TEQ SCREENING IN FISH OIL USING TWO ELISA KITS FOR DIOXIN-LIKE PCBs

Hasegawa J[1], Guruge KS[1], Seike N[2], Shirai Y[3], Yamata T[3], Yamanaka N[1], Miyazaki S[1]

[1]National Institute of Animal Health, Kannondai 3-1-5, Tsukuba, Ibaraki, 305-0856, Japan
[2]National Institute for Agro-Environmental Sciences, Kannondai 3-1-3, Tsukuba, Ibaraki 305-0856, Japan
[3]Food and Agricultural Materials Inspection Center, Shintoshin 2-1, Chuo-ku, Saitama, Saitama 330-9733, Japan

Abstract
Two commercially available ELISA kits for dioxin-like PCBs (DL-PCBs) were applied to fish oil, which used as feed ingredients, to screen dioxin toxicity equivalent (TEQ). Fish oil samples (n=16) were cleaned up by modified multilayer column and mono- and non-ortho DL-PCBs were fractionated by activated carbon column. EnBio Coplanar PCB EIA System based on monoclonal antibody against PCB 118 was applied to the mono-ortho DL-PCBs fraction, and Abraxis Coplanar PCB ELISA Kit based on polyclonal antibody against non-ortho DL-PCB congeners was applied to the non-ortho DL-PCBs fraction, respectively. The correlation coefficients between measured ELISA value and TEQ of DL-PCBs derived from HRGC/HRMS analysis were 0.94 and 0.80, respectively. In the both ELISA, almost similar correlations were observed between ELISA value and the total TEQ including PCDD/DFs corresponding to the results of only DL-PCBs. These results suggests that the both ELISA kits are useful as simple screening tool for dioxins in fish-originated feed ingredients such as fish oil and fish meal which have greater contribution of DL-PCBs to total TEQ.

Introduction
In recent years, the consumption of farmed fish notably salmon is increasing to large degree in around the world. However, farmed salmon contains higher levels of POPs including dioxins than wild salmon possibly due to contamination of fish oil and fishmeal used as feed ingredient [1]. Fish oil is also used as feed ingredient for domestic animals such as pig and chicken, and known to contain relatively high levels of PCDD/DFs and DL-PCBs compared to other feed ingredients [2]. It is important to routinely monitor these compounds in large number of samples to avoid producing feeds with elevated levels of contamination. Although the reference method for dioxin analysis using HRGC/HRMS is highly sensitive and reliable, it would be costly and time consuming, while the bio-analytical methods are relatively cost-effective and simple. CALUX assay which is one of the cell-based bioassays has been widely employed as a rapid analysis of dioxin-like toxicity in feedingstuffs [3], but the kit-based bioassays such as enzyme-linked immunosorbent assay (ELISA) have yet to gain widespread use. It is thought that an ELISA analysis would be much easier to perform widely compared to the cell-based bioassay which require a licensing for recombinant cell line and cell culture system. In almost fish-originated samples, the contribution of DL-PCBs to total TEQ is considerably high; therefore it seems that the application of ELISA which is specific for DL-PCBs to those samples would be useful for the TEQ prediction. In this study, two commercially available ELISA kits for DL-PCBs measurement were applied to fish oil used as feed ingredients and compared to the results of HRGC/HRMS analysis.

Materials and Methods
Sample preparation for ELISA
16 fish oil products were collected from several feed manufactories in Japan during 2002-05. These samples were stored at -20℃ until analysis. About 1g of fish oil sample was dissolved in 3ml of hexane. Clean-up was carried out by sulfuric acid-impregnated diatomite [4] in combination with sulfuric acid-impregnated silica gel. 10ml of concentrated sulfuric acid was dropped onto the column packed with 7g of diatomaceous earth, 10g of 44% (w/w) sulfuric acid-silica gel, and 2g of 2% (w/w) potassium hydroxide-silica gel, which are connected with activated carbon cartridge column. After sulfuric acid was dispersed on diatomite, the sample was loaded on the tandem column and eluted with hexane. Then the upper multilayer column was removed, and the carbon column was eluted with 50% dichloromethane/hexane, followed by 20% hexane/toluene. The first fraction containing mono-ortho DL-PCBs and the second fraction containing non-ortho DL-PCBs were concentrated.

ELISA analysis
Assay procedure of the two direct competitive ELISA kits for DL-PCBs was performed as described by each manufacture's instructions.

PCB 118 ELISA: EnBio Coplanar PCB EIA System was purchased from EnBio Tec Laboratories (Tokyo, Japan). Sample extracts or various concentrations of 3,3',4'-trichloro-4-methoxybiphenyl, which is a surrogate standard for PCB 118 dissolved in DMSO were mixed with a competitor–horseradish peroxidase (HRP) conjugate solution. The mixtures were added to microplate wells coated with monoclonal antibody against PCB 118, and then incubated for 30 min at room temperature with gentle shaking. After washing the plate with a wash buffer, an enzyme substrate solution containing 3,3',5,5'-tetramethylbenzidine (TMB) was added to each well and incubated for 20 min. The enzyme reaction was stopped with stopping solution and the absorbance at 450 nm was measured. The standard curves were fitted using a four-parameter logistic model and the concentrations in samples were calculated. The assay range based on PCB 118 is between 10 and 250 ng/ml.

PCB 126/169 ELISA: Abraxis Coplanar PCB ELISA Kit was purchased from Abraxis LLC (Warminster, PA, USA). An anti- DL-PCBs primary antibody solution was added to microplate wells coated with secondary antibody that capture the primary antibody. Subsequently, sample extracts or various concentrations of PCB 126 dissolved in 50% methanol/distilled water was added to the wells. After incubation for 30 min at room temperature with gentle shaking, a competitor–HRP conjugate solution was added to each well and incubated for 90 min. After incubation, the plate was washed and an enzyme substrate solution containing TMB was added to each well, and incubated for 20 min. The reaction was stopped and the absorbance at 450 nm was measured. The concentrations in samples were calculated as the former ELISA. The assay range based on PCB 126 is between 25 and 1,000 pg/ml.

Chemical analysis
PCDD/DFs and DL-PCBs were analyzed according to the provisional guideline for analytical method of dioxin related compounds in feed described by the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan [5]. About 10 g of fish oil sample was spiked with ^{13}C-labeled 2,3,7,8-substituted PCDD/DFs and DL-PCBs as internal standards, and subjected to alkaline digestion. Then the alkaline solution was extracted with hexane. The extract was cleaned up by the multilayer silica gel column, followed by separated to mono-ortho DL-PCBs fraction and non-ortho DL-PCBs and PCDD/DFs fraction using the activated carbon column. The fractionated extracts were concentrated and spiked some congeners of ^{13}C-labeled PCDD/DFs and PCBs as recovery standards. Determination of PCDD/DFs and DL-PCBs were performed by HRGC/HRMS and WHO-TEQ was calculated.

Results and Discussion
Mean WHO-TEQ derived from chemical analysis were 2.6, 9.9, and 12.5 pg/g wet weight (ww) for PCDD/DFs, DL-PCBs, and the sum, respectively. The mean and range of contribution of DL-PCBs to total TEQ was 79 (72-88) %. In all samples, the highest concentration and the highest TEQ contributor among DL-PCB congeners is PCB 118 and PCB 126, respectively. Moreover, the both congeners correlated well with the total TEQ including PCDD/DFs (r = 0.90-0.99). Hence, these congeners seem to be a useful target analyte for TEQ screening.

A highly relationship was observed between PCB 118 ELISA and HRGC/HRMS analysis (Fig.1). The correlation coefficient between the measured ELISA value and the concentration of PCB 118 analyzed by HRGC/HRMS was 0.97. Likewise, a good correlations were observed in TEQ of DL-PCBs (r = 0.94) and total TEQ including PCDD/DFs (r = 0.93). The similar results have reported in the case of retail fish samples [6]. As described above, because the residue level of PCB 118 in fish oils is relatively high among DL-PCBs, it would be easy to detect in ELISA analysis even small sample volume.

While, relatively good correlation was observed between PCB126/169 ELISA and HRGC/HRMS analysis, though the relationships are lower than the results of PCB 118 ELISA (Fig.2). The correlation coefficient between the measured ELISA value and TEQ of DL-PCBs was 0.78. A similar relationship was found in total

TEQ (r = 0.78) as well as the case of the former ELISA. It is speculated that the cross-reaction might occur with other non-ortho DL-PCB congener such as PCB 77 which is most abundant among the non-ortho DL-PCBs in fish oil samples. However, it has little contribution to the total TEQ. As the application study of this ELISA kit has limited to the highly contaminated soils in USA [7], more research using biological samples would be expected.

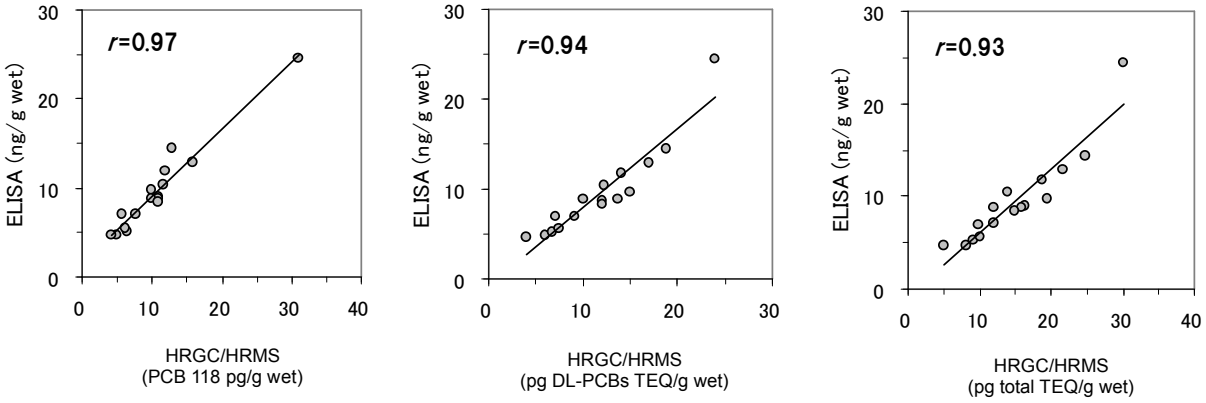

Fig. 1. Correlation between PCB 118 ELISA and HRGC/HRMS analysis in fish oil samples (n=16).

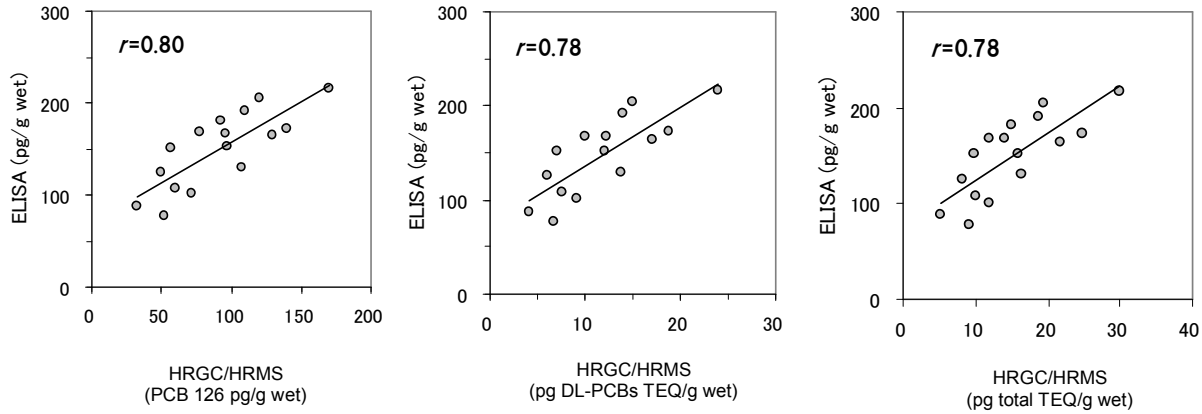

Fig. 2. Correlation between PCB 126/169 ELISA and HRGC/HRMS analysis in fish oil samples (n=16).

In conclusion, the both ELISA kits used in this study are possibly useful as rapid and simple screening tool for dioxins in fish-originated feed ingredients such as fish oil and fishmeal which have a greater contribution of DL-PCBs to total TEQ. More investigations using a large number of samples would be necessary to validate the effectiveness and reliability.

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