DIOXINS/FURANS AND DIOXIN-LIKE POLYCHLORINATED BIPHENYLS IN DUGONGS FROM THAILAND COAST

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants[1-6]. Several of the PCDD/DF congeners, particularly those substituted at 2378-positions, are persistent and bioaccumulative. Exposure to PCDDs/DFs is of concern because of the their toxicity, which include hormone-dependent cancers and reproductive effects in humans and wildlife. Although studies have examined the occurrence of PCDDs/DFs in developed countries, there is no study regarding the sources and exposure levels of aquatic wildlife in Thailand. Thailand is one of the rapidly developing countries in Southeast Asia. A rapid increase of human and industrial activities in Thailand imposes potential risk for considerable contamination by toxic substances in the marine environment. Considering the above facts in to a great advantage, we conducted monitoring of 2378-substituted PCDD/DFs and dioxin-like PCBs in dugong meat collected from Thailand coast. The toxic equivalency (TEQs) by PCDD/DFs and dioxin-like PCBs was calculated to evaluate risk posed by these chemicals. Eventually, to understand bioaccumulation factors, we also analyze seagrass, which is major diet of dugongs and major bio-accumulants in dugongs were discussed.

Materials and Methods

Samples

Two-dugong muscle tissue (that accidentally entangled in fishing nets) was obtained in Krabi (D-A) and Phangna (D-B) during Oct. 1999 and Jan. 2001, respectively with fresh condition. Samples were both oven dried and/or fixed with formaldehyde [D-A (F) and D-B (F)]. Besides, the seagrasses *Halophila* spp. the major diet (approximately 90%) of dugongs were also collected from stomach of dugong from Krabi. Further, seagrass sample was fixed in formaldehyde. The samples were exported with CITES permission to Japan, and powdered (including sea grass) for chemical analysis.

Analysis

Powdered tissues and seagrass were extracted in a Soxhlet apparatus for 15-h using dichloromethane. Fat content was measured in aliquot of extract by gravimetric method. A total of sixteen $^{13}C_{12}$-labelled 2378-chlorine-substituted PCDD/DFs and 14 of $^{13}C_{12}$-labelled dioxin-like PCBs (including 4 non-, 8 mono- and 2 di-*ortho* PCBs) were spiked in remaining samples were further

subjected to sulfuric acid treatment, moisture removal and sequence of silica gel, alumina and silica gel impregnated carbon column separations. The detailed analytical procedure for tissue and abiotic samples has been reported earlier[4-6]. The quantification and identification of PCDD/DFs and dioxin-like PCBs were performed by high-resolution gas chromatograph interfaced with a high-resolution mass spectrometer (HRGC-HRMS, Micromass, Autospec Ultima)**.** The recoveries of PCDD/DFs from dugong tissue and seagrass ranged from 59-66 % and 63 %, respectively. The concentrations for all analytes for dugong tissue expressed on pg/g fat weight and for seagrass on pg/g dry weigh basis unless otherwise specified.

Results and Discussion

In general, concentrations of 2378-PCDD/DFs were recorded in oven dried dugong tissue without formaldehyde or preserved in formaldehyde and seagrass samples (Table 1) collected in 1999. Altogether, greater concentrations were recorded in samples which is not preserved in formaldehyde (Table 1). When the levels of PCDD/DFs compared to the dugong fat samples analyzed in Queensland Australia[7], Krabi samples from Gulf of Thailand had greater levels and rest of the samples showed similar or lower levels. However, irrespective to the countries, OCDD accumulated 68 to 83 % in the dugongs and 81 % in seagrass to the total PCDD/DF load from this study. Similarly, OCDD and HpCDD were predominant congeners in Australian study with OCDD alone contributed 64.1 to 65.4 % to the total load. Further, lower levels of PCDD/DFs in formaldehyde preserved samples suggested that degradation of these compounds by formaldehyde. It should be mentioned that presence of PCDD/DF

Table 1. Concentrations and toxic equivalency of 2378-substituted PCDD/DFs, dioxin-like PCBs and organochlorine pesticides in Thailand coast dugongs (pg/g fat wt.) and seagrass (pg/g dry wt.).

Fat (%)	2.45	4.71	2.41	2.44	Nil
Sample I.D.	D-A	D- A (F)	D-B	D- B (F)	SG
2,3,7,8-D	4.4	0.23	<0.1	<0.1	<0.1
1,2,3,7,8-D	2.7	5.5	7.7	2.0	0.37
1,2,3,4,7,8-D	3.9	2.3	2.4	2.2	0.28
1,2,3,6,7,8-D	6.1	2.5	6.7	3.4	0.31
1,2,3,7,8,9-D	0.55	0.64	0.25	0.88	0.11
1,2,3,4,6,7,8-D	22	17	7.8	4.8	4.2
OCDD	346	269	133	113	32
2,3,7,8-F	4.8	3.8	4.02	4.02	0.39
1,2,3,7,8-F	0.69	0.59	1.6	1.1	0.055
2,3,4,7,8-F	2.0	0.60	2.3	0.56	0.18
1,2,3,4,7,8-F	6.2	1.0	1.8	6.5	0.27
1,2,3,6,7,8-F	3.5	1.7	2.5	2.2	0.13
2,3,4,6,7,8-F	4.2	1.3	2.6	3.0	0.091
1,2,3,7,8,9-F	4.9	2.6	2.3	3.6	0.19
1,2,3,4,6,7,8-F	9.6	4.4	5.2	3.2	0.35
1,2,3,4,7,8,9-F	1.8	2.3	<0.1	<0.1	0.32
OCDF	16	9.0	14	3.6	0.50
TEQ	12	7.9	11	5.1	0.79
Non- *ortho* PCBs					
344'5-TCB (81)	4.4	2.4	6.7	5.3	0.58
33'44'-TCB (77)	87	58	150	122	17
33'44'5-PCB (126)	10	5.0	15	7.6	0.88
33'44'55'-HxCB (169)	0.58	0.25	1.02	1.00	0.12
Mono- *ortho* PCBs					
233'44'-PCB (105)	539	282	345	406	7.7
2344'5-PCB (114)	103	45	158	175	3.9
23'44'5-PCB (118)	810	499	1644	1041	4.6
2'344'5-PCB (123)	97	23	173	75	3.2
233'44'5-HxCB (156)	164	61	580	286	4.1
233'44'5'-HxCB (157)	220	128	406	479	6.0
23'44'55'-HxCB (167)	90	77	83	141	<0.1
233'44'55'-HpCB (189)	148	35	252	321	2.3
Di- *ortho* PCBs					
22'33'44'5-HpCB (170)	307	257	145	242	0.98
22'344'55'-HpCB (180)	1015	607	779	492	2.8
TEQ	1.45	0.71	2.30	1.44	0.10

D= Dugong meat, SG= Seagrass; figures in parentheses indicates IUPAC numbers.

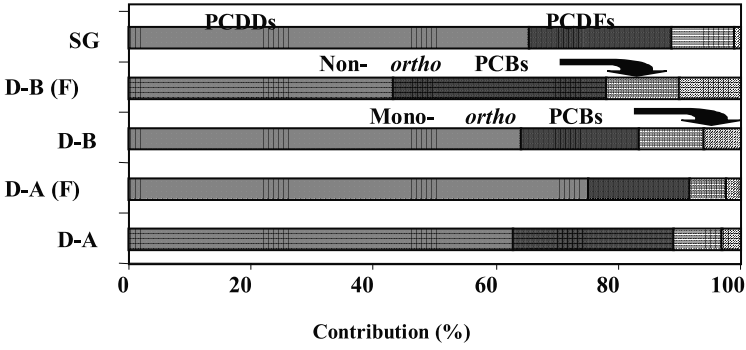

Figure 1 Toxic equivalency (TEQ) contribution by PCDD/DFs and Dioxin-like PCBs in dugong and seagrass from Thailand coast.

in Thai dugong might be the result of direct accumulation from seagrass and/or sediment during feeding. High molecular masses of PCDD/DF such as HpCDD and OCDD are considered to be least bioavailable congeners of all PCDD/DFs. However, dugong feed by cropping aboveground leaves and belowground rhizomes of selected seagrasses and this feeding habit may have resulted in the ingestion of relatively large amounts of OCDD and HpCDD deposited on seagrass and or in sediments. Biochemical formation of PCDD/DFs from precursors has been emphasized as a potential source of PCDD/DFs. This may be of particular importance as dugongs are hindgut fermentors with ingested seagrass and sediments undergoing digestion over an extended time period (140-160 hrs) in the digestive tract. In terms of toxic equivalency (TEQ) calculated after WHO-TEFs for mammals, PCDD/DF alone contributed to 5.1 to 12 pgTEQ/g on fat in dugongs and 0.79 pgTEQ/g in dry seagrass (Table 1). Comparatively, TEQ estimated in this study were lower than Queensland, Australia despite all PCDFs detected in this study.

Concentrations of dioxin-like PCBs in non-formaldehyde dugong tissues were one-fold greater when compare to formaldehyde preserved samples (Table 1). In general, D-B collected from Phangna in the year 2002 had maximum concentrations of 4739 pg/g and formaldehyde preserved D-A(F) collected from Krabi contained minimum concentrations of 2080 pg/g among dugong meat. The seagrass contained minimum concentrations of 54 pg/g dry wt. (Table 1). The observed accumulation pattern in biological samples is common all over the world[1-6]. Toxic equivalency (TEQ) by dioxin-like PCBs were also presented in Table 1. In general, TEQ levels were very low (with a ranges of 0.71 to 2.30 in dugongs and 0.10 in seagrass) when compare to PCDD/DFs. Despite low accumulation levels, non-*ortho* PCBs showed greater toxicity than mono-*ortho* PCBs. Overall, the sum of TEQ (PCDD/DF TEQ+dioxin-like PCBs TEQ) in dugongs were 6.6 to 13.7 pg/g fat and seagrass were 0.9 pg/g dry wt. On the whole, Toxicity contributed by PCDDs were predominant followed by PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs in dugongs and seagrass (Figure 1).

Threshold concentrations for TEQs in livers of aquatic mammals to elicit physiological effects has been estimated to range from 160 to 1400 (mean: 520) pg/g[8]. The mean TEQ concentration in dugong meat was approximately several magnitude less than mean threshold value of 520 pg/g, lipid wt. It should be noted that this estimate does not include safety factors that are generally applied for inter-species comparisons.

In order to understand the bio-accumulation profiles of PCDD/DFs and dioxin-like PCBs, we estimated concentrations of dugong tissue (pg/g dry wt.)/concentration in seagrass (pg/g dry wt.) collected from Andaman Sea and the results were shown in Figure 2. It is prominent that bio-

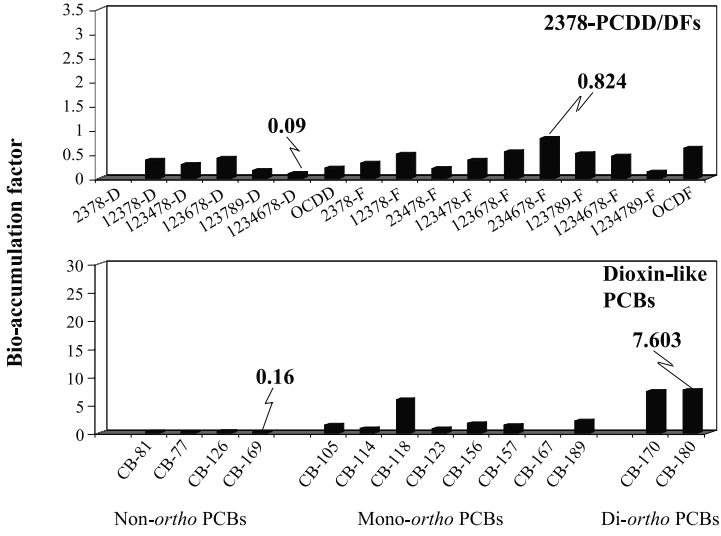

Figure 2 Bio-accumulation factors (BAF) of PCDD/DFs and Dioxin-like PCBs in dugong and seagrass from Thailand coast.

accumulation factor (BAF) decreased with in creasing chlorination for PCDDs. Elevated BAF were found for 12378-PeCDF, 234678-HxCDF and OCDF among PCDFs. As like PCDFs, dioxin-like PCBs showed similar trend with greater bio-accumulation by CB-180, 170 and 118. Less than detection limits of 2378-TCDD and CB-167 in seagrass yielded that no BAF could be discerned for this congeners (Figure 2).

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