RAPID ANALYSIS OF PCBs IN TRANSFORMER OIL BASED ON "SIMPLIFIED DIOXIN ANALYSIS SYSTEM"

Takagi Y[1], Kataoka C[2], Kitagawa H[1], Tateishi N[1], Sawadaishi K[2,3], Honda K[3]
[1]Kyoto Electronics Manufacturing Co.,Ltd., 68 Ninodan-cho, Shinden, Kisshoin, Minami-ku, Kyoto 601-8317, Japan; [2]Carbuncle BioScienTech LLC., 40-26,Tanida, Okukaiinji, Nagaokakyo, 617-0853, Japan; [3]Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, 790-8566, Japan

Abstract
In R&D to develop bioanalytical methods that would be accepted for the field of environmental analysis, we developed a "Simplified Dioxin Analysis System",[1] which we reported at the conference in 2005. This analysis system consists of an automated sample preparation device, and an automated flow biosensor based on the kinetic exclusion assay, which offers rapid analysis with a measurement accuracy of CV < 3%. It enables high purification through highly reproducible clean-up of environmental samples.[2] Here we report our rapid analysis of PCBs (polychlorinated biphenyls) in transformer oil using this system.

Introduction
In the field of environmental analysis, bioanalytical methods must output precise analytical values with high accuracy and reproducibility, in comparison with official methods. To achieve this goal, the preparation of highly purified samples with high reproducibility, reliability of analytical values based on the reactivity of the measurement system and on clear principles, and high analytical accuracy with elimination of human error[3] will be ensured. In addition, any method must be rapid and inexpensive. We created a new method enabling analysis of 3 samples within 3 hrs including clean-up steps by applying a concept on the "Simplified Dioxin Analysis System" that we developed,[1] to measure trace PCBs in transformer oil, for which rapidity is increasingly needed in Japan. Samples were prepared by direct purification through a multilayer silica gel column under heating, without extraction with dimethylsulfoxide (DMSO) and back-extraction with hexane before applying to the column, and immunosensing by a DXS-600 dioxin biosensor using a newly developed monoclonal antibody[4] that shows almost equal reactivity to a series of kanechlors (KCs). The reproducibility of analytical values of the same sample was approximately 5% in terms of CV, and the quantification range was 0.04–4 ppm (CV $\leqq$ 20% of quantified value). There was a favorable correlation (9 samples, r = 0.991) between measurements obtained using our method and measurements by HRGC/HRMS.

Materials and Methods
1. Samples for evaluation
KC300, 400, 500, and 600 were added to PCB-free insulating oil (Japan Industrial standard listing) individually or in combination at a dose of 0.01–4 mg/kg (ppm). The oil was then cleaned up before evaluation. Separately, PCB-free oil was prepared for use as a blank.

2. Sample Preparation
Transformer oil (0.2 g per sample) mixed with hexane was applied directly to a multilayer silica gel column, and the PCBs in oil were purified under heating in an automated sample preparation device. PCBs in hexane eluted from the column were concentrated and then followed by DMSO substitution (400µL), and finally washed with 200 µL hexane.

3. Measurement
To measure PCBs, we used a DXS-600 biosensor, replacing the antibody supplied in it with a monoclonal antibody [4] that shows almost equal reactivity to a series of KCs (300, 400, 500, and 600), and replacing antigen derivatives fixed to the measurement cell with a compound appropriate for PCB measurement. To 40 µL of the prepared DMSO solution, 160 µL DMSO was added and agitated, followed by 2.8 mL measurement buffer and further agitation. After addition of 1 mL fluorescence-labeled anti- PCBs monoclonal antibody solution with an optimal concentration and gentle agitation, 0.5 mL of sample was measured at a flow rate of 0.75 mL/min to the biosensor.

4. Evaluation

We analyzed the blank to evaluate the validity of the preparation and interference with the measurement system. We also evaluated the properties, detection sensitivity, and measurement accuracy of the rapid analysis system by comparing the reactivity to each KC in the presence and absence of transformer oil and analyzing oil samples containing KCs at different concentrations.

5. Correlation with HRGC/HRMS

Transformer oil obtained from 9 types of transformer were analyzed by both HRGC/HRMS and the rapid analysis system to compare measurements in order to evaluate the accuracy of the rapid analysis system.

Results and Discussion

1. Evaluation of preparation of oil samples

HRGC/HRMS of the PCB fraction eluted with hexane showed almost 100% recovery of PCBs having 3 or more chlorines (Table 1).

Table 1. Recovery of PCBs after multilayer silica gel column chromatography

	T_3CB	T_4CB	P_5CB	H_6CB	H_7CB	O_8CB	N_9CB	$D_{10}CB$
Content in stock solution containing a mixture of KC300–600 (mg/L)	1.10	1.50	1.10	0.95	0.56	0.13	0.01	N.D.
Content eluted from multilayer column (mg/L)	1.20	1.60	1.10	0.98	0.58	0.14	0.01	N.D.
Recovery (%)	109	107	100	103	104	108	100	–

N.D: data was not detectable. — : data was not determined

Measurements of several blank samples with the DXS-600 returned results of 98.5% to 100.7% of those of transformer oil-free samples, suggesting the almost complete elimination of interference with the measurement system (Table 2). Our sample preparation method is thus simple and rapid, gives stable recovery of PCBs, and purifies samples satisfactorily. Thus, it appears appropriate for preparing samples for bioassay of PCBs.

Table 2. Results of blank test

	Sample 1	Sample 2	Sample 3	Sample 4
B/B_0	0.990	0.985	1.007	1.004
CV (%)	0.66	0.60	0.99	1.09

2. Evaluation of biosensing of PCBs

Although the monoclonal antibody showed slightly less reactivity to KC600, it had almost equal affinity to all 4 KCs, suggesting that it is appropriate for measurement of KCs (Table 3). When the mixture of KC reference standards was prepared in the presence of insulating oil, sensitivity tended to decrease, owing to a decrease in the recovery of KCs eluted from the multilayer column after transfer into DMSO. But the gradient was very similar to the calibration curve in the absence of insulating oil, so this rapid analysis has adequate discrimination and a wide measurable range in the presence of insulating oil (Fig. 1).

The reproducibility of measurements of mixtures of KC reference standards at each concentration (n = 3) was approximately 5% in terms of CV, showing high accuracy. Thus, the high accuracy and reproducibility of the "Simplified Dioxin Analysis System" were also shown in the PCB analysis. Therefore, our method could be expected to be useful for rapid analysis.

When the lowest limit of quantification was 20% or less of the CV of quantified values, the rapid PCB analysis system could detect approximately 0.04 ppm, less than 1/10 of the 0.5 ppm reference value for trace PCBs in transformer oil, showing adequate discrimination and quantification.

Table 3. Specificity of anti-KC antibody

KC	IC_{50} (ppm)	
	without insulating oil	with insulating oil
KC300	0.33	0.35
KC400	0.19	0.28
KC500	0.30	0.36
KC600	0.56	0.87

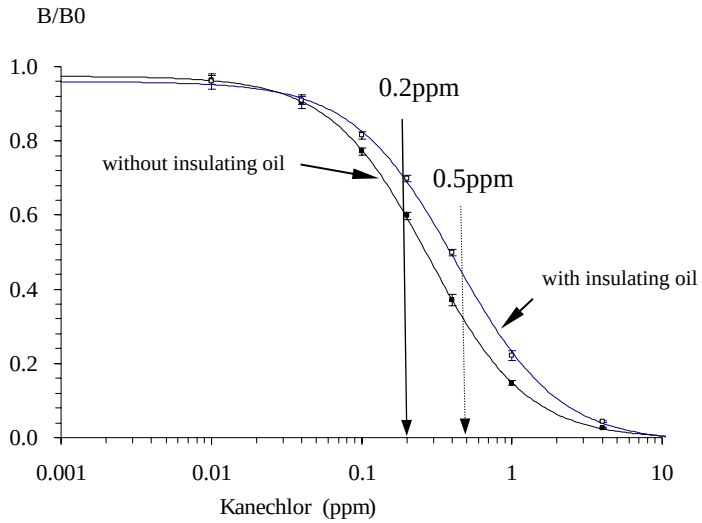

Fig. 1. Calibration curve and detectability of KCs **(N=5)**

3. Correlation with measurement using HRGC/HRMS

Comparison of the measurements of the 9 transformer oil samples between the rapid analysis system and HRGC/HRMS shows good correlation (Fig. 2).

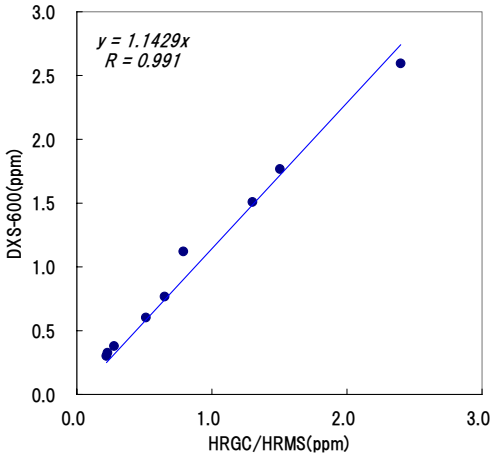

Fig. 2. Correlation between measurements using HRGC/HRMS and DXS-600

Conclusions

The rapid analysis method of PCBs in transformer oil based on "Simplified Dioxin Analysis System", was able to prepare high quality and reproducible samples, and showed reliability of measurement value. So, the PCB analysis method could be expected to contribution to rapid analysis for trace PCBs in transformer oil.

References

1 Matsuki T, Nakama E, Kishino J, Tokuda Y, Takagi Y, Kataoka C, Hamada N, Fujita H, Tateishi N, Sawadaishi K, Honda K. *Organohalogen Compounds* 2005; 67: 39.
2 Fujita H, Hamada N, Sawadaishi K, Honda K. *Journal of Environmental Chemistry* 2005; 15(3): 585.
3 Kataoka C. *Journal of Japan Society on Water Environment* 2006; 29(9): 533.
4 Takagi Y, Kataoka C, Kitagawa H, Ohmura N, Sasaki K. *Japanese Patent Application*, P2006-114732.