NEW AND RELIABLE METHOD OF MEASURING COMPLEX POLLUTION WITH DIOXINS AND PCBs BY BIOSENSOR

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Abstract

We developed a highly accurate and reproducible method of quantifying substances such as Dioxins and PCBs in environmental samples. The analytical system combines a pretreatment method and biosensors to resolve bioassay problems in environmental analysis[1]. Environmental samples often contain mixtures of various compounds or analogous congeners; therefore, use of only one measurement system may not ensure the accuracy of analytical values.

Established methods[2,3] for analyzing Dioxins in polluted soils determine the nature or source of pollution — incineration, chemicals, or PCBs — by measuring the ratios of multiple types of antibodies with different specificities in polluted samples from a mixture of sources. We applied these methods to the analysis of PCBs in insulating oil samples[4]. We obtained information on the dominant types of Kanechlor (KC300, KC400, KC500, or KC600) in samples by using two types of anti-PCB antibody with different specificity. The results indicated that our method had improved analytical precision and accuracy of analytical values.

Introduction

In May 2004, the Stockholm Convention on Persistent Organic Pollutants (POPs) entered into force for chemicals that can pose a risk to humans and ecosystems owing to their persistence, bioaccumulation, long-distance migration, and hazardous nature. Reduction in the quantities of 12 substances, including Dioxins, PCBs, and organochlorine pesticides, as well as the eventual extinction of these substances, has been addressed internationally. In May 2009, nine substances were added to the 12 already regulated, leading to an increased need for simultaneous analysis appropriate to the monitoring and survey of multiple components in multiple samples. The background to this need is the requirement for each country to voluntarily control and reduce contamination by a total of 21 different chemicals.

In Japan, in addition to surveying and monitoring for soil pollution, the efficient and reliable disposal of more than 6.5 million electrical appliances that may contain trace amounts of PCBs is an urgent task. There is a need for a determination method in which analytical precision, reproducibility, speed, and low cost are secured.

In this context, we have developed a system that enables the rapid, high-precision, and reproducible detection of Dioxins in exhaust gas and fly ash and bottom ash from incineration plants. The method is based on our Simplified Dioxin Analysis System[1], consisting of a device for highly reproducible automated sample

preparation with high recovery rates[5-8] and a biosensor that has high measurement accuracy (CV 3% or less) (Fig. 1). A device for preparing samples for GC/MS operates on the same principles as our pretreatment method and, with further improvements, is now widely used in Japan[9]. Precision profiles prepared according to the regulations of Japanese Industrial Standard (JIS) K0461 using measured values obtained from Dioxin analysis showed that the range of quantifications met the conditions of this Standard (or a CV of 10 % or less of quantified values) (Figs. 2 to 4), indicating that precision can be controlled efficiently without the need for repeated measurements.

We developed a method of rapid analysis of trace amounts of PCBs in insulating oil; the method provided reproducible analytical values from the same sample with a CV of about 5%. There was a wide quantifiable range (from 0.11 to 1.11 ppm). Application of the principle of the Simplified Dioxin Analysis System to the analysis of insulating oil samples made the procedure from preparation to measurement as short as 3 h. This allowed the screening measurement of about 70 samples a day and provided a practicable bioassay that can be used to quantify environmental samples[4]. This bioassay is specified as a quantification method under JIS K0464, "Guidelines for polychlorinated biphenyl (PCB) immunoassay."

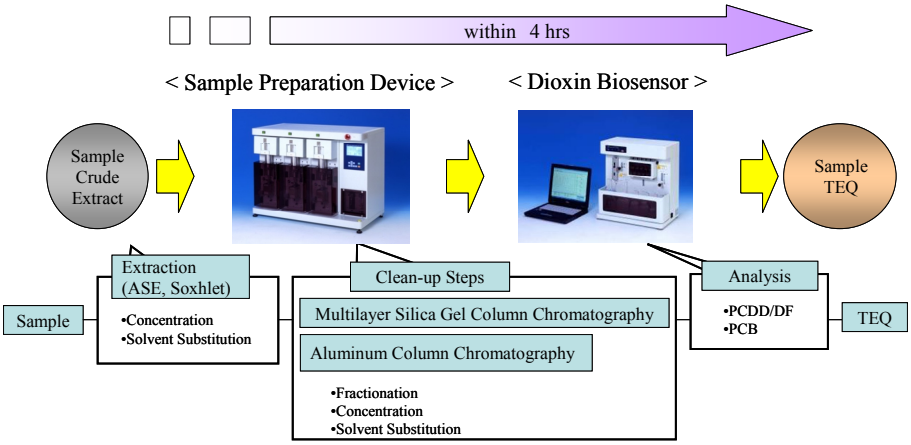

Fig. 1 Simplified Dioxin Measurement System

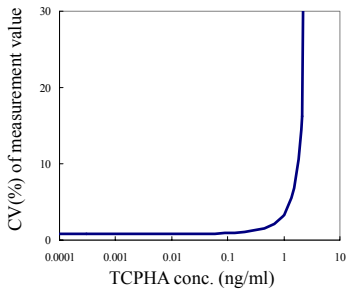

Fig. 2 Example of accuracy of measurement values

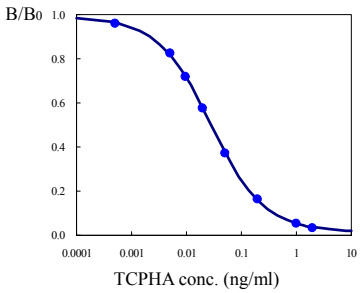

Fig. 3 Example of a calibration

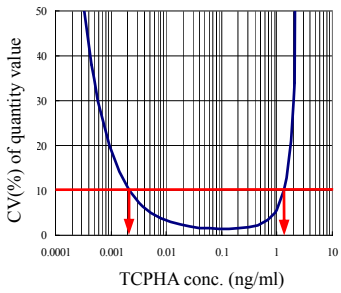

Fig. 4 Example of accuracy of quantity value

*TCPHA: 3-(6-(2,4,5-trichlorophenoxy) hexanoylamino) propionic acid
In this Dioxins measurement, TCPHA is used as a standard.

Materials and Methods

1. Samples

A total of 48 different samples of insulating oil containing PCBs at various concentrations or Kanechlor were used.

2. Reagents

Kanechlors (KC300, KC400, KC500, and KC600) were supplied by GL Sciences (Tokyo, Japan). The column set for the bioassay used for the preparation was supplied by Miura Co. Ltd. (Ehime, Japan). Fresh insulating oils (Japanese Industrial Standards listing) were purchased from Matsumura Oil Co. Ltd. (Osaka, Japan) or Japan Energy Co. (Tokyo, Japan). Cy-5 labeled $F(ab')_2$ fragment goat anti-mouse IgG (H+L) was purchased from Jackson ImmunoResearch Laboratories Inc. (PA, USA). Anti-Kanechlor monoclonal antibody (K2A A. b.) has been produced and characterized by Takagi et al.[10]. R A.b. was supplied by the RDI Division of Fitzgerald Industries Int. (MA, USA). Other reagents were supplied by Nacalai Tesque Inc. (Kyoto, Japan). The reagent for measuring PCBs was supplied by a PCB biosensor reagent kit developed by Kyoto Electronics Manufacturing Co. Ltd. (Kyoto, Japan).

3. Sample Preparation System

We prepared samples by using the procedure described by Sawadaishi et al.[11] and Takahashi et al.[12] for pretreating PCBs in insulating oil. They downsized and modified an SPD-600 preparation device for use with multiple samples (Fig. 5). First, 50 mg of insulating oil was applied directly to a column. It was then made to react at 80 °C for 1 h in a multi-layer silica gel column. After cooling, 20 mL of hexane was loaded from the top of the column. Next, the contents of the PCB-adsorbed alumina column connected below the multilayer column were dried. The PCBs were then eluted with 200 μL dimethylsulfoxide (DMSO), with heating, to prepare the sample for measurement.

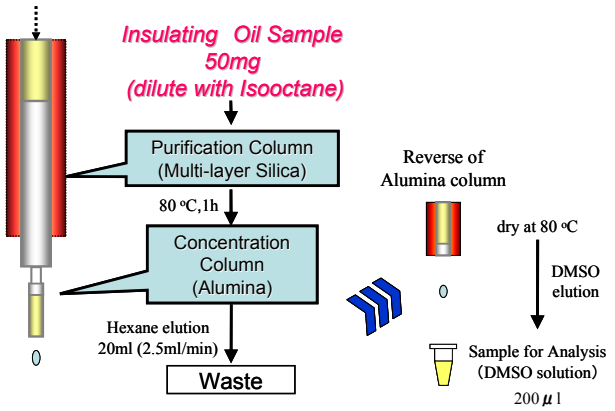

Fig. 5 Diagram of sample preparation system (PCBs)

4. Immunosensing of PCBs

To measure Dioxins, we used a DXS-600 (Kyoto Electronics Manufacturing Co., Ltd.) flow-through immunosensor that operated by Kinetic Exclusion Assay. We supplied the immunosensor with K2A A. b. monoclonal antibody, which shows almost equal reactivity to a series of KCs (KC300, 400, and 500, with the

lowest reactivity to KC600; Table 1). In the measurement cell we fixed compound antigen derivatives appropriate for PCB measurement.

Commercially available antibody (R A. b.) highly specific to KC600 was used as an antibody with specificity different from that of K2A antibody (Table 1). Fig. 6 shows the lower detection limits of each antibody.

To 15 µL of the recovered DMSO layer, 135 µL DMSO was added and the mixture agitated. This was followed by the addition of 2.1 mL of measurement buffer and further agitation. After the addition of 0.75 mL of fluorescence-labeled anti-Kanechlor monoclonal antibody (K2A A. b.) solution at optimum concentration and gentle agitation, 0.4 mL of sample was measured at a flow rate of 0.75 mL/min in the biosensor. A cell containing solidified PCB derivatives — appropriate for the commercially available R A. b. — was used as a measurement cell for this antibody.

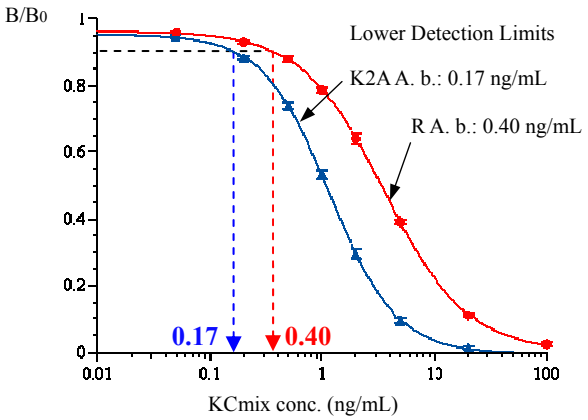

Fig. 6 Comparison of calibration curve of each antibody against KCmix.
We used KCmix (equal volumes of the Kanechlors KC300, KC400, KC500, and KC600) as a standard.

Table 1. Cross-reactivity of K2A A. b. and R A.

Kanechlor	K2A A.b.	R A.b.
KC300	0.71	0.05
KC400	1.00	0.17
KC500	0.62	0.73
KC600	0.44	1.00

Results and Discussion

Measurements of 48 insulating oil samples with K2A A. b. showed a good correlation with values from GC/MS analysis, indicating that the bioassay enabled the analysis of PCBs in insulating oil (Fig. 9).

Separately, these 48 samples were measured with two different antibodies with different specificities according to procedures used to determine the causes of soil pollution[2,3]. Plotting of the measured values obtained using R A. b. against those obtained using K2A A. b. (Fig. 7) revealed that samples could be roughly divided into four groups (A to D). Analysis of the congeners distributions in the respective groups on the basis of the results of GC/MS measurement revealed that, as a whole, Groups A, B, C, and D had KC300, KC400, KC500, and KC600, respectively, as the dominant congeners (Fig. 8).

In addition, plotting of the measured values obtained using K2A A. b. against those obtained from GC/MS

analysis yielded an almost straight line (Fig. 10(a)) for samples classified into Group B or C and another straight line (Fig. 10 (b)) for samples classified into Group A or D. Both lines passed through the origin.

This result suggests that the assay is highly precise, and that it provides results that deviate less from those of GC/MS than do those of analyses using a single type of antibody.

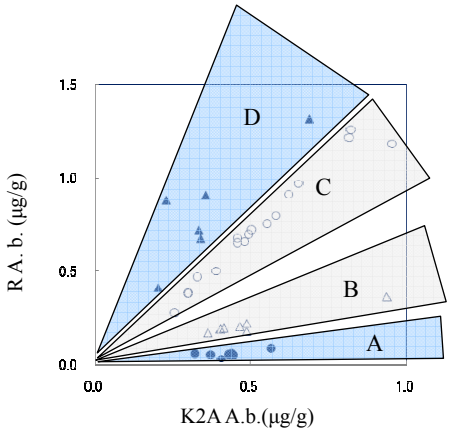

Fig. 7 Measured value of K2A A. b. and R A. b.

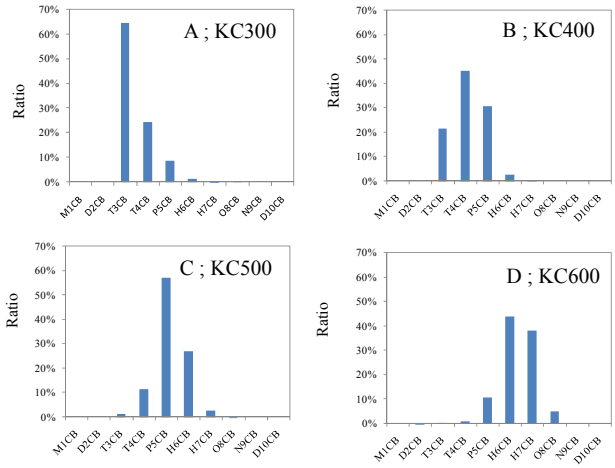

Fig. 8 Dominant congeners pattern
Groups A, B, C, and D had KC300, KC400, KC500, and KC600, respectively, as the dominant congeners.

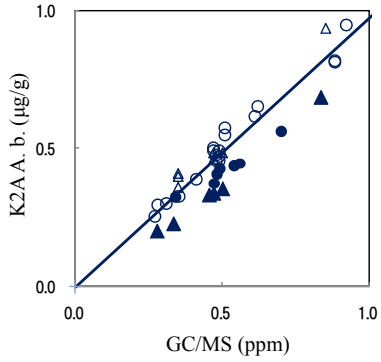

Fig. 9 Correlation diagram (K2A A. b. vs GC/MS) for 48 samples of insulating oil containing PCBs

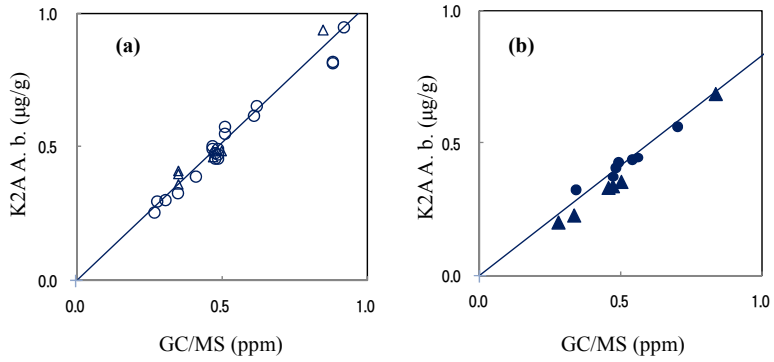

Fig. 10 Correlation diagram (K2A A. b. vs GC/MS)
(a) Groups B and C **(b)** Groups A and D

Conclusion

We evaluated a method of identifying the nature or sources of pollution by different congeners by using their ratios in measurements obtained by a biosensor using several different antibodies with different specificities. The method proved to be applicable to insulating oil samples. The presence of predominant congeners of Kanechlor

in an insulating oil sample could be predicted from the ratio of measurements obtained using two different anti-PCB antibodies with different specificities. Application of an appropriate modification factor based on the information obtained in this way enabled us to build an analytical method with higher precision than those that use a single type of antibody.

By using selected antibodies with the desired specificities to analyze food and soil samples that potentially contain multiple contaminants such as agricultural chemicals and POPs, bioassay-based methods will hopefully be able to analyze multiple components in a way analogous to congeners fractionation by chromatography. Also, simultaneous multiple analyses of the same samples are enabled by the use of differently labeled antibodies, suggesting the wider applicability of rapid determination of multiple components in multiple samples using bioassay.

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