DIOXINS, FURANS AND DIOXIN-LIKE PCBS IN EGGS OF COMMON TERNS FROM LIME ISLAND IN ST. MARY'S RIVER OF MICHIGAN, USA

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Introduction

Severe reproductive failure was noticed for colonial water birds such as common tern (*Sterna hirundo*) in past several years in many parts of the world. Only some literature reported the bill defects in these species and suggested polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dioxin-like PCBs) were the main cause[1]. Some of these effects include reproductive failure, morphological aberrations, and alterations in plasma concentrations of vitamin A and thyroid hormones, and induction of hepatic cytochrome P4501A1 activity. The Green Bay of Michigan serve as a main area for providing food and shelter for several species of colonial water birds in which toxic contaminants are severe[2]. Relatively, few documents reported highest concentrations of PCBs in common tern eggs from Great Lakes as well as from other parts of the world. Field observations indicated that bill defects and abnormal deformities has been observed in common terns from Lime Island, St. Mary's River colonies at Michigan, USA. However, there is no study have been made to relate the levels of PCDDs, PCDFs and dioxin-like PCBs until to-date. Considering the lack of information on levels of PCDDs, PCDFs and dioxin-like PCBs in eggs of this species, we measured accumulation profiles of 2378-chlorine substituted PCDD/DFs and dioxin-like PCBs. TEQs were calculated using the World Health Organization (WHO) toxic equivalency factors (TEFs) for birds[3].

Materials and Methods

Sample collection

Randomly egg (n=10) sampling was made in and around Lime Island, St. Mary's River of Michigan USA colony in 1999. The egg weight, length, breadth, embryo weight and shell weight were measured. The embryo of each egg was separated in chemically clean vials and stored at –20°C until chemical analysis.

Analysis

Embryo of egg samples were ground with sodium sulfate and then extracted using Soxhlet apparatus for 15 hrs using dichloromethane. Extracts were then subjected to concentrate to 20 ml by Kuderna-Danish (K-D) concentrator. The aliquots of extract were used for fat determination by

gravimetric method. While in the remaining extracts, 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 and further subjected to sulfuric acid treatment, moisture removal and sequence of silica gel, alumina and silica gel impregnated carbon column separations. Details of the analytical procedures have been reported in our earlier study[4]. Identification and quantification of 2378-substituted congeners of PCDD/DFs and dioxin-like PCBs (non-, mono- and di-*ortho*-substituted congeners) was performed using Hewlett Packard 6890 Series high-resolution gas chromatography interfaced with a Micromass Autospec - Ultima high-resolution mass spectrometer.

Results and Discussion

PCDD/DFs

Concentrations of PCDFs were greater in 7 of 10 eggs analyzed with the range of 170 to 680 pg/g fat wt. (Table 1). PCDDs were greater than PCDFs in 3 eggs especially highly contaminated egg (960 pg/g fat) contained greater concentration 700 pg/g fat wt. (Table 1). Altogether, 2378-PCDD/DFs concentrations in the eggs of common tern ranged from 330-960 pg/g fat wt. The ratios of PCDDs to PCDFs varied in between eggs with the range of 0.38 to 2.69. When congener specific accumulations of concern, TCDD contributed 3.1 to 10.3 % to the total 2378-PCDD/DFs. Considerably, TCDF had greater contribution with 13 to 46 % in eggs. In general, OCDD and 12378-PeCDD were predominant congeners among PCDDs and 2378-TCDF, 123478-HxCDF and 23478-PeCDF were prevalent congeners among PCDFs. Slightly varied profile in between eggs were explained by different migratory pattern of mothers during wintering season. Greater concentrations of highly toxic congeners such as 2378-TCDF, 23478-PeCDF suggested the enzyme induction. Concentrations of PCDD/DFs in this study were greater than double-crested cormorants from Green Bay, Michigan and similar to same species from The Netherlands[5,6].

Sample I.D.	Minimum	Average	Maximum	STDVEP*
Fat (%)	8.4	11	13	1.6
2,3,7,8-D	14 (1.66)	31 (3.32)	66 (6.65)	16 (1.48)
1,2,3,7,8-D	23 (2.57)	47 (5.16)	71 (8.27)	14 (1.76)
1,2,3,4,7,8-D	4.4 (0.44)	6.7 (0.74)	8.5 (1.02)	1.3 (0.18)
1,2,3,6,7,8-D	20 (2.04)	29 (3.20)	40 (5.05)	6.5 (0.86)
1,2,3,7,8,9-D	0.5 (0.06)	1.1 (0.12)	1.7 (0.22)	0.4 (0.05)
1,2,3,4,6,7,8-D	11 (1.31)	20 (2.07)	55 (4.65)	12 (0.98)
OCDD	31 (3.06)	110 (11)	530 (45)	140 (12)
2,3,7,8-F	45 (4.94)	130 (13)	280 (32)	78 (8.89)
1,2,3,7,8-F	1.4 (0.12)	11 (1.25)	27 (3.32)	8.7 (1.01)
2,3,4,7,8-F	20 (2.22)	40 (4.58)	110 (14)	27 (3.65)
1,2,3,4,7,8-F	51 (5.44)	75 (8.41)	160 (21)	31 (4.30)
1,2,3,6,7,8-F	8.2 (0.94)	13 (1.46)	34 (3.81)	7.1 (0.81)
2,3,4,6,7,8-F	6.3 (0.71)	13 (1.38)	49 (4.88)	12 (1.18)
1,2,3,7,8,9-F	<0.1 (<0.001)	<0.1 (<0.001)	<0.1 (<0.001)	
1,2,3,4,6,7,8-F	5.0 (0.50)	6 (0.65)	10 (0.84)	1.4 (0.10)
1,2,3,4,7,8,9-F	<0.1 (<0.001)	1.6 (0.11)	2.7 (0.35)	0.8 (0.11)
OCDF	2.6 (0.35)	4.7 (0.51)	7.8 (0.73)	1.3 (0.13)
2378-PCDDs	**100 (11)**	**240 (26)**	**770 (71)**	**190 (12)**
2378-PCDFs	**140 (15)**	**300 (32)**	**680 (82)**	**170 (15)**

The values rounded; Figures in parentheses indicates wet wt. basis.

* Standard deviation

PCBs

Concentrations of dioxin-like PCBs were in the ranges of 3100-9700 ng/g fat wt. (Table 2). Four non-*ortho* PCBs contributed very less to sum of dioxin-like PCBs concentrations with 0.2-0.4 %. While 2 congeners of di-*ortho* PCBs had greater contribution with 38.6-58.4 % and remaining contribution is shared by 8 mono-*ortho* PCB congeners. Overall, CB-180, 118, 105, 170, 156 and 167 were predominant congeners in tern eggs. Among, non-*ortho* PCBs, CB-126 was predominant followed by CB-77>CB-169>CB-81. In contrast to PCDD/DFs pattern, dioxin-like PCBs were similar among all analyzed eggs. The similar results have been observed in eggs of common tern from The Netherlands and Belgium. Nevertheless, concentrations of dioxin-like PCBs were similar than those to double-crested cormorants from Green Bay, Michigan, common terns from Massachusetts, USA and The Netherlands[5,6].

Table 2. Concentrations of dioxin-like PCBs (ng/g fat/wet wt.) in common tern eggs (n=10) collected from Lime Island of Great Lakes, USA.

	Minimum	Average	Maximum	STDEVP*
Non-*ortho* PCBs				
CB-81	0.32 (0.001)	0.6 (0.052)	0.87 (0.084)	0.17 (0.03)
CB-77	4.7 (0.53)	8.7 (0.91)	18 (1.56)	3.9 (0.29)
CB-126	3.2 (0.36)	4.7 (0.52)	6.5 (0.83)	1.0 (0.15)
CB-169	0.7 (0.07)	1.0 (0.11)	1.6 (0.20)	0.29 (0.04)
Mono-*ortho* PCBs				
CB-105	320 (36)	650 (71)	990 (120)	240 (27)
CB-114	18 (2.0)	41 (4.4)	64 (8.2)	16 (1.8)
CB-118	810 (89)	1700 (180)	2700 (340)	610 (75)
CB-123	25 (2.7)	52 (5.5)	76 (8.9)	19 (1.81)
CB-156	160 (18)	330 (36)	520 (66)	130 (15)
CB-157	39 (4.3)	74 (8.1)	120 (15)	25 (3.25)
CB-167	99 (11)	210 (23)	360 (46)	85 (11)
CB-189	13 (1.5)	28 (3.1)	52 (6.6)	13 (1.68)
Di-*ortho* PCBs**				
CB-170	410	710	1200	230
CB-180	1100	2500	4600	1100
Non-*ortho* PCBs	**8.9 (1.0)**	**15 (1.6)**	**27 (2.7)**	**5.4 (0.5)**
Mono-*ortho* PCBs	**1500 (160)**	**3100 (330)**	**4900 (610)**	**1100 (140)**
Di-*ortho* PCBs	**1500**	**3200**	**5800**	**1300**

The values rounded; Figures in parentheses indicates wet wt. basis; * Standard deviation

** wet wt. not calculated

Toxic equivalent (TEQs) concentrations of PCDD/DFs and dioxin-like PCBs were in the ranges of 810-1900 pgTEQ/g fat wt. (Table 3). Greater contribution by non-*ortho* PCBs was noticed followed by PCDFs, mono-*ortho* PCBs and PCDDs with slight difference in between egg samples. When the concentrations were expressed on pgTEQ/g wet weight, TEQs ranged from 91-210. The observed values were greater than LOEL for chicken and wood ducks and with in the ranges of NOEL and LOEL observed for bald eagle embryo[7]. However, lower than LD_{50}, LD_{100} observed for cormorants, Caspian terns and pheasant[7]. On the whole, lowest adverse effective level is apparent in eggs of common terns from Lime Island, St. Mary's River colony of Michigan, USA. The congeners such as 2378-TCDF, 23478-PeCDF, 2378-TCDD, 12378-PeCDD and 123478-HxCDF contributed to greater toxicity among dioxins and furans. While, IUPAC 77, 126, 105, 156 and 118 contributed to greater

toxicity among dioxin-like PCBs. The common tern is relatively sensitive to effects of *in ovo* exposure to 2378-PCDD/DFs and dioxin-like PCBs. Non-*ortho* PCBs, CB-77 and 126 alone contributed more than 50 % of total TEQ. Few investigations suggested that EROD activity was not induced to the levels greater than the background in the dose group receiving only 0.1 ng PCB-126/g, but the group receiving a dose of 1 ng PCB-126/g was induced 2.4-fold relative to background concentrations in laboratory. Hydroxylated metabolites of certain PCBs, such as PCB-77 can bind to transthyretin, which is the thyroid hormone transporting protein. Therefore, greater TEQ contribution by PCB-77 in eggs is of major concern[6].

It is worth mentioning that when common terns return from wintering grounds, the concentrations and burdens of organic chemicals will be low due to their energy spend for their return migratory flight to breeding grounds. Further, terns are known to forage in areas that are mostly within 10 km of their breeding areas. Owing to this combination of migratory and forage behavior, the body burdens in both the adult and eggs should reflect the local patterns and levels in the food. Therefore, PCBs pollution in St. Mary's river and adjacent areas is of major concern especially the greater accumulation and TEQ of non-*ortho* PCBs 77 and 126. Besides, greater concentrations of *p,p'*-DDE is probable in the eggs analyzed in this study and thus, combination of embryo toxic and etiological effects respectively by dioxin-like PCBs, PCDD/DFs and *p,p'*-DDE, may be expected.

Table 3. Toxic equibalency (TEQ) contribution by PCDD/DFs and dioxin-like PCBs (pg/g fat/wet/wt.) in common tern eggs (*n*=10) collected from Lime Island of Great Lakes, USA.

Sample I.D.	Minimum	Average	Maximum	STDVEP*
PCDDs[a]	39 (4.33)	78 (8.56)	120 (12)	25 (2.7)
PCDFs[b]	75 (8.72)	180 (19)	340 (43)	91 (11)
NO-PCBs[c]	590 (67)	960 (100)	1500 (150)	270 (25)
MO-PCBs[d]	64 (7.04)	130 (14)	200 (25)	47 (5.6)
PCDD/DFs[e]	130 (15)	260 (28)	440 (55)	110 (13)
NMO-PCBs[f]	680 (76)	1100 (120)	1700 (170)	310 (29)
Total TEQ	**810 (91)**	**1300 (140)**	**1900 (210)**	**380 (40)**

The values rounded;[a,b,c,d,e] and [f] respectively, 2378-PCDDs, 2378-PCDFs, non-*ortho* PCBs,

Figures in parentheses indicates wet wt. basis; * Standard deviation

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