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Perinatal Exposure to Purity-Controlled Polychlorinated Biphenyl 52, 138, or 180 Alters Toxicogenomic Profiles in Peripheral Blood of Rats after 4 Months

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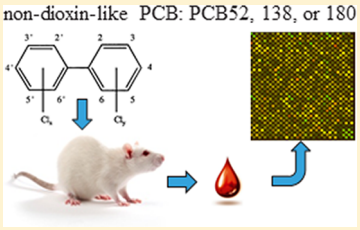
Supporting Information

ABSTRACT: It is known from controlled animal experiments and human epidemiologic studies that early life exposure to mixtures of polychlorinated biphenyls (PCBs) is a risk factor for developmental neurotoxicity. The importance of non-dioxin-like PCBs in the context of the observed effect is uncertain because of the blending with the more potent dioxin-like PCBs. Previously, a controlled rat perinatal exposure study with individual, purity-controlled, non-dioxin-like congeners (PCB52, PCB138, or PCB180) was set up. Impaired motor coordination, motor activity, and learning has been reported for the offspring at an age of approximately 4 months. Here, we report on the gene expression responses that have been observed in the blood of the same animals. ANOVA analysis called 1412 genes differentially expressed 4 months after the PCB treatment was stopped. Subsequently, each PCB exposure condition was compared to the corresponding vehicle control using a fold change analysis. The gene lists contained between 82 and 348 differentially expressed genes. Expression patterns were complex with sets of differentially expressed genes being specific for a particular PCB exposure and other sets in common between several exposure conditions. Thirty-two genes were differentially expressed under all conditions. Bioinformatic overrepresentation analysis identified enriched biological terms such as lipid metabolism, molecular transport, small molecule biochemistry, and cell signaling and proliferation. Gene lists were particularly enriched for nervous system development and function ontology. In conclusion, we have documented for the first time differential gene expression in a well-controlled animal study that reported behavioral effects of purity-controlled individual non-dioxin-like PCBs.



INTRODUCTION

Polychlorinated biphenyls (PCBs) were used commercially in numerous industrial applications because of their physical and chemical properties, such as nonflammability, chemical stability, high boiling point, low heat conductivity, and high dielectric constants. From a biological point of view, a PCB can be classified as a dioxin-like (DL) or a non-dioxin-like (NDL) congener according to its binding capacity to the arylhydrocarbon receptor. DL PCBs have, in contrast to NDL PCBs, a high affinity for the latter receptor.[1] NDL PCBs may bind to the constitutive androstane receptor and/or the pregnane X receptor.[2,3]

PCBs are persistent in the environment because of slow chemical and microbiological degradation. The dietary intake represents the main route of human exposure to PCBs, with the exception of specific cases of accidental or occupational exposure. Despite their production ban in almost all industrial countries in the late 1980s, national and international authorities still monitor the presence of these persistent organic contaminants in feed and food.[4,5]

Exposure to PCBs has been associated with a negative impact on human and animal health. PCB mixtures, as well as individual congeners, are known to cause reproductive,[6] neurological,[7] and immune dysfunctions.[8] PCB exposure during early life also became a known risk factor for neurological disorders.[9] This became evident when pregnant women, who were accidentally exposed to PCB-contaminated food, gave birth to children who showed signs of neurodevelopmental toxicity.[10,11] Epidemiological studies in the United States and several European countries have reported that low PCB exposure levels during pregnancy are correlated with decreased IQ scores of children, impaired learning and memory, decreased neuromuscular function, and lower reading compre-

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hension.[12,13] Controlled animal exposure studies support the results observed in humans.

Behavioral changes and learning deficits have been observed in primates[14] and in rodents.[15] Boix and co-workers recently reported effects on behavior when rats were exposed to individual PCB congeners (PCB52, PCB138, or PCB180) from gestational day 7 until postnatal day 21. Exposure to PCB52 impaired motor coordination possibly due to increased extracellular γ-aminobutyric acid concentration in the cerebellum. The impaired ability to learn the Y maze test following exposure to PCB138 or 180 is suggested to be related to changes in the glutamate-NO-cGMP pathway in the striatum.[16]

Here, we report on a toxicogenomics study that was performed in the context of the experiment of Boix and co-workers.[16] We investigated if (1) differential blood gene expression could be identified in the same rats 4 months after terminating the PCB exposure, (2) the response was congener-specific, and (3) gene lists were enriched for biological functions and pathways.

MATERIALS AND METHODS

Experimental Setup. The Laboratory of Neurobiology (Centro de Investigación Principe Felipe, Valencia, Spain) conducted the animal experiment in the context of the ATHON project (FOOD-CT_2006-022923). In brief, pregnant Wistar rats were treated orally with PCB52 (2,2′,5,5′-tetrachlorobiphenyl), PCB138 (2,2′,3,4,4′,5′-hexachlorobiphenyl), PCB180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl), or vehicle (corn oil) from gestational day 7 to postnatal day 21. Each dam received 1 mg of PCB/kg body weight/day via the feed. Pups were weaned at 21 days and sacrificed following behavioral and biochemical analysis at an age of 4 months. The experimental setup is described in detail by Boix and co-workers.[16]

Genome-wide gene expression analysis was performed on blood obtained from a subset of 47 pups. Six dams were used for control and PCB52 exposure experiments, 4 dams for PCB138, and 5 for PCB180. Control animals consisted of 5 male and 6 female pups, whereas 36 animals were perinatally exposed to PCBs (6 males and 6 females exposed to PCB52, PCB138, or PCB180). In total, there were eight experimental groups. During sacrifice, blood samples of 0.5 mL were collected from the tail vein in 1.3 mL RNA*later* (Qiagen, Venlo, The Netherlands). The samples were stored at −80 °C to preserve RNA quality.

RNA Extraction, Purification, and Globin Reduction. RNA was extracted from the blood samples with the RiboPure-Blood RNA isolation kit (Ambion, Foster City, USA). Blood samples were thawed at room temperature and centrifuged to pellet the blood cells. Blood cells were lysed in a guanidinium-based solution, followed by phenol−chloroform extraction, and a final purification using glass fiber filter technology. The average RNA concentration was 28.8 μg as measured with a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, USA).

A Globinclear kit (Ambion) was used to generate globin mRNA-depleted total RNA. Biotinylated globin-capture DNA oligos were added to 4 μg of total RNA, and globin mRNAs were removed by streptavidin magnetic beads. The remaining globin-reduced total RNA was purified using magnetic beads and eluted in 30 μL of elution buffer. RNA integrity was tested on an Agilent 2100 Bioanalyzer using RNA 6000 Nano Chips (Agilent Technologies, Palo Alto, USA). RNA was intact and showed two distinct bands for the 18S and 28S rRNA (ratio 28S/18S > 0.9), and the RNA integrity number (RIN) varied between 7.5 and 9.6. Samples were stored at −80 °C until further use.

RNA Amplification and Labeling. Total RNA was amplified and labeled to generate complementary RNA (cRNA) using the Low RNA input Linear Amplification kit (LRILAK PLUS) according to the instructions from Agilent Technologies. Briefly, 1 μg of globin-reduced total RNA was reverse transcribed into complementary DNA (cDNA) using T7-promotor primer and MMLV reverse transcriptase. The cDNA was transcribed into cRNA during which it was fluorescently labeled by incorporation of cyanine (Cy)3-CTP. The single-stranded, labeled cRNA was purified with RNeasy mini spin columns (Qiagen) and analyzed using a NanoDrop spectrophotometer. All samples had a yield of at least 1.65 μg of cRNA and a dye-incorporation rate of more than 8 pmol/μg.

Microarray Hybridization. A total amount of 1.65 μg of cRNA was hybridized on 4x44K Whole Rat Genome microarray slides (design 014879, Agilent Technologies) for 17 h in a hybridization oven at 65 °C.

Slides were washed according to the manufacturer's instructions and finally washed in acetonitrile for 1 min. The arrays were scanned on an Agilent DNA microarray scanner (G2565BA), and the images were processed using Agilent Feature Extraction Software (version 9.5). The use of this software included automatic grid positioning, signal intensity extraction, and quality control. Details on the data processing steps used to generate the Agilent one-color output can be found in the Agilent protocol GE1-v5_95_Feb07. All microarrays passed the quality control metrics that are implemented in Feature Extraction 9.5.

Statistical Analysis. Data were uploaded in GeneSpring 11.5.1 (Agilent Technologies) for statistical analysis. Microarray data were quantile normalized. The final data set contained only probes with an Entrez identifier and an expression signal above the 20th percentile in all samples of at least one experimental group. Two-way ANOVA was applied to determine if gender, treatment (PCB exposure), and/or an interaction between both factors contributed to differential gene expression. A Benjamini-Hochberg false discovery rate correction (FDR) was applied to correct for multiplicity. We calculated a fold change (FC) for each probe by comparing a PCB exposure condition versus vehicle control condition. Probes were called significant when they passed double filtering, i.e., FDR ≤ 0.05 and absolute FC ≥ 1.5.[17] This resulted in 6 gene lists containing differentially expressed genes (one list per experimental condition). Venn diagrams were constructed using VennPlex for a visual representation of logical relationships between the different gene lists.[18] A heatmap of differentially expressed genes was constructed using the online Matrix2png tool.[19]

Biological Interpretation and Pathway Analysis. Gene lists were submitted to functional analysis using Ingenuity Pathway Analysis (IPA, version 8.0, Ingenuity Systems). The core analysis workflow was used to identify the biological functions, ontologies, and pathways that were most significant to the different gene lists. Right-tailed Fisher's exact test was used to calculate a *p*-value determining the probability that each term assigned to that data set is due to chance alone. A heatmap of enriched pathways was constructed using Matrix2png.

RESULTS

Identification of Differentially Expressed Genes. Two-way ANOVA called 2794 probes and 1412 probes significant (FDR ≤ 0.05) for the factors gender and treatment, respectively. The probes and their annotation are in Supporting Information (file 1). No interaction effect between the fixed factors was observed at a FDR ≤ 0.05.

The 1412 genes that were called significant were used for differential expression analysis (fold change, FC). This was done for each test condition. The amount of genes that passed the double-filtering criterion (FDR ≤ 0.05 and absolute FC ≥ 1.5) varied from 82 in the case of female rats exposed to PCB180 to a maximum of 348 in the case of females exposed to PCB138. Filtering based on absolute FC ≥ 2.0 reduced the amount of differentially expressed genes considerably (Table 1). The lists with differentially expressed genes, including their biological annotation, and FCs are in Supporting Information (file 2).

Next, we constructed Venn diagrams in order to visually assess the overlap and separation between the different gene lists. When making the comparison per PCB congener, 15 to

Table 1. Amounts of Differentially Expressed Genes (DEGs) in the Blood of Rats That Were Perinatally Exposed to 1 mg of PCB/kg Body Weight/Day (PCB52, PCB138, or PCB180)[a]

	DEGs in males		DEGs in females	
PCB	absFC ≥ 1.5	absFC ≥ 2.0	absFC ≥ 1.5	absFC ≥ 2.0
52	277	86	107	24
138	263	72	348	82
180	213	51	82	16

[a]Exposure conditions were compared to vehicle controls. DEGs were identified using the double-filtering criterion: Benjamini-Hochberg False Discovery (FDR) ≤ 0.05 and an absolute fold change (abs FC) ≥ 1.5 or ≥2.0.

60% of the differentially expressed genes were changed both in male and female samples. The majority of the genes in common showed the same polarity of expression regulation (Figure 1). When making the comparison per gender, considerable numbers of genes were in common between 2 or 3 test conditions. The greatest overlap in genes was obtained when PCB52 and PCB180 test conditions were compared. Most of the genes in common had the same polarity of expression regulation. The genes in common between PCB138 and PCB52 (or PCB180) had an opposite polarity (Figure 2). Finally, an overlay of the differentially expressed gene lists identified 32 genes that were in common among the 6 conditions.

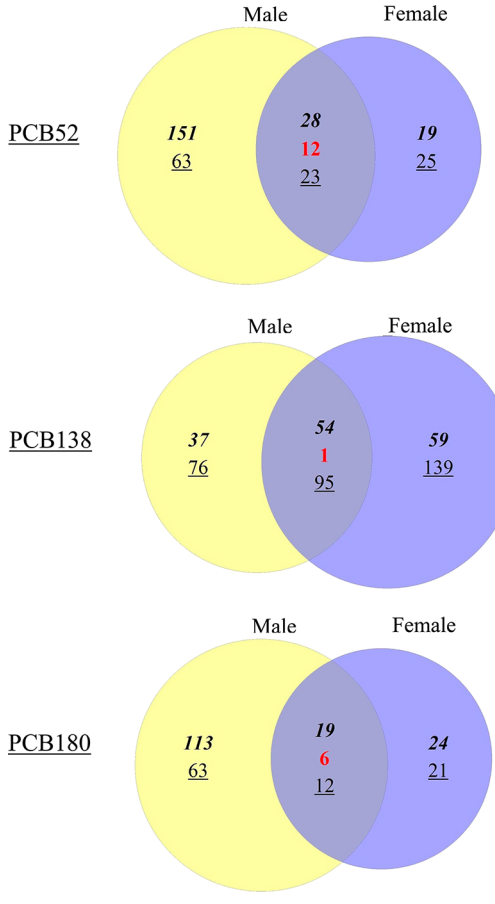

Figure 1. Two-way Venn diagrams assessing the impact of gender on the overlap and separation between the gene lists. The comparison was done separately for PCB52, PCB138, and PCB180. Gene lists were generated for each exposure condition using the double-filtering criterion (absolute FC ≥ 1.5 and FDR ≤ 0.05). Numbers in bold and italics refer to upregulated genes, numbers in red and bold to contraregulated genes, and underlined numbers to downregulated genes.

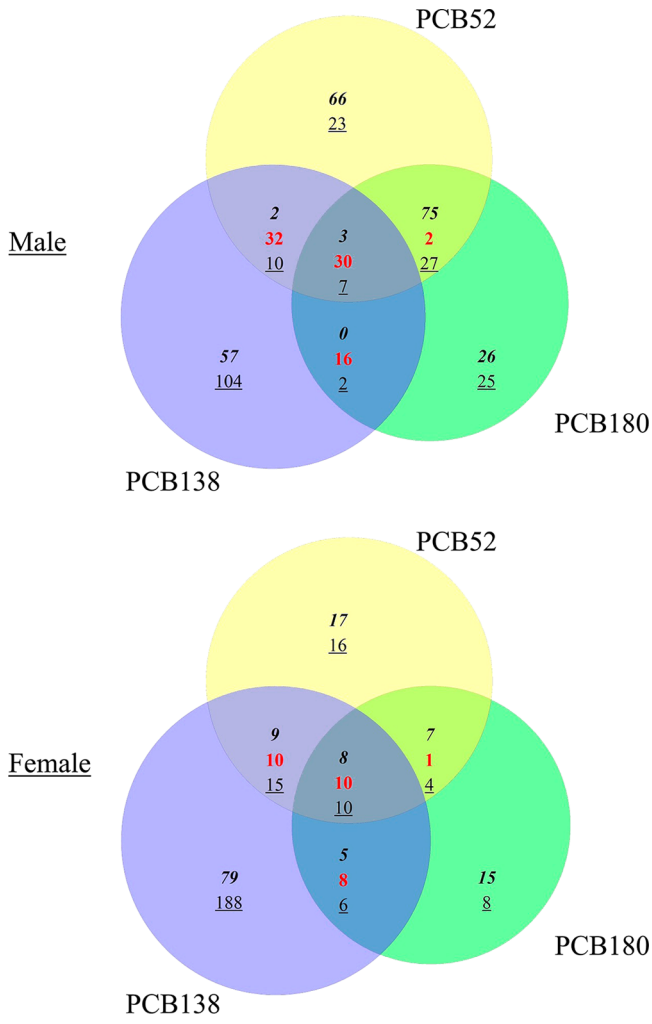

Figure 2. Three-way Venn diagrams assessing the impact of PCB exposure on the overlap and separation between the gene lists. The comparison was done separately for males and females. Gene lists were generated for each exposure condition using the double-filtering criterion (absolute FC ≥ 1.5 and FDR ≤ 0.05). Numbers in bold and italics refer to upregulated genes, numbers in red and bold to contraregulated genes, and underlined numbers to downregulated genes.

An absolute FC ≥ 2 in at least one test condition was observed for the latter genes. For example, there was a strong downregulation of fibulin 1 (FBLN1) and myxovirus resistance 2 (MX2) in all test conditions, camello-like 5 (CML5) showed a strong upregulation, and CCR4 carbon catabolite repression 4-like (CCRN4L) and pyroglutamylated RFamide peptide (QRFP) were downregulated in male samples (Figure 3).

Biological Interpretation of Differentially Expressed Genes. The top 3 enriched biological functions were identified for each gene list. Several enriched terms were shared between the different test conditions. For example, lipid metabolism was in common between PCB52 and PCB180 exposed females. Molecular transport was in common between PCB52 and

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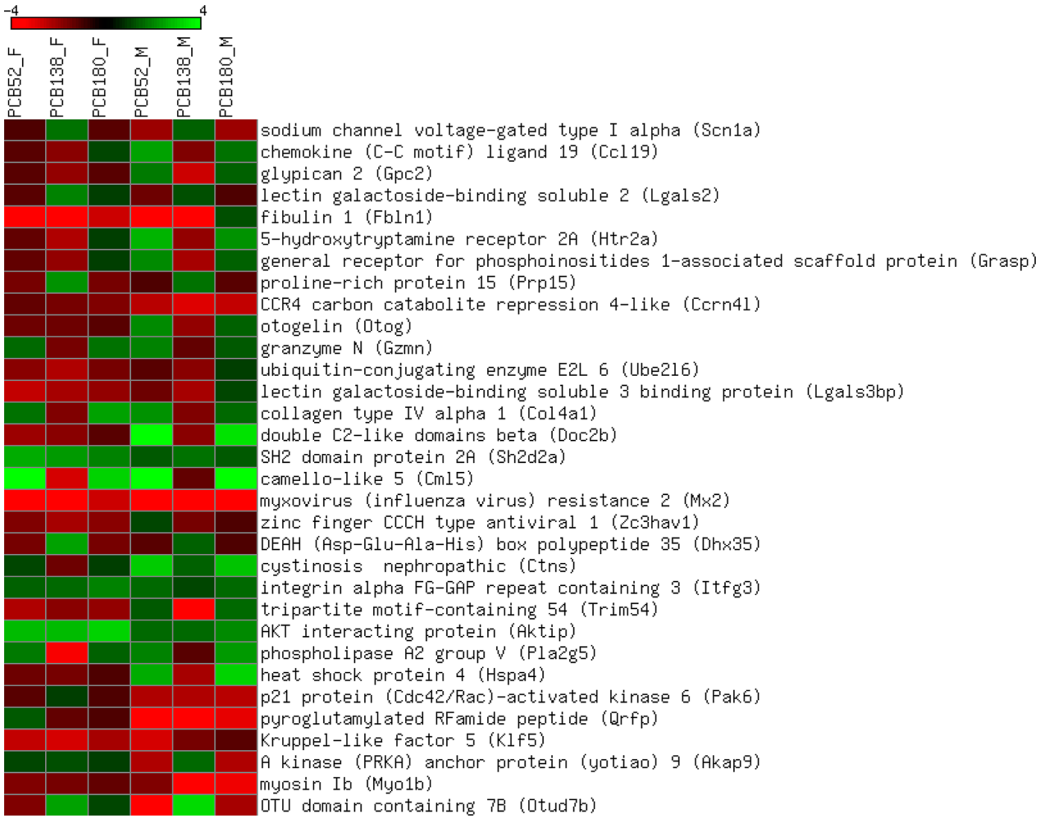

Figure 3. Heatmap of the 32 genes that were called differentially expressed for all exposure conditions (factors, PCB52, PCB138, or PCB180; and _M, male; or _F, female). The color code refers to the fold change (FC) that was obtained when comparing the exposure condition to the vehicle control, with downregulation (green) and upregulation (red).

PCB180 exposed males and females. Terms associated with PCB138 were somewhat different than the ones associated with PCB52 and PCB180 exposure. The numbers of genes that were associated with a biological term varied from 3 to 20, and only a few of these genes were in common among multiple test conditions, i.e., mesogenin 1 (MSGN1), phospholipase A2 group V (PLA2G5), soluble 1 galactoside-binding lectin (LGALS1), 5-hydroxytryptamine receptor 2A (HTR2A), and hypocretin (HCRT). An overview of the most important biological functions and associated genes is given in Table 2. None of the gene lists was significantly enriched for an IPA toxicity list related to nuclear receptor activation.

Pathway analysis revealed only weak associations of the gene lists with signaling pathways that are registered in IPA. The 10 most enriched pathways were related to immune system functioning, and the most significant pathway was type 1 diabetes mellitus signaling. The strongest associations were observed for female rats exposed to PCB52 (Figure 4). Six genes, mainly coding for subunits of major histocompatibility complexes, were associated with the type 1 diabetes mellitus signaling pathway.

PCB test conditions caused a downregulation of the latter genes (results not shown). The IPA knowledge base associated the gene lists with several pysiological system development and function ontologies: digestive system, embryonic development, endocrine system, organ development, and neurological system. The most overrepresented term was neurological system. The differentially expressed genes annotated to this term and their FC are given in Table 3. Several genes involved in neuronal differention (for example, GDF11, GPC2, and IFNA16) and neurotransmission (for example, CHRNA7, CST3, GRIA4, and GRM3) were identified.

DISCUSSION

We report on the genome-wide gene expression results that were generated from peripheral blood that was collected in the study of Boix and co-workers.[16] Our aims were to compare gene expression responses that were induced by exposure to individual NDL PCB congeners, to analyze gender dependency of the profiles, and to investigate if differentially expressed gene lists were enriched for biological terms and pathways. Our toxicogenomics study identified differential gene expression in blood of the rats almost 4 months after perinatal exposure was stopped. Boix and co-workers reported a lasting effect of PCB exposure on behavior that remained when these rats were approximately 4 months old. PCB52 impaired motor coordination but not learning. PCB138 and 180 impaired learning but not motor coordination. The observed behavioral effects were similar in males and females.[16] Additionally, Boix and co-workers found that motor activity was not affected by PCB52 exposure. PCB180 reduced it in males but not in females, and PCB138 reduced activity both in males and females.[20]

Recently, neurodevelopmental effects have been reported in an Eastern Slovakian birth cohort.[21,22] The effects were associated with blood levels of PCB138, 153, and 180.[23] Blood samples obtained from the same cohort were submitted to microarray analysis. Differential gene expression could be related to PCB exposure. Bioinformatic analysis revealed gene signatures that were enriched for biological terms related to

Table 2. Three Most Significantly Enriched Biological Functions for Each of the PCB Exposure Conditions[a]

PCB	Male	Female
52	**1. Carbohydrate metabolism** SOCS1↑,AKT2↑,SLC5A2↑,PPP1R3C↑,PTPN1↓ INS↑,HTR2A↑ **2. Molecular transport** HCRT↑,FN1↑,SLC5A2↑,PPP1R3C↑,GRIP2↑ UCP1↑,MSGN1↑,NSF↓,AQP3↑,HNRNPA3↓ PLA2G5↑,INS↑,RYR1↑,MGLL↑,SCNN1B↓ HTR2A↑,FEZ2↓,SPTBN2↑,SCN2A↓,AKT2↑ SGTA↑,VAMP2↑,CPLX1↑,SLC17A7↑ **3. Cell-to-cell signaling and interaction** NSF↓,SCN2A↑,SCN1A↑,HCRT,FN1↓,GRM3↑ CST3↓,MGLL↑,SGTA↑,INHBA↓,HTR2A↑	**1. Lipid metabolism** PLA2G5↑,MSGN1↑,LGALS1↑ **2. Molecular Transport** PLA2G5↑,ZC3HAV1↓,MSGN1↑,LGALS1↑ **3. Small molecule biochemistry** HPD↓,PLA2G5↑,CPE↓,MSGN1↑,ACHE↓ LGALS1↑
138	**1. Cell-to-cell signaling and interaction** PRL↑,CCL21↓,ACHE↓,HBEGF↑,EGF↑ PVRL1↓,INHBA↑,HTR2A↓ **2. Cellular growth and proliferation** SH2D2A↑,MYCN↓,KLK1↓,PRL↑,POU4F1↓ EGF↑,HBEGF↑,CCDC92↑,INHBA↑,FGFR3↑ PTPRJ↓,RHOA↓,KLF5↓,CCL21↓,SLC25A27↑ GCG↓,MAGI2↓,DNM2↓,HTR2A↓ **3. Cell death and survival** KLK1↓,PRL↑,POU4F1↓,CCDC92↑,EGF↑ MMP2↓,MAPK13↓,MAPK11↑,ACHE↓ INHBA↑,HSPA4↑,MAP3K10↓,HLA-C↓ RHOA↓,KLF5↓,SLC25A27↑,GCLM↓,TAP2↓ DNM2↓,HTR2A↓	**1. Cellular assembly and organization** MYCN↓,FN1↑,ATP2B1↑,CHN1↓,CDC6↓ NUMB↑,ACHE↓,TPM1↓,EGF↑,CPLX1↓ DOC2B↓,NSF↑,DMD↓,RHOA↓,GNAS↓ LGALS8↓,CHRNA7↑,ANXA7↓,DAG1↑ SNCA↓ **2. Cell-to-cell signaling and interaction** SCN1A↑,SCN2A↑,FN1↑,PRL↑,ACHE↓,EGF↑ CCL5↑,LGALS8↓,CCL21↓,CHRNA7↑,MGLL↓ PVRL1↓,SNCA↓,HTR2A↓ **3. Cellular growth and proliferation** FN1↑,SOX6↓,PRL↑,RHOA↓,CCL21↓,EGF↑ HTR2A↓
180	**1. Amino acid metabolism** HPD↓,HCRT↑,HTR2A↑ **2. Cell-to-cell signaling and interaction** SCN1A↓,HCRT↑,GRM3↑,CHRNA7↓,PVRL1↑ AKAP9↓,GRIA4↑,HTR2A↑ **3. Molecular transport** HCRT↑,PPP1R3C↑,PLA2G5↑,SLCO1A2↑ MSGN1↑,SCNN1B↓,HTR2A↑,PCYT1A↑	**1. Lipid metabolism** PLA2G5↑,MSGN1↑,LGALS1↑ **2. Molecular transport** SLC6A8↓,PLA2G5↑,ZC3HAV1↓,MSGN1↑ LGALS1↑ **3. Small molecule biochemistry** SLC6A8↓,HPD↓,PLA2G5↑,MSGN1↑ LGALS1↑

[a]Enrichment analysis was done in Ingenuity Pathway Analysis. Genes that were associated with these terms are given, as well as their expression polarity: upregulation (red arrow) or downregulation (green arrow).

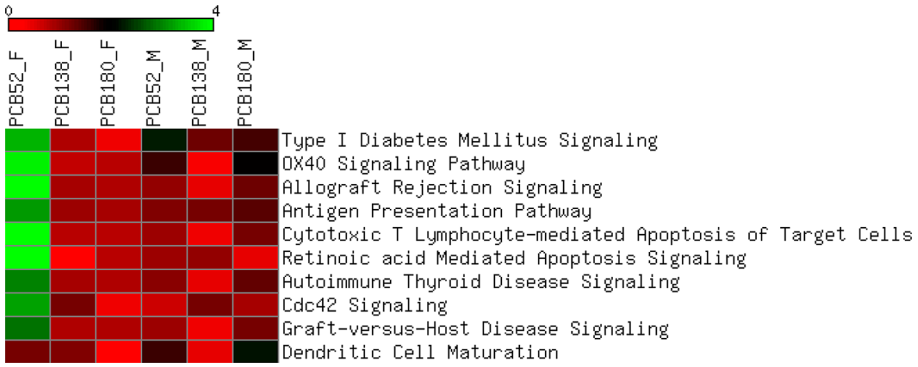

Figure 4. Heatmap of the 10 most enriched signaling pathways that were obtained from the gene lists using Ingenuity Pathway Analysis (factors, PCB52, PCB138, or PCB180; and _M, male; or _F, female). The color code is related to the $-\log_{10}$ *p*-value obtained during the overrepresentation analysis, with low significance (red) and high significance (green).

neurological diseases.[24,25] With our toxicogenomics investigation in blood, we wanted to contribute to an evidence-based identification of the mode-of-action of NDL PCBs. Furthermore, our results may have value for the future development of a blood biomarker in the context of human surveillance studies that focus on the effects of PCB exposure.

The NDL PCBs used in our study were synthesized and controlled for purity (purity ≥99.9999%) in the context of the ATHON project. The aim of ATHON, acronym for assessing the toxicity and hazard of non-dioxin-like PCBs present in food, was to increase the knowledge on the toxic effects of NDL PCBs. The latter compounds constitute a major part of the PCB mixtures found in food and human tissue. However, their biological effects are much less characterized than the more potent DL PCBs.[4,26] Minor contamination (in the range of 0.1%) of the NDL PCB congeners with potent DL-compounds is sufficient to induce toxic effects of the latter and to mask any other effects induced by NDL PCBs.[1] The use of purity-controlled NDL PCBs in the ATHON toxicological studies avoided this bias.

PCBs were chosen to represent NDL PCBs with different grades of chlorination. The level of chlorine substitution plays a role in the interaction between the individual congener and its molecular targets and hence the molecular pathways that may

Table 3. Genes Annotated to the Nervous System Development and Function Ontology Using Ingenuity Pathway Analysis[a]

Gene	Description	Annotation	PCB52		PCB138		PCB180	
			male	female	male	female	male	female
ACHE	acetylcholinesterase	Formation of dendrites, neuritogenesis, synaptogenesis		-1.5↓	-2.3↓	-1.5↓		
AKAP9	A kinase (PRKA) anchor protein 9	Calcium oscillation of spiny neurons	-2.3↓		1.6↑		-2.3↓	
CCDC92	coiled-coil domain containing 92	Differentiation of neuroglia			1.5↑	2.0↑		
CCL21	chemokine (C-C motif) ligand 21	Excitation of neurons, pain behaviour			-1.9↓	-2.3↓		
CHRNA7	cholinergic receptor, nicotinic, alpha 7	Action potential of neurons, neurotransmission				1.6↑	-1.6↓	
CPLX1	complexin 1	Exocytosis of synaptic vesicles	1.9↑			-1.9↓		
CST3	cystatin C	Neurogenesis of dentate gyrus	-1.6↓					
DAG1	dystrophin-associated glycoprotein 1)	Differentiation of neuroglia				2.1↑		
DPYSL3	dihydropyrimidinase-like 3	Differentiation of neurons, neuritogenesis, length of cortical neurons			-1.7↓			
EGF	epidermal growth factor	Expansion of dopaminergic and mesencephalic neurons, mitogenesis of nervous tissue cell lines			1.5↑	1.7↑		
FGFR3	fibroblast growth factor receptor 3	Neurogenesis of neural stem cells			1.5↑			-1.6↓
GDF11	growth differentiation factor 11	Differentiation of neurons		2.3↑	-1.9↓			
GPC2	glypican 2	Differentiation of neurons	2.0↑		-2.7↓	-1.9↓	1.6↑	
GRIA4	glutamate receptor, ionotropic, AMPA 4	Neurotransmission, function of synapse					2.5↑	
GRM3	glutamate receptor, metabotropic 3	Activation of neurons, neurotransmission, inhibition of neurons	2.4↑	1.6↑			1.8↑	
HCRT	hypocretin neuropeptide precursor	Sleep, excitation of GABAergic and noradrenergic neurons, non-rapid eye movement sleep, waking	1.8↑				1.6↑	
HTR2A	5-hydroxytryptamine receptor 2A, G protein-coupled	Sleep, excitation of neurons, excitation of pyramidal neurons	2.8↑		-1.9↓	-2.3↓	2.3↑	
IFNA16	interferon, alpha 16	Neuritogenesis , dendritic growth/branching, morphogenesis of neurites	3.6↑	-1.5↓	-4.1↓		2.0↑	
INHBA	inhibin, beta A	Proliferation of folliculo-stellate cells, quality of striatal neurons	-1.7↓		2.4↑			
KIAA1598	KIAA1598	Neuritogenesis			-2.2↓			
NUMB	numb homolog (Drosophila)	Formation of dendrites, neuritogenesis, differentiation of neuroglia, dendritic spines and neurites		-1.6↓	1.7↑	1.8↑		-1.6↓
POU4F1	POU class 4 homeobox 1	Survival of trigeminal ganglion neurons			-1.9↓			
PVRL1	poliovirus receptor-related 1	Adhesion of hippocampal neurons, synaptogenesis		-2.0↓	-2.5↓	-2.7↓	1.8↑	
RARA	retinoic acid receptor, alpha	Differentiation of neurons, survival of superior cervical ganglion neurons		-1.5↓				
RHOA	ras homolog family member A	Formation of dendrites, differentiation of neurons, neuritogenesis of hippocampal neurons			-2.4↓	-1.6↓		
SCN1A	sodium channel, voltage-gated, type I, alpha subunit	Action potential of neurons, neurotransmission	-2.1↓		1.5↑	1.9↑	-2.0↓↓	
SCN2A	sodium channel, voltage-gated, type II, alpha subunit	Action potential of neurons	-1.5↓			2.6↑		
SNCA	synuclein, alpha	Quantity of synaptic vesicles				-1.8↓		
SOX6	SRY (sex determining region Y)-box 6	Differentiation of neuroglia				-1.7↓		
SPTBN2	spectrin, beta, non-erythrocytic 2	Exocytosis of synaptic vesicles, docking of synaptic vesicles	1.9↑			-1.5↓	1.6↑	
SS18L1	synovial sarcoma translocation gene on chromosome 18-like 1	Formation of dendrites, neuritogenesis			-1.8↓			

[a]Fold changes and expression polarity (upregulation (red arrow) or downregulation (green arrow)) are given. A blank cell means that the gene was not called significant in that specific exposure condition.

be triggered. Tetrachlorinated PCB52 was chosen because it is considered to be one of the more neurotoxic compounds.[27] Hexachlorinated PCB138 and heptachlorinated PCB180 were chosen because they are among the most abundant NDL PCBs in food and human tissue.[26] Dams were given the NDL PCBs via the feed, and exposure of the offspring occurred in utero and during lactation.

Microarray gene expression analysis is probably the most enabling toxicogenomics technology. One of the promises of applying microarrays is to differentiate toxic compounds based on the gene expression responses and signatures they are able to induce in controlled experiments. Furthermore, these molecular analyses can help to unravel modes-of-action using pathway analysis.[28] Surprisingly, few toxicogenomics experiments have been performed with individual PCBs. Most of the studies have been performed with complex Aroclor mixtures containing DL compounds.[29−31]

Single congeners, and mainly DL compounds, have been used in in-vitro studies for investigating gene expression responses in hepatocytes[32−34] or blood cells.[35,36] Obviously, complex exposure scenarios reflect better the real life situation, but unraveling toxic mechanisms is complicated under these conditions because of potency differences and mixture effects. In this respect, the toxicogenomics study reported here is unique because different purity-controlled NDL PCBs were used.

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Statistical analysis identified differentially expressed genes in male and female rats. There was no indication that gender and PCB treatment mutually influenced gene expression intensities. Gene expression patterns were complex with sets of differentially expressed genes being specific for a particular PCB exposure and others in common between multiple exposure conditions. Bioinformatic overrepresentation analysis identified enriched biological terms. Lipid metabolism, molecular transport, and small molecule biochemistry were terms that were in common between PCB52 and PCB180 exposure conditions. Biological terms enriched in the case of the PCB138 exposure were more associated with cell signaling and proliferation. Addititional analyses revealed that gene lists were particularly enriched for nervous system development and function ontology. This was of interest to us because behavioral effects are documented in the same study. Genes can have different functional roles depending on the target tissue in which they are expressed. At this stage, it remains speculative to associate our observations directly to the behavioral effects. Nevertheless, peripheral gene expression signatures have been related before to organ toxicities. For example, Umbright and co-workers have shown in a rat exposure study that the known neurotoxic compound methyl parathion changed the gene expression profile of blood. The authors also identified distinct gene expression markers capable of detecting neurotoxicity.[37]

Gene expression effects identified during our research are weaker then the effects reported in other PCB exposure studies. As mentioned before, the majority of the studies used dioxins, DL PCBs, or Aroclor mixtures that contain the more potent DL PCBs. These studies observed a clear activation of the nuclear receptor pathways related to DL activity in target tissues.[29,38,39] We have observed gene expression changes in the blood of animals after a perinatal exposure to NDL PCBs. Taking into account that NDL PCBs have a lower toxic potency and that peripheral blood is in contact with several target tissues, it is reasonable to suggest that the intensity of gene expression effects may be diluted in our blood samples.

We have used Agilent microarrays and associated protocols that have been proven to be reproducible and in good agreement with other microarray platforms and real-time PCR.[40] However, our exploratory analysis could be followed up with an independent validation using other molecular biology techniques. Gene expression patterns in whole blood are known to be related to cell-type composition.[41] We did not control our results for possible differential cell count. However, we did not expect such an effect because the PCB dosages were low compared to those in other studies.[42,43] If the blood cell populations were affected 4 months after the exposure, then this might have affected our gene expression results. A parallel analysis of the blood cell population should address this uncertainty in future toxicogenomics studies.

In conclusion, we have documented for the first time differential gene expression in a well-controlled animal study using purity-controlled individual NDL PCBs. We envision that this information may complement results from other toxicological studies that aim at unraveling the toxic effects of NDL PCBs.

■ ASSOCIATED CONTENT

Supporting Information

Probes that were called significant for the factors gender and treatment (file 1) and 6 gene lists with differentially expressed genes (file 2) (there is one gene list for each PCB exposure test condition). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS

ATHON, assessing the toxicity and hazard of non-dioxin-like PCBs present in food; DEG, differentially expressed gene; DL, dioxin-like; FC, fold change; FDR, false discovery rate; IPA, Ingenuity Pathway Analysis; NDL, non-dioxin-like; PCB, polychlorinated biphenyl; PCB 52, 2,2′,5,5′-tetrachlorobiphenyl; PCB 138, 2,2′,3,4,4′,5′-hexachlorobiphenyl; PCB 180, 2,2′,3,4,4′,5,5′-heptachlorobiphenyl; RIN, RNA integrity number

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