BIOACCUMULATION PROFILES OF UNEP'S POPs IN TERRESTRIAL WILDLIFE OF JAPAN

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), and dibenzofurans (PCDFs) have not been purposely manufactured, but rather are present as impurities associated with agrochemicals in Japan[1]. While polychlorinated biphenyls (PCBs) had been used in various industrial materials, such as transformers, capacitors and non-carbon copying paper, PCDD/DFs are formed by photochemical and thermal reactions during and after municipal solid waste incinerator (MSWI) and industrial waste incinerator (IWI). A significant portion of dioxins accumulated in Japan was indicated to have originated from agrochemicals[1-2]. Considerably, the amount of waste incinerated in Japan is very high compared to other countries. Therefore, it is necessary to elucidate the significance of various sources of PCDDs/DFs on wildlife exposures.

Organochlorine pollutants (OCPs) are known for their contamination of the global environment, bio-magnification in the food chain, and long-term health effects in wildlife and humans[3-5]. Among the OCPs used, DDTs (o,p'- and p,p'-compounds of DDT, DDE and DDD), HCHs ($\alpha,\beta,\gamma,\delta$-hexachlorocyclohexane isomers), chlordane compounds (cis/trans-chlordane, cis/trans-nonachlor, oxychlordane, heptachlor), HCB (hexachlorobenzene), -drin compounds (aldrin, dieldrin, endrin), toxaphene and mirex are of significant importance[6]. These groups of compound have been identified as priority environmental contaminants posing a significant impact on human health (Stockholm Convention Meeting conducted by UNEP [United Nations Environment Programme] in May 2001) and classified all the above-mentioned chemicals are of priority (except HCH-isomers) chemicals than other organochlorine pesticides. Their exposure to humans and animals is of serious concern owing to chronic toxic effects. Since many of these chemicals have persistent properties and long-term effects, regular monitoring of these chemicals in wildlife is essential for understanding their behavior and to prevent health hazards. There is no study reported the UNEP POPs in wildlife of the Japan until to date. In this study, we investigated 10 among 12 priority UNEP-POPs in wildlife inhabiting farmlands and forests in a rural area in Ibaraki and Kanagawa prefecture, central Japan, and bat from Kyushu prefecture, southwestern part of Japan. Terrestrial mammals and freshwater birds, their food items and soil were used for the analysis.

Materials and Methods

Samples: The details of samples and their biometry were shown in Table 1. Most of the samples were livers except egg (Sample No. 80 and 81), soil (Sample No. 92). Almost all analyzed samples were adults while Sample Nos. 75, 79 and 90 were chicks and juvenile, respectively. The juvenile goshawk was road accident killed. More than one individual sample represents the pool of all individuals analyzed. Samples No. 65 to 85, 90 to 92 were collected in Ibaraki Prefecture, Sample

No. 88-89 were collected in city of mount Fuji and bat samples were collected in Kyushu Prefecture of Japan. All the collected samples were transported to Shimadzu Techno Research with dry ice and stored in freeze drier until analyzed.

Table 1. Biometry and few ecological informations of samples employed in this study**.**

Sample No.	Sample Name	Sampling[a]	Sex[b]	OCPs[c]	DXNs[d]	Fat (%)
65	Weasel	26/01/00	F	○	X	3.85
66	Weasel	??/07/1998	F	○	X	8.33
67	Raccoon dog	8/2/00	F	○	X	4.06
68	Masked palm civet	19/08/1999	M	○	X	3.29
69	Masked palm civet	24/01/1999	F	○	X	3.84
70	Wild cat	3/2/00	F	○	X	5.04
71	Greater Japanese shrew mole	15/05/1999	MMFF	○	X	3.51
72	Night heron	5/6/02	M	○	○	4.10
73	Night heron	5/6/02	F	X	○	4.60
74	Night heron	5/6/02	M	X	○	4.31
75	Night heron (chick)	24/06/02	M	○	○	3.96
76	Intermediate egret	2/6/02	M	○	○	5.20
77	Intermediate egret	3/6/02	M	X	○	5.68
78	Intermediate egret	3/6/02	M	X	○	4.86
79	Intermediate egret (chick)	24/6/2002	F	○	○	4.46
80	Intermediate egret (egg)	5/6/02	-	○	○	3.63
81	Intermediate egret (egg)	5/6/02	-	X	○	7.21
82	Carrion crow	2/4/02	M	X	○	3.77
83	Common cormorant	14/03/01	F	○	○	4.28
84	Japanese mole	NK	FF	○	○	3.45
85	Large Japanese field mouse	NK	MMM	○	○	4.48
86	Large Japanese field mouse	NK	FFF	X	○	5.54
87	Large Japanese field mouse	NK	FFF	X	○	5.98
88	Japanese mole	18/03/02	M	○	○	4.26
89	Japanese mole	21/03/02	M	○	○	7.33
29-32	Japanese mole	10-13/06/00	MMMM	○	X	4.30
90	Northern Goshawk (Juvenile)	10/10/02	F	○	○	5.87
91	Common Buzzard	10/8/02	M	○	○	5.67
92	Soil		-	○	○	-
M1-M6	Bat (Adult)		M	○	○	5.56
M7-M12	Bat (Adult)		M	○	○	5.57
F1-F6	Bat (Adult)		F	○	○	5.58
F7-F12	Bat (Adult)		F	○	○	5.13
YM1-YM6	Bat (Young)		M	○	○	5.35
YF1-YF6	Bat (Young)		F	○	○	6.49

[a]represents, day/month/year, NK=not known, M=male and F=female

[c]OCPs represents, pesticides analyzed;[d]DXNs represents, PCDD/DFs and PCBs analyzed

Analysis: The known amount of sample was ground with Na_2SO_4 and soxhlet extracted with dichloromethane (DCM) for 16 hrs. After determination of fat % from aliquot of the extract, the remaining portion was divided into two for PCDD/DF + PCBs and organochlorine pesticides analysis, respectively. Seventeen $^{13}C_{12}$-labeled 2378- substituted PCDD/DFs congeners, twelve DL-PCBs (IUPAC Nos. 81, 77, 126, 169, 105, 114, 118, 123, 156, 157, 167 and 189) internal standards were spiked for PCDD/DF and PCBs extract. While, $^{13}C_{12}$- or $^{13}C_{6}$-labeled organochlorine pesticide internal standard was spiked for OCPs extracts. For soil sample, the sulfur was removed with $AgNO_3$-silica column. The analysis of PCDD/DF and PCBs are similar to traditional method available in several literatures. For OCPs analysis, extracted solvents were passed through florisil and eluted with DCM. The eluted solvents were rotary evaporated and DMSO method adopted to remove fat and other macro-nutrients. The DMSO-cleaned samples further cleaned with florisil fractionation with 20% DCM in hexane as first fraction and DCM as second fraction. Identification and quantification of all 10 UNEP POPs were performed using a HRGC (Hewlett Packard 6890 Series) coupled with a HRMS (Micromass Autospec- Ultima).

Results and Discussion

Dioxins and Furans: The concentrations of PCDD/DFs were found in wildlife of Ibaraki prefecture and Kanagawa prefecture, central Japan, and Kumamoto prefecture, southwest Japan (Table 2). The elevated levels were noticed in liver Japanese mole (1200-6100) and large Japanese field mouse (2600-2900) on pg/g wet weight. Next to mole species, bird liver contained considerable levels. The flying mammal bat species had lesser or similar levels to those of bird chicks. PCDDs were predominant accumulants except in large Japanese field mouse and common buzzard in which the PCDFs were similar to those of PCDDs. The ratio of PCDFs to those of 1368- and 1378-TCDF was greater and we propose to explain that incineration deposition to vegetation (e.g., grass and small herbs) and that might impacted the large Japanese field mouse those in habit in underground holes and nets in small herbs like grass and plant litter. The accumulation pattern of PCDD/DF suggested that mixed incinerator sources and chloronitrophen (CNP) and pentachlorophenol (PCP) pattern[1].

Dioxin-like PCBs: Contamination of dioxin-like PCBs was elevated in birds (Table 2). Maximum concentration was noticed in common cormorants and northern goshawk (440 and 435 ppm ww, respectively) while night heron, Japanese mole had minimum concentrations. The contamination profiles of PCBs were greatly varied in animals. Particularly, non-ortho PCBs were different in bird species and mole species. Congener composition was largely different among ecosystems: high PCDD/DFs and low PCBs in land ecosystem while low PCDD/DFs and high dioxin-like PCBs in freshwater ecosystem. Concentrations of total PCBs (major PCBs) MoCB #3, DiCB #8,#15, TrCB #18, #31, #28, #33, TeCB #44, #52, #66, #70, PeCB #95, #101, #110, HxCB #138, #149, #153, HpCB #174, #187, OCB #194, #199, #203, NCB #206 and DeCB #209 were ranged from 8.2-3000 ng/g ww (data not shown). Northern goshawk and common cormorants had maximum concentrations while night heron chick and large Japanese field mouse had minimum concentrations. The homologue pattern was different in birds versus terrestrial mammals however HxCBs were prevalent followed by PeCBs or HpCBs in most of the animals.

Toxic Equivalent Quantity (TEQ): Concentrations of sum TEQs in all animals are shown in Table 2. Common cormorants, northern goshawk, large Japanese field mouse and night heron, had TEQs of more than 200 pg/g TEQ. Except large Japanese field mouse, PCDDs were predominant TEQ contributors (Figure not shown). While PCDFs were equally contributed in large Japanese field mouse, carrion crow, common buzzard. The contribution of mono-ortho PCBs was minimal while non-ortho PCBs contributed considerable levels in common cormorants, northern goshawk and all other wildlife.

Table 2. Contamination levels of PCDD/DFs, DLPCBs and their TEQ (pg/g wet wt.) in wildlife of Japan.

Sample No.	Wildlife	PCDDs	PCDFs	Non-	Mono-	TEQ
72	Night heron	660	130	1600	130000	200
73	Night heron	1400	110	490	15000	190
74	Night heron	870	120	750	19000	260
75	Night heron (chick)	230	16	17	720	13
76	Intermediate egret	230	23	170	11000	55
77	Intermediate egret	340	33	200	15000	82
78	Intermediate egret	1100	120	1300	68000	420
79	Intermediate egret (chick)	170	22	33	1700	13
80	Intermediate egret (egg)	140	8.1	34	1500	7.7
81	Intermediate egret (egg)	610	14	62	5200	18
82	Carrion crow	200	38	10	4700	9.4
83	Common cormorant	870	570	3700	440000	970
84	Japanese mole	5900	190	210	3400	140
85	Large Japanese field mouse	1400	1200	0.70	1000	270
86	Large Japanese field mouse	1500	1100	0.60	1000	250
87	Large Japanese field mouse	1700	1200	790	1700	260
88	Japanese mole	1100	78	61	360	39
89	Japanese mole	4500	370	400	80000	190
90	Northern Goshawk	1000	366	4800	430000	880
91	Nosuri	300	370	58	920	16
M1-M6	Bat	300	67	50	2000	18
M7-M12	Bat	380	61	33	2500	17
F1-F6	Bat	380	46	27	2200	15
F7-F12	Bat	170	32	14	2700	11
YM1-YM6	Bat	160	55	47	3200	17
YF1-YF6	Bat	300	48	23	1100	13
Blank	Blank	1.02	1.3	1.0	130	0.27

Toxic Threshold: Concentrations of TEQs in herons, egret falls below the range of toxic threshold values reported for chicken, pheasant or Caspian tern eggs. While cormorants and northern goshawk shows toxic thresholds concentrations. Threshold concentrations for TEQs in livers of aquatic mammals to elicit physiological effects has been estimated to range from 160 to 1400 (mean 520) pg/g on lipid weight[7]. The mean TEQ concentration in Japanese mole (2500 pg/g lipid wt.) and large Japanese filed mouse (4900 pg/g lipid wt.) was greater 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. The measured concentrations in the livers of the moles from central Japan were above the threshold values to elicit physiological effects[7].

Organochlorine Pesticides: The concentrations OCPs in all analyzed samples are shown in Table 3. HCHs were greater (with 0.2 to 62 % to the total OCPs) in liver of birds and bat with greatest concentration was noticed in northern goshawk. The β-HCH was predominant isomer in most of the samples as it is very stable to enzymatic degradation in animal body[4-5]. In the technical HCH mixtures, the proportion of isomers were in the following order; 70%-α, 15%-γ, 6%-β and 9%-δ. Only in common cormorant, Japanese mole, large Japanese field mouse, northern goshawk, bat and soil α-HCH were detected and therefore the suggest recent local hot spot source.

Table 3. Average concentrations (ng/g wet wt.) of organochlorine pesticides in wildlife of Japan.

Animals	Weasel	Racoon	MPC*	Wild cat	GJSM*	NH*	NHC*	IE*	IEC*	IEE*	CC*	JM*	LJFM*	NG*	CB*	Soil	Bat
Compounds	(n=2)	dog (n=1)	(n=2)	(n=1)	(n=1)	(n=1)	(n=2)	(n=1)	(n=1)	(n=1)	(n=1)	(n=3)	(n=1)	(n=1)	(n=1)	(n=1)	(n=6)
α-HCH	0.05	0.05	0.51	N.D.	8.2	N.D.	N.D.	N.D.	N.D.	N.D.	0.63	0.12	0.42	0.11	N.D.	0.38	0.36
β-HCH	0.89	1.7	3.6	8.2	4.1	21	2.2	10	0.98	2.0	23	3.5	1.4	67	1.0	0.66	22
γ-HCH	0.079	N.D.	0.052	N.D.	0.22	N.D.	N.D.	N.D.	N.D.	0.06	N.D.	0.05	N.D.	0.06	N.D.	0.26	0.18
δ-HCH	N.D.	N.D.	0.16	N.D.	0.74	0.07	0.08	N.D.	N.D.	N.D.	0.23	0.03	0.17	0.08	N.D.	0.30	0.03
Sum HCHs	**1.0**	**1.8**	**4.3**	**8.2**	**13**	**21**	**2.3**	**10**	**1.0**	**2.1**	**24**	**3.7**	**2.0**	**67**	**1.0**	**1.6**	**22**
HCB	**0.4**	**0.2**	**0.3**	**0.1**	**N.D.**	**21**	**1.1**	**3.2**	**1.2**	**0.69**	**130**	**N.D.**	**1.0**	**17**	**1.5**	**4.6**	**1.1**
Aldrin	N.D.	N.D.	N.D.	N.D.	N.D.	0.14	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.33	N.D.	0.05	N.D.
Dieldrin	3.7	10	7.8	0.29	62	27	3.1	1.6	2.5	0.47	34	174	19	53	3.7	1.2	1.9
Endrin	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.47	N.D.	0.12	N.M.
Sum -drin's	**3.7**	**10**	**7.8**	**0.29**	**62**	**27**	**3.1**	**1.6**	**2.5**	**0.47**	**34**	**174**	**19**	**54**	**3.7**	**1.4**	**1.9**
Heptachlor	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.17	N.D.
Heptachlor epoxide	1.0	7.7	3.6	0.10	57	59	0.92	1.9	1.3	0.26	54	59	9.0	53	3.1	0.42	1.9
Oxychlordane	25	67	25	0.14	120	200	1.5	14	1.9	2.1	44	478	11	180	11	0.06	10
trans-chlordane	N.D.	N.D.	0.04	N.D.	N.D.	0.8	N.D.	N.D.	N.D.	0.1	0.2	1.0	1.2	1.3	0.1	2.2	N.D.
cis-chlordane	0.08	0.08	0.07	N.D.	0.65	1.1	0.49	0.57	0.19	0.15	0.11	1.9	0.53	4.1	0.15	1.5	N.D.
trans-nonachlor	0.8	1.0	12	0.3	15	110	1.6	8.8	1.6	1.5	0.1	328	39	240	2.1	1.6	0.15
cis-nonachlor	0.18	0.15	0.16	0.05	2.4	26	0.63	2.5	0.43	0.27	11	32	14	53	0.28	0.59	0.45
Sum CHLs	**27**	**76**	**40**	**0.54**	**195**	**397**	**5.1**	**28**	**5.4**	**4.4**	**109**	**899**	**75**	**531**	**17**	**6.5**	**13**
op-DDE	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.13	N.D.
pp'-DDE	8.3	0.45	41	2.4	1.0	130	2.0	25	9.6	9.8	1500	496	10	1600	5.9	5.6	20
op-DDD	N.D.	N.D.	0.12	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.05	0.07	N.D.	N.D.	0.52	0.05
pp'-DDD	2.0	0.51	14	0.73	7.2	0.66	0.24	0.23	0.30	0.03	2.0	428	3.2	24	1.4	2.0	1.1
op-DDT	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.32	N.D.
pp'-DDT	N.D.	N.D.	N.D.	0.10	2.1	N.D.	N.D.	N.D.	N.D.	0.21	N.D.	62	0.25	0.25	N.D.	2.5	1.5
Sum DDTs	**10**	**1.0**	**56**	**3.2**	**10**	**131**	**2.2**	**25**	**10**	**10**	**1502**	**986**	**14**	**1624**	**7.3**	**11**	**22**
Sum OCPs	**42**	**89**	**109**	**12**	**281**	**597**	**14**	**68**	**20**	**18**	**1799**	**2062**	**110**	**2294**	**30**	**25**	**60**

*MPC, GJSM, NH, NHC, IE, IEC, IEE, CC, JM, LJFM, NG and CB, respectively indicates Masked palm civet, Greater Japanese shrew mole, Night heron Night heron-chick, Intermediate egret, Intermediate egret-chick, Intermediate egret-egg, Common cormorant, Japanese mole, Large Japanese field mouse, Northern goshawk and Common buzzard; The values rounded; ND=not detected and NM=not measured

Hexachlorobenzene was noticed in liver of birds, bat and soil whereas terrestrial animals didn't. The metabolic capacity by specialized enzymes in terrestrial animals probably influenced their elimination. Among 3 -drin compounds, only dieldrin was detected with 0.29 to 174 ng/g ww (Table 3). The aldrin and endrin was noticed at trace levels in livers of adult night heron, northern goshawk and soil. In most of biological tissues, the aldrin metabolized into dieldrin[8]. This is the first study reports considerable concentrations of dieldrin in wildlife from Japan. The results comprehended that dieldrin had predominant bioaccumulation in wildlife than aldrin and endrin. The sporadic usage of cyclodienes as seed treatments to protect cereal grains and other seeds from insects probably increased in the animals analyzed in this study. Especially, elevated concentration of dieldrin has been noticed for the mole species, which lives in ground holes of agricultural field and in forests where the dieldrin usage seems to happen. In biological samples, dieldrin retained at greater capacity and produce mortality[9]. The percentage contribution of -drin compounds was 2 to 22% with maximum level was noticed in Japanese mole and lower in wild cat and cormorants.

The concentrations of chlordanes were in the range of 0.29 to 899 ng/g ww (Table 3). Elevated contribution of chlordane compounds was noticed in terrestrial animals, bat, northern goshawk (66 to 86%) and night heron (68%). Japanese mole and northern goshawk accumulated greater chlordanes followed by night heron, greater Japanese shrew mole and common cormorant while, wild cat had lowest levels. The soil contained all chlordane compounds with equal proportion. Heptachlor epoxide was greater in common cormorants, wild cat and greater Japanese shrew mole. Oxychlordane was prevalent in weasel, racoon dog, bat and masked palm civet. Trans-nonachlor was greater in large Japanse field mouse followed by northern goshawk and Japanese mole. In biological samples, cis/trans-nonachlor could be converted as oxychlordane[10]. In vivo and in vitro studies prevailed the immediate major metabolic product of trans-nonachlor is trans-chlordane which is further converted to 1,2-dichlorochlordene and to oxychlordane[10]. Likewise, in biological samples, heptachlor will be metabolized to heptachlor epoxide during enzymatic degradations.

The concentrations of DDTs were in the range of 1.0 to 1624 ng/g ww (Table 3). Namely, Japanese mole, common cormorant and night heron accumulated greater DDTs. While, raccoon dog liver had lowest levels. The contribution of DDTs to the sum of OCPs was 1 to 88 %. The raccoon dog showed minimum contribution while, two samples of Japanese mole and common cormorants showed greater contributions. The composition of DDT metabolites found interesting that, birds had greater composition of p,p’-DDE. The raccoon dog, masked palm civet and Japanese mole had greater composition of p,p’-DDD along with DDE. One individual Japanese mole and greater Japanese shrew mole had considerable composition of p,p’-DDT. Higher composition of p,p’-DDE in most of animals clearly suggests greater ability to transform p,p’-DDT into p,p’-DDE. Relatively larger proportion of p,p’-DDT , which is the major constituent (80%) of the technical mixture of DDT in Japanese mole and greater Japanese shrew mole, suggests that recent exposure to DDT. The greater load of p,p’-DDD in greater Japanese shrew mole, and two Japanese mole suggested its feeding habit in soil as they intake food mostly from soil habitat animals (e.g., earthworm, insects etc.,) and shelter. Several studies have reported that p,p’-DDT has been converted into p,p’-DDD by anaerobic microbes living in soil and sediment.

Literature Cited

1. Masunaga, S., Takasuga, T., Nakanishi, J. (2001) Chemosphere 44, 873-885
2. Senthil Kumar, K., Iseki, N., Hayama, S.I., Nakanishi, J. and Masunaga, S. (2002) Arch Environ Contam Toxicol 42, 244-255
3. Guruge KS, Tanabe S, Fukuda M (2000). Arch Environ Contam Toxicol 38, 509-521
4. Senthil Kumar, K., Kannan, K., Sinha, R.K., Tanabe, S., Giesy, J.P. (1999). Environ Toxicol Chem, 18, 1511-1520.
5. Senthil Kumar, K., Kannan, K., Tanabe, S., Subramanian, A.N. (2001). Environ Sci Pollut Res, 8, 35-47.
6. Yamashita, M., Takemori, H., Senthil Kumar, K., Takasuga, T. (2002). The 5th Annual Meeting of Japan Society of Endocrine Disruptors Research 25-26th November 2002, p157.
7. Kannan K, Blankenship AL, Jones PD, Giesy JP (2000). Human Ecol Risk Assess 6, 181-201
8. Muralidharan, S. (1993). Ecotoxicol, 2, 196-202
9. Olsen P, Emison B, Mooney N, Brothers N (1992). Ecotoxicol, 1, 89-100
10. Tashiro, S. Matsumura, F. (1978). Arch Environ Contam Toxicol, 7, 113-127