Topic no (3-E)

ACCUMULATION PROFILES AND BURDENS OF DIOXINS, FURANS AND DIOXIN-LIKE PCBs IN COMMON CORMORANTS (*Phalacrocorax carbo*), FROM JAPAN

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Introduction

A significant of studies have put forward about sources, toxicity and formation of polychlorinated dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) in wildlife. Major sources of these chemicals are among other chemical synthetics, muncipal incineration and chlorine bleaching. PCDDs, PCDFs and non-*ortho* substituted PCBs reduce immune competence of terrestrial and marine mammals[(1)]. Similarly, studies have shown that PCDDs, PCDFs and PCBs cause toxic effects in birds. A common mechanism of action involves binding with the cytosolic *Ah*-receptor, which mediates many of these responses. Due to lipophilic properties and resistance towards metabolic breakdown, these compounds are transferred through food chain with strong biomagnification at the higher trophic levels. Considering those facts, in this study, common cormorant collected from Sagami River was used to determine specific-organ distribution of dioxins, their burden and half-lives values in their body.

Materials and Methods

Sample collection

During diet survey shooting was performed in February 1999 in and around Sagami River, one common cormorant was obtained for dioxin analysis. Immediately after collection, approximately 13 ml of blood was drawn from the heart using chemically clean syringe. Then the bird was dissected and organs such as liver, kidney and muscle was separated and packed into clean polyethylene bags. The same cormorant also contained one egg in gland that also collected for analysis. Then the samples were transported to laboratory with dry ice and stored at -30^{o}C until analysis. Same survey carried out during February 2001 in Sagami River, two common cormorants that have developing ovary was collected and treated as fore-mentioned procedure. In order to estimate half-lives four live juvenile cormorant was obtained from Lake Biwa in April 1998. The bird was taken in to a Nippon Veterinary & Animal Science University to estimate relative-absorption of PCDD/Fs. The cage with a Teflon

sheet was used as a floor to place cormorant in where the bird was feeded for one month. After feeding, this bird was sacrificed by euthanasia under anesthesia, then tissue was separated as same procedure for Sagami River birds. The feeding fish (mackerel) was purchased at market as a bulk to follow the uniformity in food. Every day fecal matter was collected then weighed. Every after collection of fecal matter the Teflon sheet was washed with tap water. Cormorant feed mackerel with five different sizes also homogenized for chemical analysis.

Determination of PCDD/Fs and dioxin-like PCBs

All the samples analyzed in this study were homogenized and freeze-dried prior to analysis. Freeze-dried, moisture content measured sample was then extracted with Soxhlet apparatus using dichloromethane for 10h. After fat content determination, sulfuric acid was treated and purified for fractionation. Silica gel and alumina was used for fractionation, in that, silica gel removed most of other pesticides like DDT (if any) and its metabolies. Alumina column removed most of mono- and di-ortho substituted PCBs. Further, in a charcoal-impregnated silica-gel mixture column fractionation step was subjected, adsorbed PCDD/Fs and dioxin-like PCBs in two fractions. The first fraction, eluted with 25% dichloromethane in hexane, consisted of mono-*ortho* substituted PCBs. The second fraction, eluted with toluene, comprised PCDDs/DF homologues and non-*ortho* substituted PCBs. Quantification and identification techniques adopted for non-*ortho* and mono-*ortho* substituted PCBs followed by HRGC-HRMS [HP6890 Hewlett Packard, Auto Spec Ultima]. The separation of PCDD/Fs was achieved using a HP 6890 machine equipped with DB-5 and DB-17 columns with splitless and solvent cut mode. Gas chromatographic separation of non-*ortho* and mono-*ortho* substituted PCBs was performed in DB-5 capillary column.

Results and Discussion

Concentrations and distribution

Concentrations of PCDD/Fs 4300, 4500, 6300, 8300 and 2600 (pg/g fat wt) in egg, muscle, kidney, blood and liver, respectively (results not shown). PCDFs were one magnitude higher than PCDDs in muscle, liver and kidney. However PCDDs were prevalent in blood when compared with PCDF.

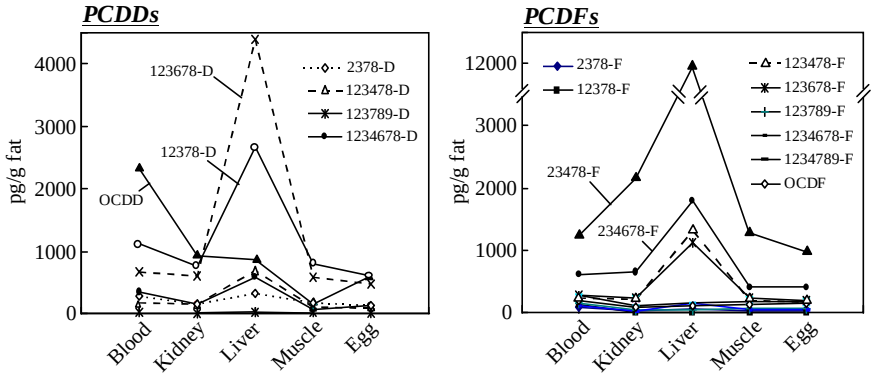

Fig.1. Concentration and distribution of PCDDs/Fs in different tissues of common cormorant.

Significantly, higher concentrations of 1,2,3,6,7,8-HxCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF were obtained (Fig. 1). Besides, the congeners, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8-PeCDD in liver and OCDD in blood seems too prevalent. Similarly, 2,3,4,7,8-PeCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF showed higher accumulation potential in cormorants.

Toxic equivalent contribution

Toxic equivalents of PCDD/Fs and dioxin-like PCBs in terms of WHO-TEFs determined in liver, egg and fish were shown (Fig.2). It is worth mentioning that toxic equivalent contribution found in cormorant liver is slightly higher and entirely different than egg and fish samples, respectively. PCDFs and chlorobiphenyl (IUPAC 126) were prevalent in liver and egg. Where as chlorobiphenyl (IUPAC 77) contributed approximately 50% of toxicity in fish. The results clearly shows that chlorobiphenyl (IUPAC 77) was easily metabolizable in liver and transferable to the reproductive cycle of birds due to its predominance in egg. It is very clear that animals belonging higher in the food chain accumulate elevated levels of dioxins and furans when compared with the fish which accumulated dioxin-like PCBs considerably. Interestingly, egg contained dioxins and dioxin-like PCBs somewhat notable levels.

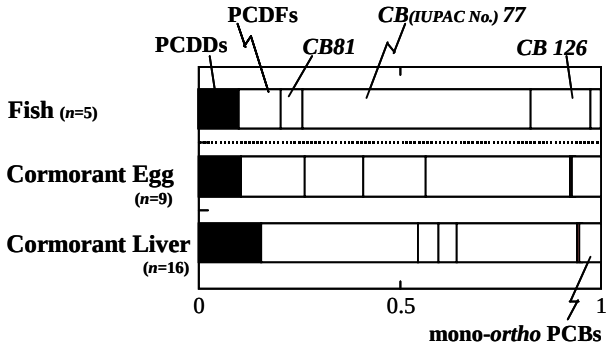

Fig.2. Toxic Equivalent Contribution(Birds TEF) of PCDD/Fs and PCBs in common cormorant and fish from Tokyo Bay, Japan.

Body burden

In order to estimate the specific accumulation and burden of PCDD/Fs, the burdens in organs such as muscle, liver, kidney, egg and blood were estimated. Muscle occupied 75% of burdens followed by blood, liver, egg and kidney. This trend is somewhat different than concentration pattern in which liver accumulated higher levels. One plausible explanation is that muscle contributed approximately 40% of the body weight than the liver (3-4%). When compared with congeners, lower chlorinated PCDDs were higher than the higher chlorinated PCDDs in muscle. In contrast, higher chlorinated PCDDs (Hepta to octa) in egg were apparent. Liver burden increased by the homologues of tetra to hexa-CDDs and down ward trend was noticed from hepta- to octa-CDDs. No specific trend was noticed for egg PCDF homologue distribution. Although, decreasing burden upto penta-CDF and increasing load was observed in case of muscle PCDF. Contrastingly, liver burdens increased from TCDF and then decrease gradually. Altogether results showed that specific accumulation of congeners in different organs. Blood and kidney burden did not showed any specific trend (results not shown). Besides, concentrations were not considerable except OCDD in blood.

Half-lives

Under the assumption of static state in a one-compartment model, the relation between concentration and half-lives could be estimated using formula shown as follows;

$$\frac{C_{Fish} \times AD \times f}{Fat_{liver}} \times \frac{T_{1/2}}{Log_e 2} \times Ratio_{liver} = C_{liver}$$

where; C is concentration, AD is administered dose, f is absorption ratio, $Log_e 2$ is 0.693 and $Ratio_{liver}$ is the burden in liver compared to those in whole body. Considering all these parameters, the ratio of weight and fat and $Ratio_{liver}$ are shown in Table 1.

The concentrations of fish and cormorant were also culled from our other field-study[(2)]. According to our estimation, half-lives of 1,2,3,4,7,8,9-HpCDF showed greatest potential (120 days) it was most dominant isomer when compared with 2,3,7,8-TCDD (43 days). Some of the other potential isomers are 1,2,3,6,7,8-HxCDF (79), 2,3,4,6,7,8-HxCDF (73), 2,3,4,7,8-PeCDF (72), 1,2,3,6,7,8-HxCDD (61), 1,2,3,7,8-PeCDD (59), 1,2,3,4,7,8-HxCDD (51) and 1,2,3,4,7,8-HxCDF (47). Relative with the above-mentioned congeners, 1,2,3,7,8,9- HxCDF (24), 1,2,3,7,8,9-HxCDD (19) 1,2,3,4,6,7,8-HpCDD (17), OCDD (14), OCDF (14), 1,2,3,4,6,7,8-HpCDF (7.8), 1,2,3,7,8-PeCDF (3.9), and 2,3,7,8-TCDF (2.6) showed lower half-lives than 2,3,7,8-TCDD in common cormorants from Japan.

Acknowledgements

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References

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Table 1. Parameters used to calculate half-lives in common cormorant.

	Symbol	
[1] *Weight ratio in whole body*		
Muscle	W_M	*0.41*
Liver	W_L	*0.029*
Kidney	W_K	*0.0090*
[2]Egg	W_E	*0.021*
[3]Blood	W_B	*0.074*
Others	W_O	*0.23*
[4] *Fat ratio in the tissues/organs*		
Muscle	F_M	*0.036*
Liver	F_L	*0.037*
Kidney	F_K	*0.028*
Egg	F_E	*0.060*
Blood	F_B	*0.0034*
Ratio liver		
2,3,7,8-TCDD		*0.075*
1,2,3,7,8-PeCDD		*0.13*
1,2,3,4,7,8-HxCDD		*0.21*
1,2,3,6,7,8-HxCDD		*0.24*
1,2,3,7,8,9-HxCDD		*0.088*
1,2,3,4,6,7,8-HpCDD		*0.24*
OCDD		*0.15*
2,3,7,8-TCDF		*0.048*
1,2,3,7,8-PeCDF		*0.026*
2,3,4,7,8-PeCDF		*0.28*
1,2,3,4,7,8-HxCDF		*0.22*
1,2,3,6,7,8-HxCDF		*0.17*
2,3,4,6,7,8-HxCDF		*0.15*
1,2,3,7,8,9-HxCDF		*0.031*
1,2,3,4,6,7,8-HpCDF		*0.14*
1,2,3,4,7,8,9-HpCDF		*0.036*
OCDF		*0.033*
Parameters		
Administrative dose	*AD*	$BW \times 0.26$
[5]Absorption ratio	f	*0.94*
Body weight	*BW*	*1885*
$Log_e 2$		*0.693*
Fish fat%		*0.07*

[1]Assumption from real value of female (n=4)
[2]Egg data measured in this study.
[3]Blood data is value from other study, unpublished.
[4]Fat ratio is value of analyzed sample in this study.
[5]Absorption ratio is using fat ratio of fecal matter/ fish.