Intergenerational transfer of Dechlorane Plus and the associated long-term effects on the structure and function of gut microbiota in offspring

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Abstract

The gut microbiota has been shown to be highly involved in many vital physiological processes that play key roles in human health. The intergenerational transfer of Dechlorane Plus (DP) and the complex interaction between DP and microbiota has been poorly studied. Additionally, the structural and functional effects of DP on the gut microbiota have not been studied. This study aimed to investigate the DP transfer in Sprague-Dawley rats during pregnancy and the effects of DP exposure on gut microbiota, as detected by 16S rRNA gene sequencing. The results showed that excretion in feces is a very important elimination pathway of orally dosed DP. The main intergenerational transfer pathway of DP might be via lactation rather than transplacental transport. The 16S rRNA sequencing revealed that DP exposure could decrease the richness and diversity of gut microbiota, especially at the genus level. Furthermore, in DP exposure groups, the gut microbiota production of metabolites of short-chain fatty acids was dramatically increased. The results demonstrated that DP exposure not only altered the gut microbiota structures, but also immensely influenced metabolic functions, causing long-term impact to offspring. This data indicates that more attention should be paid to the long-term health effects related to DP exposure.

1. Introduction

In recent years, studies have found that the gut microbiota is closely related to many physiological functions, including immunity, nutrition, metabolism, and biochemistry. The gut microbiota has been shown to be highly involved in many vital physiological processes that play key roles in human health (Khan et al., 2012). As an important organ of human metabolism, gut microbiota has various functions, such as resisting foreign pathogenic microorganisms. Recent studies have shown that environmental pollutants can affect the composition of the gut microbiota in animal and human (Jin et al., 2017; Wang et al., 2019; Zhang et al., 2015a). For example, animal studies showed that heavy metal exposure led to a decrease in microbial diversity, but there is a lack of studies on the long-term effects of DP exposure on gut microbiota. Therefore, this study aimed to investigate the DP transfer in Sprague-Dawley rats during pregnancy and the effects of DP exposure on gut microbiota, as detected by 16S rRNA gene sequencing.

Abbreviations: DP, Dechlorane Plus; KEGG, Kyoto Encyclopedia of Genes and Genomes; LD, lactation day; LOQs, limits of quantification; PCBs, polychlorinated biphenyls; ED, embryonic day; PICRUSt, phylogenetic investigation of communities by reconstructing unobserved states; POPs, persistent organic pollutants; SCFAs, short-chain fatty acids; SD, Sprague-Dawley; SPE, solid phase extraction; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran; TNF-α, tumor necrosis factor α.

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There are reports that found that some persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), 2, 3, 7, 8-tetrachlorodibenzofuran (TCDF), among others, could alter the gut microbiota in mice (Choi et al., 2013; Jin et al., 2017; Zhang et al., 2015a). This can directly or indirectly further affect the energy metabolism, nervous system, and endocrine system of the host (Buﬀe et al., 2015; Zhang et al., 2015b). The “latent early life-related regulation” model holds that exogenous environmental factors may begin at the initial stage of human development, interfere with gene regulation through long-term patterns, and gradually manifest themselves in the later stages of life, thus, causing long-term effects (Vizcaino et al., 2014). It is helpful to explore the long-term effects of prenatal exposure to contaminants on offspring.

Dechlorane Plus (DP) is a type of highly chlorinated flame retardant having the properties of POPs (Zhang et al., 2011). There are syn-DP and anti-DP isomers for technical products. The chemicals are ubiquitously found in the environment because of the large usage as a flame retardant in polymer materials including computer monitors, furniture, among others, for nearly 50 years, and can accumulate in organisms, including in humans (Sverko et al., 2011; Zhang et al., 2013). Although there were reports of DP in breast milk, placentas, and cord sera in human samples (Ben et al., 2013, 2014; Siddique et al., 2012), and many organic contaminants can be placentaally transferred (Chen et al., 2017; Gao et al., 2019; Morello-Frosch et al., 2016; Zhang et al., 2017, 2018). Currently, the investigation on the placental transfer of DP from mother to fetus is limited, with only one report available (Ben et al., 2014). For animals, although DP has been found in bird and frog eggs (Muñoz-Arnanz et al., 2012; Wu et al., 2018), information on the placental transfer of DP from mother to offspring in animal is not available.

Therefore, our hypothesis is that DP can be transferred from mother to fetal and the DP exposure can influence on the gut microbiota of the offspring. To verify the hypothesis and more thoroughly understand the intergenerational transfer of DP and the associated influence on the gut microbiota of the offspring, the present study assessed the intergenerational transfer of DP during pregnancy of rats by the measurement of DP in mother rats and their offspring. The influence of DP on the structure and function of gut microbiota in the offspring from birth to adulthood was also studied. The present study reveals the intergenerational transfer of DP and allows a better understanding of the effect of DP exposure on the structure and metabolic function of the gut microbiota combined with metabolomics analysis.

2. Material and methods

2.1. Standards and chemicals

The anti-DP and syn-DP standards were obtained from Wellington Laboratories (purity > 98%; Guelph, ON, Canada). 13C-PCB141 and 13C-PCB209, used as surrogate standard and internal standard, respectively, were acquired from AccuStandard (New Haven, Connecticut, USA). SCFAs, including 2-ethylbutyric acid, hexanoic acid, valeric acid, isovaleric acid, butyric acid, isobutyric acid, propionic acid, and acetic acid were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All solvents, including dichloromethane, hexane, acetone, and ethyl acetate, were of HPLC-grade purity (> 99%; CNW Technologies GmbH, Duesseldorf, Germany). Corn oil was purchased from Gold Arowana in Hebei Province, China. Commercial DP25 (purity > 99%) was obtained from Jiangsu Anpon Electrochemical Co., Ltd (Jiangsu, China).

2.2. Animals and DP exposure

Eighteen female and eighteen male Sprague-Dawley (SD) rats (approximately 8 weeks of age) were purchased from Southern Medical University Animal Central (Guangzhou, China). They were raised in the Specific Pathogen Free (SPF) animal laboratory with a light/dark cycle for 12/12 h at 22–24 °C and relative humidity of 50 ± 10%. After 7 days of acclimation, each female SD rat was randomly placed in a cage to mate with a male SD rat. During mating, the occurrence of...
vaginal embolism and/or spermatozoa in the vagina of female rats was recorded as Embryonic day 0 (ED 0). The pregnant rats were individually placed in a plastic cage with a sawdust pad at the bottom. A total of eighteen pregnant rats were randomly divided into three groups (six rats in each group): control group (A), pregnancy DP exposure group (B), pregnancy and lactation DP exposure group (C) (Fig. 1).

For the two DP exposure groups, pregnant rats were given a 5 mg/kg/d dose of commercial DP orally in corn oil for twenty-one days (Group B) or forty-two days (Group C). The dose used for DP exposure was selected based on the low-dose used in previous reports (Li et al., 2013). For the control group (Group A), they were dosed with the same volume of corn oil daily. Females were placed in the plastic cages with the offspring until they were euthanized (lactation day 21 [LD21]). After 21 days of lactation, the offspring (six were randomly selected from each group) were fed with feed until they reached infancy (approximately 4 weeks) and adulthood (approximately 8 weeks). Feces were collected in sterile cryopreservation tubes on days 21 and 42 (pregnant rats) and days 28 and 56 (offspring) for analysis of DP, SCFAs, and gut microbiota. The rats were euthanized after collecting feces. Finally, the feces, livers, and serum were collected and frozen at −80 °C until analysis. All experimental processes were approved by the Ethics Review Committee of Southern Medical University (L2018189).

2.3. Sample treatment protocols

For the DP analysis, 100 mg of lyophilized sample (liver or feces) was ground into powder, spiked with surrogate standard 13C-PCB141, and extracted ultrasonically three times with 30 mL 50% hexane/acetone (1:1 v/v) for 30 min. Then, concentrated extract was further cleaned up using solid phase extraction (SPE). The SPE silica gel column was activated with ethyl acetate (6 mL), dichloromethane (6 mL), and n-hexane (10 mL). The target was cleaned up with 10 mL ethyl acetate/n-hexane (1:19 v/v) and 8 mL ethyl acetate/dichloromethane (1:1, v/v). The eluate was concentrated and stored in 50 μL isooctane. The internal standard (13C-PCB209) was added for instrumental analysis. Pretreatment for serum was performed as in the previous study (Li et al., 2013).

For the analysis of SCFAs, all fecal sample treatments were performed at 4 °C to protect the volatile SCFAs. Briefly, an approximately 1 g aliquot of feces was aseptically mixed with 1 mL sterile phosphate buffer saline (pH = 7.2) in a sterile tube. After addition of the phosphate buffer saline, the mixture was ultrasonically treated for 10 min, then centrifuged at 4 °C at 13000 rpm for 10 min. Next, 1 mL of the supernatant was transferred to a 4 mL centrifuge tube, and 10 μL 50% H2SO4 solution and 0.5 g anhydrous sodium sulfate were added to the centrifuge tube. After that, 2 mL diethyl ether was added, mixed by vortexing and then centrifuged at 6000 rpm for 10 min. Finally, the supernatant solution was collected for the analysis of SCFAs using GC/MS.

2.4. Gut microbiota analysis

The genomic DNA of fecal samples was extracted as described previously (Eggers et al., 2018). The extracted DNA was quantitatively analyzed by ultraviolet spectroscopy. For 16S rRNA gene sequencing, the V3-V4 region of DNA was amplified by PCR. Amplicons were separated on 1% agarose gels and further purified using a PCR clean-up kit. Purified amplicons were validated using the Nanodrop One and pooled. Illumina MiSeq2500 platform was used for double-index amplification and sequencing, and QIIME (version 1.9.1) was used for bioinformatics analysis. Details of data quality optimization and OTU cluster analysis were described in the Supporting Information.

2.5. Instrumental analysis

The isotopes of DP were determined by a 7890B gas chromatograph (GC) system coupled with a 5977B mass spectrometer (MS) (Agilent Technologies, Santa Clara, CA) and electron capture negative ionization was used in the selected ion monitoring (SIM) mode. The chemical separation of capillary columns was performed using DB-5HT (15 m × 0.25 mm × 0.10 μm film thickness, J & W Scientific, Folsom, CA, USA). Detailed instrumental parameters of the GC/MS procedures were similar to those reported in the literature (Zhang et al., 2010). The quantitative and qualitative ions of m/z 653.8 and 651.8 were used for Syn-DP and anti-DP, respectively. The analysis of SCFAs was performed using the same instrument under electron ionization in the SIM mode. Details for instrumental analysis of SCFAs were described in the Supporting Information.

2.6. Quality assurance/quality control

One spiked sample and one blank sample were treated, as well as the conventional sample. The concentration in the actual sample was corrected according to the concentration of analyte in the procedure blank. The surrogate standard for each sample was used for the recovery control. The recovery of 13C-PCB141 for all samples was 108 ± 25%. It was not detected in the procedure blanks for syn-DP and anti-DP. Concentrations were not recovery-corrected. A signal-to-noise ratio (S/N) of 10 was selected as the criterion for the quantification of analytes. The limits of quantification (LOQs) of syn- and anti-DP were 0.046 and 0.149 pg/mL, respectively, and the LOQ of SCFAs was 2.36 μg/mL.

2.7. Statistical analysis

All data are represented as mean values and standard deviations. Statistical analysis was performed using SPSS 22.0 for Windows® (SPSS, Chicago, IL, USA) for comparisons of data between control and exposure groups. One-way analysis of variance (ANOVA) was used to determine differences in the concentrations of DP and other variances, and a Bonferroni correction was applied. Significance was set at p < 0.05.

3. Results and discussion

3.1. Concentrations of DP in serum, liver, and feces

Blood is one of the most important monitoring matrices for internal exposure of contaminants. The liver is a metabolic organ in vertebrate body, and it also produces bile in digestive system, which is associated with gastrointestinal tract. To investigate the intergenerational transfer of DP and the associated effects on the structure and function of gut microbiota in offspring, the concentrations of anti-DP and syn-DP in feces, liver, and serum were measured, and they are shown in the Supplementary Information (Table S1). In the control group, the concentration of ΣDP (sum of anti-DP and syn-DP) detected in the feces, liver, and serum was almost zero. For the exposure group samples, the highest concentrations of ΣDP were detected in feces, followed by liver and serum. Notably, the highest concentration of ΣDP detected in the feces of Group C during pregnancy reached up to 183 ± 107 μg/g dw, which was nearly 12- and 310-fold the concentration seen in liver and serum of pregnant rats, respectively (Fig. 2A). Similar results were also found a high excretion rate of DP and decabromodiphenyl ether (a similar high halogenated flame retardant) in feces (Brock et al., 2010; Feng et al., 2015). The high levels of DP in the feces indicated that the excretion in the feces is a very important elimination pathway of DP from animal body.

In a report on decabromodiphenyl ether, high level of the chemical was observed in the liver (Feng et al., 2015). To better understand whether there is similar specific accumulation of DP in liver, which associated the gastrointestinal tract, the concentration ratios of ΣDP in paired liver/serum (L/S) samples were calculated and are shown in Fig.
other POPs, the accumulation and distribution of DP in the organism can be controlled by the transport and balance of lipid reservoirs (Zeng et al., 2014). In addition, the liver is the first organ where contaminants are absorbed from the gastrointestinal tract, and is also a rich perfusion organ and a major organ for metabolizing xenobiotics, which might be very important factors for the liver-specific accumulation of DP isomers.

### 3.2. Elimination and intergenerational transfer of DP

In the feces, it was expected that there was no significant difference between the \( \Sigma DP \) of Group C and Group B during pregnancy because of the same exposure dose and duration (Fig. 2A). However, the \( \Sigma DP \) in feces of lactating rats and in the liver and serum of pregnant rats of Group C was significantly higher than those of Group B \((p < 0.01)\). The results were mainly because Group C was continuously exposed to DP, while the exposure was stopped for Group B (Fig. 2). In addition, the elimination of DP from the body by the feces because of hepatoenteral circulation is an important reason for this difference, which is also demonstrated by the significant decrease in the DP concentrations in feces, liver, and serum in adult offspring compared with immature offspring due to the termination of DP exposure (Fig. 2). Thus, the present results demonstrated that the excretion by feces was a very important pathway for DP exposure.

In our study, the total DP concentrations in feces, serum, and liver in the immature offspring of Group B were not detected (n.d.), n.d., and \(0.07 \pm 0.01 \mu g/g\) lw (lipid weight), respectively (Fig. 2). The low DP concentrations in the tissues might indicate that DP could not be transported transplacentally from mother to offspring. The present results were slightly different from the previous studies, which showed that many POPs were transferred to offspring via the placenta (Lehmann et al., 2014; Vizcaino et al., 2014; Zhang et al., 2017, 2018). The large molecular weight and steric hindrance might be the main reasons for the difficult transplacental transport of DP. In this study, the detected DP in samples of Group C might be from breastfeeding, which can be demonstrated that the concentrations of \( \Sigma DP \) in feces, liver, and serum in the immature offspring of Group C were higher than those of Group B (Fig. 2). During the lactation period, the mother rats in Group C were exposed to DP continuously. Thus, the significantly higher concentrations of DP in the immature offspring of Group C were from breastfeeding. Many studies have shown intergenerational transfer of POPs through lactation due to the occurrence of the chemicals in breast milk (Fang et al., 2015; Siddique et al., 2012; Zhang et al., 2014). Although there was a report that showed the transfer of DP by placenta in an e-waste recycling area (Ben et al., 2014), the present results demonstrated that the transfer of DP from mother to the fetus through the placenta was very limited. The main pathway of intergenerational transfer to the offspring might be through lactation. However, it should be noted that the present work did not collected milk samples and measured the levels of DP in it. Therefore, the present results can not obtain the most direct evidence on the main pathway of intergenerational transfer of DP to the offspring. It should carry out more investigation in the future to get further support.

### 3.3. Influence on the abundance and \( \alpha \)-diversity of gut microbial species

To investigate the influence of DP exposure on the gut microbial species of rats and offspring, 16S rRNA gene sequencing was used. A ward method was used to analyze the structure of gut microbial species in the exposure groups and control group. The relative abundances of the major phylum, family, and genera found in the fecal microbial communities are shown in the heatmaps (Fig. 3). At the phylum level, the abundance of Bacteroidetes in the feces of Group C decreased slightly in pregnant rats during lactation, while the abundance in the offspring did not change much from infancy to adulthood (Fig. 3A). At the family level, compared with the control group, the relative

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**Fig. 2.** Concentrations of \( \Sigma DP \) (the sum concentrations of syn- and anti-DP) in the feces (A), liver (B), and serum (C) of rats. Group A: Control group; Group B: Pregnancy DP exposure group; Group C: Pregnancy and lactation DP exposure group. Error bar indicates the standard deviation. The \( p \)-values * < 0.05 and ** < 0.01 are significantly different.

S1. In the present study, all the L/S ratios were greater than a unit, which indicated a liver-specific accumulation of DP. Similar liver-specific accumulation of DP was also reported in previous studies of the common carp and frog (Wu et al., 2018; Zeng et al., 2014). Similar to
abundance of Prevotellaceae and Streptococcaceae in pregnant rats of exposure groups during pregnancy was decreased and the immature offspring showed a decreasing trend (Fig. 3B). At the genus level, the relative abundance of Ruminococcaceae and Christensenellaceae in pregnant rats decreased, whereas the abundance of Bacteroides increased in pregnant rats during pregnancy and lactation after exposure to DP (Fig. 3C). However, for the offspring, the relative abundance of the gut microbial species generally showed a downward trend at the genera level. The taxonomic spectrum of fecal samples at the genus level showed that Lactobacillus, Lachnospiraceae, and Muribaculaceae dominated the gut bacterial community. In addition, the proportion of Muribaculaceae in pregnant rats during pregnancy and Lachnospiraceae in the immature offspring of Group C were significantly changed (Fig. 4). Thus, DP exposure had a great effect on the gut microbiota at the genus level.

Currently, although the functions of most bacteria identified in the feces of rats are unknown, several bacteria, including Ruminococcus, Alloprevotella, Roseburia, and Bacteroides are closely associated with metabolism, disease, and inflammation (Ze et al., 2012). For example, Bacteroides can decrease the liver cholesterol and triglyceride concentration in mice fed a high-fat diet, increase the production of TNF-α (tumor necrosis factor α), and improve the immune defense of mice (Gauffin Cano et al., 2012). The bacteria Alloprevotella has been associated with a reduction in cardiovascular disease in humans (Kelly et al., 2016). Similar results were available in the literature, which implicated alterations in the composition of the gut microbiota, due to exposure to contaminants, in the occurrence of many diseases (Choi et al., 2013; Xia et al., 2018; Zhang et al., 2015b). For example, oral exposure to PCBs could significantly alter the gut microbiome abundance by lowering the level of Proteobacteria in mice (Choi et al., 2013). Exposure to TCDF altered the proportion of Firmicutes compared to Bacteroidetes and increased the Flavobacteria levels, triggering significant inflammation and metabolic disorders in mice (Zhang et al., 2015a). Therefore, the present study showed that exposure to DP might indirectly influence the health of animals through the effects on the gut microbiota.

To investigate the species richness of the gut microbiota, α-diversity, which refers to the diversity of species within an ecosystem, is generally used. There are many indices to measure diversity, among which Ace and Chao 1 are the common estimation methods. In the present study, the phylogenetic diversity and species richness of the DP exposed groups decreased in pregnant rats and immature offspring based on the Ace, which is an α-diversity metric (Fig. 5A). Similarly, according to the results of the Chao 1, the species richness in the feces
from the exposed groups decreased compared to the control group in pregnant rats and immature offspring (Fig. 5B). Overall, there was a tendency towards reduction in diversity of the gut microbiota in the feces of pregnant rats and offspring in the exposure groups, which indicated that the exposure to DP could affect the gut microbial diversity.

To further understand the relationship between the gut microbiota in feces samples and DP exposure, network analysis was used, which showed that few genera were co-occurring in different groups of gut microbiota (Fig. S3). In addition, to predict microbial function, high-quality sequence-based PICRUSt (phylogenetic investigation of communities by reconstructing unobserved states) was also used. The biological metabolic pathway of 16S rRNA was analyzed using the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. The results showed that the relative abundances of cellular processes, environmental information processing, genetic information processing, and metabolism during pregnancy and genetic information processing in the offspring showed an upward trend in the DP exposed groups compared with the control group (Fig. S4). This indicated that there were some differences in gene function between the exposed groups and the control group. Therefore, DP exposure may alter the structure and function of the gut microbiota in the offspring. However, it should be noted that the pregnant rats produced an unequal number of

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**Fig. 4.** Major changes in the genus level of gut microbiota in the feces after DP exposure (the first letter P: pregnancy; L: lactation; I: immature offspring; A: adult offspring; the second letter A: Group A, the control group; B: Group B, the pregnancy DP exposure group; C: Group C, the pregnancy and lactation DP exposure group).

**Fig. 5.** Ace (A) and Chao 1 (B) of microbial α-diversities among control and exposure groups (the first letter P: pregnancy; L: lactation; I: immature offspring; A: adult offspring; the second letter A: Group A, the control group; B: Group B, the pregnancy DP exposure group; C: Group C, the pregnancy and lactation DP exposure group).
offspring, with more females than males. Therefore, gender difference can not discussed because of unequal and limited number of samples.

3.4. Gut microbiota metabolites of SCFAs

SCFAs, a kind of metabolite of gut microbiota produced in the colon are primarily absorbed into the intestinal mucosa (90%–95%) and the rest are excreted in the feces (Louis and Flint, 2017). To investigate the potential effects of DP exposure on the metabolism of gut microbiota, the concentrations of seven SCFAs, including hexanoic acid, valeric acid, isovaleric acid, butyric acid, isobutyric acid, propionic acid, and acetic acid were measured in the feces. When the composition of each SCFA compound was expressed as a percentage of the total fecal SCFAs, the proportion of isobutyric acid in samples from group C during lactation of pregnant rats was increased, while there was no significant difference in the other SCFAs in all groups (Fig. S2). The concentrations of the major SCFAs with high concentrations, including acetic acid, propanoic acid, butyric acid, and isovaleric acid are shown in Fig. 6A–D. The acetic acid concentrations were significantly increased ($p < 0.01$) in the exposed groups compared with the control group in pregnant rats and offspring, but the levels were significantly decreased in adult offspring ($p < 0.01$) (Fig. 6A).

Furthermore, the concentrations of propanoic acid were significantly increased ($p < 0.05$) in pregnant rats during lactation and immature offspring of the exposed groups compared with the control group. Similarly, the fecal butyric acid concentrations in exposed groups were significantly higher than in the control group ($p < 0.05$) (Fig. 6C) in pregnant rats and offspring. In addition, there was no significant difference in the concentration of propanoic acid in rats during pregnancy (Fig. 6B). Similar results were observed in the concentration of isovaleric acid in the adult offspring (Fig. 6D). Group C generally had the highest concentrations of SCFAs. Overall, the concentrations of SCFAs in the feces were obviously increased due to DP exposure, demonstrating that DP promotes metabolism of gut microbiota.

The effects of this promotion on animal health were unclear and needed further study. As reported, dysbiosis of gut microbiota is closely related to metabolic disorders (Xia et al., 2018), and microbiota metabolites, including SCFAs, were first affected when environmental pollutants altered the gut microbiota composition (Zhang et al., 2015a). It has previously been shown that SCFAs have multiple effects on mammalian metabolism (Lu et al., 2018). As one of the most important metabolites of gut microbiota, SCFAs are not only energy substrates for cells and gluconeogenesis, but also inhibitors of histone deacetylases (Primec et al., 2017). They can affect a series of host processes, such as energy metabolism, gut composition, and cancer, among others (Nicholson et al., 2012). Studies found that obesity was related to the composition of the gut microbiota and the production of SCFAs in humans because SCFAs provided an additional source of energy for the body (Rahat-Rozenbloom et al., 2014; Schwierz et al., 2010).

In addition, it has been proven that higher fecal SCFA concentrations were positively associated with body weight and calorie-rich diets in the mouse (Turnbaugh et al., 2006). The present results showed that the concentrations of SCFAs in the DP exposed groups were significantly increased, indicating that the exposure to DP activated bacterial fermentation, which can increase the risk of obesity in rats, although the present study did not evaluate such effects. Similarly, another POP compound, TCDF, induced significant elevation of SCFAs such as butyrate and propionate in the feces of mice (Zhang et al., 2015a). In a community-based study of adult human samples, higher levels of SCFAs in feces were found to be associated with higher gut permeability, hypertension, dyslipidemia, and central obesity (Primec et al., 2017). Furthermore, SCFAs in the feces were also associated with indicators of lower gut microbiome diversity (de la Cuesta-Zuluaga et al., 2018), which was consistent with our results. These results seem to indicate that the large excretion of SCFAs in feces is a marker of poor intestinal health and metabolic disorders. However, there was no strong evidence for this in the present study. It is necessary to further investigate the relationship between SCFAs and health, and the influence.

![Fig. 6. Concentrations of major SCFAs in feces. (A) acetic acid; (B) propionic acid; (C) butyric acid; (D) isovaleric acid. Group A: Control group; Group B: Pregnancy DP exposure group; Group C: Pregnancy and lactation DP exposure group. The p-values * < 0.05 and ** < 0.01 are significantly different.](image-url)
of DP on health via the gut microbiota.

The SD rat, an animal model with a similar structure to the human gut microbiota, begin to grow into adults from approximately the eighth week, but it is a long-term process from birth to adulthood. The present study investigated the influence of DP exposure during pregnancy and/or lactation on the gut microbiota in the adult offspring. The results have important implications for microbiological research and the associated health effects by environmental pollutants such as DP.

4. Conclusions

The present study investigated the accumulation of DP via oral ingestion and intergenerational transfer of low-dose exposure to DP by transplacental transport and lactation. The influence on the structure and function of the gut microbiota as well as metabolites of SCFAs were also studied. The results showed that many ingested DP is directly excreted in the feces, and the accumulation of the chemicals is high in the liver. DP more likely can be transferred from mother to offspring by lactation rather than transplacental transport. The present results also indicated that DP exposure not only reduced the species diversity and abundance in offspring, but also disturbed the SCFA metabolites of the gut microbiota. Although the present data resulted from animals, they remind us that the risk of DP for infants via lactation should be of concern. In addition, considering the extensive pollution of DP to the environment and the increasing usage of DP, its potential to interfere with the gut microbiota should be paid more attention, especially the influence it has on the SCFA metabolites and the associated health effects.

CRediT authorship contribution statement

Guoxia Zhang: Microbiological analysis, and draft preparation.
Qiaoqiao Ren: Chemical analysis and draft preparation.
Shengtao Ma: Methodology.
Jiguo Wu: Data analysis.
Xingfen Yang: Data analysis.
Yingxin Yu: Design, writing, reviewing & editing.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105770.

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