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# Biosensor immunoassay for the screening of dioxin-like polychlorinated biphenyls in retail fish

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ABSTRACT

Dioxin-like polychlorinated biphenyls (DL-PCBs) often make up the majority of the toxic equivalent (TEQ) contribution of dioxins found in fish samples. For the purpose of making risk assessments, it is therefore important to develop screening methods for determining TEQ concentrations of DL-PCBs in retail fish. We have developed a rapid biosensor immunoassay (BIA) for DL-PCBs that uses a surface plasmon resonance sensor (Biacore 3000). The BIA is highly specific for 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) that is generally the most abundant DL-PCB isomer found in fish. The fish extracts were first cleaned up on a multilayer silica gel column followed by an alumina column, then subjected to the assay. The quantitative limit of the assay was 1 ng PCB 118 per gram of tested sample. Dilution and recovery tests using purified fish extracts suggested that the matrix effect was minimized in the assay by diluting the analyzed samples. The assay results for retail fish samples ($n = 7$) agreed well with those obtained by an enzyme-linked immunoassay (ELISA) using the same monoclonal antibody: ELISA has been already validated for determining DL-PCBs in fish samples, so BIA performs well in this analysis. Finally, BIA results for the TEQ concentrations of DL-PCBs in retail fish samples ($n = 10$) correlated well with those obtained by high-resolution gas chromatography coupled to high-resolution mass spectrometry ($r = 0.89$). Our method is therefore useful for screening retail fish to determine the TEQ concentrations of DL-PCBs.



## 1. Introduction

Dioxin-like polychlorinated biphenyls (DL-PCBs) together with polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are collectively known as dioxins. Four non-*ortho* PCBs and eight mono-*ortho* PCBs are currently classified as DL-PCBs, and these have been assigned toxicity equivalent factors (TEFs) relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) [1,2]. People living in Japan are subject to exposure to high levels of dioxins through consumption of fish [3–5]. The DL-PCBs often make up the majority of the toxic equivalent (TEQ) contribution of dioxins in fish samples [6–9]. Therefore, it is important to determine the TEQ concentrations of DL-PCBs, in addition to those of PCDD/Fs, in retail fish.

High-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) is widely accepted as the most reliable method for determining the TEQ levels of dioxins. Although this technique is reliable and sensitive, it is time-consuming, requires expensive equipment, and must be performed by highly trained staff. Reporter-gene assays, such as the chemical-activated luciferase gene expression (CALUX) assay, are currently considered to be the best method for screening TEQ concentrations of dioxins in food [for review, see 10]. However, its drawbacks include the need for cell culture, which requires skilled personnel and elaborate

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equipment, and the probable need for a license to conduct the assay.

An enzyme-linked immunosorbent assay (ELISA) could be a simpler alternative that does not require cell culture. 2,3′,4,4′,5-Pentachlorobiphenyl (PCB 118) is generally the most abundant DL-PCB isomer found in fish [7,11,12]. On the basis of HRGC/HRMS data obtained in our national survey of dioxins in Japan [13], we found that the concentrations of PCB 118 correlates well with the TEQ concentrations of DL-PCBs in retail fish, although PCB 118 makes a relatively small contribution to the total TEQ of DL-PCBs as a result of its low TEF. Therefore, we recently developed an ELISA that uses a monoclonal antibody (Mab) that is highly specific for PCB 118 to screen retail fish for the TEQs of DL-PCBs [13]. The ELISA is simple, quick, and suitable for screening DL-PCBs in retail fish samples.

Various immunosensor techniques are growing in popularity for residue analysis because of their speed. Although electrochemical immmunosensors, like screen-printed electrodes, are widely used and cost-effective and allowing on site analysis, surface plasmon resonance (SPR) immunosensors have proved to be the most widely reported for immunosensor applications [for review, see 14]. Additionally, SPR techniques can eliminate the need for enzyme-labeled reagents and are mostly highly automated, they are quite simple methods. In particular, SPR-based biosensor immunoassays (BIAs) using Biacore instruments have been proposed, mainly for the determination of residues of veterinary drugs in foodstuffs [for reviews, see 15 and 16]. BIA has also been applied in the detection of dioxins and PCBs [17]; however, there are no previously published reports on their performance in determining these compounds in food samples. We developed an SPR-based BIA by using a Mab that is specific to PCB 118, and we assessed the performance of the BIA in screening TEQ concentrations of DL-PCBs in retail fish.

## 2. Experimental

### 2.1. Materials

Dioxin-analysis-grade acetone, dichloromethane, and *n*-hexane were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). A multilayer silica gel column filled from bottom to top with 0.9 g silica gel, 3 g of 2% (w/w) potassium hydroxide-impregnated silica gel, 0.9 g silica gel, 4.5 g of 44% (w/w) sulfuric acid-impregnated silica gel, 6 g of 22% (w/w) sulfuric acid-impregnated silica gel, 0.9 g silica gel, 3 g of 10% (w/w) silver nitrate-impregnated silica gel, and 6 g of anhydrous sodium sulfate was purchased from GL Science Inc. (Tokyo, Japan). Alumina B-Super I was obtained from ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA). Dimethyl sulfoxide (DMSO) was purchased from Sigma–Aldrich (Milwaukee, USA). An activated carbon/dispersed silica gel reversible column was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). DL-PCBs were obtained from AccuStandard Inc. (New Haven, CT, USA). PCB analogue–bovine serum albumin (BSA) conjugate, a Mab against PCB 118, and the ELISA kit using the Mab were purchased from EnBioTec Laboratories (Tokyo, Japan).

Sensor chips (CM5), HBS-EP buffer [0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant polysorbate 20; pH 7.4], and an amine coupling kit were supplied by Biacore AB (Uppsala, Sweden).

Fish samples were purchased during 2002 and 2004 from supermarkets in Tokyo, Japan. The samples (muscle tissue) were homogenized by using a food processor and stored at −20 °C until analysis.

### 2.2. Equipment

The Biacore 3000 was supplied by Biacore AB (Uppsala, Sweden). HRGC/HRMS was performed by using an HP-6890 plus gas chromatograph coupled to a JEOL JMS-700 MStation mass spectrometer supplied by JEOL Ltd. (Tokyo, Japan).

### 2.3. Purification of fish tissue for the BIA

The homogenized fish sample (20 g) was added to aqueous 2 M KOH (100 mL) and subjected to alkali digestion at room temperature for 16–20 h. The alkaline hydrolysate was added to methanol (150 mL) and extracted three times by mechanically shaking (10 min) with *n*-hexane (100 mL). The extract was washed twice with 2% (w/v) aqueous NaCl (150 mL), treated several times with concentrated sulfuric acid, and then passed through the multilayer silica gel column (see above). The eluate obtained with *n*-hexane (200 mL) was then loaded onto an alumina column (15 g) and washed with *n*-hexane (150 mL). The mono-*ortho*-PCBs containing PCB 118 was eluted with 2% (v/v) dichloromethane/*n*-hexane (150 mL). The fraction was dried by using a nitrogen stream, and the residue was dissolved in DMSO (200 μL). Alternatively, the eluate from the alumina column was loaded onto an active carbon-dispersed silica gel reversible column and washed with *n*-hexane (50 mL). The mono-*ortho*-PCBs containing PCB 118 were then eluted with 25% (v/v) dichloromethane/*n*-hexane (50 mL). The fraction was dried using a nitrogen stream and the residue was dissolved into DMSO (200 μL). The solution was centrifuged at 10,000 rpm for 5 min and the supernatant was subjected to BIA.

### 2.4. BIA

#### 2.4.1. Preparation of the biosensor chip

A PCB analogue–BSA conjugate was immobilized (yielding approximately 1500 relative response (RU)) on the surface of a CM5 sensor chip by using an amine coupling kit, following the manufacturer's instructions. Briefly, the chip surface was activated by injecting a 1:1 (v/v) mixture of 0.4 M 1-ethyl-3-(3-demethylaminopropyl) carbodiimide hydrochloride (EDC) and 0.1 M *N*-hydroxysuccinimide (NHS) at a flow rate of 10 μL min$^{-1}$ and with a contact time of 7 min. The reactants were removed and the PCB analogue–BSA conjugate (50 μg mL$^{-1}$ in 10 mM acetate buffer, pH 4.0) was injected over the surface during 10 min at a flow rate of 10 μL min$^{-1}$. To deactivate the remaining active sites, 1 M ethanolamine was injected during 7 min at a flow rate 10 μL min$^{-1}$. The sensor chip surface was washed repeatedly with 50 mM NaOH for 5 s at a flow rate of 60 μL min$^{-1}$.

#### 2.4.2. *BIA for DL-PCBs in fish*

Mixing of all the reagents, injection, and washing were performed automatically by the Biacore 3000. The running buffer was a 9:1 (v/v) mixture of HBS-EP buffer and DMSO at a flow rate of 20 μL min$^{-1}$. An aliquot of Mab against PCB 118 (5.5 μg mL$^{-1}$ in HBS-EP buffer) was mixed with the samples or with various concentrations of PCB 118 (0–600 ng mL$^{-1}$ in DMSO) in a ratio of 9:1 (v/v). The mixture was injected for 2 min over the immobilized surface and the reference (non-immobilized) surface. After the injection, the needle was washed with DMSO to eliminate the risk of carry-over of PCB 118 from a highly contaminated sample. The surface was regenerated with 50 mM NaOH for 5 s at a flow rate of 60 μL min$^{-1}$. The total run time between the samples was about 12 min. RUs were recorded on each sensorgram 15 s after the injection. All RU values were corrected by subtraction of the RUs from the reference surfaces. Duplicate injections for standard solutions were performed and the mean responses were used to construct a calibration curve. The calibration curves were fitted by using a four-parameter logistic model.

### 2.5. *ELISA for DL-PCBs in fish*

The purification procedure was performed in a similar way to that for the BIA. The ELISA kit was used according to the manufacturer's instructions (EnBioTec Laboratories, Tokyo, Japan) [18]. Briefly, samples or various concentrations of 3,3′,4-trichloro-4′-methoxybiphenyl, a surrogate standard for PCB 118, mixed with competitor–horseradish peroxidase conjugate were added to microtitre wells coated with Mab against PCB 118, incubated for 30 min at room temperature, then washed with the solution provided. An enzyme substrate solution containing 3,3′,5,5′-tetramethylbenzidine was added to each well and incubated for 20 min. The enzyme reaction was stopped with 0.5 M $H_2SO_4$ and the absorbance at 450 nm was measured. All experiments were conducted in duplicate. The calibration curves were fitted by using a four-parameter logistic model.

### 2.6. *Measurement of DL-PCBs in fish by HRGC/HRMS*

The extraction, purification, and analysis of dioxins were performed following general procedures described in a previous report [19]. Briefly, the homogenized sample with $^{13}C_{12}$-labelled internal standards was digested with aqueous KOH. The alkaline hydrolyzate was then extracted with *n*-hexane. The extracts were treated with concentrated sulfuric acid then purified on a silver nitrate/silica gel column. This was followed by further purification on an alumina column. The alumina column separated the extract into mono-*ortho* and non-*ortho* PCB fractions. The latter fraction was purified further on an activated-carbon column. Both the fractions were spiked with $^{13}C_{12}$-labelled recovery standards. Four non-*ortho* PCBs and eight mono-*ortho* PCBs were quantified by the HRGC/HRMS. The measurement of non-*ortho* and mono-*ortho* PCBs was performed in an HT-8 fused silica capillary column (SGE, TX, USA). The TEQ was calculated by using the World Health Organization (WHO) TEFs ($TEF_{1998}$) [1]. The TEQ was also calculated by using the new TEFs ($TEF_{2005}$) revised recently [2].

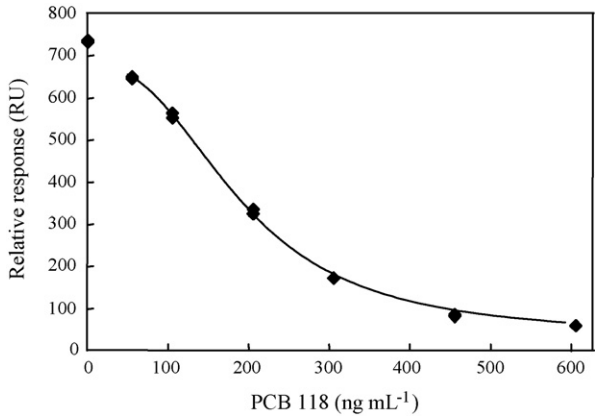


**Fig. 1 – Typical calibration curve of the BIA. Each standard solution was assayed in duplicate. The calibration curve was fitted by using a four-parameter logistic model based on the data ranging from 50 to 600 ng PCB 118 mL$^{-1}$.**

The limits of quantitation (LOQ) were around 0.1 pg g$^{-1}$ for non-*ortho* PCBs and 1.0 pg g$^{-1}$ for mono-*ortho* PCBs. Calculation of the total TEQ in a sample was carried out by assuming that all isomer concentrations below the LOQs were equal to zero.

## 3. Results and discussion

### 3.1. *BIA calibration curve*

Fig. 1 shows a typical calibration curve for BIA. To determine the accuracy and precision of the quantification, six concentrations of PCB 118 were assayed repeatedly on different days. As shown in Table 1, at PCB 118 concentrations of 100–600 ng mL$^{-1}$, the residual errors were small (−5.6 to +5.1%). Coefficients of variations (CVs) in the same concentration range were also extremely low (<2.8%). From these results, the BIA's quantitative range was selected to be 100–600 ng mL$^{-1}$ determined as tolerable concentrations defined as being less than 10% residual errors and CVs. The LOQ of 100 ng of PCB 118 per milliliter corresponds to 1 ng of PCB 118 per gram of sample, when a 20-g fish sample is tested.

**Table 1 – Accuracy and precision of the BIA calibration curve[a]**

| PCB 118 conc. (ng mL$^{-1}$) | PCB 118 found ($n$ = 6) | | |
|---|---|---|---|
| | Mean ± S.D. (ng mL$^{-1}$) | CV% | Bias% |
| 50 | 48.5 ± 14.7 | 30.4 | −3.0 |
| 100 | 101.3 ± 2.6 | 2.6 | 1.3 |
| 200 | 198.1 ± 2.7 | 1.4 | −1.0 |
| 300 | 315.3 ± 8.8 | 2.8 | 5.1 |
| 450 | 463.6 ± 7.8 | 1.7 | 3.0 |
| 600 | 566.7 ± 10.3 | 1.8 | −5.6 |

[a] The accuracy and precision were determined by analyzing each concentration of PCB 118 on 6 different days.

**Table 2 – Cross-reactivity of the BIA against DL-PCBs**

| DL-PCBs | Cross-reactivity (%) | |
|---|---|---|
| | BIA[a] | ELISA[b] |
| Non-*ortho* PCBs | | |
| PCB 77 | 40.3 | 17.8 |
| PCB 81 | 2.7 | <3.0 |
| PCB 126 | 1.5 | <3.0 |
| PCB 169 | <1.0 | <0.1 |
| Mono-*ortho* PCBs | | |
| PCB 105 | 10.3 | 2.5 |
| PCB 114 | 9.8 | 3.4 |
| PCB 118 | 100 | 100 |
| PCB 123 | <1.0 | <0.1 |
| PCB 156 | 33.2 | 7.2 |
| PCB 157 | <1.0 | <0.1 |
| PCB 167 | <1.0 | <0.1 |
| PCB 189 | <1.0 | <0.1 |

[a] The percentage of cross-reactivity relative to PCB 118 was calculated as follows: (Conc. of PCB 118 giving a certain RU)/(Conc. of DL-PCBs isomers giving the same RU) × 100.
[b] Data quoted from the EnBio Coplanar PCB EIA system instruction booklet [18].

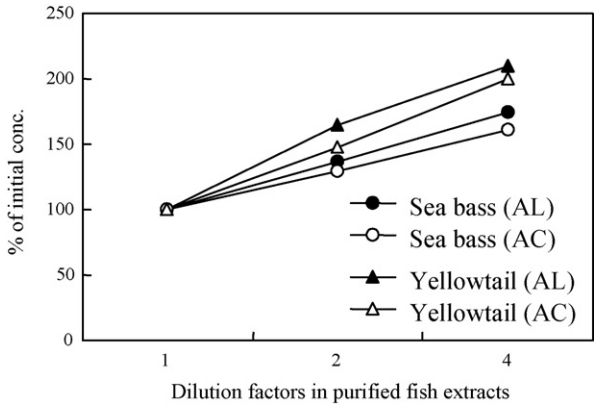


**Fig. 2 – Effect of the dilution factor on concentrations measured in retail fish. Purified extracts from two varieties of fish contaminated in the natural environment were diluted with DMSO and assayed in duplicate by the BIA. The extracts were purified by an alumina column (AL) or an activated-carbon column (AC) after a multilayer silica gel column.**

### 3.2. *Cross-reactivity of the BIA*

The cross-reactivity of the BIA to other DL-PCBs was examined. Serial dilutions of DL-PCBs were assayed and the concentrations of each compound required for around 70% of the maximal RU were compared with that of PCB 118 (Table 2). The BIA was specific to PCB 118; however, the assay cross-reacted to some extent with PCB 77, PCB 105, PCB 114, and PCB 156 (9.8–40.3% of the value for PCB 118). However these isomers are usually present in much lower concentrations than PCB 118 in fish samples. The cross-reactivity of the ELISA using the same Mab [18] is also shown in Table 2. The cross-reactivity of the BIA with some of the isomers (PCB 77, PCB 105, PCB 114 and PCB 156) appeared to be a little higher than the ELISA. This might be responsible for the difference in estimations for the cross-reactivities of both methods. The cross reactivity of the ELISA was determined by the concentrations of each compound required for 50% of the maximal binding [18]. Overall, the cross-reactivity of the BIA was similar to that of the ELISA.

### 3.3. *Effect of fish matrix on the BIA*

The effect of the fish tissue matrix on the BIA was measured by the dilution test. Purified extracts of fish samples contaminated in the natural environment were subjected to two-fold serial dilutions with DMSO before being assayed (Fig. 2). The extracts were purified by two different procedures (alumina or activated-carbon column) after the multilayer silica gel column. The concentrations in the assay appeared to increase with dilution. This shows that the dilution process can eliminate the matrix effect in the assay. No significant differences in the concentrations were found between the two purification procedures.

A recovery test using purified fish extracts was also carried out to examine in more detail the effect of the matrix on the BIA. Serial dilutions of purified extracts spiked with PCB 118 were assayed. The recovery was 51.8–77.2% and 33.4–85.4% for extracts purified by using the alumina and activated-carbon column, respectively (Table 3). The recovery tended to be greater when diluted samples were tested. For this reason,

**Table 3 – Recovery of PCB 118 from spiked purified fish extracts[a]**

| Samples | Spiked levels (ng mL$^{-1}$) | Dilution factors | Observed levels (ng mL$^{-1}$) | | Recovery (%)[b] | |
|---|---|---|---|---|---|---|
| | | | AL | AC | AL | AC |
| Procedural blank | 100 | 1 | – | – | 99.6 | 93.9 |
| | 200 | 1 | – | – | 95.4 | 93.7 |
| Yellowtail | 200 | 1 | 323 | 229 | 51.8 | 33.4 |
| | 200 | 2 | 290 | 211 | 66.6 | 46.1 |
| | 200 | 4 | 250 | 207 | 77.2 | 63.5 |
| Salmon | 200 | 1 | 320 | 342 | 66.3 | 73.3 |
| | 200 | 2 | 274 | 292 | 77.2 | 85.4 |

[a] Serial dilutions of purified extracts from fish samples were spiked with known quantities of PCB 118, and analyzed by the BIA ($n = 2$). The extracts were purified on an alumina column (AL) or an activated carbon column (AC) after a multilayer silica gel column.
[b] Recoveries were corrected by subtraction of the native levels of contamination for each sample.

**Table 4 – Reproducibility of the BIA combined with the purification procedure[a]**

| Samples | n | BIA (ng g$^{-1}$) | | CV (%) |
|---|---|---|---|---|
| | | Mean ± S.D. | Range | |
| Sea bass | 4 | 10.7 ± 0.6 | 10.1–11.4 | 5.9 |
| Salmon | 4 | 2.5 ± 0.1 | 2.3–2.6 | 4.4 |

[a] The two varieties of fish contaminated in the natural environment were extracted, cleaned up and assayed by the BIA in four separate runs on different days.

serial dilutions of fish extracts were measured in the assay, and the maximum concentration was used to minimize the matrix effect in the assay. Finally, the use of the alumina column was selected as the purification procedure after the multilayer silica gel column, although no significant differences were found between the recovery rates for the two purification procedures.

### 3.4. *Reproducibility of the BIA*

The reproducibility of the BIA in combination with the sample-preparation procedure was tested by replicate analyses of the same homogenized fish samples. The fish were extracted, cleaned, and assayed in four separate analyses on different days. The assay showed excellent results for the measured concentrations: the CVs for the two varieties of fish were 4.4–5.9% (Table 4).

### 3.5. *Comparison of the BIA with ELISA*

The performance of the BIA was investigated by using seven retail fish samples that were analyzed by both the BIA and the ELISA using the same Mab. A good correlation ($r = 0.99$) was observed between the results of the BIA and those of the ELISA (Fig. 3). The slope of the linear regression equation was

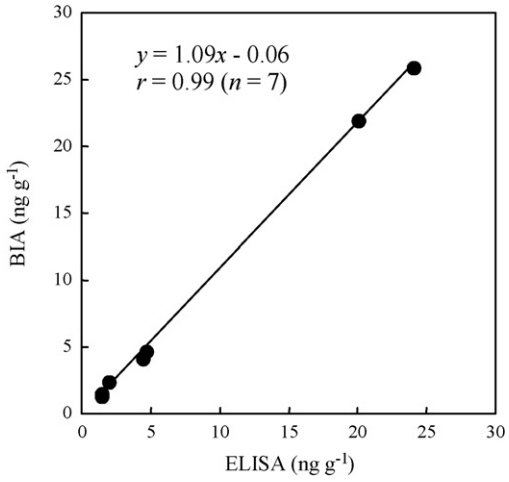


**Fig. 3 – Comparison of data from the BIA and the ELISA. Seven fish samples (one marlin, two salmon, two sea bass, and two yellowtail) were analyzed by the BIA and the ELISA using the same Mab.**

nearly 1 and y-intercept was near to zero, suggesting that the BIA gave almost the same values as the ELISA. The ELISA has been already validated for the analysis of DL-PCBs in retail fish samples, and the fish matrix did not greatly interfere with the ELISA [13]. Therefore, we concluded that the fish matrix caused no significant interference to the BIA results.

### 3.6. *Comparison of the BIA with HRGC/HRMS*

The values obtained in the BIA for ten retail fish samples tested were also compared with the results obtained by the HRGC/HRMS analysis. A good correlation ($r = 0.99$) was observed between the BIA values and the concentrations of PCB 118 obtained by HRGC/HRMS analysis [Fig. 4(a)]. At low

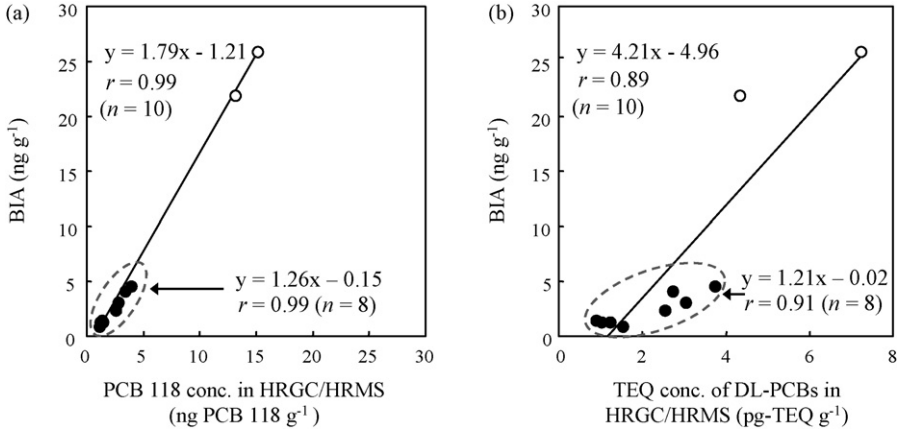


**Fig. 4 – Comparison of data from the BIA and the HRGC/HRMS analysis in retail fish. Ten samples (two mackerel, one marlin, two salmon, two sea bass, and three yellowtail) were analyzed by the BIA and HRGC/HRMS. (a) BIA values versus PCB concentrations. The sea bass samples containing significantly high levels of DL-PCBs are indicated by the open circles. The regression equation for the samples, excluding the sea bass (indicated by the dashed circle), is also shown in (a). (b) BIA values versus TEQ concentrations of DL-PCBs calculated by using $TEF_{1998}$ values. The sea bass samples are shown by the open circles. The regression equation for the samples excluding the sea bass (shown by the dashed circle) is also shown in (b).**

PCB concentrations (<5.0 ng g$^{-1}$), a good correlation ($r = 0.99$) was also obtained between the two methods. However, the slopes of the linear regression equation tended to be a little higher than 1, and this became more conspicuous when two highly contaminated samples of sea bass were included. This suggests that BIA cross-reacted with some compounds, although the positive BIA results for the retail fish samples were caused mainly by their reactivity with PCB 118. The mono-*ortho* PCBs fractions tested by the BIA usually contain PCB 105, PCB 114, and PCB 156, which can cross-react with the BIA (Table 2). However, levels of these compounds were much lower than those of PCB 118 in the samples tested by HRGC/HRMS analysis (data not shown). Therefore, the reactivity of the BIA to these compounds must be negligible. The Mab is also known to have slight cross-reactivity with other PCBs [PCB 31, PCB 66, and PCB 70; 12.9–17.8% of PCB 118] in the ELISA [18]. The presence of large amounts of these isomers could have a slight effect on the results of the BIA, although it is not clear whether these isomers were present in the samples tested in the present study.

The values obtained in the BIA for the ten retail fish samples tested were plotted against the TEQ concentrations of DL-PCBs obtained by the HRGC/HRMS analysis [Fig. 4(b)]. A good correlation ($r = 0.89$) was obtained between the BIA and TEQ values calculated by using $TEF_{1998}$. This indicates that the BIA offers a practical approach for screening TEQ levels of DL-PCBs in retail fish. However, two highly contaminated samples of sea bass, shown as open circles in Fig. 4(b), appeared to distort the correlation. These samples gave much higher values in the BIA against their corresponding TEQ concentrations. However, these samples would result in false-positive results in the BIA; they would not, therefore, cause a serious problem in a screening method. The concentrations of DL-PCBs in retail fish on the Japanese market were in the range of 0.022–4.8 pg-TEQ g$^{-1}$ ($n = 91$, median: 0.57, mean: 1.0) based on the HRGC/HRMS data produced by our national survey of dioxins in Japan [20]. The BIA will easily detect highly contaminated fish samples from the comparative data shown in Fig. 4(b). These in shellfish were in the range of 0.00012–2.6 pg-TEQ g$^{-1}$ ($n = 44$, median: 0.070, mean: 0.24). These in meat and dairy products were in the range of 0–1.2 pg-TEQ g$^{-1}$ ($n = 173$, median: 0.021, mean: 0.047). Therefore, due to the low concentrations of DL-PCBs in these samples, it is currently very difficult to apply the BIA to detect DL-PCBs in them.

TEF values have been revised recently [2]. WHO advises that the $TEF_{2005}$ are used as they replace the previous $TEF_{1998}$. We therefore compared the BIA results with the TEQ concentrations calculated by using the $TEF_{2005}$ values (Fig. 5). To eliminate any distortion of the results, the two highly contaminated samples of sea bass were excluded. A good correlation ($r = 0.87$) was observed between the BIA and TEQ values. The slope of the linear regression equation was slightly higher than the slope when the previous $TEF_{1998}$ were used for the same samples, as shown by the dashed circle in Fig. 4(b). This is mainly because the $TEF_{2005}$ of PCB 118 (0.00003) is a little lower than the $TEF_{1998}$ value (0.0001).

There are currently no internationally recognized maximum limits for DL-PCBs in foods. However, the European Union established action levels for DL-PCBs in foodstuffs including fish [21]. This directive has increased the demand for screening methods to detect these compounds. The BIA is very rapid (about 12 min per cycle) and can be automated. The BIA will enable us to screen retail fish for TEQ concentrations and will be a useful option for screening DL-PCBs in retail fish. In future assessments of the BIA technique, it will be necessary to analyze a greater number of retail samples in comparative studies.

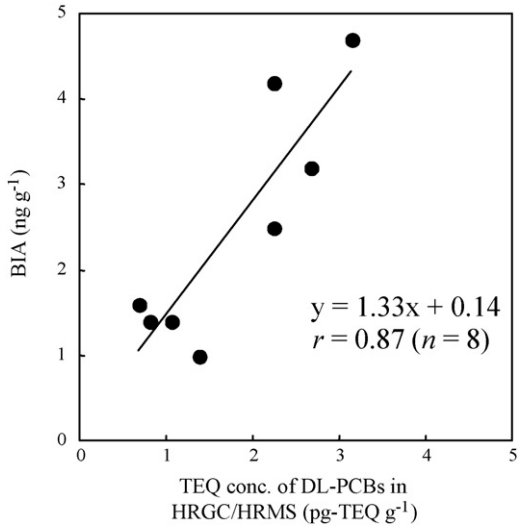


**Fig. 5 – Comparison of BIA results and TEQ concentrations calculated by using the $TEF_{2005}$ values. TEQ concentrations of DL-PCBs were calculated by using new TEFs ($TEF_{2005}$) and compared with the BIA results.**

## 4. Conclusions

The BIA performed well in the analysis of PCB 118 in retail fish. When a 20-g fish samples was tested, the quantitative limit for PCB 118 was 1 ng per gram of sample. The assay was very quick and automated. A comparative study with conventional HRGC/HRMS showed that the BIA is suitable for the screening of DL-PCBs in retail fish. Thus, the BIA will be useful for the preliminary screening of large numbers of fish samples before HRGC/HRMS analysis.

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