oil.

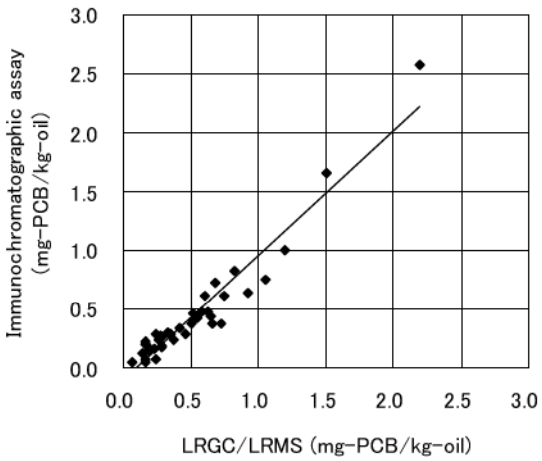


**Figure 4.** Comparison of measurements from the immunochromatographic assay and LRGC/LRMS analysis in used transformer oil.

screen PCB contamination in transformer oil at the domestic regulatory concentration of 0.5 mg-PCB/kg-oil.

***Analysis of real oil samples***

The performance of the immunochromatographic test was investigated by a comparison between immunochromatographic and instrumental (HRGC/HRMS or LRGC/LRMS) analysis. The clean-up and assay procedures were conducted for 67 used oil samples that were already analyzed by HRGC/HRMS and/or LRGC/LRMS. A good correlation ($r$ = 0.98, $n$ = 9) was observed between the immunochromatographic values and HRGC/HRMS data (Figure 3). The slope of the linear regression equation was roughly 1 (y = 1.06x - 0.055). The immunochromatographic assay data was also in good agreement with LRGC/LRMS measurements, representing high correlation coefficient ($r$ = 0.96, $n$ = 46) and reasonable regression line (y = 1.06x - 0.10) (Figure 4).

In conclusion, the newly developed clean-up method and the immunochromatographic assay had a good performance for the screening of PCB contamination in transformer oils.

**References**

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