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Congener profiles of highly toxic polychlorinated dibenzo-*p*-dioxins, dibenzofurans and dioxin-like biphenyls in biota from Sri Lanka

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Abstract

Residual pattern of highly toxic dioxins, included with polychlorinated *dibenzo -p-dioxins*, dibenzofurans and coplanar PCBs, were determined in biota from two locations in western costal area of Sri Lanka. The concentrations of PCDDs and PCDFs in samples were ranged from 0.04 to 4.5 and 0.04 to 1.3 pg/g wet wt. The non-*ortho* and mono-*ortho* coplanar PCBs concentrations were ranged 1.3 to 140 and 49 to 6000 pg/g wet wt, respectively. The observed dioxin levels in these samples were less than those are from the industrialized countries whereas within the range of most of other Asian countries. Kelani River fish accumulated 1 to 2 orders of magnitude high dioxin concentrations than fish from the Negombo Lagoon.

Key words: PCDDs, PCDFs, PCBs, TEQs, Sri Lanka, fish

1. Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are byproducts of which forms during synthesis of the primary industrial halogenated aromatics and combustion. Polychlorinated biphenyl's (PCBs) are widely used as an industrial chemical for diverse commercial applications and those technical mixtures contain several dioxin-like biphenyl congeners (DLPCBs) (Ballschmiter et al., 1989). PCDDs, PCDFs, and DLPCBs are lipophilic and stable contaminants that found in almost every environmental compartment through out the world (Zook and Rappc, 1994). Several incidents of potentially higher levels of human exposure to those chemicals have been reported (Kishimoto and Miyata, 1987). Due to their resistance to metabolism, some of those congeners cause toxic responses including immunotoxicity, dermal toxicity, carcinogenicity and adverse effects on reproduction, development, and endocrine functions (Safe, 1990; Ahlborg et al., 1995). Since these compounds are highly bioaccumulative, consumption of contaminated foods are considered to be the main route of exposure of humans. Consuming dioxin contaminated fish and seafood have elevated blood levels and body burden of dioxins (Johansen et al., 1996). Moreover, in 2000, Mocarelli et al. reported that increased probability of female births with increasing TCDD concentrations in the serum samples from the fathers. Due to strict regulations to control dioxins emissions, recent studies exhibited decreasing trend of dioxin levels in developed countries (Yamashita et al., 2000). However, data on such toxic organic pollutants in most Asian developing countries are not available. Guruge and Tanabe, 2001 reported some organo micro pollutants accumulation in fish from Sri Lanka. However, to best of knowledge, there are no reports are available for congener specific dioxin contamination in biota from Sri Lanka. In this study, congener-specific profiles of PCDDs, PCDFs and DLPCBs were examined and 2,3,7,8-TCDD equivalents were estimated in fish and benthic invertebrate collected from the western coastal area of Sri Lanka.

2. Materials and Methods

Samples are collected in 1996 and 1997 from the Kelani River mouth, Colombo harbor area and Negombo Lagoon. Sample details are given in Table 1. Total of 6 species of fish and one crab species were subjected to analysis. Fish and crab were captured by a gill net, stored in polyethylene bags and transported to Japan over dry ice, kept in -20^{0}C until the analysis.

In order to obtain representative samples, composites of mullet, jack and snapper were prepared by pooling together and homogenized while only muscle of other species were pooled and homogenized (Table 1). Then extracted, fractionated and analyzed following the methods described in elsewhere with slight modifications (Watanabe *et al.*, 2001; Minh et al., 2003). About 20 g of sample homogenized with sodium sulfate and extracted with dichloromethane (DCM) and hexane (3:1 v/v) for 16 hrs. The extract was concentrated and fat content was determined by gravimetry. An aliquot of remaining extract was spiked with ^{13}C-DDs,^{13} C-DFs and ^{13}C-DLPCBs as internal standards and treated with sulfuric acid. Then other interferences removed by passing through a multilayer acid silica gel column followed by an activated alumina column. The eluate was concentrated and passed through a packed silica gel impregnated-activated carbon for the separation of ortho-substituted PCBs from others. The first fraction contains ortho-substituted PCBs, eluted with DCM/hexane solvent mixture. Then second fraction, which eluted with toluene, contained non-*ortho*- substituted PCBs, PCDDs, and PCDFs.

The congeners were identified and quantified by high-resolution gas chromatography (HRGC), Hewlett-Packard 6890 and 7673, coupled with high- resolution mass spectrometer (HRMS), Double Focusing JEOL GC-Mate II and JEOL JMS-700D, for mono-*ortho* PCBs and non-*ortho* PCBs/DDs/DFs, respectively. A fused silica capillary (60m x *0.25* mm i.d. with 0.25[tm film thickness) of DB-1 (J&W Scientific, CA, USA) was used to separate mono-*ortho* PCBs. DB-5 (60m x 0.25 mm i.d. with 0.25 mm film thickness) was used to separate non-*ortho* PCBs and hepta- to OCDD/DFs whereas tetra- to hexachloro-DDs/DFs separation was achieved by a CP-Si188 column (60m x 0.25 mm i.d.; Supeleo, PA) with 0.1 mm film thickness. The mass spectrometers (GC-Mate II and JMS-700D) were operated in an electron impact (El) and selective ion monitoring (SIM) mode at resolution R>1400 (10% valley) and >10000 (10% valley), respectively. Oven temperature was programmed from 170°C to a final 290°C. The mass spectrometer was operated at El energy of 40eV with trap current at 600 pA. The column head pressure was kept at 200kpa. Helium was used as carrier gas. The detection limits of individual PCDDs, PCDFs, and DLPCBs congeners varied from 0.1 to 0.5 pg/g wet wt, depending on the congeners and relative responses.

3. Results and Discussion

Concentrations of PCDDs, PCDFs and DLPCBs (non- and mono-*ortho-PCBs)* congeners are shown in Figures 1 to 4. Concentration of PCDDs and PCDFs in samples ranged from 0.04 to 4.5 (11 to 280 pg/g fat wt) and 0.04 to 1.3 (6.9 to 340 pg/g fat wt) pg/g wet wt, respectively. The highest concentration of dioxin was detected in mullet from the Kelani River. However, all the toxic PCDDs and PCDFs congeners were detected only in mud crab and snapper from Kelani River and Negombo Lagoon, respectively. Over 50% samples consisted with all PCDD congeners analyzed and detection of all PCDF congeners was seen only in snapper from Negombo Lagoon. The PCDDs/PCDFs ratio was high (>1) in most samples implying that high PCDDs contamination in west coast of Sri Lanka. The concentrations of most toxic 2,3,7,8-TCDD and 2,3,7,8-TCDF congeners were ranged <0.01 to 0.09 and 0.03 to 0.34 pg/ g wet wt, and their detection frequencies were 78% and 100%, respectively. 2,3,7,8-TCDD congener was not detected from two fish species from Negombo lagoon. The concentration of dioxins in both locations was less than those recently reported data from developed countries such as USA (Geisy et al., 1999) and Japan (Iseki et al., 2001), however similar to some fish from Korea (Ok et al., 2001; Choi et al., 2001), Belgium (Focant et al., 2001), India (Senthikumar et al., 2001) and Taiwan (Peng and Weng, 2001). Concentration of 2,3,7,8-TCDD in some fresh water fish from Korea (Jeong et al., 2001) was few folds higher than that from Sri Lanka.

The concentrations of DLPCBs (coplanar PCBs), *non-ortho* PCBs and *mono-ortho* PCBs, were 1.3 to 140 (300 to 5800 pg/g fat wt) and 49 to 6000 (10000 to 194000 pg/g fat wt) pg/g wet wt, respectively. The fish from Kelani River had high DLPCBs concentrations with the highest was detected from mullet followed by catfish. Concentrations of DLPCBs in catfish and green cromide from Kelani River were 20 to 10 fold, respectively, greater than the same species from Negombo lagoon. Similar observation was reported for other organochlorine compounds such as total PCBs, DDTs and chloradane (Guruge and Tanabe, 2001) were high in Kelani River. This implies a greater input of pollutants to the Kelani River. Current DLPCBs concentration in all the species analyzed was one to two orders magnitude lower than fish from the Great Lakes (Kannan et al., 2000), Japan (Guruge et al., 2000), however, in the same range with fish from Taiwan (Peng and Weng, 2001) India (Sentilkumar et al, 2001) and Korea (Choi et al., 2001).

It is evident that toxic PCDDs, PCDFs and DLPCBs have common mechanism, involving binding to aryl hydrocarbon receptor as an initial step and start out various toxic responses in living cells (Safe, 1990). Toxic equivalent factors (TEFs), which are fractional potencies of individual congeners to most toxic 2,3,7,8-TCDD, have been used to estimate toxic potency that is dioxin toxic equivalents (TEQs) in mammal, avian and fish (Van den Berg et al., 1998). The estimated concentrations of TEQs in these biota ranged from 0.02 to 3.6 wet wt (6.5 to 155 pg/g fat wt). It was clearly seen that fish and crab from Kelani River accumulated 1 to 2 orders of magnitude high TEQs than fish from Negombo Lagoon. Currently, World Health Organization's (WHO) recommend for human Tolerable Daily Intake (TDI) of dioxins is 1 to 4 pg/Kg/day. However, most developed countries are still practicing its maximum value, while trying that the ultimate goal should be to reduce to less that 1 pg/Kg/day. The relative contribution of PCDDs, PCDFs and DLPCBs to the concentrations of TEQs in biota exhibited similar pattern from the both location, which was non-*ortho* PCB congeners contributed the highest portion. DLPCBs contributed over 80% of the total TEQs, except snapper from Negombo lagoon, which contained of 50%. The predominant of PCBs to total TEQs was similar to some Asian countries suggesting that PCB pollution in Sri Lankan aquatic environment may be widespread.

To our knowledge, this is the first report to document the extent of congener specific PCDDs, PCDFs and DLPCBs contamination in biota from Sri Lanka. This data once again highlighted the extensive contamination in Kelani River. In this study, number of samples, species locations and analytical setting were selected randomly. However, all the samples had contaminated with dioxins and suggested fish to be one of the main dioxin contamination sources for local population and wildlife. Recently, Minh et al. 2003, emphasized that open dumping sites in developing countries are hot spots of dioxins contamination. Hence, national wide monitoring is needed to identify such food items and locations of dioxin contamination and the extent of exposure to human and wildlife in Sri Lanka.

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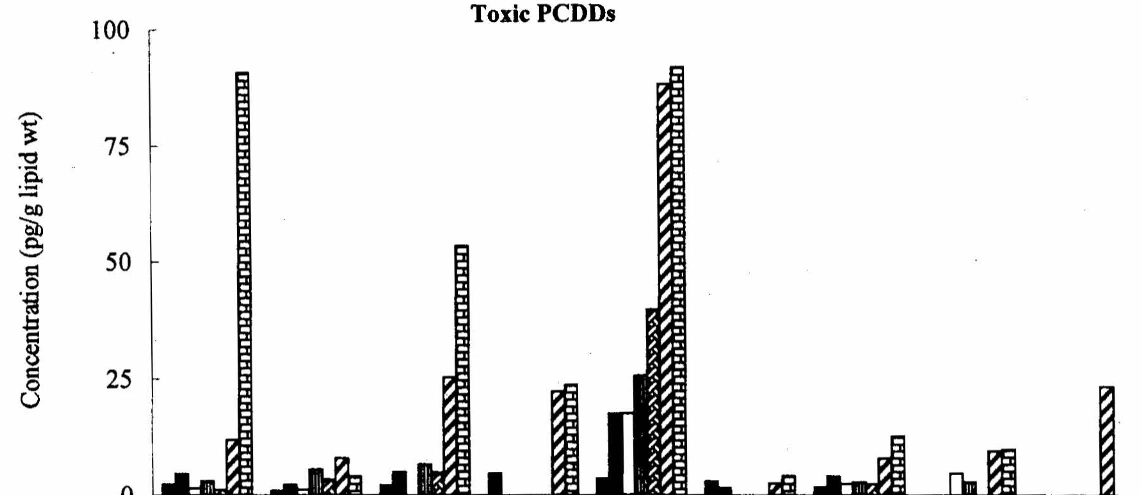
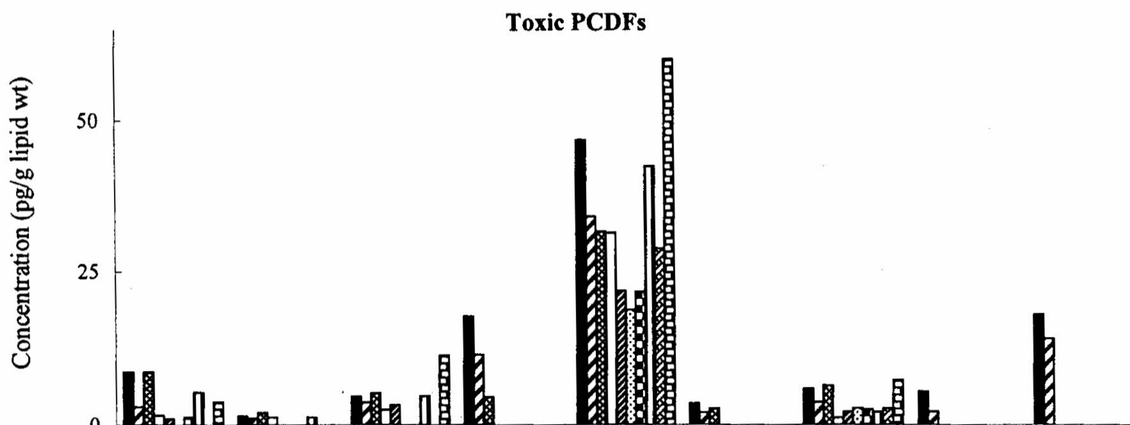


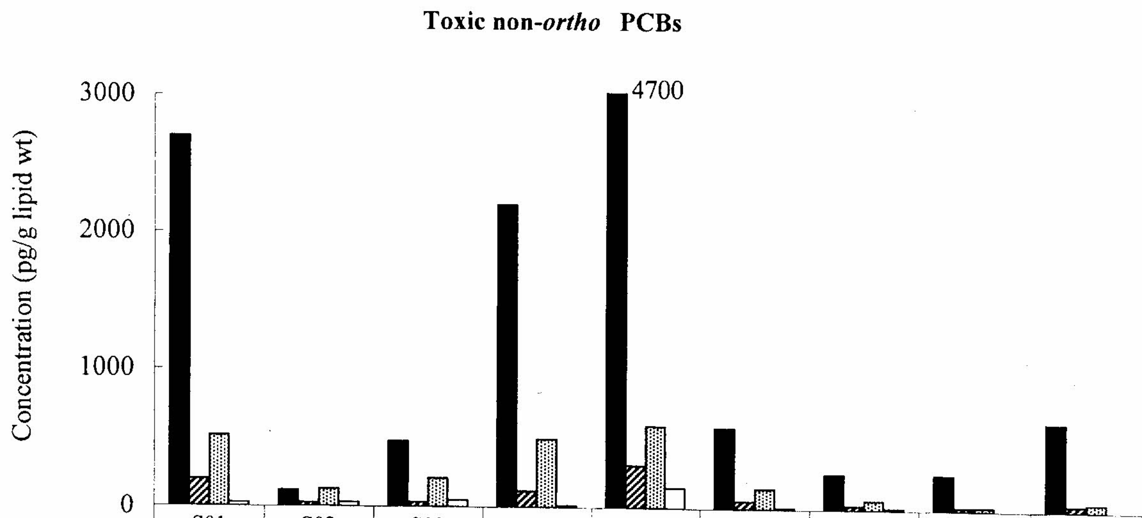
	S01	S02	S03	S04	S05	S06	S07	S08	S09
■ 2,3,7,8-TeCDD	2.26	0.81	1.99	4.58	3.50	2.85	1.64	0.00	0.00
■ 1,2,3,7,8-PeCDD	4.49	2.12	4.85	0.00	17.4	1.54	3.96	0.00	0.00
□ 1,2,3,4,7,8-HxCDD	1.38	1.18	0.00	0.00	17.5	0.00	2.31	4.57	0.00
▥ 1,2,3,6,7,8-HxCDD	2.91	5.49	6.59	0.00	25.6	0.00	2.73	2.72	0.00
▨ 1,2,3,7,8,9-HxCDD	1.11	3.27	4.84	0.00	39.7	0.00	2.23	0.00	0.00
▨ 1,2,3,4,6,7,8-HpCDD	11.9	7.87	25.2	22.2	88.1	2.41	7.95	9.43	23.1
⊞ OCDD	90.7	3.92	53.3	23.6	91.8	4.10	12.6	9.83	0.00

Fig 1 Congener specific accumulation of polychlorinated dibenzo-*p*-dioxins in biota from Sri Lanka



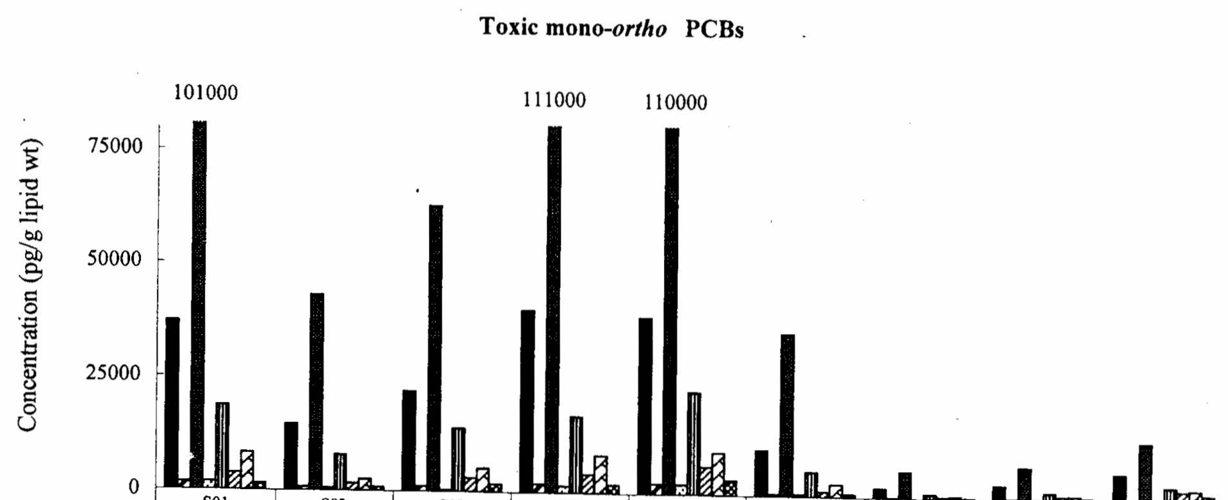
	S01	S02	S03	S04	S05	S06	S07	S08	S09
2,3,7,8-TeCDF	8.63	1.42	4.63	17.6	46.7	3.46	5.92	5.47	17.9
1,2,3,7,8-PeCDF	2.99	1.08	3.61	11.4	34.0	2.02	3.71	2.12	14.0
2,3,4,7,8-PeCDF	8.68	2.01	5.16	4.52	31.5	2.62	6.43	0.00	0.00
1,2,3,4,7,8-HxCDF	1.54	1.19	2.42	0.00	31.3	0.00	1.13	0.00	0.00
1,2,3,6,7,8-HxCDF	0.99	0.00	3.18	0.00	21.7	0.00	2.10	0.00	0.00
1,2,3,7,8,9-HxCDF	0.00	0.00	0.00	0.00	18.6	0.00	2.67	0.00	0.00
2,3,4,6,7,8-HxCDF	1.20	0.00	0.00	0.00	21.6	0.00	2.45	0.00	0.00
1,2,3,4,6,7,8-HpCDF	5.33	1.18	4.66	0.00	42.3	0.00	2.09	0.00	0.00
1,2,3,4,7,8,9-HpCDF	0.00	0.00	0.00	0.00	28.7	0.00	2.72	0.00	0.00
OCDF	3.78	0.00	11.3	0.00	60.0	0.00	7.33	0.00	0.00

Fig 2 Congener specific accumulation of polychlorinated dibenzofurans in biota from Sri Lanka



	S01	S02	S03	S04	S05	S06	S07	S08	S09
■ 3,3',4,4'-TeCB	2700	120	480	2200	4700	590	260	260	640
▨ 3,4,4',5-TeCB	200	27	33	120	310	57	30	25	44
▦ 3,3',4,4',5-PeCB	520	130	210	500	600	150	69	26	58
□ 3,3',4,4',5,5'-HxCB	27	29	51	13	150	10	13	0	0

Fig 3 Congener specific accumulation of non-*ortho* substituted polychlorinated biphenyls in biota from Sri Lanka



	S01	S02	S03	S04	S05	S06	S07	S08	S09
2,3,3',4,4'-PeCB	37200	14500	22000	40000	38600	10000	2100	3100	5800
2,3,4,4',5-PeCB	1700	770	1100	1900	2300	520	96	170	190
2,3',4,4',5-PeCB	101000	43000	62700	111000	110000	35600	5700	7100	12800
2',3,4,4',5-PeCB	1800	560	420	1500	2200	570	20	110	230
2,3,3',4,4',5-HxCB	18600	7900	13900	16800	22500	5400	1000	1600	3200
2,3,3',4,4',5'-HxCB	3700	1600	3100	4100	6100	1100	370	870	2400
2,3',4,4',5,5'-HxCB	8300	2500	5100	8300	9300	2800	620	920	2600
2,3,3',4,4',5,5'-HpCB	1500	870	1700	1900	3250	630	180	600	1500

Fig 4 Congener specific accumulation of mono-*ortho* substituted polychlorinated biphenyls in biota from Sri Lanka

Table 1. Biometry of samples from Sri Lanka

Year	Location/Species	Sample no	n	Mean body Weight (g)	Mean body Length (cm)
1996 April	Kelani River mouth				
	Mullet, *Lisa dusumari*	S01 † **	6	78	21
	Cat fish, *Macronus gulio*	S02 ‡	3	224	29
	Jack (Juvenile), *Crangidae sp.*	S03 †	8	12	11
	Green cromide, *Etroplus suratensis*	S04 ‡	3	278	21
	Mud crab, *Scyllar serrata* *	S05 ‡	2	172	nd
	Colombo Harbour				
	Rabbit fish, *Siganus vermiculatus*	S06 ‡	3	238	20
1997 April	Negombo Lagoon				
	Snapper, *Lutjanus decussatus*	S07 †	7	20	11
	Cat fish, *Macronus gulio*	S08 ‡	2	170	22
	Green cromide, *Etroplus suratensis*	S09 ‡	2	350	22

*Soft tissue

nd: not determined

† Pooled and whole body homogenized

‡ Pooled and homogenized muscle

**Two composites each with 3 fish