Study design, objectives, hypotheses, main findings, health consequences for the population exposed, rationale of future research

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Introduction

In Slovakia, the Chemko Chemical Company, based in Strazske, in the Michalovce district, produced PCBs between 1959 and 1984, in the amount of more than 21,000 tons of commercial mixtures (Delor 103, 104, 105, 106, Delotherm DK and DH, Hydelor 137). PCBs were used for similar industrial purposes as in the west. Improper disposal from the Chemko plant via release of effluent directly into the Laborec River resulted in long-term contamination of sediment. As a result eastern Slovakia, the Michalovce district in particular, is recognized as one of the areas all over the world most heavily polluted with PCBs. Historical studies show that blood and adipose PCB levels were higher in Czechoslovakia than elsewhere in the 1970's and 1980's[1]. Current data indicate that persons who eat locally raised food – pork, beef, poultry, eggs – in this district have elevated serum concentrations of PCBs[2]. Environmental exposure to organochlorines in the Michalovce district indicate association with higher rates of certain cancers[3], but an inverse association with risk of breast cancer[4]. An increased prevalence of thyroid disorders in the polluted area was also reported[5].

This "experimental setting in nature" has attracted international scientific teams and two projects in the area are ongoing: Evaluating Human Health Risk from Low-dose and Long-term PCB Exposure, 5th FP Project QLK4-2000-00488, 2001-

2004; PCBRISK (http://www.pcbrisk.sk/) and Early Childhood Development and PCB Exposures in Slovakia, NCI/NIH, # R01-CA96525 University of California, Davis, USA.

This paper is serving as an introduction to papers of a session reporting on various health outcomes associated with PCB exposure.

The objectives of the PCBRISK project were targeted at an evaluation of the human health risks of low-dose and long-term exposure to a group of persistent organochlorine pollutants, including polychlorinated biphenyls (PCBs) and their metabolites, organochlorine pesticides, polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) within a population that has been exposed to these chemicals as a result of environmental pollution. The hypotheses tested are closely related to these objectives. The basic hypothesis that can be further developed for a particular compound of interest and health outcome is as follows: „Is there any association between a certain type of health outcome investigated and the organochlorine exposure?" Any of the project objectives was targeted at the place of residence as a predictor of any health outcome.

Methods and Materials

The project was a cross-sectional epidemiological study and consisted of the following elements:

Examination of 2478 subjects (2047 adults, 50.7 % females and 49.3 % males, and 431 8-9 years old children) living in a PCB-polluted area. Adult subjects were recruited by help of the primary health care physicians by random selection from an alphabetical list of their clients. Children were recruited on the basis of classes of the elementary schools. Outpatient clinics and schools were approached in an aerial distance and direction from the Chemko plant as indicated in the Tables 1 and 2. In all subjects blood samples were analysed for PCBs and organochlorine pesticides, hormones, antibodies, biomarkers and questionnaires on biomedical data were completed. In all adults with no contraindication (1200) was performed the oral glucose tolerance test. In 300 of the adult subjects were done additional estimations of OH-PCBs, $MeSO_2$–PCBs, PCDDs, PCDFs, coplanar PCBs, heavy metals (Cd, Hg, Pb, Mn, Zn, Se), bioassays of dioxin-like activity and xenoestrogenic activity. In 431 children were examined cognitive and hearing functions, dental status and determined organochlorine and toxic metal levels in serum and thyroid morphology and function. Statistical treatment was targeted at assessment of associations between organochlorine levels and signs of human health disorders.

Results and Discussion

Recruitment of subjects. Table 1 is summarizing the information on the residence of the primary health care physician's offices, its aerial distance and direction from the PCB plant in Strážske, number of adult subjects recruited and data on their PCB serum concentration and Table 2 is showing the same data for the children and their schools. The village Nacina Ves is 6 km in SSE direction from the plant. The educational level of subjects turned out to be a very important predictor of health outcomes and therefore it was included into Table 1.

Table 1. The sites of outpatient clinics at which adult subjects were recruited and data on their PCB serum concentration and educational level.

			Sum of PCBs without LODs [ng/g serum lipids]			Educational level [%]		
Site of outpatient clinic	Distance and direction from Strazske	n	Mean	Median	SD	Elementary school	High school	Univer -sity
Michalovce	15 km SSE	693	2750.4	1703.0	5609.5	11.7	68.7	19.6
Strazske	0	229	4289.0	2607.2	5357.6	15.2	66.9	17.8
Zaluzice	17 km SE	72	2681.7	1945.8	2772.4	11.6	72.5	15.9
Stropkov	41 km NNW	360	908.8	756.7	640.0	17.9	74.0	8.1
Svidnik	53 km NNW	400	897.8	754.5	1006.3	15.7	66.5	17.8
Okruhle	32 km NW	102	848.3	741.8	526.8	68.0	27.0	5.0
Giraltovce	37 km NW	119	618.4	555.3	311.8	29.9	62.4	7.7
Total		1975	1988.7	1065.0	4048.6	18.0	66.7	15.3

Table 2. Sites of schools where children were recruited and data on their breast feeding and fish consumption.

Sites of schools and number of children		Sum of PCBs without LODs [ng/g serum lipids]			Breast fed [%]	Mean length of breast feeding [weeks] (S.D.)	Consumption of fish [%]
	n	Mean	Median	SD			
Michalovce	135	511.2	404.6	467.3	93.8	22.6 (24.9)	4.0
Strážske	59	916.2	684.3	989.3	91.8	16.0 (16.3)	7.9
Nacina Ves	22	1692.8	1423.9	1146.3	86.4	10.7 (11.6)	13.6
Svidník	121	318.6	209.1	330.4	95.6	22.6 (26.0)	5.0
Stropkov	50	364.1	248.1	365.7	98.0	23.7 (33.4)	7.7
Giraltovce	44	257.5	204.6	265.5	94.0	22.0 (23.1)	4.0
Total	431	529.9	321.0	655.8	94.2	21.2 (24.8)	5.7

When recruiting the subjects the main criterion was to obtain a large exposure range to PCBs. After analyzing all samples for PCBs, we came to the conclusion that although this goal of the study was reached, the most important epidemiological information, an assessment of the total size of the population segment having an exposure exceeding a certain level, was missed. It is obvious from Table 1 that the outpatient clinics in the north-west direction from the Strazske plant were prioritized and the data on population living in other directions, especially to the south are scarce. Even though from the basic demographic data and our limited data on PCB exposure and a certain degree of extrapolation, a tentative conclusion may be drawn that the mean over 1000 ng of sum of PCBs/g serum lipids is characteristic for a population of approximately several tens of thousands adult citizens living in an area about 50 by 30 km. The PCB serum concentrations in our subjects show a very skewed distribution toward the higher values. Therefore median or other suitable percentile may be of higher informative value with regard to health outcomes. For adult subjects the quartiles of PCB serum concentration in ng/g serum lipids ranged as follows: 1. quartile 124-675, 2.quartile 675-1065, 3. quartile 1065-1978, 4. quartile 1978-101411. Thus the subjects belonging to the upper percentiles incur disproportionately higher health risk compared to subjects of the lower percentiles. This estimate of the size of the population segment at risk is very preliminary, but if confirmed, the health outcomes of such an extent of exposure and large number of subjects exposed may have very serious health and social consequences. However the definitive information on the size of population segment at higher risk from PCBs can be obtained by a well designed study using geographic information system.

Exposure assessment. We have been aware of the drawbacks of many environmental epidemiological studies and wanted to avoid them: (1) low exposure level, (2) narrow range of exposures, (3) indirect exposure assessment. (1) Previous data have shown that the exposure levels in eastern Slovakia will be very high compared to other PCB exposure settings reported. (2) In order to have a broad range of exposures the subjects were recruited from several subregions at various distances from the pollution source as shown in Tables 1 and 2. We have realized after analyzing serum samples from the most distant region that, in terms of risk assessment currently used[6,7], even this region has to be considered less but still significantly polluted. Therefore considering the relevant regions studied in this project as “background” or “un-polluted” is not appropriate. (3) Exposure assessment in our study was based on determination of the compounds of interest in blood/serum of subjects exposed. Having this primary source of information on

individual exposure to organochlorines, we considered data on residence, environment, nutrition and lifestyle as secondary.

The data set obtained in the PCBRISK project is very large and complex. Presently the main findings are reported as separate sets of data. On the other hand identification of associations within various health outcomes found in this study will be the future stage of their exploitation. The following are two examples of such proceeding in which associations between impairments of neurobehavioral performance and hearing were looked for. As reported[8], there was an association between the extent of exposure to PCB and several parameters of the neurobehavioral performance in the group of 8-9 years old school children studied. In the same group of children the hearing was also examined. The results of two of the hearing tests, the pure tone audiometry and the transient evoked otoacoustic emissions also have shown deficits associated with PCB exposure. The parameters of neurobehavioral performance and of the hearing functions were correlated in the same group of children.

Pure tone audiometry vs. simple reaction time. As shown[8] there was an association between hearing thresholds at several frequencies tested and the extent of exposure to PCB. One may logically ask if in a particular child neurobehavioral deficits and increased hearing thresholds coincide. From neurobehavioral parameters examined the simple reaction time to visual stimulus was chosen and related to hearing thresholds at 8 various tone frequencies tested. It can be seen from Table 3 that for all frequencies the reaction time was positively associated with the hearing threshold and for 5 of the 8 frequencies this association was significant

Table 3. Spearman's correlation between the hearing thresholds [dB] (mean for the right and left ear) at various frequencies [kHz] and simple reaction time [ms] in 448 8-9 years old children exposed to PCBs. Correlation significant at $p<0.05$ is marked by *. The data on mean PCB serum level in children can be found in Table 2.

	Pure tone frequencies tested [kHz]							
	0.25	0.5	1	2	3	4	6	8
Correlation coefficient	0.139*	0.088	0.142*	0.100*	0.072	0.096*	0.094*	0.078
Sigma	0.003	0.061	0.003	0.034	0.127	0.042	0.047	0.100

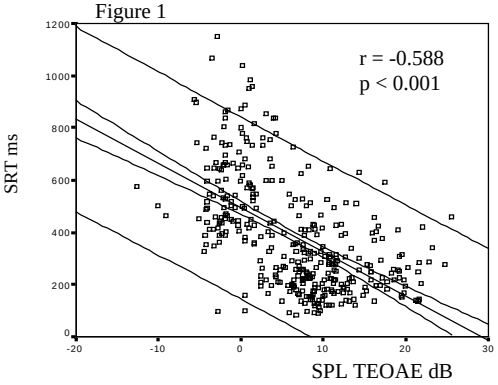
Transient evoked otoacoustic emissions vs. battery of neurobehavioral tests targeted at complex mental processes: Benton recognition test, Digit span (forward, backward), Digit symbols, Cube-hand coordination, Simple reaction time, Raven nonverbal intelligence test and Scale of behavior (at home, at school). It was shown[8] that the sound pressure level of transient evoked otoacoustic

emissions significantly decreased with increasing exposure of children to PCBs. The data in Table 4 document that in our exposed cohort of children the sound pressure level of transient evoked otoacoustic emissions was significantly associated with results of all applied neurobehavioral tests with the exception of Raven intelligence test.

Table 4. Spearman's correlation between the sound pressure level of transient evoked otoacoustic emissions [dB] (mean for the right and left ear) and various neurobehavioral tests in 369 8-9 years old children exposed to PCBs. Correlation significant at p<0.05 is marked by *.

	Neurobehavioral tests applied						
	Digit span-forward	Digit span-backward	Raven test	Digit symbols	Benton test	Scale of behavior at home	Simple reaction time
Correlation coefficient	0.169*	0.225*	0.097	-0.238*	0.214*	-0.180*	-0.588*
Sigma	0.001	<0.001	0.066	<0.001	<0.001	0.001	<0.001

Figure 1



The relation between the simple reaction time (SRT) and sound pressure level (SPL) of transient evoked otoacoustic emissions (TEOAE) is shown in Figure 1. It is showing that deficits in function of the outer hair cells of the cochlea are accompanied by deficits of the brain function manifested as an increased reaction time.

These associations found between the results of neurobehavioral and hearing examination show that in a population group of children highly exposed to PCBs, there is a great probability that in a particular child a neurobehavioral deficit will be accompanied by subclinical but identifiable hearing impairment.

It follows from the data obtained in the PCBRISK project that there is a pressing need for further action to avoid environmental and adverse health effects from PCBs in the studied eastern Slovakian area.

Conclusions: The results of the PCBRISK project confirm that the Michalovce area in eastern Slovakia belongs to the most heavily polluted areas with PCB all over the world. Very robust associations were found between exposures to PCB and from other organochlorines especially to HCB and several health outcomes. In adults these were thyreopathies, diabetes mellitus, signs of autoimmune processes

and in children hearing impairment, dental enamel defects and neurobehavioral deficits. Our data confirm the statement of an EC document[6] that "the toxic properties seem to have been underestimated and new epidemiological, toxicological and mechanistic data have emerged which indicate that dioxins and some PCBs have a broader impact on health than previously assumed".

A follow-up study in the Michalovce region should be targeted at the following issues: (1) By longitudinal observations of the relevant health outcomes to obtain data on their development in time. (2) To elucidate the impact of genetic predisposition in genes regulating metabolism and detoxification of xenobiotics on organochlorine levels in this population, particularly with regard to hydroxylated and methylsulfonated-PCB metabolites. (3) To apply the geographic information system for assessment of risk to the population exposed to PCB in the Michalovce region.

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References

1 Kocan A., Petrik J., Drobna B. and Chovancova J. (1994) Chemosphere 29, 2315.

2 Kocan A., Petrik J., Jursa S., Chovancova J. and Drobna B. (2001) Chemosphere 43, 595.

3 Pavuk M., Cerhan J.R., Lynch C.F., Schecter A., Petrik J., Chovancova J. and Kocan A. (2004)
Chemosphere 54, 1509.

4 Pavuk M., Cerhan J.R., Lynch C.F., Kocan A., Petrik J. and Chovancova J. (2003) J. Expo.
Anal. Environ. Epidemiol. 13, 267.

5 Langer P., Tajtakova M., Fodor G., Kocan A., Bohov P., Michalek J. and Kreze A.
Eur J Endocrinol. 1998 Oct;139(4):402-9.

6 Commission of the European Communities: Community Strategy for Dioxins, Furans and
Polychlorinated Biphenyls, Brussels 24. 10. 2001 COM (2001) 593 final.

7 Commission of the European Communities: White Paper on Food Safety, Brussels 12. 1. 2000
COM (1999) 719 final.

8 Sovcikova E., Trnovec T., Hustak M., Petrik J., Kocan A., Drobna B., Wimmerova S. and
Wsolova L. DIOXIN 2004 Short Paper submitted.