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PCBs contamination in seafood species at the Eastern Coast of Thailand

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ABSTRACT

Polychlorinated biphenyls (PCBs) are a large group of persistent organic substances spread throughout the world. The most toxic PCBs are those that are dioxin-like (dl-PCBs). Environmental studies on PCBs in Thailand are limited, especially with regards to dl-PCBs. This study is one of the first in this country that demonstrates contamination of seafood with PCBs and determines the levels of PCBs and total dioxin like activity in mussels, oysters and shrimp, from the Eastern Coast of Thailand. Sixty pooled samples of mussels and twenty-seven pooled samples of oysters were collected from cultivation farms and twenty-one pooled samples of shrimp were collected from fisherman piers. Qualitative and quantitative measurements of 49 PCB congeners was obtained by HRGC-ECD analysis and total dioxin-like activity using the CAFLUX bioassay. Total PCB concentrations varied between three species, ranging between 19 and 1100 ng g^{-1} lipid adjusted weight, and the levels of PCBs in shrimp was three time higher than that in mussels and oysters. With respected to the pattern of PCB congeners, it implied that the source of PCBs exposure in this area could be from the regional contamination. The calculated CAFLUX bioanalytical equivalents (BEQs) values ranged between 0.8 and 18 pg BEQ g^{-1} lipid adjusted weight, and showed a good relationship with the chemical-derived TEQs. Therefore, the CAFLUX bioassay can be used for effective screening of dioxin-like activity in marine species effectively.

1. Introduction

Polychlorinated biphenyls (PCBs) are one of the largest groups of persistent organic pollutants (POPs). They comprise a total of 209 possible chlorine-substituted biphenyl congeners, but PCBs in samples analyzed for environmental and regulatory purposes usually consist of complex mixtures of about 130 congeners originating from technical mixtures (e.g. Aroclors, Clophens, Kanechlors etc.) (Safe, 1990). Many PCBs are considered to be resistant to biological or chemical degradation and are persistent in the environment (IPCS, 1993). PCBs were produced and used as lubricants in transformers and capacitors, stabilizers in paints, polymers and as adhesives (Kimbrough and Jensen, 1989). At present, PCBs are still being released into the environment from hazardous waste sites, illegal or improper disposal of industrial wastes, old consumer products, leaking electrical transformers and incineration of some chemical waste. In addition, other sources of PCBs exposure appear to result from redistribution of PCBs previously introduced into the environment (IPCS, 1993). As a result, these compounds are still environmentally ubiquitous and bioaccumulate in the lipids of exposed organisms. Furthermore, biomagnification of PCBs through the (human) food chain has also been well documented in literature (Voogt et al., 1990; IPCS, 1993; de Boer et al., 2001; Borga et al., 2001).

Exposure of PCBs is still of concern due to their potential adverse health effects to humans and the environment at relatively low dose levels. These compounds have been linked to reproductive effects, immunotoxicity and carcinogenicity (Fernandez et al., 2004). In spite of the fact that levels have decreased over the last several decades, there are still concerns that PCBs continue to have an impact on normal endocrine function and reproduction in fish and wildlife (Okumura et al., 2004; Sapozhnikova et al., 2004). Additionally, transfer of PCBs to infants, either *in utero* or postnatally via human milk still occurs and may result in growth delay, developmental defects and neurocognitive deficits (Wang et al., 2004).

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From a toxicological point of view, most attention is being paid to those PCB congeners that produce a similar toxicity to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and they are commonly known as dioxin-like PCBs (dl-PCBs). Given that these specific congeners lack multiple *ortho*-chlorines, but contain adjacent *meta*- and *para*-substituted chlorine atoms, they have also been called "coplanar" PCBs. Some of these PCBs show particularly high "dioxin-like" toxicity, especially the PCBs with IUPAC Nos. 77, 126, and 169 (Safe, 1990; van den Berg et al., 1998). In addition, considerable dioxin-like toxicity is also attributed to some mono–*ortho*-chlorinated PCBs (MO-PCBs) (e.g. PCBs 105, 114, 118, 156 and 189). Based on similarities of their toxic and biochemical potencies to that of TCDD, the relative toxicity of PCB congeners is directly compared with TCDD and expressed as toxic equivalency factors (TEFs) (van den Berg et al., 2006). Given the relatively high concentrations of MO-PCBs in various food and wildlife samples, their contribution to the total dioxin-like activity is likely significant compared to other dioxin-like compounds e.g. PCB Nos. 77, 126, and 169, the chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) (Jiang et al., 2007; Durand et al., 2008; Zhang et al., 2008).

To measure the concentration of dioxin-like compounds, high resolution gas chromatography combined with high resolution mass spectrometry (HRGC/HRMS) is the preferred technique. However, this analytical method needs highly skilled operators, expensive instrumentation, is time consuming, very costly and is not suitable for high throughput screening. Therefore, alternative bioanalytical screening methods have been developed (Denison et al., 1993; Nagy et al., 2002; Denison et al., 2004). The chemically activated luciferase expression (CALUX) assay and chemically activated fluorescence expression (CAFLUX) assay are recently developed highly sensitive bioanalytical techniques to detect and measure dioxin-like compounds. These techniques take advantage of the specific mechanism of action of dioxin-like compounds, namely binding to the Ah-receptor, nuclear translocation of the ligand-bound AhR and its dimerization with the Ah-receptor nuclear translator (ARNT) protein, followed by binding of the ligand:Ah-receptor:ARNT complex its specific DNA recognition site (dioxin response elements (DREs) resulting in stimulation of transcription of adjacent genes, including luciferase or green fluorescent protein genes in the CALUX or CAFLUX recombinant cell lines (Nagy et al., 2002; Denison et al. 2004)). Results are expressed as dioxin bioanalytical equivalents (BEQs), i.e. the ability of the mixture of dioxin-like compounds to induce luciferase or fluorescent protein expression, relative to TCDD (Eljarrat et al., 2002; Gizzi et al., 2005; Jeong et al., 2005). BEQs are directly related to the overall toxic equivalents (TEQs) determined in *in vivo* toxicity studies (van den Berg et al., 2006). Use of these bioassays allows the detection and relative quantification of dioxin like compounds in various extracts of environmental or food matrices at a sensitivity that approaches that of instrumental analysis methods.

The concentrations of PCBs and dioxin-like compounds are usually higher in food samples with higher lipid content and generally associated with food of animal origin. Thus, lipid rich food consumption is an important pathway for human exposure to PCBs and it has been estimated that the major route of exposure to these compounds is still from ingestion of contaminated food (Safe, 1990; Schecter et al., 1997; Bocio and Domingo, 2005; Charnley and Doull, 2005; Huwe and Larsen, 2005; Llobet et al., 2008; Zhang et al., 2008). Several studies of persistent organochlorine compounds, including PCBs, have by been reported from South East Asia (Monirith et al., 2003). However, specific studies with PCBs from the Gulf of Thailand are much more limited (Monirith et al., 2003) and only two studies have been conducted to date that to assess the distribution of PCBs in this region (Thao et al., 1993; Kanatireklap et al., 1997). Moreover, information about dioxin-like compounds from this area is even more limited. This is surprising given that the Eastern area of Thailand is one of the main industrialized areas along the Gulf of Thailand. In addition, the coast of this region has also a very rich marine and estuarine ecosystem, with seafood being the main source of food for many people. Thus, given the high amount of seafood (e.g. shellfish and shrimp) consumption, it is essential to have adequate knowledge about the levels of PCBs and other dioxin like compounds in these materials. Without this information the possible risks for humans of PCBs and related compounds via consumption of local seafood can not be determined.

The present study was focused on assessing contamination levels of PCBs and total dioxin like activity in mussels, oysters and shrimp from the Eastern Coast of Thailand. Total dioxin-like activity was determined by using an AhR-CAFLUX bioassay (Nagy et al., 2002) and HRGC-ECD was used to obtain qualitative and quantitative PCB congener specific information in these three types of human food sources. The TEQ results of the chemical analyses of were compared with the BEQ values obtained with the AhR-CAFLUX assay.

2. Materials and methods

2.1. Chemicals and materials

The analytical grade solvents *n*-pentane, dichloromethane, *n*-hexane and isooctane were purchased from Merck (Darmstadt, Germany). Acetone and toluene used for rinsing glassware were also purchased from Merck, Germany. Aluminium oxide 60 active basic (Activity I) and anhydrous sodium sulfate (Merck, Darmstadt, Germany) were activated at 550 °C for 5 h prior to use. Silica gel 60 (0.063–0.200 nm) for column chromatography (70–230 mesh ASTM) (Merck, Darmstadt, Germany) was activated at 220 °C 8 h prior to use. All chemical standards were purchased from Accu-Standard Inc. (New Haven, CT USA) with following purity; 99.1% for TCDD, 100% for PCB 29, 99.8% for PCB 112. PCBs congeners used for identification by HRGC-ECD and their purity are presented in Table 1.

2.2. Study area and sample collection

The provinces Chonburi and Rayong that cover the Eastern Coast of Thailand were selected for this study. The selection of green mussels (*Perna viridis Linneaus*), oysters (*Saccostrea commercialis*) and shrimp (*Penaeus merguiensis*) was based upon their availability at the sampling locations, their common use for human consumption, their high economic value and importance in the local ecosystem. These marine benthic organisms are very suitable as sentinel species for local contamination, since they tend not to migrate. All samples were collected during January to February of 2006 and 2007. The locations of mussel sampling (M1, M2, M3 and M4) are shown in Fig. 1 and are still existing mussel farms. Oysters were collected at commercial farms as well and these farms (O1 and O2) are connected to the open sea and therefore are representative for ecosystem exposure. Since there were no commercial shrimp farms in the mussel and oyster collection areas, shrimp samples were collected at the fisherman piers along the coast of these 2 provinces at sites located close to mussels and oysters farms. The number of individual animals in each pooled sample varied from 20 to 30 for mussels, 30–40 for oysters and 5–10 for shrimp. After collection, all biotic samples were transported in ice to the laboratory and stored at −80 °C until use. All samples were freeze dried before analysis.

Table 1
PCB congeners used for this study and their purity.

IUPAC No.	Chlorine substitution pattern	Purity %	IUPAC No.	Chlorine substitution pattern	Purity %
8	2,4′	99.6	132	2,3,4,2′,3′,6′	99.8
17	2,4,2′	99.0	138	2,3,4,2′,4′,5′	99.8
18	2,5,2′	99.5	149	2,4,5,2′,3′,6′	98.9
28	2,4,4′	100.0	151	2,3,5,6,2′,5′	100.0
31	2,5,4′	100.0	153	2,4,5,2′,4′,5′	99.2
33	3,4,2′	99.0	156	2,3,4,5,3′,4′	99.0
37	3,4,4′	99.7	158	2,3,4,6,3′,4′	100.0
44	2,3,2′,5′	99.0	166	2,3,4,5,6,4′	99.0
49	2,4,2′,5′	99.0	169	3,4,5,3′,4′,5′	100.0
52	2,5,2′,5′	99.0	170	2,3,4,5,2′,3′,4′	99.0
60	2,3,4,4′	99.0	171	2,3,4,6,2′,3′,4′	99.6
66	2,4,3′,4′	99.0	177	2,3,5,6,2′,3′,4′	100.0
67	2,4,5,3′	99.0	179	2,3,5,6,2′,3′,6′	100.0
70	2,5,3′,4′	99.0	180	2,3,4,5,2′,4′,5′	99.7
74	2,4,5,4′	100.0	183	2,3,4,6,2′,4′,5′	99.0
77	3,4,3′,4′	100.0	187	2,3,5,6,2′,4′,5′	99.0
82	2,3,4, 2′,3′	99.0	189	2,3,4,5,3′,4′,5′	100.0
87	2,3,4,2′,5′	99.5	191	2,3,4,6,3′,4′,5′	99.8
95	2,3,6,2′,5′	100.0	194	2,3,4,5,2′,3′,4′,5′	100.0
99	2,4,5,2′,4′	99.0	195	2,3,4,5,6,2′,3′,4′	99.8
101	2,4,5,2′,5′	99.7	199	2,3,4,5,6,2′,3′,6′	100.0
105	2,3,4,3′,4′	99.0	201	2,3,4,6,2′,3′,5′,6′	100.0
114	2,3,4,5,4′	100.0	205	2,3,4,5,6,3′,4′,5′	99.6
118	2,4,5,3′,4′	99.8	206	2,3,4,5,6,2′,3′,4′,5′	100.0
126	3,4,5,3′,4′	100.0	208	2,3,4,5,6,2′,3′,5′,6′	99.8
128	2,3,4,2′,3′,4′	99.7	209	2,3,4,5,6,2′,3′,4′,5′,6′	100.0

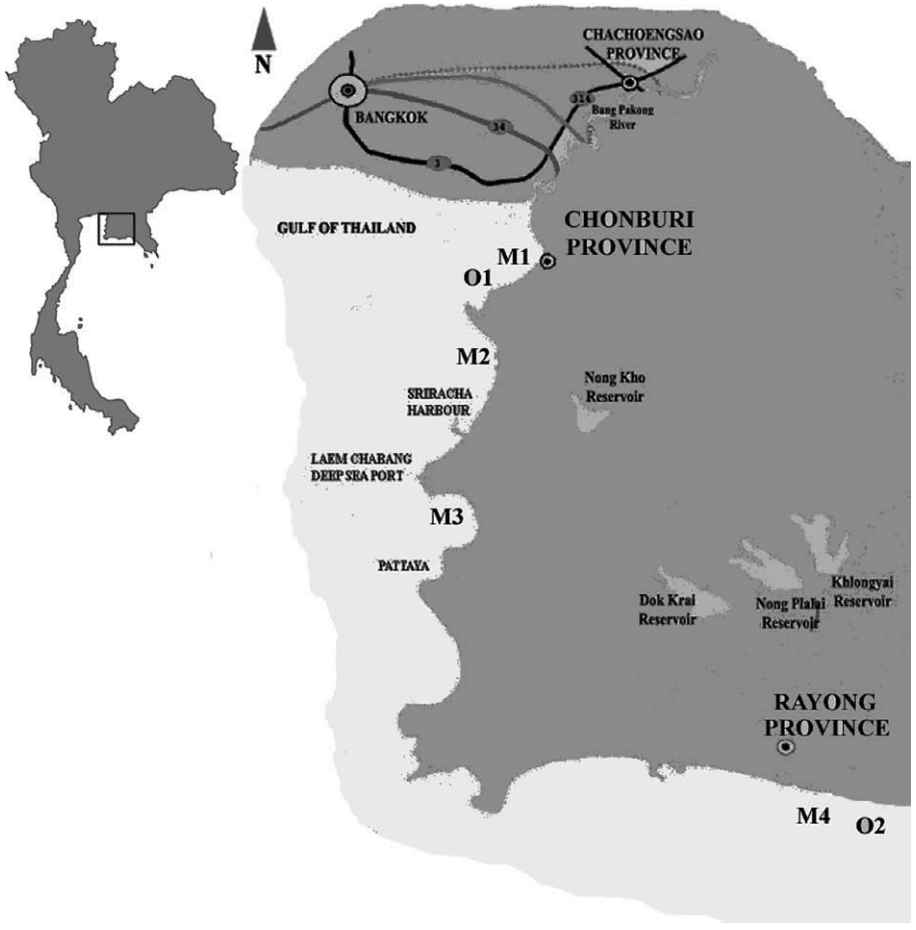

Fig. 1. Sampling locations along the east coast of the Gulf of Thailand.

2.3. Sample preparation

Prior to analysis, the samples were thawed and pooled samples of each species were composed. The soft edible part was homogenized with a vertical blender. Sample extraction was performed by using a Milestone (ETHOS SEL) Microwave Solvent Extraction Labstation according to the following procedure. Five gram sub-samples were accurately weighed into microwave extraction vessels. One extraction set included 5 samples and 1 blank. Approximately 2 g of anhydrous sodium sulfate was mixed with the samples and the surrogate standard (PCB 29; 0.08 $\mu g\, g^{-1}$) was added into all vessels. This was followed by the addition of a 30 mL of *n*-pentane and dichloromethane (1:1, v/v) solvent mixture. The extraction vessels were assembled according to the manufacturer's instructions and the extraction was conducted at 120 °C for 15 min at 70% power. The clean-up procedure was adapted from the method described earlier (de Boer, 1988). After the extraction, vessels were cooled to room temperature and the supernatant from the extraction vessels was carefully transferred to the top of a 15 g $Al_2O_3 \cdot 8\%H_2O$ column (basic Al_2O_3 activity I, i.d. 2 cm). Elution took place with 120 mL of hexane (the extraction vessels rinsed twice with 20 mL and final elution with 80 mL hexane). This elute was concentrated to 2 mL by rotary evaporation after adding 2 mL isooctane as a keeper. The extract was then transferred to the top of a 2.2 g of $SiO_2 \cdot 2\%H_2O$ column (silica gel 60, 0.063–0.200 mm, i.d. 0.8 cm) and eluted with 10 mL of isooctane and the fraction containing PCBs was collected and concentrated to final solution at 1 mL by N_2 gas. This final solution was divided into 2 parts; 0.5 mL of the internal standard (PCB 112; 0.04 $\mu g\, g^{-1}$) was added to the first part for HRGC-ECD analyses, while the second part (0.5 mL) was stored at −20 °C prior to CAFLUX analysis. All samples were analyzed in duplicate. It should be noted that the surrogate standard (PCB 29) does not contribute to the BEQ or TEQ concentrations for PCBs. However, after this clean-up procedure each sample could still have included PCDDs and PCDFs or other halogenated compounds, which were not separated by this clean-up procedure.

2.4. Chemical analysis

The PCB congeners presented in Table 1 were targeted for qualitative and quantitative HRGC-ECD analysis. The quantification of these 49 PCB congeners was performed by GC-μECD (Hewlett-Packard 6890). Two capillary columns, Factorfour VF5 ms and VF1701 ms, 50 m length × 0.15 mm i.d. × 0.25 μm film thickness (Varians, Palo Alto, CA USA), were used to enhance identification and confirmation of PCB congeners in the GC chromatograms. Two microlitre of final elute sample was injected in splitless mode. Injector and detector temperatures were set at 270 °C and 300 °C, respectively. The temperature program for both columns was similar (initially set at 90 °C for 3 min, raised by 20 °C/min to 215 °C and held for 30 min, then raised again by 5 °C/min to 270 °C, and held for 23 min. Helium was used as carrier gas with velocity at 4.6 mL/min. Nitrogen was used as make up gas for the detector with a velocity at 60 mL/min in the constant flow mode.

All extracts were analyzed in batches that also included blank samples and identification of the congener specific standard calibration curves. Multi-level calibration curves in the linear response interval of the detector were created for the quantification of 49 PCB congeners and a good correlation ($r^2 > 0.99$) was achieved. The identification of 49 congeners was based on their retention times relative to the internal standard (PCB 112) used for quantification on GC-μECD. The limit of detection (LOD) for these PCBs was approximately 4 pg g^{-1}. Results are reported as 'not detected' (ND) when the peak area of a specific congener was lesser than the peak area of the LOD. TEQ concentrations of the mono–*ortho* PCBs 105, 114, 118, 156 and 189 were calculated using the WHO 2005 TEF values (van den Berg et al., 2006).

2.5. CAFLUX bioassay

The AhR-CAFLUX cells were cultured in phosphate-buffered saline medium with l-glutamine and supplemented by 10% fetal calf serum and 1% of penicillin/streptomycin (45.5/45.5 units mL^{-1}) at 37 °C and in a 5% CO_2 atmosphere saturated with water. After culturing, cells were harvested by trypsinization and 50 μL cell suspension was removed for cell counting. The other cells were seeded in 96-wells plates with 200 μL of cell suspension at a density of 1×10^4 cells/well and placed in the incubator for 24 h. Prior to dosing on the plate, the second half of sample final solutions (0.5 mL) were transferred in 4 μL of DMSO using a centrifuge under vacuum, and 400 μL of medium was added to each extract in DMSO. After incubation, the medium was removed and the cells were seeded with 200 μL medium containing standard TCDD, DMSO, or a sample extract dissolved in DMSO. A standard solution series for TCDD was added to each plate at concentrations of 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.0025, 0.001, and 0.0005 nM. The limit of detection of the CAFLUX bioassay was about 1 pM. On each plate, nine DMSO solutions of TCDD, DMSO alone, and sample extract were analyzed in 2 wells (2 μL/well). The mean of these results was used for calculation of TCDD bioanalytical equivalents. DMSO only was added to the outer wells of the plate. After 48 h of incubation, the medium was replaced by PBS and EGFP fluorescence measured in a Microplate Reader (Spectra MAX Genuini XS) as previously described (Nagy et al., 2002). All tissue culture media were purchased from Gibco/Innovation (Grand Island, NY, USA).

2.6. MTT measurement

The MTT assay was performed on the wells with sample extracts and the three highest concentrations of TCDD to determine possible cytotoxicity. After removal of the medium, the cells were incubated for 30 min with 200 μL serum-free medium containing 1 mg MTT mL^{-1}. Medium was then removed and replaced by 200 μL iso-propanol per well, followed by a 15 min incubation and measurement of absorbance at 595 nm in a spectrophotometer (SPECTRAmaxPLUS^{384}).

2.7. Lipid analysis

The lipid content of the samples was determined by the method developed by Bligh and Dyer (Honeycutt et al., 1995; Smedes and Askland, 1999). Briefly, one gram of freeze dried sample was mixed for 30 s with 20 mL methanol, 10 mL chloroform, and 80 μL deionized water. Another 10 mL chloroform and 10 mL deionized water were added and the solution mixed. The mixture was centrifuged for 10 min at 1000 rpm, followed by the removal of the upper methanol layer. A 10 mL aliquot of the lower chloroform layer was collected, weighted, and evaporated, after which the weight of the remaining fat residue was determined.

2.8. Statistical analysis

The quantification of unknown samples was calculated from using the fitted concentration–response curve obtained using a sigmoidal dose–response nonlinear regression curve fit (GraphPad Prism 3.0, GraphPad Software Inc., San Diego, CA). Chemo-analytical results were processed using Principle Components Analysis (PCA). Spearman's Rank correlation coefficient was using for chemo-analytical and bio-analytical dioxin equivalents (BEQ) values. All statistical calculations were processed by SPSS version 12.0 for Windows, SPSS Inc. (Chicago, IL USA).

3. Results

3.1. General sample characteristics

General sample information (size, weight and lipid content) is presented in Table 2. The size of mussels from location M2 and M4 was larger than that from location M1 and M3 and this could be due to differences in age of the mussels. The mussels from M2 and M4 were about 1 year old, while those from M1 and M3 were about 6–8 months old. Lipid content of the shrimp, mussels and oysters varied between species (see Table 2).

3.2. Total PCB concentrations

The average total concentrations of 49 PCBs (∑PCB) in mussels, oysters and shrimp samples are presented in Table 2 and were expressed either on a dry weight, wet weight or lipid adjusted weight. Total PCB concentrations varied between species and ranged between 19 and 1100 ng g^{-1} lipid adjusted weight (lw). Among the three seafood species studied, the highest concentrations of ∑PCB were observed in shrimp with average concentrations of 775 ± 230 ng g^{-1} lw. For mussels and oysters, the highest concentrations were found at the 3rd location (M3) and the 2nd location (O2) being respectively 454 ± 125 and 304 ± 65 ng g^{-1} lw. In Fig. 2 the quantitative differences between mussels, oysters and shrimp are expressed either on a dry or wet weight basis or lipid adjusted weight. From these results it can be concluded that based on dry or wet weight there is a clear overlap in PCB concentrations for the mussels and oysters. However, when adjusted for lipid, PCB concentrations in the shrimp were approximately three times higher than those in the mussels and oysters.

3.3. Congener specific profiles

Most PCB congeners studied could be detected in the three seafood species. However, some congeners present in the reference mixture could not be detected in any species, these include PCBs 194, 195, 205, 206, 208 and 209. In addition, no attempt was made to identify and quantify the potent dioxin like PCBs 77, 126 and 169, as concentrations are generally much lower than the more common non dioxin-like congeners like PCBs 28, 52, 101 and 138. For reliable identification and quantification of PCBs 77, 126

Table 2
PCB and dioxin-like compound concentrations in mussels, oysters and shrimp collected at various locations from the eastern coast of the Gulf of Thailand.

Parameter	Seafood species						
	Mussels from 1st location, namely Sapan laung-jao (M1) (n[a] = 15)	Mussels from 2nd location, namely Koh loy (M2) (n = 15)	Mussels from 3rd location, namely Laem-chabang (M3) (n = 15)	Mussels from 4th location, namely Ban pae (M4) (n = 15)	Oysters from 1st location, namely Au lungdam (O1) (n = 12)	Oysters from 2nd location, namely Pak prasae (O2) (n = 15)	Shrimp (n = 21)
Size, cm.	7.60 ± 0.80[b] (6.40–9.00)[c]	9.40 ± 0.70 (8.20–10.50)	5.80 ± 1.20 (5.50–7.20)	9.40 ± 0.70 (7.20–8.40)	3.60 ± 0.80 (2.10–5.20)	2.80 ± 0.90 (2.00–4.80)	12.30 ± 0.84 (10.40–14.00)
Moisture content, %	82.08 ± 0.78 (80.62–83.50)	82.13 ± 0.59 (81.00–82.91)	82.33 ± 0.82 (80.82–84.46)	82.08 ± 0.40 (81.28–82.85)	65.11 ± 0.48 (64.38–65.79)	65.18 ± 0.83 (63.75–66.84)	64.64 ± 0.79 (61.81–66.12)
Lipid content, % wet weight	1.17 ± 0.11 (0.92–1.38)	1.17 ± 0.07 (1.00–1.26)	1.17 ± 0.07 (1.06–1.32)	1.17 ± 0.09 (1.08–1.40)	2.50 ± 0.22 (2.06–2.72)	2.51 ± 0.13 (2.34–2.68)	0.70 ± 0.40 (0.20–1.60)
Sum of 49 PCBs concentrations							
Dry weight, ng g^{-1} dw.	9.35 ± 8.22 (1.04–27.57)	21.11 ± 2.60 (17.80–26.60)	24.51 ± 6.77 (8.08–34.57)	4.29 ± 1.93 (1.80–7.87)	9.05 ± 4.39 (1.54 – 15.93)	14.29 ± 3.05 (9.76–21.51)	5.42 ± 1.61 (0.14–7.70)
Wet weight, ng g^{-1} ww.	1.68 ± 1.48 (0.19–4.96)	3.80 ± 0.47 (3.20–4.79)	4.41 ± 1.22 (1.45–6.22)	0.77 ± 0.35 (0.32–1.42)	3.17 ± 1.53 (0.54–5.57)	5.00 ± 1.07 (3.41–7.53)	1.90 ± 0.56 (0.05–2.70)
Lipid adjusted weight, ng g^{-1} lw.	173.22 ± 153.20 (19.18–510.56)	390.94 ± 48.18 (329.59–492.53)	453.82 ± 125.44 (149.59–640.23)	79.43 ± 35.77 (33.28–145.74)	192.61 ± 93.31 (32.76–338.87)	304.00 ± 64.86 (207.57–457.59)	774.61 ± 229.37 (19.95–1,100.57)
Dioxin-like compounds concentrations[d]							
Dry weight, pg BEQ g^{-1} dw.	0.32 ± 0.16 (0.09–0.62)	0.43 ± 0.15 (0.26–0.68)	0.62 ± 0.22 (0.17–0.99)	0.32 ± 0.13 (0.20–0.55)	0.27 ± 0.20 (0.04–0.74)	0.36 ± 0.17 (0.18–0.72)	0.05 ± 0.01 (0.02–0.07)
Wet weight, pg BEQ g^{-1} ww.	0.06 ± 0.03 (0.02–0.11)	0.08 ± 0.03 (0.05–0.12)	0.11 ± 0.04 (0.01–0.06)	0.06 ± 0.02 (0.04–0.10)	0.09 ± 0.07 (0.01–0.26)	0.12 ± 0.06 (0.06–0.25)	0.02 ± 0.01 (0.01–0.03)
Lipid adjusted weight, pg BEQ g^{-1} lw.	5.93 ± 2.90 (1.62–11.54)	8.01 ± 2.70 (4.83–12.64)	11.47 ± 4.06 (3.14–18.39)	5.95 ± 2.33 (3.78–10.24)	5.75 ± 4.34 (0.84–15.64)	7.57 ± 3.69 (3.82–15.31)	7.57 ± 1.91 (3.13–10.45)
CAFLUX-derived BEQ, pg BEQ g^{-1} lw.	7.84 ± 3.76 (1.62–18.39)				6.42 ± 3.68 (0.84–15.31)		7.57 ± 1.91 (3.13–10.45)
Mono-PCBs-derived TEQ[e], pg TEQ g^{-1} lw.	0.94 ± 0.89 (ND–2.75)				0.54 ± 0.33 (ND–0.19)		1.47 ± 0.68 (ND–3.06)

ND = Not detectable.
[a] The number of pooled samples.
[b] Mean value is expressed as mean ± standard deviation.
[c] Range (min – max).
[d] Dioxin-like compounds as determined with the AhR-CAFLUX assay.
[e] Mono-PCBs as analyzed by HRGC-ECD are comprised with PCBs Nos. 105, 114, 118, 156 and 189.

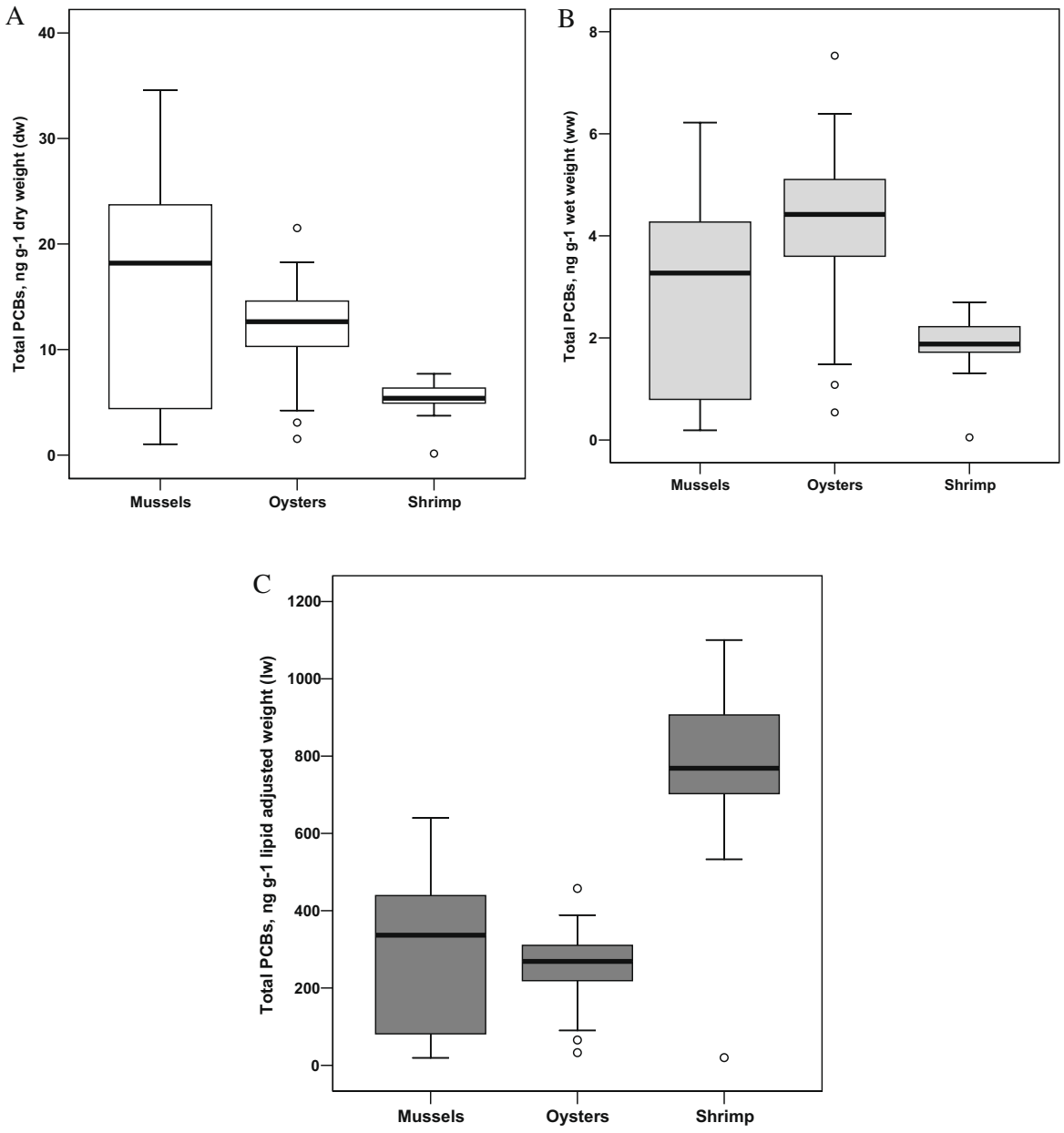

Fig. 2. Box plots of total PCB concentrations in mussels, oysters and shrimp collected at various locations from the Eastern Coast of the Gulf of Thailand, expressed either as dry weights (A), wet weights (B) or lipid adjusted weights (C). Each box represents the median and 25–75th percentiles. The whiskers represent the 10th & 90th percentiles. Circles represent the outliers.

and 169 in environmental samples HRGC/HRMS is needed for analysis. Differences in pattern distribution of the PCB congeners that were studied in mussels, oysters and shrimp are visualized in Fig. 3. A relatively large variability in individual concentrations can be observed for all congeners. The major difference between the three species is the fact that the shrimp was found to contain a much higher proportion of lower chlorinated PCBs than oysters and mussels. In addition, it was found that neither of the three species were retaining octa- and nona-chlorinated PCBs at concentrations higher than the established limit of detection (4 pg g^{-1}). In mussels and oysters penta- and hexachlorinated biphenyls represented the major portion of ∑PCB, with PCB 153 being the quantitatively the most significant congener. In contrast, the tri- and tetrachlorinated congeners were the most abundant PCBs in shrimp with PCB 28 being the most dominant congener from a quantitative point of view.

In Fig. 3(D), the isomer distributions of PCBs relative to ∑PCB in the oysters, mussels and shrimps are shown. Furthermore, it can be observed that the distributions of isomer groups from tri- to octachlorobiphenyls are relatively similar for oysters and mussels. The high contribution of Cl_3 and Cl_4 PCBs, almost 60% of the total, in shrimp is apparent and clearly a downward trend in PCB retention can be observed with increasing number of chlorine atoms.

3.4. Principal component analysis

Principal component analysis (PCA) has been used extensively for explaining the polluted situation of PCBs in the environmental metrics (Howel, 2007). In this study the total variance of the fist two components were used to explain the original variables. PCA was applied to the homologous congener profiles of all samples. The first two components of mussels, oysters, and shrimp ac-

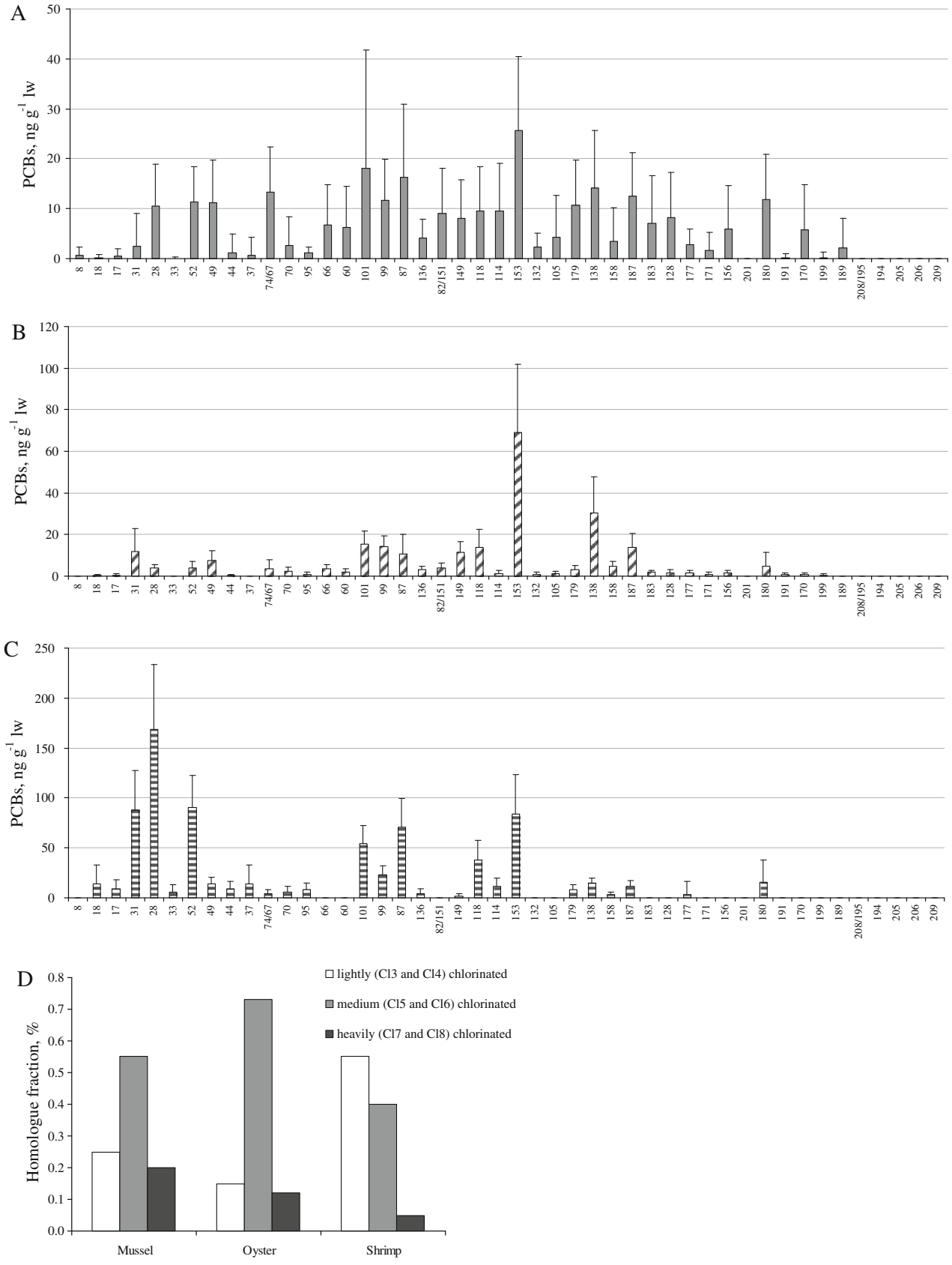

Fig. 3. Congener specific distributions and concentrations (mean ± SD) of individual PCB congeners in mussels (A), oysters (B) and shrimp (C); and their isomer groups distribution (D). Isomer groups have been divided in lightly (Cl_3 and Cl_4), medium (Cl_5 and Cl_6) and heavily (Cl_7 and $Cl_{8)}$) chlorinated.

counted for 52.29%, 55.83% and 64.63% of total variation, respectively. Therefore, the majority of variables were attributable to the first two components. The score plot of principle component PC1 and PC2 for three species (Fig. 4) had shown that the highest

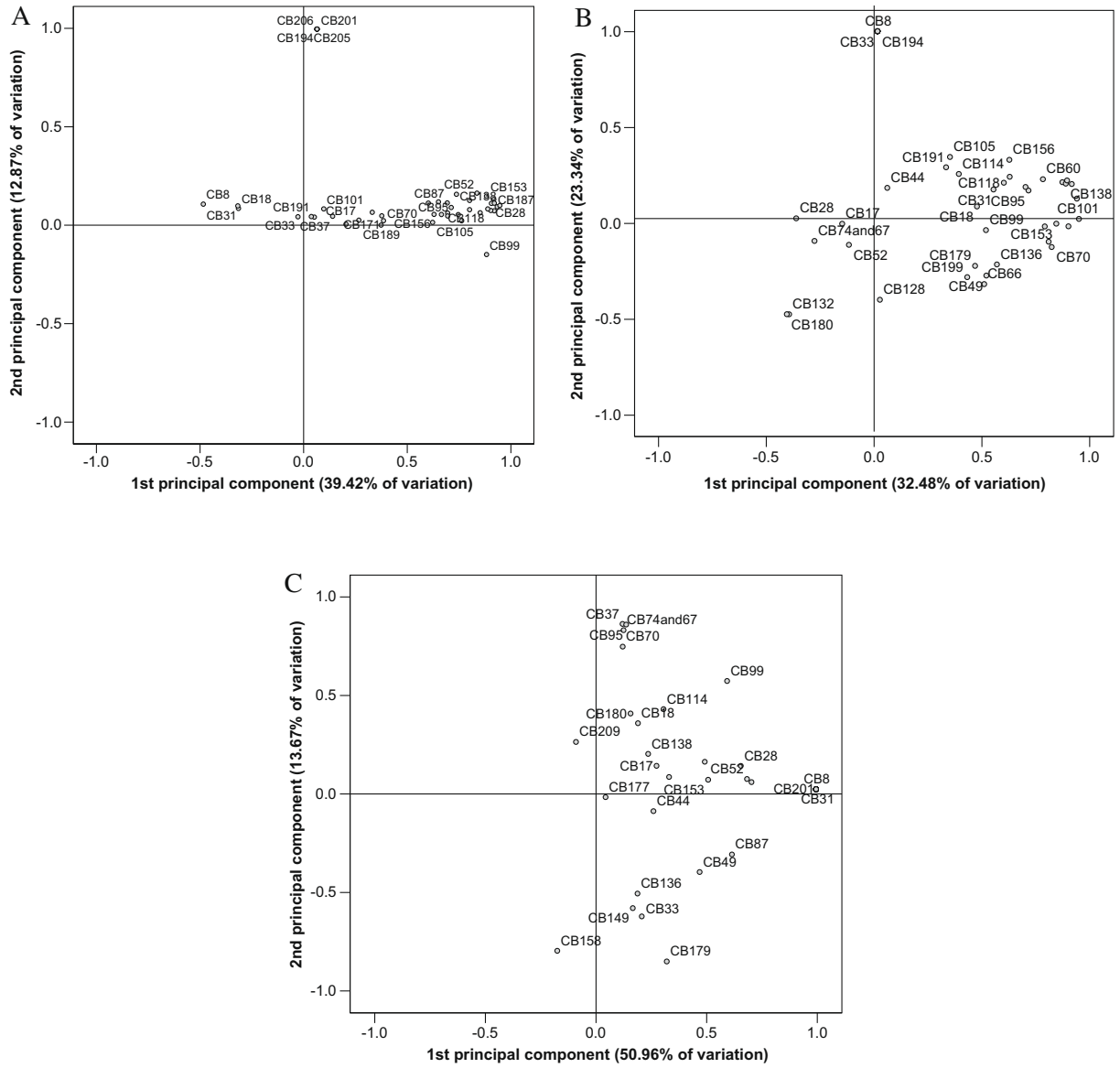

Fig. 4. Score plot of principal component analysis of mussels (A), oysters (B) and shrimp (C) collected at various locations from east coast of the Gulf of Thailand.

values of the 1st principal component (PC1) in mussels sample (39.42% of variation) were PCB nos. 138, 179 and 180, the highest values of PC1 in oysters sample (32.48% of variation) were PCB Nos. 101, 149 and 158, and the highest values of PC1in shrimp (50.96% of variation) were PCB nos. 8, 31, 66 and 60. Therefore, the PCA results demonstrated the similar dominant congeners as observed with the congener specific profiles in Fig. 3.

3.5. Bioassay derived dioxin bioanalytical equivalencies (BEQs)

The AhR-CAFLUX cell bioassay was used to screen for the presence of dioxin-like chemicals in mussels, oysters and shrimp and BEQs determined. BEQ values are directly related to instrumental TEQs as they are based on the same Ah-receptor mechanism of action. These results are shown in Table 2. As with the chemical analysis, the results in BEQs have been expressed either on dry or wet weight and lipid adjusted. The highest concentrations in BEQs (11.5 ± 4.1 pg BEQ g^{-1} lw) were detected in mussels from the 3rd location (M3), followed by those from the 2nd location of mussels (M2) (8.0 ± 2.7 pg BEQ g^{-1} lw). The lowest BEQ concentrations were measured in oysters (5.8 ± 4.3 pg BEQ g^{-1} lw).

3.6. Relationship between CAFLUX-derived BEQ and chemically-derived TEQ values for MO-PCBs

The average AhR CAFLUX-derived BEQ values of mussels, oysters and shrimp were 7.8 ± 3.8, 6.4 ± 3.7 and 7.6 ± 1.9 pg BEQ g^{-1} lw, respectively. The trend observed between the three species was found to be similar to the average $\sum$PCB levels determined by chemical analysis. Therefore, the results of CAFLUX bioassay were directly compared to TEQ levels of MO-PCBs as determined by chemical analysis. These chemically-derived values were calculated by using only the MO-PCBs detected in this study (PCB 105, 114, 118, 156 and 189) multiplied by their most recent TEF value which was 0.00003 for all of congeners (van den Berg et al., 2006) (Table 2). The chemically-derived TEQ values of mussels, oysters and shrimp were 0.9 ± 0.9, 0.5 ± 0.3, and 1.5 ± 0.7 pg TEQ g^{-1} lw. These comparisons reveal that all AhR CAFLUX BEQ values were consistently higher than the chemically-derived TEQ values with the same sample.

However, a highly significant linear relationship between both BEQ and chemically-derived TEQ values was obtained (Fig. 5) for mussels ($R^2 = 0.72$, $p < 0.0001$), oysters ($R^2 = 0.71$, $p < 0.0001$), and shrimp ($R^2 = 0.70$, $p < 0.0001$). A relationship between CAFLUX-de-

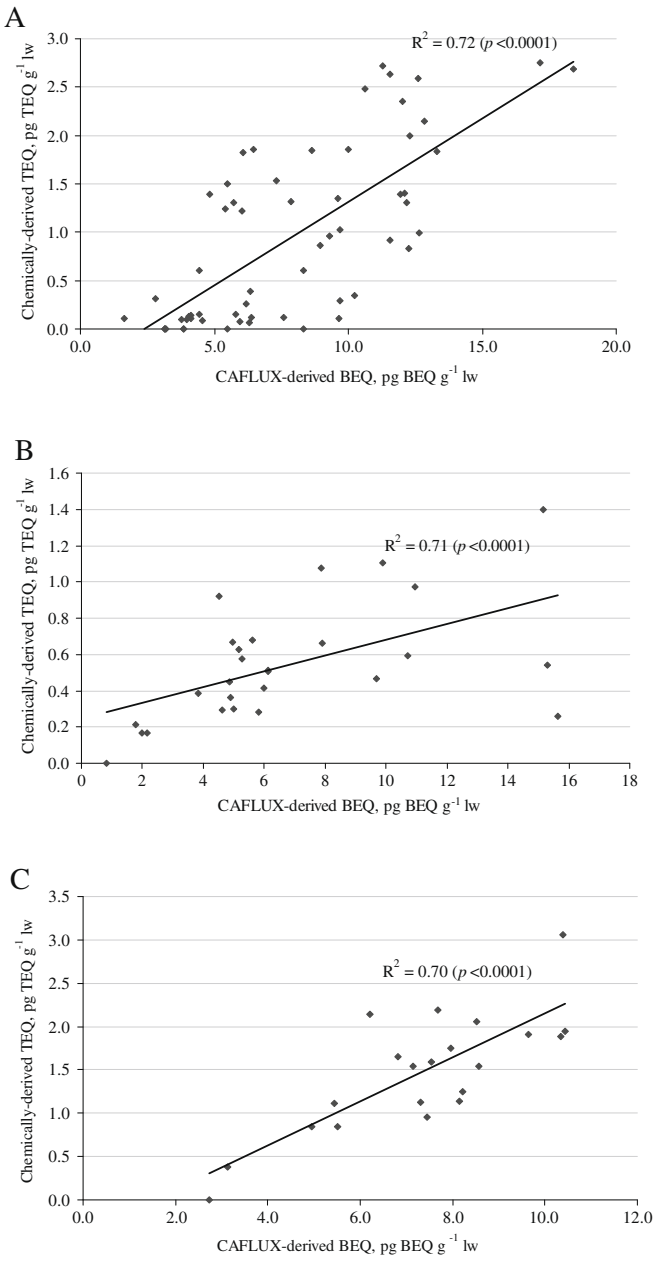

Fig. 5. Statistical relationship between HRGC-ECD-derived MO-PCB TEQs and CAFLUX-derived BEQs of mussels (A), oyster (B) and shrimp (C) collected at various locations from east coast of the Gulf of Thailand.

rived BEQs and total PCBs was also found in mussels, oysters and shrimp with $R^2 = 0.6$ ($p < 0.001$), $R^2 = 0.4$ ($p < 0.001$) and $R^2 = 0.4$ ($p < 0.001$), respectively. Based on the relationships presented in Fig. 5, it can be seen that the AhR CAFLUX had a good predictive value. With respect to the differences observed between both methods it should be kept in mind that the chemically determined TEQs in this study were only based on MO-PCBs and did not include other persistent and more potent dioxin like compounds (PCDDs, PCDFs and PCBs), which could not be analyzed by HRGC-ECD. Including these additional chemicals would result in a significantly higher potency value for the chemically determined TEQs.

4. Discussion and conclusions

In this study, edible mussels, oysters and shrimps from the east coast of Thailand have been analyzed for PCBs. The concentrations of total PCBs in these seafood products from this area were rather similar with those observed from other Asiatic areas (e.g. China, Hong Kong, Japan, Malaysia etc (Tsutsumi et al., 2001; Monirith et al., 2003; Jiang et al. 2007)). Expressed on wet or dry weight, the variation of total PCBs within each seafood species was rather low. For mussels and oysters this can be explained by the fact that both species have similar feeding behavior. In addition, the mussels and oysters used in this study were from the cultivation farms and equally exposed to seawater, in which PCBs and other organochlorine compounds have a better mobility compared to soil or sediment. Based on wet or dry weight shrimps contained significantly less PCBs than mussels and oysters. However, when expressed on a lipid weight basis, shrimp contain significantly higher levels of PCBs than mussels. The much lower lipid content in shrimp might contribute to this observation. Our results are in agreement with studies from Western Europe (Roose et al., 1998; Voorspoels et al., 2004). Many countries have used different approaches to determine the limits of exposure of non dioxin-like PCBs, i.e. Germany is using congeners specific limits for PCB Nos. 28, 52, 101, 138, 153 and 180 in food of animal origin at 8 ng g^{-1} wet weight or 600 ng g^{-1} fat, Belgium is using the sum of seven PCBs congeners; PCB Nos. 28, 52, 101, 118, 138, 153 and 180 in bovine, pork, poultry, animal fats and oils, eggs and derived products, if >2% fat, at 200 ng g^{-1} fat, or the Netherlands are also using the seven PCBs for foods of animal origin at 20 ng g^{-1} wet weight or 2000 ng g^{-1} fat (European Commission, 2000). In our study, the sum of these indicators PCB were 101 ± 61, 142 ± 54, and 446 ± 148 ng g^{-1} fat and 1.0 ± 0.6, 2.3 ± 0.9, and 1.1 ± 0.4 ng g^{-1} wet weight, in mussels, oysters and shrimp, respectively. It should be noticed that these seven PCBs together represented the major proportion, approximately 50%, of total PCB concentrations in this study. If we use these seven indicator PCBs as limit values for non dioxin-like activity our results indicated levels in all three food species below those limit values.

The congener patterns in our study show that the penta- and hexachlorinated isomers comprised the major fraction of the ∑PCB in mussels and oysters, more than 50% of the total content, followed by the tri- and tetrachlorinated isomers. For the shrimps the pattern is clearly different with a dominance of the lower chlorinated PCBs, especially those with three or four chlorines. This difference can most likely be explained by a possible difference in rate of metabolism of PCBs among these three species (mussels, oysters and shrimp), as well as differences in diet, behavior and trophic level. In view of the similarities in PCB pattern observed in oysters, mussels and shrimp between different locations, our results might indicate that the PCB contamination in this marine environment is more likely to be a result from diffuse long range or regional transport than directly from local point sources.

A good statistical relationship was found between chemically-derived TEQs and bio-assay derived BEQ values. However, the CAFLUX-derived BEQs were significantly higher than TEQs calculated on the basis of analytical data using the WHO 2005 TEFs (van den Berg et al., 2006). The AhR activity measured in this bioassay does not permit to identify the specific dioxin like chemicals. The most likely explanation for this difference is the presence of additional dioxin-like compounds in the mussels, oysters and shrimp. In our study, the only PCBs with dioxin like properties were the MO-PCBs. In many biological samples it has been observed that these congeners can contribute significantly (e.g. 50–60%) to the dioxin like content of a sample extract (Schoeters et al., 2004; So et al., 2005; Jiang et al., 2007). However, chlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like non-*ortho* PCBs (PCB 77, 126 and 169) can be major contributors to the total BEQs/TEQs found in biological samples (Baars et al., 2004; Schoeters et al., 2004; Jiang et al., 2007). Thus, it not surprising that there is a consistent difference between the bioassay BEQs and chemically-derived TEQs in our study.

The CALUX and CAFLUX bioassays are increasingly being used for quantitative screening of dioxin-like compounds in various marine samples, including fishes, mussels, oysters, starfishes, sea birds, and marine mammals (Schoeters et al., 2004; Gizzi et al., 2005; Jeong et al., 2005; So et al., 2005). The results of our study show that the AhR CAFLUX bioassay can be used effectively for initial screening of dioxin like activity in marine food samples. In spite of the fact that we did no chemical analysis of all known dioxin like compounds, the bioassay derived TEQs showed good correlations with either ∑PCBs or MO-PCBs. The fact that the chemically-derived TEQs based on MO-PCBs consistently underestimated the AhR CAFLUX BEQs can easily be explained by the presence of other dioxin-like compounds that were not measured. Obvious candidates for these compounds include the chlorinated dibenzofurans and non-*ortho* PCBs 77, 126 and 169, which have significantly higher potencies than the MO-PCBs (van den Berg et al., 2006). The AhR CAFLUX bioassay applied here, like that of the CALUX assay, can be utilized for screening large numbers of food and environmental samples. It can also be used for estimation of the human daily intake of potential dioxin-like compounds and assessment of the potential health risks for human consumers due to food consumption. However, it should be emphasized that at levels above a regulatory cutoff level (e.g. a Tolerable Daily Intake (TDI) or Maximum Residue Limit), final confirmation of the identity of the responsible chemical(s) should come from congener specific chemical analytical techniques.

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