Human exposure to PCBs and some other persistent organochlorines in eastern Slovakia as a consequence of former PCB production

Anton Kocan[1], Beata Drobna[1], Jan Petrik[1], Stanislav Jursa[1], Jana Chovancova[1], Kamil Conka[1], Branko Balla[1], Eva Sovcikova[1], Tomas Trnovec[1]

[1]Slovak Medical University, Bratislava

Introduction

Out of estimated 1.3 million tonnes of PCBs totally produced in the world, about 21 500 t were manufactured in 1959-1984 by the chemical plant Chemko situated at the town of Strazske, Michalovce District, eastern Slovakia. Chemko PCB products called Delor 103, Delor 105, Delor 106, Delotherm and Hydelor were used inside former Czechoslovakia chiefly as heat transfer fluids, capacitor and transformer oils, and paint additives. Because of poor production technology, especially in the 1960s and early 1970s, high amounts of PCB waste discharged, into an open, several km long, muddy Chemko effluent canal causing the increased contamination of the nearby Laborec River and Zemplinska Sirava Lake. A higher PCB content in water sediment, surface water, soil, and air has resulted in high exposure of wild fish and food products coming from cattle, swine and poultry kept in the polluted area and fed with locally produced feed [1,2].

Two previous pilot studies realized in 1993 and 1997-98 indicated that mean PCB levels measured in the blood, adipose tissue and milk of the human population living in the polluted area (Michalovce District) are several times higher if compared with other Slovak districts [3-8].

The objective of this exposure oriented part of the EU's 5th Framework Programme PCBRISK project (*www.pcbrisk.sk*) [9] was to evaluate the PCB and selected organochlorine pesticide exposure of minimally 2 000 adults (equally men and women) and 400 children (equally boys and girls, 8/9 years old) living in the towns and villages of the Michalovce District adjacent to the Laborec River downstream the Chemko plant where higher PCB human levels could be expected and in the towns and villages of the Svidnik and Stropkov Districts lying several

tens km upstream the Laborec where lower PCB exposure was expected. The exposure of at least 300 adults out of those 2 000 to dioxins, furans and dioxin-like PCBs was evaluated as well. The assessment of health effects and other factors presented in further PCBRISK presentations within the DIOXIN 2004 Symposium is based on this exposure data.

Materials and Methods

Two study samples (adults and children) within this cross sectional study were chosen at random from the study population that was defined by lists of the adult health insured kept by general practitioners selected from the study area and by lists of 8 – 9 year-old children from selected schools in the area.

The basic inclusion criterion for adult subjects was long-term permanent residence in the study area. The presence of mild chronic controlled illness (e.g., rheumatism, hypertension, diabetes, thyroid disorders, non-morbid obesity, allergy, etc.) did not cause a subject to be excluded from the study. Pregnancy, and acute injuries and illnesses, including all stages of tuberculosis caused exclusion from the study sample. Children involved in the study had to be born and living in the study area. Children's mothers should have permanently lived in the respective area for at least 5 years before the child's birth.

In August 2001 – February 2002 whole blood was taken from the fasting subjects into anticoagulant-free Vacutainer™ tubes (S-Monovette, Sarstedt, Germany) and after clotting centrifuged at 3 000 rpm for 15 minutes. The serum were subdivided into several vials and stored frozen at –18 C in glass vials until chemical analysis and bioassays.

Each melted serum sample (0.5 – 5 ml) intended for PCB/pesticide analysis was spiked with a PCB-174 congener 24 hours prior to sample processing in order to check the recovery of a cleanup procedure. After the serum had been treated with 1-propanol PCBs and organochlorine pesticides were isolated by solid phase extraction (SPE) on a 1-g C_{18}-column (Altech Inc., USA). An SPE hexane:CH_2Cl_2 extract was purified using an H_2SO_4/silica column. An eluate was concentrated, dissolved in a solution of PCB-103 congener (a syringe standard to adjust the final volume) and injected onto a gas chromatograph 6890N (Agilent Technologies, USA) equipped with a 60 m × 0.25 mm × 0.25 μm DB-5 capillary column (J&W Scientific, USA), a micro electron capture detector, and an Agilent Chemstation software. The quantification of 15 PCB congeners (# 28, 52, 101, 123^{+149}, 118, 114, 153, 105, 138^{+163}, 167, 156^{+171}, 157, 180, 170, and 189) and 6 organochlorine pesticides (α-HCH, β-HCH, γ-HCH, HCB, pp'-DDT, and pp'-DDE) was based on a multilevel calibration curve constructed by five standard congener mixtures of

the 21 analytes, and 2 spiking PCB congeners. An analysis batch consisted of 10 serum samples, one solvent blank and one spiked porcine serum in-house reference material. A certified reference material (CRM) was analyzed on a regular basis. The laboratory analyzing the samples has successfully participated in German round robin tests (sheep serum) since 1997.

Each melted serum sample (5 – 30 ml) intended for the analysis of PCDDs, PCDFs, dioxin-like and other PCBs was spiked with $^{13}C_{12}$-labeled extraction/internal standards (15 PCDDs/Fs, 12 dioxin-like non- and mono-*ortho* PCBs, and 11 non-dioxin-like PCBs) 24 h prior to sample processing. After the serum had been treated with diluted formic acid the analytes were SPE isolated using 10-g C_{18}-column (UCT Inc., UK). A hexane extract was cleaned-up on a Power-Prep^{TM} semi-automated cleanup system (FMS Inc., USA) with pre-packed disposable silica, alumina and carbon columns. A toluene eluate fraction containing seventeen 2378-substitted PCDDs/Fs and non-ortho PCBs (# 77, 81, 126, 169) was concentrated and then diluted with a $^{13}C_{12}$-labeled recovery/syringe standard. The analytes were separated on a 30 m × 0.25 mm × 0.25 µm DB-5ms capillary column (J&W Scientific, USA) and quantified by high-resolution MS (MAT 95XL, Germany). An analysis batch consisted of 14 serum samples, one solvent blank and one spiked porcine serum in-house reference material. A human serum CRM was analyzed in each 3rd batch. The analysis of a Power-Prep fraction containing mono-*ortho* and other potentially (neuro)toxic non-dioxin like PCB congeners is in progress and therefore results are not presented here yet.

An enzymatic method [10] based on the determination of total cholesterol, free cholesterol, phospholipids and triglycerides was used to determine total lipids in all the serum samples analyzed. Then, the levels of the analytes could be adjusted to the lipid weight basis.

Results and Discussion

Out of 2 049 blood samples taken from adults (1 011 in the Michalovce District and 1 038 in the Svidnik/Stropkov Districts) and 460 ones taken from children (231 in the Michalovce District and 229 in the Svidnik/Stropkov Districts), PCBs and selected organochlorine pesticides were determined in 2 047 ones (836 men and 1 211 women) and 434 ones (221 boys and 213 girls) respectively.

Out of 328 blood samples taken from adults (143 in the Michalovce District and 185 in the Svidnik/Stropkov Districts), 2378-substituted PCDD/Fs and non-*ortho* PCBs were determined in 320 ones (199 males and 121 females).

If PCB congeners and organochlorine pesticides were quantified in more than two thirds of the samples analyzed their median, mean, minimum, 10-percentile,

90-percentile and maximum levels are reported in Table 1 (adults) and Table 2 (children). The arithmetic means and medians for any individual PCB congener were calculated with half LOD values and LOD ones respectively. The sum of all the 15 individual PCB congeners analyzed, including the ½ LODs represents "PCBs (15 cong)".

Table 1: PCB and some organochlorine pesticides in 2 047 human blood serum samples taken from adults living in eastern Slovakia.

Compound	Content, ng/g, lipid weight basis						
	Median	Mean	Min	10 perc	90 perc	Max	%
PCB-28			< 1.7			565	38.7
PCB-52			< 1.6			625	8.9
PCB-101			< 1.3			253	15.6
PCB-105			< 1.4			447	45.8
PCB-114			< 1.3			152	27.8
PCB-118	31.4	62.6	< 4.3	10.1	120	3 539	94.1
PCB-123^{+149}			< 1.2			75.9	9.8
PCB-138^{+163}	205	366	8.5	92.4	691	14 050	100.0
PCB-153	335	585	38.8	156	1 112	25 089	100.0
PCB-156^{+171}	38.5	69.3	< 6.8	17.5	124	4 066	99.9
PCB-157			< 1.3			385	44.8
PCB-167	9.9	18.0	< 2.1	2.8	35.3	709	81.2
PCB-170	125	241	16.3	55.3	428	27 481	100.0
PCB-180	312	575	42.4	140	1 043	44 673	100.0
PCB-189			< 1.7			1 497	55.6
PCBs (15 cong)	1 087	1 972	149	504	3 452	101	
HCB	663	921	21.7	127	1 929	17 928	100.0
α-HCH			< 0.8			17.5	6.7
ß-HCH	46.2	57.3	<2.8	15.4	111	782	97.0
γ-HCH			< 0.6			269	22.6
pp'-DDE	1 770	2 448	54.0	558	5 016	22 382	100.0
pp'-DDT	48.9	75.6	<3.6	20.0	157	940	99.5

The most abundant PCB congeners quantified in all the samples from adults were #153, 138^{+163}, 180 and 170. Out of the sum of all the 15 congeners analyzed, #153 and the sum of #153, 138^{+163} and 180 represented, on average, 30.6 % (s_x = 2.3 %) and 77.5 % (s_x = 3.0 %) respectively. The mean mutual ratio of #153 : #138^{+163} : #180 was 39 : 24 : 37. Similar numbers were found in the children's samples. The mean DDE/DDT ratio in the adults was 39.9 (min – max: 2.6 – 360). However, lower analytes levels in children's blood and, in many cases, insufficient sample volume caused that less abundant congeners could not be quantified (see

Table 2). Because of high correlation between #153 levels and the sum of PCB congeners in this study (r = 0.94 and 0.99 in the case of adults and children respectively), one can work with the #153 values as this congener was quantified in all the children's samples but one. It is interesting that mean PCB levels were statistically significantly higher in adult male blood lipids (2 383 vs 1 688 ppb; $p < 0.0001$; Mann-Whitney test) while HCB levels were higher in adult female lipids (688 vs 1 088 ppb; $p < 0.0001$). On the contrary, 8 – 9 year-old boys had higher mean HCB levels than girls (133 vs 109 ppb; $p = 0.004$). There was no significant difference in mean p,p'-DDE content in adult male and female blood lipids (2 525 vs 2 395 ppb).

The median, mean, minimum, 10-percentile, 90-percentile and maximum levels of PCDDs, PCDFs and non-*ortho* PCBs expressed as summed WHO TEQs are given in Table 3. If some congener was present at a concentration lower than its limit of detection half of the LOD was used for TEQ calculation. Whereas a mean ratio of TEQ_{PCDDs} and TEQ_{PCDFs} in many other countries is greater than 1, this ratio was 0.41 (min – max: 0.02 – 3.5) in the Slovak human general population studied, i.e. the contribution of TEQ_{PCDFs} to the total TEQ was, on average, about 2.5-times higher than that of TEQ_{PCDDs}. It can be caused by higher PCB levels found in the Slovak population and consequently higher PCDF levels as PCDFs are present in PCB formulations. On the other hand, lower mean TEQ_{PCDDs} can be caused by the reduced use of chlorinated phenols and other PCDD precursors in Slovakia in the past. There was no statistically significant difference between the levels of PCDDs, PCDFs and coplanar PCBs expressed as TEQs in men and women.

Table 2: PCB and some organochlorine pesticides in 434 human blood serum samples taken from 8 – 9 year-old children living in eastern Slovakia.

Compound	Content, ng/g, lipid weight basis						
	Media	Mean	Min	10 perc	90 perc	Max	%
PCB-28			< 3.2			103	7.4
PCB-52			< 2.9			153	3.5
PCB-101			< 3.3			55.0	11.8
PCB-105			< 1.6			19.3	7.4
PCB-114			< 1.6			7.2	2.3
PCB-118			< 4.8			118	49.8
PCB-123^{+149}			< 3.1			13.2	3.7
PCB-138^{+163}	68.0	107	< 9.9	24.4	223	1 011	98.8
PCB-153	109	172	< 25	38.1	387	1 757	99.8
PCB-156^{+171}			< 3.4			239	60.1
PCB-157			< 1.5			20.3	6.2
PCB-167			< 3.0			76.3	23.3
PCB-170			< 4.1			1 056	91.9
PCB-180	88.5	150	< 8.6	25.7	346	2 285	99.1
PCB-189			< 1.6			38.9	9.4
PCBs (15 cong)	377	568	66.6	150	1 208	6 495	
HCB	89.9	121	<	44.9	219	2 279	99.3
α-HCH			< 1.6				0.0
ß-HCH			< 5.3			121	49.3
γ-HCH			< 1.6			49.0	4.4
pp'-DDE	473	677	31.0	157	1 378	11 733	100.0
pp'-DDT			< 5.7			2 970	60.1

Table 3: PCDDs, PCDFs and non-ortho PCBs (#77, 81, 126, and 169) in 320 human blood serum samples taken adults living in eastern Slovakia.

Compound	Content, pg WHO TEQ/g, lipid weight basis					
	Median	Mean	Min	10	90 perc	Max
PCDDs	3.1	3.8	0.9	1.5	7.1	15.8
PCDFs	9.3	11.2	1.4	5.3	17.7	88.6
non-*ortho* PCBs	8.9	15.1	0.3	3.6	29.4	256.
PCDDs+PCDFs	12.8	15.0	3.3	7.3	22.6	90.2
PCDDs+PCDFs+non-*ortho*	21.9	30.1	4.9	12.0	50.8	298.

As can be seen in Figures 1 and 2, the levels of the POPs increased with the age of subjects. It proves that metabolic POPs degradation is lower than their body intake.

The proximity of variables (TEQ_{PCDDs}, TEQ_{PCDFs}, TEQ_{cPCBs}, PCBs as a sum of 15 congeners, HCB and p,p'-DDE) is shown in Figure 3 representing a principal component analysis biplot constructed using the statistical program SPSS 7.5 for Windows – simply said, the smaller angles are between vectors the stronger connection is between the variables and vice versa. Thus, there is a good correlation between DDE and HCB human blood lipid levels as well as among PCB, coplanar PCB and PCDF ones unlike PCDDs implying dissimilarity in the origin of the human exposure.

Figure 1: Median PCB-153 congener, HCB, and p,p'-DDE levels in blood serum vs age groups.

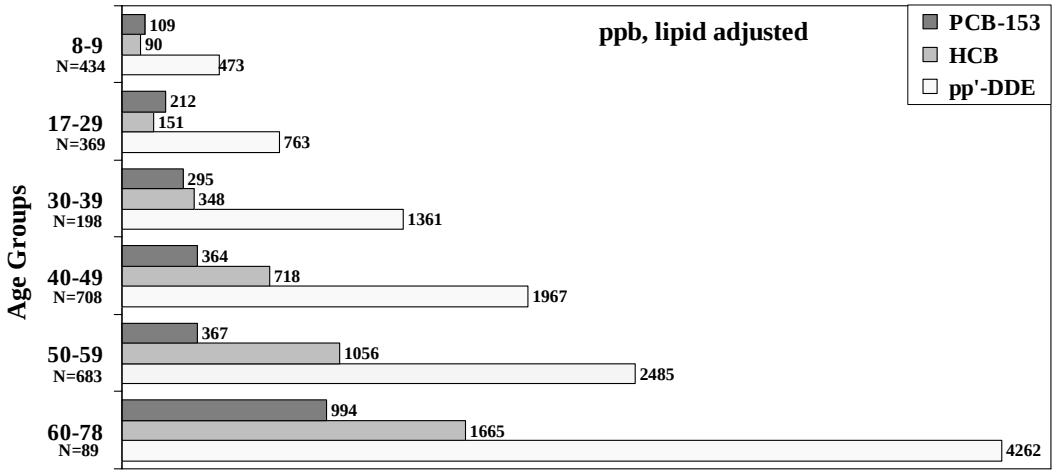


Figure 2: Median WHO TEQ calculated from PCDDs, PCDFs and non-*ortho* PCBs in blood serum vs age groups.

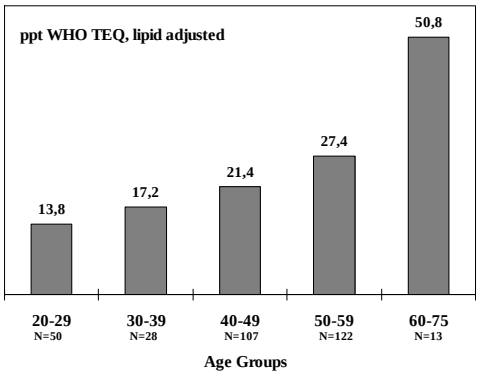
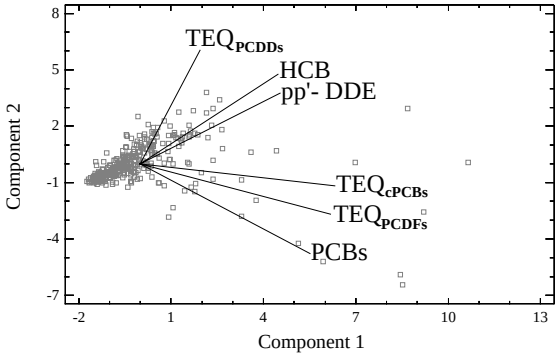


Figure 3: A PCA biplot for PCDD, PCDF, cPCB, HCB and DDE levels found in 320 adults living in eastern Slovakia.



If the median PCB-153 congener, HCB and p,p'-DDE blood lipid levels found in this study are compared with the median levels of those pollutants found in blood lipids taken in 1998 from 420 people living in the same area (Michalovce and Stropkov Districts) one can observe a decline of 36 % for PCB-153, 29 % for p,p'-DDE and even 50 % for HCB.

Acknowledgment

The authors would like to thank Juraj Koska, Zofia Radikova, Lucia Krinantova, Richard Imrich, Katarina Buckova, and Henrieta Stefankova for collecting blood specimens and Jarmila Paulikova, Jarmila Salajova, and Juraj Loksa for their skilled laboratory assistance.

This study was supported by the European Commission's 5th Framework Programme: Quality of Life and Management of Living Resources under the project "Evaluating Human Health Risk from Low-dose and Long-term PCB Exposure" (PCBRISK, QLK4-2000-00488).

References

1. Kocan A., Petrik J., Jursa S., Chovancova J. and Drobna B. (2001) Chemosphere 43, 595.
2. Petrik J., Kocan A., Jursa S., Drobna B., Chovancova J. and Pavuk M. (2001) Fresenius Environ. Bull. 10, 375.
3. Petrik J., Drobna B., Kocan A., Chovancova J. and Pavuk M. (2001) Fresenius Environ. Bull. 10, 342.
4. Kocan A., Petrik J., Drobna B. and Chovancova J. (1994) Chemosphere 29, 2315.
5. Kocan A., Petrik J., Drobna B. and Chovancova, J. (1994) Organohalogen Compounds 21, 147.
6. Kocan, A., Drobna, B., Petrik, J., Chovancova, J., Patterson, Jr., D.G. and Needham, L.L. (1995) Organohalogen Compound 26, 186.
7. Pavuk M., Cerhan J.R., Lynch C.F., Kocan A., Petrik J. and Chovancova J. (2003) J. Exp. Anal. Environ. Epidem. 13, 267.
8. Pavuk M., Cerhan J.R:, Lynch C.F., Schecter A., Petrik J., Chovancova J. and Kocan A. (2004) Chemosphere 54, 1509.
9. Trnovec T., Kocan A., Langer P., Sovcikova E., Tajtakova M., Bergman A., van den Berg M., Brouwer A., Machala M., Winneke G., Sampson B., Brunekreef B., Pavuk M. and Bencko V. (2000) Endocrine Regulations 34, 167.
10. Akins J.R., Waldrep K. and Bernert J.T.Jr. (1989) Clin. Chim. Acta 184, 219.