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Dioxins/Furans and Polychlorinated Biphenyls (PCBs) in Dugongs from the Thailand Coast

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The pollution of aquatic ecosystems by polychlorinated diaromatic hydrocarbons (PCDHs), including polychlorinated biphenyls (PCBs), is of major concern. Particularly, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants (Senthil Kumar et al. 1999a,b, 2001a,b, 2002a,b). Several of the PCDD/DF congeners, particularly those substituted at the 2,3,7,8-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. Several studies have reported the occurrence of PCDDs/DFs and PCBs in human tissues and wildlife, from various parts of the world (Senthil Kumar et al. 2001a,b, 2002b). While various industrial practices have been attributed as sources of PCDDs/DFs in the environment, stringent regulations to control their emissions in several developed countries have decreased exposures and thereby concentrations in humans and wildlife in recent years. 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. The rapid increase in human and industrial activities in Thailand imposes potential risk for considerable contamination by toxic substances in the marine environment (Hungspreugs et al. 1989; Kan-atireklap et al. 1997).

The dugong (*Dugong dugon*) which belongs to the family Dugonidae, order Sirenia, is an herbivorous mammal living completely in and around the marine environment. Its lifespan is up to 73 years (Marsh et al. 2001) and it feeds almost exclusively on a number of genera of seagrasses, especially *Halophila* and *Halodule* (Preen and Marsh 1995). Dugongs are listed as endangered or exterminated over much of their range and the species is considered to be vulnerable to extinction. Considering this, we conducted monitoring of 2,3,7,8-substituted PCDD/DFs and dioxin-like PCBs in Dugong muscle tissue collected from the Thailand coast. The toxic equivalency (TEQs) contributed by PCDD/DFs and dioxin-like PCBs was calculated to evaluate the risk posed by these chemicals. To understand bioaccumulation factors, we also analyzed seagrass, which is a major food of dugongs.

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MATERIALS AND METHODS

Two dugong muscle tissue (that accidentally entangled in fishing nets) was obtained in Krabi (D-A) and Phangna (D-B) in fresh condition (October 1999 and January 2001 respectively). Samples were analyzed as dried (room temperature [D-A and D-B]) and after fixing with formaldehyde [D-A (F) and D-B (F)] to find out any effect of PCDD/DF and dioxin-like PCBs concentration. A seagrass sample, was collected from the stomach of the dugong from Krabi. The seagrass sample was fixed in formaldehyde. The samples were exported with CITES permission to Japan, and powdered (including sea grass) for chemical analysis.

Powdered dugong muscle tissues and seagrass were extracted in a Soxhlet apparatus for 15-h using dichloromethane. Fat content was measured in an aliquot of the extract by gravimetric method. A total of sixteen $^{13}C_{12}$-labelled 2,3,7,8-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. Samples were subjected to sulfuric acid treatment, moisture removal and the 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 (Senthil Kumar et al. 2001b, 2002a,b). 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). The recoveries of PCDD/DF from dugong muscle ranged from 59-66% and from seagrass were 63%. The concentrations for all analytes for dugong meat expressed on pg/g fat weight and for seagrass on pg/g dry weigh basis unless otherwise specified.

RESULTS AND DISCUSSION

Concentrations of 2,3,7,8-PCDD/DFs were detected in the dugong muscle either fresh or preserved in formaldehyde, and seagrass samples (Table 1). Only the congeners 2,3,7,8-TCDD and 1,2,3,4,7,8,9-HpCDF were lower than the detection limit in dugongs (D-B, D-B[F]) collected from Phangna and 2,3,7,8-TCDD in seagrass. Altogether, greater concentrations were recorded in samples not preserved in formaldehyde (Table 1) and collected in 1999. PCDD homologues were greater in dugong muscle (ranges 81-92%) and seagrss (94%) for the total PCDD/DF contribution and thus, the ratio (PCDDs to PCDFs) ranged from 4 to 11 in dugong and 15 in seagrass. When the levels of PCDD/DFs were compared to the dugong fat samples analyzed from Queensland Australia (Haynes et al. 1999), the Krabi samples collected in 1999 (un-preserved with formaldehyde) had greater levels and the rest of the samples showed similar or lower levels. All PCDF congeners were detected at or near detection limits in Australian dugongs. However, irrespective of the countries, OCDD accumulated 68 to 83% in dugongs and 81% in seagrass compared to the total PCDD/DF load from the Thailand coast. Similarly, OCDD and HpCDD were predominant congeners in Australian samples with OCDD alone contributing 64.1 to 65.4% of the total load. Notably, the Australian study demonstrated that PCDDs accumulation was increased with

increasing chlorination. Lower occurrence of PCDFs was explained by selective metabolism of these congeners.

Table 1. Concentrations and TEQs of 2378-substituted PCDD/DFs and dioxin-like PCBs 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 muscle, SG= Seagrass; figures in parentheses indicates IUPAC numbers.
A= Dugong-1 and B= Dugong-2; (F)* indicates formaldehyde preserved samples.

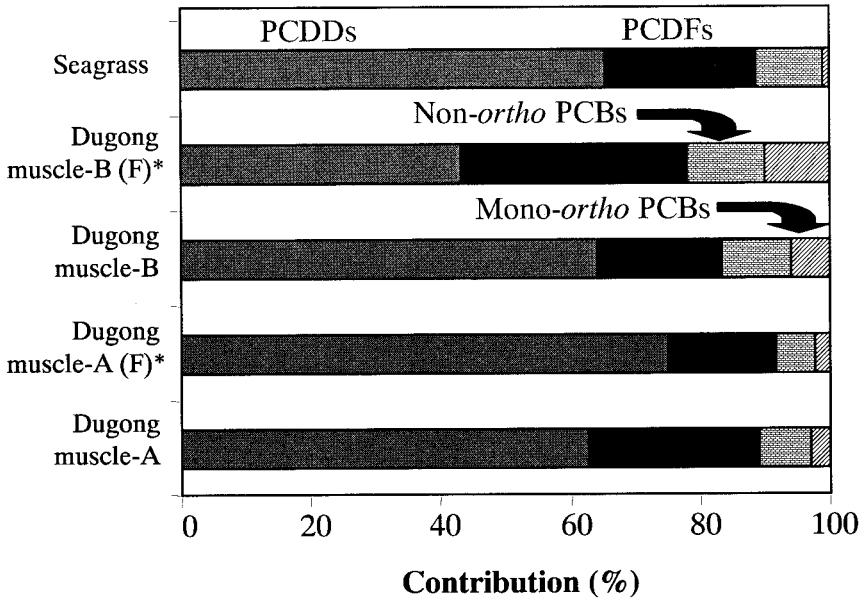

Figure 1 Toxic equivalency (TEQ) contribution by PCDD/DFs and Dioxin-like PCBs in dugong and seagrass from Thailand coast. *(F) indicates formaldehyde preserved samples.

Further, lower levels of PCDD/DFs in formaldehyde preserved samples suggest degradation of these compounds. It should be mentioned that presence of PCDD/DF in Thai dugong might be the result of direct accumulation from seagrass and/or sediment during feeding. High molecular masses of PCDD/DF congeners such as HpCDD and OCDD are considered to be the least bioavailable of all PCDD/DFs. However, dugong feed on aboveground leaves and belowground rhizomes of selected seagrasses (Preen, 1995) 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 (Lanyon and Marsh, 1995).

In terms of toxic equivalency (TEQ) calculated using WHO-TEFs for mammals (Van den Berg et al. 1998), PCDD/DF alone contributed from 5.1 to 12 pgTEQ/g fat weight in dugongs and 0.79 pgTEQ/g in dry weight in seagrass. Comparatively, TEQs estimated in this study were lower than for Queensland were were, Australia (Haynes et al. 1999).

Concentrations of dioxin-like PCBs in fresh dugongs were two-fold greater when compared to formaldehyde preserved samples (Table 1). Dioxin-like PCBs in dugong collected in 2001 had greater levels than samples collected in 1999. In general, D-B had

maximum concentration of 4739 pg/g and formaldehyde preserved D-A(F) contained the minimum concentration of 2080 pg/g. The seagrass contained minimum concentrations of 54 pg/g dry wt. (Table 1). Eight congeners of mono-*ortho* (mainly CB-118, 105, 157) PCBs accumulated greatly followed by two di-*ortho* PCBs and four highly toxic non-*ortho* PCBs. Among non-*ortho* PCBs, CB-77 was most prevalent followed by CB-126, CB-81 and CB-169. For two di-*ortho* PCBs, CB-180 was the most predominant congener followed by CB-170. The observed accumulation pattern in biological samples is similar the world over (Senthil Kumar et al. 1999a,b, 2002a,b,c). Seagrass showed a slightly different accumulation pattern with greater accumulation of CB-105 followed by CB-157 and CB-118 among mono-*ortho* PCBs. Non-*ortho* PCBs and di-*ortho* PCB accumulation patterns were similar to the dugong pattern. These observations suggest that CB-118 is more highly concentrated in the animal body than CB-105 and 157. Previous studies related to isomer/congener-specific accumulation of PCBs in biota from the Thai coast. Kannan et al. (1997) reported 6.6 to 240 ng/g fat wt. total PCBs in various fish species from Thailand. Comparatively, Hungspreugs (1988) reported 14-41 ng/g wet wt PCBs in Thai coastal fish. Kan-atireklap et al. (1997) reported <0.01-20 ng/g wet total PCBs in green mussel (*Perna viridis*) from entire coastal regions of Thailand.

Toxic equivalency (TEQ) for dioxin-like PCBs (only for non- and mono-*ortho* PCBs due to unavailability of TEF for 2 di-*ortho* PCBs) were calculated and presented in Table 1. In general, TEQ levels were very low (with ranges of 0.71 to 2.30 pg/g fat weight in dugongs and 0.10 pg/g dry weight in seagrass) when compared 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 0.9 pg/g dry wt. in seagrass. On the whole, toxicity contributed by PCDDs were predominant, followed by PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs in dugongs and seagrass (Fig. 1).

Threshold concentrations for TEQs in livers of aquatic mammals needed to elicit physiological effects has been estimated to range from 160 to 1400 (mean: 520) pg/g lipid wt. (Kannan et al., 2000). The mean TEQ concentration in dugong muscle was approximately 2 orders 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 muscle (pg/g dry wt.)/concentration in seagrass (pg/g dry wt.) and the results are shown in Fig. 2. The bio-accumulation factor (BAF) decreased with increasing chlorination of PCDDs. Elevated BAFs were found for 1,2,3,7,8-PeCDF, 2,3,4,6,7,8-HxCDF and OCDF among PCDFs. As with PCDFs, dioxin-like PCBs showed a similar trend with greater bio-accumulation by CB-180, 170 and 118. Since 2,3,7,8-TCDD and CB-167 levels were below detection limit in seagrass, no BAF could be discerned for these congeners (Fig.2)

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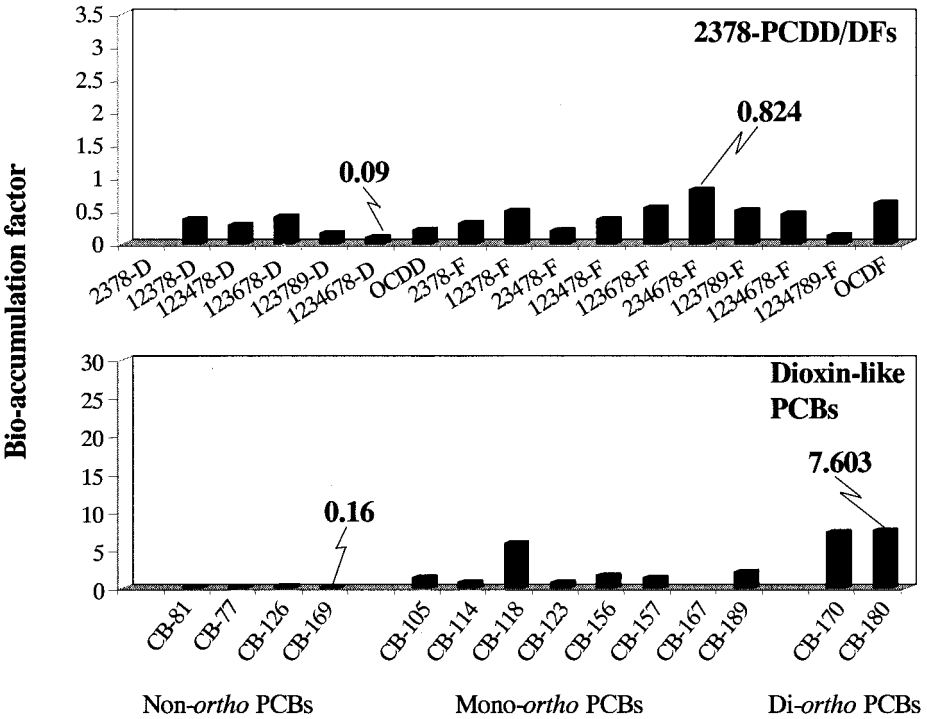

Figure 2. Bio-accumulation factors (BAFs) of PCDD/DFs and dioxin-like PCBs in dugong and seagrass from Thailand coast.

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