A simplified pretreatment method for the determination of polychlorinated biphenyls in transformer oil by enzyme-linked immunosorbent assay

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Introduction

Polychlorinated biphenyls (PCBs) were used for various applications such as thermal and transformer oils. Due to health-related concerns and potential environmental impacts the use of PCBs was banned and the storage and disposal have been regulated in many countries. To prevent further release of PCBs in the environment some specific disposal methods, e.g. high temperature incineration and degradation with alkali metals, catalyses, supercritical water, ultraviolet radiation and bacterial enzymes have been developed. When field engineers monitor the disposal process of PCBs, rapid and cost-effective screening methods are required to provide them with on-site and real-time information. Moreover, such screening methods are able to reduce the number of samples requiring laboratory confirmation by GC/ECD [1] or GC/MS [2].

Two types of field test methods are currently available for PCB screening. One method involves chemical dehalogenation of the PCBs followed by analysis with a colorimetric reaction or chloride-specific electrode [3, 4]. The other method relies on immunoassay [5]. The immunoassay system is based on enzyme-linked immunosorbent assay (ELISA) in which a competitive reaction between PCBs and a PCB conjugate is used to determine the PCBs concentration in soil samples [6]. However, it is difficult to apply immunoassay kits to PCB screening in waste oil because of hydrocarbon co-contaminations [7].

Determination of PCBs by the GC or screening methods often requires extensive cleanup procedures to remove interferences contributed by the matrix. It is particularly difficult to separate PCBs from the interfering compounds derived from the mineral oil because of their very similar and chemical characteristics. To simply and rapidly analyze PCBs in waste oil, simplified pretreatment methods using a combination of dimethylsulfoxide (DMSO) / hexane partition and solid phase extraction have been developed [8, 9]. These methods are appropriate for the GC method and not for the screening method. In this study, simplified pretreatment methods for determination of PCBs in transformer oil by ELISA were examined. To develop the on-site pretreatment method for ELISA, our objectives were set on (1) final solvent was DMSO due to the determination of PCBs by ELISA, (2) cleanup procedure was more simple as possible and enough to eliminate interferences in transformer oil for the PCB screening by ELISA, and (3) evaporation procedure by an evaporator or a N_2 purge was not used for the simple and rapid screening.

Material and Methods

Reagents and materials

Dimethylsulfoxide (DMSO) for biochemistry (Wako Pure Chemical Industries, Japan) and *n*-hexane for PCB analysis (Kanto Chemical Co., Inc., Japan) desiccated through Na_2SO_4 anhydrous for PCB analysis (Kanto Chemical Co., Inc., Japan) were used as solvents for immunoassay, extraction by DMSO/hexane partition and cleanup by 10% $AgNO_3$/44%H_2SO_4 (AS) cartridge (Supelco, Sigma-Aldrich Japan Co., Japan).

Kanechlors mixture (KC) was purchased from GL Sciences Inc. (Japan). The composition of KC was KC-300 ~25% (w/w), KC-400 ~25% (w/w), KC-500 ~25% (w/w) and KC-600 ~25% (w/w), and the total concentration was 400µg/ml-hexane (100 ± 10µg/ml each). KC standards were prepared by diluting KC with hexane.

Sun-Ohm MU (Oil) was kindly supplied by Kansai Tech Co. (Japan). The specific gravity of Oil was 0.88 and the composition was Paraffin ~53.5%, Naphthene ~38.5% and Aromatic ~8.0%. Matrix standards were prepared by diluting Oil with hexane.

Quantitative hydrocarbon *n*-Paraffin (C_{10-20}) mixture was purchased from GL Sciences Inc. (Japan). Hydrocarbon standards were prepared by diluting the *n*-Paraffin mixture with hexane.

Waste oil samples contaminated with PCBs were prepared by adding KC to Oil. The following waste oil samples were used for final conformation:

53.9µg-KC300/g-transformer oil

52.9µg-KC300/g-liquid paraffin

These values were determined by a conservative method (EPA method 8082) of capillary-column GC/ECD analysis.

GC/MS analysis

Qualitative and quantitative measurements of KC and Oil for recovery and elution tests were carried out using GC/MS. The GC/MS analytical system was comprised of an HP 6890 series unit (Hewlett-Packard, Agilent Technologies, USA) equipped with a GC-Mate (JEOL Ltd., Japan). A DB-5MS fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Agilent Technologies, USA) was employed for separation of each constituent in KC and Oil. The column temperature for KC was held at 60ºC for 1 min, increasing to 180ºC at 40ºC/min, to 260ºC at 4ºC/min, to 280ºC at 40ºC/min, and was then held isothermal for 1 min. The column temperature for Oil was held at 60ºC for 1 min, increasing to 180ºC at 40ºC/min, to 220ºC at 4ºC/min, to 280ºC at 40ºC/min, and was then held isothermal for 1 min. The injection temperature was 260ºC and 1µl of each final sample solution and the calibration standards were injected into the column using split-less mode. The carrier gas (helium) flow rate was 1.0ml/min and the ion source temperature was maintained at 270ºC. The ionization energy and current were 70eV and 300µA, respectively. The resolution of the mass spectrometer was set at ~1,000. The qualitative determination of KC and Oil was carried out by scan mode, of which monitoring mass (*m/z*) range was 30-500. The quantitative determination of KC was performed according to selected ion monitoring (SIM) mode by switching accelerating voltage. The monitoring masses (*m/z*) for KC were 256 (3CBs), 292 (4CBs), 326 (5CBs) and 360 (6CBs). The concentrations of KC were determined by superior 3 peaks of each 3-6CBs (total 12 peaks) which were major components in KC. The quantitative determination of Oil was performed using SIM mode by switching magnetic field. The monitoring masses (*m/z*) of selected ions for Oil were 43 ($C_3H_7^+$), 57 ($C_4H_9^+$), 71 ($C_5H_{11}^+$) and 85 ($C_6H_{13}^+$). The concentrations of Oil were determined by superior 3 peaks of *n*-Paraffin ($C_{16\text{-}18}$) which were dominant constituents in Oil. The hydrocarbon standards were used for identification of Oil. The KC and matrix standards were used for calibration of KC and Oil.

Enzyme-linked immunosorbent assay (ELISA)

EnBio Screen kit for co-PCB, Kanechlor 500 (Aroclor 1254) Amersham Biosciences, UK was employed as the immunoassay system for PCB screening. The kit is based on competitive enzyme-linked immunosorbent assay (ELISA) utilizing monoclonal antibody against 2,3',4,4',5-pentachlorobiphenyl (PCB#118).

PCB samples (or standards) in DMSO and horseradish peroxidase (HRP) conjugate PCB competitors in TBS (20mM Tris, 150mM NaCl; pH 7.5) with 0.05% Tween 20 and 1% BSA were mixed in the ration of 1:3. 50 µl of the mixture were added to each well of 96-well microtiterplates coated with purified monoclonal antibody against PCB#118 and incubated for 30min at room temperature (20 - 28ºC) with shake. The wells were washed three times and 50µl of tetramethylbenzidine (TMB) solution (Amersham Biosciences, UK) were added and incubated for 20min. The color development was stopped with 1N H_2SO_4 and measured at 450nm with a microplate reader (Bio-Rad Laboratories, USA). Results were expressed as the ratio of B (OD_{450} for the PCB sample) to B_0 (OD_{450} for the blank). The dose-response curves (B/B_0 vs. logarithmic concentration of the analyte) were fitted by the four-parameter logistic model using the computer software (Microplate Manager Ver. 5.1, Bio-Rad Laboratories, USA). The KC, true and false (hapten) PCB#118 standards were used for calibration of PCBs.

Simplified pretreatment method for ELISA

1. Cleanup by sulfuric acid / silica gel cartridge

Sulfuric acid / silica gel (AS) cartridge is used as a popular cleanup method, because the cleanup using AS cartridge is a simple and effective method for the elimination of poly aromatic hydrocarbons (PAHs). Addition and recovery test of PAHs standards for AS cartridge showed almost PAHs were eliminated. GC/MS scan analysis of cleanup samples by AS cartridge showed that the monitoring mass (*m/z*) of 202, which interpreted as fluoranten or piren in Oil, was removed mainly. Cross reactivity test of PAHs for the ELISA kit showed weak responses [10]. Therefore, we think that a pretreatment method for the ELISA kit need hardly remove PAHs by AS cartridge. However, the presence of PAHs similarly to hydrocarbons subjected the ELISA kit to negative interferences due to white muddiness of the buffer. After all, the determination of PCBs by the ELISA kit needs to eliminate PAHs by AS cartridge.

Elution test of PCBs for AS cartridge with hexane indicated that 0-5ml and 0-10ml fractions totaled up to ~86% and ~97%, respectively. Elution test of blank for AS cartridge with hexane indicated contaminations like hydrocarbons, of which the total ion chromatogram (TIC) was different from that of Oil. The contaminations exhibited a peak in 10-20ml fraction which was equivalent to approximately 20ng/ml in terms of *n*-Paraffin (C_{15}), and decreased 1/10 in 0-10ml and 1/3 in 190-200ml. Then, the samples were directly added to AS cartridge and eluted with 5 or 10ml hexane. Elution volume of hexane is preferred to be fewer because the PCB concentrations in solutions were diluted without evaporation procedure by an evaporator or a N_2 purge.

2. Extraction by DMSO/hexane partition

DMSO/hexane partition is a simple and effective method to extract PCBs form oil matrix such as hydrocarbons [7]. Appropriate conditions of DMSO/hexane partition for the screening of PCBs in waste oil by ELISA are (1) more

effective PCBs extraction to detect PCBs by ELISA and (2) fewer volume of solvents due to condensation without evaporation procedure. To construct a simplified pretreatment for the screening, the ratio of DMSO/hexane partition was examined by the following procedure:

4ml-KC standards (5 and 0.5µg-KC/ml-hexane)
↓
Add 0.05, 0.1, 0.2, 0.5, 1, 2 and 4ml-DMSO
↓ Shake (by mixer for test tube) and Remove hexane layer (by pipette)
Collect aliquots of DMSO layer
↓
ELISA

The result of DMSO/hexane partition is shown in Figure 1. The top of Figure 1 means the volume ratios of DMSO to hexane (DMSO/hexane) versus the recovery ratios of additional KC (Rec.), which indicates the ratios of KC transferred from hexane to DMSO layer. The bottom of Figure 1 means DMSO/hexane versus the condensation factors of additional KC (Con.), which is defined as Rec./(DMSO/hexane). The value of Con. appropriate for a simplified pretreatment for the screening is preferred to be relatively high and constant. Figure 1 indicates that DMSO/hexane = 0.05 (= 0.2ml-DMSO/4ml-hexane) at Rec. = 5.7% and Con. = 114% would be appropriate for the screening.

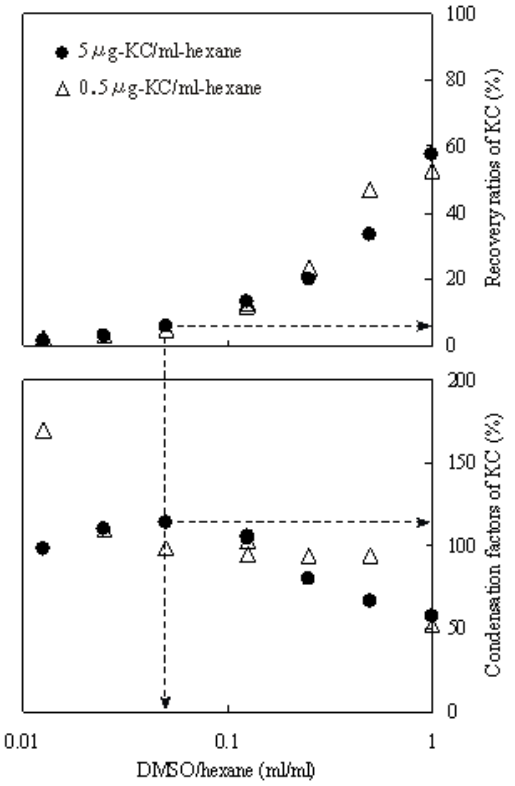

Figure 1: Recovery and condensation by DMSO/hexane partition

3. Interference by the remains of oil matrix

ELISA system is subject to negative interference in the presence of hydrocarbon co-contaminations [7]. To construct a simplified pretreatment for the screening, oil matrix must be eliminated enough to detect PCBs by ELISA. Next, the interference in the remains of oil matrix was examined by the following:

0.05-0.5g waste oil samples (50µg-KC/g-Oil)
↓
AS cartridge
↓ Elute with 5 ml hexane (to 10 ml centrifuge tubes with 0.1ml scale and stopper)
Add 0.5-2.5ml DMSO
↓ Shake and Remove hexane layer
Collect aliquots of DMSO layer → ELISA
↓
Add equivalent volume of hexane and water to DMSO → GC/MS analysis

The result of interference in the remains of oil matrix is showed in Figure 2, which means the concentrations of remaining oil matrix determined by GC-MS versus the ratios of PCB concentrations determined by ELISA to GC-MS. Figure 2 indicates that an increase in the remains of oil matrix resulted in a decrease in PCB concentrations by ELISA and more than 200µg/ml concentrations of remaining oil brought about severe negative interference. In the experiment a great quantity of sample or DMSO addition led to a lot of remaining oil while the recovery ratio of PCB was higher. Therefore, an appropriate volume of waste oil sample for the screening would be less than 0.2g.

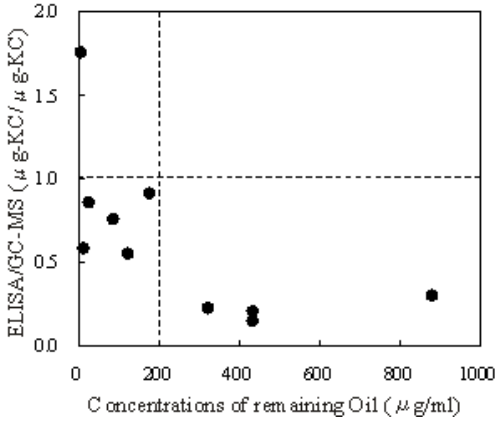

Figure 2: Negative interference in ELISA by the remains of oil matrix

4. Construction of a simplified pretreatment for ELISA

A simplified pretreatment appropriate for the screening by ELISA was constructed in consideration of the results which were (1) direct addition of waste oil sample to AS cartridge and elution with ~5ml hexane, (2) DMSO/hexane = ~0.05 and (3) sample volume < ~0.2g. Hence, a following simplified pretreatment procedure was proposed and examined:

Pretreatment-A (~40 times dilution)

0.1g waste oil samples (0, 10 and 50µg-KC/g-Oil, 53.9µg-KC300/g-transformer oil and 52.9µg-KC300/g-liquid paraffin)
↓
AS cartridge
↓ Elute with 5ml hexane
Add 0.2ml DMSO
↓ Shake and Remove 5.1ml top (5ml hexane + 0.1ml DMSO) layer
0.1ml DMSO
↓
ELISA

To be compared with the pretreatment-A, we examined a following pretreatment procedure which is a reverse to the pretreatment-A but a normal:

Pretreatment-B (~5 times dilution)

0.1g waste oil samples (0, 2 and 10µg-KC/g-Oil, 53.9µg-KC300/g-transformer oil and 52.9µg-KC300/g-liquid paraffin)
↓
Add 1ml hexane and 1.5ml DMSO
↓ Shake and Remove 1.5ml top (1ml hexane + 0.5ml DMSO) layer
Collect 1ml aliquots of DMSO layer
↓
Add 1 ml hexane and 3ml Milli-Q water
↓
Collect 0.6ml aliquots of hexane layer
↓
AS cartridge
↓ Elute with 5ml hexane
Add 0.1ml DMSO
↓ Evaporate 5ml hexane by N_2 purge
0.1ml DMSO

↓
ELISA

Results and Discussion

PCB concentrations determined in according to the pretreatment-A and -B are shown in Figure 3. ELISA values (µg-KC/g-Oil) of the pretreatment-A and -B were corrected by multiplying dilution factors of 40 and 5, respectively. The dilution factors mean physical (due to solvents addition) and chemical (due to DMSO/hexane partition) dilution by each pretreatment procedure. For the calibrations using true or false PCB#118 standards, the ELISA values of KC-mix and KC-300 were corrected by multiplying content ratios of 20 and 13.6. The content ratios mean that KC(-mix) and KC-300 include 5% and 7.4% PCB#118, respectively.

Figure 3 indicates relatively good correlations between ELISA values and KC standards or GC/ECD (EPA-8082) values in both pretreatment procedures, while ELISA response to KC contents were more sensitive in pretreatment-A (~50%) than -B (~20%). This means that interferences in ELISA by contaminations such as hydrocarbons or PAHs would be larger in pretreatment-B than -A. Detection ranges in pretreatment-A and -B were 10-500 µg-KC/g-Oil and 3-125 µg-KC/g-Oil, respectively, because the detection range of the ELISA kit was 6.5-250 ng-PCB#118/ml-DMSO. These detection limits are not sufficient for the ban limits regulated by some countries, however, these detection ranges are enough for the PCB screening. The pretreatment-A is more appropriate for the on-site screening of PCBs in waste oil by ELISA, because the pretreatment-A is much simpler than -B.

In this study, we suggested the pretreatment-A which was composed of a cleanup by AS cartridge and an extraction by DMSO / hexane partition on a scale of 10ml centrifuge tube without evaporation procedure. The pretreatment time was ~10min and detection range was 10-500 µg-KC/g-Oil if EnBio Screen kit for co-PCB, Kanechlor 500 (Aroclor 1254) Amersham Biosciences, UK was used as a detection method for PCB screening.

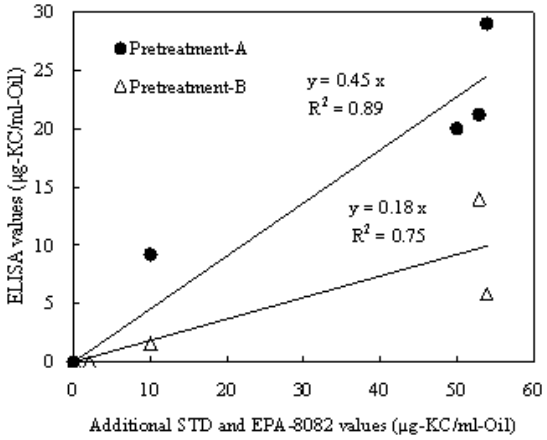

Figure 3: ELISA response to KC contents in waste oil samples pretreated by -A and -B

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