CONTAMINATION OF DIOXINS AND ORGANOCHLORINE PESTICIDES IN CROWS FROM A DUMPING SITE IN SOUTH INDIA, AND THEIR EFFECTS ON THE HEPATIC XENOBIOTIC-METABOLIZING ENZYMES

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Introduction

Open landfill dumping areas for municipal wastes in Asian developing countries are ubiquitous and have recently received particular attention with regard to environmental pollution. Because of the uncontrolled burning of solid waste, contamination by various toxic chemicals including dioxins and related compounds in these dumping sites has been speculated. Although the use of organochlorine pesticides (OCs) has already been restricted in most of developed countries since the 1970's due to their persistence and high toxicity, they are still used for public health and agriculture in developing countries. However, only a few studies are available on the contamination by these chemicals in dumping sites in developing countries.

The induction of cytochrome P450 (CYP) enzymes is a responsive mechanism elicited by exposure to xenobiotic chemicals. Exposure to planar halogenated aromatic hydrocarbons (PHAHs) activates aryl hydrocarbon receptor (AhR), and stimulates the transcription of CYP1A and the other target genes. On the other hand, exposure to phenobarbital-type chemicals, DDT and *ortho*-chlorine substituted PCBs change the expression of CYP2B/2H/2C/3H subfamilies through constitutive androstane receptor (CAR). CYP expression level in wildlife is considered as a biomarker of dioxin and/or OC accumulation and their toxic responses.

In this study, we investigated the contamination by dioxins and OCs in crows from a dumping site in south India, and their effects on the hepatic xenobiotic-metabolizing enzymes.

Materials and Methods

House crows (*Corvus Splendens*) were collected from Perangudi (dumping site) and Muttukadu (reference site) of India in August-September 2000. Jungle crows (*C. macrorhynchos*) were collected only from the dumping site in the same period. Crows were immediately dissected after measurement of biometry. Muscle samples were stored in a freezer at -20℃ until chemical analysis. Liver samples were frozen in liquid nitrogen, and stored at -80℃ until microsome preparation.

Chemical analysis of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and coplanar PCBs (Co-PCBs) was carried out following the method of the Environmental Agency of Japan[1] with some modifications. About 20g of muscle samples were ground with anhydrous sodium sulfate and extracted in a Soxhlet apparatus using dichloromethane. The extract, after adding an internal standard (cleanup spike), was then applied to a gel permeation chromatography for lipid removal, and then the extract was passed through activated silica gel packed in a glass column. Separation of PCDD/DFs and Co-PCBs was performed by passing through activated alumina and activated carbon (activated carbon-dispersed silica gel) packed in a glass column. An internal standard (syringe spike) was spiked to the analytical solution. Identification and quantification of PCDDs, PCDFs, and non-*ortho* Co-PCBs was performed using high-resolution

gas chromatography (HRGC:Hewlett-Packard 6890) / high-resolution mass spectrometry (HRMS:JEOL JMS-700D), and mono-*ortho* Co-PCBs using HRGC (Hewlett-Packard 6890) / HRMS (JEOL GC mate). Toxic equivalencies (TEQs) were calculated using toxic equivalency factors determined for bird species[2]. PCBs and other organochlorines (DDTs, HCHs, chlordane compounds, HCB, heptachlor epoxide, *tris*(4-chlorophenyl)methane and *tris*(4-chlorophenyl)methanol were analyzed following the method reported by Kajiwara *et al*[3].

Hepatic microsomal fractions were prepared according to the method of Guengerich[4]. Protein content was measured with the bicinchonic acid assay. Measurements of methoxy-(MROD), ethoxy-(EROD), pentoxy-(PROD) and benzyloxyresorufin-*O*-dealkylation (BROD) activities were done by the method of Kennedy *et al*[5]. Immunoblotting of liver microsome fraction was performed as described previously[6].

Correlations between CYP activities and concentrations of organochlorine compounds were examined by Spearman rank correlation. The Mann-Whiteney *U*-test was used for the detection of statistical differences among groups.

Results and Discussion

PCDD/DFs and Co-PCBs were detected in all the muscle homogenates of house crows and jungle crows from India (Table 1). Concentrations of TEQs in jungle crows and house crows from the dumping site ranged from 37 to 81 and from 20 to 110 pg/g fat wt, respectively. On the other hand, the concentrations of house crows from the reference site ranged from 7.7 to 33 pg/g fat wt. 2,3,7,8-T_4CDD, 1,2,3,7,8-P_5CDD, 2,3,4,7,8-P_5CDF, PCB77 exhibited significant contributions of total TEQs in Indian crows. Mean total TEQs concentrations in Indian crows were less than those reported in the tissues of birds from Japan[7], the United States and the North Pacific[8]. The concentrations of 2,3,7,8-substituted PCDDs and PCDFs in jungle crows and house crows from the dumping site were almost similar, whereas those of house crows from the reference site were significantly low (p<0.05). The distribution pattern of PCDD/DF congeners in crows varied according to sites and species. Notably, H_6CDD, H_7CDD, O_8CDD, H_6CDF and H_7CDF in the muscle of crows from the dumping site were significantly higher than those in crows from the reference site. This result indicates that crows in the dumping site are exposed to PCDD/DFs probably released during combustion of wastes in the dumping site.

OC pesticides and PCBs were also detected in all the muscle homogenates of crows from India (Table 2). Residue levels of OCs in jungle crows analyzed in this study followed the order HCHs > DDTs ≥ PCBs > CHLs > HCB ≥ heptachlor epoxide. Among the OCs analyzed, concentration of HCHs in jungle crows was the highest, having a range of 600-3400 ng/g fat wt. DDTs were the second highest, ranging from 370 to 920 ng/g fat wt. This residue pattern is similar to that in other bird species of our earlier study[9, 10]. Higher concentrations of HCHs and DDTs in jungle crows may be explained by the continuous usage of these pesticides for agricultural and vector control operations. The residue patterns of OCs in house crows were different from those of jungle crows. Residue levels of OCs in house crows from India were in the order of PCBs ≥ HCHs ≥ DDTs > CHLs > HCB. Concentrations of HCHs in crows analyzed in this study were much lower than our earlier study. Ramesh *et al*[11] reported elevated concentrations of HCHs during wet season in the Vellar river water in India. Samples analyzed in this study were collected during dry season, which may be one of the reasons of low HCH concentration. Besides, the Indian government has banned technical HCH usage for crops since 1990, and also had taken a decision to phase out a production of 30 kt of HCH per annum[12]. Comparing OC contaminations in the house crows between the dumping site and the reference site, the concentrations of HCHs and heptachlor epoxide in house crows from dumping site were significantly higher than those from the reference site. In both the species, the levels of MROD and EROD activities were higher than PROD and BROD activities

Table 1. Concentrations (pg/g fat wt) (mean and range) of PCDDs/DFs and Co-PCBs in the muscles of house crow and jungle crow collected from a dumping site and a reference site in India.

	Dumping site		Reference site
	Jungle crow (*n* =5)	House crow (*n* =8)	House crow (*n* =5)
fat content (%)	**2.8** (1.9-3.4)	**3.1** (2.4-4.3)	**2.8** (2.4-3.4)
dioxins			
2,3,7,8-T_4CDD	**3.8** (2.3-5.3)	**4.3** (<0.025-13)	**2.2** (<0.025-6.1)
1,2,3,7,8-P_5CDD	**13** (7.4-22)	**13** (<0.025-33)	**6.5** (1.6-13)
1,2,3,4,7,8-H_6CDD	**9.2** (5.6-15)	**12** (<0.025-25)	**4.2** (1.4-11)
1,2,3,6,7,8-H_6CDD	**32** (23-43)	**36** (6.2-64)	**9.7** (4.6-20)
1,2,3,7,8,9-H_6CDD	**2.9** (< 0.025-4.8)	**5.3** (<0.025-14)	**1.0** (<0.025-4.1)
1,2,3,4,6,7,8-H_7CDD	**44** (19-77)	**61** (25-120)	**6.6** (2.6-13)
O_8CDD	**57** (29-100)	**95** (37-220)	**9.6** (4.7-21)
furans			
2,3,7,8-T_4CDF	**<0.025** -	**0.40** (<0.025-3.2)	**<0.025** -
1,2,3,7,8-P_5CDF	**<0.025** -	**1.1** (<0.025-8.0)	**<0.025** -
2,3,4,7,8-P_5CDF	**19** (11-32)	**13** (0.63-31)	**3.2** (1.4-6.0)
1,2,3,4,7,8-H_6CDF	**11** (6.0-20)	**16** (3.7-45)	**2.0** (<0.025-5.7)
1,2,3,6,7,8-H_6CDF	**7.5** (4.2-15)	**11** (3.1-27)	**1.6** (<0.025-4.5)
1,2,3,7,8,9-H_6CDF	**<0.025** -	**<0.025** -	**<0.025** -
2,3,4,6,7,8-H_6CDF	**6.4** (3.1-13)	**5.3** (< 0.025-9.2)	**1.1** (0.87-2.7)
1,2,3,4,6,7,8-H_7CDF	**5.6** (2.8-8.8)	**15** (4.7-38)	**1.6** (<0.05-7.9)
1,2,3,4,7,8,9-H_7CDF	**<0.05** -	**5.2** (< 0.05-42)	**<0.05** -
O_8CDF	**<0.05** -	**<0.05** -	**<0.05** -
non-ortho PCBs			
3,3',4,4'-T_4CB(77)	**67** (44-87)	**140** (19-450)	**32** (24-43)
3,4',4,5'-T_4CB(81)	**5.2** (2.8-8.0)	**16** (2.3-44)	**2.6** (1.4-3.6)
3,3',4,4',5-P_5CB(126)	**19** (10-32)	**31** (5.0-62)	**6.8** (2.0-14)
3,3',4,4',5,5'-H_6CB(169)	**37** (21-58)	**36** (13-64)	**35** (21-59)
mono-ortho PCBs			
2,3,3',4,4'-P_5CB(105)	**13,000** (5700-24000)	**15,000** (2300-45000)	**2,500** (810-3800)
2,3,4,4',5-P_5CB(114)	**2,500** (990-4100)	**4,400** (1200-9100)	**1,600** (990-2400)
2,3',4,4',5-P_5CB(118)	**70,000** (31000-130000)	**79,000** (14000-230000)	**22,000** (11000-42000)
2',3,4,4',5-P_5CB(123)	**1,100** (450-2100)	**1,300** (310-3500)	**320** (120-720)
2,3,3',4,4',5-H_6CB(156)	**20,000** (7300-44000)	**28,000** (8100-66000)	**18,000** (9000-25000)
2,3,3',4,4',5'-H_6CB(157)	**4,600** (2100-9800)	**7,000** (25000-13000)	**5,200** (2800-7200)
2,3',4,4',5,5'-H_6CB(167)	**3,200** (1600-5400)	**3,800** (870-9300)	**1,900** (1100-3000)
2,3,3',4,4',5,5'-H_7CB(189)	**1,600** (930-3000)	**3,100** (1100-6300)	**3,800** (1500-5400)
total PCDDs	**160** (94-260)	**220** (89-420)	**40** (15-89)
total PCDFs	**49** (31-86)	**66** (20-120)	**9.5** (1.6-19)
total non-*ortho* PCBs	**130** (78-170)	**230** (40-600)	**77** (53-92)
total mono-*ortho* PCBs	**120,000** (50000-200000)	**140,000** (33000-340000)	**56,000** (40000-87000)
PCDDs-TEQs	**18** (11-28)	**19** (0.61-49)	**9.1** (1.7-20)
PCDFs-TEQs	**22** (13-37)	**17** (7.2-35)	**3.7** (1.6-7.3)
non-*ortho* PCBs-TEQs	**5.8** (3.5-7.8)	**12** (1.8-33)	**2.6** (1.8-3.4)
mono-*ortho* PCBs-TEQs	**4.8** (1.9-8.6)	**6.3** (1.8-13)	**3.0** (1.9-4.3)
total TEQs	**50** (37-81)	**55** (20-110)	**18** (7.7-33)

(Table 3). EROD and MROD activities, which are catalytic activities of CYP1A/1B subfamilies induced by dioxin compounds in rodents, showed no correlation with TEQ, and had no difference between the dumping site and the reference site. However, positive correlations between DDTs, HCB and TCPMe concentrations with EROD, PROD and BROD activities were found. These activities inferred to be catalyzed by one CYP enzyme, because the three catalytic activities were closely correlated among themselves. To determine whether crows expressed hepatic CYP1A-, CYP1B-, and CYP2B-like proteins, western blot analyses were conducted using anti-rat CYP1A1, CYP1B1 and CYP2B1 polyclonal antibodies. All CYP isozymes investigated were detected, and

especially high expression of CYP2B-like isoform was observed. However, the relative staining intensities of CYP2B-like protein revealed no correlation with OCs concentration, which indicates that crow may express CYP isozymes which are different from mammalian CYPs.

Table 2. Concentrations (ng/g fat wt) (mean and range) of organochlorines in the muscles of house crow and jungle crow from a dumping site and a reference site in India.

	Dumping site		Reference site
	Jungle crow (n=5)	House crow (n=5)	House crow (n=5)
fat content (%)	**3.0** (2.0-3.4)	**2.9** (2.4-4.3)	**2.8** (2.1-3.6)
PCBs	**570** (300-880)	**1300** (910-1700)	**910** (380-1300)
DDTs	**590** (370-920)	**660** (100-1000)	**740** (470-1100)
HCHs	**1400** (600-3400)	**970** (550-1500)	**450** (270-1000)
CHLs	**70** (12-260)	**34** (15-59)	**21** (13-37)
HCB	**6.0** (4.4-7.4)	**5.7** (3.1-7.9)	**7.4** (5.4-9.2)
HP epoxide	**7.6** (<0.22-14)	**14** (2.6-28)	**2.7** (1.0-4.1)
TCPMe	**2.0** (<0.054-2.4)	**5.3** (2.6-9.5)	**8.8** (3.5-13)
TCPMOH	**6.6** (<2.1-6.6)	**7.9** (<2.1-11)	**13** (4.4-21)

Table 3. CYP enzyme activities in crows from a dumping site and a reference site in India.

	Dumping site		Reference site
	Jungle crow (n=11)	House crow (n=20)	House crow (n=18)
MROD	103 ± 82	46 ± 36	59 ± 46
EROD	58 ± 46	71 ± 46	72 ± 58
PROD	1.6 ± 0.94	2.7 ± 1.4	2.4 ± 1.5
BROD	1.3 ± 0.90	2.7 ± 2.0	2.2 ± 1.8

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