TRANSCRIPTOMICS IDENTIFIES DIFFERENCES BETWEEN ULTRAPURE NON-DIOXIN-LIKE POLYCHLORINATED BIPHENYLS (PCBs) AND DIOXIN-LIKE PCB126 IN CULTURED PERIPHERAL BLOOD MONONUCLEAR CELLS

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Introduction

Polychlorinated biphenyls (PCBs) remain ubiquitously present in human lipids despite the ban on their production and use. PCBs bioaccumulate and biomagnify in biota because of their stability and lipophilic character. Their presence can be chemically monitored in peripheral blood samples of the general population. Depending on the molecular structure of the PCBs, different processes and enzymes are induced in the body. We tested whether *in vitro* exposure to different PCB congeners induced different gene expression profiles in peripheral blood cells.

Materials and methods

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood of 8 healthy individuals. Cells were exposed *in vitro* to ultra-pure individual non-dioxin-like (NDL)-PCB congeners (PCB52, 138 or 180, 10µM) or dioxin-like (DL)-PCB congener PCB126 (1µM) during 18h. Differential gene expression response was measured using Agilent Whole Human Genome microarrays. Two-way ANOVA analysis was used to assess if gender and PCB exposure contributed to differential gene expression. Differentially expressed transcripts (congener-specific) were identified by performing t-tests for each PCB congener and each gender, with significance levels of $p < 0.05$ and a cut-off value for the fold change (FC) of at least 1.5. Functional analysis was performed using GO and Ingenuity Pathway Analysis.

Results and discussion:

Two-way ANOVA analysis, with a factor gender and PCB exposure, revealed that factors are important in influencing gene expression responses in blood cells (Table 1). More than 3000 genes were differentially expressed ($p<0.05$) between male and female donors and in relation to PCB treatment, 861 genes could be identified with a p-value of 0.05. 147 genes showed a significant modulation of gene expression with a more stringent p-value cut-off ($p < 0.001$).

Effects	Differentially expressed genes		
p-value	**< 0.05**	**< 0.01**	**< 0.001**
Gender	3330	1725	546
Treatment	861	373	147
Interaction	261	47	8

Table 1: Number of differentially expressed genes influenced by gender, treatment or an interaction of both factors using different p-values as cutoffs (Two-way ANOVA).

The exposure-related genes were used for hierarchical clustering analysis, which revealed that DL-PCB126 induced a different gene expression response compared to the NDL-PCBs. The genes within the gene clusters are linked to ion binding, immune and inflammatory response, induction or regulation of cell death or apoptosis, lipid and cholesterol homeostasis (Figure 1).

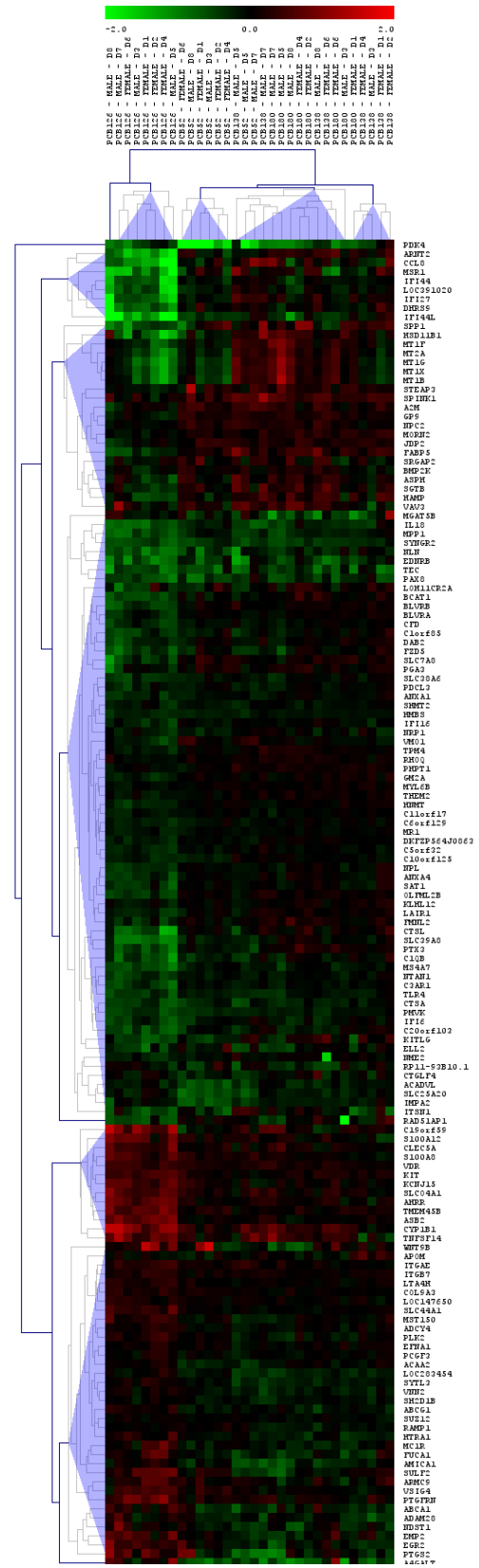

Figure 1: Hierarchical clustering analysis. Depicted here are gene expression values of genes influenced by treatment from analyses of 8 PBMC samples exposed to 4 different PCB congeners (Two-way ANOVA, p < 0.001). Ratios are a measure of relative gene expression in each experimental sample. Samples and genes are clustered using complete linkage and Euclidean distance as the distance metric. The genes in cluster 1 are not linked to a specific process, cluster 2 genes are linked to ion binding, cluster 3 to immune and inflammatory response, cluster 4 to induction or regulation of cell death or apoptosis and cluster 5 to lipid and cholesterol homeostasis.

Differentially expressed genes per PCB congener and per gender were identified using a one-class Student t-test. Gene lists were compared using 4-way Venn diagrams. Based on these differentially expressed gene lists ($p < 0.05$ and $FC > 1.5$), all PCB exposed samples were significantly related to nuclear receptor activation or signalling, hypoxia (HIF signalling), cell cycle (G2/M and G1/S transition), acute phase response and hepatic toxicity. PCB126 showed a more dioxin-like response by AhR signalling, while PCB52, PCB138 and PCB180 gene lists were enriched for other nuclear receptors that all form a heterodimer with the retinoid X receptor (RXR).

PCBs are known to bind to nuclear receptors which trigger other biological responses. Three nuclear receptors acting as regulators of detoxification and elimination of xenobiotics (Nakata *et al.*, 2006) were selected and all direct and indirect downstream interacting biological molecules according to IPA were added to an interaction network. The colored molecules are those that are also present in the gene lists obtained by t-testing for each PCB congener. In green, the genes are shown that show a changed gene expression pattern after exposure to PCB126 (CYP1B1, ESR1, MAF), the red symbols refer to genes significantly influenced by NDL-PCB exposure (ABCB10, MDM2, SERPINB2) and the molecules in red/green were present in differentially expressed gene lists from both PCB126 and one or more NDL-PCB (CDK2, HSP90AB1, PTGS2, TP53). The colored molecules connected with blue lines are interacting directly or indirectly with the receptors and are induced in our *in vitro* experiment, but were not recognized by IPA as induced by the receptor via activation or transcription. Some of the molecules (e.g. ESR1) interact with more than one nuclear receptor, indicating that the pathways are connected. When overlapping the networks generated for each pathway with biological functions and diseases, the most relevant function (according to IPA) related to CAR/PXR is lipid metabolism, with 11 molecules in the network related to this function. The AhR receptor network was associated with cancer, which related to 15 molecules in the network.

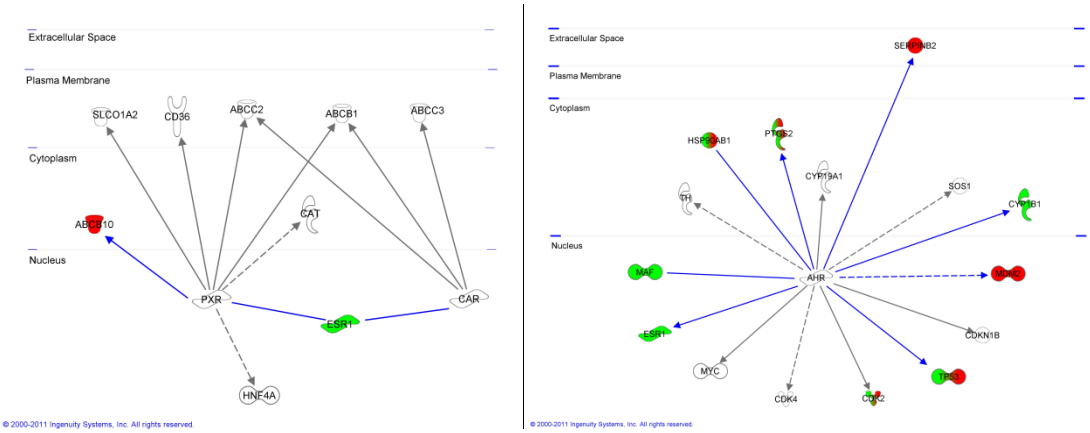

Figure 2: Interaction network of 3 nuclear receptors with direct and indirect activation or transcription relationships (downstream) with other biological molecules present in blood and/or PBMC. Interacting molecules with A: CAR and PXR, B: AhR. The colored molecules refer to genes significantly influenced by PCB exposure; green refers to PCB126 exposure, red to NDL-PCB exposure. These networks were prepared using Path Designer in Ingenuity Pathway Analysis.

This study is the first one to report on genome-wide transcriptomics using ultrapure NDL-PCB congeners. A differential gene expression response could be induced in *ex vivo* exposed peripheral blood cells from healthy

donors. The data indicate that blood cells originating from male donors responded differently from cells of female donors. Hierarchical clustering analysis showed a congener-specific response pattern. The PCBs induced a weak, but significant, effect in resting primary PBMC. PCB126 clearly induced the AhR signaling pathway and organ toxicity pathways, whereas the induction of nuclear receptor pathways by the NDL-PCBs was limited but significant. Gene expression analysis in PBMC may yield biomarkers for exposure to PCBs and may relate to some of the known *in vivo* systemic effects.

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