(5) Precision profile

濃度を調整した標準液に対して、2 重測定で 6 回測定を行い、各濃度における吸光度とその SD 値から%CV を求めプロットしたものです。

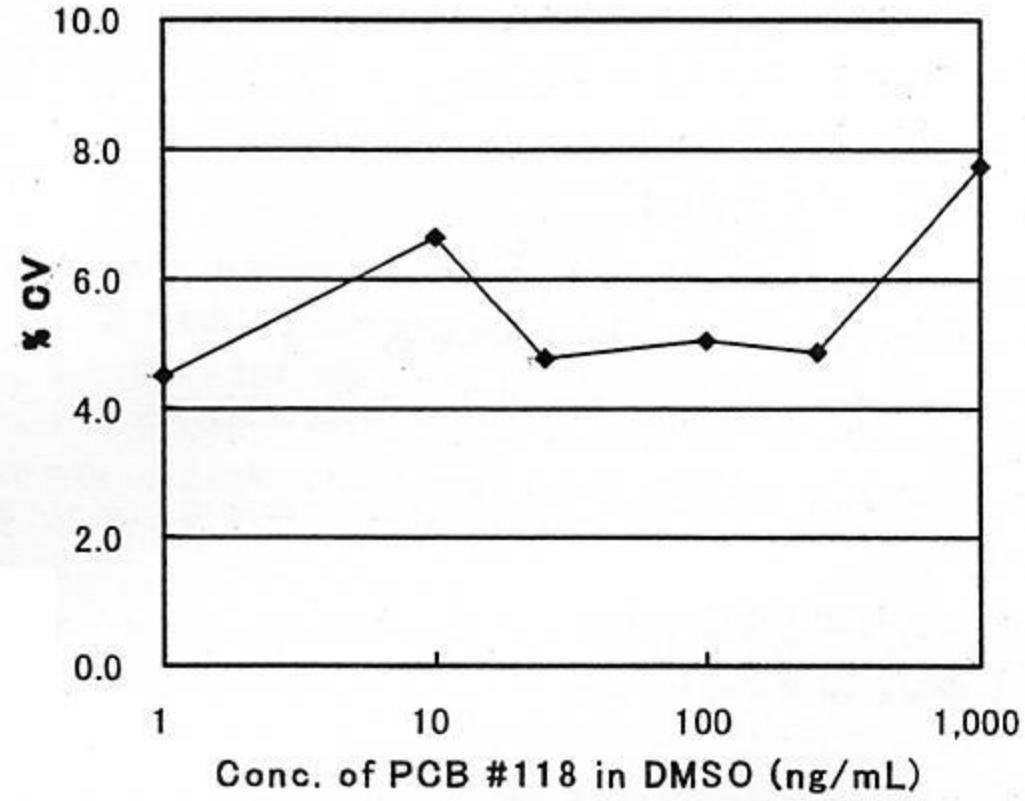


図 6　Precision profile

Claim 1 **Determination of Sensitivity**

AIM

To determine the sensitivity of the assay.

METHOD

The sensitivity, defined as three standard deviations below the mean optical density of 8 zero standard replicates was determined. The corresponding concentration was calculated from a standard curve ranging between 1 to1,000 ng/ml set up in 8 replicates. The grand mean zero and standard values were then used to calculate the sensitivity. The data was plotted as mean ±SD as a 4-parameter plot and calculated values by an attached calculation soft wear of a micro-plate reader. .

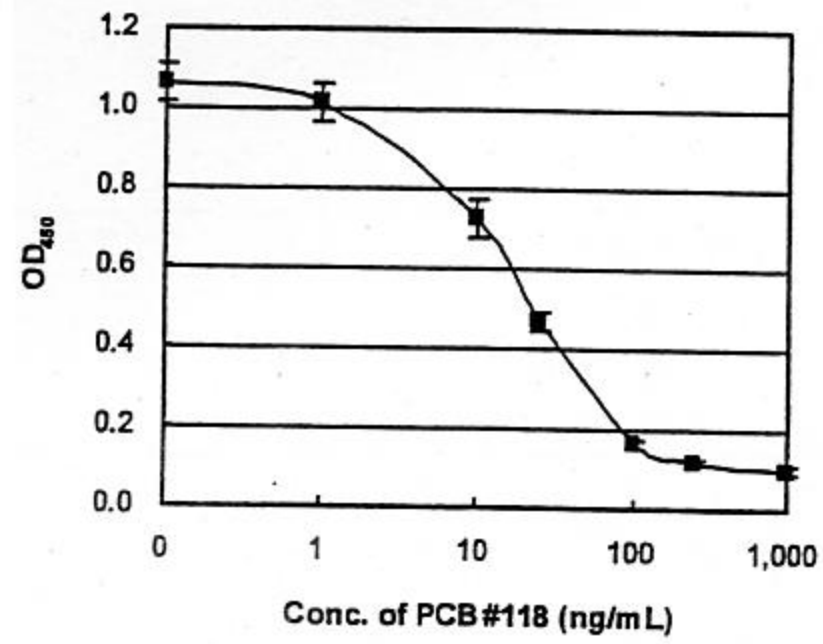
The sensitivity, defined as the concentration on the standard curve equivalent to 3 standard deviation below zero standard (n=10), was determined to be 6.5 ng/ml of PCB #118 in DMSO.

RESULTS

This was determined as 6.5 ng/ml. No cross ranging was found between the absorbance of zero-standard below 3SD.

Table 2. Intra-assay of the absorbancies (OD450) for PCB #118 (n=8)

ng/ML	Mean	SD	CV	Mean+3SD	Mean-3SD
0	1.061	0.048	4.5	1.20	0.92
1	1.010	0.048	4.8	1.16	0.87
10	0.725	0.048	6.6	0.87	0.58
25	0.467	0.022	4.8	0.53	0.40
100	0.163	0.008	5.1	0.19	0.14
250	0.117	0.006	4.9	0.13	0.10
1,000	0.100	0.008	7.7	0.12	0.08



Claim 2 **Demonstration of Within-Assay Precision**

AIM

To determine the within-assay precision of measurement of controls in the assay.

METHOD

Low, medium and high controls were prepared from a stock standard. The results were obtained by using one batch of performance trial kit.

RESULTS

Sample	Mean (ng/mL) ± SD	CV (%)	n
High	98.4 ± 5.04	5.1	8
Med	55.5 ± 3.90	7.0	8
Low	18.5 ± 1.66	8.9	8

CONCLUSIONS

The within assay precision was shown to be within acceptable limits.

Claim 3 **Demonstration of Between-Assay Precision**

AIM

To demonstrate the between-assay precision of measurement of controls in the assay.

METHOD

Low, medium and high controls were prepared from a stock standard. The results were obtained by using one batch of performance trial kit.

RESULTS

Sample	Mean (ng/mL) ± SD	CV (%)	n
High	83.7 ± 9.66	11.5	6
Med	45.4 ± 7.92	17.5	6
Low	17.5 ± 2.88	16.4	6

CONCLUSIONS

The between assay precision was shown to be within acceptable limits.

Claim 4 **Determination of Stability**

AIM .

To demonstrate the stability of reagents when stored at the recommended temperature of 4°C.

METHOD

The standard curves were obtained using reagents from the 4°C stability trial at time points at 0, 3 and 6 months using one batch of kit. Low and high controls were prepared from a stock standard.

RESULTS

Typical assay data for a standard curve of 0 - 1,000ng/ml surrogate standard.

PCB STD (ng/ml)	OD450		
	T=0	T=3 months	T=6 months
0	1.599	1.597	1.157
2.5	1.575	1.485	1.251
10	1.510	1.415	1.125
25	1.359	1.178	0.992
100	0.885	0.755	0.460
250	0.498	0.413	0.276
1,000	0.164	0.189	0.098

Sample	Mean (ng/mL) ± SD	CV (%)	n
High	101 ± 10.7	10.6	3
Low	25.8 ± 3.24	12.5	3

CONCLUSIONS

The reagents give a good response after 6 months storage at 4°C. The 6 months period precision was shown to be within acceptable limits.

Claim 5 **Demonstration of Between-plate Precision**

AIM

To demonstrate the between-plate precision of measurement of controls in the assay.

METHOD

Low and high controls were prepared from a stock standard. The results were obtained by using six batch of performance trial kit.

RESULTS

Sample	Mean (ng/mL) ± SD	CV (%)	n
High	49.4 ± 3.6	9.1	6
Low	21.5 ± 1.9	7.2	6

CONCLUSIONS

The between plate precision was shown to be within acceptable limits.

Claim 6 **Cross-reactivitiy of the Assay**

AIM

To investigate the degree of cross-reactivity of the predominant PCB congener in PCB products (e.g., Aroclor and Kanechlor).

METHOD

Standard solution of each congener were prepared in DMSO.

RESULTS

The cross-reactivities (% CR) are described as ratios between the IC50 value of each compound and that of PCB #118.
This was done for significant cross-reactants only.

PCB products	% CR	
Kanechlor 300 (equivalent to Aroclor 1242)	3.87	(42.3)*
Kanechlor 400 (Aroclor 1248)	7.36	(80.5)
Kanechlor 500 (Aroclor 1254)	9.14	(**100**)
Kanechlor 600 (Aroclor 1260)	1.69	(18.5)

* Ratios between IC_{50} value of each PCB product and that of Kanechlor 500 are described in parenthesis.

Predominant PCBs	IUPAC #	% CR
2,3-DiCB	5	< 0.1
2,4'-DiCB	8	< 0.1
2,2',5-TriCB	18	< 0.1
2,3,3'-TriCB	20	< 0.1
2,4,4'-TriCB	28	3.5
2,4',5-TriCB	31	12.9
2',3,4-TriCB	33	2.6
2,2',3,5'-TeCB	44	< 0.1
2,2',5,5'-TeCB	52	< 0.1
2,3',4,4'-TeCB	66	15.2
2,3',4',5-TeCB	70	14.9
2,2',3,5',6-PeCB	95	< 0.1
2,2',4,5,5'-PeCB	101	< 0.1
2,3,3',4,4'-PeCB	105	2.5
2,3,3',4',6- PeCB	110	0.88
2,3',4,4',5-PeCB	118	**100**
2,2',3,4,4',5'-HexCB	138	< 0.1
2,2',3,4',5',6-HexCB	149	< 0.1
2,2',4,4',5,5'-HexCB	153	< 0.1
2,2',3,3',4,4',5-HepCB	170	< 0.1
2,2',3,3',4,5,6'-HepCB	174	< 0.1
2,2',3,4,4',5,5'-HepCB	180	< 0.1

PAHs	% CR
Acenaphtene	< 0.1
Acenaphtherene	< 0.1
Anthracene	< 0.1
Benzo(a)anthracene	< 0.1
Benzo(a)pyrene	< 0.1
Benzo(b)fluoranthene	< 0.1
Benzo(ghi)perylene	< 0.1
Benzo(k)fluoranthene	< 0.1
Chrysene	< 0.1
Dibenzo(ah)anthracene	< 0.1
Fluoranthene	< 0.1
Hexachlorobenze	< 0.1
Indeno(123cd)pyrene	< 0.1
Naphthalene	< 0.1
Phenanthrene	< 0.1
Pyrene	< 0.1
Hexachlorobenze	< 0.1
	< 0.1
	< 0.1
	< 0.1

Other related compounds	% CR
Biphenyl	< 0.1
1,2-dichlorobenzene	< 0.1
3,4-dichloroaniline	< 0.1
3,4-dichloroanisole	< 0.1
3,4-dichloronitro-benzene	< 0.1
3,4-dichlorophenol	< 0.1
3,4-dichlorotoluene	< 0.1
1,2,3-trichlorobenzene	< 0.1
3,4,5-trichloroaniline	< 0.1
3,4,5-trichlorophenol	< 0.1
2,3,7,8-TCDD	< 0.1
2,3,7,8-TCDF	< 0.1