Dioxins in the adipose tissue of the Slovenian brown bear (Ursus arctos)

Žiga Bolta[1], Boštjan Križanec[2], Ernest Vončina[3], Marko Jonozovič[4]

[1]National Chemicals Bureau
[2]University of Maribor, Faculty of Mechanical Engineering
[3]Environmental Protection Institute
[4]Slovenia Forest Service

Introduction

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) are ubiquitous environmental contaminants and toxic to the living beings. The toxicity is extensively described elsewhere [1,2]. The PCDD/Fs most threatening characteristic may be the bio-accumulation up the food chain, where the bear as well as human stand at the top and are therefore exposed to relatively highly contaminated nutrition with the PCDD/Fs. These facts are raising the concern and the demand for improved knowledge of the PCDD/Fs levels in the nature.

Solid scientific evidence on contamination with PCDD/Fs was presented for the polar bear (*Ursus maritimus*) [3-5], while PCDD/Fs levels in the brown bear (*Ursus arctos*) population, which once lived throughout the entire European continent, has remain undetermined. Slovenia is one among the few European countries with preserved viable indigenous brown bear (*Ursus arctos*) population[6]. The forested south-east of country shelters the core habitat of the Slovene bear population[6]. The area presents the essential connection of the large habitat ranging form the Greek Pindus Mountains over Dinaric Mountains into the Alps and central Europe.

The "Management Strategy of the Brown Bear (*Ursus arctos*) in Slovenia"[7] 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. Our investigation of the PCDD/Fs contents in adipose tissues of the brown bear presents the first information of the PCDD/Fs in the wild life of Slovenia and provides valuable data of the PCDD/Fs contamination level in the central Europe.

Methods and Materials

Sample collection and preparation: Samples of adipose tissue were collected from the brown bear culled in the south-eastern part of Slovenia in March 2004. The samples have been taken from kidney fat and subcutaneous skin fat from the upper leg of more than two years old male bear, weighing 135 kg. The tissue samples were homogenized and divided into adequate portions. The portions were frozen in -20^{o}C until the analysis.

Extraction, Clean-up and Analysis: Isotope dilution method used 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, Palo Alto, CA, USA) coupled to a Finnigan MAT 95PL (Finnigan, Bremen, Germany) high resolution mass spectrometer. The column used was a JW-DB-5MS+DG (60m x 0.25mm I.D., 0.25µm film thickness) capillary column. The mass spectrometer operates at the electron impact ionization mode using selected ion monitoring (SIM) at a minimum resolution of 10.000 (10% valley). In addition to daily sensitivity and relative response factor (RRF) checks, the mean RRF are regularly re-evaluated for each congener. 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. Samples were analyzed for the PCDDs and PCDFs. TEQs were calculated using WHO TEFs[8].

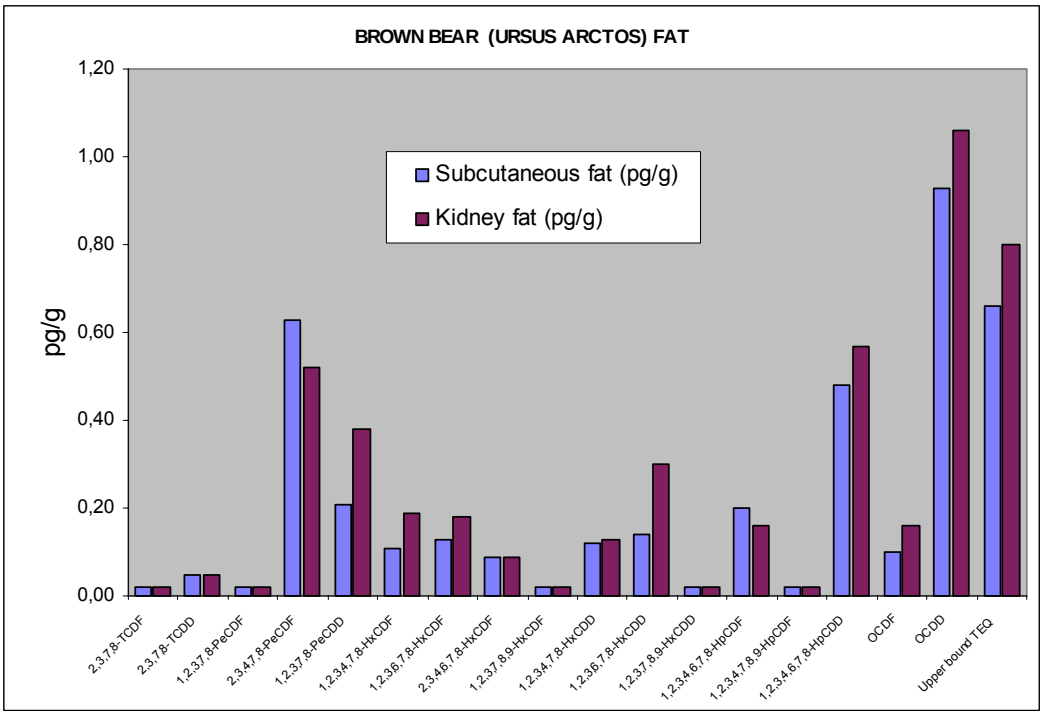
Results and Discussion

Estimation of the brown bear population in Slovenia is 450-550 animals[7] in the range of 5300 km^2. To sustain the long-term quality of the living conditions for the bear, its long-term conservation and safe coexistence bear and man, the encroachments into the bear population have been foreseen under "The Management Strategy of the Brown Bear (*Ursus arctos*) in Slovenia"[7]. The scope of the brown bear extraction, foreseen in the encroachment action plans, has created a valuable source of a regular number of the brown bear tissue samples and thus enabled a high quality investigation. The results in Table 1 present the analyses of the first set of the brown bear adipose tissue samples.

Table 1: Concentration levels of PCDD/Fs in adipose tissue of the brown bear (*Ursus arctos*).

Congeners	WHO TEFs[8]	Subcutaneous fat (pg/g)	Kidney fat (pg/g)
2378 TCDD	1	<0.05	<0.05
12378 PnCDD	1	0.21	0.38
123478 HxCDD	0.1	0.12	0.13
123678 HxCDD	0.1	0.14	0.30
123789 HxCDD	0.1	<0.05	n.d.(<0.02)
1234678 HpCDD	0.01	0.48	0.57
OCDD	0.0001	0.93	1.06
2378 TCDF	0.1	n.d.(<0.02)	n.d.(<0.02)
12378 PnCDF	0.05	n.d.(<0.02)	n.d.(<0.02)
23478 PnCDF	0.5	0.63	0.52
123478 HxCDF	0.1	0.11	0.19
123678 HxCDF	0.1	0.13	0.18
234678 HxCDF	0.1	0.09	0.09
123789 HxCDF	0.1	n.d.(<0.02)	n.d.(<0.02)
1234678 HpCDF	0.01	0.20	0.16
1234789 HpCDF	0.01	n.d.(<0.02)	n.d.(<0.02)
OCDF	0.0001	<0.1	0.16
Upper bound sum TEQs (pg/g)		0.66	0.80
Middle bound sum TEQs (pg/g)		0.63	0.77
Lower bound sum TEQs (pg/g)		0.59	0.74

Figure 1: Contribution of Tetra- to Octa homologues of 2,3,7,8-substituted PCDD/F congeners in brown bear (*Ursus arctos*) and TEQs for subcutaneous and kidney fat samples. PCDD/Fs non-detected were set to LOD (0.02pg/g).



Analysis of the subcutaneous and kidney fat shows a considerable quantity of PCDD/Fs. The PCDD/Fs quantities measured are well above the quantification levels. Higher PCDD/Fs levels have been detected in the kidney fat (Figure 1). Compared to the levels of PCDD/Fs published for the polar bear[3,4,6] the analysis results for the brown bear in our study stand in the lower range of the reported values. Lower PCDD/Fs detected in the adipose tissue of the brown bear could be explained through its different nourishment; while the polar bear primarily feeds on meat, brown bear can seasonally consume up to 95% of vegetal food, depending on availability of the food in the living area[7]. The quantities of the PCDD/Fs measured in the brown bear tissue samples from our study, lie inside the range of the PCDD/Fs detected in the foodstuffs[9]. According to the results obtained, regular control of the persistent toxic substances in the wild life should be performed with careful consideration of the tissue sample source for the analysis.

Acknowledgements

The collection of the brown bear tissue samples has been initiated by the Ministry of health of the Republic of Slovenia under UNEP GEF project (No. GF/2732-02-4463) for the preparation of the National Implementation Plan for Persistent Organic Pollutants. The brown bear fat samples have been provided by the Slovenia Forest Service.

References

1 Safe S. (1990) Crit. Rev. Toxicol. 21, 51.

2 Tanabe S., Iwata H. and Tatsukawa R. (1994) Sci. Tot. Environ. 154, 163.

3 Norstrom R.J., Simon M. and Muir D.C.G. (1990) Environ. Pollut. 66, 1.

4 Oehme M., Biseth A., Schalbach M. and Wiig Ø. (1995) Environ. Pollut. 90, 401.

5 Kumar K.S., Kannan K., Corsolini S., Evans T., Giesy J.P., Nakanishi J. and Masunaga S. (2003) Environ. Pollut. 119, 151.

6 Jerina K., Debeljak M., Džeroski S., Kobler A. and Adamič M. (2003) Ecological Modelling 170, 453.

7 The Management Strategy of the Brown Bear (*Ursus arctos*) in Slovenia. Adopted by Slovene Government on Feb. 24th 2002, www.sigov.si/mkgp/slo/1_603_rjavi_medved.php.

8 Van den Berg M., Birnbaum L., Bosveld A.T.C., Brunstrom B., Cook P., Feeley M., Giesy J.P., Hanberg A., Hasegawa R.,Kennedy S.W., Kubiak T.J., Larsen J.C., Rolaf van Leeuwen F.X., Liem A.K.D., Nolt C., Peterson P.E., Poellinger L., Safe S., Schrenk D., Tillitt D., Tysklind M., Younes M., Waern F. and Zacharewski T. (1998) Environ. Health Perspect. 106, 775.

9 Programme of the official survey of the foodstuffs, products and substances in contact with foodstuffs - final report for the year 2003, p25.