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Comparative Toxicity and Liver Transcriptomics of Legacy and Emerging Brominated Flame Retardants following 5-Day Exposure in the Rat

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Abstract

The relative toxicity of three legacy and six emerging brominated flame retardants* was studied in the male Harlan Sprague Dawley rat. The hepatocellular and thyroid toxicity of each flame retardant was evaluated following five-day exposure to each of the nine flame retardants (oral

*three legacy brominated flame retardants: polybrominated diphenyl ether 47 (PBDE 47), decabromodiphenyl ether (decaBDE), and hexabromocyclododecane (HBCD); **six “emerging” brominated flame retardants: 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), bis(2-ethylhexyl) tetrabromophthalate (TBPH), tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (TBBPA-DBPE), 1,2-bis(tribromophenoxy)ethane (BTBPE), decabromodiphenylethane (DBDPE), hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO).

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Electronic supplementary material: Supplementary material to be placed in journal online folder

Declaration of competing interests

The authors declare that they have no conflict of interest.

Supplements

1. Clinical chemistry findings
2. Hematology findings
3. Liver enzyme levels

The toxicogenomic files and the benchmark dose files can be found in the accompanying Data in Brief Article (Shockley KR et al. 2020). The toxicogenomic data can be found on the Gene Expression Omnibus (GEO) database under accession number [GSE153366](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE153366).

gavage in corn oil) at 0.1 – 1000 $\mu\text{mol/kg}$ body weight per day. Histopathology and transcriptomic analysis were performed on the left liver lobe. Centrilobular hypertrophy of hepatocytes and increases in liver weight were seen following exposure to two legacy (PBDE-47, HBCD) and to one emerging flame retardant (HCDBCO). Total thyroxine (TT4) concentrations were reduced to the greatest extent after PBDE-47 exposure. The PBDE-47, decaBDE, or HBCD liver transcriptome was characterized by upregulation of liver disease-related and/or metabolic transcripts. Fewer liver disease or metabolic transcript changes were detected for the other flame retardants studied (TBB, TBPH, TBBPA-DBPE, BTBPE, DBDPE, or HCDBCO). PBDE-47 exhibited the most disruption of hepatocellular toxic endpoints, with the Nrf2 antioxidant pathway transcripts upregulated to the greatest extent, although some activation of this pathway also occurred after decaBDE, HBCD, TBB, and HCBCO exposure. These studies provide information that can be used for prioritizing the need for more in-depth brominated flame retardant toxicity studies.

Graphical abstract

Keywords

Legacy brominated flame retardants; Emerging brominated flame retardants; Liver and thyroid toxicity; Liver transcript change

1. Introduction

The use of flame retardants, including brominated flame retardants (BFRs), was initiated in response to fire safety regulations in the last half of the 20th century (Jans 2016), and exposure to these chemicals can occur in the home or in the workplace. Flame retardants are used in a variety of consumer and commercial products including polyurethane foam and plastics (particularly in electric equipment), textiles, and wire coating. Brominated flame

retardants can be additive or bonded into the polymers. Additive flame retardants are mixed directly into the polymer without directly reacting with the polymer molecule, and due to the lack of a chemical bond, they can be released into the environment. Because most brominated flame retardants are hydrophobic with a low vapor pressure, they can accumulate in lipids (Jans 2016). They also have been detected in breast milk, air, food and water (LaKind et al. 2018; Lehmann et al. 2018).

Flame retardants fall into two categories: the “legacy flame retardants” - flame retardants in use for many years - and “emerging flame retardants”, which are being developed to replace toxic legacy flame retardants (U. S. Environmental Design for the Environment 2014a; U. S. Environmental Design for the Environment 2014b; U. S. Environmental Design for the Environment 2014c). The toxicity of BFRs and brominated chemicals has been a concern for many years (de Boer and Stapleton 2019; Dunnick et al. 1997). Production of bromine has increased more than two-fold over the past several decades (U. S. Geologic Survey accessed 2019). The legacy flame retardants in this study [penta BDE (PBDE-47), decabromodiphenyl ether (decaBDE), and hexabromocyclododecane (HBCD)], are among those targeted by the U. S. EPA as candidates for replacement. The emerging flame retardants [2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB); bis(2-ethylhexyl) tetrabromophthalate (TBPH); decabromodiphenylethane (DBDPE); tetrabromobisphenol A-bis(2,3-dibromopropyl ether (TBBPA-DBPE); 1,2-bis(tribromophenoxy)ethane (BTBPE); hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO)] are those identified by the U.S. EPA as candidate replacements (U. S. Environmental Design for the Environment 2014a; U. S. Environmental Design for the Environment 2014b; U. S. Environmental Design for the Environment 2014c). Exposure to both the legacy and emerging flame retardant chemicals may be widespread because they are found in the environment and in the home (Gannon et al. 2019; Ma et al. 2012; Percy et al. 2020; Qu et al. 2013; Sjödin et al. 2019). The Center for Disease Control provides data on human tissue levels of the polybrominated diphenyl ethers, but not on the other flame retardants (Cowell et al. 2019; Sjödin et al. 2019).

Male Harlan Sprague Dawley rat studies were used to compare the liver and thyroid toxicity of the nine brominated flame retardants described above. We chose liver and thyroid tissues for study, because both are target organs for toxicity after exposure to other brominated chemicals (Dunnick et al. 1997; National Toxicology Program 2015). Toxicogenomic data after a five-day exposure scenario provides information on mechanisms, disease pathways and biomarkers that can be used for predicting long term toxicity (Mezencev and Auerbach 2019; National Academy of Sciences 2005). The goals of the current study are to provide information for comparing the toxicity of legacy and emerging flame retardants and to prioritize the need for more in-depth flame retardant toxicity studies.

2. Materials and Methods

2.1 Flame retardant chemicals

This study compared the toxicity of three legacy flame retardants and 6 emerging flame retardants (Table 1). Chemical identities were confirmed, and chemical purities were determined prior to use (Table 1). The oral dose formulations were prepared in corn oil

vehicle (Spectrum, New Brunswick, New Jersey) and were determined to be within 10% of the target concentration (Table 2). Prior to study initiation and at the end of the study the formulations were within 10% of the day 0 value.

2.2 Experimental design

Flame retardant chemicals were administered to seven-week-old male Harlan Sprague Dawley rats once daily via oral gavage for 5 consecutive days using 6 rats per dose level per chemical. The animals were randomized to study group by body weight so that group body weights were similar. The animals were given NTP-2000 diet (Zeigler Brothers, Gardners, PA) and tap water ad[ISP-CHECK] libitum. The flame retardants were given in corn oil at 5 ml/kg body weight to deliver doses of: 0, 0.1, 1, 10, 100, 1000 $\mu\text{mol/kg}$ body weight per day per flame retardant (Table 2). The animal studies were conducted according to the guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and approved by the local Animal Care and Use Committee.

Necropsy was conducted after five days of dosing (one day after the last dose). Animals were anesthetized with CO_2/O_2 anesthesia (approximately 70/30% mixture). Blood was collected from the vena cava or aorta. Samples for hematology analysis were collected into tubes containing tripotassium ethylenediaminetetraacetic acid (K3 EDTA), and samples for clinical chemistry and thyroid hormone analysis were collected into serum collection tubes without anticoagulant, centrifuged, and the serum harvested. The endpoints for this study included hematology, clinical chemistry, body and organ weights, liver enzyme levels, thyroid hormone concentrations, liver histopathologic analysis, and liver transcriptomics.

2.3 Clinical chemistry/hematology

The following hematology parameters were evaluated on an Advia 120 hematology analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY): red blood cell count, hemoglobin concentration, hematocrit, manual hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count and differential, absolute reticulocyte count, platelet count, nucleated erythrocyte count. A qualitative evaluation of morphological features in all cellular components was performed on Wright-Giemsa stained blood smears. The following clinical chemistry parameters were evaluated on a Cobas chemistry analyzer (Roche Diagnostics, Indianapolis, IN): alanine aminotransferase, sorbitol dehydrogenase and creatine kinase activities; and total and direct bilirubin, alkaline phosphatase, blood urea nitrogen, creatinine, glucose, sodium, potassium, chloride, total protein, albumin, cholesterol, triglyceride, and bile acid salts/concentrations.

2.4 Thyroid hormone assays

Serum total thyroxine (TT4) was measured using a Cobas chemistry analyzer (Roche Diagnostics, Indianapolis, IN), and serum total triiodothyronine (TT3) and serum thyroid stimulating hormone (TSH) concentrations were measured using a radioimmunoassay kit (MP biomedical, Santa Ann, California).

2.5 Cytochrome P450 assays

Frozen liver from the remaining right and caudate lobes of the liver was also collected and flash frozen at approximately -70°C for determination of cytochrome P450 CYP1A1, CYP1A2, CYP2B1, and UDP glucuronosyl transferase liver enzyme levels (UGT1A1). Microsomal suspensions were prepared and the concentrations of protein in each suspension were determined using a BCA protein assay kit. The microsomal suspensions were used to determine CYP1A1, CYP1A2, CYP2B1, and UDP glucuronosyl transferase levels (UGT1A1) using commercially available ELISA kits [CYP1A1, Rat CYP1A1 ELISA Kit, LifeSpan Biosciences (Seattle, WA); CYP2B1, Rat Cytochrome P450 2B1 (Cyp2b1) ELISA Kit, MyBioSource (San Diego, CA); CYP1A2, Rat CYP1A2 ELISA Kit, LifeSpan Biosciences (Seattle, WA); UGT1A1, Rat UGT1A / UGT1A1 ELISA Kit, LifeSpan Biosciences (Seattle, WA)].

2.6 Liver and Thyroid Gland Histopathology

At necropsy, a portion of the left lobe of the liver and the thyroid glands were fixed in 10% neutral buffered formalin, trimmed, paraffin embedded, sectioned, and stained with hematoxylin and eosin for microscopic evaluation. After fixation in NBF, tissues were trimmed, processed, embedded in paraffin, sectioned at a thickness of 5 microns, stained with hematoxylin and eosin (H&E) and examined microscopically by experienced board-certified veterinary pathologists and peer-reviewed by a third pathologist. Histopathologic evaluation was performed on all livers of animals from chemicals with treatment effects and the highest exposure group from chemicals without a treatment effect. Next, 1-2 livers from the 0, 100, and 1000 $\mu\text{mol/kg}$ and thyroid glands from 0 and 1000 $\mu\text{mol/kg}$ exposures groups from all nine chemicals were randomly selected for review by two additional pathologists. Lesions were graded for severity using a 4-point scale.

2.7 RNA Collection

At necropsy, the remaining left lobe of the liver was processed for RNA isolation. Tissue was placed in a cryotube containing RNAlater™ and stored at $2-8^{\circ}\text{C}$ overnight. After overnight storage the RNAlater™ was removed and the samples stored at -60°C to -80°C until processed for RNA isolation for use in microarray analysis.

RNA isolation was performed on all liver tissue samples preserved in RNAlater™, using a Qiagen RNeasy Mini Kit (Qiagen Inc., Valencia, CA) with a deoxyribonucleic acid (DNA) digestion step. The concentration and purity of each RNA sample was calculated from UV absorbance readings (A_{260} and A_{280}) obtained using a NanoDrop Spectrophotometer (ND-1000, NanoDrop Products; Thermo Scientific, Wilmington, DE). All samples were also evaluated for RNA integrity using an Agilent RNA 6000 Nano Chip kit with an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). The RIN was based on the amount of degradation in a sample and was presented as a number between one and ten with one being the most degraded and ten being the most intact RNA.

2.8 Microarray Analysis

Microarray expression analysis was performed using total RNA isolated from liver samples. RNA meeting the following acceptance criteria were used for microarray expression

analysis: RNA samples with a concentration 35 ng/μL and purity between 1.80 to 2.20 and an RNA integrity number (RIN) 7.0. The RNA was amplified through cDNA synthesis, *in vitro* transcription and biotin labeling using GeneChip® 3' IVT PLUS Reagent Kit (Affymetrix, Santa Clara, CA). One hundred nanograms of total RNA was amplified as directed in the Affymetrix 3' IVT Plus kit protocol. Fifteen micrograms of amplified biotin-aRNAs was fragmented, and 12.5 μg was hybridized to each array for 16 h at 45 °C in a rotating hybridization oven using the Affymetrix Eukaryotic Target Hybridization Controls and protocol. The amplified RNA (aRNA) was fragmented then hybridized to Affymetrix Rat Genome 230 2.0 arrays (Affymetrix, Santa Clara, CA). Arrays were stained and then washed using the GeneChip Hybridization, Wash and Stain Kit according to the user manual. Each GeneChip® array was scanned using an Affymetrix GeneChip® Scanner 3000 7G to generate microarray image data (.DAT files) and raw expression level data (.CEL files).

All samples within a single flame retardant group were randomly sorted into a processing order to prevent batch effects. Also, throughout the RNA isolation, *in vitro* transcription, hybridization, fluidics and scanning procedures, a single operator handled all samples within each flame retardant group.

2.9 Microarray Data Normalization

Probe intensity data from Rat Genome 230 version 2 Affymetrix GeneChip® arrays were read into the R software environment (<http://www.R-project.org>) directly from .CEL files using the R/affy package (Gautier et al. 2004). Each data set was comprised of 6 independent samples from 6 different treatment groups (0, 0.1, 1, 10, 100, or 1000 μmol/kg body weight) for a total of 36 samples in each data set (except for the 100 μmol/kg BTBPE and the control TBB groups which had 5 samples/group). Probe-level data quality was assessed using image reconstruction, box plots of raw signal intensity, and histograms of raw signal intensities. Normalization was carried out using the robust multi-array average (RMA) method separately for each data set (Irizarry et al. 2003). Briefly, the RMA method adjusts the background of perfect match (PM) probes, applies a quantile normalization of the corrected PM values, and calculates final expression measures using the Tukey median polish algorithm. RMA scatterplots were used as an additional quality control measure.

2.10 Statistical analysis

Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (Dunnett 1955) and Williams (Williams 1971; Williams 1972). Hematology and clinical chemistry endpoints were analyzed using the nonparametric multiple comparison methods of Shirley (Shirley 1977) (as modified by Williams (Williams 1986)) and Dunn (Dunn 1964). Jonckheere's test (Jonckheere 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test).

2.11 Statistical assessment of differential gene expression

Statistical contrasts were used to find pairwise gene expression differences between the control group and each dose group using the R/maanova package (Wu et al. 2003). For each flame retardant, the model

$$Y_i = \mu + \text{DOSE} + \varepsilon_i \quad (1)$$

was used to fit the $\log_2$ transformed gene expression measures Y_i , where μ is the mean for each array, DOSE is a six-level factor representing the dose effect (0, 0.1, 1, 10, 100, or 1000 $\mu\text{mol/kg}$ body weight) and ε_i captures random error for probe set i . A total of five different comparisons were tested for each probe set (0 vs 0.1 $\mu\text{mol/kg}$, 0 vs 1 $\mu\text{mol/kg}$, 0 vs 10 $\mu\text{mol/kg}$, 0 vs 100 $\mu\text{mol/kg}$, and 0 vs 1000 $\mu\text{mol/kg}$). All statistical tests were performed using F_s , a modified F-statistic incorporating shrinkage estimates of variance components (Cui et al. 2005). P-values were calculated by permuting sample labels 1000 times. In order to reduce the number of false positives, p-values were adjusted for multiple hypothesis testing corresponding to all probe sets on the array using the Benjamin-Hochberg false discovery rate (FDR) procedure implemented using the `p.adjust()` function in R. This correction controls the expected proportion of errors among the significant results (Benjamini and Hochberg 1995). Unless otherwise noted, an FDR threshold of 0.05 was used for statistical significance. $\log_2$ fold changes were calculated by subtracting the control (0 $\mu\text{mol/kg}$) and dose treated (0.1 $\mu\text{mol/kg}$, 1 $\mu\text{mol/kg}$, 10 $\mu\text{mol/kg}$, 100 $\mu\text{mol/kg}$, or 1000 $\mu\text{mol/kg}$) relative expression values from model (1) above (Churchill 2004).

Over-represented gene sets were determined from the gene list obtained above by testing for association with gene pathway relationships (www.ingenuity.com). Enrichment of pathway members among differentially expressed probe sets were assessed using the one-tailed Fisher exact test for 2×2 contingency tables.

2.12 Benchmark dose analysis

The benchmark dose (BMD) is defined as the dose corresponding to a predetermined change in response referred to as the benchmark response (BMR). Liver transcriptomic data were used to calculate the BMD and the lower bound of the 95% confidence interval of the BMD using BMDEExpress version 2.0 (Phillips et al. 2019). All BMD calculations were performed within the BMDEExpress framework separately for each chemical.

The data corresponding to the control-AFFX probe sets were first removed from each data set. Then, a classical one-way ANOVA was used to filter the remaining RMA-normalized probe set intensities to find transcripts that were differentially expressed across dose groups with a P value < 0.05 and $|\text{fold-change}| \geq 1.5$. In this way, probe sets that did not respond to treatment were removed from the analysis. Next, the Hill, power, linear, second-degree polynomial, third-degree polynomial, and a set of four exponential models were fit to the data for each remaining probe set. The BMR level was set to 1.349 standard deviations above or below the control group, representing a 10% increase over control response rate that is standard in BMD analysis (Yang et al. 2012). For the linear, second-degree polynomial, third-degree polynomial cases, a nested likelihood ratio test was used to select

the best model fit. The more complex model was selected if the fit was improved ($P < 0.05$), but the less complex model was selected if the fit was not improved ($P \geq 0.05$). The lowest Akaike information criterion (AIC) was used to select the best fitting model comparing the remaining models with the best nested model. The power parameter was restricted to ≤ 1 for all model fitting to avoid infinite slope at the origin. Hill model fits were not selected if the estimated dose at half maximal response was less than 1/3 of the lowest positive dose, and the next best model was selected instead.

The calculated BMD values are used as input data for Gene Ontology (GO) analyses. When more than one probe set mapped to the same Entrez ID, the BMD values were averaged across probe sets to obtain a single expression value for each Entrez ID. Probe sets that mapped to more than one Entrez ID were removed from the analysis. The resulting Entrez IDs were matched to Biological Process GO terms as a basis for gene set definitions. The output consists of a range of summary exposure levels (mg/kg/day) representing the central tendencies and variability of BMD and BMDL based on the calculated BMD and BMDL values for the genes in a category.

3 Results

3.1 Mortality, body weights, clinical signs, and thyroid hormone levels

In the male Sprague-Dawley rat, there were no statistically significant treatment-related effects on mortality or body weight after five days of exposure to any of the nine flame retardants. Evaluation of other study endpoints showed that of the nine flame retardants tested, PBDE-47 exhibited the most pronounced effects on TT4. At the top two PBDE-47 dose groups (1000 or 100 $\mu\text{mol/kg}$), TT4 concentration was significantly decreased to 14% or 26% of the hormone concentration in the control group, respectively (Fig. 1A). TBBPA-DBPE also showed a significant decrease in TT4 in the high dose group (74% of hormone concentration of in the control group). For all other exposure groups, TT4 concentrations were not significantly different from the concurrent controls (Fig. 1A). The T3 level was significantly reduced in the top two PBDE-47 exposure groups compared to control; for all other exposure groups and chemicals there was no significant difference between the T3 level and the concurrent control. There were no treatment-related effects on TSH levels for any of the chemicals tested.

After PBDE-47 exposure the liver was enlarged and the absolute liver weight was approximately 1.5 or 1.25 times the weight of controls, for the 1000 and 100 $\mu\text{mol/kg}$ groups, respectively (Fig. 1B). The liver weight for HCDBCO was 1.2 times the weight of controls in the 1000 $\mu\text{mol/kg}$ group; and the liver weight for HBCD group was 1.2 times the weight of the control liver weight in the 1000 $\mu\text{mol/kg}$ group. For all other dose groups and flame retardants the liver weight was within 11% of the weight of controls and not significantly different from control liver weights (Fig. 1B).

3.2 Clinical chemistry, hematology, and liver enzymes

Most of the clinical chemistry endpoints measured were unchanged compared to concurrent controls (Supplement 1). The A/G ratios in the 1000 and 100 $\mu\text{mol/kg}$ PBDE-47 dose groups

were significantly decreased compared to controls (80% and 87% of the control, respectively), which was associated with an increase in globulin or a decrease in albumin concentration, respectively. Alanine aminotransferase activity was significantly increased in the PBDE-47 high dose group compared to control and the TBBPA-DBPE dose groups at 10, 100, and 1000 $\mu\text{mol/kg}$ compared to controls; these increases were mild being less than a 2-fold change compared to concurrent controls. Creatine kinase activity was mildly increased in the top three dose groups of TBBPA-DBPE, and the top two dose groups of HCDBCO, compared to controls. Bile acid concentrations were decreased in the top two dose groups of PBDE-47 compared to controls.

The hematology endpoints in treated groups did not show significant changes compared to concurrent control groups for all nine flame retardants at the top two dose groups (Supplement 2). UDP GT1a1 liver enzyme levels were significantly increased in the two highest dose groups after PBDE-47, decaBDE, or HBCD exposures (Fig. 1C. Supplement 3).

3.3 Histopathology

Minimal to mild centrilobular hypertrophy of hepatocytes was observed after PBDE-47, HBCD or HCDBCO exposures (Table 3, Fig. 2). Exposure to all three of these chemicals caused increases in liver weight (Fig. 1B). Affected hepatocytes were enlarged with abundant cytoplasm with a pale eosinophilic, ground-glass appearance. The normal eosinophilic granular cytoplasm was displaced to the periphery of the cells (Fig. 2). Small numbers of multinucleate cells and occasional mitotic figures were present. No histological changes were present in the thyroid gland.

3.4 Liver transcriptomic findings

For the PBDE-47, decaBDE, HBCD, TBB, or HCDBCO exposures, there were 10 or more significant liver transcript changes ($\text{FDR} < 0.05$) at the highest dose group tested compared to controls (Table 4). Selected significant liver transcript changes after PBDE-47 (Table 5), decaBDE (Table 6), HBCD (Table 7), TBB (Table 8), DBDPE (Table 9) or HCDBCO (Table 10) exposures helped to define the comparative toxicity across these chemicals. PBDE-47 flame retardant exposure produced the greatest number of liver transcriptomic changes of the 9 flame retardants investigated (Table 4), many of which occurred at the top three exposure levels. There was no significant liver disease-related transcript changes ($\text{FDR} < 0.05$) after TBPH, TBBPA-DBPE, and BTBPE exposures. (Complete TGMX findings for the 9 flame retardants studied are found in the Data in Brief article (Shockley KR et al. 2020)).

The transcript changes after PBDE-47, decaBDE, HBCD, TBB, HCDBCO flame retardants were associated with Nrf2 pathway, metabolic, and/or liver disease transcript changes based on Ingenuity pathway analysis (Table 11). Compared to controls, amyloid precursor protein transcript (APP) was increased after exposure to PBDE-47 at the top three exposure levels (Table 5), and after HCDBCO exposure at the top dose level (Table 10).

The Data in Brief Article contains detailed information for the liver transcript changes after exposure to each of the nine flame retardants (Shockley KR et al. 2020).

3.5 Benchmark dose analysis

In order for a BMDL value to be calculated for a GO Biological Process category, the category had to contain at least 5 differentially expressed genes, where at least 5% of the genes in a category are differentially expressed, and the one-sided Fisher's exact test for overrepresentation of differentially expressed genes in a category was significant at the 0.05 level. Using these criteria, a total of 400 BMDL values were calculated for PBDE47, while no BMDL values could be calculated for TBPH, TBBPA-DBPE, BTBPE, DBPE, or HCDBCO. The transcriptomic BMDL for PBDE-47 was 7.53 mg/kg; for deca BDE, 14.36 mg/kg; for HBCD 38.11 mg/kg; and for TBB, 110.77 mg/kg (Shockley KR et al. 2020). The transcriptomic BMDL values reported here were based on functional categories rather than individual genes since many genes are typically activated in response to a chemical perturbation and changes in a single genes are often subject to biological compensatory systems (Thomas et al. 2007).

The Data in Brief Article contains detailed information for the benchmark dose calculations after exposure to each of the nine flame retardants (Shockley KR et al. 2020).

4.0 Discussion

The hepatocellular and thyroid toxicity of three legacy brominated flame retardants and six emerging brominated flame retardants was studied in the Harlan Sprague Dawley rat after a five-day oral exposure. Of the nine flame retardants studied, the most treatment-related effects after PBDE-47 included increases in liver weight, decreases in serum TT4 concentrations, liver transcript changes, and minimal centrilobular hypertrophy of hepatocytes compared to controls. Increases in liver weight and the occurrence of centrilobular hypertrophy also occurred after HBCD and HBDBCO exposure. With PBDE-47 and HBCD there were also increases in liver enzyme levels including UDP GT1A1 (Figure 1), an enzyme involved in the conjugation and elimination of the PBDEs and thyroid hormones. PBDEs compete with thyroid binding proteins because of the similarity in structures of PBDE and thyroid hormones (Hoffman et al. 2017; McDonald 2002; Richardson et al. 2008). In other longer-term exposure studies, the finding of hepatocellular hypertrophy may occur in combination with hepatocellular necrosis and may help to predict carcinogenic process (Allen et al. 2004).

The toxicity of the flame retardants may be influenced several of factors. For the three flame retardants that caused centrilobular hypertrophy of hepatocytes (Figure 2) (and increases in liver weight (PBDE47, HBCD, and HBDBCO), the Kow (octanol-water partition coefficient) was in the 5-7 range, suggesting some degree of lipid solubility that would confer the ability to enter/cross the cell membrane. However, for TBB and TBPH with a Kow within the 67 range there were no hepatocellular lesions. This may be due in part to steric hinderance from the side chain preventing optimal absorption through the cell membrane. For the remaining flame retardants with a Kow > 8, there were no hepatocellular lesions (decaBDE, TBBPA-DBPE, BTBPE, DBDPE). It should be noted that as animals age the membrane structure becomes more disorganized (Grinna 1977; Noble et al. 1999) possibly allowing for different xenobiotic absorption patterns. Other factors that may

influence toxicity include molecular weight, 3-D structure, number of bromine atoms, and the nature of the side chains.

Thyroid hormone changes may be early markers for the onset of disease processes related to thyroid hormone dysregulation. In this study, PBDE-47 exposures caused the most reduction in TT4 concentrations, and this is of concern because of the essential need for thyroid hormone during development (Gibson et al. 2018; Lam et al. 2017). TBBPA-DBPE also decreased TT4 concentrations relative to controls but to a lesser extent than PBDE-47.

The mechanism for this thyroid effect may be related to the upregulation of UDP-glucuronosyltransferase transcripts, which code for enzymes that facilitate the glucuronidation and excretion of thyroid hormones (Szabo et al. 2009; Tong et al. 2007). Additionally, PBDEs share a similar structure to T4 and T3, and studies have suggested that they competitively bind to thyroid serum transporter proteins (e.g., transthyretin) leading to lower TT4 circulating levels (Hoffman et al. 2017). PBDEs inhibit sodium iodine symporter-mediated iodide uptake in rat thyroid follicular cells (Hoffman et al. 2017; Wu et al. 2016). In this study, there was not a compensatory increase in TSH in response to the observed decreases in TT4. This finding is consistent with other studies whereby compensatory increases in TSH were only observed after longer PBDE exposure times (Stoker et al. 2004). Brominated flame retardant toxic effects, including effects on thyroid function, are reported to occur in humans at low exposures (Gaylord et al. 2020; Ji et al. 2019; Makey et al. 2016) compared to levels needed to cause these effects in animals (National Toxicology Program 2015), suggesting that the brominated flame retardants may be more toxic in humans than in animals. Exposure to combinations of drugs and chemicals that lower thyroid hormone levels by a variety of mechanisms (Burch 2019), may result in a greater disease-risk than exposure to a single agent.

PBDE-47 liver transcript changes provide disease alerts (Dunnick et al. 2012; Dunnick et al. 2018), including alerts for carcinogenic activity (Smith et al. 2016; Thomas et al. 2013). This included liver and metabolic transcript changes after PBDE-47 exposure, and Nrf2 antioxidant pathway changes (representing an adaptive response to oxidative stress (Osburn and Kensler 2008). Continued exposures could lead to other toxicities including mutations in critical genes (Cooke et al. 2003; Evans et al. 2004). PBDE-47 induction of Nrf2 pathway transcripts (e.g. *GSTA3*, *NQO1*, *AKR1b7*, *SULT2A1*), metabolic transcripts (e.g. *CYP1a1*, *CYP2b6*, *Ces2c*), and conjugating enzyme transcripts (e.g. UDP GT1a1, *UGT2B11*, *UGT2B17*, and *UBT2A1*), are potential candidate markers for early prediction of longer-term toxic effects. Upregulation of the metabolic transcripts (e.g. *CYP1a1*) is often associated with ligand activation of the aryl hydrocarbon receptor, and this receptor can be activated by dioxin and non-dioxin like ligands (Sadar et al. 1996a; Sadar et al. 1996b; Schulz et al. 2012; Tolson and Wang 2010).

PBDE-47 induced transcript changes included down regulation of lipid efflux pump transcripts (*ABCG5/8*). *ABCG5/8* proteins function as heterodimers to regulate lipid content in cells (Patel et al. 2018). Down regulation of these lipid efflux pumps also occurred at PND 22 in rats after PBDE-47 and PBDE mixture (DE-71) in utero/postnatal exposure (Dunnick et al. 2018). Lipid secretion to the bile is impaired in *ABCG5/8* genes null mice

(Yu et al. 2002). These findings together suggest that decreased lipid efflux function could contribute to liver lipid accumulation after PBDE exposure (Dunnick et al. 2012). Accumulation of liver lipids may also be a factor in nonalcoholic fatty liver disease (Eslam et al. 2018; Younossi 2019; Younossi et al. 2016). PBDE-47 exposure also upregulated *ABCC3* and *ABCB1* membrane transcripts (Ghanem and Manautou 2018; Hodges et al. 2011). Recent studies suggest that *ABCC3* plays a role in cell proliferation and cancer (Adamska et al. 2019; Carrasco-Torres et al. 2016). Other brominated chemicals exposures also alter the expression of membrane transcripts in model systems (Cannon et al. 2019; Trexler et al. 2019)..

DecaBDE caused an increase in liver enzyme transcript levels and NrF2 pathway transcripts, and like PBDE mixture (DE-71) (National Toxicology Program 2015), caused hepatocellular tumors in a 2-year rodent study (National Toxicology Program 1986). In these five-day studies, HBCD and HCDBCO also caused increases in liver enzyme transcript levels and some NrF2 pathway transcripts, as well as increases in liver weights and lesions. These results suggest that HBCD and HCDBCO should be considered for further toxicity studies, especially because there have been no adequate cancer studies for either chemical. There were fewer liver transcript changes noted after TBB, TBPH, TBBPA-DBPE, BTBPE, or DDBPE five-day exposures. This study provides initial information to compare the toxicity of legacy and some of the emerging brominated flame retardants (Zuiderveen et al. 2020). Of the nine flame retardants studied, PBDE-47 was the most toxic after a five-day oral exposure in the male Harlan Sprague Dawley rat.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Toxicity of legacy and emerging brominated flame retardants
- 5-Day Toxicogenomic studies
- Liver toxicity
- Benchmark dose analysis using toxicogenomic data

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Figure 1.

A. Total thyroxine (T4) (ug/dL) after five days of dosing

Mean $\pm$ SEM are shown for each dose group. Statistical significance for the control group indicates a significant trend test. Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group.

* Statistically significant at P = 0.05

** Statistically significant at P = 0.01

B. Absolute liver weights (grams) after five days of dosing

Mean $\pm$ SEM are shown for each dose group. Statistical significance for the control group indicates a significant trend test. Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group.

* Statistically significant at P = 0.05

** Statistically significant at P = 0.01

C. UDP GT1A1 (ng/mL) after five days of dosing

Mean $\pm$ SEM are shown for each dose group. Statistical significance for the control group indicates a significant trend test. Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group.

* Statistically significant at P = 0.05

** Statistically significant at P = 0.01

Figure 2.

Hepatocellular changes with PBDE-47 exposure. Figure a: Displays a normal liver from a control rat. Figure b: Affected liver from rat exposed to PBDE-47 shows a mild tinctoral change. The affected liver from rats exposed to HBCD and HCDBCO display similar features. The hepatocytes throughout the lobule are enlarged and have cytoplasm that has a pale, ground glass appearance. (CV, central vein)

Table 1.

Flame retardants used in the 5-day toxicity studies

Chemical Information ^a	Structure	Supplier, Lot Number, Purity
Legacy flame retardants		
Polybrominated diphenyl ether 47 (PBDE 47) CAS RN: 5436-43-1 Molecular Weight: 485.82 Molecular Formula: C ₁₂ H ₆ Br ₄ O Log K _{ow} : 6.5		Cerilliant, ER081208-02, Purity by Gas chromatography-Mass spectrometry detection 99.5%
Decabromodiphenyl oxide (Deca BDE; BDE-209) CAS RN: 1163-19-5 Molecular Weight: 959.2 Molecular Formula: C ₁₂ H ₁₀ Br ₁₀ O Log Kow: 10.1		Gojira Fine Chemicals, LLC, 0427501, Purity by Gas chromatography-Mass spectrometry detection > 99%
1,3,5,7,9,11-Hexabromocyclododecane (HBCD) CAS RN: 25637-99-4; 3194-55-6 Molecular Weight: 641.70 Molecular Formula: C ₁₂ H ₁₈ Br ₆ Log Kow: 7.5		Gojira Fine Chemicals, LLC, 0422503, Purity by High performance liquid chromatography-Evaporative light scattering detection 96%
Emerging flame retardants		
2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) CAS RN: 183658-27-7 Molecular Weight: 549.9 Molecular Formula: C ₁₅ H ₁₈ Br ₄ O ₂ Log Kow: 7.5		Toronto Research Chemicals, Inc., LUZ-193-8, Purity by Gas chromatography-Mass spectrometry 97.9%
bis(2-ethylhexyl) tetrabromophthalate (TBPH) CAS RN: 26040-51-7 Molecular Weight: 706.145 Molecular Formula: C₂₄H₃₄Br₄O₄ Log Kow: 5.7		Gojira Fine Chemicals, LLC, 1027502, Purity by Gas chromatography-Mass spectrometry 99%
Tetrabromobisphenol A-bis(2,3-dibromopropyl ether) (TBBPA-DBPE) CAS RN: 21850-44-2 Molecular Weight: 943.619 Molecular Formula: C ₂₁ H ₂₀ Br ₈ O ₂ Log Kow: 12 (est)		Archway Sales (Distributor for Great Lakes Chemical Corp) 534106, Purity by Gas chromatography-Mass spectrometry 94.5%

<p><u>1,2-bis(tribromophenoxy)ethane (BTBPE) (Firemaster 680)</u> CAS RN: <u>37853-59-1</u> Molecular Weight: 687.639 Molecular Formula: C₁₄H₈Br₆O₂ Log Kow: 8.9</p>		<p>Gojira Fine Chemicals, LLC, 0427502 Purity by Gas chromatography-Mass spectrometry > 99%</p>
<p><u>Decabromodiphenylethane (DBDPE)</u> CAS RN: <u>84852-53-9</u> Molecular Weight: 971.2 Molecular Formula: C₁₄H₄Br₁₀ Log Kow: 14 (est)</p>		<p>Ivy Fine Chemicals, IF15070, Purity by Gas chromatography-Flame ionization detection 94%</p>
<p><u>Hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO)</u> CAS RN: <u>51936-55-1</u> Molecular Weight: 540.764 Molecular Formula: C₁₃H₁₂Br₂Cl₆ Log Kow: 6.7</p>		<p>Chem Services, 4522700, Purity by Gas chromatography-Mass spectrometry 99%</p>

^a<https://comptox.epa.gov/dashboard/dsstoxdb/results?search=51936-55-1#properties>

Table 2.

Dose formulation development and analysis

Flame Retardant	Dose (μmol/kg)	Dose (mq/kg)	Analytical System Details	Method Qualification Range ^a
Legacy flame retardants				
Polybrominated diphenyl ether 47 (PBDE 47)	0 0.1 1 10 100 1000	0 0.0485 0.485 4.85 48.5 485	Method: GC-ECD^b Column: Rtx-5 (1-μm, 30m×0.32mm ID), Restek, Bellefonte, PA Oven Program: 8°C to 200°C, 20°C/min; 200°C to 300°C, 10°C/min	0.08-2.56 μg/mL
Decabromodiphenyl oxide (Deca BDE; BDE-209)	0 0.1 1 10 100 1000	0 0.1 0.959 9.59 95.9 959	Method: UPLC-UV (228 nm) Column: Acquity XBridge C18 (3.5 μm, 2.1 x 100 mm), Waters, Milford, MA Mobile Phase and gradient: A: Water, B: Acetonitrile; 70% B for 2 min; 70 to 100% B in 3 min; 100% B for 2 min	0.01-0.24 mg/mL
1,3,5,7,9,11-Hexabromocyclododecane (HBCD)	0 0.1 1 10 100 1000	0 0.06 0.641 6.41 64.1 641	Method: UPLC-CAD. Column: Waters SunFire C18 (3.5 μm, 4.6 x 100 mm) Waters, Milford, MA Mobile Phase and gradient: A: Water, B: Acetonitrile; 80% B for 2 min; 80 to 100% B in 5 min; 100% B for 1 min, 8	0.008-0.16 mg/mL
Emerging flame retardants				
2-ethylhexyl-2,3,4,5-Tetrabromobenzoate (TBB)	0 0.1 1 10 100 1000	0 0.05 0.55 5.50 55 550	Method: GC-ECD Column: Phenomenex Inferno ZB-5HT (30 m × 0.250 mm i.d., 0.1-μm; Phenomenex, Torrance, CA Oven Program: 50 °C (2 min); 50 to 280 °C at 20° C/min; 280 to 320 °C at 5 °C/min	0.007-0.183 mg/mL
bis(2-ethylhexyl) tetrabromophthalate (TBPH)	0 0.1 1 10 100 1000	0 0.07 0.71 7.06 70.6 706	Method: GC-ECD Column: Phenomenex Inferno ZB-5HT (30 m × 0.250 mm i.d., 0.1-μm, Phenomenex, Torrance, CA Oven Program: 50 °C (2 min), 50 to 280 °C at 20° C/min; 290 to 320 °C at 5 °C/min	0.007-0.18 mg/mL
Tetrabromobisphenol A- bis(2,3-dibromopropyl ether) (TBBPA-DBPE)	0 0.1 1 10 100 1000	0 0.1 0.94 9.4 94.3 943	Method: HPLC-UV(215 nm) Column: InertClone, ODS-2 (5 μm, 250 × 4.6 mm) Phenomenex, Torrance, CA Mobile Phase and gradient: Acetonitrile:Water (95:5, v:v), isocratic	0.02-188.6 mg/mL
1,2-bis(tribromophenoxy)ethane (BTBPE) (Firemaster 680)	0 0.1 1 10 100 1000	0 0.07 0.69 6.88 68 680	Method: GC-ECD, Agilent (Santa Clara, CA) 6890 Column: Inferno ZB-5HT (30 m × 0.250 mm i.d., 0.1-μm), Phenomenex, Torrance, CA Oven Program: 50 °C (2 min), 50 to 280 °C at 20 °C/min, 280 to 320 °C at 5 °C/min	0.007-0.18 mg/mL
Decabromodihentylethane (DBDPE)	0 0.1 1 10 100 1000	0 0.1 0.97 9.71 97.1 970	Method: UPLC-UV (225 nm) Column: XBridge C18 (2.1 × 100 mm, 3.5 μm) Mobile Phase and gradient: A: Water, B: Acetonitrile; 90% B to 100% B in 5 min, hold at 100% B for 1 min	0.01-0.301 mg/mL

Flame Retardant	Dose ($\mu\text{mol/kg}$)	Dose (mg/kg)	Analytical System Details	Method Qualification Range ^a
Hexachlorocyclopentadi enyl- dibromocyclooctane (HCDBCO)	0 <u>0.1</u> <u>1</u> <u>10</u> <u>100</u> <u>1000</u>	0 0.05 0.54 5.41 54.1 541	Method: GC-ECD Column: Phenomenex Inferno ZB-5HT (0.1- μm , 30 m $\times$ 0.250 mm i.d.), Phenomenex, Torrance, CA Oven Program: 100 °C to 260° C at 40 °C/min; 260 to 300 °C at 4 °C/min; 300 to 340 °C at 20 °C/min	0.007-144 mg/mL

^a Analytical method qualification criteria were; $r = 0.99$; precision $\pm 5\%$; accuracy, $\pm 10\%$

^b GC-ECD: Gas Chromatography- Electron Capture Detection, UPLC-PDA: Ultra Performance Liquid Chromatography-Photodiode Array, CAD: Charged Aerosol Detector, LC-UV: Liquid Chromatography-Ultraviolet

Table 3.

Incidence of histopathologic findings after flame retardant exposure

Dose Level ($\mu\text{mol/kg}$)						
	0	0.1	1	10	100	1000
PBDE-47						
Centrilobular hypertrophy of hepatocytes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6/6 (1.0)	6/6 (2.0)
HBCD						
Centrilobular hypertrophy of hepatocytes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4/6 (1.0)
HCDBCO						
Centrilobular hypertrophy of hepatocytes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4/6 (1.8)	6/6 (1.5)

Six animals were examined for histopathologic lesions per dose group. Treatment-related histopathologic lesions were present in the liver at 100 and 1000 $\mu\text{mol/kg}$ with PBDE-47, HBCD, and HCDBCO. Average severity grade in parenthesis (1.0 = minimal, 2.0 = mild). No treatment-related liver histopathologic alterations were present with decaBDE, TBB, TBPH, TBBPA-DBPE, BTBPE, or DBDPE.

Table 4.

Number of significant ^{*} liver transcriptome changes after legacy or emerging flame retardant 5-day exposure

Dose $\mu\text{mol/kg}$	0.1	1	10	100	1000
Legacy Flame retardants					
PBDE 47 $\uparrow$	23	13	59	759	1829
PBDE47 $\downarrow$	21	15	52	240	1366
decaBDE $\uparrow$	0	10	25	24	29
decaBDE $\downarrow$	0	1	2	1	1
HBCD $\uparrow$	1	1	1	23	184
HBCD $\downarrow$	0	0	0	0	63
Emerging Flame retardants					
TBB $\uparrow$	0	0	1	2	46
TBB $\downarrow$	1	0	1	0	20
TBPH $\uparrow$	0	0	0	0	0
TBPH $\downarrow$	0	0	0	0	0
TBBPA-DBPE $\uparrow$	0	0	0	0	0
TBBPA-DBPE $\downarrow$	0	0	0	0	0
BTBPE $\uparrow$	0	0	0	0	0
BTBPE $\downarrow$	0	0	0	0	0
DBDPE $\uparrow$	0	0	0	2	3
DBDPE $\downarrow$	0	0	0	0	0
HCDBCO $\uparrow$	0	0	0	8	15
HCDBCO $\downarrow$	0	0	0	4	4

^{*} Number of transcripts significantly changed at a false discovery rate (FDR) < 0.05

Table 5.

Selected significant hepatic transcripts following 5-day PBDE-47 exposure

Dose $\mu\text{mol/kg} \sim \text{mg/kg}$	PBDE-47 0.1 0.0485	PBDE-47 1 0.485	PBDE-47 10 4.85	PBDE-47 100 48.5	PBDE-47 1000 485
Metabolic enzymes					
<i>CYP1A1</i> 1370269_at	-1.00	1.16	1.31	6.11	65.53
<i>Cyp1A2</i> 1387243_at	-1.11	-1.07	1.02	1.15	1.23
<i>CYP2B6</i> 1371076_at	-1.07	1.32	4.51	10.93	12.51
<i>CYP2C8</i> 1370241_at	1.06	-1.11	-1.13	-1.08	1.28
<i>CYP3A5</i> 1387118_at	-1.04	-1.01	1.37	2.21	2.84
<i>CYP4A22</i> 1368607_at	-1.09	-1.09	1.10	3.32	4.36
<i>POR</i> 1387109_at	1.06	1.06	1.09	2.24	3.85
<i>Ces2c</i> (includes others) 1368905_at	1.17	-1.01	1.68	6.00	18.53
Nrf2 pathway					
<i>AKR7A2</i> 1367843_at	-1.00	1.02	1.11	1.21	1.39
<i>DNAJA3</i> 1378392_at	1.10	1.05	1.07	1.19	1.24
<i>EPHX1</i> 1387669_a_at	1.06	1.04	1.26	1.65	2.13
<i>GSTA1</i> 1368180_s_at	1.06	1.02	1.26	1.56	2.11
<i>GSTA3</i> 1371089_at	1.39	1.34	1.67	3.98	13.87
<i>GSTM5</i> 1386985_at	1.03	1.03	1.13	1.44	1.86
<i>PRDX1</i> 1367613_at	-1.03	-1.02	-1.00	1.08	1.27
<i>NQO1</i> 1387599_a_at	1.04	-1.02	1.39	2.64	6.22
<i>Akr1b7</i> 1368569_at	-1.05	-1.11	-1.02	1.02	6.57
<i>SULT2A1</i> 1387006_at	1.42	1.12	-1.18	-1.18	4.74
<i>ALDH1a7</i> 1368718_at	-1.00	-1.12	1.23	9.69	15.45
<i>ALDH1A1</i> 1387022_at	1.20	1.16	1.47	2.49	4.04
Conjugating enzymes					
<i>UGT2A1</i> 1369850_at	1.15	1.10	1.24	1.32	2.06

Dose $\mu\text{mol/kg} \sim \text{mg/kg}$	PBDE-47 0.1 0.0485	PBDE-47 1 0.485	PBDE-47 10 4.85	PBDE-47 100 48.5	PBDE-47 1000 485
<i>UGT2B10</i> 1380278_at	1.02	1.02	1.16	1.50	1.92
<i>UGT2B17</i> 1370698_at	1.18	1.09	1.69	2.38	2.73
<i>UGT2B11</i> 1381852_at	-1.15	-1.20	1.08	1.44	3.26
<i>UGT2B7</i> 1368397_at	1.03	-1.00	1.07	1.13	1.20
Membrane protein					
<i>ABCC3</i> 1369698_at	1.21	1.35	2.09	9.62	23.41
<i>ABCB1</i> 1370464_at	1.14	1.08	1.18	1.87	3.45
<i>ABCG5</i> 1369455_at	2.00	-1.03	-1.17	-1.89	- 3.99
<i>ABCG8</i> 1369440_at	-1.06	-1.09	-1.17	-1.95	- 4.66
<i>SLC35E3</i> 1389391_at	-1.04	-1.05	1.04	1.25	2.03
<i>SLC22A1</i> 1368191_a_at	1.00	1.05	1.12	1.28	1.49
<i>SLC26A1</i> 1368600_at					
<i>MMGT1</i> 1395325_s_at	1.00	1.07	1.26	1.95	2.55
<i>MMGT1</i> 1376168_at	-1.08	-1.01	1.20	1.81	2.43
<i>ABCD2</i> 1368561_at	1.24	1.24	1.24	1.98	2.54
<i>APP</i> 1371572_at	1.11	1.07	1.32	2.43	3.05

Significant transcripts at FDR < 0.05 (bold numbers) where gene annotations are based

Table 6.

Selected significant hepatic transcripts following 5-day DecaBDE exposure

Dose $\mu\text{mol/kg} \sim \text{mg/kg}$	decaBDE 0.1 0.1	decaBDE 1 0.959	decaBDE 10 9.59	decaBDE 100 95.9	decaBDE 1000 959
Metabolic enzymes					
<i>CYP1A1</i> 1370269_at	1.06	−1.01	1.00	3.10	26.30
<i>Cyp1A2</i> 1387243_at	1.01	1.05	1.04	1.15	1.44
<i>CYP2B6</i> 1371076_at	1.40	4.21	5.86	5.86	6.057
<i>CYP2C19</i> 1370580_a_at	1.06	1.23	1.26	1.32	1.34
<i>CYP3A5</i> 1387118_at	1.02	1.33	1.49	1.51	1.56
<i>CYP3A7</i> 1370387_at	1.07	1.70	1.84	2.67	2.64
<i>POR</i> 1387109_at	1.05	1.35	1.41	1.30	1.50
Ces2c (includes others) 1368905_at	1.05	1.40	1.93	1.95	1.94
<i>Aldh1a7</i> 1368718_at	−1.51	−1.54	2.63	2.51	5.02
<i>INMT</i> 1373975_at	1.08	1.63	1.51	1.89	2.41
Nrf2 pathway					
<i>EPHX1</i> 1387669_a_at	−1.00	1.15	1.32	1.28	1.31
<i>NQO1</i> 1387599_a_at	1.03	1.24	1.23	1.32	1.98
Conjugating enzymes					
<i>UGT2B17</i> 1370698_at	1.09	1.52	1.96	1.89	1.87
<i>Ugt2b17</i> 1387955_at 1370698_at	1.35	1.71	2.43	2.12	2.25
Membrane protein					
<i>ABCC3</i> 1369698_at	1.35	1.71	2.43	2.12	2.25
<i>MMGT1</i> 1395325_s_at	1.12	1.45	1.51	1.55	1.58
<i>MMGT1</i> 1376168_at	1.17	1.46	1.61	1.59	1.59
<i>APP</i> 1371572_at	1.14	1.33	1.55	1.62	1.44
Other					
<i>CDH17</i> 1369224_at	1.83	1.53	2.41	2.37	3.35
<i>RETREG1</i> 1373011_at	1.83	1.53	2.41	2.37	2.29

Dose $\mu\text{mol/kg}$ ~ mg/kg	decaBDE 0.1 0.1	decaBDE 1 0.959	decaBDE 10 9.59	decaBDE 100 95.9	decaBDE 1000 959
<i>MMGT1</i> 1376168_at	1.17	1.46	1.61	1.59	1.59
<i>CNDP2</i> 1372132_at	1.10	1.28	1.49	1.50	1.55

Significant transcripts at FDR < 0.05 (bold numbers) where gene annotations are based on Ingenuity

Table 7.

Selected significant hepatic transcripts following 5-day HBCD exposure

Dose $\mu\text{mol/kg} \sim \text{mg/kg}$	HBCD 0.1 0.05	HBCD 1 0.55	HBCD 10 5.50	HBCD 100 55	HBCD 1000 550
Metabolic enzymes					
<i>CYP2B6</i> 1371076_at	1.29	1.36	1.78	2.63	4.00
<i>CYP3A5</i> 1387118_at	1.05	1.07	1.15	1.82	2.45
<i>Ces2c</i> (includes others) 1368905_at	1.19	1.31	1.45	2.07	4.41
Nrf2 pathway					
<i>AKR7A3</i> 1368121_at	-1.07	-1.01	-1.06	1.28	2.30
<i>GSTA3</i> 1371089_at	-1.05	1.25	1.40	2.00	5.23
<i>SULT2A1</i> 1387006_at	1.18	1.31	1.45	1.67	3.46
<i>ALDH1a7</i> 1368718_at	4.56	2.82	-1.33	6.95	34.89
<i>ALDH1A1</i> 1387022_at	1.13	1.15	1.32	1.66	2.48
<i>EPHX1</i> 1387669_a_at	1.10	1.12	1.12	1.36	1.73
Conjugating enzymes					
<i>UGT2A1</i> 1369850_at	1.03	1.10	1.10	1.40	1.97
<i>UGT2B17</i> 1370698_at	1.12	1.20	1.25	1.70	2.13
Membrane protein					
<i>ABCC3</i> 1369698_at	1.02	-1.11	-1.01	1.72	5.26
<i>ABCB1</i> 1370464_at	1.03	1.14	1.20	1.34	2.16
<i>SLC6A6</i> 1374531_at	-1.18	-1.09	-1.15	-1.39	-3.59
<i>SCD</i> 1370355_at	1.28	1.12	1.01	-1.55	-3.90
Other					
<i>crystallin lambda 1</i> 1376051_at	1.03	1.23	1.17	1.36	2.49
<i>Slco1a4</i> 1387094_at	1.08	1.19	1.27	1.52	2.29

Significant transcripts at FDR < 0.05 (bold numbers) where gene annotations are based on Ingenuity

Table 8.

Selected significant hepatic transcripts following 5-day TBB exposure

Dose $\mu\text{mol/kg} \sim \text{mg/kg}$	TBB 0.1 0.05	TBB 1 0.55	TBB 10 5.50	TBB 100 55	TBB 1000 550
Metabolic enzymes					
<i>CYP1A1</i> 1370269_at	-1.04	1.00	1.16	19.11	161.69
<i>CYP1A2</i> 1387243_at	-1.04	-1.02	1.05	1.39	2.05
Nrf2 pathway					
<i>GSTA3</i> 1371089_at	-1.07	1.13	1.22	1.32	3.46
<i>NQO1</i> 1387599_a_at	-1.18	-1.14	-1.11	1.12	3.39
Membrane protein					
<i>SLC39A4</i> 1374366_at	-1.08	1.05	-1.01	1.11	1.78
Other					
<i>Acot1</i> 1398250_at	1.23	-1.12	-1.04	1.12	8.95
<i>EHHADH</i> 1368283_at	1.15	1.08	1.11	1.09	2.09
<i>EC11</i> 1367659_a_at	1.08	-1.03	1.10	1.15	1.95
<i>AIG1</i> 1375845_at	1.01	-1.01	1.14	1.04	1.85
<i>RDH5</i> 1379587_at	-1.07	-1.05	-1.03	-1.09	-1.62
<i>ACOT2</i> 1388211_s_at	1.01	-1.12	-1.01	-1.03	2.46
<i>A2M</i> 1367794_at	1.20	1.07	-1.10	-1.08	-3.06

Significant transcripts at FDR < 0.05 (bold numbers) where gene annotations are based on Ingenuity

Table 9.

Selected significant hepatic transcripts following 5-day DBDPE exposure

Dose $\mu\text{mol/kg}$ ~ mg/kg	DBDPE 0.1 0.1	DBDPE 1 0.97	DBDPE 10 9.71	DBDPE 100 97.1	DBDPE 1000 970
Metabolic enzymes					
<i>CYP1A1</i> 1370269_at	1.02	1.12	1.22	11.20	75.23
<i>CYP1A2</i> 1387243_at	1.10	1.08	1.10	1.42	1.82

Significant transcripts at FDR < 0.05 (bold numbers) where gene annotations are based on Ingenuity

Table 10.

Selected significant hepatic transcripts following 5-day HCDBCO exposure

Dose $\mu\text{mol/kg} \sim \text{mg/kg}$	HCDBCO 0.1 0.07	HCDBCO 1 0.69	HCDBCO 10 6.88	HCDBCO 100 68	HCDBCO 1000 680
Metabolizing Enzymes					
<i>CYP2B6</i> 1371076_at	1.04	−1.12	1.31	2.33	4.58
<i>CYP3A5</i> 1387118_at	−1.06	1.06	1.03	1.30	1.59
<i>CYP2C19</i> 1370580_a_at	1.01	−1.01	1.03	1.19	1.27
<i>Ces2c</i> (includes others) 1368905_at	−1.07	1.08	1.05	1.61	2.83
Nrf2 pathway					
<i>GSTA3</i> 1371089_at	−1.01	1.40	1.09	2.11	2.52
Membrane protein					
<i>ABCC3</i> 1369698_at	1.22	1.31	1.45	2.88	4.42
<i>UGT2B17</i> 1370698_at	1.04	−1.02	1.20	1.80	2.02
<i>APP</i> 1371572_at	1.07	−1.01	1.16	1.33	1.51
Other					
<i>PIR</i> 1377662_at	1.02	1.02	1.16	1.25	1.61
<i>KLF9</i> 1370209_at	−1.26	−1.24	−1.21	−1.53	−1.70
<i>ZDHHC2</i> 1370828_at	1.12	1.05	1.10	1.34	1.65

Significant transcripts at FDR < 0.05 (bold numbers) where gene annotations are based on Ingenuity

Table 11.Pathway changes^a

Chemical	↑ NRF2-mediated Oxidative Stress Response [*]	↑ Metabolic Pathway Transcripts [*]	↑ Activation of Liver Disease Pathways ^{**}
PBDE-47			
decaBDE	-		+/-
HBCD	-		+/-
TBB	-	-	+/-
TBPH	-	-	-
TBPA-DBPE	-	-	-
BTBPE	-	-	-
DBDPE	-	-	+/-
HCDBCO	-	-	+/-

^aPathway changes based on Ingenuity analysis of differentially expressed transcripts^{*} = >10% of pathway transcripts activated; +/- = 3-10% of pathway transcripts activated^{**} = > 37 -875 liver disease transcript changes; +/- =1-12 liver disease transcript changes