Determination of polychlorinated biphenyls (PCBs) in insulating oil by a Cocktail PCB ELISA

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Introduction

Polychlorinated biphenyls (PCBs) have been demonstrated to cause cancer and other adverse health effects on the immune system, reproductive system, nervous system and endocrine system[1)]. In Japan, a special measures law on promotion of waste PCBs management requiring that waste PCBs be treated within 15 years was enacted in 2001. Insulating oils contaminated with PCBs are great concerns, because millions of transformers suspected of containing PCB are stockpiled, and those oils need to be measured for appropriate treatment. For determination of PCBs in insulating oil, instrumental analysis such as high-resolution gas chromatography mass spectrometry (GC-MS) and GC with an electron capture detector (GC-ECD) are generally employed[3)]. These analytical methods are highly reliable. However, they have several potential drawbacks including expensive instrumentation, large sample volume, extensive purification and technical expertise in operation. Due to these shortcomings, the analysis of a large number of samples may be both cost and time prohibitive. Therefore there is a strong need for rapid, simple, and cost-effective methods for quantitative analysis of PCBs in insulating oils, such as enzyme-linked immunosorbent assay (ELISA). In Japan, PCBs were marketed under the trade name, Kanechlor (KC), and KC-300, 400, 500 and 600, practically used PCBs, have a broad range of chlorination (Table 1). In this paper, we report a performance of newly developed cocktail PCB ELISA, which gives fairly even reactivity from KC-300 to KC-600, and a simple pretreatment method for determination of PCBs in insulating oils.

Table 1. Composition ratio of PCB homologues in Kanechlor (% w/w)

Kanechlor(KC)	Number of chlorine in PCBs									
	1	2	3	4	5	6	7	8	9	10
KC-300		12	55	27	5	1				
KC-400			17	51	28	3				
KC-500			2	10	52	32	3			
KC-600			1	1	9	42	39	7		

Materials and Methods

Reagents

General PCB ELISA and LC (Lower Chlorinated) PCB ELISA were purchased from Abraxis LLC (PA, USA). Standard PCBs, namely KC-300, 400, 500 and 600 were obtained from GL Sciences Inc. (Tokyo, Japan). All chemical reagents were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Pretreatment of insulating oil for ELISA analysis

Oil samples added with hexane and dimethyl sulfoxide (DMSO) were mixed vigorously. Then the hexane layer was removed and freshly prepared hexane was added. After repetition of this washing step, the DMSO layer was diluted with aqueous solution and extracted with hexane. After being dehydrated, the hexane layer was sulfonated with sulfuric reagent and washed with aqueous solution. The hexane layer was evaporated and reconstituted to DMSO.

Immunoassay Procedure

Standard or pretreated PCBs were dissolved and diluted in 100% DMSO, and added to distilled water to give a final concentration of 40 % DMSO solution prior to the ELISA assay. Two hundred (200) uL of sample, 250 uL of PCB-HRP (horseradish peroxidase) conjugate, and 500 uL of anti-PCB antibody coupled with magnetic particles were added to a polystyrene test tube for the assay and incubated for 30 min at room temperature. The magnetic rack was used to magnetically separate the reaction mixture. After separation, the magnetic particles were washed twice with 1.0 mL of washing buffer. The colored product was developed for 20 min at room temperature by the addition of 500 uL of a coloring reagent. The colored product was stopped by addition of 500 uL of a stop reagent, and absorbance was measured at 450nm.

$B/B_0(\%)$=(absorbance at sample) / (absorbance at PCB=0) * 100

Results and Discussion

*Cross reactivity of General and LC PCB ELISA against PCB conge*ners

General and LC PCB ELISA preferably reacted with 4 to 5 chlorinated PCBs and 3 to 5 chlorinated PCBs respectively as shown in Figure 1. These cross reactivity patterns were relatively broad, and it seemed that over and under estimation was inevitable in determination of unknown KC samples due to the broad range of chlorination of KC series.

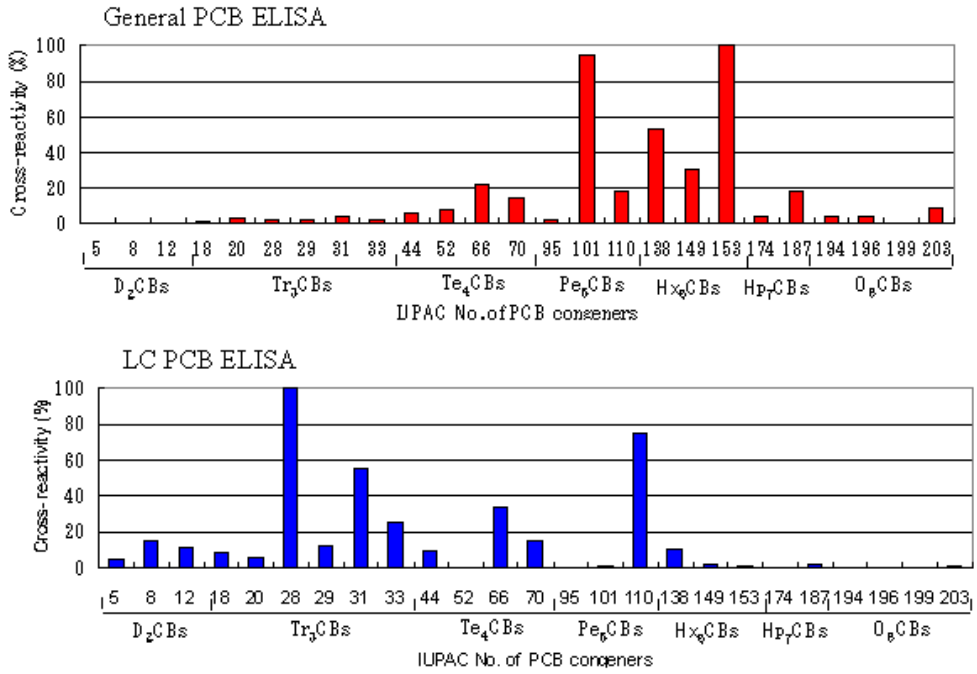


Figure 1. Cross reactivity of General and LC PCB ELISA against PCB congeners

Modification of cross reactivity by mixing General and LC PCB ELISA reagents

Kit reagents of General and LC PCB, such as anti-PCB antibody coupled with magnetic particles and PCB-HRP conjugates, were mixed at an optimized rate to modify the cross reactivity against series of KC (Cocktail PCB ELISA). The cross reactivity patterns of General, LC and Cocktail PCB ELISAs against KC series were shown in Table 2. General and LC PCB ELISA highly reacted with KC-500 to 600 and KC-300 to 400, respectively and their cross reactivity against KC series reflected the cross reactivity results of PCB congeners. On the other hand, Cocktail PCB ELISA reacted with KC 300 to 500 almost equally and moderately reacted with KC-600. These data indicate that the cross reactivity patterns of Cocktail PCB ELISA against KC series were well improved to determine PCBs whose range of chlorinations were from 3 to 7.

Table 2 Cross reactivity of PCB ELISAs against Kanechlor series

Kanechlor	Dominant PCB homologues	Cross reactivity(%)		
		General	LC	Cocktail
KC-300	Tr3CB	49	102	101
KC-400	Te4CB	100	100	100
KC-500	Pe5CB	217	58	118
KC-600	Hx6CB, Hp7CB	217	27	55

Assay working range

The assay working ranges of each ELISA based on KC-400 were determined in DMSO and insulating oil. The lowest and highest quantitative limits in DMSO were defined based on the range between 90 and 15 %B/B_0. The assay working ranges in insulating oil were determined by calculating the dilution and recovery rate of pretreatment methods for insulating oil, except LC. As shown in Table 3, these ELISAs were highly sensitive , and both General and Cocktail PCB ELISA could determine around 1 mg/Kg level of PCBs in insulating oil.

Table 3 Assay working range based on KC-400

Sample	Quantitative limits	General	LC	Cocktail
DMSO	Low (ug/Kg)	1.0	5.0	3.0
	High (ug/Kg)	1000	1000	1000
Insulating Oil	Low (mg/Kg)	0.4	NT	1.0
	High (mg/Kg)	160	NT	320

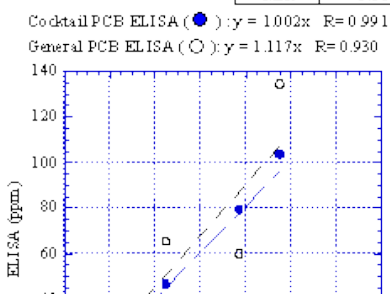
NT: Not Tested

Comparison between PCB ELISAs and GC-ECD in determination of PCBs in real insulating oils

Twenty-six insulating oils contaminated with PCBs were determined by PCB ELISAs (Cocktail and General) and GC-ECD. For ELISA analysis, samples were pretreated and assayed as described in Materials and Methods. On the other hand, the values of GC-ECD were obtained according to Notification No.192 (1992) of the Ministry of Health and Welfare of Japan. As shown in Table 4 and Figure 2, the values of both Cocktail and General PCB ELISA were well correlated to those of GC-ECD both in lower and higher concentration of PCB contaminated samples, and Cocktail PCB ELISA was more approximated to GC-ECD values, based on the ELISA / GC ratios (Cocktail / GC; average: 0.89, SD: 0.16, max: 1.19 and min: 0.61, General / GC; average: 1.08, SD: 0.35, max: 1.97 and min: 0.47), slopes (Cocktail: 1.002, General: 1.117) and correlation coefficients (R) (Cocktail: 0.991, General: 0.930). These data suggest that combining Cocktail PCB ELISA and developed pretreatment method gives a rapid, simple and highly accurate analytical method for determination of PCBs in insulating oils.

Table 4 Comparison of PCB ELISA and GC-ECD in determination of PCBs in insulating oils

Sample No.	GC-ECD (mg/Kg)	ELISA (mg/Kg)		ELISA / GC ratio		Dominant Kanechlor (KC)
		Cocktail	General	Cocktail/GC	General/GC	
1	1.0	1.1	1.0	1.08	0.96	KC-400
2	3.4	2.3	2.5	0.70	0.74	KC-300/400
3	12.8	8.8	21.3	0.68	1.66	KC-500
4	13.7	13.3	17.8	0.97	1.30	KC-500
5	13.2	12.9	25.9	0.98	1.97	KC-500
6	2.3	2.2	3.0	0.95	1.32	KC-300 ~ 600
7	44.6	46.4	64.8	1.04	1.45	KC-500
8	20.2	16.0	20.7	0.79	1.02	KC-400/500
9	31.0	28.1	37.1	0.91	1.20	KC-500
10	8.6	9.0	9.9	1.04	1.14	KC-300/400
11	77.2	79.1	59.5	1.02	0.77	KC-300/500
12	95.2	103.5	133.8	1.09	1.41	KC-500
13	4.2	2.9	3.6	0.69	0.87	KC-500
14	5.7	6.8	9.5	1.19	1.68	KC-500
15	15.1	11.3	12.3	0.75	0.82	KC-300/500
16	42.1	29.1	19.8	0.69	0.47	KC-300
17	1.7	1.7	1.2	1.02	0.73	KC-500
18	4.6	4.4	4.2	0.95	0.91	KC-500
19	2.5	1.8	2.0	0.73	0.82	KC-500
20	1.0	1.0	1.0	0.96	0.97	KC-500
21	2.9	1.8	3.0	0.61	1.04	KC-500
22	22.2	22.0	15.8	0.99	0.71	KC-300/400
23	7.2	5.0	8.7	0.69	1.22	KC-500
24	21.6	19.2	24.9	0.89	1.15	KC-300/400
25	20.1	17.0	15.2	0.84	0.75	KC-300/400
26	6.8	6.3	7.6	0.93	1.12	KC-500
			Average	0.89	1.08	
			SD	0.16	0.35	
			Max	1.19	1.97	
			Min	0.61	0.47	



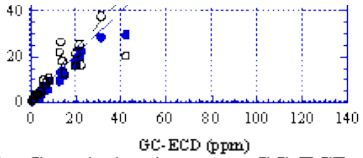


Figure 2. Correlation between GC-ECD and ELISA

References

1) US EPA homepage, http://www.epa.gov/opptintr/pcb/effects.html

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