Molecular response mechanism in *Escherichia coli* under hexabromocyclododecane stress

Kunliang Yang\(^a\), Qiao Zhong\(^a\), Huaming Qin\(^a\), Yan Long\(^a\), Huase Ou\(^a\), Jinshao Ye\(^a,\ast\), Yanfen Qu\(^b\)

\(^a\) Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 510632, Guangdong, China
\(^b\) Zhongji Ecological Science & Technology Co., Ltd., Guangzhou 511443, Guangdong, China

**Highlights**
- Cell metabolism under HBCD stress at molecular and network levels was explored.
- Glycogen storage, glycolysis and oxidative phosphorylation were down-regulated.
- The ability of cell recognition and transport was suppressed by HBCD.
- HBCD exposure caused DNA damage and inhibited cell growth.
- Chemotaxis and nucleotide excision repair were the main mechanism to resist HBCD.

**Abstract**

The effects of hexabromocyclododecane (HBCD) on the relationship between physiological responses and metabolic networks remains unclear. To this end, cellular growth, apoptosis, reactive oxygen species, exometabolites and the proteome of *Escherichia coli* were investigated following exposure to 0.1 and 1 lM HBCD. The results showed that although there were no significant changes in the pH value, apoptosis and reactive oxygen species under HBCD stress, cell growth was inhibited. The metabolic network formed by glycolysis, oxidative phosphorylation, amino acids biosynthesis, membrane proteins biosynthesis, ABC transporters, glycogen storage, cell recognition, compound transport and nucleotide excision repair was disrupted. Cell chemotaxis and DNA damage repair were the effective approaches to alleviate HBCD stress. This work improves our understanding of HBCD toxicity and provides insight into the toxicological mechanism of HBCD at the molecular and network levels.

**1. Introduction**

Hexabromocyclododecane (HBCD) is used as a non-aromatic additive flame retardant in expanded and extruded polystyrene foams, textiles and electronic appliances (Wang et al., 2019a,b). The excellent flame-retardant property of HBCD led to its increased mass production from 16,700 t in 2001 to 31,000 t in 2011 (Cao et al., 2018).

HBCD has been found in environmental media, biota samples and human tissues worldwide (Chen et al., 2015; Desborough et al., 2016; Jo et al., 2017; Nostbakken et al., 2018; Cathrine et al., 2007; Malarvannan et al., 2013; Rawn et al., 2014). The results showed that HBCD can be enriched in the human body through dust inhalation and a normal diet (Roosens et al., 2009). Due to its persistence, bioaccumulation, toxicity and long-range transport, in May 2013, HBCD was listed in Annex A of the...
Stockholm Convention as a new persistent organic pollutant, but it can still be used in building materials until 2024 (Koch et al., 2015).

HBCD is a genotoxic compound (Lu et al., 2018) with diverse subchronic effects (Song et al., 2016; Wang et al., 2016a). Traditional research methods showed that exposure to HBCD caused oxidative stress and cell apoptosis in nematodes by reactive oxygen species (ROS) accumulation (Wang et al., 2018). Oral administration of HBCD disrupted lipid and glucose homeostasis in mice, resulting in increased body weight and liver weight (Yanagisawa et al., 2014).

Gene profiling assay revealed that genes related to oxidative stress, general cell lesions and DNA damage in Escherichia coli were significantly changed after exposure to HBCD (Krivoshiev et al., 2015). The complete genome sequence of Bacillus sp. HBCD-sjtu revealed 80 genes involved in motility and chemotaxis (Shah et al., 2018). High-throughput transcriptome sequencing studies found that HBCD also affected the two-component system consisting of ribosome assembly and glyoxylate and dicarboxylate metabolism in Rhodopseudomonas palustris (Wang et al., 2019a,b). At the systematic level, the metabolic pathways and global proteomic responses of cells exposed to HBCD remain unclear and require further study.

E. coli is a model organism for molecular biology research. The simple cell structure of E. coli can help researchers better understand cellular response processes. The research results from this species can be used as a reference for different species. Therefore, E. coli is widely used in studies of the mechanisms of toxicity of pollutants such as flame retardants, pesticide and antibiotics (Bhatti et al., 2019; Su et al., 2014; Viveiros et al., 2007). In this study, iTRAQ-based proteomic analysis and exometabolomics techniques were utilized to characterize the proteomic and metabolic profiles of E. coli following exposure to different concentrations of HBCD. Differentially expressed proteins and metabolites were analyzed to study the metabolic process of E. coli under HBCD stress using bioinformatics. These findings will provide new insights into the molecular response mechanism of HBCD at the metabolic and network levels. The clarification of these mechanisms is a basis for the application and management of HBCD.

2. Materials and methods

2.1. Strain and chemicals

E. coli ATCC 8739 was obtained from the Guangdong Microbial Culture Collection Center in China. HBCD was purchased from Sigma–Aldrich (St. Louis, MO, USA). The bacterial culture medium consisted of 3 g L\(^{-1}\) beef extract, 10 g L\(^{-1}\) peptone and 5 g L\(^{-1}\) NaCl. The concentrations of Na\(_2\)HPO\(_4\), KH\(_2\)PO\(_4\), NaCl, NH\(_4\)Cl, MgSO\(_4\), CaCl\(_2\) and glucose in the mineral salt medium for HBCD resistance were 12.8, 3.0, 0.5, 1.0, 0.492, 0.438 and 4 g L\(^{-1}\), respectively.

2.2. Microbial culture and HBCD treatment

E. coli was cultivated in 250 mL Erlenmeyer flasks with 150 mL culture medium at 37 °C on a rotary shaker at 130 r min\(^{-1}\). After 12 h, the cells were separated by centrifugation at 6,000 r min\(^{-1}\) for 10 min and were washed three times with sterile distilled water. Subsequently, HBCD methanol standard solution was added into 20 mL of MS containing 1 g L\(^{-1}\) of cells with varying concentrations (0.01, 0.1, 0.5, 1, 5 and 10 μM) of pollutant. The MS containing the same concentration of methanol was used as the control group. These examples were incubated in the dark at 37 °C on a rotary shaker at 130 r min\(^{-1}\) for 12 h. The culture medium with 0, 0.1 and 1 μM of HBCD was investigated from 0 to 96 h to observe the physiological effect of HBCD. Each treatment had three biological replicates.

2.3. Analysis of physiological effects

Growth was monitored by measuring optical density at 600 nm. After centrifugation at 6,000 r min\(^{-1}\) for 10 min, the pH value of the supernatant was measured by the PHS-3C pH meter (Reox, China). The cells were used to determine cell viability and reactive oxygen species concentrations using the Annexin V-FITC Apoptosis Detection Kit (KeyGEN Biotech, China) and the Reactive Oxygen Species Assay Kit (BD, San Jose, US) according to the manufacturers’ instructions. Cell viability was measured by a FACSAria flow cytometer (BD, San Jose, US), and then, 10,000 cells were acquired per sample at a flow rate of 10 mL min\(^{-1}\), and ROS was measured by a FACSAria flow cytometer with 50,000 cells acquired per sample at a flow rate of 20 mL min\(^{-1}\). According to the results of the basic physiological effects, the cells exposed to 0.1 μM or 1 μM of HBCD for 12 h were analyzed by exometabolomics and proteomics.

2.4. Exometabolomics analysis

Exometabolite analysis was performed using a published method (Lu et al., 2019). After exposure to 0, 0.1 and 1 μM of HBCD for 12 h, the samples were centrifuged at 14,000 r min\(^{-1}\) for 2 min. Then, 100 μL of sample supernatant was transferred to an Eppendorf tube, and 10 μL of labeled internal standard mixture (0.05 mM 13C glucose, 0.05 mM 13C xylene, 0.05 mM 13C vanilllic acid and 0.025 mM 13C cellobiose) was added. After lyophilization, each tube was shaken at 1400 r min\(^{-1}\) at 30 °C for 90 min with the addition of 10 μL of methoxyamine hydrochloride solution. Then, each sample was shaken at 1400 r min\(^{-1}\) at 30 °C for 30 min with 90 μL of FAMEs/MSTFA. After centrifugation at 14,000 r min\(^{-1}\) for 10 min, the supernatant was transferred to the sampling vial to be analyzed with an Agilent 7890 GC connected to an Agilent 5977 MS.

A Rtx5Sil-MS column (30 m × 0.25 mm ID with a 0.25 μm film thickness) was used for chromatographic separation. Two microelectrodes of each sample were injected into the injector port with an initial temperature of 50 °C, which was increased to 270 °C at a rate of 12 °C s\(^{-1}\) (held for 3 min). The initial oven temperature was programmed from 50 °C, increased at 5 °C min\(^{-1}\) to 65 °C (held for 0.2 min), increased at 15 °C min\(^{-1}\) to 80 °C (held for 0.2 min) and was finally increased at a rate of 15 °C min\(^{-1}\) to 310 °C (held for 12 min). The injection source and MS transfer line temperature were 230 °C and 250 °C, respectively. The electron ionization was 70 eV, and the mass spectra were acquired in the full scan mode at 8 spectra per second.

For data analysis, the retention times were measured using FAMEs. The raw data were deconvoluted into individual chemical peaks via Agilent MassHunter Unknows Analysis software v. B.07.00 for molecular feature extraction. The compounds were identified by comparing the mass spectra and retention times of all deconvoluted peaks with an Agilent Fiehn Metabolomics Library (2013 version) containing the spectra profile. A required minimum spectra match score of 75 indicated a putative hit. The list of metabolites was further curated by comparing putatively identified metabolites to reference standards and by cross-checking mass spectra with the NIST metabolite database.

2.5. Protein preparation

After exposure to HBCD, the cells were separated at 6,000 r min\(^{-1}\) for 5 min, washed using pure water and suspended in 1 mL of lysis buffer (7 M urea, 1% w/v dithiothreitol and 4% w/v 3-[(3-Cholamidopropyl)dimethylammonio]propanesulfonate, 2 M...
thiourea, 15 mM Tris–HCl) with 0.2 g L⁻¹ phenylmethylsulfonyl fluoride, 2% v/v IPG buffer and 0.6 g L⁻¹ dithiothreitol. After vibration for 10 s three times, the samples were frozen in liquid nitrogen three times for 15 min each, and subsequently treated by ultrasonication for 20 min. A nuclease mix at a final concentration of 1% v/v was added to the lysate, incubated at 4 °C for 30 min and the cellular debris was removed at 4 °C by centrifugation at 13,500 r min⁻¹ for 1 h. The concentration of the protein after centrifugation was determined using the Bradford method.

2.6. Protein digestion

The proteins from each sample were reduced with 2 μL of reducing reagent at 37 °C for 1 h and then the cysteine residues were blocked by adding 1 μL of cysteine-blocking reagent for 10 min at room temperature in the dark. The protein samples were added in 10 KD Amicon Ultra-0.5 centrifugal filter devices, followed by centrifugation at 12,000 r min⁻¹ for 20 min. After washing three times with 100 μL of dissolution buffer, the samples in the filter devices were digested with 50 μL of trypsin (Promega, USA) at 4% w/w overnight at 37 °C. Subsequently, the samples were centrifuged at the same conditions, and 1 μg of trypsin was added to each filter for 2 h. After centrifugation, liquid in the collection tube was collected. The concentration of tryptic peptides was measured using the Bradford method.

2.7. iTRAQ labeling, desalination and protein identification

The resultant peptide samples were labeled using an iTRAQ reagent multiplex kit according to the manufacturer’s instructions. Reagents 115, 116 and 117 indicate the control sample, and the samples exposed 0.1 μM or 1 μM of HBCD, respectively. Ethanol (150 μL) was added to each tube with iTRAQ reagent. After vortexing, the tryptic peptides were transferred to a new tube followed by incubation for 2 h. Water (100 μL) was added to each sample to stop the reaction. One microliter of each sample was analyzed by ABI 4800 MALDI TOF/TOF (Applied Biosystems, Foster City, CA) to determine the labeling efficiency. Subsequently, the iTRAQ-labeled samples were mixed, vortexed, centrifuged, desalted with Strata-X (Phenomenex, USA), and separated by strong cation exchange chromatography. The labeled peptides were dried in a vacuum concentrator. The samples were then dissolved in solution (2% v/v acetonitrile, 0.1% v/v formic acid), centrifuged at 12,000 r min⁻¹ 3 times (20 min per time), and analyzed by an AB Sciex TripleTOF 5600 mass spectrometer (AB Sciex, Framingham, MA, USA) equipped with a Nanospray III source (AB Sciex). The examined proteins were identified if the unused scores were above 2 and the peptides (>95%) were larger than 1. The fold changes of 116:115 and 117:115 greater than 1.2-fold were set to be significant with p-value = 0.058). These differentially expressed metabolites are involved in novobiocin biosynthesis, tyrosine metabolism, citrate cycle, phenylalanine, tyrosine and tryptophan biosynthesis, the pentose phosphate pathway, glyoxylic and dicarboxylate metabolism, starch and sucrose metabolism and purine metabolism (Fig. 2D). Xanthine is a weak alkaline substance. Compared with the control group, reduction in xanthine and the efflux of citrate and p-hydroxyphenylpyruvic acid resulted in a decrease pH value in the culture medium following exposure to 0.1 and 1 μM HBCD for 12 h.

2.8. Database analysis

One-way analysis of variance of pH, cell viability and ROS data was performed using IBM SPSS Statistics Version 20 (IBM Corp, Armonk, NY, USA). Exometabolomics data were further analyzed by MetaboAnalyst 4.0 (https://www.metaboanalyst.ca/). Metabolic pathway enrichment was carried out by KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/) (Ai and Kong, 2018). The bubble diagram was drawn by RStudio Version 1.1.447 (RStudio, Inc., Boston, MA, USA) and other diagrams were drawn by OriginPro 9.2 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Physiological effects of HBCD

Cell growth was investigated by measuring the optical density of the cellular mixture at 600 nm. The results showed that after being cultured for 1 h, cell growth was inhibited following exposure to 0.1 or 1 μM of HBCD compared with the control group (Fig. S1). Intracellular ROS and pH play an important role in cell signaling and homeostasis (Ozcan and Ogun, 2015). There is a cell adaptation phase for cells cultured in new environment. During culture for 6 h, the pH value decreased and intracellular ROS significantly increased compared with 0 h due to cell metabolism (Fig. 1A and B). Compared with the control group, the intracellular ROS and cell viability did not significantly change due to exposure to 0.1 and 1 μM of HBCD (Fig. 1B and C). However, the pH value changed following 0.1 and 1 μM HBCD stress for 12 h (Fig. 1A). In addition, HBCD treatment for 12 h did not cause apoptosis. The pH value significantly decreased following exposure to different doses (0.01, 0.1, 0.5, 1, 5 and 10 μM) of HBCD for 12 h, but intracellular ROS was not significantly affected by these doses except for 10 μM HBCD (Fig. 1D).

3.2. Exometabolite profiling in the presence of HBCD

To reveal the cellular metabolic response to HBCD, exometabolomics was performed after exposure for 12 h (Table S1). Exometabolite profiling was analyzed by orthogonal partial least-squares discriminant analysis. The results (R²X = 0.752, Q²Y = 0.78 between the control group and the 0.1 μM of HBCD treatment group, and R²X = 0.785, Q²Y = 0.686 between the control group and the 1 μM of HBCD treatment group) indicated that the discriminative and predictive ability of the orthogonal partial least-squares discriminant analysis was good. Differentially expressed metabolites were identified by setting a threshold of variable importance in the projection (VIP greater than 1 and p-value ≤ 0.05) (Fig. 2A and B).

Four differentially expressed metabolites were identified, including citrate, maltose, xanthine and p-hydroxyphenylpyruvic acid. Following exposure to 0.1 μM of HBCD, the concentration of citrate increased, whereas the content of maltose and xanthine significantly decreased. Following exposure to 1 μM of HBCD, the concentration of xanthine decreased, while the content of p-hydroxyphenylpyruvic acid significantly increased. The content of ribose also increased slightly following exposure to 1 μM of HBCD (p-value = 0.058). These differentially expressed metabolites are involved in novobiocin biosynthesis, tyrosine metabolism, citrate cycle, phenylalanine, tyrosine and tryptophan biosynthesis, the pentose phosphate pathway, glyoxylic and dicarboxylate metabolism, starch and sucrose metabolism and purine metabolism (Fig. 2D). Xanthine is a weak alkaline substance. Compared with the control group, reduction in xanthine and the efflux of citrate and p-hydroxyphenylpyruvic acid resulted in a decrease pH value in the culture medium following exposure to 0.1 and 1 μM HBCD for 12 h.

3.3. Differentially expressed proteins after HBCD exposure

To clarify the molecular mechanism of the E. coli response to HBCD stress, an iTRAQ-based proteomics experiment was performed after exposure to 0.1 and 1 μM HBCD. Among the identified proteins, the expression levels of 124 and 148 proteins were significantly up- and downregulated in the 0.1 μM HBCD treatment
group (116/115), respectively, while 106 and 143 proteins were significantly up- and downregulated in the 1 μM HBCD treatment group (117/115), respectively (Table S2 and Fig. 3A). From this analysis, 73 proteins were significantly upregulated, and 79 proteins were significantly downregulated, in the both 0.1 and 1 μM HBCD treatment groups. Additionally, 11 differentially expressed proteins were significantly upregulated in the 0.1 μM HBCD treatment group, but significantly downregulated in the 1 μM HBCD treatment group. Five proteins were significantly downregulated in the 0.1 μM HBCD treatment group, but significantly upregulated in the 1 μM HBCD treatment group. A good correlation between the 0.1 μM and 1 μM HBCD treatment groups was observed using the Pearson correlation test (R = 0.6568, p-value = 0), suggesting that cells showed the same trend in protein expression due to exposure to 0.1 μM and 1 μM HBCD (Fig. 3B).

3.4. Metabolic pathway enrichment of differentially expressed proteins

Metabolic pathway enrichment analysis of the differentially expressed proteins was performed using KOBAS 3.0 (p-value < 0.05). The result showed that 13 metabolic pathways were significantly enriched in all the treatment groups, which included secondary metabolite biosynthesis, ribosomes, glycolysis, metabolic pathways, antibiotic biosynthesis, carbon metabolism, amino acid biosynthesis, the pentose phosphate pathway, microbial metabolism in diverse environments, ABC transporters, pyruvate metabolism, amino sugar and nucleotide sugar metabolism and nucleotide excision repair (Fig. 4A and B). Additionally, 9 significantly enriched metabolic pathways including methane metabolism, purine metabolism, base excision repair, alanine, aspartate and glutamate metabolism, starch and sucrose metabolism, porphyrin and chlorophyll metabolism, pyrimidine metabolism, glycine, serine and threonine metabolism and butanoate metabolism occurred only in the 0.1 μM HBCD treatment group. Four metabolic pathways, including quorum sensing, citrate cycle, glutathione metabolism and valine, and leucine and isoleucine degradation were significantly enriched only in the 1 μM HBCD treatment group.

4. Discussion

HBCD can bind to the Ca2+ ATPase and inhibit its activity, resulting in decreased ATP level in cells (Al-Mousa and Michelangeli, 2014). Proteins involved in energy metabolism were significantly downregulated following exposure to HBCD. The PTS system...
glucose-specific EIIA component (Crr) controls the intake and phosphorylation of glucose. A low abundance of Crr resulted in inefficient glucose intake and glucose conversion to glucose-6P following exposure to 0.1 μM and 1 μM HBCD (Fig. 5). These results provided direct evidence to regarding why Na+/K+-ATPase activity and glucose transport were affected under HBCD stress (Wang et al., 2016b). Normally, glucose-6P is further converted to fructose-6P by the catalysis of glucose-6-phosphate isomerase (Pgi) for glycolysis, or used for glycogen storage and accumulation under the catalysis of phosphoglucomutase (Pgm). The downregulation of Pgi and Pgm indicated that the transformation of glucose into glycogen storage and glycolysis were inhibited. Maltose is involved in glycogen storage and was significantly reduced following exposure to 0.1 μM HBCD (p-value = 0.02). Additionally, 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase (GpmA) and pyruvate dehydrogenase (AccE) are involved in glycolysis and were downregulated in all the treatment groups, further suggesting that glycolysis was inhibited under HBCD stress.

Fig. 2. OPLS-DA of metabolites in *E. coli* after treatment with 0.1 μM HBCD (A) and 1 μM HBCD (B). A: R2X = 0.752, R2Y = 0.918, Q2Y = 0.78 and B: R2X = 0.785, R2Y = 0.933, Q2Y = 0.686. Differential metabolites in *E. coli* exposed to HBCD at 0.1 and 1 μM (C). Metabolic pathway analysis of differentially expressed metabolites (D). *, P < 0.05; **, P < 0.01.
findings indicated that the transmembrane transport of glucose was limited and the energy produced by glycolysis would be reduced under HBCD stress.

Oxidative phosphorylation is another major means for cells to obtain energy. During oxidative phosphorylation, NADH:quinone oxidoreductase II (Ndh) and succinate:quinone oxidoreductase (SdhB) are important proteins in the electron transport chain. Ndh catalyzes the transfer of electrons from NADH to quinone (Marreiros et al., 2016). The reaction is divided into main two parts: FAD reduction and FAD oxidation. First, FAD is reduced to FADH₂ by hydride transfer from NADH. The hydrides are then transferred from FADH₂ to quinone to form quinol (Marreiros et al., 2017). It is noteworthy that FADH₂, the intermediate of the above reaction, is also the catalytic substrate of SdhB. Electrons are transferred from FADH₂ to ubiquinone to form ubiquinol via the iron-sulfur centers [2Fe-2S], [4Fe-4S], and [3Fe-4S] under the catalysis of SdhB (Horsefield et al., 2006). The proton-motive force is generated via an electron transport chain and coupled to the

Fig. 3. (A) The number of differentially expressed proteins in E. coli exposed to HBCD at 0.1 and 1 μM. (B) Pearson correlation analysis of proteomic profiling. ([116/115] fold-change of proteins following exposure to 0.1 μM HBCD, [117/115] fold-change of proteins following exposure to 1 μM HBCD.)

Fig. 4. Metabolic pathway enrichment analysis of differentially expressed proteins in E. coli exposed to 0.1 μM (A) and 1 μM (B) HBCD for 12 h.
catalysis of ATP synthase to produce ATP for cell metabolic activities. The downregulation of Ndh, SdhB and AtpA would lead to a decreased electron transfer rate and ATP synthesis rate in the cell respiratory chain. Moreover, Glu172 of Ndh plays a key role in proton transfer to the quinone pocket. This modification affects the binding of NADH at the active site and hinders the reduction of quinone (Marreiros et al., 2017). Glutamate dehydrogenase (GdhA) catalyzes the conversion of α-ketoglutaric acid to glutamic acid. In this study, the downregulation of GdhA involved in glutamate metabolism in all treatment groups resulted in a decrease in glutamate synthesis. This finding is consistent with the downregulation of Ndh. The Lack of glutamic acid might affect the functional expression of Ndh and cause the inhibition of its activity. Obviously, both the glycolysis pathway and oxidative phosphorylation pathway were inhibited under HBCD stress. The decreased energy metabolism did not result in apoptosis, but it affected various metabolic activities and inhibited cell growth.

Quorum sensing can influence the construction and tolerance of biofilm (Whiteley et al., 2017). The fluidity of the biofilm, as well as signal proteins and channel proteins on the biofilm, are closely related to the transmembrane transport of substances. Quorum sensing was significant following exposure to 1 μM of HBCD (p-value = 0.0003). Eleven proteins related to quorum sensing were differentially expressed. Although the alteration in quorum sensing did not reach a significant level following exposure to 0.1 μM of HBCD, there were still 7 differentially expressed proteins, of which 5 proteins were upregulated. Under starvation conditions, putative D, D-dipeptide ABC transporter periplasmic binding protein (DdpA) might be used to import D-alanyl-D-alanine as an energy source (Lessard et al., 1998). Cells would resist the negative effect of ATP deficiency caused by HBCD via the upregulation of DdpA. In addition, the upregulation of autoinducer-2 ABC transporter periplasmic binding protein (LsrB) could bind to autoinducer-2 in the cytoplasm to induce bacterial chemotaxis (Hegde et al., 2011). Chemotaxis is very important for bacteria to flee from toxicants. This process is a beneficial means for E. coli to alleviate the toxicity of HBCD. Signal recognition particle receptor (FtsY), as a receptor protein for the signal recognition particle, mediates the cotranslational targeting of integral membrane proteins. The number of membrane-associated ribosomes and proteins are dramatically reduced in FtsY-depleted cells (Herskovits and Bibi, 2000; Ido et al., 2010). Protein translocation ATPase (SecA) plays a key role in protein transport by forming a discrete anti-parallel dimer (Banerjee et al., 2017). The downregulation of FtsY and SecA reduced the amount of proteins in the cell membrane and limited cell recognition and membrane transport of substances. These inhibitory effects were more obvious under exposure to the high concentration of HBCD (1 μM). The ABC transport pathway was significantly enriched in all treatment groups (p-value < 0.01). Maltose, amino acids and Fe-enterobactin-related transport proteins (MaLE, HisJ, HisP, LivK and FepB) were significantly downregulated, further demonstrating that cell transport was inhibited.

Studies have shown that brominated flame retardants can cause damage to gene (Pereira et al., 2016; Sharma et al., 2018). Similarly, HBCD exhibited genotoxicity toward cells at the molecular level, p-Hydroxyphenylpyruvic acid, which can cause DNA damage (van Dyk and Pretorius, 2005), was significantly increased following exposure to 1 μM HBCD in the exometabolomics experiment (p-value = 0.045). Nucleotide excision repair, an important mechanism of DNA repair, was remarkably enriched in all the treatment groups (p < 0.05) and is unique in its ability to maintain genomic stability. The complex formed by excision nuclease subunit A (UvrA) and excision nuclease subunit B (UvrB) first binds to DNA and recognizes damage (Fig. S2). Then, UvrA is separated. Excision nuclease subunit C (UvrC) binds to the complexes of UvrB and DNA and mediates the incisions on both the 3′- and 5′- sides of DNA. Finally, excision and filling were performed under the action of DNA helicase II (UvrD), DNA polymerase I (PolA) and DNA ligase (LigA). UvrC is the least abundant of the Uvr proteins and is a key component in opening DNA incisions (Truglio et al., 2006). The upregulation of UvrC, PolA and LigA indicated that cellular DNA was damaged during HBCD stress. E. coli accelerated DNA repair through the activation of nucleotide excision repair. The upregulation of exodeoxyribonuclease III (XthA) was involved in base excision repair, which is another mechanism to repair damaged DNA. XthA is responsible for the cleavage of the damaged DNA from the 3′- to 5′- direction, further suggesting that DNA damage repair is activated. In addition, the upregulation of broad specificity 5′(3′)-nucleotidase and polynucleotidase (UmpP) catalyzes the formation of purines and pyrimidines. This result is consistent with the metabolomics results (Fig. 2). Xanthine, a metabolite of purine, was significantly reduced in both the treatment groups (p < 0.01). As one of the substrates for DNA synthesis, ribose was slightly increased following exposure to 1 μM HBCD. The increase in purines, pyrimidines, and ribose provided a sufficient source for the DNA repair process. In general, HBCD caused an increase in p-
hydroxypyruvic acid, which damages DNA. In addition, the cells activated and accelerated DNA damage repair mechanisms to resist the toxic effects of HBCD.

5. Conclusions

Although HBCD at 0.1 and 1 μM did not lead to cellular apoptosis and increased ROS, it inhibited glycolysis, transport, cell recognition, glycan storage, oxidative phosphorylation, and membrane protein biosynthesis. As a result, metabolic activities and cell growth were disturbed. Cell chemotaxis and DNA damage repair mechanism were means to alleviate HBCD stress. This work improved our understanding of HBCD toxicological mechanism at the pathway and network level.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the Guangdong MEPP Fund (No. GDOE[2019]A09) and the National Natural Science Foundation of China (No. 21876064) for their financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135199.

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