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Mechanisms of Toxicity of Hydroxylated Polybrominated Diphenyl Ethers (HO-PBDEs) Determined by Toxicogenomic Analysis with a Live Cell Array Coupled with Mutagenesis in *Escherichia coli*

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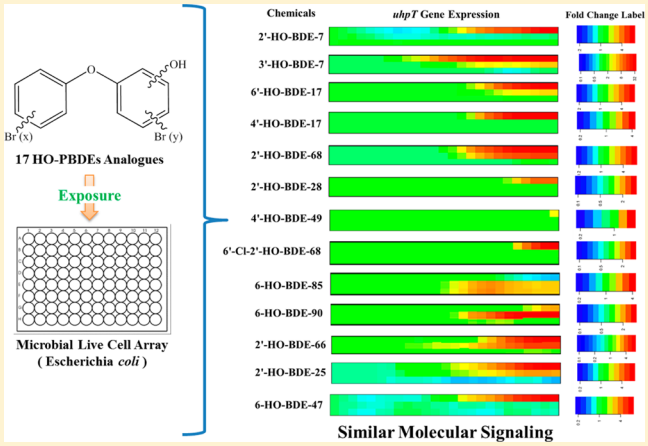
Supporting Information

ABSTRACT: Results of previous studies have indicated that 6-HO-BDE-47, the addition of the hydroxyl (HO) group to the backbone of BDE-47, significantly increased the toxicity of the chemical compared to its postulated precursor analogues, BDE-47 and 6-MeO-BDE-47. However, whether such a result is conserved across polybrominated diphenyl ether (PBDE) congeners was unknown. Here, cytotoxicity of 32 PBDE analogues (17 HO-PBDEs and 15 MeO-PBDEs) was further tested and the underlying molecular mechanism was investigated. A total of 14 of the 17 HO-PBDEs inhibited growth of *Escherichia coli* during 4 or 24 h durations of exposure, but none of the MeO-PBDEs was cytotoxic at the concentrations tested. 6-HO-BDE-47 and 2-HO-BDE-28 were most potent with 4 h median effect concentrations (EC_{50}) of 12.13 and 6.25 mg/L, respectively, which trended to be lesser with a longer exposure time (24 h). Expression of 30 modulated and validated genes by 6-HO-BDE-47 in a previous study was also observed after exposure to other HO-PBDE analogues. For instance, *uhpT* was upregulated by 13 HO-PBDEs, and three rRNA operons (*rrnA*, *rrnB*, and *rrnC*) were downregulated by 8 HO-PBDEs. These unanimous responses suggested a potential common molecular signaling modulated by HO-PBDEs. To explore new information on mechanisms of action, this work was extended by testing the increased susceptibility of 182 mutations of transcriptional factors (TFs) and 22 mutations as genes modulated by 6-HO-BDE-47 after exposure to 6-HO-BDE-47 at the 4 h IC_{50} concentration. Although a unanimous upregulation of *uhpT* was observed after exposure to HO-PBDEs, no significant shift in sensitivity was observed in *uhpT*-defective mutants. The 54 genes, selected by cut-offs of 0.35 and 0.65, were determined to be responsible for "organic acid/oxoacid/carboxylic acid metabolic process" pathways, which supported a previous finding.



INTRODUCTION

Some hydroxylated polybrominated diphenyl ethers (HO-PBDEs) have been identified as potential transformation products of polybrominated diphenyl ethers (PBDEs) and have been detected in human bodies,[1,2] which has raised concern about their potential toxicity and a need for knowledge about modes of molecular toxicity, so that accurate assessments of risk can be conducted.[3] Results of several *in vivo* or *in vitro* studies[4−6] suggested that PBDEs might undergo biotransformation. For example, biotransformation of BDE-100 to mono-HO-PBDEs was observed in both rats[5] and mice,[4] and two mono-HO-PBDEs were identified in human hepatocytes exposed to BDE-99.[6] However, not all studies have found HO-PBDEs to be biotransformation products of PBDEs. After exposure to BDE-47 via their diet in Japanese medaka, no HO-PBDEs were detected.[7] Results of the sequential study suggested that demethylation of 6-MeO-BDE-47 was the

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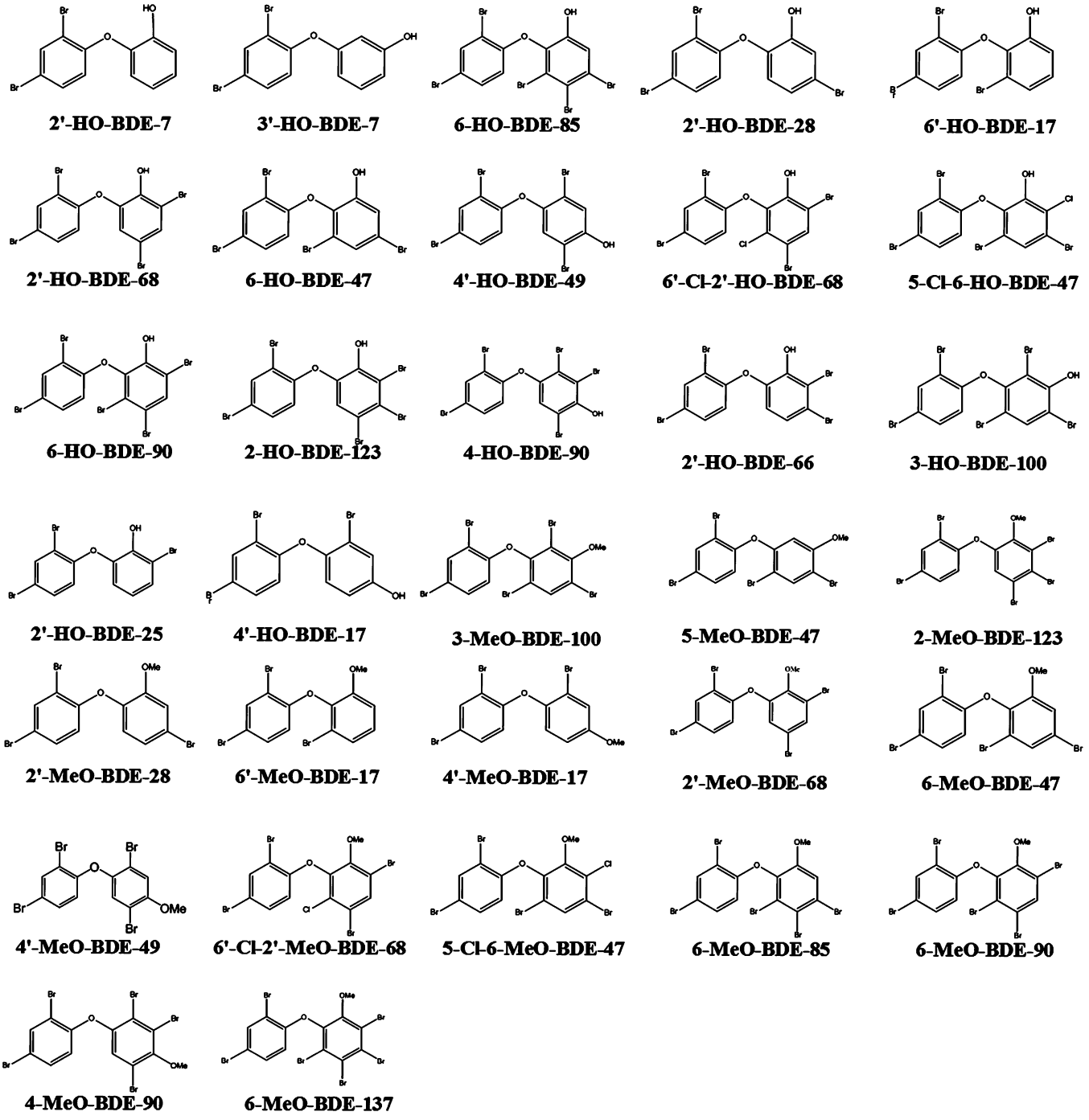

Figure 1. Structures of 17 HO-PBDE and 15 MeO-PBDE analogues.

primary pathway leading to formation of 6-HO-BDE-47 in medaka, while the previously hypothesized formation of HO-PBDEs from synthetic BDE-47 did not occur.[8] Because HO-PBDEs can have a natural origin or be derived from synthetic brominated flame retardants, more attention is being paid to the HO-PBDEs, some of which are more potent for end points than the postulated precursor PBDEs and corresponding methoxylated polybrominated diphenyl ethers (MeO-PBDEs).[3] The fact that HO-PBDEs, including 6-HO-BDE-47 were detected in human blood,[1] has led to interest in the potential of HO-PBDEs to modulate gene expression and led to toxicity. Assessment of PBDEs and their analogues has mostly focused on their nuclear-hormone-receptor-mediated potency[3,9−11] or effects on reproduction[12] or neurotoxicity.[13] However, the generic toxic potency and the underlying molecular mechanisms of these brominated compounds had not been investigated previously.

Genome-wide transcriptional investigations, such as whole cell arrays,[14,15] are high-throughput methods to determine toxicological mechanisms of target chemicals and could serve as a tool for a number of reverse genetic approaches. Unlike microarray technology, the live cell array can avoid complex protocols of pretreatment, high-cost experimental materials, interferences, and lack of temporal resolution but can achieve comparable results, such as toxicity identification and pollutant-specific molecular fingerprints.[16] From the use of genome-wide

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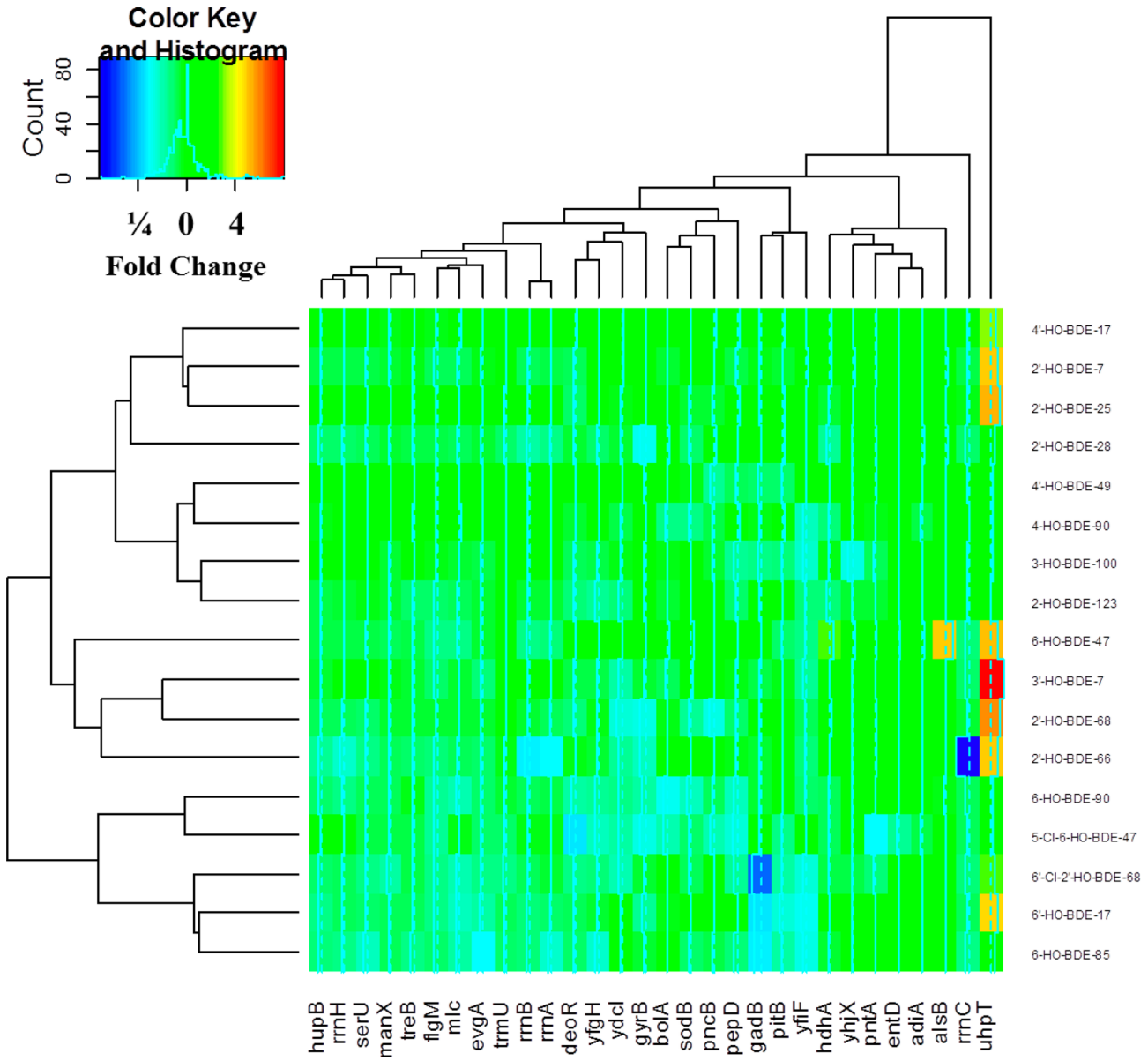

Figure 2. Quantitative expressions of 30 genes in *E. coli* after a 4 h exposure to 17 HO-PBDE analogues. The fold change of gene expression is indicated by the color gradient on the right. The chemical and gene identifiers can be found on the right and bottom of the plot, respectively.

live cell arrays, cytotoxicity of 6-HO-BDE-47 and its two analogues, BDE-47 and 6-MeO-BDE-47, and the associated molecular mechanisms had already been assessed in previous studies, which indicated that potency of 6-HO-BDE-47 to inhibit growth of *Escherichia coli* was greater than its postulated precursor BDE-47 and corresponding 6-MeO-BDE-47 and identified potential pathways and transcriptional networks modulated by 6-HO-BDE-47.[15] However, whether such a mechanism is conserved across PBDE congeners was unknown. Recently, the gene loss-of-function screen assay provides another new resource for not only systematic analyses of unknown gene functions and gene regulatory networks but also genome-wide testing of mutational effects in *E. coli* K-12.[17] Changes in sensitivities of mutants offers another way of further characterizing mechanisms of action and might be a good addition to newly developed high-throughput studies.[18]

In previous studies,[15] the addition of the hydroxyl (HO) group to the backbone of BDE-47 normally caused stronger potency that that of the methoxy (MeO) functional group. To investigate whether this effect occurs among various PBDE congeners, cytotoxicity of 32 PBDE analogues (17 HO-PBDEs and 15 MeO-PBDEs) was tested. The results of those studies suggested that only HO-PBDEs can cause toxicity to *E. coli* at concentrations that do not exceed solubility. To explore possible common molecular signaling among different HO-PBDE congeners, 30 genes modulated by 6-HO-BDE-47 were also assessed for transcriptional responses of *E. coli* to 16 other HO-PBDEs. Then, using 6-HO-BDE-47 as a model chemical, 182 gene knockout mutants, including all transcriptional factors (TFs) and modulated genes by 6-HO-BDE-47, were assessed to determine shifts in sensitivity after exposure to 6-HO-BDE-47 at the IC_{50}. On the basis of shifts in patterns of sensitivity, an assessment of transcriptional networks, including all TFs, was conducted to determine possible mechanisms of toxicity of 6-HO-BDE-47.

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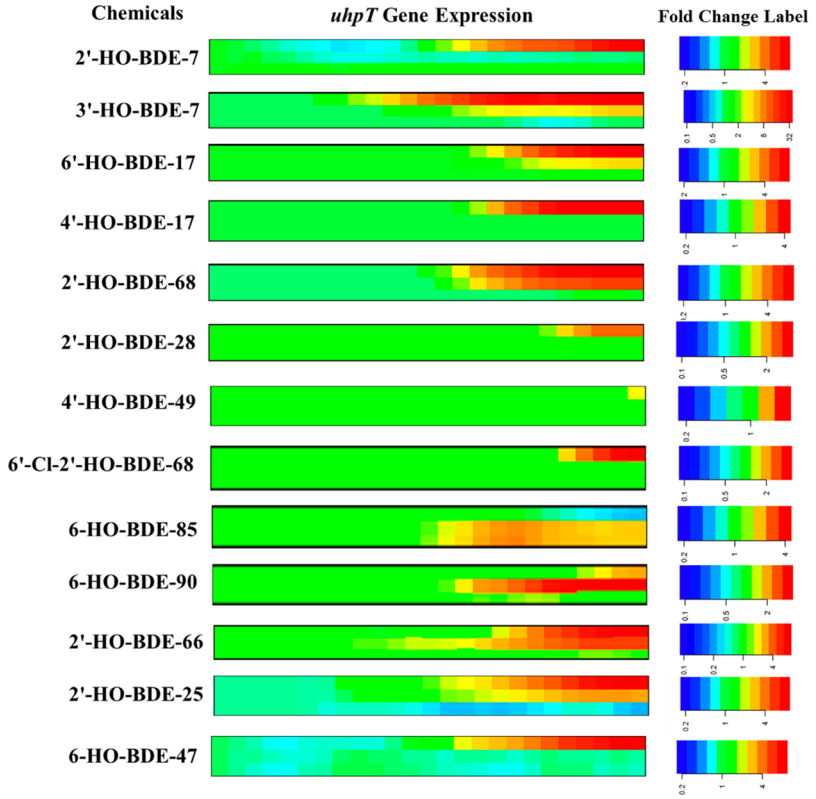

Figure 3. Real-time, quantitative expression of *uhpT* in *E. coli* after exposure to various HO-PBDE analogues. Exposure to lesser, moderate, and greater concentrations of target compounds was represented by the lower, middle, and upper bands in each gene column. The fold change of gene expression is indicated by the color gradient on the right, and the time course of expression changes is indicated from left to right.

MATERIALS AND METHODS

Chemicals and Reagents. Analogues of PBDEs, including 17 HO-PBDEs (Figure 1) and 15 MeO-PBDEs (see Figure S1 of the Supporting Information), were synthesized in the Department of Biology and Chemistry of City University of Hong Kong following previously published methods.[19] Purities of the synthesized compounds were determined to be greater than 98%. The results of proton nuclear magnetic resonance (^{1}H NMR) and electrospray liquid chromatography−tandem mass spectrometry (LC−MS/MS) were used to determine that unwanted intermediates and end products were not produced during the synthesis. In particular, there were no detectible polybrominated dibenzo-*p*-dioxins/furans.[8]

Live Cell Array and *E. coli* Keio Collection. The microbial live cell array collection, including more than 1800 of 2500 promoters in the entire genome of *E. coli* K-12 strain MG1655, was used to assess differential expression or genes by use of previously described methods.[15] Each of the reporter strains was coupled with a bright, fast-folding green fluorescent protein (GFP) fused to a full-length copy of an *E. coli* promoter in a low-copy plasmid. This enabled measurement of expression of genes within minutes with high accuracy and reproducibility. The tested knockout clones (strain background: *E. coli* K-12 BW25113) carried a deletion of a single gene, with a kanamycin resistance gene serving as the replacement, which were developed as a collaboration between the Institute for Advanced Biosciences, Keio University (Tokyo, Japan), Nara Institute of Science and Technology (Ikoma, Japan), and Purdue University (West Lafayette, IN). All clones from either live cell array or gene knockout collections were grown at 37 °C in lysogeny broth (LB)−Lennox media plus 25 mg/L kanamycin.

Cytotoxicity. Stock solutions of test chemicals (2000 mg/mL) were prepared in dimethyl sulfoxide (DMSO, Tedia, Fairfield, OH), and other stock solutions were made by serial dilution with DMSO. Eight different concentrations of HO-PBDE analogues (100, 25, 6.4, 1.6, 0.39, 0.098, 0.024, or 0.006 mg/L) (n = 3) were used in the *E. coli* cytotoxicity test. After 4 or 24 h of incubation at 37 °C, growth of *E. coli* was determined by measurement of optical density (OD) at 600 nm, by use of a Synergy H4 hybrid microplate reader (BioTek Instruments, Inc., Winooski, VT). In parallel, 10 μL of Alamar blue (Beijing CellChip Biotechnology, Inc., Beijing, China) was added to 150 μL of LB medium for each well to assess cell viability after 3 h of incubation, according to the ratio. Alamar blue was known to be nontoxic to cells. After cells were stained with Alamar blue for 1 h, blue−red fluorescence was detected by a Synergy H4 hybrid microplate reader (excitation/emission, 545/590 nm) (BioTek Instruments, Inc., Winooski, VT).

Expression of Genes. Exposure was performed with a slight modification of previously described methods.[20] Strains of *E. coli* were inoculated into a fresh 96-well plate from a 96-well stock plate by use of disposable replicators (Genetix, San Jose, CA). Cells were incubated at 37 °C for 3.0 h in a 96-well plate and then transferred into a 384-well plate. Finally, 3.79 μL of DMSO (solvent control) or chemical stock solutions were added into individual wells on the 384-well plate to make a final concentration of 0, 1, 10, and 100 mg of chemical/L, except for 2′-HO-BDE-28 and 6-HO-BDE-47. These two chemicals, 2′-HO-BDE-28 and 6-HO-BDE-47, killed *E. coli* at a concentration of 100 mg/L, and their exposure concentration were

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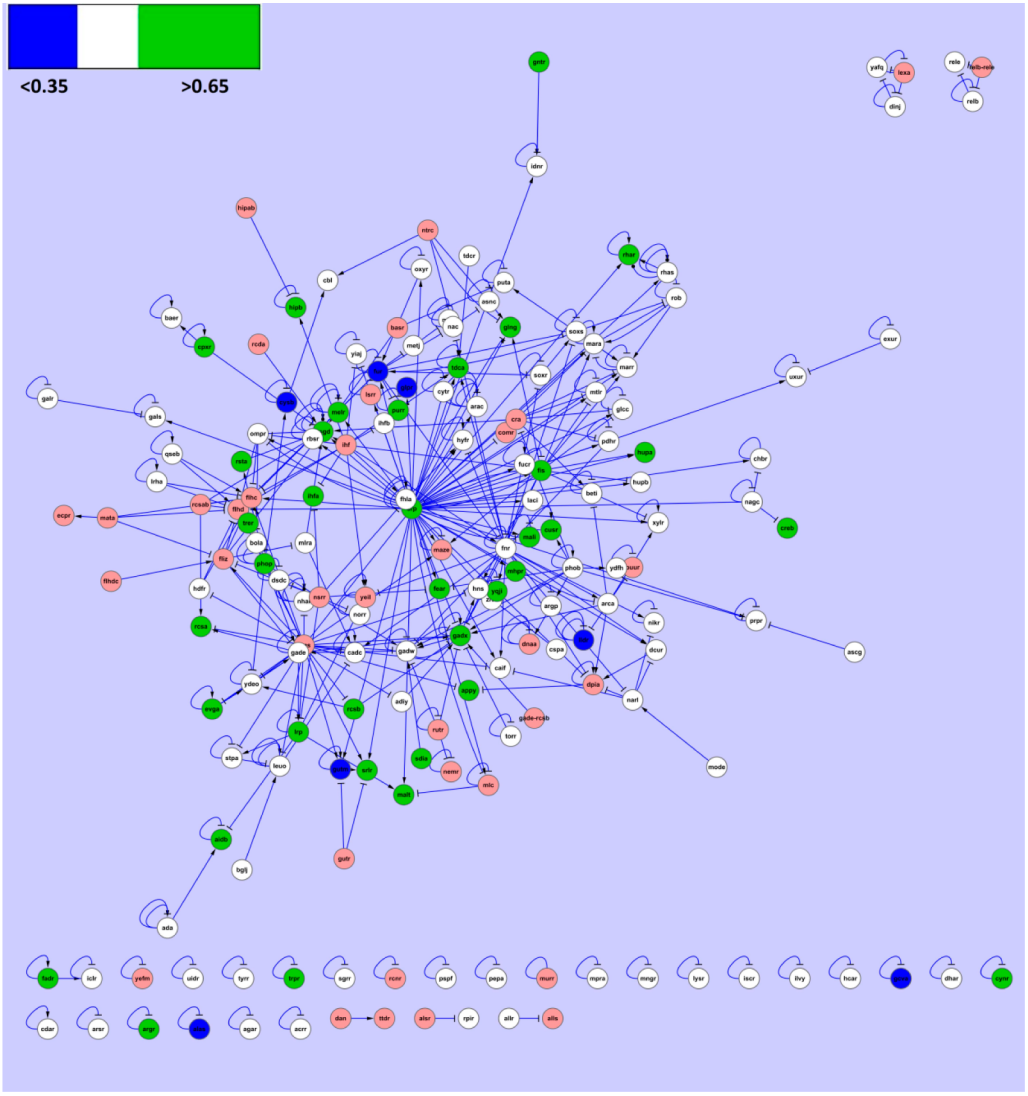

Figure 4. Shifts in sensitivity of TF-defective mutants by exposure to 6-HO-BDE-47 and their regulated network in *E. coli*. Each gene is displayed by a circular node, and the transcriptional interaction between TF−TF is indicated by an arrow edge. Different colors in the node represent the *E. coli* growth against the 4 h IC_{50} concentration of 6-HO-BDE-47. Green nodes represented resistant strains using a cutoff of 0.65; blue nodes represented sensitive strains using a cutoff of 0.35; and the white nodes represented the strains with no significant sensitivity changes. The pink nodes represent genes that are absent in the *E. coli* Keio collection.

made up to be 0, 0.01 × EC_{20}, 0.1 × EC_{20}, and EC_{20}, respectively. GFP intensity of each well was consecutively monitored every 10 min for 4 h by a Synergy H4 hybrid microplate reader (excitation/emission, 485/528 nm) (BioTek Instruments, Inc., Winooski, VT).

Assessment of Shifts in Sensitivities. To characterize the sensitivity of specific gene knockout strains, clones of interest were screened by comparing to the 4 h IC_{50} concentration of 6-HO-BDE-47 obtained in the initial wide-type screen in two rounds. After thawing, clones of interest were inoculated from the stock plates into a 15 mL centrifuge tube with fresh medium and pre-cultured overnight for the sensitivity screening. In each clone of each round, there was also a "control" group in two replicates without target chemical addition to ensure its robust growth. All conditions were duplicated. Plates were incubated at 37 °C for a 3 or 23 h period for a total of 4 or 24 h exposure, and then 4 μL of Alamar blue was added to 75 μL of LB medium for each well to assess cell viability. After 1 h of incubation with Alamar blue, the blue−red fluorescence was detected by a Synergy H4 hybrid microplate reader (excitation/emission, 545/590 nm).

Statistical Analysis and Data Visualization. Structures of HO-PBDE analogues were generated with ChemBioDraw Ultra 11.0 (Figure 1). A gene expression heat map of *uhpT* was conducted by use of R software (Figure 2). For temporal gene expression, a linear regression model was applied to select promoter reporters, of which expression was significantly differentiated relative to exposure to the chemicals (Figure 3). Genes were considered to be altered significantly only when their p values were less than 0.001. The TF−TF interactions were looked up in a RegulonDB database, and the visualized transcriptional network was conducted by use of Cytoscape, version 2.6.0 (Figure 4).[21] Gene set enrichment analysis (GSEA) was performed on R version 3.0.2 using the "GOstats" package, with R script tailored to *E. coli*.

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Table 1. Cytotoxicity End Points of 17 HO-PBDEs after a 4 and 24 h Exposure to *E. coli*[a]

compounds	24 h EC_{50}[b] (mg/L)	4 h EC_{50} (mg/L)	24 h LOEC[c] (mg/L)	4 h LOEC (mg/L)	24 h MII[d] (%)	4 h MII (%)
4-HO-BDE-90	NA[e]	NA	100	NA	13	NA
3-HO-BDE-100	NA	NA	100	NA	13	NA
2′-HO-BDE-66	NA	NA	100	100	23	21
2′-HO-BDE-68	NA	NA	25	NA	24	NA
6-HO-BDE-85	NA	NA	25	100	27	14
2-HO-BDE-123	NA	NA	6.25	NA	27	NA
5-Cl-6-HO-BDE-47	NA	NA	25	NA	29	NA
6-HO-BDE-90	NA	NA	6.25	NA	31	NA
6′-Cl-2′-HO-BDE-68	NA	NA	25	NA	32	NA
2′-HO-BDE-28	4.28	6.52	6.25	6.25	100	100
6-HO-BDE-47	9.61	12.13	1.56	6.25	100	100
6′-HO-BDE-17	NA	NA	NA	100	NA	11
4′-HO-BDE-17	NA	NA	NA	25	NA	14
3′-HO-BDE-7	NA	NA	NA	100	NA	16
2′-HO-BDE-7	NA	NA	NA	NA	NA	NA
4′-HO-BDE-49	NA	NA	NA	NA	NA	NA
2′-HO-BDE-25	NA	NA	NA	NA	NA	NA

[a]Vaules are shown as the mean of three replicates. [b]"EC_{50}" means "median effect concentration". [c]"LOEC" means "lowest observed effect concentration". [d]"MII" means "maximum inhibition induction". [e]"NA" means "not achieved".

RESULTS AND DISCUSSION

Cytotoxicity. After a 4 or 24 h exposure, no inhibition of cell division was observed for 15 MeO-PBDEs at concentrations ranging from 0 to 25 mg/L. However, cell division of *E. coli* cells was significantly inhibited after exposure to 14 of 17 HO-PBDEs, especially for 6-HO-BDE-47 and 2′-HO-BDE-28, which showed a concentration-dependent effect including total inhibition of growth of bacterial at 100 mg/L (Table 1). This finding indicated that HO-PBDEs were more cytotoxic to *E. coli* cells than MeO-PBDEs. The more cytotoxic potency HO-PBDEs was also reported in previous publications involving H295R cells[10] or zebrafish embryos.[22] Differences between the two functional groups, HO or MeO, were presumed to be the main reason for the observed differential toxic potencies. Hydroxyl groups can form hydrogen bonds with water molecules to enhance water solubility and also play an important role in enzymatic reactions. Among the 17 tested HO-PBDEs, 6-HO-BDE-47 and 2′-HO-BDE-28 were most cytotoxic, with median effect concentrations (EC_{50}) of 12.13 and 6.25 mg/L with a 4 h exposure, respectively. When the duration of exposure was 24 h, the EC_{50} values of 6-HO-BDE-47 and 2′-HO-BDE-28 were 9.68 and 4.28 mg/L, respectively, which indicated that longer term exposure to HO-PBDEs resulted in greater toxicity to *E. coli*. Both 6-HO-BDE-47 and 2′-HO-BDE-28 have been shown to be produced naturally,[2,23,24] and humans might be exposed to these chemicals via consumption of sea food.[25]

Profiles of Gene Expressions. Expression of 30 genes that had been shown previously to be modulated by 6-HO-BDE-47 in the microbial reporter stains was also observed after exposure to 16 other HO-PBDE analogues and exhibited both time- and concentration-dependent responses (see Figure S4 of the Supporting Information). Each specific HO-PBDE analogue induced a different pattern of expression of genes, which suggested complex mechanisms of effects of HO-PBDEs on toxicogenomic responses. However, common molecular signals were also observed in profiles of expression of genes. A total of 8 of 30 genes, *evgA*, *hupB*, *rrnB*, *flgM*, *rrnA*, *rrnC*, *serU*, and *uhpT*, can be modulated by more than 10 HO-PBDE analogues (see Figure S2 of the Supporting Information), which indicated that *E. coli* might undergo several similar molecular pathways while exposed to different HO-PBDE analogues. After a 4 h exposure, fold changes of 30 genes were reported in a heat map (Figure 2), which showed that exposure to HO-PBDEs results in fewer upregulated gene reporter stains than downregulated strains. The gene *uhpT* was classified into one group from the other 29 genes and mainly showed an upregulated profile.

Three rRNA operons (*rrnA*, *rrnB*, and *rrnC*) were consistently downregulated following exposure to HO-PBDEs, while *uhpT* was upregulated by 14 HO-PBDE analogues (Figure 3 and see Figure S3 of the Supporting Information). In *E. coli*, the *uhpT* gene, a P_i-linked hexose phosphate antiport carrier,[26] is a member of the major facilitator superfamily, which can catalyze active transport of sugar phosphates by an obligatory exchange mechanism. Expression of *uhpT* is controlled by a two-component regulatory system consisting of histidine kinase *uhpB* and the response regulator *uhpA*,[27] which are normally involved in responses of bacteria to environmental stimuli.[28] Fosfomycin is known to be transported into cells via the *glpT* and *uhpT* transporters,[29] which indicated that disturbance of glucose-6-phosphate acquisition might be a common signaling response during exposure to HO-PBDEs. In mammals, the alignment of the sequence of amino acids suggested that *uhpT* belongs to a family of transporters of phosphorylated metabolites, including the glucose-6-phosphate transporter,[30] which also posed an indication related to membrane transporter disease after exposure to HO-PBDEs in mammals. The three rRNA operons (*rrnA*, *rrnB*, and *rrnC*) belong to 16S rRNA, which is a component of the 30S small subunit of prokaryotic ribosomes. Because of its high conservation between different species of bacteria and archaea, these genes were usually used for phylogenetic studies.[31] The 16S rRNAs also play an important role in subunit association and translational accuracy in bacteria. Downregulation of the rRNA operons by HO-PBDEs might be another common signaling adaptive response.

Shifts in Sensitivities of Mutants. The gene loss-of-function screen assay provides another new method for genome-wide testing of mutational effects for specific cytotoxic contaminants,[17] such as zinc-containing nanoparticles.[18]

Considering that 6-HO-BDE-47 was strongly cytotoxic to *E. coli*, a mutant library, including 182 mutations in TFs and 22 mutations as genes modulated by 6-HO-BDE-47, was constructed to assess shifts in their sensitivities after exposure to 6-HO-BDE-47 at the IC_{50} for 4 h. Some genes that are essential for growth of bacteria are not available in the *E. coli* Keio collection. For each mutated strain, cytotoxicity was determined in two cycles. Information on inhibition collected in two rounds was fitted to a linear function ($y = x$) with adjusted R^2 values of 0.9530 or 0.9158 after 4 or 24 h exposure, respectively, which ensured its robustness (see Figure S5 of the Supporting Information). Differences in cytotoxicity between 4 and 24 h exposure groups were also compared via their 95% confidence ellipses of mean or prediction over the collected data points (see Figure S5 of the Supporting Information). Responses observed after a 24 h exposure tended to be less than those after a 4 h exposure, which indicated that the mutants would exhibit stronger inhibition after a longer duration of exposure.

Great concerns existed on the relationships among different high-throughput screening technologies. For example, correlations between "sensitivity profiles" and results of studies with microarrays have been proposed previously, but few correlations were observed between profiles of genes during global genome monitoring and sensitivity of mutants of *E. coli*.[17,32] To explore potential relationships between expression genes and profiles of sensitivities of mutants, 22 selected genes that were modulated by the model chemical 6-HO-BDE-47 were also assessed to determine sensitivities of mutant cell lines after prior exposure to the IC_{50} concentration of 6-HO-BDE-47 for 4 h. Here, growth of 22 mutation strains, expressed as the ratio between the exposed and control, ranged from 0.34 ± 0.03 to 0.69 ± 0.04. Although expression of *uhpT* can be modulated by most of the tested HO-PBDE analogues, growth of its mutation strain was not significantly different from growth of the wide-type strain. After exposure to 6-HO-BDE-47, no clear evidence of a relationship between expressions of genes and shifts in sensitivity of growth of mutation strains was observed in this research.

A mutation library consisting of a whole transcriptional network in *E. coli* was also introduced during the study, the results of which are presented here to study changes in sensitivity to exposure to 6-HO-BDE-47 against the 4 h IC_{50} concentration (Figure 4). In molecular biology and genetics, a TF is usually regarded as one of the most common mechanisms used by cells to control when genes are switched on or off.[33] TFs are also known as one of the groups of proteins that read and interpret the genetic "blueprint" in DNA, by binding to DNA and helping to initiate a program of increased or decreased gene transcription. RegulonDB, the primary reference database of the best-known regulatory network of any free-living organisms, has summarized 202 TFs and their associated TF−TF transcriptional relationships (see Table S1 of the Supporting Information), which make it easier to determine how a particular TF interacts with multiple other TFs while in up- or downregulated situations.[34] On the basis of their observed interactions among all TFs, changes in sensitivity were observed over the entire transcriptional network. Based on growth cutoffs of 0.35 and 0.65, 8 and 46 strains were selected as those carrying the most sensitive and resistant genes to 6-HO-BDE-47. Because of their important roles in development, intercellular signaling, and cell cycle, mutations in TFs are associated with specific diseases in organisms.[35] Here, the 54 mutant strains affected by 6-HO-BDE-47 were also taken as selected genes for a hypergeometric-based test, and this gene ontology (GO) term association analysis strongly suggested that 6-HO-BDE-47 caused toxicity to bacteria through an "organic acid metabolic process", an "oxoacid metabolic process", and a "carboxylic acid metabolic process" ($p < 0.05$; see Table S2 of the Supporting Information). HO-PBDEs contain the phenol group, which can confer weak acidity and be generally referred to as organic acids. This finding supported a previous report that 6-HO-BDE-47 was difficult to be transformed into other corresponding products during *in vitro* microsomal exposure studies.[7]

Implications. PBDEs and their metabolites (HO-PBDEs and MeO-PBDEs) are of interest because of their potential effects on people,[36−38] especially HO-PBDEs, which showed a more potent receptor activity compared to those of the parent PBDEs and corresponding MeO-PBDEs.[3,15,25] Previous studies showed that HO-PBDEs were detected even in human blood,[1] which indicated that formation of HO-PBDEs from PBDEs might occur via endogenous transformation. Studies also showed that HO-PBDEs can be formed from naturally occurring MeO-PBDEs[7,8,23] and can be detected in various marine organisms,[25,39] which also posed another route of dietary exposure to HO-PBDEs by humans, this observation resulted in considerable concern about the toxic mechanism of these accumulative transformation products of naturally occurring and synthetic PBDEs. In our study, HO-PBDEs inhibited growth of *E. coli* rather than MeO-PBDEs, which ranked HO-PBDEs to an even greater risk. We also found that several similar molecular signalings would be modulated after exposure to different HO-PBDEs. For example, *uhpT* can be upregulated by 13 of the 17 HO-PBDEs, and three rRNA operons (*rrnA*, *rrnB*, and *rrnC*) can be downregulated by 8 HO-PBDEs, which proposed possible mechanism of toxicity of HO-PBDEs. Further work should be conducted to uncover connections between the molecular responses in prokaryotic and eukaryotic cells and to investigate the toxicity potencies of HO-PBDEs in human cells.

■ ASSOCIATED CONTENT

Supporting Information

Transcriptional relationships between TFs summarized by RegulonDB (RegulonDB, version 8.0), also known as the primary reference database of the best-known regulatory network of any free-living organisms (Table S1), gene set enrichment analysis on 54 sensitive-shift genes to 6-HO-BDE-47 against the 182 TF mutations in the Keio collection (Table S2), structures of 15 MeO-PBDE analogues (Figure S1), altered genes by different HO-PBDEs (Figure S2), real-time, quantitative expression of *rrnA/B/C/H* in *E. coli* after exposure to various HO-PBDE analogues (Figure S3), real-time, quantitative determination of mRNA abundances as measures of 30 selected genes in *E. coli* after exposure to 17 HO-PBDE analogues (Figure S4), and linear fitting of data points collected in two cycles to demonstrate shifts in sensitivities of mutants pre-exposed for 4 h to the IC_{50} concentration of 6-HO-BDE-47 (Figure S5). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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