Environmental Pollution xxx (xxxx) xxx

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Mechanisms of transplacental transport and barrier of polybrominated diphenyl ethers: A comprehensive human, Sprague-Dawley rat, BeWo cell and molecular docking study☆

Yingxin Yu [a, b], Xiaojing Li [b], Junjie Hu [c], Zi'an Jiang [b], Xiaolan Zhang [b], Guiying Li [a], Shengtao Ma [a], Bingli Lei [b], Xiangming Fang [d], Ruifang Fan [e], Taicheng An [a, *]

[a] Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, Guangzhou Key Laboratory of Environmental Catalysis and Pollution Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou, 510006, PR China
[b] Institute of Environmental Pollution and Health, School of Environmental and Chemical Engineering, Shanghai University, Shanghai, 200444, PR China
[c] School of Environment and Civil Engineering, Dongguan University of Technology, Dongguan, 523808, Guangdong, PR China
[d] Shanghai Huangpu Maternity & Infant Health Hospital, Shanghai, 200020, PR China
[e] School of Life Science, South China Normal University, Guangzhou, 510631, PR China

ARTICLE INFO

Article history:
Received 24 September 2020
Received in revised form
25 October 2020
Accepted 12 November 2020
Available online xxx

Keywords:
Polybrominated diphenyl ethers
Sprague-dawley rats
BeWo cell monolayer model
Molecular docking study
Transplacental transport mechanisms
P-glycoprotein

ABSTRACT

Although studies have reported that polybrominated diphenyl ethers (PBDEs) can transfer from mothers to fetuses, the underlying transplacental transport and barrier mechanisms are still unclear. Therefore, we conducted a series of comprehensive experiments in humans, Sprague-Dawley rats, and a BeWo cell monolayer model, as well as a molecular docking study. PBDEs in mothers can transfer to fetuses with a ratio of approximately 0.46, suggesting that the placenta could not efficiently acts as a barrier to PBDE transplacental transport. Similar results were observed in pregnant rats, although varying times were required for different congeners to reach a steady-state in fetuses. The transport ratios at pregnancy day 14 in rats were generally higher than those at pregnancy day 18, which demonstrated that the barrier capacity of immature placentas was lower than that of mature placentas. None concentration-dependent transplacental transport was observed in BeWo cells with efflux ratios of 1.73–2.32, which suggested passive diffusion mechanisms govern the influx of PBDEs through placenta. The accumulated ratios of PBDEs and the inhibitor assay indicated that the effluent channel of P-glycoprotein was partially inhibited by PBDEs. Using molecular docking studies, three pocket sites were identified for different congeners in P-glycoprotein, which demonstrated that the inhibition of P-glycoprotein efflux pump through the pocket sites.

Author statement

Yingxin Yu, Design, Methodology, Formal analysis, and draft preparation. Xiaojing Li, Methodology and experiment. Junjie Hu, Methodology and experiment. Zi'an Jiang, Methodology and experiment. Xiaolan Zhang, Formal analysis. Guiying Li, Formal analysis. Shengtao Ma, Formal analysis. Bingli Lei, Methodology. Xiangming Fang, Sample collection. Ruifang Fan, Methodology. Taicheng An, Design, reviewing & editing

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of persistent organic pollutants (POPs) that have been widely used as flame retardants in fire safety (Zota et al., 2018). PBDEs are not bound to polymers by chemical bonds and can be easily released into the environment during the life cycle of their products (Frederiksen et al., 2010a, 2010b; Yu et al., 2012a). As a result, the ubiquitous occurrence of PBDEs has been observed in all kinds of environmental matrices (An et al., 2011; Boon et al., 2002; Han et al., 2016; Mazdai et al., 2003; Schecter et al., 2006; Tang et al., 2014; Vizcaino et al., 2011, 2014a; Xiong et al., 2016; Yu et al., 2012b, 2019).

☆ This paper has been recommended for acceptance by Professor Christian Sonne.

* Corresponding author.
E-mail address: antc99@gdut.edu.cn (T. An).

https://doi.org/10.1016/j.envpol.2020.116091
0269-7491/

Although PBDEs are beneficial to humans and have saved countless lives, researchers have demonstrated that such chemicals have many negative effects on human health, including neurotoxicological and reproductive effects (Albert et al., 2018; Eriksson et al., 2006; Zhang et al., 2018b). Furthermore, the high lipophilicity and low degradability of PBDEs in the environment, as well as their difficult biotransformation, leads to their accumulation in organisms. Consequently, PBDEs have been listed as priority-controlled pollutants by the Stockholm Convention aimed to protect human health and the ecological environment.

In addition to their accumulation in environmental matrices, the large and widespread usage and high lipophilicity of PBDEs lead to their accumulation in human bodies. For instance, their occurrences in breast milk, blood, and hair were observed in multiple studies (Mazdai et al., 2003; Tang et al., 2014; Vizcaino et al., 2011, 2014a; Gascon et al., 2012; Zhang et al., 2014). PBDEs have been detected in umbilical cord blood and fetal tissue, suggesting that they can pass through the placenta to the fetus (Vizcaino et al., 2011; 2014a). Placenta is an important organ for the exchange of gases and nutrients as well as waste products between a mother and her fetus (Baker et al., 1981; Jeong et al., 2018). Placenta is regarded as a barrier that protects fetuses against toxicants circulating in maternal blood. However, the occurrence of PBDEs in fetuses indicates that the placental barrier is finite (Jiang et al., 2019; Zheng et al., 2017). Fetuses are more vulnerable and have lower immune and metabolic capabilities to toxicants than adults (Vizcaino et al., 2014b). Thus, prenatal life is the most sensitive developmental stage for humans.

Many studies have shown that POPs can pass through the placenta from mother to fetus, and this is influenced by multiple factors, including the physico-chemical properties of the chemicals, protein-binding affinity with the chemicals, and placental permeability (Jeong et al., 2018; Morello-Frosch et al., 2016; Vizcaino et al., 2014b; Wu et al., 2010; Zhang et al., 2018a). Both the active and passive transport of xenobiotics between maternal and fetal blood may influence the absorption and distribution of molecules in fetuses (Aylward et al., 2014). For example, membrane transporters include a large number of efflux and influx pumps, which can pump xenobiotics back to the mother or into fetuses. Pacyniak et al. showed that the uptake of PBDEs in the liver was associated with the specific binding of organic anion transporting polypeptides (influx transporters) (Pacyniak et al., 2010, 2011). In addition, a study of understanding PBDE absorption in the intestine found that the efflux transporters of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated protein (MRP) may participate in the transepithelial transport of PBDEs in Caco-2 cells (Yu et al., 2017).

Despite the importance of early life exposure to environmental toxicants, the transplacental transport and barrier mechanisms of most environmental chemicals are still not fully understood. Thus, there is an urgent need to conduct a systematic investigation aimed to explore the transplacental transport characteristics and unveil the underlying mechanisms of PBDE transport. The present study hypothesized that the underlying mechanisms of transplacental transport and barrier of PBDEs can be revealed using a comprehensive human, Sprague-Dawley (SD) rats, BeWo cell and molecular docking study. To achieve this, paired samples of maternal serum, umbilical cord serum, and placentas collected from a local hospital in Shanghai, China, were analyzed. The influence of placental maturity (according to the echo intensity of the placental basal plate, parenchyma and chorionic plate, the placental images were divided into 0–III grades. In the present study, the duration of pregnancy was used to reflect the effects of placental maturity) on transplacental transport was observed in the animal experiments but was difficult to observe in human samples. Thus, animal experiments were also conducted using pregnant SD rats to further probe the transplacental transport characteristics of PBDEs. To further understand the underlying transplacental transport mechanisms, the roles of membrane transporters (including P-gp, BCRP, and MRP) expressed on placentas were investigated using BeWo cells, a choriocarcinoma cell line from human trophoblasts, and act an effective tool to investigate the factors associated with the rate-limiting barrier for maternal-fetal exchange. The confluent BeWo cell monolayers include the maternal-facing plasma membrane and the basal membrane, which make contact with maternal blood and fetal circulation, respectively. The transplacental transport of PBDEs can be determined according to the transported PBDEs from apical (AP) to basolateral (BL) chambers in Transwell inserts. In addition, possible binding sites between the membrane transporters and PBDE congeners were analyzed using a theoretical molecular docking software. To our knowledge, this is the first report providing comprehensive insights into the underlying transplacental transport and barrier mechanisms of organic contaminants.

2. Materials and methods

2.1. Materials and reagents

Pregnant SD rats were purchased from JOINN Laboratories (Suzhou, China) (license number: SCXK (Su) 2013—0003). The BeWo placental choriocarcinoma cell line was purchased from Shanghai Aolu Biological Technology Co., Ltd. (Shanghai, China) and used for transplacental transport experiments. The cells were cultured in RPMI1640 supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a 5% CO_2 atmosphere, and then were sub-cultured using 0.25% ethylenediaminetetraacetic acid-trypsin when they reached 70%—90% confluence. To obtain sufficient amounts of PBDEs for animal dosing, corn oil containing 3—7 brominated congeners were prepared as described in the Supporting Information (SI) in detail. In the present study, the aimed PBDE congeners including BDE17, 28, 71, 47, 66, 100, 99, 85, 154, 153, 138, 183, 190, and 209. Those congeners with interferences were not discussed as indicated in the section of quality assurance and quality control in the Supporting Information. Other materials and reagents are also given in the SI.

2.2. Paired samples of maternal serum, umbilical cord serum, and placentas

Paired samples of maternal serum, umbilical cord serum, and placentas were collected in Shanghai, China between 2013 and 2014, the detailed description of these samples was reported in our previous studies (Zhang et al., 2017; 2018a). In this study, 32 paired samples collected in 2013 were randomly selected and used for this investigation. As described in our previous studies, volunteers were randomly enrolled at the local hospital. The Ethics Committee of Dongguan University of Technology approved this study, and informed consent was collected from all volunteers. Detailed information is listed in Table S1. The sample treatment protocols are similar to a previous study and are given in the SI (Hovander et al., 2000). The instrumental analysis can also be found in the SI.

2.3. Dosing and treatment of SD rats

Generally, pregnant rats were housed in cages (one per cage) with access to water and food ad libitum, under a 12/12 h light/dark cycle. On gestation at a given day (date sperm-plug positive = GD0), pregnant rats were randomly divided into four groups, which included three experimental groups with three rats per group and

one control group. Because the pregnant period of rats is approximately 21 days, in the present study, GD14 and GD18 were used to investigate immature and mature placenta influence. In addition, 14 and 18 days of pregnancy in the SD rats are similar to that at the second and third trimester of pregnancy in humans, respectively. To study the factors influencing the transplacental transport of PBDEs, three groups of animal experiments were performed, including one group with a single dose of corn oil containing different PBDE concentrations (low, medium, and high) (Table S2) at GD14; one group with a single dose of corn oil containing a given PBDE concentration with different sampling times (6, 12, 24, 72, and 120 h) at GD14; and one group with a single dose of corn oil at different pregnancy stages (GD14 and GD18. Each rat was administrated 4 mL of corn oil containing PBDEs by gavage. The control group received 4 mL of corn oil without PBDEs by gavage and was used as the reference. It should be noted that since the pregnant rats were commercially purchased, they were judged by hand touching rather than observing the occurrence of vaginal embolism and/or spermatozoa in the vagina of female rats to judge whether they were pregnant. Therefore, although the purchased pregnant rats were sent to our laboratory at GD7 (as the supplier said), there might be an error of about 2 days during pregnancy. In the present study, we took the purchased rats plus 7 days as GD14. In order to prevent the error of 2 days, the mice had been born, and only add 11 days, i.e., GD18 as the mature placenta after the pregnant rats were sent to the laboratory. Actually, in our pre-experiment, pregnant rats were born on the 11th day, that is, the GD18 after the pregnant rats were sent to our laboratory from the supplier.

After dosed, maternal blood was collected and then centrifuged at 3500 rpm for 15 min. The serum obtained (approximately 3 mL) was transferred to a brown glass vial and stored for further analysis. The fetuses (including blood and other tissues) and placentas were carefully removed, rinsed with saline water, and stored at −20 °C in the absence of light. The Ethics Committee of Dongguan University of Technology approved all animal experiments, which were performed in accordance with the guidelines of the Care and Use of Laboratory Animals.

2.4. Transplacental transport experiments using a BeWo cell monolayer model

Before this experiment, a cell viability assay was performed, which is given in the SI. Then, BeWo cells were seeded at 1×10^5 cells/cm^2 on Transwell inserts (0.4 μm, 1.12 cm^2, polyester). The AP and BL chambers contained 0.5 and 1.5 mL of medium (pH = 7.4), respectively, which were changed daily from day 2 post-seeding until monolayer formation. The barrier-forming capacity of the BeWo cell layer was examined using a light microscope. Monolayer formation was visualized and the transepithelial electrical resistance (TEER) was measured daily using an EVOM2 Cell Potentiometer. Generally, a TEER value of 35 Ω/cm^2 is considered to be a tightly formed monolayer in BeWo cells. Before and after the experiment, the TEER values were detected. To explore the transport characteristics of PBDEs in BeWo cell monolayers, experiments were performed as described in our previous study, which tested transepithelial transport using a Caco-2 cell monolayer (Yu et al., 2017). The detailed experiment and sample treatment information, and all other quality assurance and quality control, calculations and statistical analysis are given in the SI.

2.5. Homology modeling and molecular docking

Molecular docking studies were carried out to probe the molecular interactions between the transporter protein (P-pg) and different PBDE congeners. The chemical structures of the PBDE congeners were prepared using the structure sketch drawing tool. Ligand flexibility was then generated by ligand conformations before docking. The 3D conformations of PBDE congeners were processed using AutoDock Vina, allowing the rotation of the ligand to provide the final and reacted forms. Finally, 3D structures and stereoisomers of the ligands with the 'reacted' electrophile were built by OpenBabe. The structural template for P-gp modeling was selected by submitting the amino acid sequence of P-gp (NCBI Q99527) to the Swiss-Model web server (https://swissmodel.expasy.org/interactive) to generate 20 three-dimensional (3D) protein structures using homology modeling with 4LSG as the template. The constructed protein models were evaluated with Ramachandran plots and the best models with 95% of the residues in favorable regions were selected. The Autodock Vina program was used to perform molecular docking, and the grid box was set to cover the complete protein to detect any possible binding pocket(s) by blind docking. For each congener, the generated docking poses were inspected and those with the lowest free energy were chosen as the final conformations with which to construct the tertiary structures of P-gp in complex with PBDE congeners.

3. Results and discussion

3.1. Concentrations of PBDEs in paired maternal serum, umbilical cord serum, and placentas samples

A total of 32 paired samples of maternal serum, umbilical cord serum, and placentas were measured (Table S3). Among the quantified 12 PBDE congeners with no interferences, only three congeners including BDE28, 47, and 153 were observed in all the three types of matrices with detection frequencies higher than 50%. The lipid-adjusted concentrations of Σ_{12}PBDEs (sum of 12 PBDE congeners) in placenta, and maternal and umbilical cord serum samples were obtained as 0.09–3.92, 0–13.7, and 0.04–17.4 ng/g lw (lipid weight), respectively. Among the 12 PBDE congeners, the concentration of BDE153 was found to be the highest in placenta and maternal serum, while BDE47 concentrations were highest in umbilical cord serum (Fig. 1A). The highest lipid-adjusted concentration was observed in umbilical cord serum (17.4 ng/g lw), followed by maternal serum (13.7 ng/g lw), while placentas (3.92 ng/g lw) had the lowest PBDE concentrations (Table S3). The median concentrations followed a similar sequence of umbilical cord serum (4.45 ng/g lw), maternal serum (3.57 ng/g lw), and placental samples (0.88 ng/g lw). Using paired samples of maternal serum, umbilical cord serum, and placentas, we found that PBDEs can be transported from mother to fetus with the highest lipid-adjusted concentration in umbilical cord serum followed by maternal serum and placentas. Similar results have also been observed in previous studies (SI), although the available data were very limited.

The correlations among concentrations of toxicants in umbilical cord serum, maternal serum, and placentas can be used to evaluate the potential barrier functions of the placenta. The higher coefficient suggested higher the relative amount of contaminants transported to fetuses through placentas (Li et al., 2013; Zhao et al., 2013). There was a non-linear correlation with Spearman's correlation coefficient of 0.37 ($p < 0.04$) between the PBDE concentrations in placentas and maternal serum (Fig. 1B). Positive linear relationships between umbilical cord and maternal sera for the total concentrations of PBDEs were found, with a linear slope of 0.46 and R^2 of 0.33 ($p = 0.01$) (Fig. 1B). Similar results were reported in previous study, in which positive correlations of total PBDE concentrations were observed between umbilical cord and maternal sera (Kim et al., 2012). These results revealed simultaneous increases of PBDE concentration in umbilical cord and maternal blood. For individual congeners, statistically significant

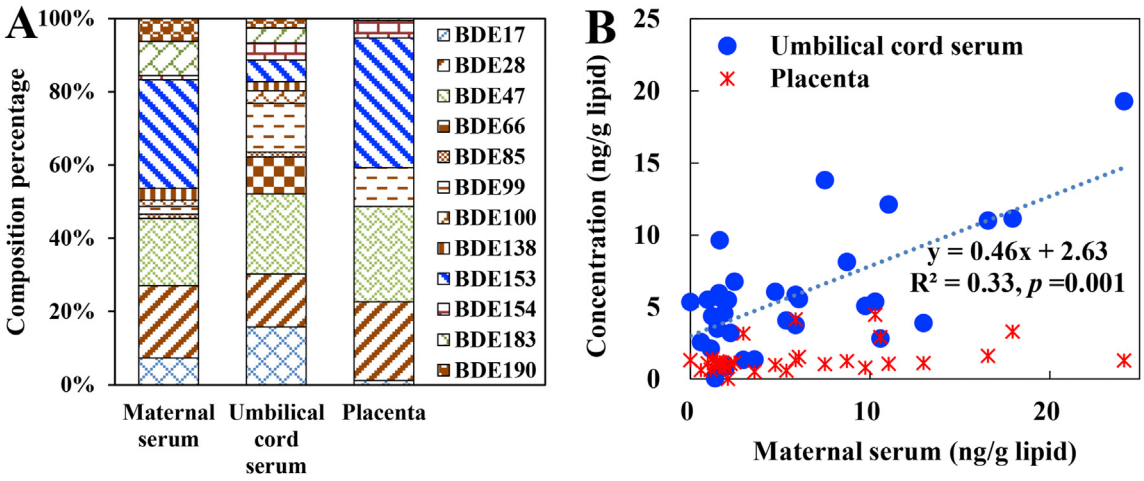

Fig. 1. Composition profiles (A) of PBDEs in maternal serum, umbilical cord serum, and placenta samples and the relationships (B) of PBDEs maternal sera vs. placenta or umbilical cord sera.

linear relationships were observed between umbilical cord and maternal sera for BDE28, 47, and 153 with *p*-values of 0.01, 0.03, and <0.001, respectively. The present results were not consistent with a study by Jakobsson et al., who found that there were no correlations among the individual PBDE congeners (Jakobsson et al., 2012). Moreover, the detection of PBDEs in umbilical cord serum suggests the net influx of PBDEs into placentas and failure of protecting fetuses.

Several studies have assessed paired maternal and umbilical cord sera in Europe, Asia, and North America. The transplacental transport of individual PBDE congeners in those reports are listed in Table S4. A large range of the concentration ratios between the fetus (umbilical cord serum) and maternal blood samples (F/M: 0.31–2.88) for different congeners were observed. A decrease in transplacental transport was observed with increasing degrees of bromination (Frederiksen et al., 2010a; Meijer et al., 2008), although our data did not show the same trend because of the low detection frequencies of most PBDE congeners and the limited data available for statistical analysis. In fact, there were no clear explanations for the variability in the literature. Thus, the data gaps and inconsistencies in the literature hamper the evaluation and comparison of findings from other studies, and additional studies based on human tissues or animal experiments are warranted.

3.2. Characterization of transplacental transport of PBDEs in SD rats

An experiment of five consecutive days following a single medium concentration dose of PBDEs at GD14 was conducted. The results showed that the F/M and P/M (concentration ratios between placenta and maternal serum samples) values varied from 0.01 to 0.59 and from 0.12 to 0.71 for different congeners, respectively (Table S5). In general, F/M and P/M ratios increased with increases in the sampling time (Fig. 2A). A steady-state was reached after approximately 24–72 h exposure with the ratios of F/M around 0.4–0.5 with the exception of BDE28 which reached the steady-state quickly because of lower molecular sizes and LogK_{OW} for the transepithelial transport. For the tetra- and penta-BDEs, a steady-state was reached at 24 h, while the steady-states for hexa- and hepta-brominated congeners were reached at 72 h.

We found in animal tests that the F/M ratios of PBDEs decreased with increases in the degree of bromination during sampling time leading up to when the steady-state was reached (Table S5). Similarly, some studies have found that low brominated congeners with lower molecular sizes and LogK_{OW} had higher F/M ratios in humans than those of highly brominated congeners with higher molecular sizes and LogK_{OW} (Frederiksen et al., 2010a; Meijer et al., 2008; Zhao et al., 2013). However, we found an interesting phenomenon that a congener-specific shift in PBDE compositions (from low-to high-brominated congeners) occurred between maternal and umbilical cord serum. Thus, there were no remarkable differences among the F/M ratios for all PBDE congeners when the steady-state was reached (72 and 120 h); however, as shown in Fig. 2B, differences were observed before the steady-state was reached (<24 h). The increase in the linear slopes from 6 to 24 h (−0.19, −0.13, and −0.16) to 72–120 h (0.04 and −0.03) clearly indicated this observation. Similar results were also observed for the P/M ratios (Fig. 2C). The results demonstrated that low brominated congeners with lower molecular sizes and LogK_{OW} more easily crossed the placenta than highly brominated congeners, which have higher molecular sizes and LogK_{OW}. Conversely, once the highly brominated congeners transported into the fetuses, they were difficult to be eliminated from fetuses to maternal blood, or by metabolization, due to protein binding on either side of the placenta and the immature metabolic capacities of the fetuses (Chen et al., 2014). The phenomenon also indicated that the placenta did not act as an effective barrier to PBDE transplacental transport, and in particular, the degree of congener bromination did not influence the composition distribution to the fetuses once a steady-state was reached.

The F/M values ranged from 0.07 to 0.79 and the P/M values varied from 0.30 to 0.72 for different PBDE congeners (Table S6), while similar F/M and P/M values were observed for the three levels of exposure for the same congener. With the increase in the number of bromines in PBDE congeners, the F/M and P/M values decreased. To better understand the results, the data were further analyzed. In addition, to avoid the influence of molecular weight on the results, the pmol amounts of PBDEs were considered. The results are shown in Table S7. With the exception of BDE28, significant linear correlations were observed between the PBDEs transported to fetal rats and placentas from maternal serum, with linear slopes of 0.04–0.21 and 0.06–0.60, and R^2 of 0.84–0.99 and 0.68–0.89, respectively. The average data for tri-to hepta-BDEs are shown in Fig. 3A and B, and had linear slopes of 0.15 and 0.38, with R^2 of 0.94 and 0.83 for the transport to fetal rats and placentas, respectively. It should be pointed out that a very large deviation seemed to be observed in Fig. 3A and B, which is due to the different concentrations of the congeners (Table S7).

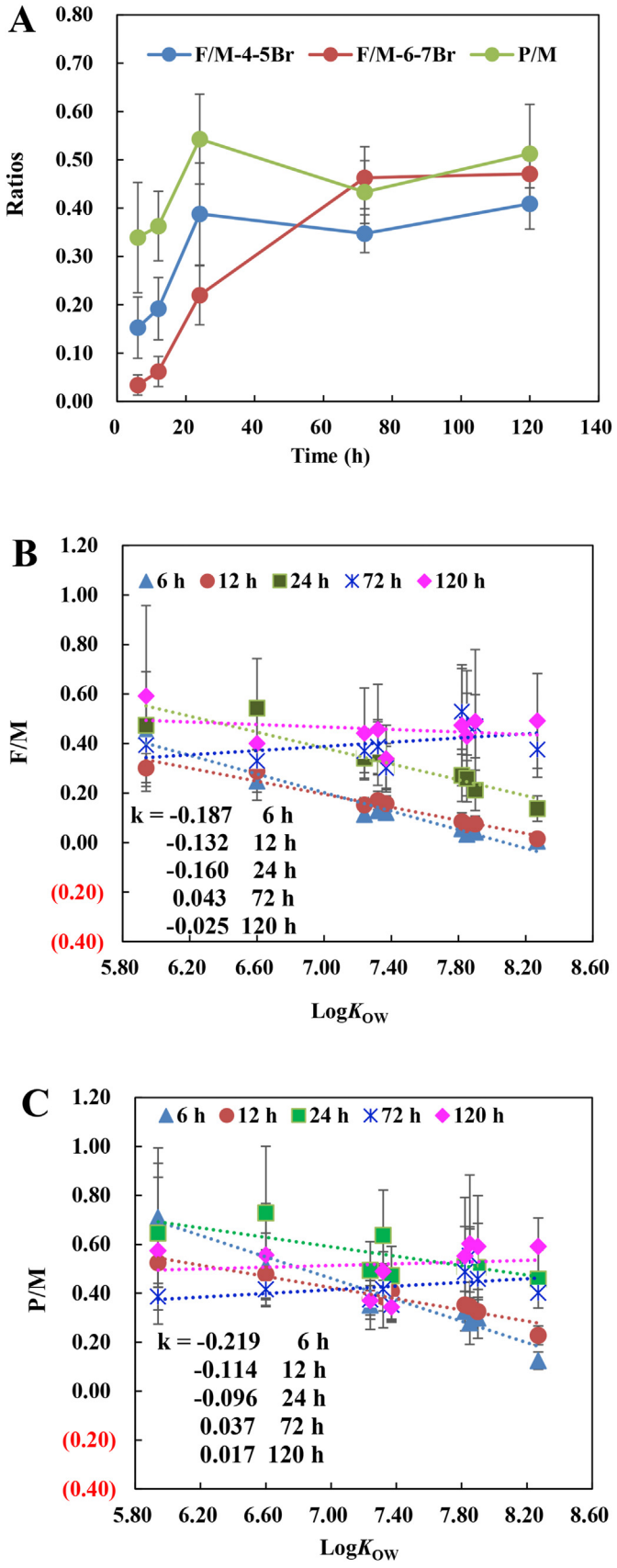

Fig. 2. Influences of sampling time and LogK_{OW} on PBDE transport in female SD rats. The data in A for P/M are the average ratios of 4–7 brominated congeners.

According to the present study, significant linear correlations were observed among the PBDE congeners transported to fetal rats or placentas from maternal blood with the exception of BDE28. The linear correlations indicated that a passive diffusion mechanism governed the transplacental transport of PBDEs. However, we do not believe that passive diffusion was the sole mechanism since the partition ratios between matrices of highly lipophilic organic chemicals should be close to 1 after adjustment by lipid contents if transport is driven solely by the lipid content in the tissues (Vizcaino et al., 2014a). As indicated by Vizcaino et al., active transplacental transport of lipophilic organic chemicals is potentially associated with their transport through membranes (Vizcaino et al., 2014a). For example, it was suggested that the human plasma protein, transthyretin, could bind PBDEs and facilitate placental transport (Chen et al., 2014). Our study suggested that passive diffusion mechanisms govern the transplacental transport of PBDEs, while other mechanisms are also likely to be involved.

To more clearly understand the transplacental transport of PBDEs, the influence of the duration of pregnancy (reflecting the effects of placental maturity) was further studied and the results are shown in Fig. 3C and Table S8. In the present study, 14 and 18 days of pregnancy were studied. The F/M ratios of PBDE exposures on pregnancy day 14 were from 0.14 to 0.54 for different congeners, and they varied between 0.11 and 0.42 for those on pregnancy day 18. However, for P/M, the ratios of PBDE exposure at pregnancy day 14 ranged from 0.46 to 0.73, while those on pregnancy day 18 ranged from 0.28 to 0.48. The F/M ratios of PBDE exposures on pregnancy day 14 were generally comparable with those on day 18 for the lower brominated congeners, while the ratios were higher for the higher brominated congeners. Similar transports were observed for the P/M ratios. These results suggested that the transplacental transport of the low brominated congeners are similar. In other words, the placental barrier to the low brominated congeners is limited, whether in mature placenta or immature placenta. However, for high brominated congeners, the transplacental transport through mature placenta is hindered compared with that of immature placenta, which effectively protects the fetus. Therefore, the present study demonstrated that the barrier capacity of mature placentas was higher than that of immature placentas, although neither were effective barriers to PBDE transplacental transport. In addition, as is well known, the pregnancy of SD rats lasts for approximately 21 days. Thus, 14 and 18 days of pregnancy in the SD rats are similar to that at the second and third trimester of pregnancy in humans, respectively. Therefore, this result also shows that early exposure to high or low brominated congeners is more likely to be transported to the fetus, and thus affects the fetus. This indicates that early exposure to pollutants is harmful to fetal health.

3.3. Transplacental transport of PBDEs using BeWo cell monolayer model

A BeWo cell monolayer model was used to further investigate the transplacental transport mechanism of PBDEs. The transport of PBDEs at different concentrations (molecular weight-based) are shown in Fig. 4A and detailed data are presented in Tables S9. The concentration of PBDEs detected in the basolateral side of the Transwell (transported) and in cells (accumulated) increased from 0.43 to 3.58 pmol and from 0.24 to 1.64 pmol within 12 h exposure to PBDEs ranging from 2.5 to 20 ng/mL, respectively. This revealed that both the transported and accumulated PBDEs were linearly correlated with the exposure concentrations. The linear relationships indicated that (1) they exhibited an unsaturated function because they did not reach a steady-state; (2) there was a concentration-dependent mechanism of PBDE transport and

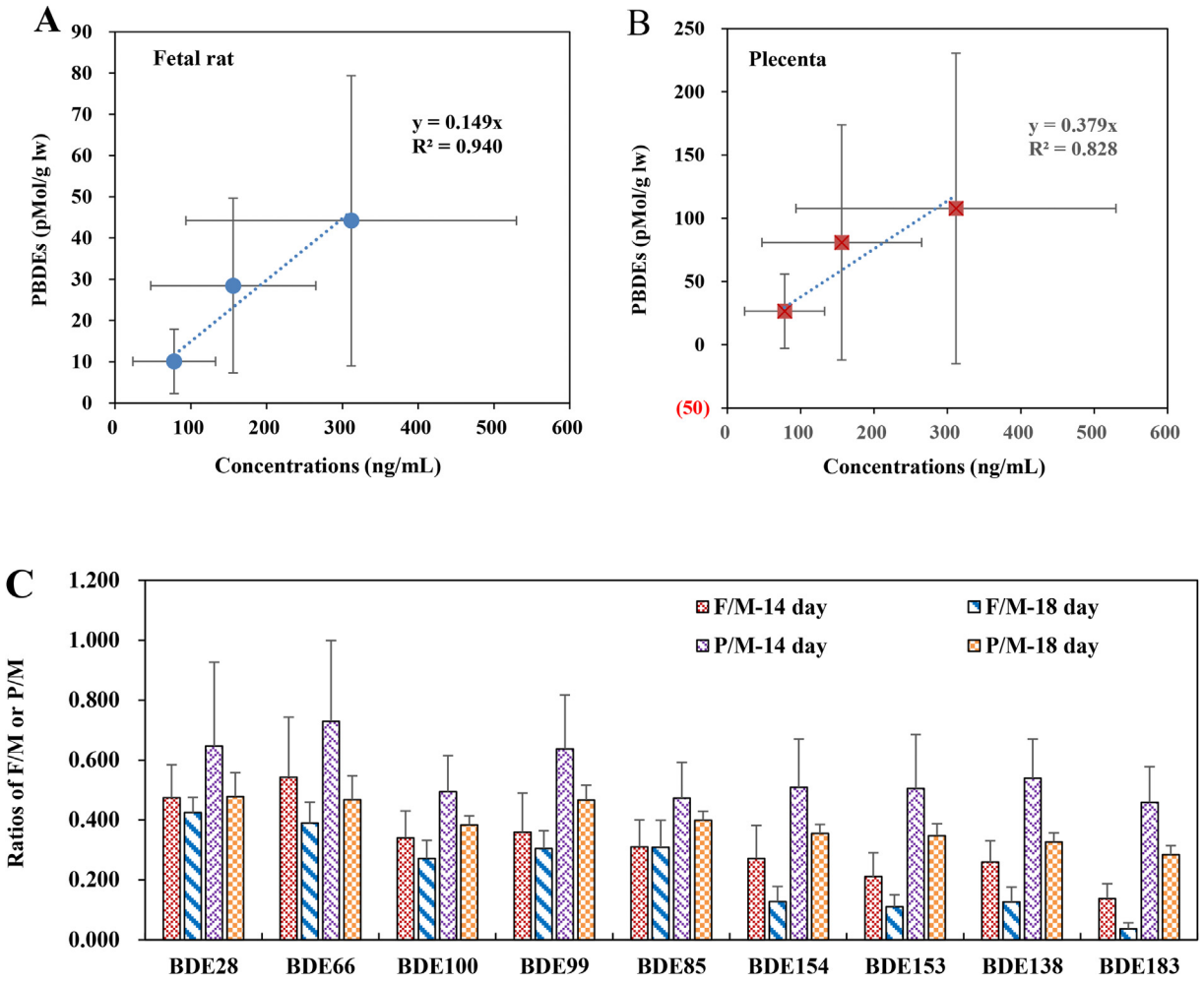

Fig. 3. Influences of concentrations and placental maturity on PBDE transport in female SD rats (A and B are the concentration influence; C is placental maturity influence). The error bars of x-axis are the deviations of BDE28, 66, 85, 99,100, 138, 153, 154, and 183 concentrations; the error bars of y-axis are the deviations of transported PBDEs of the congeners.

accumulation. Thus, these results indicated that the transportation of PBDEs occurred by passive diffusion. However, it may be that passive diffusion dominated and was supplemented by a small fraction of active transport, leading to the transporter effect (Kimura et al., 2014). Thus, confirmation of whether transporters were involved in the transport of PBDEs was necessary.

Regarding the influence of time, the transported and accumulated PBDEs increased with increases in the sampling time, although large variations were observed among different congeners (Table S10). The average ratios of transported PBDEs in BeWo cells increased from 2.5% to 11.3% when the sampling time increased from 1 to 24 h. However, the average ratios of accumulated PBDEs in cells increased from 7.1% to 25.6% during 1–12 h exposures, whereas no increases were observed from 12–24 h exposure. Further analyses revealed that there was a linear increase in transported PBDEs over the whole sampling time ($R^2 = 0.98$) (Fig. 4B), while there was an approximately linear tendency for PBDEs to accumulate intracellularly within the first 12 h, which then remained steady at 12–24 h. This phenomenon implied that transporters in BeWo cells were involved in the transplacental transport of PBDEs.

To obtain further evidence, the application of the apparent permeability coefficient (P_{app}) and efflux ratio (ER) were used to further explain the transplacental transport mechanism. The bidirectional transport of PBDEs from AP to BL and BL to AP were assessed at a PBDE concentration of 10 ng/mL for 12 h. As shown in Table S11, the $P_{app(AP-BL)}$ had an average of 20.3×10^{-7} cm/s, which was significantly higher than the $P_{app(BL-AP)}$ with a mean of 10.0×10^{-7} cm/s. These data revealed that AP-BL transport was the main process. We also found that the ERs from 1.73 to 2.32 were higher than a unit. The ER value is an indicator of whether a transporter participates in transport. When the ER value is greater than 1.5, it is generally considered that transport is directional and that transporters might assist in the PBDE transport process. In the present study, all the ER values of the PBDE congeners were above 1.5, indicating that protein efflux transporters may be involved in transportation.

Moreover, we also found that low brominated congeners tended to have higher permeation values than highly brominated congeners, which was similar with the phenomenon in animal experiments. However, a parabolic curve was observed between the accumulated intracellular PBDEs and LogK_{OW}, where the most highly accumulated PBDEs were those with a LogK_{OW} of approximately 7.4 (Fig. 4C). Moreover, this finding was consistent with the finding that PBDE congeners with LogK_{OW} values of approximately 7 had the greatest biomagnification potential in the food chain (Yu et al., 2012b). One explanation for this phenomenon is that low brominated congeners readily cross the phospholipid bilayer into

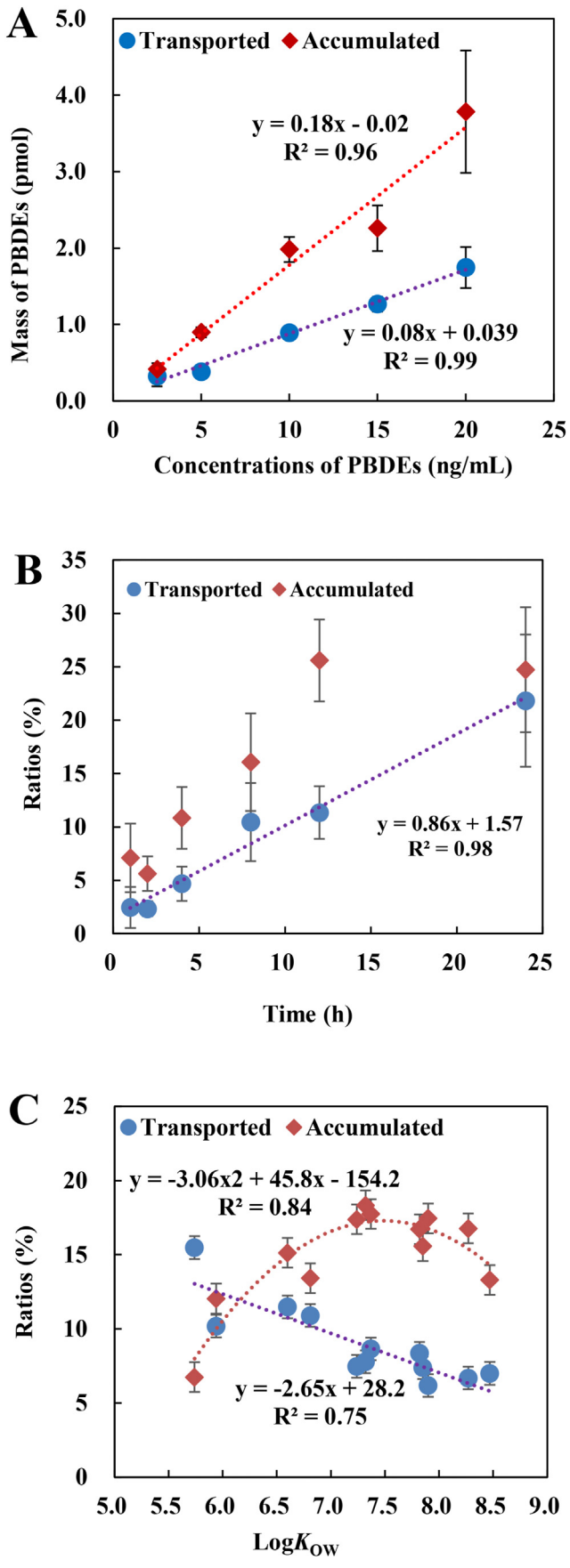

Fig. 4. Transported and accumulated PBDEs in BeWo cells. Influence factors included PBDE concentrations (A), sampling time (B), and LogK_{OW} (C).

cells and flow out of the cells into the basolateral chamber by passive diffusion. However, highly brominated congeners have difficulties crossing or penetrating the cell membrane, which may require the assistance of carrier proteins. Thus, there was a tendency for the accumulation to increase and then subsequently decrease, which was with the highest tendency for the congeners with a LogK_{OW} of approximately 7.4. Thus, these results indicate the transport dynamic equilibrium of various brominated congeners in cells or organisms.

3.4. Transporters involved in the transplacental transport of PBDEs

To further explore whether efflux transporters are involved in the transport of PBDEs, the role of three efflux pumps (P-gp, MRPs, and BCRP) were assessed. The transported and accumulated ratios of different PBDE congeners ranged from 2.2% to 17.6% and from 23.6% to 45.1% before and after the addition of the P-gp inhibitor, verapamil (100 μM), respectively (Fig. 5A and B). Compared with the control group, significant decreases in the transported PBDEs and increases in intracellular accumulations were observed. However, no significant changes were observed for either the transported or accumulated ratios of PBDEs when 20 μM of MK571 (an inhibitor of MRPs) or 20 μM of KO143 (an inhibitor of BCRP) were added. Thus, the results suggest that the P-gp efflux pump participate in the efflux of PBDEs from placenta, while MRPs and BCRP might participate in the transport but with very lower efficiency. The accumulation of PBDE congeners were found in BeWo cells in control group, and the phenomenon was even found at lower exposure dose, which indicated that the function of P-gp was partially inhibited by PBDEs.

P-gp, MRPs, and BCRP are expressed in the trophoblast of the human placenta and have been widely used to study the transplacental transport processes of xenobiotics (Vähäkangas *et al.*, 2009). P-gp is distributed in the villi of the brush-like border facing the mother, and is an ATP-dependent pump that extrudes exogenous substances from the trophoblast tissue in the fetal-to-maternal direction; thus, decreasing the fetal absorption of such substances (Vähäkangas et al., 2009). Verapamil is a specific inhibitor of P-gp and is also a P-gp substrate that might interact with the pump to attenuate efflux functions. As a result, the intracellular accumulation of PBDEs significantly increased by the addition of the inhibitor. This was potentially due to verapamil limiting the ability of P-gp to efflux PBDEs, indicating that such transport in the BeWo monolayer required the active transport mechanism of P-gp. However, BCRP and MRPs were involved in the transplacental transport of some chemicals (such as positively and negatively charged polystyrene nanoparticles, and 4-nitrophenol and acetaminophen) which have different structural and physicochemical properties, as reported previously (Kloet et al., 2015; Mitra et al., 2010; Seelig et al., 1998). Similar results were not observed in the present study, indicating that BCRP and MRPs were not involved in the transport of PBDEs, or they might participate in the transport but with very lower efficiency, or their expression in BeWo cells was too low to cause significant changes.

3.5. Molecular docking of P-gp with PBDEs

To further explore the inhibition of PBDE congeners on P-gp pump, the interactions between PBDEs and P-gp were evaluated. No such information was available in the published literature, and therefore, molecular docking studies between PBDE congeners and human P-gp were performed. The results are shown in Fig. 6. Three pocket sites that PBDEs bind to were identified within P-gp. The first pocket site was surrounded by Phe 163 (π-π stacked

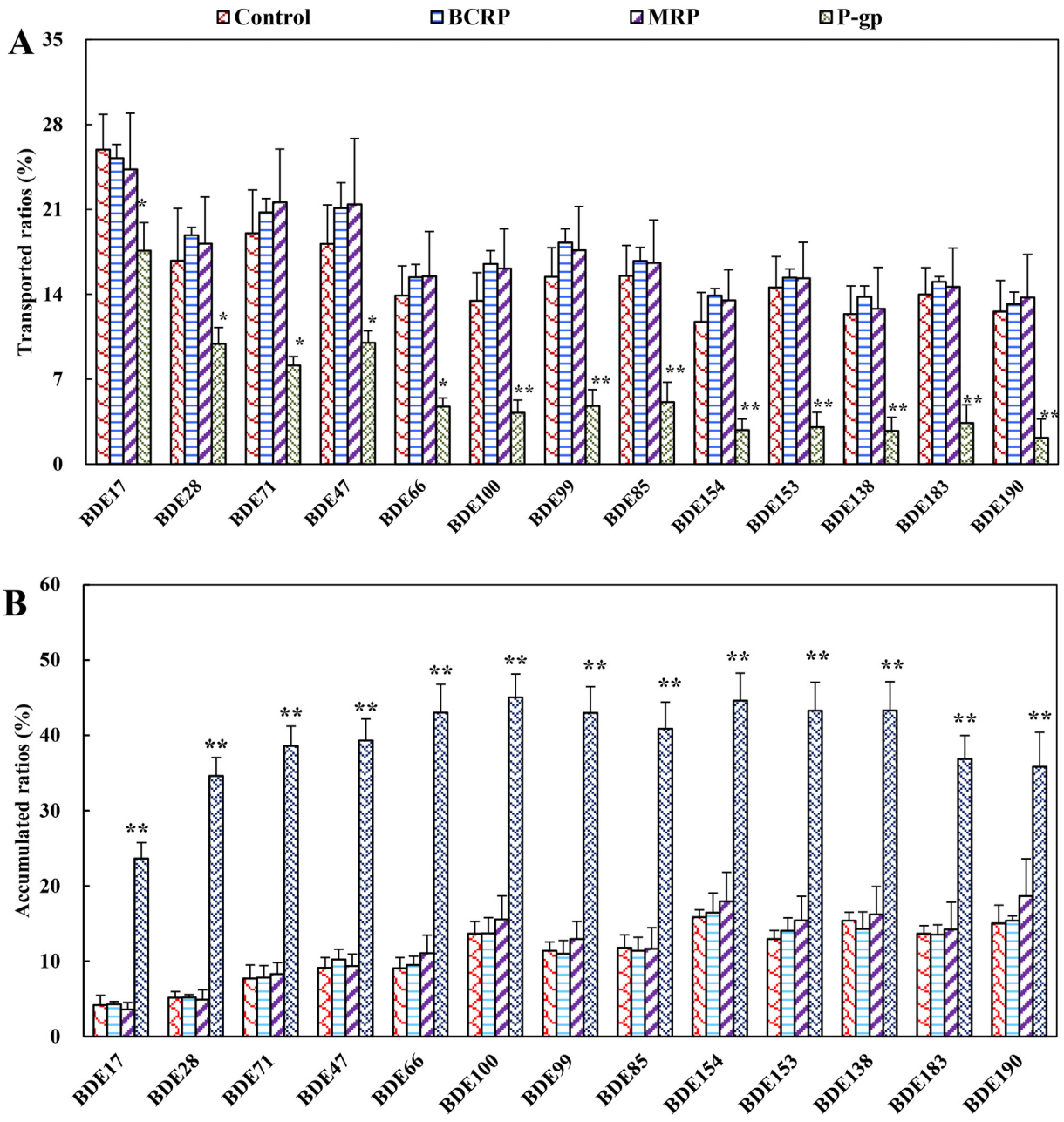

Fig. 5. Transport and accumulation ratios of PBDEs with the addition of inhibitors. *$p < 0.05$; **$p < 0.01$.

interaction), Asp 164, Val 168, Glu 902, Phe 904, Arg 905, Val 1168 (π-alkyl interaction), Asp 1171, Lys 1172, and Thr1174 as shown in Fig. 6A and Fig. S1A, with the strongest binding to BDE17, BDE28, BDE47, BDE66, and BDE71. The second pocket site was surrounded by Gln 678, Lys 681, Thr684, Val 695, Arg 699, Ile 700, Ala 1006, Ile 1009, Met 1010 (hydrophobic interaction), and Lys 1014 (Fig. 6B and Fig. S1B), with the strongest binding to BDE85, BDE100, BDE138, BDE153, and BDE154. The third pocket site was surrounded by Ser 180, Lys181, Glu 184, Val 185, Glu 353, Ala 354, Asn 357, Ala 823, Gln 824, Lys 826, and Gly 827 (Fig. 6C and Fig. S1C), and BDE99, BDE183, and BDE190 were caved in this pocket. In addition, the complex of P-gp with BDE99 and BDE190 contained one hydrogen bond, which formed a bridging oxygen of the two congeners with a side chain of Lys181. Conversely, we did not observe hydrogen bonds or any strong interactions in the induced fit docking of other PBDE congeners.

The computational docking results suggested that the binding sites of PBDE congeners within P-gp depended, in part, on stereoisomerism. Low brominated congeners such as BDE17 and BDE28 had the lowest space-resistance, and thus, could access the innermost binding pockets. However, for highly brominated congeners, including BDE183 and BDE190, hindrance from the bromine atoms caused them to enter the surface pockets. Not only the number of bromines but also the position of the bromine substitution in PBDEs could influence the binding affinity. For example, BDE99 and BDE100 belong to pentabromodiphenyl ethers, but the strongest binding sites of the two congers are different. Although the active sites for P-gp have not been fully revealed in the literature, the four main functional domains were identified, including those at residues 49–350, 386–622, 693–980, and 1015–1253 (Aller et al.,

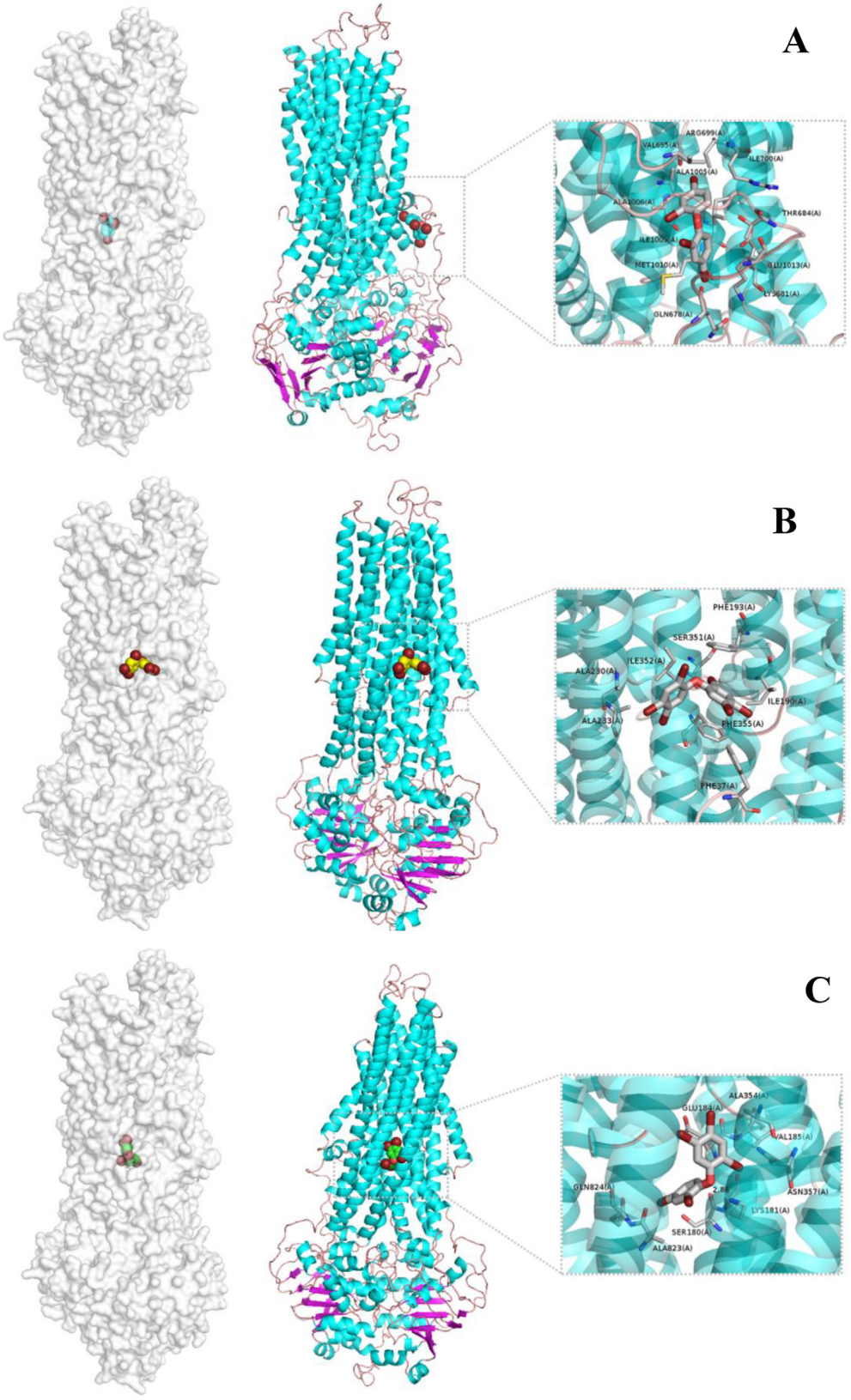

Fig. 6. Binding modes of BDE47, BDE153, and BDE99 with P-gp. The binding poses and interactions of BDE congers with P-gp were performed using Autodock Vina v0.10.1.2. (A: interaction between BDE47 with P-gp; B: interaction between BDE153 with P-gp; C: interaction between BDE99 with P-gp. Glu, glutamic acid; Lys, lysine; Asn, asparagine; Ser, serine; Ala, alanine; Val, valine; Phe, phenylalanine; Arg, arginine; Val, valine; Thr, threonine; Ile, isoleucine; Gln, glutarnine, and Met, methionine).

2009; Chufan et al., 2013). In addition, P-gp had multiple transport-active binding sites, for which the activities differed (Chufan et al., 2013). In the present study, although various binding sites for PBDE congeners within P-gp were found, these sites were located at the main functional domains of P-gp and could transfer PBDE congeners across the cell membrane. Therefore, it was not unexpected that despite the binding sites of PBDE congeners being different in P-gp, the corresponding docking scores were similar as shown in Table S12. Additionally, the interactions and docking scores among congeners generally agreed with the results of P-gp efflux assays in BeWo cells.

4. Conclusions

The underlying transplacental transport and barrier mechanisms were investigated in the present study on the basis of the a comprehensive study via PBDEs in paired human placenta, maternal and umbilical cord blood samples, pregnant SD rats, BeWo cells, and molecular docking. The results showed that PBDEs could transfer from mother to fetus. Similar results were observed from SD rats. The barrier capacity of mature placenta is higher than immature placenta, although both of them does not act as effective barrier to transplacental transport of PBDEs. Moreover, there were varied time to reach a steady-state for different brominated congeners. Low brominated congeners can more easily pass across the placenta than high brominated congeners; while once the high brominated congeners transported into fetuses, they are difficult eliminated from fetuses to maternal blood or by metabolization. Passive diffusion mechanism governs the transplacental transport of PBDEs, although the efflux transporter of P-gp participates the transport. Three different pocket sites in the efflux transporter for the transplacental transport of PBDEs were found and the binding sites of PBDE congeners with P-gp partial depends on the stereoisomerism according to the theoretical molecular docking study. The results firstly provides the underlying mechanism of PBDE transplacental transport and can offer more insights into transplacental transports of more persistent organic pollutant.

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.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (21677094, 41991310, and 41977303), and Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2017BT01Z032).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.116091.

References

Albert, O., Huang, J.Y., Aleksa, K., Hales, B.F., Goodyer, C.G., Robaire, B., Chevrier, J., Chan, P., 2018. Exposure to polybrominated diphenyl ethers and phthalates in healthy men living in the greater Montreal area: a study of hormonal balance and semen quality. Environ. Int. 116, 165–175.

Aller, S.G., Yu, J., Ward, A., Weng, Y., Chittaboina, S., Zhuo, R.P., Harrell, P.M., Trinh, Y.T., Zhang, Q.H., Urbatsch, I.L., Chang, G., 2009. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. Science 323 (5922), 1718–1722.

An, T.C., Zhang, D.L., Li, G.Y., Mai, B.X., Fu, J.M., 2011. On-site and off-site atmospheric PBDEs in an electronic dismantling workshop in south China: gas-particle partitioning and human exposure assessment. Environ. Pollut. 159 (12), 3529–3535.

Aylward, L.L., Hays, S.M., Kirman, C.R., Marchitti, S.A., Kenneke, J.F., English, C., Mattison, D.R., Becker, R.A., 2014. Relationships of chemical concentrations in maternal and cord blood: a review of available data. J. Toxicol. Environ. Health 17 (3), 175–203. Part B.

Baker, H., Frank, O., Deangelis, B., Feingold, S., Kaminetzky, H.A., 1981. Role of placenta in maternal-fetal vitamin transfer in humans. Am. J. Obstet. Gynecol. 141 (7), 792–796.

Boon, J.P., Lewis, W.E., Tjoen-A-Choy, M.R., Allchin, C.R., Law, R.J., de Boer, J., ten Hallers-Tjabbes, C.C., Zegers, B.N., 2002. Levels of polybrominated diphenyl ether (PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. Environ. Sci. Technol. 36 (19), 4025–4032.

Chen, Z.J., Liu, H.Y., Cheng, Z.C., Man, Y.B., Zhang, K.S., Wei, W., Du, J., Wong, H., Wang, H.S., 2014. Polybrominated diphenyl ethers (PBDEs) in human samples of mother–newborn pairs in South China and their transplacental transport characteristics. Environ. Int. 73, 77–84.

Chufan, E.E., Kapoor, K., Sim, H.M., Singh, S., Talele, T.T., Durell, S.R., Ambudkar, S.V., 2013. Multiple transport-active binding sites are available for a single substrate on human P-glycoprotein (ABCB1). PloS One 8 (12), e82463.

Eriksson, P., Fischer, C., Fredriksson, A., 2006. Polybrominated diphenyl ethers, a group of brominated flame retardants, can interact with polychlorinated biphenyls in enhancing developmental neurobehavioral defects. Toxicol. Sci. 94 (2), 302–309.

Frederiksen, M., Thomsen, C., Frøhaug, C., Vorkamp, K., Thomsen, M., Becher, G., Knudsen, L.E., 2010a. Polybrominated diphenyl ethers in paired samples of maternal and umbilical cord blood plasma and associations with house dust in a Danish cohort. Int. J. Hyg Environ. Health 213 (4), 233–242.

Frederiksen, M., Vorkamp, K., Mathiesen, L., Mose, T., Knudsen, L.E., 2010b. Placental transfer of the polybrominated diphenyl ethers BDE-47, BDE-99 and BDE-209 in a human placenta perfusion system: an experimental study. Environmental Health 9, 32.

Gascon, M., Fort, M., Martinez, D., Carsin, A.E., Forns, J., Grimalt, J.O., Marina, L.S., Lertxundi, N., Sunyer, J., Vrijheid, M., 2012. Polybrominated diphenyl ethers (PBDEs) in breast milk and neuropsychological development in infants. Environ. Health Perspect. 120 (12), 1760–1765.

Han, W.L., Fan, T., Xu, B.H., Feng, J.L., Zhang, G., Wu, M.H., Yu, Y.X., Fu, J.M., 2016. Passive sampling of polybrominated diphenyl ethers in indoor and outdoor air in Shanghai, China: seasonal variations, sources, and inhalation exposure. Environ. Sci. Pollut. Control Ser. 23 (6), 5771–5781.

Hovander, L., Athanasiadou, M., Asplund, L., Jensen, S., Wehler, E.K., 2000. Extraction and cleanup methods for analysis of phenolic and neutral organohalogens in plasma. J. Anal. Toxicol. 24 (8), 696–703.

Jakobsson, K., Fång, J., Athanasiadou, M., Rignell-Hydbom, A., Bergman, A., 2012. Polybrominated diphenyl ethers in maternal serum, umbilical cord serum, colostrum and mature breast milk. Insights from a pilot study and the literature. Environ. Int. 47, 121–130.

Jeong, Y., Lee, S., Kim, S., Park, J., Kim, H.J., Choi, G., Choi, S., Kim, S., Kim, S.Y., Kim, S., Choi, K., Moon, H.B., 2018. Placental transfer of persistent organic pollutants and feasibility using the placenta as a non-invasive biomonitoring matrix. Sci. Total Environ. 612, 1498–1505.

Jiang, Y.F., Yuan, L.M., Lin, Q.H., Ma, S.T., Yu, Y.X., 2019. Polybrominated diphenyl ethers in the environment and human external and internal exposure in China: a review. Sci. Total Environ. 696, 133902.

Kim, T.H., Bang, D.Y., Lim, H.J., Won, A.J., Ahn, M.Y., Patra, N., Chung, K.K., Kwack, S.J., Park, K.L., Han, S.Y., Choi, W.S., Han, J.Y., Lee, B.M., Oh, J.E., Yoon, J.H., Lee, J., Kim, H.S., 2012. Comparisons of polybrominated diphenyl ethers levels in paired South Korean cord blood, maternal blood, and breast milk samples. Chemosphere 87 (1), 97–104.

Kimura, O., Ohta, C., Koga, N., Haraguchi, K., Kato, Y., Endo, T., 2014. Carrier mediated uptake of nobiletin, a citrus polymethoxyflavonoid, in human intestinal Caco-2 cells. Food Chem. 154, 145–150.

Kloet, S.K., Walczak, A.P., Louisse, J., van den Berg, H.H.J., Bouwmeester, H., Tromp, P., Fokkink, R.G., Rietjens, I.M.C.M., 2015. Translocation of positively and negatively charged polystyrene nanoparticles in an in vitro placental model. Toxicol. Vitro 29 (7), 1701–1710.

Li, L.X., Chen, L., Meng, X.Z., Chen, B.H., Chen, S.Q., Zhao, Y., Zhao, L.F., Liang, Y., Zhang, Y.H., 2013. Exposure levels of environmental endocrine disruptors in mother-newborn pairs in China and their transplacental transport characteristics. PloS One 8 (5), e62526.

Mazdai, A., Dodder, N.G., Abernathy, M.P., Hites, R.A., Bigsby, R.M., 2003. Polybrominated diphenyl ethers in maternal and fetal blood samples. Environ. Health Perspect. 111 (9), 1249–1252.

Meijer, L., Weiss, J., van Velzen, M., Brouwer, A., Bergman, A., Sauer, P.J.J., 2008. Serum concentrations of neutral and phenolic organohalogens in pregnant women and some of their infants in The Netherlands. Environ. Sci. Technol. 42 (9), 3428–3433.

Mitra, P., Audusa, K.L., 2010. MRP isoforms and BCRP mediate sulfate conjugate efflux out of BeWo cells. Int. J. Pharm. 384 (1/2), 15–23.

Morello-Frosch, R., Cushing, L.J., Jesdale, B.M., Schwartz, J.M., Guo, W.H., Guo, T., Wang, M.M., Harwani, S., Petropoulou, S.S.E., Duong, W., Park, J.S., Petreas, M., Gajek, R., Alvaran, J., She, J.W., Dobraca, D., Das, R., Woodruff, T.J., 2016. Environmental chemicals in an urban population of pregnant women and their newborns from San Francisco. Environ. Sci. Technol. 50 (22), 12464–12472.

Pacyniak, E., Hagenbuch, B., Klaassen, C.D., Lehman-McKeeman, L., Guo, G.L., 2011. Organic anion transporting polypeptides in the hepatic uptake of PBDE congeners in mice. Toxicol. Appl. Pharmacol. 257 (1), 23–31.

Pacyniak, E., Roth, M., Hagenbuch, B., Guo, G.L., 2010. Mechanism of polybrominated diphenyl ether uptake into the liver: PBDE congeners are substrates of human hepatic OATP transporters. Toxicol. Sci. 115 (2), 344–353.

Schecter, A., Papke, O., Harris, T.R., Tung, K.C., Musumba, A., Olson, J., Birnbaum, L., 2006. Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of US food and estimated PBDE dietary intake by age and sex. Environ. Health Perspect. 114 (10), 1515–1520.

Seelig, A., 1998. A general pattern for substrate recognition by P-glycoprotein. Eur. J. Biochem. 251 (1/2), 252–261.

Tang, Z.W., Huang, Q.F., Cheng, J.L., Yang, Y.F., Yang, J., Guo, W., Nie, Z.Q., Zeng, N., Jin, L., 2014. Polybrominated diphenyl ethers in soils, sediments, and human hair in a plastic waste recycling area: a neglected heavily polluted area. Environ. Sci. Technol. 48, 1508–1516.

Vähäkangas, K., Myllynen, P., 2009. Drug transporters in the human blood-placental barrier. Br. J. Pharmacol. 158, 665–678.

Vizcaino, E., Grimalt, J.O., Fernández-Somoano, A., Tardon, A., 2014a. Transport of persistent organic pollutants across the human placenta. Environ. Int. 65, 107–115.

Vizcaino, E., Grimalt, J.O., Glomastad, B., Fernández-Somoano, A., Tardón, A., 2014b. Gestational weight gain and exposure of newborns to persistent organic pollutants. Environ. Health Perspect. 122 (8), 873–879.

Vizcaino, E., Grimalt, J.O., Lopez-Espinosa, M.J., Llop, S., Rebagliato, M., Ballester, F., 2011. Polybromodiphenyl ethers in mothers and their newborns from a non-occupationally exposed population (Valencia, Spain). Environ. Int. 37 (1), 152–157.

Wu, K.S., Xu, X.J., Liu, J.X., Guo, Y.Y., Li, Y., Huo, X., 2010. Polyhrominated diphenyl ethers in umbilical cord blood and relevant factors in neonates from Guiyu, China. Environ. Sci. Technol. 44 (2), 813–819.

Xiong, J.K., Li, G.Y., An, T.C., Zhang, C.S., Wei, C.H., 2016. Emission patterns and risk assessment of polybrominated diphenyl ethers and bromophenols in water and sediments from the Beijiang River, South China. Environ. Pollut. 219, 596–603.

Yu, Y.X., Lou, S.F., Wang, X.X., Lu, S.Y., Ma, S.T., Li, G.Y., Feng, Y., Zhang, X.Y., An, T.C., 2019. Relationships between the bioavailability of polybrominated diphenyl ethers in soils measured with female C57BL/6 mice and the bioaccessibility determined using five in vitro methods. Environ. Int. 123, 337–344.

Yu, Y.X., Pang, Y.P., Li, C., Li, J.L., Zhang, X.Y., Yu, Z.Q., Feng, J.L., Wu, M.H., Sheng, G.Y., Fu, J.M., 2012a. Concentrations and seasonal variations of polybrominated diphenyl ethers (PBDEs) in in- and out-house dust and human daily intake via dust ingestion corrected with bioaccessibility of PBDEs. Environ. Int. 42, 124–131.

Yu, Y.X., Wang, M.M., Zhang, K.Q., Yang, D., Zhong, Y.F., An, J., Lei, B.L., Zhang, X.Y., 2017. The transepithelial transport mechanism of polybrominated diphenyl ethers in human intestine determined using a Caco-2 cell monolayer. Environ. Res. 154, 93–100.

Yu, Y.X., Zhang, S.H., Huang, N.B., Li, J.L., Pang, Y.P., Zhang, X.Y., Yu, Z.Q., Xu, Z.G., 2012b. Polybrominated diphenyl ethers and polychlorinated biphenyls in freshwater fish from Taihu Lake, China: their levels and the factors that influence biomagnification. Environ. Toxicol. Chem. 31 (3), 542–549.

Zhang, X.L., Li, X.J., Jing, Y., Fang, X.M., Zhang, X.Y., Lei, B.L., Yu, Y.X., 2017. Transplacental transfer of polycyclic aromatic hydrocarbons in paired samples of maternal serum, umbilical cord serum, and placenta in Shanghai, China. Environ. Pollut. 222, 267–275.

Zhang, X.L., Wu, X., Lei, B.L., Jing, Ye, Jiang, Z.A., Zhang, X.Y., Fang, X.M., Yu, Y.X., 2018a. Transplacental transfer characteristics of organochlorine pesticides in paired maternal and cord sera, and placentas and possible influencing factors. Environ. Pollut. 233, 446–454.

Zhang, X.L., Zhang, K.Q., Yang, D., Ma, L., Lei, B.L., Zhang, X.Y., Zhou, J., Fang, X.M., Yu, Y.X., 2014. Polybrominated biphenyl ethers in breast milk and infant formula from Shanghai, China: temporal trends, daily intake, and risk assessment. Sci. Total Environ. 497/498, 508–515.

Zhang, Y.N., Mao, P., Li, G.Y., Hu, J.J., Yu, Y.X., An, T.C., 2018b. Delineation of 3D dose-time-toxicity in human pulmonary epithelial Beas-2B cells induced by decabromodiphenyl ether (BDE209). Environ. Pollut. 243, 661–669.

Zhao, Y.X., Ruan, X.L., Li, Y.Y., Yan, M.C., Qin, Z.F., 2013. Polybrominated diphenyl ethers (PBDEs) in aborted human fetuses and placental transfer during the first trimester of pregnancy. Environ. Sci. Technol. 47 (11), 5939–5946.

Zheng, M.Y., Li, X.H., Zhang, Y., Yang, Y.L., Wang, W.Y., Tian, Y., 2017. Partitioning of polybrominated biphenyl ethers from mother to fetus and potential health-related implications. Chemosphere 170, 207–215.

Zota, A.R., Mitro, S.D., Robinson, J.F., Hamilton, E.G., Park, J.S., Parry, E., Zoeller, T.R., Woodruff, T.J., 2018. Polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDE metabolites (OH-PBDEs) in maternal and fetal tissues, and associations with fetal cytochrome P450 gene expression. Environ. Int. 112, 269–278.