

# **The Effects of Prenatal GenX (HFPO-DA) Exposure on Gut Microbiota and Metabolic Function in Pregnant Rats and Their Offspring**

<sup>#</sup>Wenchao Han <sup>a,\*</sup>, <sup>#</sup>Xinpeng Zhang <sup>b</sup>, Dan Li <sup>a</sup>, Wendong Liu<sup>a</sup>, Yanxia He <sup>c</sup>, Haitao Shang<sup>d</sup>, Zhugen Yang <sup>e</sup>, Hongyun Liu<sup>f</sup>, Wenjing Zhu <sup>g,\*</sup>

<sup>a</sup> Department of Pediatrics, Qingdao Hospital, University of Health and Rehabilitation Sciences (Qingdao Municipal Hospital), Qingdao 266071, China

<sup>b</sup> Graduate School of Dalian Medical University, Dalian, China

<sup>c</sup> Department of Pediatrics, Luoyang Central Hospital, Affiliated to Zhengzhou University, Henan Province, China

<sup>d</sup> Department of Digestive, Qingdao Hospital, University of Health and Rehabilitation Sciences (Qingdao Municipal Hospital), Qingdao 266071, China

<sup>e</sup> Faculty of Engineering and Applied Sciences, Cranfield University, Cranfield, MK43 0AL UK

<sup>f</sup> Pathology Department, Qingdao Hospital, University of Health and Rehabilitation Sciences (Qingdao Municipal Hospital), Qingdao 266071, China

<sup>g</sup> Medical Research Department, Qingdao Hospital, University of Health and Rehabilitation Sciences(Qingdao Municipal Hospital), Qingdao

266071, China

#Wenchao Han and #Xinpeng Zhang contributed equally to this manuscript.

\*Corresponding authors: Wenchao Han, E-mail address:  
hanwenchaodr@outlook.com (W. Han). Department of Pediatrics,  
Qingdao Municipal Hospital, No. 5 Donghai Middle Road, 266071  
Qingdao, China.

\*Corresponding author: Wenjing Zhu, zhuwj@uor.edu.cn Medical  
Research Department, Qingdao Hospital, University of Health and  
Rehabilitation Sciences (Qingdao Municipal), Qingdao 266071

## **Abstract**

Hexafluoropropylene oxide dimer acid [(HFPO-DA), GenX] is listed as Substances of Very High Concern (SVHC) by the Commission of Member States (MSC) in 2019. The threat of GenX to human health, especially to sensitive populations such as pregnant women, infants and children cannot be ignored. We aimed to investigate the effects of GenX on the intestinal barrier function and gut microbiota of both pregnant rats and their offspring. We subsequently performed a metabolomic analysis of the maternal gut to investigate the metabolic disruptions associated with GenX exposure. Pregnant Sprague-Dawley rats (n = 12/group) received GenX once daily by gavage at 0, 1, 10, or 100 mg/kg body weight from gestational day (GD) 0.5 through GD 19.5. We observed intestinal tissue damage and changes in the gut microbiota in both pregnant rats and their offspring. Additionally, we performed untargeted LC/MS metabolomic analysis on the intestinal tissues of the pregnant rats. The intestinal barrier function of the pregnant rats was impaired. The results of 16S rRNA gene sequencing indicate that GenX alters the gut microbiota diversity and composition in both pregnant rats and their offspring. The metabolites of pregnant rats were also found to be significantly altered, and these metabolites are mainly associated with amino acid metabolism, carbohydrate metabolism, and lipid metabolism. Collectively, Exposure to GenX disrupts the intestinal barrier function of pregnant rats, exacerbates microbiome toxicity, and disturbs the gut

microbiome-metabolome homeostasis.

**Keywords:** Hexafluoropropylene oxide dimer acid, Pregnancy rat, Gut Microbiota, Metabolomics.

## 1.Introduction

Perfluorooctanoic acid (PFOA), a typical compound of per- and polyfluoroalkyl substances, is widely used in industrial and commercial application(Butenhoff et al., 2006). However, it is considered a global environment contaminant in recent years owing to its negative environmental effects and various toxicities (Darrow et al., 2014; Fei et al., 2009; La Rocca et al., 2014). Thus, PFOA was restricted in many countries and even added to the Stockholm Convention as a Persistent Organic Pollutant in 2019. Hexafluoropropylene oxide dimer acid (HFPO-DA), trade name GenX, as a replacement compound to PFOA, has been produced since 2010. Compared to PFOA, it was a shorter-chain analogues with shorter half-life. In Europe, the current consumption of GenX was estimated to be 10 - 100 tons per year(Sun et al., 2019). To date, GenX has been widely detected in multiform environmental matrices all over the world(Feng et al., 2021; Gebbink & van Leeuwen, 2020; Pan et al., 2018). Of note, it have since been discovered in drinking water and presented a higher challenge in potable

water treatment than PFOA(Dixit et al., 2020). This implies that if GenX release will not be stopped, it will continue to accumulate in drinking water, thus becoming global environmental pollutants potentially threatening the human health and wildlife. Based on this, the MSC (Commission of Member States) of the European Chemicals Agency (ECHA) identified HFPO-DA as SVHC (Substances of Very High Concern) in 2019 (*Homepage - ECHA*, n.d.). The Department of Health and Human Services in North Carolina has set a drinking water equivalent level of 140 ng/L as a drinking water health goal for GenX. Despite such efforts, GenX was detectable in humans serum and might lead adverse healthy effect(Calafat et al., 2019; Petriello et al., 2022).

Several studies have revealed the further toxic effects of GenX. Oral exposure to 30mg/kg GenX in pregnant Sprague-Dawley rats caused increased neonatal mortality, lower maternal serum thyroid hormone and lower offspring body weight(Conley et al., 2021, 2022). In CD-1 mice, pregnant dams were dosed GenX(10 mg/kg/d) via oral gavage showed placental weight was significantly increased, embryo: placenta weight ratios were significantly reduced, as well as histopathological changes in placentas.(Blake et al., 2020). Furthermore, after a 90-day subchronic toxicity study of GenX conducted in mice, Caspase-3 immunohistochemical analysis indicated that the liver cells primarily affected were hepatocytes, characterized by increased cytoplasmic staining,

fine stippling, and formation of apoptotic bodies, with occasional nuclear staining. These effects were dose-dependent. Additionally, Kupffer cells and leukocyte aggregates also exhibited mild cytoplasmic staining, indicating low-level immune activity, but the extent of damage was notably less than that observed in hepatocytes (Chappell et al., 2020). GenX , exposing to fertile chicken eggs via air cell injection, may induce thinned right ventricular wall and elevated heart rates, and steatosis(X. Xu et al., 2021). In addition, two *in vitro* studies' represent that the exposure of differentiated rat thyroid cells (FRTL-5) to GenX produces acute cyto-toxic and geno-toxic effects. A modeling research on male rats indicated that GenX showed a higher toxicity score in modeled serum and liver concentrations than PFOA(Coperchini et al., 2020; S. Zhang et al., 2021). According to these studies, the safety of GenX as an alternative to PFOA needs to be further assessed. Further studies examining the toxicological effects of GenX are warranted.

It is generally known that oral intake of PFASs is an important route for its exposure. The gut is considered a vital recipient for environmental pollution after oral exposure. The gut microbiota possesses diverse metabolic activities and exhibit a pivotal role in maintaining organism health(Stiemsma & Michels, 2018; Tanaka & Nakayama, 2017; Thomas et al., 2017; Zmora et al., 2019). PFOA can induce gut barrier dysfunction and gut microbiota disturbance in mammals, and even related to human

ulcerative colitis(Barry et al., 2013; Steenland et al., 2013). A recent study indicates the potential gut toxicity of 200 $\mu$ g /L GenX, as male mice were orally exposed to it for 6 weeks to evaluate this effect. The results showed that GenX exposure caused colonic inflammation, the dysfunction of gut barrier, and caused the imbalance of cecal gut microbiota and changes of cecal microbiota diversity(Xie et al., 2021a). However, there are few studies that investigated GenX in gut toxicity on susceptible population (pregnant women and newborns). In this study, we orally administered GenX to pregnant Sprague-Dawley rats for 20 d to evaluate the effects on gut. We hope that these results can bring new insight into the potential risks of GenX exposure to human health, especially to pregnant women.

## **2. Materials and Methods**

### **2.1 Animals & Chemicals**

We have described this part in our previous article(Lv et al., 2024).

Ten-week-old SPF-grade SD rats (half male, half female) were obtained from Jinan Pengyue Laboratory Animal Breeding Co., Ltd. They were housed in polypropylene cages with sterilized bedding at  $23 \pm 1$  °C on a 12 h light/dark cycle (lights on 06:00–18:00) and provided LabDiet 5001 chow and autoclaved distilled water in polypropylene bottles ad libitum.

GenX ammonium salt (CAS 13252-13-6, lot C12467627; Shanghai Macklin Biochemical Co.,  $\geq 97$  % purity) was dissolved in deionized water

supplied by the hospital's central laboratory.

## **2.2 Experimental Design**

The ten-week-old Sprague-Dawley rats were acclimated for 1 week after arrival at the SPF Animal Laboratory. Each female rat was mated with one male overnight, and the presence of a vaginal plug was defined as gestation day 0.5 (GD 0.5). The pregnant rats were sorted by body weight in ascending order and then numbered sequentially. A computer software was used to generate a random number table, and the rats were randomly assigned to four groups according to the randomly generated numbers, with 12 rats in each group ( $n = 48$ ): 0 (deionized water only), 1, 10 and 100 mg/kg GenX. GenX was orally administered daily via gavage from GD 0.5 to GD 19.5. At the day of GD 19.5, Using the same random method, eight pregnant rats were selected from each group ( $n = 32$ ) and were placed into a carbon dioxide anesthesia chamber. The carbon dioxide valve was opened, and once the rats lost consciousness, the  $\text{CO}_2$  concentration was increased to 100%. After the animals showed no signs of consciousness (e.g.:no corneal reflex), the gas flow was continued for an additional 2 minutes to ensure death. Then, the gut (ileum and colon) of the dams and fetuses were collected and fixed in paraformaldehyde solution (4% PFA, Sigma), while the feces were collected and stored at  $-80^\circ\text{C}$  immediately. The rest pregnant rats ( $n=16$ ) spontaneously delivered. On postnatal day 7 (PND 7), the offspring rats were sorted by body weight and numbered. Using a random

number table, two offspring rats were randomly selected from each litter (n = 32). Fetuses obtained by cesarean section at gestational day 19.5 were already dead after placental separation. Pups at PND7 were euthanized by decapitation. Similarly, the intestines and feces of the pups (PND7) were collected and stored accordingly. For the pregnant rat and offspring control groups and the 100 mg/kg GenX treatment group, five samples of intestinal contents from each group were used for 16S rRNA gene sequencing. Four samples from the pregnant rat control group and the 100 mg/kg treatment group were used for untargeted metabolomics.

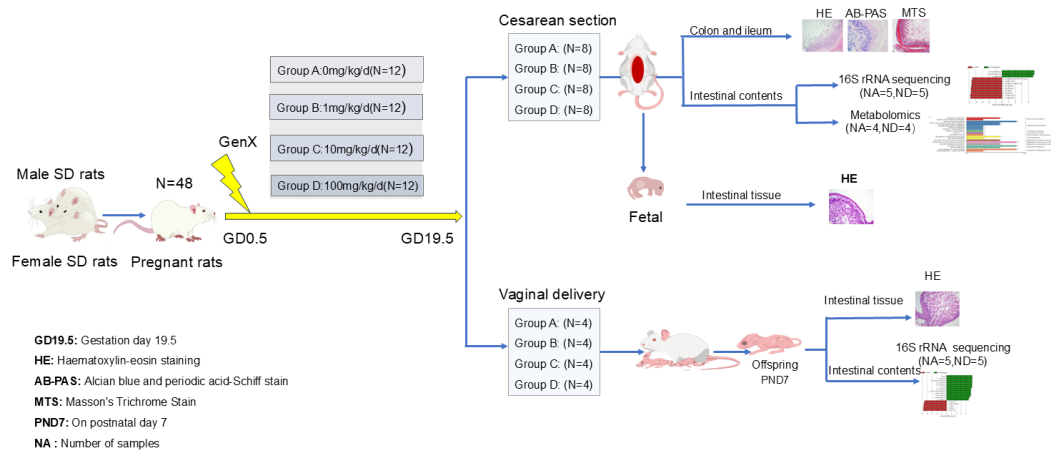


**Figure1: Sample sizes for each part of the experiment and the overall experimental design process**

### 2.3 Haematoxylin-eosin staining

All gut tissues (ileum and colon) were fixed in a paraformaldehyde solution (4% PFA, Sigma). Three randomly selected gut tissues from each group (both dam and fetal rats) were dehydrated by rising in ethanol, cleared in xylene, and impregnated in paraffin. Tissue sections of 5µm were prepared and stained with hematoxylin & eosin (H&E) for histochemical

analysis(Xie et al., 2021a).

## **2.4 Masson's Trichrome Stain**

The prepared tissue sections were dewaxed with graded ethanol and xylene. Next, they were treated with Weigert's iron hematoxylin for 5-10 minutes. After thorough washing with water, the sections were treated with Masson's trichrome fuchsin solution for 5-10 minutes. Subsequently, they were immersed in 2% glacial acetic acid and stained with 1% phosphomolybdic acid. The sections were then counterstained with aniline blue. After another wash with weak acid water, the sections were dehydrated with ethanol, cleared with xylene, and mounted with neutral resin. Finally, the sections were examined under a microscope for image acquisition.

## **2.5 Alcian blue and periodic acid-Schiff staining**

The sections were treated with Alcian Blue dye for 5 minutes and then rinsed with running tap water for 2 minutes. Next, after dewaxing the sections with graded ethanol and xylene, they were treated with 1% periodic acid for 5 minutes, followed by rinsing with distilled water. Subsequently, the sections were stained with Schiff reagent for 30 minutes in the dark. After rinsing, the sections were stained with hematoxylin. Finally, the sections were dehydrated, cleared, and mounted. The sections were then examined under a microscope for image acquisition(L.-L. Xu et al., 2022).

## **2.6 16S rRNA gene sequencing**

To detect the main alternation in the gut microbiota, 8 samples extracted from the colonic contents were randomly selected from the control group and 100mg/kg GenX-treated group for 16S rRNA gene sequencing. This samples from this study were processed on Illumina Nova seq platform by generating PE reads targeted regions. These short reads are assembled, clustered and de-noised to obtain tags, which can be used for species annotation and abundance analysis. Further analysis includes alpha diversity, beta diversity, differential analysis between groups, correlation analysis, function prediction, etc.

Alpha was performed to identify the complexity of species diversity of each sample utilizing QIIME2 software. Beta diversity calculations were analyzed by principal coordinate analysis (PCoA) to assess the diversity in samples for species complexity. One-way analysis of variance was used to compare bacterial abundance and diversity. Linear discriminant analysis (LDA) coupled with effect size (LEfSe) was applied to evaluate the differentially abundant taxa.

## **2.7 Detection of untargeted metabolites**

The LC/MS system for metabolomics analysis is composed of Waters Acquity I-Class PLUS ultra-high performance liquid tandem Waters Xevo G2-XS QToF high resolution mass spectrometer. The column used is

purchased from Waters Acquity UPLC HSS T3 column (1.8um 2.1\*100mm).

The raw data collected using MassLynx V4.2 is processed by Progenesis QI software for peak extraction, peak alignment and other data processing operations, based on the Progenesis QI software online METLIN database and Biomark's self-built library for identification, and at the same time, theoretical fragment identification and mass deviation All are within 100ppm.

Principal component analysis and Spearman correlation analysis were used to judge the repeatability of the samples within group and the quality control samples. The R language package ropls was used to perform OPLS-DA modeling, and 200 times permutation tests was performed to verify the reliability of the model. The method of combining the difference multiple, the P value and the VIP value of the OPLS-DA model was adopted to screen the differential metabolites. The screening criteria are  $FC > 1$ ,  $P \text{ value} < 0.05$  and  $VIP > 1$ .

## **2.8 Statistical analysis**

Data processing and analysis were performed using SPSS 28.0 and GraphPad Prism 8.0 software. Data are expressed as the mean  $\pm$  SEM. For independent sample experiments, the Shapiro-Wilk test was used to assess normality. The t-test was used to evaluate differences between two groups, and one-way ANOVA was used to assess differences among three groups.

259 A P-value of less than 0.05 was considered statistically significant

260

261

262

263

264

265

266

267

268

269

270

271

272

273

274

275

276

277

278

279

280

## **3. Results**

### **3.1 Histopathological changes**

In ileum of the dams, the intestinal villi shrank and decreased in all GenX-treated groups, and the intestinal epithelial cells of 100mg/kg/d GenX-treated group were significantly shed, showing obvious erosion, and the intestinal villus atrophy and reduction was more obvious (Fig.2). In colon of the maternal, the intestinal villus structure is damaged in all GenX-treated groups while inflammatory cells and lymphoid nodule was observed in 10 and 100mg/kg/d GenX-treated groups (Fig.3). There were no obvious signs of pathological injury in gut of fetal rats and offspring rats (Supplementary.Fig.1.2). In the ileum and colon of dams, the number of Alcian blue (AB)-positive goblet cells in the intestinal mucosa significantly decreased in GenX-treated pregnant rats relative to the control group, indicating a reduction in intestinal acidic/neutral mucus and a weakened intestinal mucosal barrier (Fig.4.5). According to the Masson's trichrome staining results of the ileum and colon in the pregnant rats, no significant changes were observed in the collagen fibers and muscle fibers in the colon and ileum, indicating no obvious fibrosis was detected (Supplementary.Fig.3).

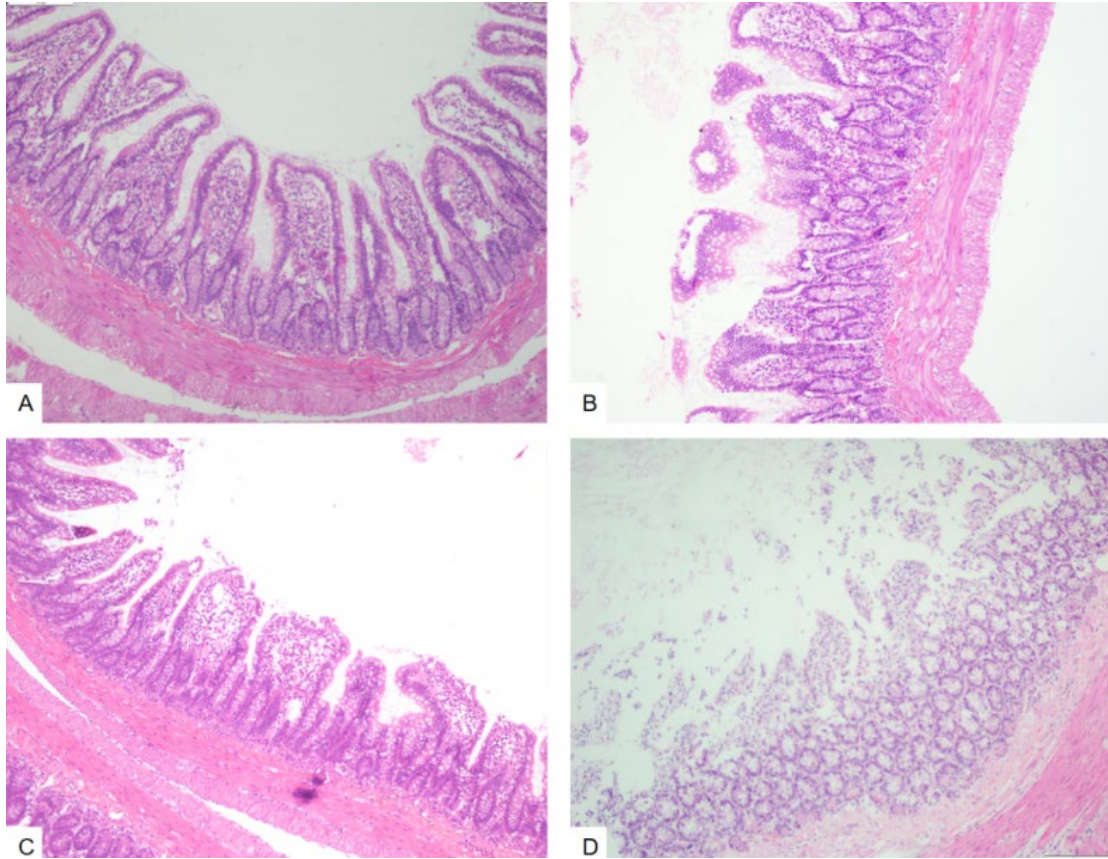


**Fig.2. Histomorphology of ileum in the dams (H&E staining, ×100).** A: CON; B: 1mg/kg/d; C: 10mg/kg/d; D: 100mg/kg/d.

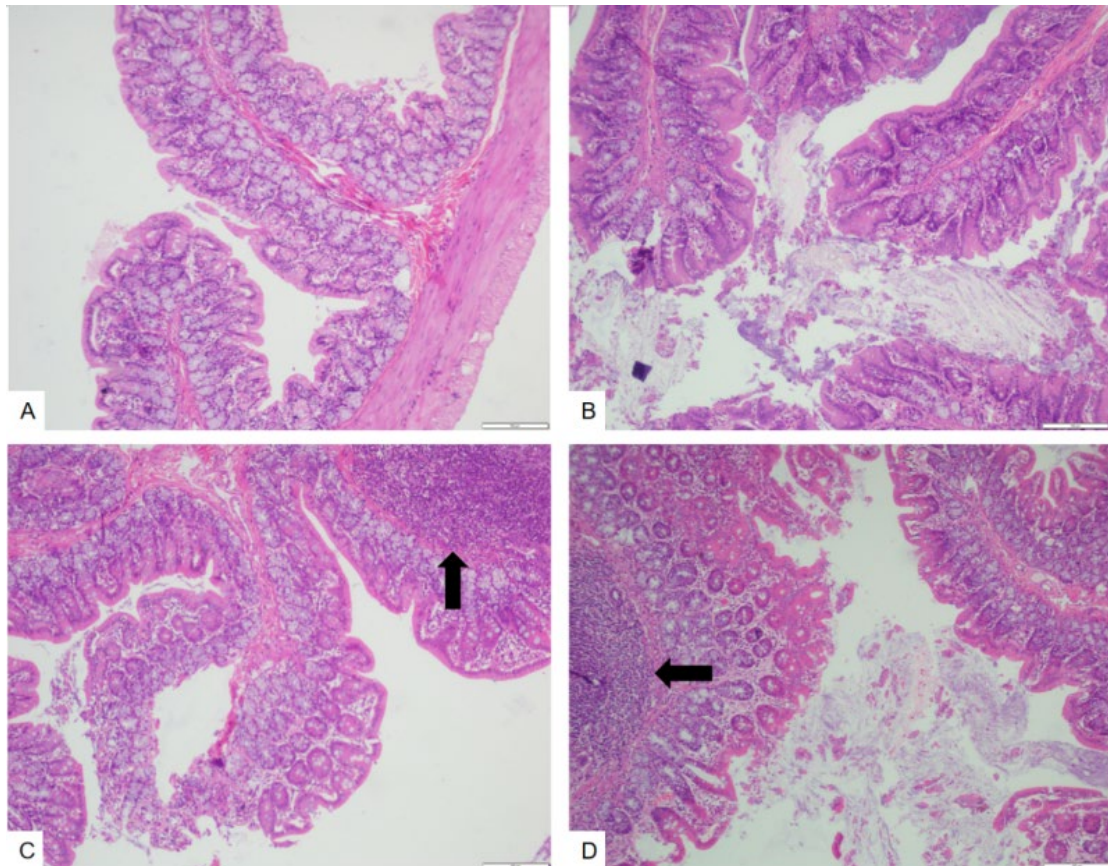


**Fig.3. Histomorphology of colon in the dams (H&E staining, ×100).** A: CON; B: 1mg/kg/d; C: 10mg/kg/d; D: 100mg/kg/d. Black arrows: Focal hyperplasia in local lymphoid tissue

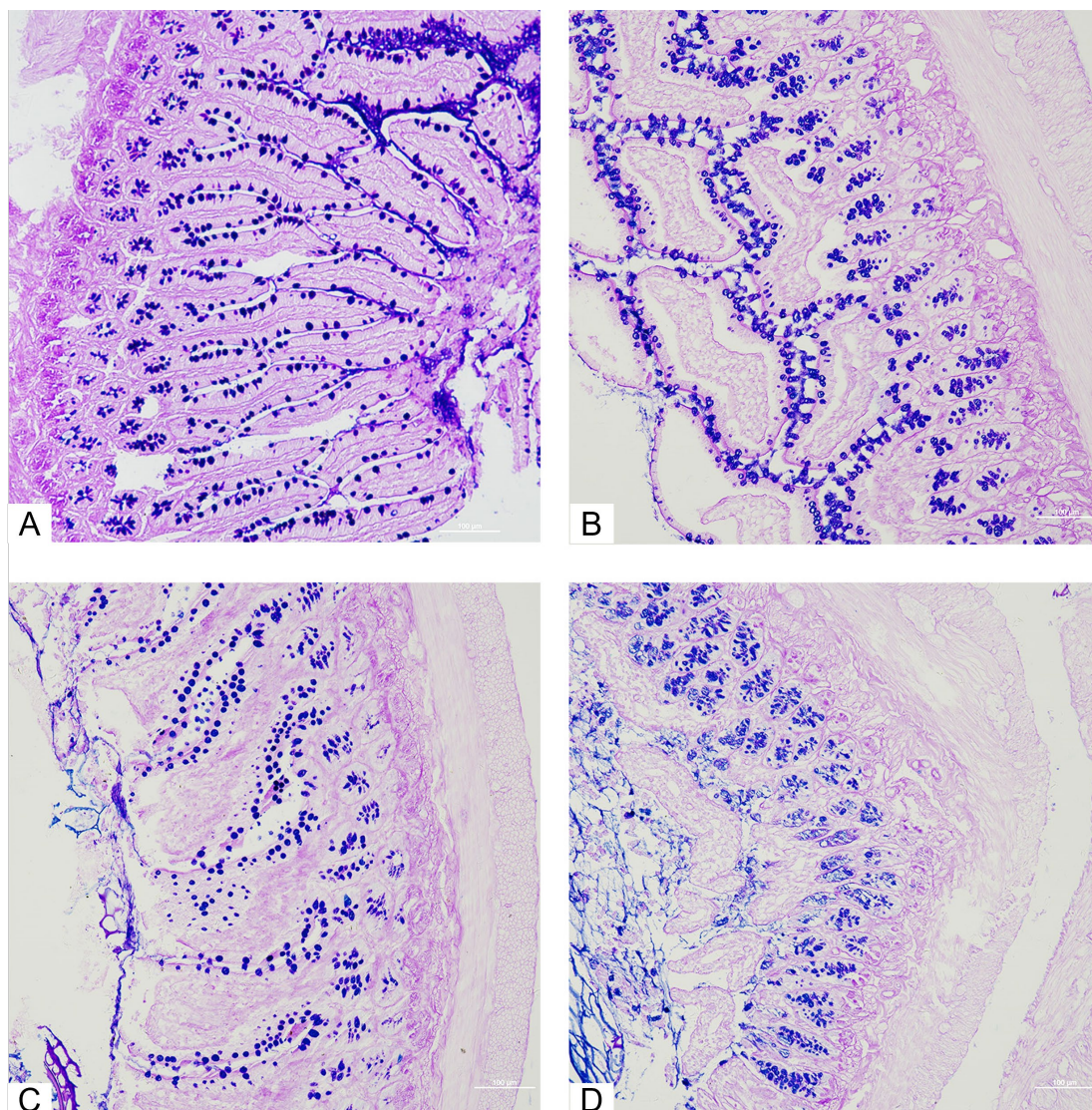


**Fig.4. Histomorphology of ileum in the dams (Alcian blue and periodic acid-Schiff staining, ×200).** A: CON; B: 1mg/kg/d; C: 10mg/kg/d; D: 100mg/kg/d.

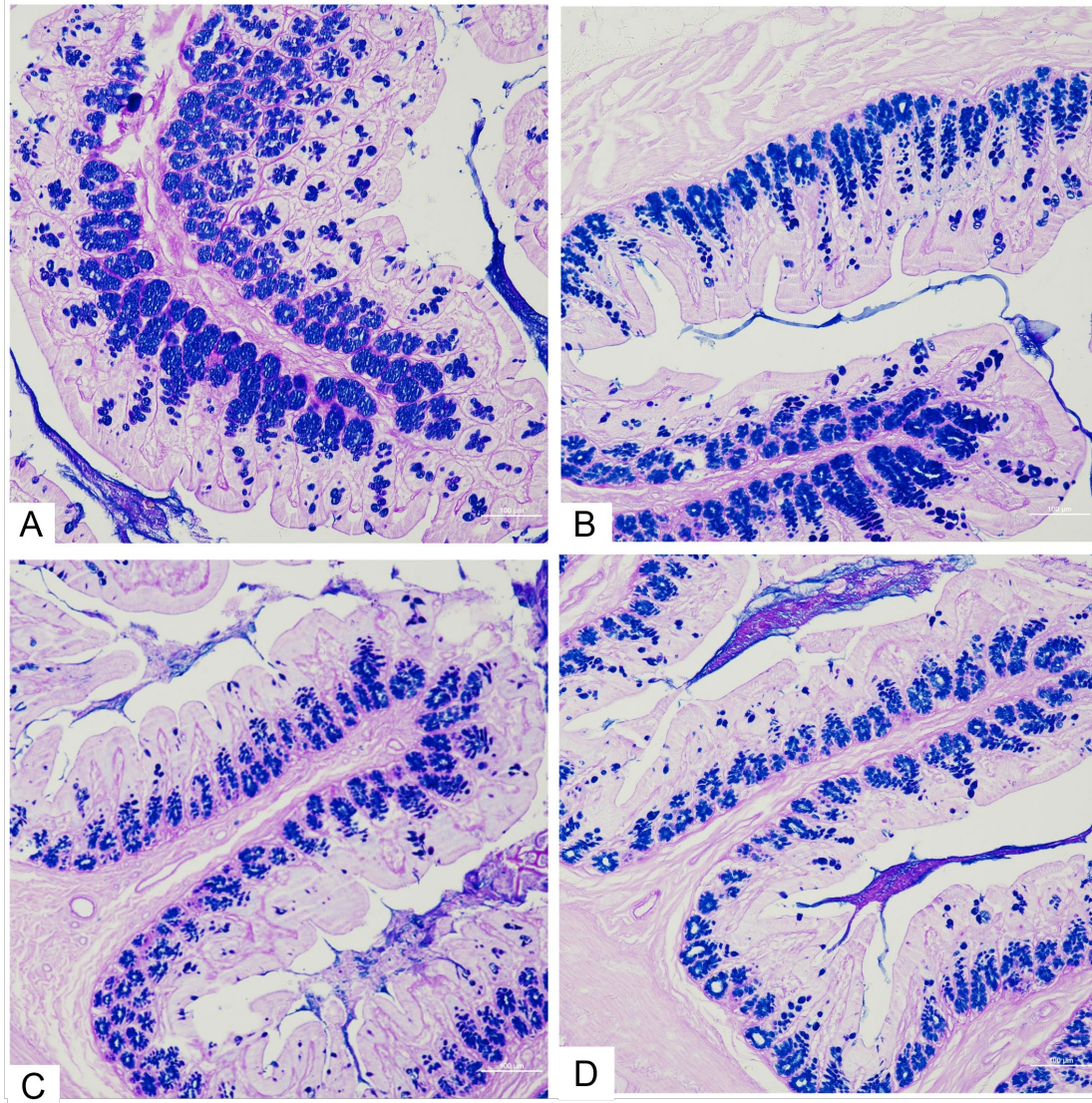


**Fig.5. Histomorphology of colon in the dams (Alcian blue and periodic acid-Schiff staining,  $\times 200$ ). A: CON; B: 1mg/kg/d; C: 10mg/kg/d; D: 100mg/kg/d.**

## **3.2 Gut microbiota changes**

### **3.2.1 Gut microbiota changes in GD19.5 pregnant rats**

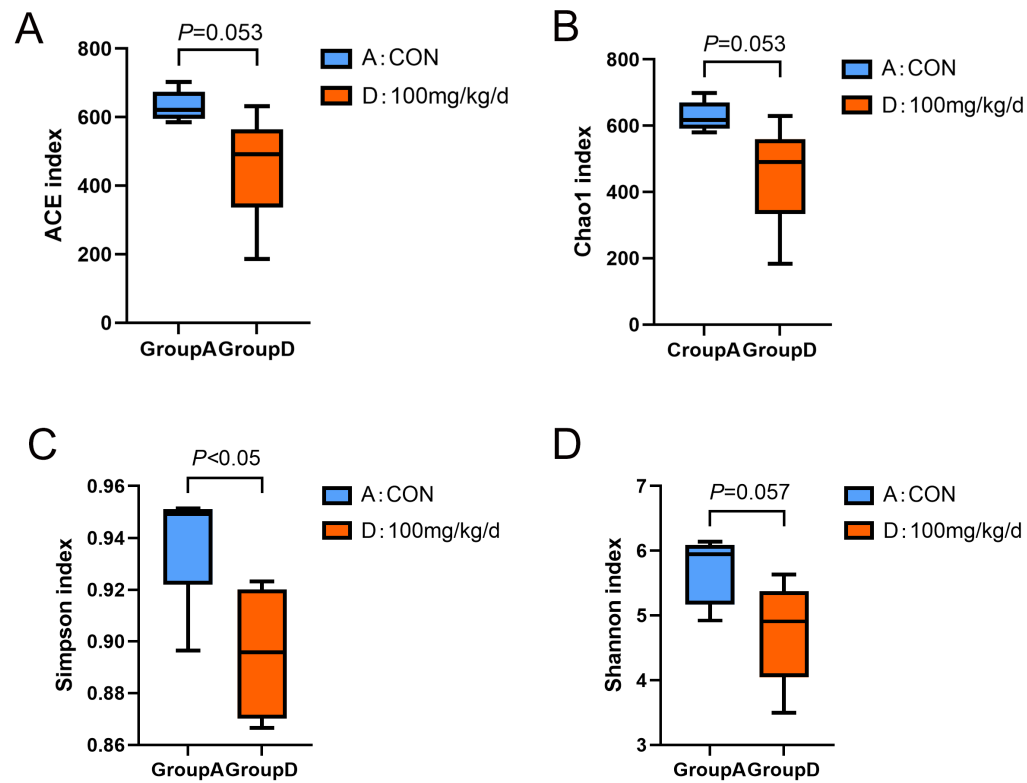
Alpha diversity measures species abundance and diversity. As Alpha diversity analysis, Simpson index of 100mg/kg GenX-treated group in pregnant rats was significantly lower than the control group ( $P < 0.05$ ), implying that GenX exposure has influence on the species diversity of intestinal microflora (Fig.6C). Both Chao1 index and Ace index had a

downward trend in 100mg/kg GenX-treated group (Fig.6A-B). In addition, our observation of  $\beta$  diversity analysis by PCoA showed that the intestinal microbiota of the control group and 100mg/kg GenX-treated group were clearly distinguishable (Fig.6G).

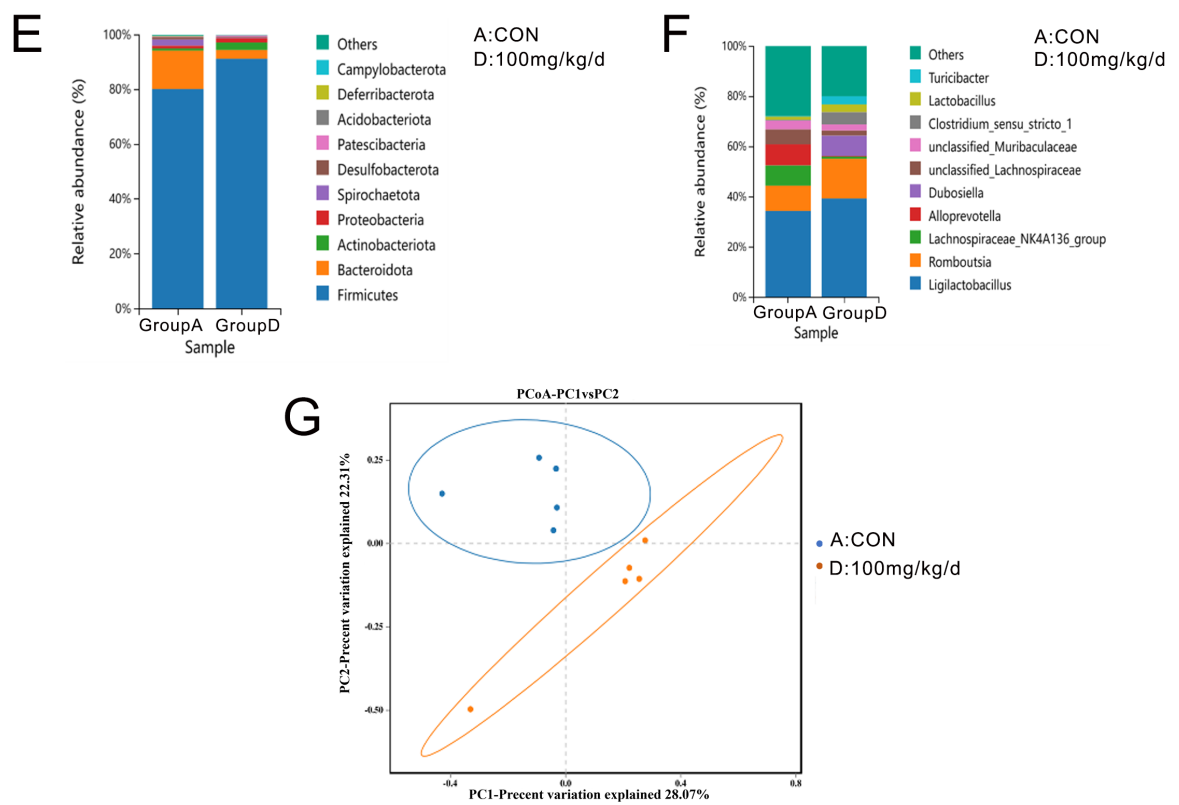
At the phylum level, the dominant bacteria in the intestinal microbiota in control group and 100mg/kg GenX-treated group were both Firmicutes, accounting for more than 80% (Fig.6E-F). Compared with the control group, the relative abundance of Bacteroides of 100mg/kg GenX-treated group was significantly reduced ( $P < 0.05$ ). At the genus level, the dominant bacteria in the intestinal flora of control and 100mg/kg GenX-treated pregnant rats were both *Ligilactobacillus*. Compared with the control group, a total of 17 species in 100mg/kg GenX-treated group ( $P < 0.05$ ), including 14 species with decreased relative abundance and 3 species with increased relative abundance. In the top ten species of intestinal microbiota in pregnant rats, the relative abundance of *Alloprevotella* of 100mg/kg GenX-treated group was significantly decreased ( $P < 0.01$ ).

The results of LEfSe analysis showed that, the species enriched in the intestinal microbiota of control group included Bacteroidetes, Bacteroidia, Bacteroidales, Lachnospirales, Prevotellaceae, Lachnospiraceae, Lachnospiraceae\_NK4A136\_group, Prevotella, unclassified\_Alloprevotella. The species enriched in the intestinal

341 microbiota of 100mg/kg GenX-treated group were Firmicutes,  
342 Erysipelotrichales, and Erysipelotrichaeae (Fig.6H-I).



343



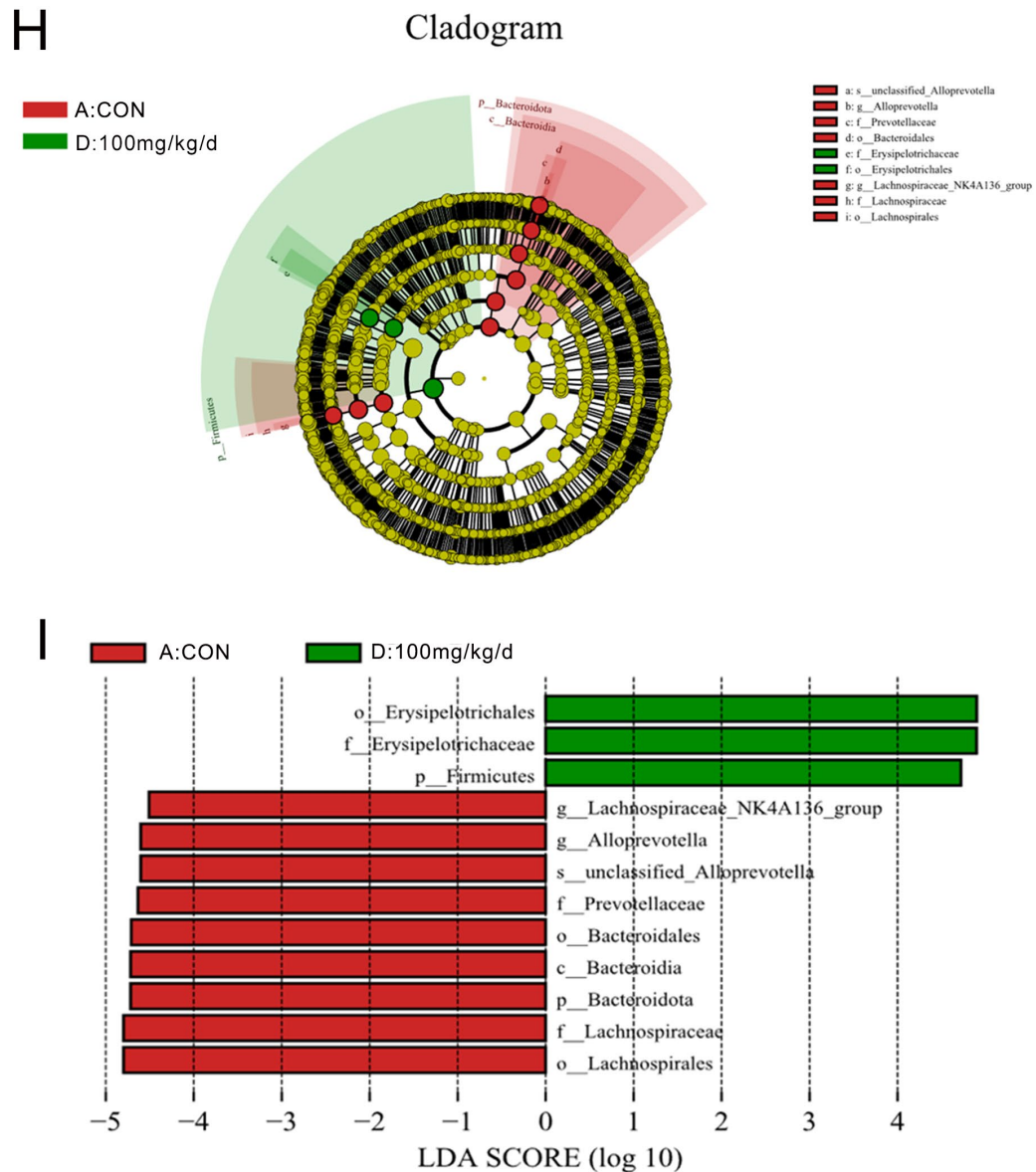


**Fig.6. GenX exposure caused the change of gut microbiota diversity in GD19.5 pregnant rats (n = 5 per group).** A-D: The ACE Shannon index, Chao1 index, Simpson index and Shannon index of  $\alpha$ -diversity in the gut microbiota. E-F: The variation of gut microbiota at the phylum level and genus level. G: PCoA analysis of gut microbiota. (H-I) The LefSe analysis and LDA SCORE bar chart.

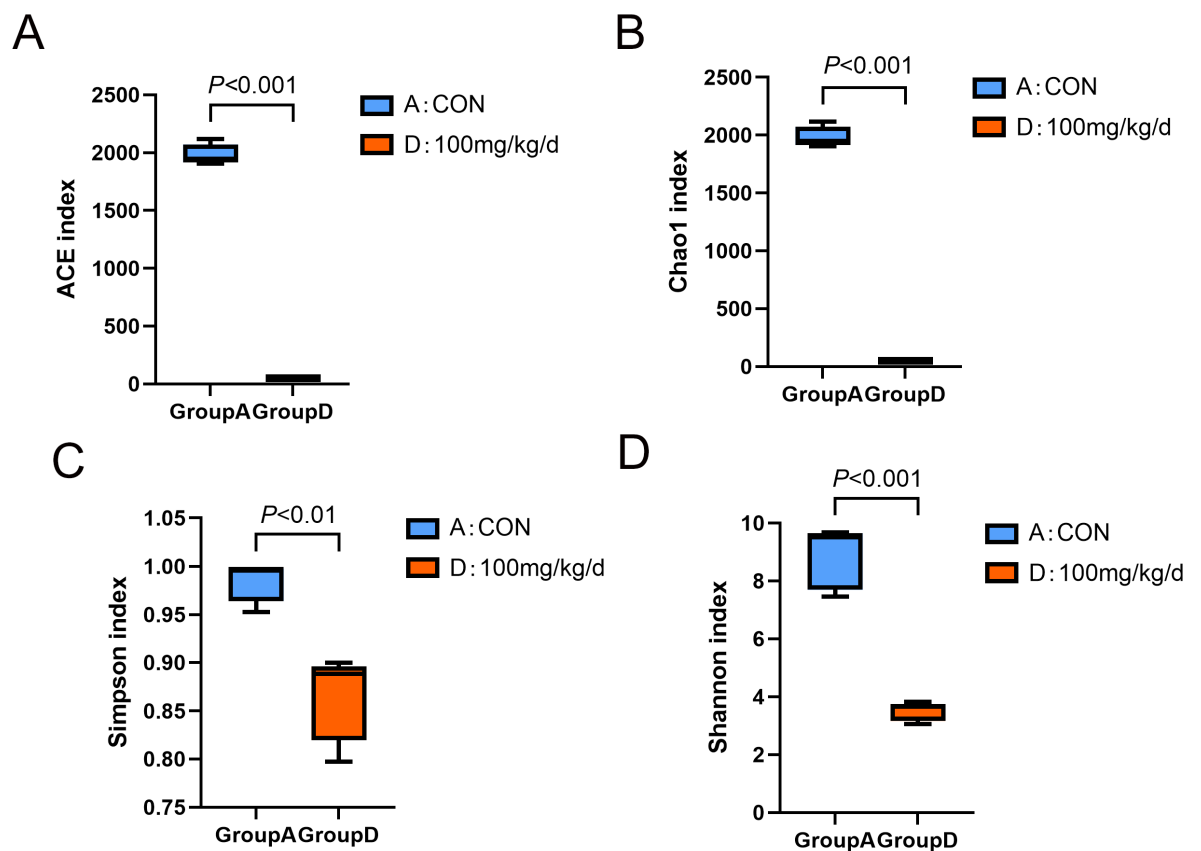
### 3.2.2 Gut microbiota changes in PND7 offspring rats

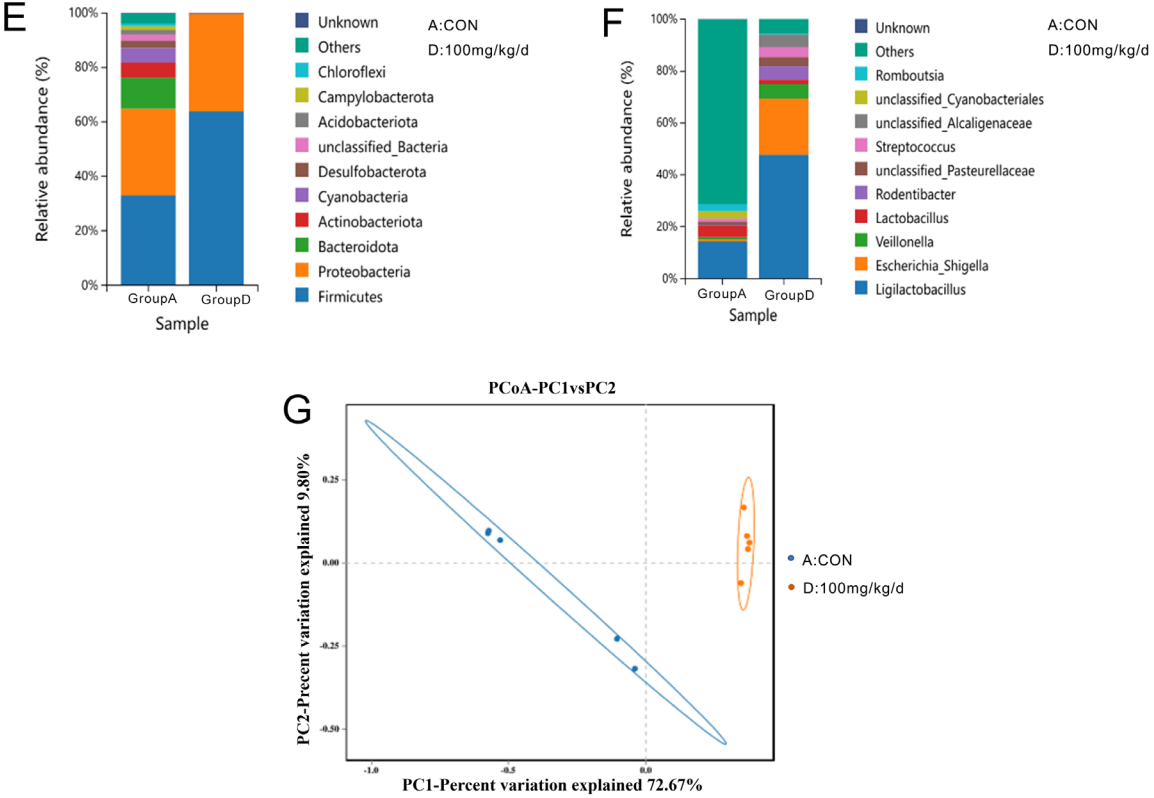
In offspring rats, Chao1, Ace, Simpson and Shannon index of 100mg/kg GenX-treated group were significantly lower than the control group, which suggested the reduced diversity of the gut content (Fig.7A-

D). Similar to pregnant rats,  $\beta$  diversity analysis by PCoA showed that the intestinal microbiota were clearly distinguishable in two groups (Fig.7 G).

At the phylum level, the dominant bacteria in the intestinal microbiota in control group and 100mg/kg GenX-treated group were both Firmicutes and Proteobacteria, with the sum more than 60%, especially over 95% in 100mg/kg GenX-treated group (Fig.7E-F). Compared with the control group, the relative abundance of Bacteroidetes and Actinobacteria in the 100mg/kg GenX-treated group was both significantly decreased ( $P < 0.01$ ), while the relative abundance of Firmicutes increased ( $P < 0.01$ ). As with pregnant rats, the dominant genus in offspring rats was *Ligilactobacillus*. Compared with the control group, a total of 529 species in 100mg/kg GenX-treated group had significantly different abundance ( $P < 0.05$ ), including 516 species showed decreased relative abundance (469 species were detected in 0 in the HFPO-DA exposed group), and 13 species showed increased relative abundance, suggesting that the exposure affected the abundance and diversity of GenX during pregnancy. At the level of the main species, relative abundance of *Bacteroides* and *Lachnospiraceae\_NK4A136\_group* in 100mg/kg GenX-treated group was significantly lower compared with the control group ( $P < 0.05$ ). Besides, the relative abundance of *Lactobacillus*, *Escherichia-Shigella*, *Streptococcus* and *Veillonella* was significantly increased compared with the control group ( $P < 0.01$ ).

Similar to the results of pregnant rats, LEfSe analysis showed the species enriched in the control group included Bacteroidetes, Alphaproteobacteria and Clostridia. The species enriched in the 100mg/kg/d GenX-treated group were Firmicutes, bacilli, Gammaproteobacteria, Lactobacillales, Enterobacteriales, Lactobacillaceae, Enterobacteriaceae, Pasteurellaceae, Combination Lactobacillus, Escherichia coliShigella, unclassified\_Ligilactobacillus, and unclassified\_Escherichia-Shigella (Fig.7H-I).





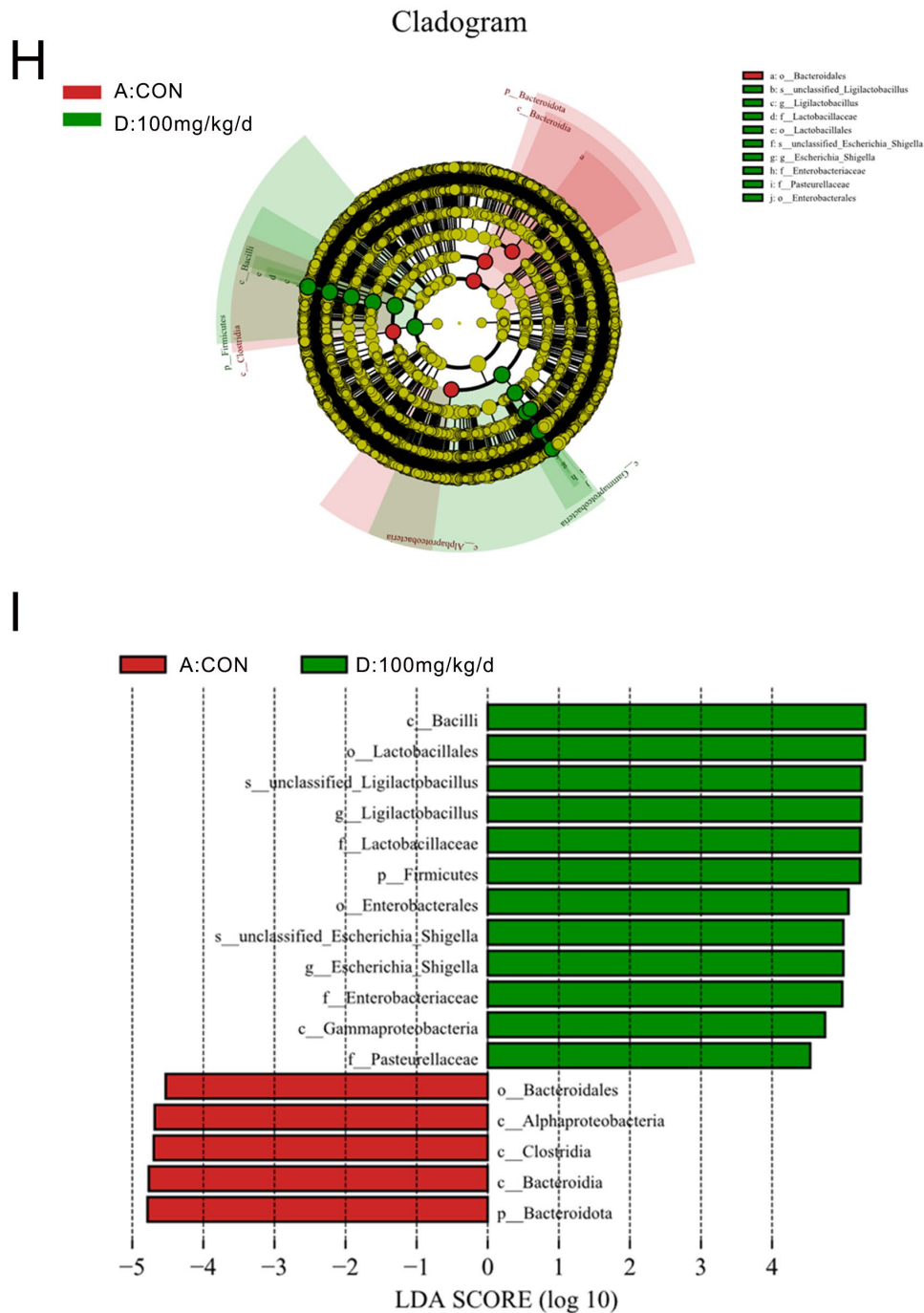


**Fig.7. GenX exposure caused the change of gut microbiota diversity in PND7 offspring rats (n = 5 per group).** A-D: The ACE Shannon index, Chao1 index, Simpson index and Shannon index of  $\alpha$ -diversity in the gut microbiota. E-F: The variation of gut microbiota at the phylum level and genus level. G: PCoA analysis of gut microbiota. H-I: The LEfSe analysis and LDA SCORE bar chart.

### 3.3 Effects of GenX on the Gut Metabolome of GD19.5 Pregnant Rats

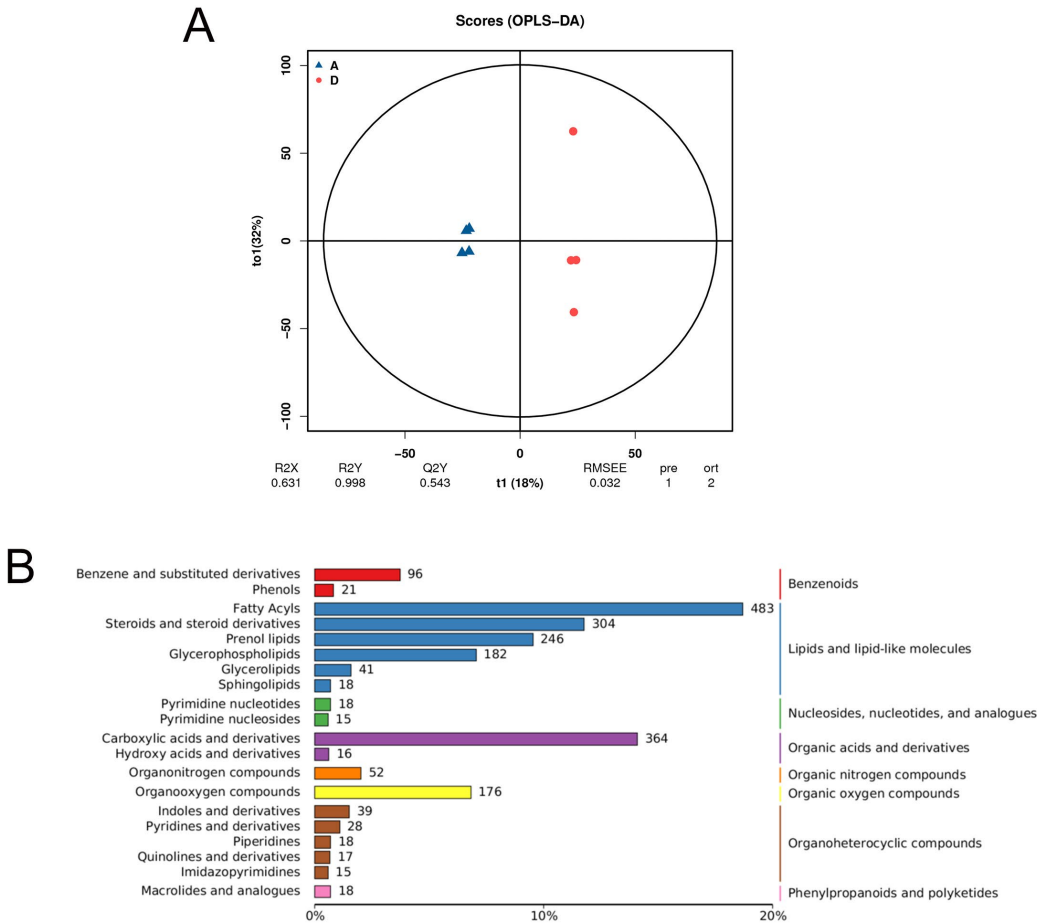
A total of 3,549 metabolites were identified from eight samples.

396 According to the OPLS-DA model, there was a clear separation between  
397 the control group and the 100 mg/kg GenX-treated group, and the sample  
398 model was effective ( $Q^2Y > 0.5$ ) (Fig. 8A). Most of these metabolites were  
399 classified as Lipids and lipid-like molecules and Organic acids and  
400 derivatives (Fig. 8B).

401 In comparison to the control group, 106 metabolites in the 100 mg/kg  
402 GenX-treated group showed significant differences ( $P < 0.05$ ), with 28  
403 metabolites increased and 78 decreased. The decreased metabolites  
404 included Nona-4,6-dienedioylcarnitine, D-glycero-beta-D-manno-Heptose  
405 1,7-bisphosphate, and 5-(2-Aminopropyl) indol, while the increased  
406 metabolites included 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine  
407 and 2-Hydroxymyristic acid (Fig. 8C).

408 Enrichment analysis of the differential metabolites based on the  
409 KEGG database identified the top 20 pathways annotated with the most  
410 differential metabolites. Among these, the pathway most associated with  
411 Carbohydrate Metabolism was the most prominent (Fig. 8D). The top three  
412 most notable pathways among the top 20 were Drug metabolism - other  
413 enzymes, Pentose phosphate pathway, and Phosphonate and phosphinate  
414 metabolism (Fig. 8E). In the 100 mg/kg GenX-treated group, metabolites  
415 associated with these three pathways, except for  
416 Monoethylglycinexylidide (which increased), showed a decrease. These  
417 included 6-Methylthiopurine 5'-monophosphate ribonucleotide, 6-

418 Thioxanthine 5'-monophosphate, 5-Fluorodeoxyuridine monophosphate,  
419 D-Glyceraldehyde 3-phosphate, D-Glucono-1,5-lactone 6-phosphate, 2-  
420 Deoxy-D-ribose 5-phosphate, Hydroxymethylphosphonate, CMP-2-  
421 aminoethylphosphonate, and 2-Phosphinomethylmalate. This suggests that  
422 GenX may affect multiple metabolic pathways, including Drug  
423 metabolism - other enzymes, Pentose phosphate pathway, and Phosphonate  
424 and phosphinate metabolism.



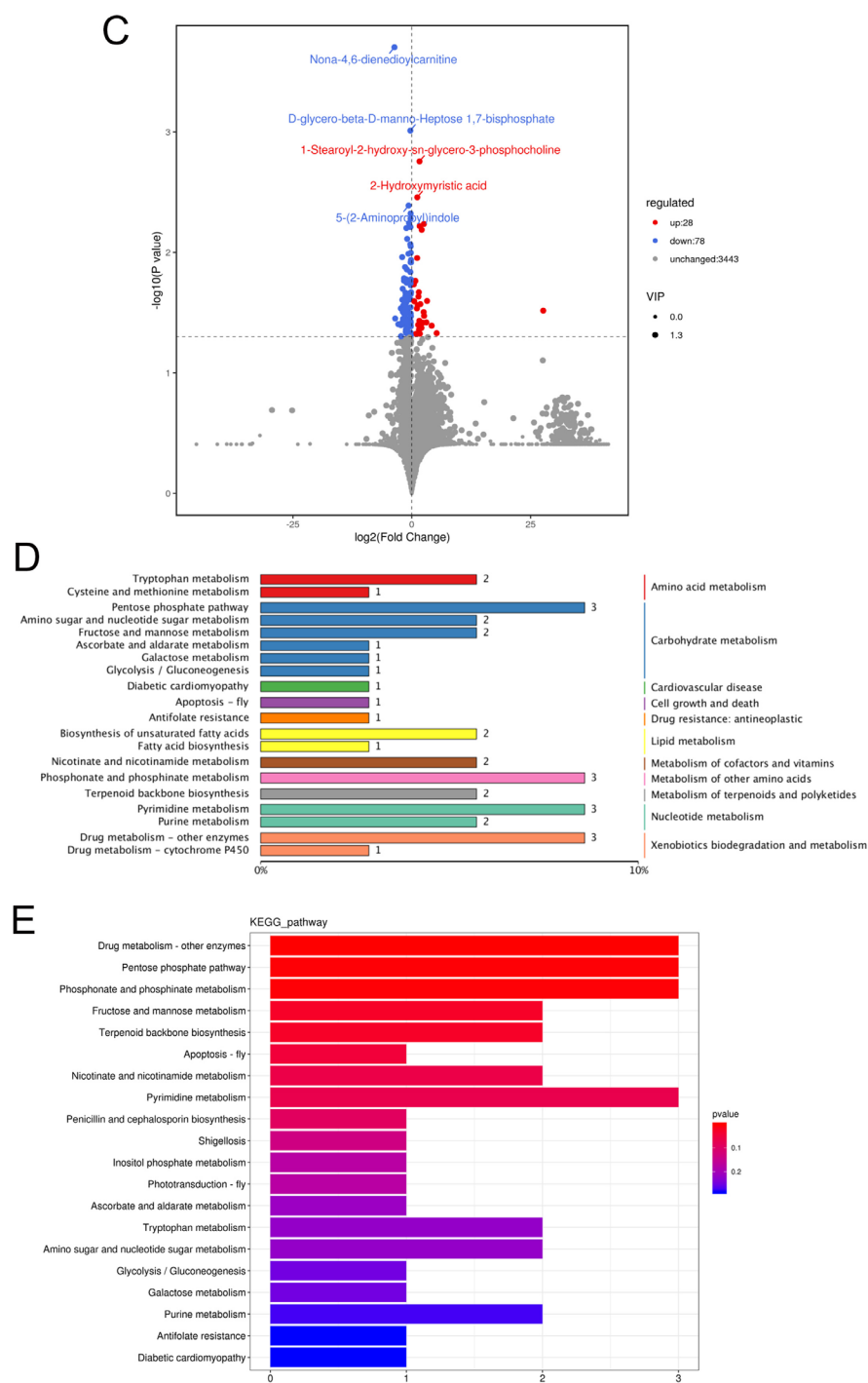


**Fig. 8. Effects of GenX on the Gut Metabolome of GD19.5 Pregnant Rats (n = 4 per group).** A: Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA). B: Classification of all metabolites based on the HMDB database. C: Volcano plot of differential metabolites. D: KEGG database annotation of differential metabolites, selecting the top 20 pathways annotated with the most differential metabolites, and creating a summary bar chart. E: Pathway enrichment analysis of differential metabolites.

#### 4. Discussion

PFOA has been shown to have adverse effects on the environment and human health(Li et al., 2017; Negri et al., 2017; Olsen et al., 2007). The gut is the organ that has the most external contact, therefore has a great risk of being attacked by toxic substances(Tanaka & Nakayama, 2017). Intestinal barrier impairment and dysbiosis have been observed in mice with PFOA exposure as low as 0.5 mg/kg/day(Shi et al., 2020). Similarly, prenatal PFOS exposure at doses ranging from 0.3–3 mg/kg/day was shown to compromise mucosal barrier integrity and disrupt microbiota in offspring mice(Yi et al., 2024). GenX, a novel alternative to PFOA, has been detected in various environmental media, including drinking water and food, posing a potential threat to human health (Galloway et al., 2020; Gebbink & van Leeuwen, 2020; Strynar et al., 2015). A study by Xie et al. demonstrated that subchronic exposure to 200 µg/L GenX via drinking water for six weeks was sufficient to induce significant disturbances in the intestinal microbiota of mice(Xie et al., 2021). In the present study, we found that exposure to a low concentration of GenX (1 mg/kg/day) during pregnancy also significantly reduced intestinal microbiota abundance and diversity in both maternal and offspring rats. This finding provides further evidence that the gut is highly susceptible to disruption by PFASs.

Bacteroidetes is one of the main bacterial phylum of the intestinal flora, which is mainly involved in many important metabolic activities in

the human colon and can maintain intestinal homeostasis through the production of short-chain fatty acids(Z.-Y. Chang et al., 2022). Compared with control rats, GenX exposure during pregnancy resulted in a significant decrease in the abundance of Bacteroidetes, and the Firmicutes / Bacteroidetes abundance ratio (F / B) increased significantly in the intestinal flora of both pregnant and their offspring rats. Of note, previous studies have shown that increased F / B is associated with intestinal inflammatory disease(C.-J. Chang et al., 2015; Frank et al., 2007; Yin et al., 2025). As a butyrate-producing strain, Lachnospiraceae\_NK4A136\_group was associated to be negatively associated with the occurrence of intestinal permeability and intestinal inflammation(Hu et al., 2019; Ma et al., 2020). The abundance of Lachnospiraceae\_NK4A136\_group showed a decreasing trend in the intestinal microflora of the GenX exposed group. Notably, Escherichia-Shigella, Vironella, Streptococcus and Lactobacillus, showed significant increases in the HFPO-DA exposure group. Previous studies have shown that Escherichia-Shigella is increased in patients with ulcerative colitis(Alberca et al., 2022; He et al., 2021). Furthermore, the genus Escherichia-Shigella acts as a TLR 4 agonist or ligand for the pathway of inflammasome activation and leading to the pro-inflammatory state of cirrhosis. Both Escherichia-Shigella and Streptococcus were associated with an increase in serum inflammatory cytokine IL-6. As a Gram-negative

bacterium, *Veillonella* can produce LPS and then induce an inflammatory  
respons(*Gedgaudas et al., 2022*) . The study of *Bogert et al* showed that a  
combination of *Streptococcus* and *Veillonella* could trigger different  
immune responses and enhanced levels of IL-8, IL-6, IL-10 and TNF-  
 $\alpha$ (*van den Bogert et al., 2014*). In addition, the results of LefSe analysis in  
pregnant rats showed that gut microbes in the GenX exposed group were  
enriched in *Dantofilamentosa* and *Dansipelicaceae*, which is considered to  
be closely associated with inflammation-related gastrointestinal diseases  
and metabolic disorders.

At the genus level, *Ligilactobacillus* was the dominant bacteria in the  
gut microbiota of both control and 100 mg/kg GenX-treated pregnant rats,  
suggesting a stable core role during pregnancy. *Ligilactobacillus salivarius*  
has been reported to improve conception rates and full-term pregnancy  
outcomes in women with infertility or recurrent miscarriage (*Huerga*  
*López et al., 2025*), and to enhance short-chain fatty acid production,  
exerting anti-inflammatory effects (*Huang et al., 2025*) and supporting host  
immunity(*Indo et al., 2021*). These findings indicate that *Ligilactobacillus*  
may contribute to maintaining gut stability, regulating immune responses,  
and supporting maternal metabolic adaptation. Its dominance under GenX  
exposure indicates that the chemical mainly affects non-dominant taxa and  
overall community function, without displacing the core genus.

The species with changed abundance in the intestinal microbiota of

501 pregnant and their offspring rats compared with the control suggested a  
502 correlation with the occurrence of intestinal inflammation. Based on this,  
503 we performed H&E staining on the intestinal (colon and ileal) tissues of  
504 pregnant rats, fetal rats (pnd0) and offspring rats to observe whether the  
505 histomorphology had inflammation-related manifestations. The results  
506 showed that GenX exposure caused obvious shedding of intestinal  
507 epithelial cells, and obvious erosion; impaired integrity of intestinal ileum,  
508 and local lymphoid focal hyperplasia, suggesting inflammatory changes.  
509 In light of these findings, Alcian Blue-Periodic Acid-Schiff (AB-PAS)  
510 staining was conducted on the ileum and colon tissues of pregnant rats. The  
511 results demonstrated a marked decrease in the number of AB-PAS-positive  
512 goblet cells and the quantity of intestinal mucus within the ileal and colonic  
513 mucosa. Intestinal mucus functions as a crucial barrier, separating the  
514 epithelial layer from the gut microbiota and facilitating the harmonious  
515 coexistence of symbiotic bacteria with the host organism. The reduction in  
516 intestinal mucus is associated with increased intestinal barrier permeability,  
517 which may potentially compromise the integrity of the mucosal defense  
518 mechanism(Pelaseyed et al., 2014). The findings in male mice by Xie et al.  
519 suggest that GenX exposure causes inflammation in the colon and ileum  
520 and impairs the colonic intestinal barrier function (Xie et al., 2021a). In our  
521 study, there was no obvious change in the intestinal tissue morphology of  
522 offspring rats, and the altered species abundance and diversity of offspring

rats may be related to the changes in the intestinal flora of pregnant mice. Previous studies have shown that the structure of intestinal flora in pregnant women with inflammatory bowel disease is changed and can further affect the intestinal flora composition of neonates, suggesting that exposure to adverse environmental factors during pregnancy can directly affect the intestinal microbial diversity of offspring(Ferretti et al., 2018; Torres et al., 2020). In metabolomics studies on pregnant mice, GenX has been found to affect the metabolism of various substances, including amino acid metabolism, carbohydrate metabolism, and lipid metabolism(J.-J. Zhang et al., 2025; Q.-Y. Zhang et al., 2024). Additionally, GenX exerts effects on carbohydrate metabolism and lipid metabolism. Relevant reports have indicated that HFPO-DA can activate the PPAR- $\alpha$  pathway, leading to alterations in hepatic lipid metabolism(Attema et al., 2022). In the correlation analysis between metabolites and gut microbiota in pregnant mice, it was found that the Firmicutes and Bacteroidetes phyla, which showed significant changes in the gut of pregnant mice and are closely related to intestinal inflammation, exhibited extremely high correlations with the vast majority of differential metabolites. Some related studies have indicated that the Firmicutes phylum is associated with the digestion of fats and proteins and can regulate nutrient absorption(Simpson & Campbell, 2015). Bacteroidetes are involved in carbohydrate metabolism, bile acid and steroid metabolism, and promote the formation of intestinal

mucosa(Schutzowski et al., 2019). This suggests that GenX may alter the composition and abundance of gut microbiota, thereby changing the metabolism of substances. Similarly, the metabolic, inflammatory, and immune changes caused by GenX in the body may also be the reasons for the disruption of gut microbiota. A study has have also shown that GenX can affect the gut microbiota and alter its metabolic pathways(Rashid et al., 2023).

Our results showed that exposure to GenX during pregnancy led to a decrease in microbial diversity, species abundance, and overall microbial richness in pregnant rats. Moreover, the species with different abundance differences in the intestinal microflora of pregnant rats and their offspring rats were mostly correlated with the development of intestinal inflammation. Further studies found that GenX exposure during pregnancy led to impaired ileal barrier, intestinal villus atrophy, reduction, and the occurrence of colonic inflammation in pregnant rats. As a novel surrogate of the typical perfluorinated compound PFOA, there are still few studies on the intestinal toxicity of GenX. The results of this study suggest that exposure to GenX during pregnancy may lead to the occurrence of intestinal inflammation and the disorder of the intestinal flora of pregnant and their offspring rats. Notably, intestinal structural alterations were observed in this study even at the lowest dose of 1 mg/kg/day. At this dose, the serum GenX concentration in rats was approximately 0.15µg/mL (Lv

et al., 2024). Using the rat-to-human conversion factor of 0.16, this level is higher than the human equivalent dose(Nair & Jacob, 2016). Reported serum levels of GenX in highly exposed human populations range from 0.13 to 1.72µg/L(Pan et al., 2017), suggesting that our lowest effective dose is roughly 13.9-fold higher than environmental exposure levels. This comparison, however, should be interpreted with caution, as the present study involved only 19.5 days of exposure and may not fully reflect the effects of chronic human exposure. More animal experiments and population epidemiological studies are needed to further explore the effects of exposure to GenX during pregnancy on the intestinal flora.

## **5. Conclusion**

Our study indicates that GenX exposure during pregnancy decreases gut microbial diversity and abundance in both pregnant rats and their offspring, impairs intestinal barrier integrity, reduces intestinal villus height, induces inflammatory changes in colonic tissue, and disrupts gut microbiome–metabolome homeostasis. These findings highlight the potential health risks of GenX and underscore the need for continued environmental monitoring and control.

## **Author contributions**

All authors reviewed, revised, and approved the final manuscript.

## **Data availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## **Funding**

This work was supported by the National Natural Science Foundation of China (Grant No. 81903271), the Shandong Province Medical and Health Science and Technology Project (Grant No. 202306031042), the Shandong Provincial Natural Science Fund for Young Scientists (Grant No. ZR2023QH301), Shandong Province Natural Science Foundation (No. ZR202111120048), 2022 Shinan District Science and Technology Plan Project (2023-2-015-YY), 2024 Qingdao Science and Technology Plan Park Cultivation Plan Project(24-1-5-yqpy-23-qy) and Qingdao Outstanding Health Professional Development Fund.

## **Compliance with ethical standards**

### **Ethical approval**

The experiment followed the institutional and national guidelines for the Care and Use of Laboratory Animals and was approved by the Ethics Committee of the Qingdao Municipal Hospital.

### **Conflict of interest**

The authors declare they have no actual or potential competing financial

611 interest.

612

613

614

615

616

617

618

619

620

621

622

623

624

625

626

627

628

629

630

631

632

## References

- Alberca, G. G. F., Cardoso, N. S. S., Solis-Castro, R. L., Nakano, V., & Alberca, R. W. (2022). Intestinal inflammation and the microbiota: Beyond diversity. *World Journal of Gastroenterology*, 28(26), 3274–3278. <https://doi.org/10.3748/wjg.v28.i26.3274>
- Attema, B., Janssen, A. W. F., Rijkers, D., van Schothorst, E. M., Hooiveld, G. J. E. J., & Kersten, S. (2022). Exposure to low-dose perfluorooctanoic acid promotes hepatic steatosis and disrupts the hepatic transcriptome in mice. *Molecular Metabolism*, 66, 101602. <https://doi.org/10.1016/j.molmet.2022.101602>
- Barry, V., Winquist, A., & Steenland, K. (2013). Perfluorooctanoic Acid (PFOA) Exposures and Incident Cancers among Adults Living Near a Chemical Plant. *Environmental Health Perspectives*, 121(11–12), 1313–1318. <https://doi.org/10.1289/ehp.1306615>
- Blake, B. E., Cope, H. A., Hall, S. M., Keys, R. D., Mahler, B. W., McCord, J., Scott, B., Stapleton, H. M., Strynar, M. J., Elmore, S. A., & Fenton, S. E. (2020). Evaluation of Maternal, Embryo, and Placental Effects in CD-1 Mice following Gestational Exposure to Perfluorooctanoic Acid (PFOA) or Hexafluoropropylene Oxide Dimer Acid (HFPO-DA or GenX). *Environmental Health Perspectives*, 128(2), 27006. <https://doi.org/10.1289/EHP6233>
- Butenhoff, J. L., Olsen, G. W., & Pfahles-Hutchens, A. (2006). The

655 applicability of biomonitoring data for perfluorooctanesulfonate to  
656 the environmental public health continuum. *Environmental Health*  
657 *Perspectives*, 114(11), 1776–1782. <https://doi.org/10.1289/ehp.9060>

658 Calafat, A. M., Kato, K., Hubbard, K., Jia, T., Botelho, J. C., & Wong, L.-  
659 Y. (2019). Legacy and alternative per- and polyfluoroalkyl  
660 substances in the U.S. general population: Paired serum-urine data  
661 from the 2013-2014 National Health and Nutrition Examination  
662 Survey. *Environment International*, 131, 105048.  
663 <https://doi.org/10.1016/j.envint.2019.105048>

664 Chang, C.-J., Lin, C.-S., Lu, C.-C., Martel, J., Ko, Y.-F., Ojcius, D. M.,  
665 Tseng, S.-F., Wu, T.-R., Chen, Y.-Y. M., Young, J. D., & Lai, H.-C.  
666 (2015). Ganoderma lucidum reduces obesity in mice by modulating  
667 the composition of the gut microbiota. *Nature Communications*, 6(1),  
668 7489. <https://doi.org/10.1038/ncomms8489>

669 Chang, Z.-Y., Liu, H.-M., Leu, Y.-L., Hsu, C.-H., & Lee, T.-Y. (2022).  
670 Modulation of Gut Microbiota Combined with Upregulation of  
671 Intestinal Tight Junction Explains Anti-Inflammatory Effect of  
672 Corylin on Colitis-Associated Cancer in Mice. *International Journal*  
673 *of Molecular Sciences*, 23(5), 2667.  
674 <https://doi.org/10.3390/ijms23052667>

675 Chappell, G. A., Thompson, C. M., Wolf, J. C., Cullen, J. M., Klaunig, J.  
676 E., & Haws, L. C. (2020). Assessment of the Mode of Action

677 Underlying the Effects of GenX in Mouse Liver and Implications for  
678 Assessing Human Health Risks. *Toxicologic Pathology*, 48(3), 494–  
679 508. <https://doi.org/10.1177/0192623320905803>

680 Conley, J. M., Lambright, C. S., Evans, N., McCord, J., Strynar, M. J., Hill,  
681 D., Medlock-Kakaley, E., Wilson, V. S., & Gray, L. E. (2021).  
682 Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) alters  
683 maternal and fetal glucose and lipid metabolism and produces  
684 neonatal mortality, low birthweight, and hepatomegaly in the  
685 Sprague-Dawley rat. *Environment International*, 146, 106204.  
686 <https://doi.org/10.1016/j.envint.2020.106204>

687 Conley, J. M., Lambright, C. S., Evans, N., Medlock-Kakaley, E., Hill, D.,  
688 McCord, J., Strynar, M. J., Wehmas, L. C., Hester, S., MacMillan,  
689 D. K., & Gray, L. E. (2022). Developmental toxicity of Nafion  
690 byproduct 2 (NBP2) in the Sprague-Dawley rat with comparisons to  
691 hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) and  
692 perfluorooctane sulfonate (PFOS). *Environment International*, 160,  
693 107056. <https://doi.org/10.1016/j.envint.2021.107056>

694 Coperchini, F., Croce, L., Denegri, M., Pignatti, P., Agozzino, M., Netti, G.  
695 S., Imbriani, M., Rotondi, M., & Chiovato, L. (2020). Adverse  
696 effects of in vitro GenX exposure on rat thyroid cell viability, DNA  
697 integrity and thyroid-related genes expression. *Environmental*  
698 *Pollution (Barking, Essex: 1987)*, 264, 114778.

699           <https://doi.org/10.1016/j.envpol.2020.114778>

700     Darrow, L. A., Howards, P. P., Winquist, A., & Steenland, K. (2014). PFOA  
701           and PFOS serum levels and miscarriage risk. *Epidemiology*  
702           (*Cambridge, Mass.*), 25(4), 505–512.  
703           <https://doi.org/10.1097/EDE.0000000000000103>

704     Dixit, F., Barbeau, B., Mostafavi, S. G., & Mohseni, M. (2020). Efficient  
705           removal of GenX (HFPO-DA) and other perfluorinated ether acids  
706           from drinking and recycled waters using anion exchange resins.  
707           *Journal of Hazardous Materials*, 384, 121261.  
708           <https://doi.org/10.1016/j.jhazmat.2019.121261>

709     Fei, C., McLaughlin, J. K., Lipworth, L., & Olsen, J. (2009). Maternal  
710           levels of perfluorinated chemicals and subfecundity. *Human*  
711           *Reproduction (Oxford, England)*, 24(5), 1200–1205.  
712           <https://doi.org/10.1093/humrep/den490>

713     Feng, X., Chen, X., Yang, Y., Yang, L., Zhu, Y., Shan, G., Zhu, L., & Zhang,  
714           S. (2021). External and internal human exposure to PFOA and  
715           HFPOs around a mega fluorochemical industrial park, China:  
716           Differences and implications. *Environment International*, 157,  
717           106824. <https://doi.org/10.1016/j.envint.2021.106824>

718     Ferretti, P., Pasolli, E., Tett, A., Asnicar, F., Gorfer, V., Fedi, S., Armanini,  
719           F., Truong, D. T., Manara, S., Zolfo, M., Beghini, F., Bertorelli, R.,  
720           De Sanctis, V., Bariletti, I., Canto, R., Clementi, R., Cologna, M.,

721 Crifò, T., Cusumano, G., ... Segata, N. (2018). Mother-to-Infant  
722 Microbial Transmission from Different Body Sites Shapes the  
723 Developing Infant Gut Microbiome. *Cell Host & Microbe*, 24(1),  
724 133-145.e5. <https://doi.org/10.1016/j.chom.2018.06.005>

725 Frank, D. N., St Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz,  
726 N., & Pace, N. R. (2007). Molecular-phylogenetic characterization  
727 of microbial community imbalances in human inflammatory bowel  
728 diseases. *Proceedings of the National Academy of Sciences of the*  
729 *United States of America*, 104(34), 13780–13785.  
730 <https://doi.org/10.1073/pnas.0706625104>

731 Galloway, J. E., Moreno, A. V. P., Lindstrom, A. B., Strynar, M. J., Newton,  
732 S., May, A. A., & Weavers, L. K. (2020). Evidence of Air Dispersion:  
733 HFPO-DA and PFOA in Ohio and West Virginia Surface Water and  
734 Soil near a Fluoropolymer Production Facility. *Environmental*  
735 *Science & Technology*, 54(12), 7175–7184.  
736 <https://doi.org/10.1021/acs.est.9b07384>

737 Gebbink, W. A., & van Leeuwen, S. P. J. (2020). Environmental  
738 contamination and human exposure to PFASs near a fluorochemical  
739 production plant: Review of historic and current PFOA and GenX  
740 contamination in the Netherlands. *Environment International*, 137,  
741 105583. <https://doi.org/10.1016/j.envint.2020.105583>

742 Gedgudas, R., Bajaj, J. S., Skieceviciene, J., Varkalaite, G., Jurkeviciute,

743 G., Gelman, S., Valantiene, I., Zyklus, R., Pranculis, A., Bang, C.,  
744 Franke, A., Schramm, C., & Kupcinskas, J. (2022). Circulating  
745 microbiome in patients with portal hypertension. *Gut Microbes*,  
746 14(1), 2029674. <https://doi.org/10.1080/19490976.2022.2029674>

747 He, X.-X., Li, Y.-H., Yan, P.-G., Meng, X.-C., Chen, C.-Y., Li, K.-M., & Li,  
748 J.-N. (2021). Relationship between clinical features and intestinal  
749 microbiota in Chinese patients with ulcerative colitis. *World Journal*  
750 *of Gastroenterology*, 27(28), 4722–4737.  
751 <https://doi.org/10.3748/wjg.v27.i28.4722>

752 Homepage—ECHA. (n.d.). Retrieved August 21, 2025, from  
753 <https://echa.europa.eu>

754 Hu, S., Wang, J., Xu, Y., Yang, H., Wang, J., Xue, C., Yan, X., & Su, L.  
755 (2019). Anti-inflammation effects of fucosylated chondroitin  
756 sulphate from *Acaudina molpadioides* by altering gut microbiota in  
757 obese mice. *Food & Function*, 10(3), 1736–1746.  
758 <https://doi.org/10.1039/c8fo02364f>

759 Huang, Y., Yang, C., Fu, B., Guo, H., Chen, Y., & Xu, D. (2025). Impact of  
760 *Ligilactobacillus salivarius* Li01 on benzo[a]pyrene-induced colitis,  
761 based on host-microbiome interactions in Mongolian gerbils.  
762 *Frontiers in Nutrition*, 12, 1494525.  
763 <https://doi.org/10.3389/fnut.2025.1494525>

764 Huerga López, C., Sánchez Martín, M. J., Herráez Moreta, A., Calvo

765 Urrutia, M., Cristóbal García, I., Díaz Morillo, C., Blanco-Rojo, R.,  
 766 Sáez, M. E., Olivares, M., Arroyo, R., Herranz, C., Alba, C.,  
 767 Rodríguez, J. M., & Fernández, L. (2025). *Ligilactobacillus*  
 768 *salivarius* CECT5713 Increases Term Pregnancies in Women with  
 769 Infertility of Unknown Origin: A Randomized, Triple-Blind,  
 770 Placebo-Controlled Trial. *Nutrients*, 17(11), 1860.  
 771 <https://doi.org/10.3390/nu17111860>

772 Indo, Y., Kitahara, S., Tomokiyo, M., Araki, S., Islam, M. A., Zhou, B.,  
 773 Albarracin, L., Miyazaki, A., Ikeda-Ohtsubo, W., Nochi, T.,  
 774 Takenouchi, T., Uenishi, H., Aso, H., Takahashi, H., Kurata, S.,  
 775 Villena, J., & Kitazawa, H. (2021). *Ligilactobacillus salivarius*  
 776 Strains Isolated From the Porcine Gut Modulate Innate Immune  
 777 Responses in Epithelial Cells and Improve Protection Against  
 778 Intestinal Viral-Bacterial Superinfection. *Frontiers in Immunology*,  
 779 12, 652923. <https://doi.org/10.3389/fimmu.2021.652923>

780 La Rocca, C., Tait, S., Guerranti, C., Busani, L., Ciardo, F., Bergamasco,  
 781 B., Stecca, L., Perra, G., Mancini, F. R., Marci, R., Bordi, G., Caserta,  
 782 D., Focardi, S., Moscarini, M., & Mantovani, A. (2014). Exposure  
 783 to endocrine disrupters and nuclear receptor gene expression in  
 784 infertile and fertile women from different Italian areas. *International*  
 785 *Journal of Environmental Research and Public Health*, 11(10),  
 786 10146–10164. <https://doi.org/10.3390/ijerph111010146>

787 Li, K., Gao, P., Xiang, P., Zhang, X., Cui, X., & Ma, L. Q. (2017).  
788 Molecular mechanisms of PFOA-induced toxicity in animals and  
789 humans: Implications for health risks. *Environment International*,  
790 99, 43–54. <https://doi.org/10.1016/j.envint.2016.11.014>

791 Lv, D., Liu, H., An, Q., Lei, C., Wang, Y., Sun, J., Li, C., Lin, Y., Dong, Q.,  
792 Yang, Z., Che, K., Liu, W., & Han, W. (2024). Association of adverse  
793 fetal outcomes with placental inflammation after oral gestational  
794 exposure to hexafluoropropylene oxide dimer acid (GenX) in  
795 Sprague-Dawley rats. *Journal of Hazardous Materials*, 461, 132536.  
796 <https://doi.org/10.1016/j.jhazmat.2023.132536>

797 Ma, L., Ni, Y., Wang, Z., Tu, W., Ni, L., Zhuge, F., Zheng, A., Hu, L., Zhao,  
798 Y., Zheng, L., & Fu, Z. (2020). Spermidine improves gut barrier  
799 integrity and gut microbiota function in diet-induced obese mice.  
800 *Gut Microbes*, 12(1), 1–19.  
801 <https://doi.org/10.1080/19490976.2020.1832857>

802 Nair, A. B., & Jacob, S. (2016). A simple practice guide for dose conversion  
803 between animals and human. *Journal of Basic and Clinical*  
804 *Pharmacy*, 7(2), 27–31. <https://doi.org/10.4103/0976-0105.177703>

805 Negri, E., Metruccio, F., Guercio, V., Tosti, L., Benfenati, E., Bonzi, R., La  
806 Vecchia, C., & Moretto, A. (2017). Exposure to PFOA and PFOS  
807 and fetal growth: A critical merging of toxicological and  
808 epidemiological data. *Critical Reviews in Toxicology*, 47(6), 482–

809 508. <https://doi.org/10.1080/10408444.2016.1271972>

810 Olsen, G. W., Burris, J. M., Ehresman, D. J., Froehlich, J. W., Seacat, A.  
811 M., Butenhoff, J. L., & Zobel, L. R. (2007). Half-Life of Serum  
812 Elimination of Perfluorooctanesulfonate, Perfluorohexanesulfonate,  
813 and Perfluorooctanoate in Retired Fluorochemical Production  
814 Workers. *Environmental Health Perspectives*, 115(9), 1298–1305.  
815 <https://doi.org/10.1289/ehp.10009>

816 Pan, Y., Zhang, H., Cui, Q., Sheng, N., Yeung, L. W. Y., Guo, Y., Sun, Y.,  
817 & Dai, J. (2017). First Report on the Occurrence and  
818 Bioaccumulation of Hexafluoropropylene Oxide Trimer Acid: An  
819 Emerging Concern. *Environmental Science & Technology*, 51(17),  
820 9553–9560. <https://doi.org/10.1021/acs.est.7b02259>

821 Pan, Y., Zhang, H., Cui, Q., Sheng, N., Yeung, L. W. Y., Sun, Y., Guo, Y.,  
822 & Dai, J. (2018). Worldwide Distribution of Novel Perfluoroether  
823 Carboxylic and Sulfonic Acids in Surface Water. *Environmental*  
824 *Science & Technology*, 52(14), 7621–7629.  
825 <https://doi.org/10.1021/acs.est.8b00829>

826 Pelaseyed, T., Bergström, J. H., Gustafsson, J. K., Ermund, A.,  
827 Birchenough, G. M. H., Schütte, A., van der Post, S., Svensson, F.,  
828 Rodríguez-Piñeiro, A. M., Nyström, E. E. L., Wising, C., Johansson,  
829 M. E. V., & Hansson, G. C. (2014). The mucus and mucins of the  
830 goblet cells and enterocytes provide the first defense line of the

831       gastrointestinal tract and interact with the immune system.  
832       *Immunological Reviews*, 260(1), 8–20.  
833       <https://doi.org/10.1111/imr.12182>

834       Petriello, M. C., Mottaleb, M. A., Serio, T. C., Balyan, B., Cave, M. C.,  
835       Pavuk, M., Birnbaum, L. S., & Morris, A. J. (2022). Serum  
836       concentrations of legacy and emerging per- and polyfluoroalkyl  
837       substances in the Anniston Community Health Surveys (ACHS I and  
838       ACHS II). *Environment International*, 158, 106907.  
839       <https://doi.org/10.1016/j.envint.2021.106907>

840       Rashid, F., Dubinkina, V., Ahmad, S., Maslov, S., & Irudayaraj, J. M. K.  
841       (2023). Gut Microbiome-Host Metabolome Homeostasis upon  
842       Exposure to PFOS and GenX in Male Mice. *Toxics*, 11(3), 281.  
843       <https://doi.org/10.3390/toxics11030281>

844       Schutkowski, A., König, B., Kluge, H., Hirche, F., Henze, A., Schwerdtle,  
845       T., Lorkowski, S., Dawczynski, C., Gabel, A., Große, I., & Stangl,  
846       G. I. (2019). Metabolic footprint and intestinal microbial changes in  
847       response to dietary proteins in a pig model. *The Journal of*  
848       *Nutritional Biochemistry*, 67, 149–160.  
849       <https://doi.org/10.1016/j.jnutbio.2019.02.004>

850       Shi, L., Zheng, J., Yan, S., Li, Y., Wang, Y., Liu, X., & Xiao, C. (2020).  
851       Exposure to Perfluorooctanoic Acid Induces Cognitive Deficits via  
852       Altering Gut Microbiota Composition, Impairing Intestinal Barrier

853 Integrity, and Causing Inflammation in Gut and Brain. *Journal of*  
854 *Agricultural and Food Chemistry*, 68(47), 13916–13928.  
855 <https://doi.org/10.1021/acs.jafc.0c05834>

856 Simpson, H. L., & Campbell, B. J. (2015). Review article: Dietary fibre-  
857 microbiota interactions. *Alimentary Pharmacology & Therapeutics*,  
858 42(2), 158–179. <https://doi.org/10.1111/apt.13248>

859 Steenland, K., Zhao, L., Winquist, A., & Parks, C. (2013). Ulcerative  
860 Colitis and Perfluorooctanoic Acid (PFOA) in a Highly Exposed  
861 Population of Community Residents and Workers in the Mid-Ohio  
862 Valley. *Environmental Health Perspectives*, 121(8), 900–905.  
863 <https://doi.org/10.1289/ehp.1206449>

864 Stiemsma, L. T., & Michels, K. B. (2018). The Role of the Microbiome in  
865 the Developmental Origins of Health and Disease. *Pediatrics*,  
866 141(4), e20172437. <https://doi.org/10.1542/peds.2017-2437>

867 Strynar, M., Dagnino, S., McMahan, R., Liang, S., Lindstrom, A.,  
868 Andersen, E., McMillan, L., Thurman, M., Ferrer, I., & Ball, C.  
869 (2015). Identification of Novel Perfluoroalkyl Ether Carboxylic  
870 Acids (PFECAs) and Sulfonic Acids (PFESAs) in Natural Waters  
871 Using Accurate Mass Time-of-Flight Mass Spectrometry (TOFMS).  
872 *Environmental Science & Technology*, 49(19), 11622–11630.  
873 <https://doi.org/10.1021/acs.est.5b01215>

874 Sun, S., Guo, H., Wang, J., & Dai, J. (2019). Hepatotoxicity of

875 perfluorooctanoic acid and two emerging alternatives based on a 3D  
876 spheroid model. *Environmental Pollution*, 246, 955–962.  
877 <https://doi.org/10.1016/j.envpol.2018.12.065>

878 Susic, D., Davis, G., O’ Sullivan, A. J., McGovern, E., Harris, K., Roberts,  
879 L. M., Craig, M. E., Mangos, G., Hold, G. L., El-Omar, E. M., &  
880 Henry, A. (2020). Microbiome Understanding in Maternity Study  
881 (MUMS), an Australian prospective longitudinal cohort study of  
882 maternal and infant microbiota: Study protocol. *BMJ Open*, 10(9),  
883 e040189. <https://doi.org/10.1136/bmjopen-2020-040189>

884 Świrkosz, G., Szczygiał, A., Logoń, K., Wrześniewska, M., & Gomułka,  
885 K. (2023). The Role of the Microbiome in the Pathogenesis and  
886 Treatment of Ulcerative Colitis—A Literature Review.  
887 *Biomedicines*, 11(12), 3144.  
888 <https://doi.org/10.3390/biomedicines11123144>

889 Tanaka, M., & Nakayama, J. (2017). Development of the gut microbiota in  
890 infancy and its impact on health in later life. *Allergology*  
891 *International: Official Journal of the Japanese Society of*  
892 *Allergology*, 66(4), 515–522.  
893 <https://doi.org/10.1016/j.alit.2017.07.010>

894 Thomas, S., Izard, J., Walsh, E., Batich, K., Chongsathidkiet, P., Clarke, G.,  
895 Sela, D. A., Muller, A. J., Mullin, J. M., Albert, K., Gilligan, J. P.,  
896 DiGuilio, K., Dilbarova, R., Alexander, W., & Prendergast, G. C.

897 (2017). The Host Microbiome Regulates and Maintains Human  
 898 Health: A Primer and Perspective for Non-Microbiologists. *Cancer*  
 899 *Research*, 77(8), 1783–1812. [https://doi.org/10.1158/0008-](https://doi.org/10.1158/0008-5472.CAN-16-2929)  
 900 5472.CAN-16-2929

901 Torres, J., Hu, J., Seki, A., Eisele, C., Nair, N., Huang, R., Tarassishin, L.,  
 902 Jharap, B., Cote-Daigneault, J., Mao, Q., Mogno, I., Britton, G. J.,  
 903 Uzzan, M., Chen, C.-L., Kornbluth, A., George, J., Legnani, P.,  
 904 Maser, E., Loudon, H., ... Peter, I. (2020). Infants born to mothers  
 905 with IBD present with altered gut microbiome that transfers  
 906 abnormalities of the adaptive immune system to germ-free mice. *Gut*,  
 907 69(1), 42–51. <https://doi.org/10.1136/gutjnl-2018-317855>

908 van den Bogert, B., Meijerink, M., Zoetendal, E. G., Wells, J. M., &  
 909 Kleerebezem, M. (2014). Immunomodulatory properties of  
 910 *Streptococcus* and *Veillonella* isolates from the human small  
 911 intestine microbiota. *PloS One*, 9(12), e114277.  
 912 <https://doi.org/10.1371/journal.pone.0114277>

913 Xie, X., Zhou, J., Hu, L., Shu, R., Zhang, M., Xiong, Z., Wu, F., & Fu, Z.  
 914 (2021). Exposure to hexafluoropropylene oxide dimer acid (HFPO-  
 915 DA) disturbs the gut barrier function and gut microbiota in mice.  
 916 *Environmental Pollution (Barking, Essex: 1987)*, 290, 117934.  
 917 <https://doi.org/10.1016/j.envpol.2021.117934>

918 Xu, L.-L., Chen, Y.-K., Zhang, Q.-Y., Chen, L.-J., Zhang, K.-K., Li, J.-H.,

919       Liu, J.-L., Wang, Q., & Xie, X.-L. (2022). Gestational exposure to  
920       GenX induces hepatic alterations by the gut-liver axis in maternal  
921       mice: A similar mechanism as PFOA. *Science of The Total*  
922       *Environment*,                               820,                               153281.  
923       <https://doi.org/10.1016/j.scitotenv.2022.153281>

924       Xu, X., Ni, H., Guo, Y., Lin, Y., Ji, J., Jin, C., Yuan, F., Feng, M., Ji, N.,  
925       Zheng, Y., & Jiang, Q. (2021). Hexafluoropropylene oxide dimer  
926       acid (HFPO-DA) induced developmental cardiotoxicity and  
927       hepatotoxicity in hatchling chickens: Roles of peroxisome  
928       proliferator activated receptor alpha. *Environmental Pollution*  
929       *(Barking, Essex: 1987)*,               290,               118112.  
930       <https://doi.org/10.1016/j.envpol.2021.118112>

931       Yi, W., Shi, J., Wang, L., Wang, D., Wang, Y., Song, J., Xin, L., & Jiang, F.  
932       (2024). Maternal PFOS exposure in mice induces hepatic lipid  
933       accumulation and inflammation in adult female offspring:  
934       Involvement of microbiome-gut-liver axis and autophagy. *Journal*  
935       *of Hazardous Materials*,           470,           134177.  
936       <https://doi.org/10.1016/j.jhazmat.2024.134177>

937       Yin, Y., Yang, T., Tian, Z., Shi, C., Yan, C., Li, H., Du, Y., & Li, G. (2025).  
938       Progress in the investigation of the Firmicutes/Bacteroidetes ratio as  
939       a potential pathogenic factor in ulcerative colitis. *Journal of Medical*  
940       *Microbiology*, 74(1). <https://doi.org/10.1099/jmm.0.001966>

941 Zhang, J.-J., Chen, Y.-K., Chen, Y.-Q., Zhang, Q.-Y., Liu, Y., Wang, Q., &  
 942 Xie, X.-L. (2025). Gestational GenX Exposure Induces Maternal  
 943 Hepatotoxicity by Disrupting the Lipid and Bile Acid Metabolism  
 944 Distinguished from PFOA-Induced Pyroptosis. *Toxics*, 13(8), 617.  
 945 <https://doi.org/10.3390/toxics13080617>

946 Zhang, Q.-Y., Xu, L.-L., Zhong, M.-T., Chen, Y.-K., Lai, M.-Q., Wang, Q.,  
 947 & Xie, X.-L. (2024). Gestational GenX and PFOA exposures induce  
 948 hepatotoxicity, metabolic pathway, and microbiome shifts in  
 949 weanling mice. *The Science of the Total Environment*, 907, 168059.  
 950 <https://doi.org/10.1016/j.scitotenv.2023.168059>

951 Zhang, S., Chen, K., Li, W., Chai, Y., Zhu, J., Chu, B., Li, N., Yan, J., Zhang,  
 952 S., & Yang, Y. (2021). Varied thyroid disrupting effects of  
 953 perfluorooctanoic acid (PFOA) and its novel alternatives  
 954 hexafluoropropylene-oxide-dimer-acid (GenX) and ammonium 4,8-  
 955 dioxa-3H-perfluorononanoate (ADONA) in vitro. *Environment*  
 956 *International*, 156, 106745.  
 957 <https://doi.org/10.1016/j.envint.2021.106745>

958 Zmora, N., Suez, J., & Elinav, E. (2019). You are what you eat: Diet, health  
 959 and the gut microbiota. *Nature Reviews. Gastroenterology &*  
 960 *Hepatology*, 16(1), 35–56. [https://doi.org/10.1038/s41575-018-](https://doi.org/10.1038/s41575-018-0061-2)  
 961 0061-2



**DSPACE**

<https://dspace.org/>

## **The effects of prenatal GenX (HFPO-DA) exposure on gut microbiota and metabolic function in pregnant rats and their offspring**

**Han, Wenchao; Zhang, Xinpeng; Li, Dan; Liu, Wendong; He, Yanxia ...**

**2026-01-15**

Elsevier

<https://doi.org/10.1016/j.envpol.2025.127363>

Han W, Zhang X, Li D, et al., (2026) The effects of prenatal GenX (HFPO-DA) exposure on gut microbiota and metabolic function in pregnant rats and their offspring. Environmental Pollution, Volume 389, January 2026, Article number 127363