ACCUMULATION OF CHLORINATED AND BROMINATED PERSISTENT TOXIC SUBSTANCES (PTS) AND THEIR RELATIONSHIP TO TESTOSTERONE SUPPRESSION IN NORWAY RATS FROM JAPAN

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Introduction

Contamination of chlorinated/brominated persistent toxic substances (PTS) such as polychlorinated, -dibenzo-p-dioxins (PCDDs), -dibenzofurans (PCDFs), -biphenyls (PCBs), -organochlorine pesticides (OCPs) {e.g., aldrin, dieldrin, endrin, chlordane compounds [*cis/trans*-chlordane, *cis/trans*-nonachlor, oxychlordane, heptachlor, heptachlor epoxide], hexachlorobenzene (HCB), 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDTs) and its metabolities [*o,p/p,p'*-DDD and DDE] and hexachlorocyclohexane (HCHs)} and -brominated diphenyl ethers (PBDEs) are considered to important class of chemicals due to persistence in nature, bioaccumulation potential and adverse health effects in wildlife and humans. Among South East Asian countries, Japan reported to contaminated with aforesaid chemicals with considerable amounts[1-4].

There is no document reports contamination of PTS in wild animals, which in-habit near humans. Norway rat (NR) inhabits not only near human environment but also distributed worldwide. Especially, NR feeds on human waste and shelter in and around human environment and thus exposure of toxic contaminants in this animal considered to similar with those in humans. In addition, rats have unique physiology that match with humans (e.g., they have similar pathogens as humans have). Therefore, analysis of toxic contaminants in NR considered as indirect measure in humans. Considering those facts, in this study, we analyzed NR collected from urban area, rural area, waste dumping or land fill site and isolated remote island from Japan. Particularly several chlorinated and brominated organic contaminants such as PCDDs, PCDFs, PCBs, DDTs, HCHs, chlordane compounds, heptachlor, heptachlor epoxide, HCB, aldrin, dieldrin, endrin and PBDEs were analyzed in rat livers by isotope dilution technique using HRGC-HRMS. In addition, laboratory Wistar rats (WR) were used as control.

Materials and Methods

Norway rats (NR) or brown rat (*Rattus norvegicus*) are considered to indicator species because they are one of the most common rats that encountered around the world. Norway rat lives both as a commensal in close association with man. Consequently, in this study, NR (several individuals in each location) were collected and their age and sex was determined. The individuals that we analyzed in this study were determined as 7-week old males. For each location, 3 individual rat liver was extracted and combined for cleanup and analysis. Furthermore, NR analyzed in this study were collected from two highly urbanized localities of Tokyo (Shinjuku "Tokyo-Snj" and Ikebukuro "Tokyo-Ikb"), Osaka (Umeda "Osaka-Umd" and Nanba "Osaka-Nnb"), waste dumping or landfill site of Sapporo, "Landfill-Sp" and remote Teuri Island, Hokkaido "Teuri-Is-Hk" in 2003. In addition, 3 laboratory raised Wistar rat (WR) used as control that analyzed individually but their average data were compared to NR. Immediately after collection, all rats were dissected and liver was removed and stored at de-freezer for chemical analysis. For chemical analysis, liver was homogenized with Na_2SO_4 spiked with internal standards such as $^{13}C_{6\text{-}12}$-labeled OCPs, $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs, $^{13}C_{12}$-PCBs and $^{13}C_{12}$-PBDEs and Soxhlet extracted with dichloromethane (DCM) for 16-h. The details of sample clean were described in our previous reports[2-3]. The identification and quantification was performed using high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS)[5-6]. Two methods blank samples were also analyzed. The blank doesn't contain quantifying amount of any target contaminants. Eventually, testosterone suppression experiment was determined by gene chip method. Particularly the bio-marker enzymes such as StAR, CYP1A1/2A1, NQ01, HO1, MT, HSP70, UGT, GST, ST were measured in control WR and NR from Teuri-Is-Hk and Tokyo-Snj.

Results and Discussion

Concentrations of organochlorine pesticides (OCPs) are presented in Table 1. In control WR, contamination of OCPs was in the order of HCB>HCHs=Chlordanes=DDTs>cyclodienes. On the other hand, NR contained elevated DDTs followed by chlordanes, HCB, HCHs and cyclodienes. Particularly, NR from Tokyo-Snj contained maximum total OCPs concentrations followed by Landfill-Sp. Minimum concentrations of OCPs in control WR might be contamination of their laboratory fed diet.

Table 1. Average concentrations (pg/g lipid) of organochlorine pesticides in Norway and Wistar rats.

Rat	Wistar Rat	Norway Rat					
Sample Name	Lab. Control	Terri-Is-Hk	Landfill-Sp	Tokyo-Snj	Tokyo-Ikb	Osaka-Umd	Osaka-Nnb
	n=3	n=1	n=3	n=3	n=3	n=3	n=3
α-HCH	350	1,100	940	1,200	2,800	790	1,000
β-HCH	2,700	8,900	17,000	25,000	23,000	5,300	33,000
γ-HCH	930	320	370	370	960	560	540
δ-HCH	43	22	31	<10	65	60	49
Sum HCHs	4,000	10,000	18,000	27,000	27,000	6,700	35,000
HCB	4,700	10,000	15,000	640,000	27,000	6,200	20,000
Aldrin	<10	<10	<10	25	<10	<10	<10
Dieldrin	1,300	3,300	4,100	1,300	14,000	6,600	4,600
Endrin	70	33	22	<10	42	1,100	41
Sum -drin's	1,300	3,300	4,100	1,300	14,000	7,700	4,600
Heptachlor	380	170	120	58	280	81	110
Heptachlor epoxide	620	3,000	6,900	6,700	9,300	1,200	4,900
Oxychlordane	1,500	12,000	14,000	62,000	22,000	4,500	15,000
trans-Chlordane	650	390	370	360	1,000	450	650
cis-Chlordane	420	10,000	190	260	730	300	410
trans-Nonachlor	370	4,300	470	1,000	17,000	3,200	3,800
cis-Nonachlor	57	300	37	71	1,500	360	300
Sum Chlordanes	4,000	30,000	22,000	70,000	52,000	10,000	25,000
o,p'-DDE	77	260	240	2,100	2,600	340	270
p,p'-DDE	1,100	14,000	190,000	2,200,000	65,000	12,000	51,000
o,p'-DDD	49	130	370	2,300	6,700	300	380
p,p'-DDD	1,400	6,500	120,000	140,000	56,000	3,600	79,000
o,p'-DDT	110	82	170	2,600	1,000	200	180
p,p'-DDT	1,300	14,000	220,000	1,100,000	81,000	8,500	190,000
Sum DDTs	4,000	35,000	530,000	3,400,000	210,000	25,000	320,000

Detection limit 10 pg/g; The values rounded.

Compositions of HCHs were predominated by β isomers (>80%) in NR and WR (<80%). Dieldrin was predominant among cyclodienes irrespective to rat species. Composition of chlordane compounds in NR was differed depending upon sampling locations. Particularly, slightly greater cis-chlordane in Teuri-Is-Hk was attributed to the source from migratory birds. Teuri Island located near Hokkaido however, human activities are less (<500 people) this leads to arrival of about 1-million migratory birds for wintering. There is no government record of chlordane usage in and around Hokkaido. Perhaps, imported chlordane probably used by the in habitants in and around Hokkaido might have influenced their occurrence in Landfill-Sp. DDTs and its metabolites were major contaminants among OCPs with contribution ranged from 22% to 90% to total OCPs load. Greater DDTs contributions in urbanized regions such as Tokyo and Osaka suggest prolonged persistency of these chemicals in soil.

Concentrations of PCBs were greater than the organochlorine pesticides (Table 2). The average concentrations of PCBs were elevated with a contribution range from 34% to 55% to the total POPs/PBDE load. Again, NR from Tokyo-Snj had maximum PCBs concentrations whereas; minimum PCBs was observed in control WR. HxCBs were predominant homologues followed by HpCBs and PeCBs. Particularly, dioxin-like PeCB-118 was predominant contaminants in most rat while, HxCB-156 was greater in Landfill-Sp. Norway rat feed cables, electrical appliances, plastic and therefore PCB can easily ingested when they feed in and around electrical transformers/capacitors that runs in under grounds in Tokyo Metropolitan.

Table 2. Average concentrations (pg/g lipid) of PCBs in Norway and Wistar rats.

Rat	Wistar Rat	Norway Rat					
Sample Name	Lab. Control	Terri-Is-Hk	Landfill-Sp	Tokyo-Snj	Tokyo-Ikb	Osaka-Umd	Osaka-Nnb
Coplanar PCBs							
TeCB #81	<10	39	41	270	260	83	150
TeCB #77	73	30	20	18	150	75	65
PeCB #126	45	1,100	2,000	53,000	3,700	1,100	3,000
HxCB #169	14	330	1,000	23,000	790	180	920
PeCB #123	9.5	40	22	580	620	83	81
PeCB #118	840	5,300	7,500	190,000	26,000	8,100	17,000
PeCB #105	290	1,300	1,700	23,000	8,100	2,600	5,400
PeCB #114	13	120	330	5,900	560	210	430
HxCB #167	30	360	330	10,000	2,200	560	1,100
HxCB #156	72	970	15,000	160,000	4,200	1,700	6,200
HxCB #157	28	290	4,500	44,000	1,300	410	1,600
HpCB #189	14	190	2,400	36,000	620	240	1,200
Sum Coplanar PCBs	1,400	10,000	30,000	550,000	49,000	15,000	37,000
Total PCBs							
MCBs	58	16	20	9.6	48	21	22
DiCBs	920	230	330	220	590	340	430
TrCBs	1,500	500	770	530	3,100	960	1,500
TeCBs	2,000	4,200	3100	19,000	31,000	5,100	7,600
PeCBs	5,300	26,000	57000	1,000,000	120,000	30,000	54,000
HxCBs	4,500	36,000	350000	4,100,000	170,000	56,000	180,000
HpCBs	1,600	16,000	170000	1,600,000	73,000	30,000	150,000
OCBs	330	3,500	33000	340,000	14,000	8,300	51,000
NCBs	57	790	5100	53,000	3,700	1,700	6,200
DeCB	53	520	6900	45,000	1,900	470	1,200
Total PCBs	16000	88,000	630,000	7,200,000	420,000	130,000	450,000

Detection limit 10 pg/g; The values rounded

Table 3. Average concentrations (pg/g lipid) of PCDDs and PCDFs in Norway and Wistar rats.

Rat	Wistar Rat	Norway Rat					
Sample Name	Lab. Control	Terri-Is-Hk	Landfill-Sp	Tokyo-Snj	Tokyo-Ikb	Osaka-Umd	Osaka-Nnb
PCDD/DFs							
2378-TeCDD	<1	5.5	22	110	24	7.7	22
12378-PeCDD	2.9	32	110	660	130	38	140
123478-HxCDD	2.4	36	180	180	120	51	220
123678-HxCDD	26	120	770	630	420	210	760
123789-HxCDD	4.9	56	140	150	120	62	180
1234678-HpCDD	190	680	2,000	1,000	1,400	850	3,000
OCDD	470	4,200	2,700	2,500	5,600	2,400	6,200
Sum PCDDs	**700**	**5,100**	**5,900**	**5,200**	**7,800**	**3,600**	**11,000**
2378-TeCDF	2.1	9.4	9.2	48	100	26	41
12378-PeCDF	1.3	5.9	5.9	25	48	16	33
23478-PeCDF	8.7	170	1,100	2,200	960	410	1,700
123478-HxCDF	9.7	140	710	490	530	280	1,300
123678-HxCDF	6.1	140	900	560	620	320	1,500
123789-HxCDF	<1	1.9	2.0	4.1	7.3	2.8	7
234678-HxCDF	5.7	160	570	430	590	280	1,400
1234678-HpCDF	21	300	1,300	480	810	510	2,500
1234789-HpCDF	3.2	63	190	60	110	71	350
OCDF	12	130	130	73	140	94	260
Sum PCDFs	**28**	**1,100**	**5,300**	**4,400**	**3,900**	**2,000**	**9,100**
Sum PCDD/DFs	**730**	**6,200**	**11,000**	**9,600**	**12,000**	**5,600**	**20,000**

Detection limit 1 pg/g; The values rounded.

The contamination of PCDDs and PCDFs were minimum (Table 3) with contribution of <2% to the total POPs/PBDE load. Maximum PCDD/DF concentrations have been noticed in NR from Osaka-Nnb followed by Tokyo-Ikb and Landfill-Sp while, Tokyo-Snj samples showed moderate concentrations. OCDD was greater in WR, while toxic congeners such as 23478-PeCDF, 1234678-HpCDD, 1234678-HpCDF was abundant in NR. The incinerator fly ash may be possible explanation for PCDD/DFs in NR. Furthermore varied food intake and/ or composition seems to probably explanation for slight geographical variation.

Greater concentrations of PBDEs also noticed in NR (Table 4). Particularly, NR from Tokyo-Snj had maximum concentrations followed by Landfill-Sp. The contribution of PBDEs was 1%-28% to the total POPs/PBDE load. DeBDE was prevalent homologue in WR and NR from Teuri Is-Hk and Osaka. HxBDE-153 was greater in Landfill-Sp and Tokyo-Snj. The plastic samples dumped in landfill probably influenced greater HxBDE-153. The other predominant PBDE congeners in this study were TeBDE-47, DeBDE-209, HpBDE-183, PeBDE-99, -100 which, is somewhat different than other countries due to greater usage of DeBDE in Japan, while PeBDE was mainly used in other countries.

Table 4. Average concentrations (pg/g lipid) of PBDEs in Norway and Wistar rats.

Rat	Wistar Rat	Norway Rat					
Sample Name	Lab. Control	Terri-Is-Hk	Landfill-Sp	Tokyo-Snj	Tokyo-Ikb	Osaka-Umd	Osaka-Nnb
TrBDE#28	46	45	350	77	370	110	130
TeBDE#49	38	<1	37	14	45	<1	<1
TeBDE#47	970	1,200	13,000	21,000	17,000	1,800	4,600
PeBDE#100	260	300	3,900	11,000	5,900	410	1,400
PeBDE#119	<1	<1	<1	1,600	170	<1	<1
PeBDE#99	330	160	2,900	2,600	3,900	530	1,200
PeBDE#85	38	N.D.	350	<1	310	34	81
HxBDE#154	<1	24	470	1,500	370	47	260
HxBDE#153	280	290	41,000	88,000	6,500	710	6,800
HxBDE#139	<1	<1	410	<1	270	24	110
HxBDE#138	<1	<1	1,100	340	370	32	200
HpBDE#183	100	160	12,000	1,800	1,100	750	5,700
HxBDE#197	180	290	22,000	2,100	1,200	1,300	4,800
HxBDE#196	<1	35	6,300	390	180	640	1,700
HxBDE#208	<1	110	3,600	700	340	1,600	2,000
HxBDE#207	470	310	8,400	2,000	660	4,100	3,100
DeBDE#209	6,000	3,600	27,000	7,500	11,000	73,000	26,000
Sum Major PBDEs	**8,700**	**6,500**	**140,000**	**140,000**	**47,000**	**77,000**	**46,000**
MBDEs	<1	<1	<1	<1	<1	<1	<1
DiBDEs	<1	<1	31	16	<1	<1	<1
TrBDEs	84	50	390	77	370	120	150
TeBDEs	970	1,200	13,000	21,000	17,000	1,800	4,600
PeBDEs	700	550	8,400	19,000	13,000	1,200	3,300
HxBDEs	330	350	43,000	95,000	7,900	880	8,400
HpBDEs	100	200	18,000	9,100	1,700	1,000	9,800
OBDEs	180	760	17,000	29,000	2,800	3,800	16,000
NBDEs	470	420	4,300	2,900	1,000	6,200	5,100
DeBDE	6,000	3,600	27,000	7,500	11,000	73,000	26,000
Total PBDEs	**8,900**	**7,100**	**130,000**	**180,000**	**55,000**	**88,000**	**73,000**

Detection limit 1 pg/g; The values rounded.

Since, Norway rats live in close association with humans, contamination pattern in this species indirectly reflects the levels in humans since they feed mostly on human waste. NR will eat nearly any type of food. When given a choice, they select a nutritionally balanced diet, choosing fresh, wholesome items over stale or contaminated foods. They prefer cereal grains, meats and fish, nut, and some types of fruit. Food items in household garbage offer a fairly balanced diet and also satisfy their moisture needs. Based on this information's humans from Tokyo-Snj and humans lives near Landfill-Sp expected to intake comparatively greater organic chemicals. The result also reveals that NR could be used as bio-indicator species in order to understand the occurrence of toxic contaminants in and around human environment.

Table 5. Average WHO-TEQ concentrations (pg/g lipid) of PCDDs, PCDFs and dioxin-like PCBs in wild Norway rat and laboratory control Wistar rats from Japan.

Rat	Wistar Rat	Norway Rat					
Sample Name	Lab. Control	Terri-Is-Hk	Landfill-Sp	Tokyo-Snj	Tokyo-Ikb	Osaka-Umd	Osaka-Nnb
Sum PCDDs TEQ	8.6	66	260	880	240	87	310
Sum PCDFs TEQ	7.1	130	780	1,300	680	310	1,300
Sum NO[1] PCBs TEQ	4.7	110	210	5,500	380	110	310
Sum MO[2] PCBs TEQ	0.17	1.4	11	130	6.6	2.3	6.5
Total TEQ	21	310	1,300	7,800	1,300	510	1,900

The values rounded; [1]non-ortho PCBs; [2]mono-ortho PCBs.

The WHO-TEQ (mammalian TEF) was calculated in this study (Table 5). The TEQ concentrations (pg/g lipid) were in the decreasing order of 7,800 (Tokyo-Snj), 1,900 (Osaka-Nnb), 1,300 (Tokyo-Ikb, Landfill-Sp), 510 (Osaka-Umd), 310 (Teuri-Is-Hk) and 21 (Wistar rat). Except Tokyo-Snj NR, PCDD/DFs were greater TEQ contributors in rats from other places and control WR. The highest mean TEQ (7,800 pg-TEQ/g lipid, with coplanar PCBs comprise >70%) in livers of Tokyo-Snj NR. The observed TEQ is comparable to those in fish-eating mammals (e.g., seals and whales) which were reported to possess high concentrations of lipophilic chemicals. Greater proportion of non-ortho PCBs TEQ in NR is localized. Perhaps, we do not know weather all three NR from Tokyo-Snj have similar concentration or only one individual among 3 mixed extracts had elevated levels and thus, further study is suggested with individual rats that collected from various locations.

Testosterone effect in NR was measured using mRNA bio-marker enzymes such as StAR, CYP1A1/2A1, NQ01, HO1, MT, HSP70, UGT, GST, ST that measured in WR and NR from Teuri-Is-Hk and Tokyo-Snj, respectively by gene chip method. The mRNA expression levels of cytochrome P450 1A subfamily in NR from urban and landfill areas were higher than that of control WR. Therefore we assume that reproductive effect might be probable in Tokyo-Snj NR. Particularly, our results show a significant ($p>0.01$) suppression of mRNA expression of testosterone synthetic enzymes and StAR in testes of Tokyo-Snj NR which had higher TEQ. Consequently, the testosterone levels in Tokyo-Snj were significantly lesser (Figure 1) than Teuri-Is-Hk and control WR and therefore, the current coplanar PCBs level seems have negative implications on testis in NR from Tokyo-Snj region. Furthermore, humans lives in this regions expected to intake greater TEQ.

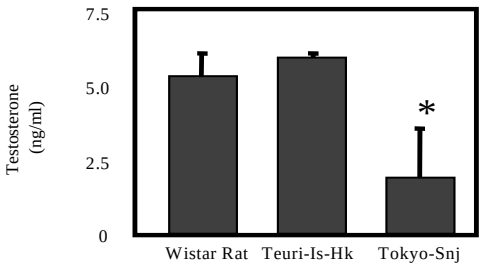

Figure 1. Testosterone levels in Norway rat and Wistar rat from Japan (*denotes significantly different/reduced).

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