

## Levels of PCDDs, PCDFs, and PCBs in the blood of the non-occupationally exposed residents living in the vicinity of a chemical plant, comparison with a background level for the Czech population

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### Introduction

The Czech Republic ranks among the countries with a relatively high body burden of PCBs that used to be produced in former Czechoslovakia, more precisely in Slovakia, until 1984 when the production was abolished<sup>1,2</sup>. In 1994, nation-wide Environmental Health Monitoring System was implemented in the Czech Republic<sup>3</sup>. Indicator PCB congeners and selected chlorinated pesticides started to be monitored in human body fluids and tissues of the Czech population. The indicator PCB levels in breast milk samples showed a significant downward trend in time<sup>4</sup> with regional inter- and intra-individual variability. However, to measure the population body burden of dioxins, blood is considered to be more appropriate than other body fluids. Little has been known about body burden of PCDDs, PCDFs and dioxin-like PCBs in the Czech population. Sporadic data are only available on level of dioxins in the breast milk<sup>4-7</sup>, or for several pooled blood samples analyzed within the Environmental Health Monitoring System.

More than 35 years ago, approximately 80 Czech workers were occupationally exposed to 2,3,7,8-TCDD in a chemical factory producing chlorinated herbicides and pesticides in Central Bohemia. Still in 1996, they showed a mean 2,3,7,8-TCDD plasma level of 256 pg/g fat<sup>8</sup>. It was supposed that also the residents living surrounding the plant might be at increased exposure risk.

The objective of this study was (a) to investigate concentrations of PCDDs/PCDFs/PCBs in blood samples from non-occupationally exposed residents living in the vicinity of a chemical plant and (b) to compare the results with the background concentrations available for PCDDs/PCDFs/PCBs in blood samples from the evidently non-exposed Czech population.

### Methods and Materials

**Study population and blood collection:** Altogether 60 residents from three small cities- Neratovice, Libiš, Tišice - (twenty subject from each) situated near a chemical plant were chosen by a random procedure. Twenty blood donors living in a city at about 80 km from the chemical plant were taken as controls. All study subjects were asked to complete a questionnaire to obtain

data on age, body mass index, clinical history, occupation, lifestyle habits, and consumption of locale animal products. Each of the study subjects was sampled by the trained medical staff who collected 50 ml of blood into five 10 ml vacutainers containing heparin as an anticoagulant. The samples were shipped in dry ice to the analytical laboratory.

**Dioxin analyses:** The concentrations of PCDDs, PCDFs, non-ortho-and mono-ortho PCBs were determined in the whole blood samples using high-resolution GC/MS as described in <sup>9</sup>. Briefly, the mixture of isotope labeled standards was added to 30 g of whole blood. The sample was diluted with 50 ml of water and treated with 10 ml of ammoniac solution and 50 ml of ethanol, then extracted 3 times with an n-hexane:diethylether mixture (1:1). The lipid content was determined gravimetrically after evaporating the solvents. The sample was cleaned-up on a 3-column system (combined silica gel, combined alumina – to separate the PCB fraction from PCDD/F fraction – and carbo). The PCB fraction adjusted to 100µl and the PCDD/F fraction adjusted to 25 µl were injected into GC/HRMS system (Finnigan MAT95XP, USA), the isotope dilution method was used for quantitation.

**Statistics:** The toxicity equivalents (TEQs) were calculated using the toxicity equivalency factors (TEFs) recommended by the WHO <sup>10</sup>. For descriptive statistics, concentrations below the limit of determination were considered to be half the limit of determination. Logarithmically transformed data were statistically analyzed using ANOVA. P-values lower than 0.05 were considered statistically significant.

## Results and Discussion

Descriptive characteristics of the study subjects are presented in Table 1.

**Table 1.** Descriptive characteristics of the study enrollees

Characteristics	Neratovice	Libiř	Tiřice	Beneřov (Control group)	Total
N subjects	20	20	20	20	80
Males	10	4	8	8	30 (37.5%)
Females	10	16	12	12	50 (62.5%)
Mean age (y) -	44.6	41.4	42.8	43.3	43.0
<b>Mean length of residence in locality (y) -</b>	30.6	26.9	28.2	33.8	28.9 (5-58)
Distance range from residence to plant (km)	1 – 2	1 – 2	2 – 5		1 - 5
Mean BMI (kg/m <sup>2</sup> ) -	26.3	26.6	26.8	30.6	27.5

Table 2 presents basic statistical data on WHO-TEQ levels. In 79 % of samples 2,3,7,8-TCDD was below the limit of determination ranging from 1.2 to 3.8 pg/g fat. The maximum detected concentration was 9.0 pg/g fat. Some other PCDD and PCDF congeners were detected in less than 50% of analyzed samples (Table 3). The correlation of PCDD, PCDF, and PCB with age was observed with the higher values in the older age groups. A small but significant difference between males and females was observed only for PCB TEQ only.

Significantly higher TEQ levels were found in all three exposed groups compared with controls. However, the dioxin-like PCBs with the prevalence of PCB congeners 156 and 126 contributed from 60 to 69% to the total TEQ value and were responsible for the differences between the exposed and control groups. A similar relationship in PCDDs:PCDFs:PCBs contribution to the WHO-TEQ value was also observed for Czech breast milk samples <sup>4,7</sup>. This finding confirms that the Czech population is still at a higher exposure risk to PCBs. On the other hand, the TEQ values for PCDDs and PCDFs reported in the present study are comparable with the relevant European data for the general population <sup>11, 12</sup> and do not indicate that ambient exposure to PCDDs/PCDFs might be an important contributor to dioxin body burden. It is well known fact that food consumption, which is particularly true of fatty animal foods, accounts for 95 –98% of total human exposure to dioxins. Higher I-TEQ values for PCDDs/PCDFs were found among subjects consuming locally produced eggs and beef <sup>13</sup>. Likewise in this study the multifactorial analysis shows association between the consumption of locally produced eggs and WHO-TEQ values in the blood. However, the levels of WHO-TEQ in the blood of the study groups are about by two orders of magnitude lower than those obtained in the population with observed exposure-related adverse health effects <sup>14</sup>.

In conclusion, the present study is the first study of dioxins blood levels in the Czech population and points out the existence of areas with potential higher exposure to dioxins. Further studies are needed to obtain reference values for blood dioxin levels in the Czech general population.

**Table 2.** Descriptive statistical data on PCDDs, PCDFs, and PCBs expressed as WHO-TEQ values in pg/g fat

Locality	Parameters	PCDD	PCDF	PCB	Total
Neratovice N=20	Mean±SD	5.6±3.0	12.3±5.1	33.4±19.1	51.2±24.3
	CI	4.3-6.9	10.1-14.5	25.0-41.7	40.5-61.9
	<b>GM</b>	<b>5.0</b>	<b>11.4</b>	<b>29.2</b>	<b>46.3</b>
	Median	4.5	11.8	28.9	49.5
	Ranges 10% –90%	3.3-8.9	7.0-17.5	15.1-51.7	26.9-72.9
	Min-max	2.7-15	5.6-24.9	14.7-92.6	24-118
Libiš N=20	Mean±SD	5.1±2.6	11.8±5.0	40.5±19.5	57.4±22.7
	CI	4.0-6.3	9.6-14.0	31.9-49.1	47.4-67.4
	<b>GM</b>	<b>4.7</b>	<b>10.9</b>	<b>36.7</b>	<b>53.5</b>
	Median	4.6	10.3	37.8	54.0
	Ranges 10% –90%	3.0-6.9	6.4-16.1	19.9-66.3	31.7-82
	Min-max	2.3-13.3	5.2-27.2	18.5-93.6	29-116
Tišice N=20	Mean±SD	5.3±6.2	14.8±13.3	34.4±28.0	54.5±46.4
	CI	2.6-8.0	8.9-20.6	22.1-46.6	34.2-74.8
	<b>GM</b>	<b>4.2</b>	<b>11.9</b>	<b>28.3</b>	<b>45.0</b>
	Median	3.6	10.7	27.8	42.0
	Ranges 10% –90%	2.5-6.3	7.2-21.2	15.4-52.4	24.9-73.6
	Min-max	2.2-30.9	5.3-58	11.7-134	22-223
Benešov N=20	Mean±SD	3.3±1.6	6.6±2.2	15.4±6.3	25.4±8.9
	CI	2.6-4.0	5.7-7.6	12.6-18.2	21.2-29.3
	<b>GM</b>	<b>3.0</b>	<b>6.3</b>	<b>14.3</b>	<b>24.0</b>
	Median	2.7	6.2	13.4	24.0
	Ranges 10% –90%	2.2-6.6	4.0-9.0	8.1-26.2	16.0-37.4
	Min-max	1.6-7.1	2.8-11.8	7.6-27	13.0-45.0

CI = confidence interval

**Table 3.** PCDD and PCDF congeners with levels below the limit of determination of the used method in more than 50% samples.

PCDD	< LOD (%)	PCDF	< LOD (%)
2,3,7,8-TCDD	79	2,3,7,8-TCDF	72,5
1,2,3,7,8- PCDD	71	1,2,3,7,8-PeCDF	91
1,2,3,4,7,8-HxCDD	84	2,3,4,6,7,8-HxCDF	76
1,2,3,7,8,9-HxCDF	72,5	1,2,3,7,8,9-HxCDF	100
		1,2,3,4,7,8,9-HpCDF	96
		OCDF	65

LOD = limit of determination

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### References

1. WHO/ECEH (1996). Environmental Health in Europe Series 3, 121
2. Černá M. and Bencko V. (1999) *Centr. Eur. J. Publ. Health.* 7, 67.
3. Kliment V., Kubínová R., Kazmarová H., Kratzer K., Šišma P., Ruprich J., Černá M. and Gregůrková M. (2000) *Centr. Eur. J. Publ. Health.* 8, 198.
4. Černá M., Šmíd J., Svobodník J., Grabic R., Crhová Š. and Kubínová R. (2003) *FEB* 12, 203.
5. Van Leeuwen F.X.R. and Malisch R. (2003) *Organohalogen Compounds*, 56, 311.
6. Bencko V., Skulová Z., Krečmerová M. and Djien Liem A.K. (1998) *Toxicology Lett.* 96-97, 341.
7. Bencko V., Černá M., Jech L. and Šmíd J. (2004) *Environ. Toxicol. Pharmacol.* in press.
8. Pelclová D., Fenclová Z., Preiss J., Procházka B., Spáčil J., Dubská Z., Okrouhlik B., Lukáš E. and Urban P. (2002) *Int. Arch. Occup. Environ. Health* 75, S60.
9. Grabic R., Novák J. and Pacáková V. (2000) *J. High Resol. Chromatogr.* 23, 595.
10. Van den Berg M., Birnbaum L., Bosveld A.T.C., Brunström B., Cook P., Feeley P., Giesy J.P., Hanberg A., Hasegawa R., Kennedy S.W., Kubiak T., Larse J.C., van Leeuwen F.X.R., Djien Liem A.K., Nolt C., Peterson R.E., Poellinger L., Safe S., Schrenk D., Tillitt D., Tysklind M., Younes M., Waern F. and Zacharewski T. (1998). *Environ. Health Perspect.* 106, 775.
11. Wittsiepe J., Schrey P., Ewers U., Wilhelm M., Selenka F. (2000) *Environmental Res.* 83, 46.
12. Buckley-Golder D. (1999) Report produced for European Commission DG Environment AEAT/EEQC/0016.
13. Goldman L.R., Harnly M., Flaterry J., Patterson D.G.,JR., and Needham L.L. (2000) *Environ. Health Perspect.* 108, 13.
14. ATSDR ToxProfiles (2002) Agency for Toxic Substances and Disease Registry, Atlanta, GA.