POLYCHLORINATED DIBENZO-P-DIOXINS AND FURANS, POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN THE BROW BEAR POPULATION IN SLOVENIA

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Introduction

The brown bear (*Ursus arctos*) is the most widespread bear species in the world[1]. Its distribution covers a vast range of habitats from northern arctic tundra to dry desert. The Slovenian brown bear population is one among the few European countries with preserved viable indigenous brown bear population[2].

The dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are man made compounds, highly persistent, toxic and globally transported from the point sources. Since these compounds are accumulated in the living beings mostly with food intake man and bear who stand close at the top of the food chain are highly exposed to their bio-accumulation effects[3,4]. The PCDD/Fs, PCBs and OCPs levels have been studied in the polar bear (*Ursus maritimus*)[3-5] population, while only very limited information on these compounds was presented for the brown bear[6]. The present data is a result of continuation of the investigation of the PCDD/Fs, PCBs and OCPs contents in adipose tissues of the brown bear in Slovenia. It provides valuable data on the PCDD/Fs, PCBs and OCPs contamination level in the wild life for the central European area.

Sample collection and preparation: Samples of adipose tissue (15 samples) were collected from the individual brown bears culled in Slovenia according to the designated Large Beast Management Plan in the year 2004 and in the spring of 2006. The subcutaneous skin fat samples have been taken from lateral thigh. Samples were homogenized and frozen at -20^{o}C until the analysis.

Extraction, Clean-up and Analysis (PCDD/Fs, PCBs): Used isotope dilution method was based on US EPA 1613B protocol. The clean-up was carried out firstly with fat saponification, than multilayer silica column, gel permeation chromatography, carbon columns using Carbopack C. Purified extracts were analyzed on a HP 6890 GC (Hewlett-Packard) coupled to a Finnigan MAT 95PL (Finnigan) high resolution mass spectrometer. The column used was a JW-DB-5MS+DG (60m x 0.25mm I.D., 0.25µm film thickness). The mass spectrometer operates at the electron impact ionization mode (EI) using selected ion monitoring (SIM) at a minimum resolution of 10.000 (10% valley). In this validated method, reference materials, blanks (both instrumental and method) and the "in-house" quality control samples were included in the analysis scheme to ensure the control of the analysis. TEQs were calculated using WHO TEFs[7].

Extraction, Clean-up and Analysis (OCPs): A DMSO based matrix solid-phase dispersion extraction and clean-up column chromatography procedure was used. A Pasteur mini-column, packed with the sample-Celite mixture powder, is joined to the second column of 5g 15% water deactivated slurry filled Florisil. For the elution of organochlorine pesticides from the mini column DMSO is used. After the liquid-liquid extraction in the upper part of the second Florisil column the adsorption chromatography in the lower part goes on. The pesticides are then eluted with 75 ml mixture of hexane/diethyl ether (70:5, v/v). A gas chromatograph HP 5890 Serial II equipped with

a split/splitless injector is used and a dual column chromatography coupled to two ECD follows. Two capillary columns of different polarities, HP-5MS and DB-1701 are simultaneously used to obtain a higher level of confidence in analyte identification.

Results and Discussion

The latest estimate of the brown bear (*U. arctos*) population size for Slovenia is that about 500-700 bears[8] live in range of 5300 km^2. The "Management Strategy of the Brown Bear in Slovenia"[9] with its underlying action plans for preserving and control of the brown bear population has created a unique opportunity for collecting the brown bear tissue samples and the PCDD/F, PCB and OCP assessment. The study includes adipose tissue samples of 15 animals of different age and gender.

Analyses of residue concentrations of persistent OCPs in the samples shows (Table 1) that no contamination status of pesticides in brown bear from Slovenia can be proved. The concentration level for all twenty six OCPs in the 15 adipose tissue samples was less than the limit of determination (0.01 mg kg^{-1} lipid wt.) for each OCP.

Table 1. Concentrations of organochlorine pesticides from brown bear adipose tissues.

Pesticides	Concentration (mg kg^{-1} lipid wt.)	Pesticides	Concentration (mg kg^{-1} lipid wt.)
HCB	<0.01	chlordane-cis	<0.01
alpha-HCH	<0.01	chlordane-trans	<0.01
beta-HCH	<0.01	o,p'-DDE	<0.01
gamma-HCH (lindane)	<0.01	p,p'-DDE	<0.01
tecnazene	<0.01	o,p'-DDD	<0.01
quintozene	<0.01	p,p'-DDD	<0.01
heptachlor	<0.01	o,p'-DDT	<0.01
heptachlor epoxide-cis	<0.01	p,p-DDT	<0.01
heptachlor epoxide-trans	<0.01	endosulfan I	<0.01
aldrin	<0.01	endosulfan II	<0.01
dieldrin	<0.01	o,p'-methoxychlor	<0.01
endrin	<0.01	p,p –methoxychlor	<0.01
isodrin	<0.01	mirex	<0.01

The average concentration of the PCCD/F detected in the 15 adipose tissue samples was 0,57 pg TEQ/g fat. Compared to the levels of PCDD/Fs published for the polar bear (*U. maritimus*) [3-5] the analysis results in our study (Table 2) are 10 times lower than the reported values for polar bear. Also the PCBs quantities detected in our study were significantly lower from those reported in polar bears; the PCBs mean concentration was 1,3 pg TEQ/g fat which is aprox. 1.000 below the values typically reported for the polar bear. One of the reasons for lower quantities of PCDD/Fs and PCBs detected in the adipose tissue of the brown bear could be the differences in the environment and the nourishment habits of both species; while the polar bear primarily feeds on meat, brown bear consumes up to 95% of vegetal food, depending on the season and availability of the food in the living area[7].

Table 2. Concentrations of PCDD/Fs, non-*ortho* and mono-*ortho* PCBs in Slovenian brown bear (*Ursus arctos*) fat tissue samples

PCDD/Fs PCBs Concentration (pg g^{-1} lipid wt.)	(male, 154,5,03/06)[a]	(male, 65,1,04/04)[a]	(female, 56,2,04/04)[a]	(male, 41,2,04/04)[a]	(male, 210,8,03/06)[a]	(male, 75,2,03/06)[a]	(female, 105,8,05/04)[a]	(female, 74,1,04/04)[a]	(male, 80,2,10/04)[a]	(male, 161,5,10/04)[a]	(female, 95,10,05/04)[a]	(female, 83,3,11/04)[a]	(male, 114,4,11/04)[a]	(female, 105,3,11/04)[a]	(female, 117,6,11/04)[a]	average
2,3,7,8-TCDD	0,11	<0,05	<0,05	0,06	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
1,2,3,7,8-PCDD	0,36	0,27	0,24	0,19	0,14	0,17	0,19	0,13	0,08	0,10	0,10	0,08	0,06	0,06	0,05	0,15
1,2,3,4,7,8-HxCDD	1,1	1,2	0,15	0,06	0,16	0,09	0,10	0,11	<0,05	0,09	0,12	<0,05	<0,05	0,08	<0,05	0,22
1,2,3,6,7,8-HxCDD	1,1	1,2	0,55	0,46	0,37	0,43	0,40	0,25	0,18	0,23	0,16	0,12	0,12	0,14	0,09	0,39
1,2,3,7,8,9-HxCDD	0,36	0,26	0,11	0,20	0,08	0,09	0,08	0,07	<0,05	<0,05	0,05	<0,05	<0,05	<0,05	<0,05	0,09
1,2,3,4,6,7,8-HpCDD	1,8	8,0	1,4	0,88	1,0	1,0	0,90	1,30	0,50	0,48	0,48	0,49	0,44	0,40	0,23	1,3
OCDD	2,3	42	0,84	1,0	1,2	2,6	1,4	2,2	0,70	0,34	1,2	1,3	0,56	0,66	0,42	3,9
2,3,7,8-TCDF	0,30	<0,05	<0,05	0,06	0,06	0,09	<0,05	0,06	0,21	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	0,05
1,2,3,7,8-PCDF	0,08	<0,05	<0,05	0,09	<0,05	<0,05	<0,05	<0,05	0,07	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
2,3,4,7,8-PCDF	1,1	0,58	0,64	0,64	0,58	0,49	0,43	0,43	0,40	0,34	0,22	0,27	0,24	0,15	0,15	0,44
1,2,3,4,7,8-HxCDF	0,18	0,22	0,18	0,14	0,13	0,09	0,11	0,13	0,09	0,08	0,06	<0,05	0,06	0,06	<0,05	0,10
1,2,3,6,7,8-HxCDF	0,33	0,38	0,30	0,26	0,21	0,18	0,19	0,23	0,10	0,12	0,09	0,07	0,10	0,05	<0,05	0,17
2,3,4,6,7,8-HxCDF	0,39	0,41	0,27	0,20	0,17	0,16	0,12	0,13	<0,05	0,09	0,08	<0,05	<0,05	<0,05	<0,05	0,13
1,2,3,7,8,9-HxCDF	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
1,2,3,4,6,7,8-HpCDF	0,49	6,6	0,27	0,23	0,22	0,31	0,19	0,35	0,14	0,12	0,17	0,20	0,20	0,09	0,07	0,64
1,2,3,4,7,8,9-HpCDF	<0,05	0,20	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
OCDF	0,13	6,3	<0,1	<0,1	<0,1	0,15	<0,10	0,39	<0,10	<0,1	0,1	0,17	<0,10	<0,1	<0,1	0,48
[UB]TEQ PCDD/Fs	1,4	1,1	0,80	0,73	0,62	0,60	0,58	0,52	0,42	0,41	0,34	0,32	0,29	0,25	0,23	0,57
PCB 81	1,6	0,37	1,6	0,63	0,71	0,11	0,21	< 0,1	0,14	0,15	<0,1	< 0,1	< 0,1	<0,1	<0,1	0,37
PCB 77	2,2	2,4	3,0	1,2	2,0	1,0	0,95	1,4	2,1	0,66	0,68	0,67	0,56	0,55	0,29	1,3
PCB 126	38	25	6,1	12	8,6	2,6	5,5	2,0	1,0	4,1	1,1	0,93	0,73	0,53	0,66	7,3
PCB 169	35	14	9,1	9,0	10	8,7	5,0	4,2	2,7	5,9	6,2	5,3	4,2	3,7	0,76	8,2
PCB 105	1100	440	400	350	330	210	230	110	52	190	110	96	45	55	30	250
PCB 114	150	84	45	39	39	21	44	11	5,7	28	15	13	6,1	6,2	4,0	34
PCB 118	4800	2450	1500	1400	1500	720	1500	370	170	1000	380	320	150	170	150	1100
PCB 123	66	28	26	22	17	4,9	14	4,1	2,5	11	4,3	3,5	2,3	2,3	2,1	14
PCB 156	2000	1180	670	790	510	570	340	460	190	420	400	370	270	220	40	560
PCB 157	280	227	150	170	99	140	77	140	57	91	91	93	71	58	8,9	120
PCB 167	640	378	94	170	110	68	86	36	19	63	24	18	16	15	11	120
PCB 189	320	283	110	150	92	120	50	8,4	2,6	56	84	81	66	44	6,9	98
[UB]TEQ PCBs	6,0	3,7	1,3	2,0	1,5	0,82	1,0	0,60	0,28	0,86	0,48	0,44	0,32	0,26	0,12	1,3
[UB]TEQ PCDD/Fs+PCBs	7,4	4,9	2,1	2,7	2,1	1,4	1,58	1,1	0,70	1,3	0,82	0,76	0,61	0,50	0,34	1,9

[UB] Upper bound, [a] (gender, weight, approximate age, time)

The brown bear tissue samples analyzed show significant pattern in the distribution of PCDD/Fs and PCBs according to the gender of the sampled specimen and the period of the sample uptake. The PCDD/Fs levels between the samples taken from male bears (8 samples) are in average 1,6 times larger than the PCDD/Fs quantities in female bears (7 samples). The difference is even more pronounced for PCBs, where the average quantity detected in male subjects exceeds the female ones for 3,2 times. Higher contents PCDD/Fs and PCBs levels in male subject has also been observed and explained by other authors[3,10].

The differences can also be observed between PCDD/Fs and PCBs contents of the samples collected in spring and those collected in autumn. The spring samples (8) collected form March till May exceed the autumn ones (7 samples) for 2,3 times in case of PCDD/Fs contents and for over 5 times when PCBs contents are compared. The difference is due to the mass of subcutaneous fat accumulation towards the autumn period which dilutes the compounds within.

Some levels of PCDD/Fs and PCBs in the adipose tissues of the species at the top of the food chain are inevitable according to the constant presence of these substances in the environment and their persistent nature. The present study demonstrates that the quantities of the PCDD/F, PCB and OCP measured in the adipose tissue samples of the Slovenian brown bear (*U. arctos*) stand well bellow the levels of these compounds in the polar bear (*U. maritimus*). The study also revealed that sampling origin and time in case of adipose tissue collection can have significant influence on the results obtained.

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