The Performance of Immunoassay Screening Method by Portable Instrument for Polychlorinated Biphenyls in Transformer Oil

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Abstract

We report the performance of a rapid and simple immunoassay system for the detection of polychlorinated biphenyls (PCBs) in transformer oil. The assay is implemented using a rapid pretreatment column and a portable instrument using disposable detection cell. With the anti-PCB antibody chosen, the assay shows equal response towards the commercially used PCB mixtures (Kanechlor) in Japan. The recovery and coefficient of variation (CV) of sample preparation for the assay are 50% and 3.6%, respectively. This system reached to 0.2mg/kg sensitivity within 30% CV. Measurements of transformer oil using both the immunoassay and high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) showed a good correlation for the range from 0.1 mg/kg to 5.0 mg/kg PCBs. Additionally, 274 samples measured by gel permeation chromatography-low resolution gas chromatography-electron capture detection (GPC-LRGC-ECD) were tested by the immunoassay. In the case that a regulatory concentration of total PCB was set at 0.5mg/kg, the proportion of false negative and false positive in all the samples were only 0.4% and 8%, respectively at 0.4mg/kg cutoff. These results suggest that the described immunoassay can provide a fast and effective alternative to other procedures such as GC-ECD for screening transformer oil for PCBs.

Introduction

PCBs were widely used as electrically insulating heat conductors prior to their prohibition in 1973 in Japan. Even though production and use has been prohibited for a long period, low level quantities of PCBs were mixed into insulating oil used in electrical machinery, mainly transformers, as recognized by the Japanese government in 2003. The suspected number of contaminated transformers may extend to approximately six million transformers in Japan. The necessity of testing such a large number of transformers for PCB contamination has stimulated urgent interest in development of measurement techniques. Presently, the main method for PCB detection is GC-MS (high resolution gas chromatography-low resolution mass spectrometry (HRGC-LRMS) or HRGC-HRMS) or GC-ECD but the long time and the high cost of these procedures has resulted in a search for quicker and cheaper alternatives.

Recently, immunoassay, which utilizes an antigen antibody reaction for detection, has seen increasing application in environmental analysis.[1] Immunoassays for the detection of PCBs in transformer oil have been reported.[2,3] However, the performance of these assays is not still enough to identify PCB contamination because the regulatory concentration of degraded PCBs in Japan is very low at less than 0.5ppm. In addition, immunoassay has a fundamental hurdle for application to PCB measurement because the antibody used in assay may show different reactivity, depending upon PCB congeners. Regulations specify total weight of PCBs, resulting in a situation where the ideal assay response is total PCB irrespective of the mix of individual congeners that are present in a particular transformer oil. In order to overcome these problems for sensitivity and antibody cross reactivity in immunoassay for PCBs in transformer oil, we developed rapid clean-up procedure with an automated instrument and a portable assay reader.[4,5,6] We also developed anti-PCB antibody that reacts broadly and equally with commercial PCB mixtures (Kanechlors) used in insulating oil in Japan[7]. In this report, the performance of the developed assay system including a rapid clean-up, a portable reader, and an antibody showing broad cross reactivity towards PCB congeners is defined and then applied to approximately 300 used transformer oil to investigate its screening capacity for PCBs.

Materials and Methods

Chemicals: Commercial mixtures of PCBs (Kanechlor; KC-300, 400, 500, and 600) came from GL Sciences (Tokyo, Japan). Monoclonal anti-PCB antibody was purchased from Kyoto Electric Manufacturing (Kyoto, Japan) and labeled with gold colloid by previously reported method[8]. Phosphate-buffered saline (PBS; consisting of 137mM NaCl, 3mM KCl, 20mM Na_2HPO_4 and 1.5mM KH_2PO_4, pH 7.4) was prepared in-house. Bovine

serum albumin (BSA) came from Sigma Aldrich (St. Louis, MO). PCB-free insulating oil for electric transformers came from Mobil Sekiyu (Tokyo, Japan).

Sample Preparation for Immunoassay: Extraction and clean-up procedures for PCBs followed the method reported previously[5,6]. Briefly, oil samples (0.25g) were applied to a multi-layer chromatography column consisting of anhydrous sodium sulfate, silica containing fuming sulfuric acid, followed by silica, mainly for removal of aromatic and polar compounds in oil. The fraction containing PCBs was eluted with *n*-hexane (10mL) after several minutes. The eluate was then concentrated by evaporation and solvent exchanged into 0.25mL of Dimethyl sulfoxide (DMSO). Finally, the DMSO phase was partitioned with oil after centrifugation, and then diluted in PBS for the immunoassays. The total time for preparation of one sample was less than fifteen minutes.

Immunoassay System: Immunoassay system used in this work was based on the theory and operation which have been described by ourselves[9] and others[10]. The system consists of disposable detection cell with membrane solid phase and a commercial portable instrument called IMNY (Sibata Scientific Technology LTD., Tokyo, Japan). Briefly, 0.05ml of pretreatment solution described above was added to the antibody solution to allow binding of the antibody. Antibody was prepared in PBS supplemented with 0.1% bovine serum albumin (PBS-BSA). The antibody concentration was fixed in all experiments so the degree of binding varies with PCB concentration, allowing the PCB concentration to be estimated from a measurement of the free antibody remaining in solution. The measurement liquid was flowed through the membrane of the detection cell and a portion of the free antibody (not bound to PCB in the measurement liquid) bound to the antigen analog on the membrane. The captured antibody was quantified by measuring the optical absorption of the colloidal gold label and the measurement was used to calculate the concentration of PCB in the pretreatment liquid. The total time for immunoassay of one sample was about two hours, but the real operating time was only about ten minutes.

GPC-LRGC-ECD, HRGC-LRMS, HRGC-HRMS: Quantification of PCBs for evaluation of both sample preparation and immunoassay system was performed using instruments of LRGC-ECD, HRGC-LRMS (EI-SIM mode) and HRGC-HRMS (EI-SIM mode). The methods and procedures followed were as previously reported.[11,12,13] PCBs would be detected less than 0.01mg/kg with high accuracy using HRGC-HRMS measurement by internal standard method that sample oils were spiked with surrogate solution, which has been approved in regulation for decomposed transformer oil in Japan[13]. GPC (CLNpak EV-G/CLNpak EV-2000, Showa Denko), following sulfuric acid treatment was used as clean-up method for the measurement by LRGC-ECD. The recovery of PCBs using this method is almost 100%, which give high correlation between GPC-LRGC-ECD and HRGC-HRMS[11]. Major congeners (3Cl~8Cl) of commercial PCB (Kanechlor) were analyzed with HRGC-LRMS measurement according to sample preparation of HRGC-HRMS method.

Results and Discussion

Sample Preparation: PCB-free insulating oil which was spiked with equal weight mixture of the four Kanechlors (KC-mix) at 1mg/kg was independently extracted 5 times (n=5) using the method for immunoassay described above. PCBs in prepared solution were quantified using HRGC-LRMS, and then recovery of PCBs and repeatability of sample preparation were determined. Sample preparation procedure for immunoassay showed 50% of PCB recovery with 47% average and 3.6% CV.

Antibody Response to Kanechlor Mixtures: It is expected that the PCB contamination in used transformer oil is of Kanechlor origin so initial evaluation of the system performance began with the four Kanechlors (KC300, KC400, KC500 and KC600). Calibration curves constructed for each Kanechlor and KC-mix showing the relative response as a function of PCB concentration for Kanechlor concentration are shown in Figure 1. Every response curves were overlapped each other, and IC_{50} values (PCB concentration giving relative response of 50%) were respectively 1.2, 0.93, 0.96 and 1.6mg/kg for KC-300, 400, 500 and 600. Equal response to the four Kanechlors suggested that this assay system had a great potential to determine total PCB concentration in transformer oil containing the four Kanechlors in any mixed ratios. Relative response of 10% and 90% in KC-mix can show the sensitivity to detect PCBs ranging from 0.1mg/kg to 10mg/kg.

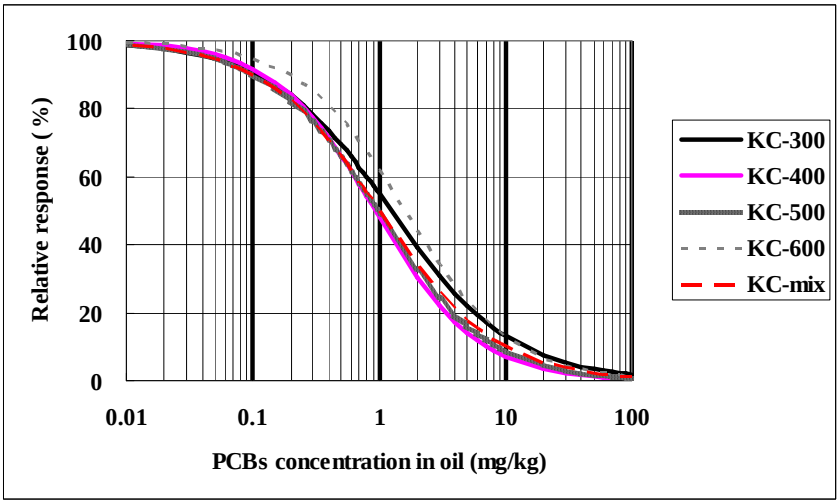

Figure 1. Assay response for Kanechlors

Precision and Sensitivity of Measurement: Figure 2 shows precision at various PCBs concentrations. CV of determinations at for PCBs concentrations were calculated from 5 times independent pretreatment and measurements (n=5) for PCB-free oil spiked with KC-mix. The lower detection limit corresponding to 20%-30% of CV along International Organization for Standardization (ISO 15089) specified as quality-guidelines for immunoassay[14] was estimated to be 0.2mg/kg in this assay. In the same way, the upper limit could be estimated as greater than 5mg/kg.

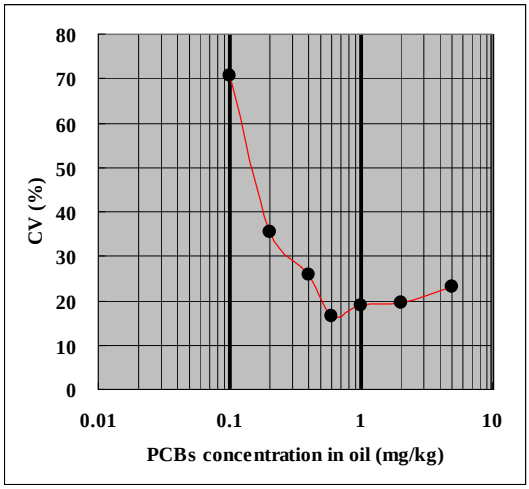

Figure 2. Dependence of CV on PCB concentration in assay

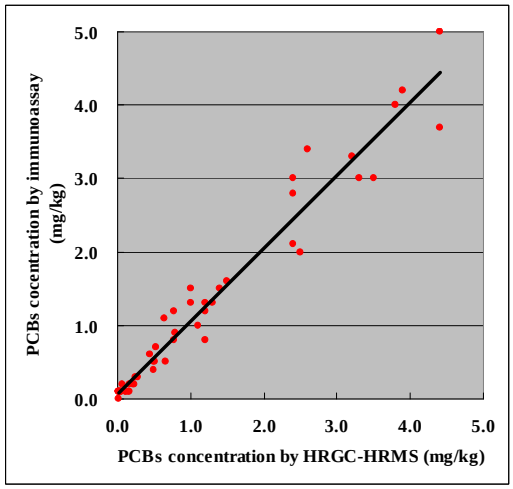

Figure 3. Relationship between assay and HRMS

Transformer Oil Samples: Fifty samples of used transformer oil were analyzed by immunoassay and HRGC-HRMS. In Figure 3, immunoassay estimates are plotted as a function of HRGC-HRMS estimates ranging from 0mg/kg to 5.0mg/kg. The slope, intercept, and correlation coefficient (r^2) of the best fit line between the two sets of data were 0.99, 0.069 and 0.96, respectively. Immunoassay showed a good correlation with HRGC-HRMS for the determination of total PCB concentration.

This good relationship encouraged us to investigate screening performance of this assay with 274 used transformer oil. Total PCB concentrations of all samples were determined by immunoassay and GPC-LRGC-ECD as shown in Figure 4. Presently, there is no official guideline to identify low level PCB contamination in transformer, but there is regulatory concentration for breaking PCBs down to harmless constituents in oil is 0.5 mg/kg in Japan[13]. This study therefore assumed 0.5 mg/kg as regulatory concentration.

In this role, all samples measuring greater than 0.5 mg/kg by GPC-LRGC-ECD are regarded as positives while those measuring 0.5mg/kg or less are negatives. In terms of the screening immunoassay, these are further broken down into true positives, false positives, true negatives and false negatives. Positive samples that measure positive by immunoassay (i.e. are greater than or equal to the cut off concentration) are true positive while positive samples that measure negative by immunoassay are false negatives. In similar manner, negative samples that measure negative by immunoassay are true negatives while negative samples that measure positive by immunoassay are false positives. Figure 5 shows the frequency of false negative and false positive samples when cutoff concentration was varied. In screening at 0.4mg/kg cutoff, only one sample (0.4% in all the samples) was confirmed as false negative with 23 samples (8% in all the samples) as false positive. In screening at 0.2mg/kg cutoff, there was no false negative sample with sixty one false positive samples (20% in all the samples). This excellent performance of immunoassay in screening could be explained from distribution of total PCB concentrations of all samples in Figure 4. Total PCB concentrations of 220 samples were measured by GPC-LRGC-ECD at equal to or less than 0.2mg/kg (Fig. 4). 184 out of the same 220 samples were measured by immunoassay at equal to or less than 0.2mg/kg. These results strongly supported that the developed immunoassay has an excellent screening performance for assumed regulation at 0.5 mg/kg. The assay described here is a powerful tool to rapidly screen PCB contamination in transformer oil, at least in the case that a majority of total samples are expected to be less than 0.5mg/kg.

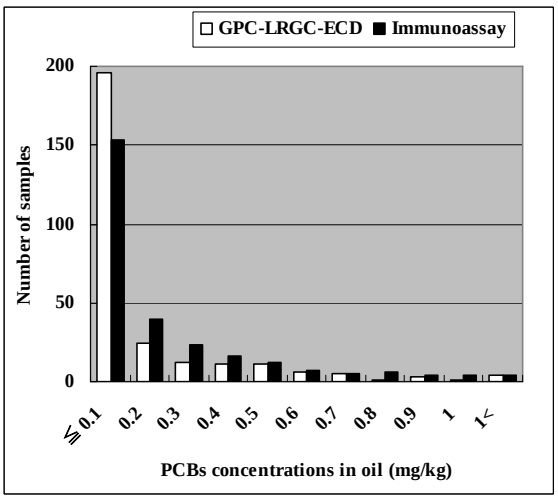

Figure 4. Concentrations measured by assay and ECD

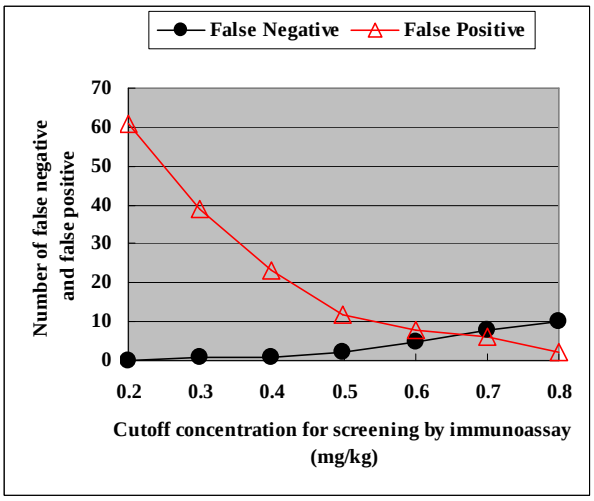

Figure 5. Frequency of false negative and false positive

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