Early Disease Biomarkers of PCB-exposed Human Population

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ABSTRACT

Polychlorinated biphenyls are widespread persistent hazardous residual environmental contaminants, which have been shown to cause diseases in humans due to oxidative stress, endocrine disruption, and mitochondrial poisoning (Trnovec et al. 2003 and Hertz-Picciotto et al. 2003).This project is a collaborative effort by Howard University and CNMC **(DC Group)**, Slovak Medical University **(Slovak group)** and University of California at Davis **(UC group)** to improve our ability to study the process of developing diseases in the early stage before the clinical sign arises. These personalized measures of exposures will be combined with genomic information to decipher environmental and genetic risk factors for disease development and progression. We have pinpointed three specific aims; **Aim # 1**: *To refine and confirm prior observation on the human genome-wide gene expression pattern upon exposure of PCBs / OH-PCB metabolites in Human Peripheral Blood Mononuclear Cells (PBMC) in vitro to correlate risk*, **Aim # 2**: *To obtain genomic biomarkers of diseases induced /caused by PCBs or their metabolites in early childhood*, and **Aim # 3**: *To validate the candidate disease biomarkers in randomized population studies.* We have selected an unique study population consisting of mother-child pairs (cohort design) from two districts of Slovakia: **Michalovce**, with high PCB contamination (22,000 tons of industrial waste flowed to rivers), and **Svidnik**, with 'background' levels of PCBs during the years 2002-2005 (as a part of the work by Dr. Hertz-Picciotto/Trnovec's NIH RO1 project # RO1-CA96525) to achieve early disease biomarkers for PCB-exposed human population.

DC group has partially published their continuing work funded by NIH grants to show that the over expression of MT1K (Metallothionein) and CYP1A1 P450 (Cytochrome P450), can be associated with human liver disease. We have identified two most potentially significant biomarker genes, CYP1A1 (69.81 up-regulation) and MT1K (14.66 up-regulation), showing highest over-expression at *p*-value ‹0.005 (Dutta et al. 2007). These were selected out of several hundred genes induced *in vitro*, using PCB exposed human liver (HepG2) cells. Over expression of the CYP1A1 (Cytochrome P450) gene was specific to PCB-77 and MT1K (Metallothionien) to PCB-153 (Table 1). In another studies we have shown that apoptosis was the most significant cellular process as a result of oxidative stress but each of these congeners has a unique gene expression signature which was further validated by Taqman RT-PCR and immunoblotting studies, and the pathways involved leading to the common apoptotic effect was completely different. PCB-153 probably acted through TNF receptor, leading to oxidative stress through the involvement of metallothionin gene families causing apoptosis mainly by mitochondrial pathway. In contrast PCB-77 acted through aryl hydrocarbon receptor, leading to oxidative stress through the involvement of cytochrome P450 (CYP1A1) and thereby causing apoptosis mainly by nuclear pathway (De et al, 2007). We have also been able to establish that chronic exposure to PCB-153 could lead to an altered protein expression in human liver cells (HepG2) by altering several apoptotic and tumor suppressor proteins (Ghosh et al. 2007). In this project, we are continuing gene-profiling studies on PCB exposure using human PBMC cell from healthy blood donors. We have completed screening for the research participants for PBMC cells donor and already selected 15 such candidates for the drawing of blood. We have identified PCB 153 and PCB 138, the most abundant PCBs in the blood samples of our selected cohort from Michalovec (Highest Exposure Group) and Svidnik District (background exposure group). The PBMC cells will be exposed with the human equivalence level of this two groups and Microarray experiment will be done to see the global gene expressions.

A. List of gene expression by PCB-153 in HepG2 cells *in vitro*

Gene Symbol	Gene ID	Gene Name	Fold Change (Up-regulation)	Remarks*
217546_at	MT1K	Metalothionein 1K	14.66	Polycystic Liver Disease (Confirmed by RT-PCR)
201008_s_at	TXN1P	Thioredoxin interacting protein	11.91	Polycystic Liver Disease
229800_at	DCAMKL1	Doublecortin and CaM kinase-like 1	9.79	-
210472_at	MT1G	Metalothionein 1G	8.47	Polycystic Liver Disease
205749_at	CYP1A1	Cytochrome P450, family 1A1	6.70	Fatty Liver, hepatocellular carcinoma, fibrosis,cirrhosis (Confirmed by RT-PCR)

B. List of gene expression by PCB-77 in HepG2 cells *in vitro*

Gene Symbol	Gene ID	Gene Name	Fold Change** (Up-regulation)	Remarks
205749_at	CYP1A1	Cytochrome P450, family 1A1	69.81	Fatty Liver, hepatocellular carcinoma, fibrosis,cirrhosis (Confirmed by RT-PCR)
205681_at	BCL2A1	BCL-2 related protein A1	9.89	-
229967_at	SUI1	Putative translation initiating factors	8.30	Chronic Active Liver Disease
207097_at	SLC17A2	Solute carrier family 17 A2	6.69	Polycystic Liver Disease
209921_at	SLC7A11	Solute carrier family 7, member 11	5.94	Polycystic Liver Disease

Table 1. Comparative list of the top five (5) genes which have been reported to have disease connections are shown in this table. Out of 188 and 125 genes up-regulated by PCB 153 and PCB 77 respectively, the data represent the top five (5) genes whose fold changes increased more that 5.5 with a p-value <0.001.(Dutta et al., Environ. Toxicol. Pharmacol., 2007; *in press*) Disease connections are adopted after US National Toxicology Program, 2006.

The Slovak and UC Davis groups have completed the acquisition of the children's data from birth through 16 months of age, including questionnaire administrations, hearing examinations, pediatric examinations, and developmental examinations. The initial cohort consists of over 1000 mother-infant pairs). Mothers of newborns, aged 18-42 years, reached the median PCB value 430 ng/g serum lipids (mean 620 ng/g serum lipids). As expected, the PCB serum concentrations in our subjects showed skewed distribution toward the higher values. The maternal PCB contents (Figure 1) demonstrate that PCB 153, 138, 170 and 180 are the most predominant congeners,similar to other studies (Hovander et al, 2006). This same pattern of 153, 138, 170 and 180 being the most abundant can be seen in the cord PCBs also. The 6 month and 16 month PCBs continue the trend seen in the maternal and cord PCBs. For the OH-PCB data, OH-PCB 187 congener is the most abundant. Our published data show that the hydroxylated metabolites correlate strongly with their parent compounds (Park et al 2007), and appear to be preferentially passed across the placenta (Park et al, in press, Chemosphere).

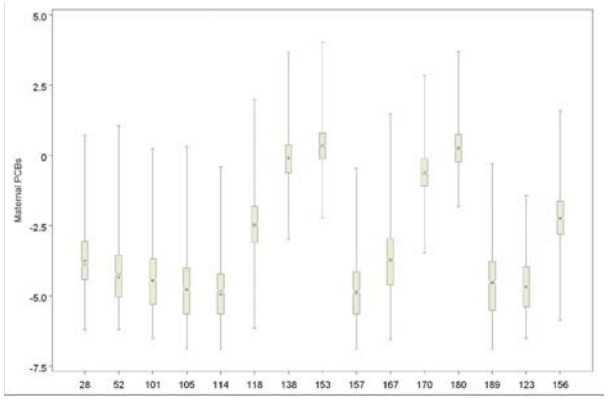

Figure 1. log maternal PCBs ng/ml

Additionally, the methylsulfone metabolites of PCBs can be detected in both maternal and cord samples, indicating placental transfer of these compounds as well, albeit at much lower concentrations (Linderholm et al, 2007). We attempted to identify the major sources of these PCBs through diet. Each mother completed a food frequency questionnaire regarding the items she ate, the amounts, and the frequency during the pregnancy. We also asked her to describe, semi-quantitatively, where she obtained individual food items: at retail outlets or through local production. We focused attention on eight high-fat food items. Results showed that total consumption of fat from locally produced sources was a strong predictor of PCB body burdens in the pregnant mothers, but fat from similar food items purchased from retail outlets was not (Sonneborn et al, in press, J Expos Sci. Env. Epidemiol.).

With regard to growth and development, the UC Davis/Slovak collaboration demonstrated that prenatal PCB exposures were not associated with a global reduction in birth weight in the Slovak newborns, but did appear to induce deficits in intrauterine growth for Romani boys (Sonneborn et al, in press, Paediatr Perinat Epidemiology). This finding mirrors several studies that showed male neonates to be at higher risk for PCB-induced intrauterine growth restriction (Rylander et al 1996, Hertz-Picciotto et al 2005), and moreover, suggests that other environmental factors, including those mediated through social disadvantages, may modify the impact of PCB exposures.

Results on neurobehavioral testing conducted at 16 months of age indicate that PCBs may influence cognitive development through more than one mechanism. Each child was examined using the Bayley Scales of Infant Development. Two scores were determined: the MDI or Mental Development Index, and PDI or Psychomotor Development Index. Based on maternal PCB concentrations, higher prenatal exposures to the dioxin-like PCBs, specifically, the sum of congeners 118 and 156, are related to deficits in the MDI (Park et al, doctoral dissertation, 2007). These analyses were adjusted for other relevant factors such as maternal IQ, the home environment, child's sex and district, and were replicated using cord serum PCBs, which was measured in a subset of the samples. Non-dioxin-like PCBs (sum of 138, 153, 170 and 180) in the prenatal period were not associated with the Bayley scores, but postnatal exposures to this class of PCBs were associated with deficits in the MDI, even after controlling for prenatal PCB exposures. Further analyses demonstrated that OH-PCB 107 in the prenatal period, but none of the other hydroxy-metaboites of PCBs are associated with lower MDI scores. Notably, OH-PCB 107 is principally the metabolite of PCB-118. .

Finally, the impact of PCBs on immune development was assessed in multiple ways. The thymic index, a measure of the volume of the thymus based on ultrasonography, was evaluated during the hospital stay of these newborns. The values were then regressed against the sum of the congeners of PCBs. After multivariate adjustment for child's sex, birth weight, and gestational age at birth; and maternal education, ethnicity, smoking, alcohol consumption, and respiratory infections during pregnancy, a higher PCB concentration was associated with reduced thymic index (Park et al in press). The magnitude of the association was similar to that of one week of prematurity.

In the adult population, higher PCB exposure was associated with alterations of thyroid gland (higher thyroid volume, more frequent hypoechogenicity), increased levels of anti-TPO antibodies and alterations of TSH levels (mainly in males), and more frequent prevalence of dysglycemic states. School-aged children with PCB exposure in the highest tertile had decreased hearing thresholds at lower frequencies and decreased amplitudes of otoacoustic emissions (Fig. 2) not related to thyroid hormone levels. It was concluded that long-term environmental exposure to PCBs is associated with subclinical but diagnosable hearing deficits (Trnovec et al. 2007).Furthermore, performance of children in psychological tests was worse, if compared to children with lower PCB exposure. Proportion of dental enamel defects increased with increasing PCB exposure. In newborns, increased prenatal exposure to PCBs was related to smaller thymus and changes in lymphocyte subpopulations. In general, the subjects belonging to the upper percentiles of PCB exposure incur disproportionately higher health risk compared to subjects of the lower percentiles. Estimation of the size of population segment at risk is preliminary, but if confirmed using geographic information system, the health outcomes of such an extent of exposure and large number of subjects exposed may have very serious public health consequences.

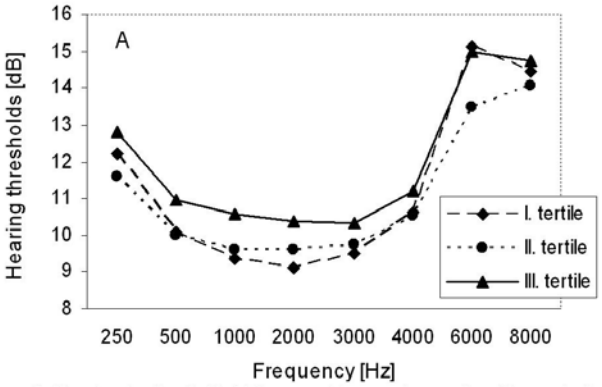

Figure 2. Mean hearing thresholds (dB) assessed by pure tone audiometric examinatio of 428 8- 9-years old children residing in an area of Eastern Slovakia polluted by PCB grouped into tertiles with regard to PCB serum concentrations. Data for left ears.

References:

De S., Ghosh S., Chen Y-Q, Moses L., Kesari A., Hoffman EP, Dutta SK, Identification of PCB Induced Congener Specific Oxidative Stress Response Pathways by Microarray Analysis using Human Liver Cell Line (Communicated, 2007).

Dutta SK., **Ghosh S.**, De S. Hoffman EP. CYP1A1 and MT1K are Congener Specific Biomarker Genes for Liver Diseases Induced by PCBs. *Environ. Toxicol. Pharmacol., 2007* (Accepted and in Press. Published online: doi:10.1016/j.etap.2007.10.009).

Ghosh S., De S., Dutta S. K., Altered Protein Expressions in Chronic PCB-153 Induced Human Liver (HepG2) cells. *Int. J. Toxicol.*, 26, 203-212, 2007.

Hovander L, Linderholm L, Athanasidou M, Bignert A, Fangstrom B, Kocan A, Petrik J, Tronvec T, Bergman A, Levels of PCB and their Metabolites in the Serum of Residents of Highly Contaminated Area in Eastern Slovakia. Environ. Sci. Technol. 40(12), 3696-3703, 2006

Hertz-Picciotto I, Trnovec T, Kočan T, Charles MJ, Čižnar P, Langer P, Sovčikova E, James R. PCB's and early childhood development in Slovakia: Study design and background. Fresenius Environmental Bulletin 12:208-214, 2003.

Hertz-Picciotto I, Charles MJ, James (Baker) RA, Keller JA, Willman E, Teplin S (2005). *In utero* polychlorinated biphenyl exposures in relation to fetal and early childhood growth. Epidemiology Sept 2005;16(5):648-656.

Linderholm L, Park J-S, Kočan A, Trnovec T, Athanasiadou M, Bergman Å, Hertz-Picciotto I. Maternal and cord serum exposure to PCB and DDE methyl sulfone metabolites in eastern Slovakia. Chemosphere 2007 Sep;69(3):403-10 PMID: 17574648.

Park H-Y, Hertz-Picciotto I, Petrik J, Palkovičova L, Kočan A, Trnovec T. Prenatal PCB exposure and thymus size at birth. Environ. Health. Persp. (*in press*) published online August 14, 2007, http://www.ehponline.org/members/2007/9769/9769.pdf.

Park J-S, Linderholm L, Charles MJ, Athanasiadou M, Petrik J, Kočan A, Drobna B, Trnovec T, Bergman A, Hertz-Picciotto I (2007). Polychlorinated biphenyls and their hydroxylated metabolites (OH-PCBs) in pregnant women from Eastern Slovakia. Environ Health Persp 115(1):20-27. PMID: 17366814

Park J-S, Bergman Å, Linderholm L, Athanasiadou M, Kočan A, Petrik J, Drobna B, Trnovec T, Charles MJ, Hertz-Picciotto I. Placental transfer of polychlorinated biphenyls, their hydroxylated metabolites, and pentachlorophenol in pregnant women from eastern Slovakia. Chemosphere (In press).

Rylander L, Stromberg U, Hagmar L. Dietary intake of fish contaminated with persistent organochlorine compounds in relation to low birth weight. Scand J Work Environ Health 1996;22(4):260-6

Sonneborn D, Park H-Y, Petrik J, Kočan A, Palkovičova L, Trnovec T, Nguyen DV, Hertz-Picciotto I. Prenatal PCB exposures in Eastern Slovakia modify effects of social factors on birthweight. Paediatr Perinatal Epidemiol (in press)

Trnovec T., Kokan A., Langer P., Sovcikova E., Tajtakova M., Bergman A., Berg van den M., Brouwer A., Machala M., Winneke G., Sampson B., Brunekreef B. Gene expression patterns in human placenta. PNAS, 100(8), 4748-4753, 2003.

Trnovec T, Sovčíková E, Hust'ák M, Wimmerová S, Kočan A, Jurečková D, Langer P, Palkovičová Ľ, Drobná B Exposure to polychlorinated biphenyls and hearing impairment in children . *Environ. Toxicol. Pharmacol., 2007* (Accepted and in Press. Published online: DOI:10.1016/j.etap.2007.10.030).

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