TEMPORAL CHANGES IN THE HEPATIC GENE EXPRESSION PROFILES OF FEMALE RATS EXPOSED TO TCDD, PECDF, PCB126, AND PCB153 FOR 14 OR 53 WEEKS

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Introduction

Toxic Equivalency Factors (TEFs) are being used as an interim procedure for estimating the risks associated with exposure to mixtures of highly persistent dioxin-like halogenated aromatic hydrocarbons (HAHs). Significant questions remain regarding how well this approach works to predict the cancer risk associated with exposures to dioxin-like polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs), particularly in real world exposures which include higher concentrations of nondioxin-like PCBs and other compounds.

The TEF approach is supported by the common mechanism for the biological and toxicological actions of dioxin-like compounds which initially involves high affinity, specific binding to the cellular aryl hydrocarbon receptor (AhR) (Safe 1990).[1] The current TEFs for dioxin-like PCDDs, PCDFs and PCBs were derived from *in vitro* and *in vivo* animal models where dose-response studies and AhR binding assays were generally conducted on individual chemicals (Van den Berg et al., 1998).[2] The relative potency of each chemical was then compared with that of 2,3,7,8-TCDD, which was assigned a TEF of 1.0. It assumes that the relative potency (TEF) of a given congener remains relatively constant for each of the biological and toxicological responses associated with that agent. While experimental data supports the assigned relative potency (TEF) of these agents for several biological and/or toxicological responses, there is little direct experimental data supporting the use of the current TEFs to reflect the relative carcinogenic potency of these agents. The National Toxicology Program's toxic equivalency factor evaluation of dioxin like substances addressed this uncertainty by investigating the carcinogenic potency of 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, 3,3',4,4',5-PeCB (PCB 126), and 2,2',4,4',5,5'-HxCB (PCB 153), individually and in several defined mixtures. These agents are representative of a wide range of dioxin-like and nondioxin-like (PCB 153) HAHs that are regularly detected in the environment and human tissues. Since the liver is a target organ for the non-cancer and cancer responses of HAHs, the current study investigated temporal- and chemical-specific alterations in gene expression profiles in the liver of rats from the NTP study.

Materials and Methods

As part of the National Toxicology Program's toxic equivalency factor evaluation of dioxin-like substances, female Sprague Dawley Rats were administered *toxicologically equivalent* doses of TCDD (3, 10, 22, 46, 100 ng/kg/day), PeCDF (6, 20, 44, 92, 200 ng/kg/day), PCB126 (10, 30, 100, 175, 300, 550, 1000 ng/kg/day), PCB153 (10, 100, 300, 1000 ug/kg/day), or corn oil (vehicle control) by gavage for 2 years. Subgroups of additional rats were also sacrificed after 14, 31, or

53 weeks for mechanistic studies. Target organs from these rats were then removed, flash frozen in liquid nitrogen and stored at –70ºC.

The present study utilized liver tissue from rats treated for 14 or 53 weeks with the highest dose of each of these compounds. Frozen tissues were disrupted by homogenization with a rotor stator homogenizer and isolated according to the manufacturer's instructions for the Qiagen RNA mini-kit. There were a total of 6 animals in each dose group. Animals within each dose group were randomly paired and the RNA from these animals was pooled, thus reducing the sample size to $n = 3$ for each group while preserving biological variability. RNA was reverse transcribed, labeled with Cyanine 3 or Cyanine 5 esters, and co-hybridized to TIGR 26K rat chips with labeled RNA from treated and age- and time-matched controls. The TIGR 26K chip (http://pga.tigr.org/RatText.shtml) was developed from several independent expression libraries and represents 11 different tissues and 2 developmental states (fetus and adult), including rat fetus, placenta, ovary, heart, lung, liver, spleen, kidney, skeletal muscle, adult brain, and pheochromocytoma cells treated with and without nerve growth factor. These arrays also contained probes for a variety of housekeeping genes as well as spike-in exogenous controls to evaluate the efficiency of cDNA labeling reactions and ensure that samples were derived from high quality RNA. Arrays were scanned with the Axon Genepix Pro 4.0 scanner and high quality spots were filtered with associated software. The LOcally WEighted Scatterplot Smoothing (LOWESS) regression algorithm was performed in the TIGR Microarray Data Analysis System (MIDAS) to eliminate intensity- and block-dependent bias in the quantification of gene features. Gene expression in treated animals was calculated as the $\log_2$ fold-change relative to age- and time-matched controls. Gene expression patterns were identified by TIGR Microarray Experiment Viewer (MEV) software with Pavlidis Template Matching (PTM)[3] and Principle Component Analysis (PCA)[4]. GenMapp[5] was utilized to map biochemical pathways affected by TCDD. Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was employed to determine the expression level of selected genes.

Results and Discussion

Although animals at the 14 wk time point demonstrate few symptoms of hepatic neoplasia, several animals at the 53 wk time point exhibit toxicant-induced hepatocyte hypertrophy, multinucleate hepatic foci, and bile duct hyperplasia (http://ntp-server.niehs.nih.gov/htdocs/LT&ST_Pages/TR520_T&C.html). These toxic responses serve as predictive biomarkers of neoplastic transformation in rat liver tissues at 53 wks. TCDD, PeCDF, PCB126, and PCB153-treated animals were evaluated by PCA to determine whether changes in gene expression were associated with histopathological differences between 14 and 53 wk time points. The PCA plot indicated that the gene expression profile associated with 14 or 53 wk treatment with PCB153 was substantially different than 14 or 53 wk treatment with TCDD, PeCDF, or PCB126. Unlike the other toxicants, PCB153 demonstrates poor affinity for the AhR and this disparity may be reflected in the global gene expression profiles of animals treated with these compounds. Furthermore, the global expression profiles of animals treated for 14 wks with TCDD, PeCDF, and PCB126 translocated towards the same PCA quadrant after 53 wks treatment. In contrast, the gene expression profile of animals treated for 53 wks with PCB153 shifted in the opposite direction than that of the dioxin-like compounds. These differences may reflect the fact that chronic (53 wk) treatment with dioxin like compounds activated genes involved in neoplastic transformation, while these same genes were unchanged by 53 wk treatment with PCB153.

In an attempt to delineate genes that may be involved in the chronic toxicity of TCDD and other dioxin-like compounds, PTM was utilized to identify genes that were differentially expressed between animals treated for 14 wk and 53 wks with dioxin like toxicants, but were unchanged during this period by PCB153. Considering that all genes were paired to age-and treatment-matched controls, these genes specifically reflect genetic adaptation to chronic treatment and not merely age-related changes in gene expression. PTM mapping revealed several genes that may serve as sources or symptoms of neoplastic transformation during the 14-53 wk time period. These genes include members of the oxidative stress response pathway, regulators of cell growth and division, and mediators of angiogenesis.

The gene expression data for animals treated with TCDD, PeCDF, PCB126, or PCB153 was analyzed using GenMapp to identify biochemical pathways that may play a role in histopathological signs of toxicity. Mitochondrial long-chain fatty acid beta-oxidation and fatty acid synthesis were among those pathways affected by toxicant exposure. Toxicant-mediated perturbation of these pathways may contribute to fat redistribution, chronic weight loss, and hepatic steatosis, all of which have previously been associated with TCDD toxicity.

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