Ecotoxicology and Environmental Safety 279 (2024) 116488

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Exposure to high concentrations of triphenyl phosphate altered functional performance, liver metabolism and intestinal bacterial composition of aquatic turtles

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ARTICLE INFO

Edited by: Dr R Pereira

Keywords:
Aquatic turtle
Triphenyl phosphate
Physiological performance
Antioxidant response
Hepatic metabolite
Intestinal microbiota

ABSTRACT

Organophosphorus flame retardants, such as triphenyl phosphate (TPhP), exist ubiquitously in various environments owing to their widespread usage. Potential toxic effects of residual flame retardants on cultured non-fish species are not concerned commonly. TPhP-induced physiological and biochemical effects in an aquatic turtle were evaluated here by systematically investigating the changes in growth and locomotor performance, hepatic antioxidant ability and metabolite, and intestinal microbiota composition of turtle hatchlings after exposure to different TPhP concentrations. Reduced locomotor ability and antioxidant activity were only observed in the highest concentration group. Several metabolic perturbations that involved in amino acid, energy and nucleotide metabolism, in exposed turtles were revealed by metabolite profiles. No significant among-group difference in intestinal bacterial diversity was observed, but the composition was changed markedly in exposed turtles. Increased relative abundances of some bacterial genera (e.g., *Staphylococcus*, *Vogesella* and *Lawsonella*) probably indicated adverse outcomes of TPhP exposure. Despite having only limited impacts of exposure at environmentally relevant levels, our results revealed potential ecotoxicological risks of residual TPhP for aquatic turtles considering TPhP-induced metabolic perturbations and intestinal bacterial changes.

1. Introduction

Organophosphorus flame retardants (OPFRs) that are synthesized from phosphorous substances are being increasingly widely used in various consumer products (van der Veen and de Boer, 2012). Currently, OPFRs can be ubiquitously detected in a variety of environments, and bioaccumulative in organisms that are frequently exposed to these compounds, or through the food chain (Andresen et al., 2004; Sala et al., 2021). Despite supposed lower toxicities, growing evidence has indicated that OPFRs can cause a series of toxicological effects, including neurotoxicity, hepatotoxicity, reproductive and developmental toxicity, etc. (Chen et al., 2019; Ramesh et al., 2020; Sun et al., 2022). A most common OPFR, triphenyl phosphate (TPhP) is largely used in polymer industries related to textiles, building materials, plastic packaging, etc., and dispersed into various environments, including air, water, soil environments, during its processes of production, utilization, and waste treatment (Xing et al., 2018). Consequently, the detectable levels of TPhP as well as its derived byproducts in these environments have been shown to be increasing with the increased production and usage (Tan et al., 2016).

The detection rates of TPhP can be almost 100% in water samples collected from various sites worldwide, and the maximum detected concentration reaches up to 7.9 μg/L in river surface water (e.g., reported concentrations of 10–200 ng/L in Germany, Andresen et al., 2004; 0.9–5.2 ng/L in China, Zhang et al., 2018; 41–360 ng/L in US, Sutton et al., 2019; up to 7.9 μg/L in Denmark, Li et al., 2019; Zhang et al., 2022), and up to 14 μg/L in the effluent of sewage treatment plants (Lu et al., 2021; Umamaheswari et al., 2021). TPhP can enter and accumulate in the body of aquatic organisms via skin absorption, food intake and others, and accordingly has been easily detected in various tissues of aquatic organisms (Kim et al., 2011). As a typical OPFR, similar toxic effects due to TPhP exposure are observed in aquatic

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https://doi.org/10.1016/j.ecoenv.2024.116488
Received 8 January 2024; Received in revised form 26 March 2024; Accepted 19 May 2024

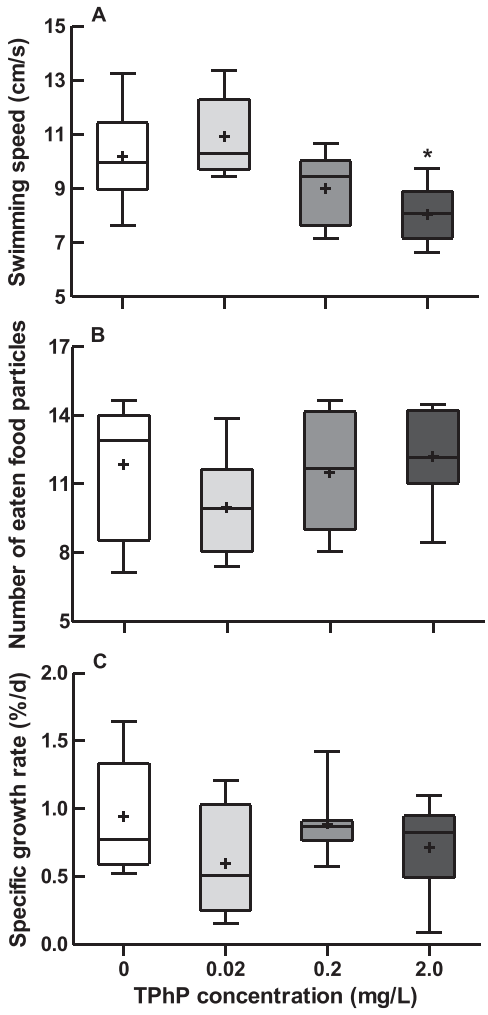

Fig. 1. Swimming speed (A), daily food intake (B) and specific growth rate (C) of *Pelodiscus sinensis* hatchlings exposed to 0 (control), 0.02, 0.2 and 2.0 mg/L TPhP. Asterisk above the box and whisker plot indicated a significant difference from the control group (*t* test, $^*P < 0.05$).

organisms, including invertebrates, fish and amphibians (Yuan et al., 2018; Ramesh et al., 2020; Lu et al., 2021; Chen et al., 2022). For example, long-term TPhP exposure would lead to an obvious accumulation in various tissues, and thus cause oxidant/antioxidant imbalance, biochemical alteration and morphological abnormality in fish (Ramesh et al., 2020; Yao et al., 2021). In recent years, increasing studies on toxic effects of TPhP (and other OPFRs) have been conducted in non-model aquatic organisms (Lu et al., 2021; Wang et al., 2022), but our understanding of its potential impacts in these species is still limited (no studies conducted on turtle species to date).

The liver and gut are two important organs responsible for metabolizing various compounds and maintaining homeostasis, and can interact with each other (termed as the gut-liver axis). Through the portal vein, intestine-derived bacterial products are transported to the liver, and alter its metabolic or physiological state, while liver-synthesized primary bile acids are carried to the intestine, and can shape intestinal bacterial community (Tripathi et al., 2018). Investigating the changes in some physiological parameters of these two organs has become a commonly used measure in toxicology testing. Under pollutant stress, the balance of hepatic oxidative/antioxidant system and intestinal microbiota might be disrupted due to excessive production of free radicals in the liver during the metabolism of exogenous toxic chemicals (Chen et al., 2022), and the change in the composition of bacterial community (Zhang et al., 2017). Exposure to TPhP (or other OPFRs), even at low environmental concentrations, might cause distinct hepatic antioxidant (enzymatic and non-enzymatic) responses (Yan et al., 2017; Ding et al., 2022), and intestinal microbiota disorders in aquatic organisms (Wang et al., 2022; Zhang et al., 2022). A recent study showed that TPhP exposure altered intestinal bacterial composition and gene expression along gut-liver axis, and disturbed hepatic lipid metabolism in mice (Liu et al., 2023).

The Chinese soft-shelled turtle (*Pelodiscus sinensis*) is a reptile species that widely distributed in China, as well as in other parts of East Asia and South-East Asia. It can serve as a good experimental model for toxicological studies in aquatic reptiles (Meng et al., 2023). In this study, a series of physiological indicators (including feeding, swimming and growth performances, hepatic biochemical and metabolomic profiles, and intestinal microbiota) were measured from TPhP-exposed *P. sinensis* hatchlings (at concentration levels of 0.02, 0.2, or 2 mg/L for 30 days). TPhP-induced physiological and metabolic changes have been revealed by microbiomic and metabolomic analysis in some aquatic species (e.g., hepatic metabolite changes in amphibians, Lu et al., 2021; intestinal microbiota changes in fish, Wang et al., 2022; Zhang et al., 2022). Based on these study findings, TPhP-induced physiological, bacterial and metabolic changes in *P. sinensis* could be expected.

2. Material and methods

2.1. Chemical reagent and testing kit

Triphenyl phosphate (TPhP > 99% purity, CAS no. 115–86–6) and chemical reagents required for sample processing and liquid chromatography-mass spectrometry (LC-MS) detection was purchased from the Sinopharm Chemical (or Aladdin) Reagent Co., Ltd. (Shanghai, China). TPhP was dissolved in dimethyl sulfoxide (DMSO) to create concentrated stock solutions (Lu et al., 2021). Assay kits for detecting the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and the content of reduced glutathione (GSH) in hepatic tissue of turtles were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Experimental animal and treatment

New-laid *P. sinensis* eggs ($N = 120$) were collected from a Huzhou private farm, and incubated at 28 ℃. Hatchling turtles were kept individually. Approximately 40 days after hatching, 56 healthy hatchlings (14 individuals in each treatment group) were selected for the TPhP exposure experiment. Egg incubation, hatchling collection and rearing, and experimental exposure process were similar to those described in our previous studies (Kang et al., 2022; Lu et al., 2022; Meng et al., 2023). TPhP exposure concentrations were set at 0, 0.02, 0.2, or 2 mg/L. The concentration of 0.02 mg/L was used here because it closely approximated the maximum concentration of TPhP reported in water samples from sewage treatment plants, and we took a possible increase of TPhP level into consideration due to its increased usage over the last few years (Lu et al., 2021; Zhang et al., 2024), while concentrations of 0.2 and 2 mg/L TPhP represent 10- and100-fold above the level, respectively. The quality parameters of rearing water were as follow: pH, 7.6 ± 0.26; dissolved oxygen, 6.3 ± 0.15 mg/L; conductivity, 141.6 ± 7.61 μS/cm; ammonia-nitrogen, 0.14 ± 0.08 mg/L.

2.3. Animal functional performance

Thirty-two hatchlings (8 individuals in each treatment group) were used to measure the food intake and growth rate (during exposure), and swimming performance (after a 30-day exposure). The measurements of the feeding, growth and swimming performance were conducted as described in our previous studies (Kang et al., 2022; Lu et al., 2022; Meng et al., 2023).

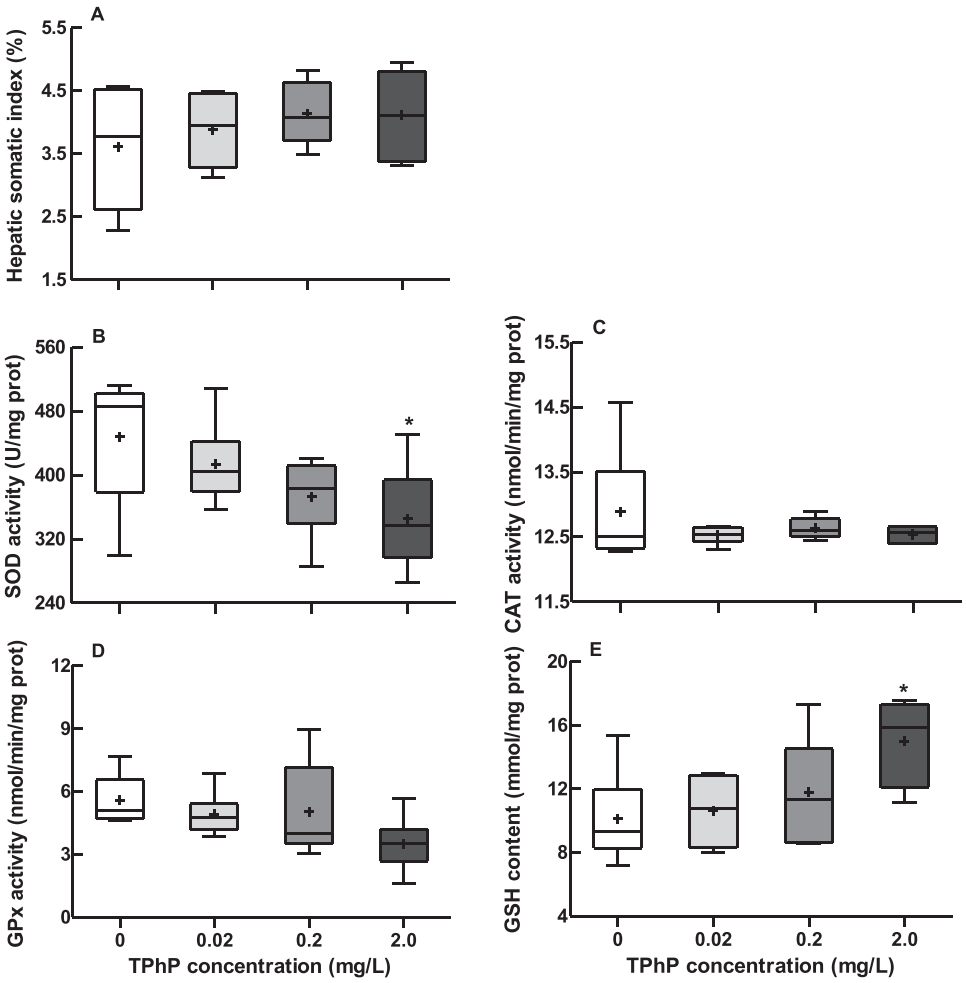

Fig. 2. Hepatic somatic index (A), SOD (B), CAT (C) and GPx (D) activities, and GSH content (E) of *Pelodiscus sinensis* hatchlings exposed to 0 (control), 0.02, 0.2 and 2.0 mg/L TPhP. Asterisks above the box and whisker plots indicated significant differences from the control group (*t* test, $^*P < 0.05$).

2.4. Hepatic antioxidant activity and metabolomic analysis

After a 30-day exposure, remaining 24 hatchlings (6 individuals in each treatment group) were sacrificed for liver and intestinal tissues. The liver tissue from each individual turtle was divided into two portions. One portion was used to measure the antioxidant activities (SOD, CAT and GPx enzymatic activities, and nonenzymatic GSH content), and another portion (approximately 100 mg) was used to measure the metabolite profiles. Detailed procedures for sample collection, processing and preparation, hepatic antioxidant activity determination, and non-targeted liquid chromatography-mass spectrometry (LC-MS) based metabolomic analysis were performed as described in our previous studies (Lu et al., 2022; Meng et al., 2023). The hepatic metabolomic analysis was completed by the Suzhou PANOMIX Biomedical Tech Co., Ltd. (Suzhou, China).

2.5. Intestinal microbiomic analysis

Intestinal samples were used to analyze the microbiota profiles, and detailed procedure for microbiomic analysis was performed as described in our previous studies (Kang et al., 2022; Meng et al., 2023). The intestinal microbiomic analysis was completed by the Hangzhou Kaitai Biotechnology Co., Ltd. (Hangzhou, China).

2.6. Statistical analysis

Differences in turtle swimming speed, food intake, specific growth rate, hepatic somatic index, antioxidant parameters (SOD, CAT, GPx activities, and GSH content) and metabolites (their spectral areas), alpha diversity index of intestinal bacterial community (Chao index) among treatment groups were performed using one-way analysis of variance (ANOVA). The functional metagenomes of intestinal bacteria were predicted using PICRUSt 2. Differences in relative abundances of intestinal bacteria and predicted KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways at different levels among groups were performed using nonparametric Kruskal-Wallis test. Further comparisons between the exposure and the control group were performed using student's *t* test or nonparametric Mann-Whitney test. Among-group difference in hepatic metabolite profiles was also performed using partial least squares discriminant analysis (PLS-DA), and difference in intestinal bacterial abundance and similarity of bacterial composition were performed using principal coordinates analysis (PCoA) and analysis of similarities (ANOSIM) based on Bray-Crutis distance, respectively. Before parametric analyses, normality and homogeneity of variances were confirmed using Kolmogorov–Smirnov test and Levene's test.

3. Results

3.1. Hatchling performances during exposure

No deaths occurred during the exposure period. The 2 mg/L TPhP-exposed hatchlings exhibited a poorer locomotor performance than those of control and low-concentration exposure groups ($F_{3,\ 28} = 6.54$, $P < 0.01$, Fig. 1A). However, no apparent impacts on feeding (number of

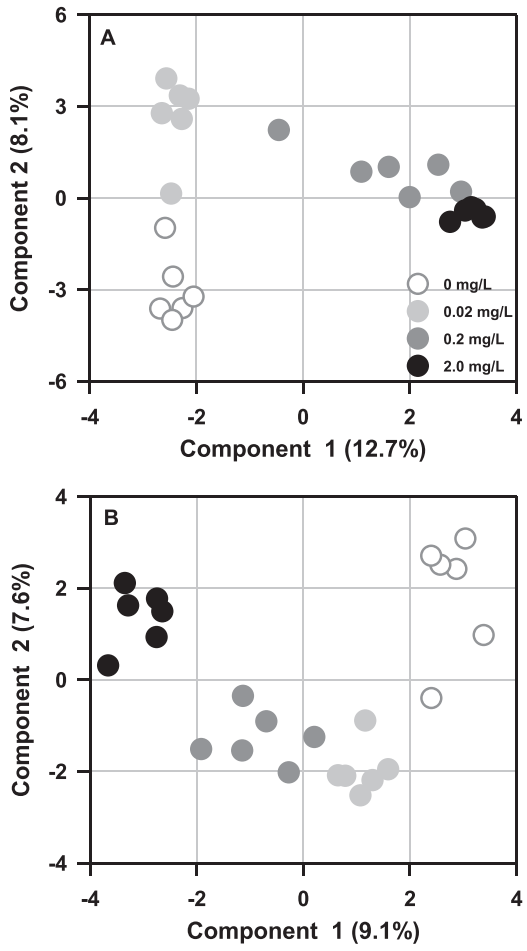

Fig. 3. Score plots for partial least squares discriminant analysis (PLS-DA) in the positive (A) and negative (B) ion mode for hepatic metabolite profiles in *Pelodiscus sinensis* hatchlings exposed to 0 (control), 0.02, 0.2 and 2.0 mg/L TPhP.

eaten food particles, $F_{3,\ 28} = 1.29$, $P = 0.296$, Fig. 1B) and growth performance (specific growth rate, $F_{3,\ 28} = 1.65$, $P = 0.200$, Fig. 1C) were found in hatchlings following TPhP exposure.

3.2. Hepatic antioxidant responses

After exposure, the relative liver size of turtles was not significantly different among groups (hepatic somatic index: $F_{3,\ 20} = 0.73$, $P = 0.545$, Fig. 2A). Hepatic antioxidant responses of hatchlings were shown to be changed by TPhP exposure. The SOD activity ($F_{3,\ 20} = 3.05$, $P = 0.052$, Fig. 2B) of liver tissue appeared to be lower, but GSH content ($F_{3,\ 20} = 3.72$, $P = 0.028$, Fig. 2E) in the 2 mg/L TPhP-exposed group was higher than that in the control group, although no among-group differences in CAT ($F_{3,\ 20} = 0.83$, $P = 0.495$, Fig. 2C) and GPx activities ($F_{3,\ 20} = 1.96$, $P = 0.152$, Fig. 2D) were found here.

3.3. Hepatic metabolite profile

PLS-DA analysis of metabolite profiles revealed a distinct among-group separation, with 20.8% (in the positive ion mode, Figs. 3A) and 16.7% (in the negative ion mode, Fig. 3B) of cumulative variances from the first two latent components, respectively. Some key metabolites were identified, and exhibited significant differences among groups (all $P < 0.05$, Table 1). Identified metabolites were involved in amino acid, energy and nucleotide metabolism, etc. Some amino acids (such as leucine, citrulline, arginine, etc.) were shown to increase after TPhP exposure. Similarly, some hepatic metabolites (such as glutathione, niacinamide, uridine, dopamine, arabinose, etc.) increased, but others (such as nicotinamide adenine dinucleotide-NAD, adenosine diphosphate-ADP, uridine diphosphate-UDP, norepinephrine, etc.) decreased after TPhP exposure (Table 1).

3.4. Intestinal microbiota

In this study, a total of 2 660 293 raw reads and 1 572 415 clean reads were obtained (Table S1). Based on the rarefaction curve of the Chao index, the sequencing depth would be sufficient (Fig. S1). A total of 950 operational taxonomic units (OTUs) were generated from the sequences. Among these, 312 OTUs were shared among different groups, and 46, 87, 88 and 70 OTUs were exclusive to the 0, 0.02, 0.2 and 2.0 mg/L TPhP-exposed groups, respectively (Fig. S2). There was no among-group difference in the bacterial community diversity (Chao index, $F_{3,\ 20} = 0.43$, $P = 0.735$, Fig. 4A). Among-group separation could not be identified by PCoA analysis (Fig. S3). However, ANOSIM analysis revealed a significant among-group difference in intestinal bacterial community ($R = 0.21$, $P < 0.01$, Fig. S3).

The relative abundance of the intestinal bacterial community varied after TPhP exposure (Fig. S4). For example, at the genus level, the relative abundances of *Halomonas* (15.2 ± 2.0%, $H = 7.77$, $P = 0.051$), *Chryseobacterium* (8.8 ± 1.2%, $H = 8.60$, $P = 0.035$), *Pelagibacterium* (4.7 ± 0.6%, $H = 8.45$, $P = 0.038$), *Hyphomicrobium* (2.5 ± 0.3%, $H = 7.97$, $P = 0.047$), *Clostridium_sensu_stricto_1* (2.2 ± 0.3%, $H = 6.90$, $P = 0.075$) and *Deinococcus* (2.2 ± 0.3%, $H = 12.34$, $P < 0.01$) were > 2%, and differed significantly among groups (Fig. 4B, C). Similarly, some genera accounted for a low proportion in bacterial community (e.g., *Vogesella*, 0.040 ± 0.001%; *Nocardioides*, 0.015 ± 0.004%; *Lawsonella*, 0.015 ± 0.005%; *Arcicella*, 0.010 ± 0.003%), but they increased significantly or were mainly observed in those TPhP-exposed groups (Fig. 4C).

PICRUSt 2 analysis identified 176 KEGG pathways at three hierarchical levels. At the top level, there were no between-group differences in relative abundances of these primary pathways (e.g., metabolism, genetic information processing, cellular processes). At the second (e.g., amino acid metabolism, terpenoids and polyketides metabolism, transcription) and third levels [e.g., biosynthesis of ansamycins, valine leucine and isoleucine degradation, lipoic acid metabolism, tricarboxylic acid (TCA) cycle], relative abundances of genes related to some pathways differed significantly among groups (all $P < 0.05$, Fig. S5). Relative abundances of genes related to valine leucine and isoleucine degradation, lipoic acid metabolism, TCA cycle, were lower in higher-concentration (0.2 and 2.0 mg/L TPhP) exposure groups (Fig. S6).

4. Discussion

Growing evidence indicates that, as an emerging contaminant, exposure to TPhP, as well as to other OPFRs, could cause remarkable metabolic disorders and other toxic symptoms in aquatic organisms (Chen et al., 2022; Wiegand et al., 2023). Relatively high contents of OPFRs can already be detected in turtle tissues (Sala et al., 2021). However, relevant results from aquatic turtle species under exposure to OPFRs are not available currently. Here, we evaluated for the first time the potential toxic impacts of chronic TPhP exposure in an aquatic turtle using multiple endpoints. A marked growth-inhibitory effect could be observed in some fish species following TPhP exposure (Wang et al., 2022). Contrarily, it was not found in *P. sinensis* even exposed to TPhP at concentrations much higher than environmentally relevant levels.

TPhP has been documented to have endocrine disrupting effects, and may alter the physiological properties and function of aquatic organisms (Li et al., 2019). Decreased locomotion ability in the highest-concentration exposure group might be associated with physiological dysfunction (e.g., impairment of visual acuity, malformation of muscle tissue) caused by TPhP exposure (Shi et al., 2019; Zhang et al.,

Table 1
Some differential hepatic metabolites, and their logarithmic (base 2) fold changes (FC) against the control group, and associated *P*-values (*t* test, * $P < 0.05$, ** $P < 0.01$) in different TPhP treatment (0.02, 0.2 or 2.0 mg/L) groups.

	0.02 mg/L *vs* 0 mg/L		0.2 mg/L *vs* 0 mg/L		2.0 mg/L *vs* 0 mg/L	
Metabolites	Log_2(FC)	*P*	Log_2(FC)	*P*	Log_2(FC)	*P*
Amino acid metabolism						
Leucine	0.30	*	0.43	*	0.40	*
Citrulline	0.65		0.03		3.27	**
Arginine	1.03	*	0.39	*	0.47	
Cysteine	-0.49		0.62		1.19	*
Aminoadipic acid	-0.53		-0.47		2.83	**
Antioxidant system						
Glutathione	0.59		0.58		1.13	*
Energy metabolism						
Niacinamide	0.61		0.68	*	0.56	
NAD	0.81		-0.12		-1.47	**
NADH	0.98		1.38	*	0.16	
Nucleotide metabolism						
ADP	-0.39		-1.66	*	-1.35	*
Uridine	0.52	*	0.05		2.61	**
UDP	-0.53	*	-0.37		-0.09	
D-Ribose	1.05		0.43		1.20	**
Carbohydrate metabolism						
D-Arabinose	1.45		1.98	*	2.63	*
D-Glucose	0.77		-0.48		1.2	*
D-Mannose	0.75		0.40		0.77	*
Related to lipid metabolism and neurotransmission						
Myristoleic acid	0.03		0.34	*	0.19	
Stearolic acid	0.04		2.28	*	1.42	
Norepinephrine	-0.35		-0.69		-1.59	**

2024). Certainly, these aspects of potential physiological dysfunction in TPhP-exposed turtles need to be evaluated in future studies.

TPhP exposure would significantly affect the antioxidant defense response of *P. sinensis* hatchlings, although no significant changes in CAT and GPx activities were shown in TPhP-exposed groups. SOD is involved in the process of antioxidation and integrated detoxification, and plays a function of scavenging superoxide free radicals for organisms when exposed to contaminated environments (Chen et al., 2022). Decreased hepatic SOD activity in 2 mg/L TPhP-exposed turtles might result from the excessive consumption during the process of scavenging free radicals. Moreover, GSH can play a protective role from oxidative damage in living cells (Mehdi et al., 2021). Increased hepatic GSH content in 2 mg/L TPhP-exposed turtles (also indicated by identified GSH level from metabolomic data) might be a protective compensatory response against the oxidative damage when exceeding the capacity of scavenging free radicals of antioxidant enzymes.

Comparative analysis with those results from previous studies indicated that the sensitivity of antioxidant responses to TPhP exposure in *P. sinensis* might be lower than in other aquatic species. For example, significantly altered hepatic SOD and/or CAT activities were shown when the TPhP exposure concentrations were $\geq$ 4 μg/L in *Labeo rohita* fingerlings (Umamaheswari et al., 2021), 20 μg/L in *Danio rerio* (Ramesh et al., 2020), 50 and 100 μg/L in *Polypedates megacephalus* (Chen et al., 2022) and *Polypedates megacephalus* tadpoles (Ding et al., 2022), and 0.5 mg/L in *Mytilus galloprovincialis* (Meng et al., 2020), whereas those did not occur under these exposure concentrations in *P. sinensis*. Similarly, the GPx activity was changed in *M. galloprovincialis* exposed to 0.5 mg/L TPhP (Meng et al., 2020), but that was not evident in *P. sinensis* even at a concentration of 2 mg/L.

Some liver metabolites were identified to be differentially-expressed in *P. sinensis* hatchlings after TPhP exposure. Of them, some amino acids were altered significantly in TPhP-exposed turtles, probably reflecting metabolic disorders in amino acids. Unexpectedly, the levels of liver leucine, citrulline and arginine showed upward trends in TPhP-exposed turtles. Previous studies have documented that the exogenous supply of these amino acids would contribute to improving the immune and antioxidant ability in fish (Zhou et al., 2020). Whether increased levels of these amino acids were compensatory responses under TPhP-induced oxidative stress requires further confirmation. Similarly, increased liver leucine level was found in TPhP-exposed zebrafish (*D. rerio*, up to 0.3 mg/L, Du et al., 2016) and amphibian larvae (*Rana zhenhaiensis*, up to 0.1 mg/L, Lu et al., 2021), although an opposite trend was shown in TPhP-exposed crustacean, *Daphnia magna* (Scanlan et al., 2015). The change trend of other liver amino acids could be different between studies. Glutamine and glutamate levels were decreased in TPhP-exposed fish (Du et al., 2016), while glutamine level was increased in crustacean (Scanlan et al., 2015). Conversely, some degree of changes in glutamine and glutamate levels were not found in this study.

TPhP-induced interference of the tricarboxylic acid (TCA) cycle, being reflected by decreased levels of some TCA intermediates, has been reported in other aquatic organisms, such as crustacean and fish (Scanlan et al., 2015; Du et al., 2016). However, such changes were also not found in TPhP-exposed turtles, although some metabolites involved in energy metabolism (e.g., niacinamide, nicotinamide adenine dinucleotide-NAD and its reduced form-NADH, ADP) were shown to change markedly. Previous studies have evidenced that TPhP has neurotoxicity, and its exposure can cause behavioral abnormality and locomotor decline in fish (Hong et al., 2018; Zhang et al., 2022, 2024). Besides decreased locomotor ability in 2 mg/L TPhP-exposed turtles, a downward trend in the liver norepinephrine level was also found in this study. However, to date those studies conducted on aquatic organisms exposed to various environmental pollutants have not showed a consistent change trend in brain neurotransmitter (e.g., dopamine, norepinephrine) levels (Øverli et al., 2005). As reported in crustacean and fish (Scanlan et al., 2015; Du et al., 2016), TPhP-induced disturbances in carbohydrate, lipid and fatty acid, and nucleotide metabolism could be observed in this study, which were reflected by noticeable changes of some related metabolites, such as glucose, ribose and uridine, in TPhP-exposed *P. sinensis*.

Metabolic disorders in various tissues, including liver, brain etc., might be associated with a disrupted intestinal microbiota (Wiertsema et al., 2021; Zheng and Wang, 2021; Zhang et al., 2022). The intestinal bacterial diversity was decreased significantly by exposure to a high concentration (3.5 mg/L) of TPhP in a fish species, *Cyprinus carpio* (Wang et al., 2022), but did not by lower concentrations of TPhP (up to 100 μg/L) in another fish species, *Oryzias melastigma* (Zhang et al.,

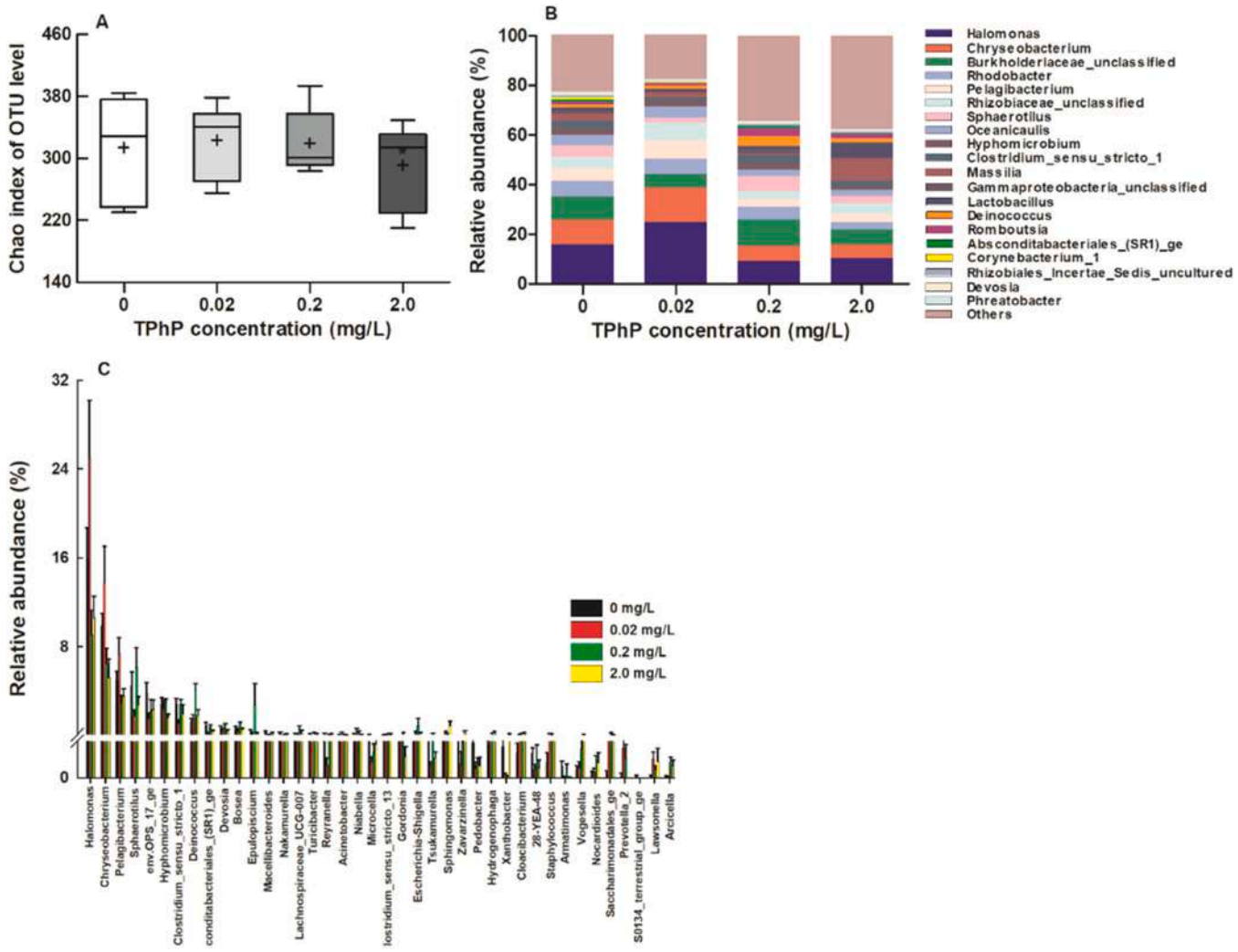

Fig. 4. The alpha diversity index-Chao index (A) and relative abundances of intestinal microbiota at the genus level (B), and significantly-changed bacterial genera (C) in *Pelodiscus sinensis* hatchlings exposed to 0 (control), 0.02, 0.2 and 2.0 mg/L TPhP.

2022). Certainly, the TPhP levels in the natural water environment (< 20 μg/L) are far lower than the concentrations that used in this (2.0 mg/L) and a previous study (Wang et al., 2022). TPhP exposure impact on intestinal bacterial diversity of aquatic organisms would be limited at environmentally realistic concentrations.

The bacterial composition changed significantly in TPhP-exposed turtles, which was similar to above mentioned studies conducted on fish. The relative abundances of some genera (e.g., *Halomonas*, *Chryseobacterium*, *Pedobacter*) decreased, while those of others (e.g., *Staphylococcus*, *Vogesella*, *Saccharimonadales_ge*) increased in higher-concentration exposure groups, indicating a disrupted intestinal microbiota in TPhP-exposed turtles. The bacterial genus *Halomonas*, having protease-producing ability, plays a vital role in protein degradation (Liu et al., 2016). Predictably, decreased abundance of *Halomonas* in higher-concentration exposure groups might modify the rate of protein degradation, and thus potentially affecting the generation of a variety of amino acids in the intestine. Somewhat inconsistently, our results showed that the levels of some hepatic amino acids (e.g., leucine, citrulline, arginine) were not lowered in higher-concentration exposure groups. Some bacterial genera, such as *Staphylococcus*, *Vogesella* and *Lawsonella*, are considered as potentially (or opportunistic) pathogenic bacteria, and can cause various infections (Kamala and Sivaperumal, 2023). Increased abundances of these bacterial genera in TPhP-exposed groups might already generate an adverse outcome for these turtles. *Chryseobacterium* species are also identified as pathogenic bacteria of aquatic organisms (Loch and Faisal, 2015). However, increased abundance of this bacterial genus was only observed in the low concentration exposure group (0.02 mg/L) in this study. Whether *Pedobacter* species could exhibit beneficial properties to the hosts still remains unclear, but a previous study indicated the abundance of *Pedobacter* was significantly reduced in unhealthy fish (Wang et al., 2018). Accordingly, decreased abundance of *Pedobacter* in TPhP-exposed turtles might be associated with their unhealthy status. *Prevotella* species have been documented to associate with carbohydrate consumption and propionate production, which might further affect the levels of some metabolites, such as glucose and cholesterol (Ley, 2016; Precup and Vodnar, 2019). Unexpectedly, increased abundances of the bacterial genus, *Prevotella*, were observed in the guts of 0.02 and 0.2 mg/L exposed turtles, but no obvious changes in liver glucose and cholesterol levels were found in these two groups.

5. Conclusion

This study was the first to investigate potential toxic effects of TPhP exposure in a freshwater turtle species. Physiological and biochemical disorders caused by TPhP exposure were reflected from the changes in locomotor performance, hepatic antioxidant activity and metabolite profile, and intestinal microbiota in TPhP-exposed turtles. Decreased

locomotor ability and hepatic SOD activity was observed only in turtles exposed to an unrealistically high concentration of TPhP. TPhP-induced adverse effects on the growth, survival and locomotor performance of *P. sinensis* would be slight at environmentally relevant levels.

The analysis of hepatic metabolomic profiles and intestinal microbiota revealed obvious metabolic disturbances and microbial dysbiosis (e.g., increased abundances of pathogenic bacteria) caused by TPhP, even at our lowest exposure concentration. Despite having limited impacts on the functional performance of *P. sinensis*, potential adverse consequences of metabolic disorders and microbial dysbiosis caused by exposure to low environmental levels of TPhP could not to be ignored and remained to be further investigated.

Overall, our results suggested potential toxic effects of TPhP in *P. sinensis* at higher concentration levels, and provided further insight into the toxicity mechanism of TPhP exposure in aquatic organisms.

CRediT authorship contribution statement

hongliang Lu: Supervision. **Jin-Hui Zhang:** Data curation. **Jia-Hui Liu:** Software. **Jia-Meng Yang:** Writing – original draft. **An-Ni Yang:** Data curation. **Zhi-Hao Cao:** Writing – review & editing. **Huo-Bin Tang:** Investigation.

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

Data availability

The data that has been used is confidential.

Acknowledgments

This study was supported by grants from the Natural Science Foundation of Zhejiang Province (LY21C030007).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116488.

References

Andresen, J.A., Grundmann, A., Bester, K., 2004. Organophosphorus flame retardants and plasticisers in surface waters. Sci. Total Environ. 332, 155–166.

Chen, J.-Y., Hu, H.-L., Feng, L., Ding, G.-H., 2022. Ecotoxicity assessment of triphenyl phosphate (TPhP) exposure in *Hoplobatrachus rugulosus* tadpoles. Chemosphere 292, 133480.

Chen, R., Hou, R., Hong, X.-S., Yan, S.-H., Zha, J.-M., 2019. Organophosphate flame retardants (OPFRs) induce genotoxicity in vivo: a survey on apoptosis, DNA methylation, DNA oxidative damage, liver metabolites, and transcriptomics. Environ. Int. 130, 104914.

van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119–1153.

Ding, G.-H., Wang, Z.-Y., Chen, J.-Y., Wu, Q.-Q., Zhou, Y.-N., 2022. Comprehensive assessment of the ecological risk of exposure to triphenyl phosphate in a bioindicator tadpole. Chemosphere 308, 136242.

Du, Z.-K., Zhang, Y., Wang, G.-W., Peng, J.-B., Wang, Z.-Y., Gao, S.-X., 2016. TPhP exposure disturbs carbohydrate metabolism, lipid metabolism, and the DNA damage repair system in zebrafish liver. Sci. Rep. 6, 21827.

Hong, X.-S., Chen, R., Hou, R., Yuan, L.-L., Zha, J.-M., 2018. Triphenyl phosphate (TPHP)-induced neurotoxicity in adult male Chinese rare minnows (*Gobiocypris rarus*). Environ. Sci. Technol. 52, 11895–11903.

Kamala, K., Sivaperumal, P., 2023. Prevalence of enterotoxin genes of *Staphylococcus* sp. isolated from marine fish to reveal seafood contamination. Mar. Pollut. Bull. 195, 115464.

Kang, C.-Q., Meng, Q.-Y., Dang, W., Shao, Y.-J., Lu, H.-L., 2022. Effects of chronic exposure to the fungicide vinclozolin on gut microbiota community in an aquatic turtle. Ecotoxicol. Environ. Saf. 239, 113621.

Kim, J.W., Isobe, T., Chang, K.H., Amano, A., Maneja, R.H., Zamora, P.B., Siringan, F.P., Tanabe, S., 2011. Levels and distribution of organophosphorus flame retardants and plasticizers in fishes from Manila Bay, the Philippines. Environ. Pollut. 159, 3653–3659.

Ley, R., 2016. *Prevotella* in the gut: choose carefully. Nat. Rev. Gastroenterol. Hepatol. 13, 69–70.

Li, Y., Chen, R.-C., He, J.-W., Ma, H.-J., Zhao, F.-R., Tao, S., Hu, J.-Y., 2019. Triphenyl phosphate at environmental levels retarded ovary development and reduced egg production in Japanese medaka (*Oryzias latipes*). Environ. Sci. Technol. 53, 14709–14715.

Liu, H., Guo, X.-W., Gooneratne, R., Lai, R.-F., Zeng, C., Zhan, F.-B., Wang, W.-M., 2016. The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. Sci. Rep. 6, 24340.

Liu, X.-S., Lin, J.-T., Chen, Y.-T., Jiang, M.-Z., Liu, Q., Zhang, J., Lu, X.-X., Hong, J.-B., Sun, W.-J., Sun, Y.-Q., Guo, L.-X., 2023. Gestation and lactation triphenyl phosphate exposure disturbs offspring gut microbiota in a sex-dependent pathway. Food Chem. Toxicol. 172, 113579.

Loch, T.P., Faisal, M., 2015. Emerging flavobacterial infections in fish: a review. J. Adv. Res. 6, 283–300.

Lu, H.-L., Hu, Y.-C., Xu, C.-Q., Dang, W., Lin, Z.-H., 2021. Developmental and liver metabolite changes induced by TPhP exposure in brown frog (*Rana zhenhaiensis*) tadpoles. Asian Herpetol. Res. 12, 135–142.

Lu, H.-L., Kang, C.-Q., Meng, Q.-Y., Hu, J.-R., Melvin, S.D., 2022. Functional and hepatic metabolite changes in aquatic turtle hatchlings exposed to the anti-androgenic fungicide vinclozolin. Ecotoxicol. Environ. Saf. 231, 113220.

Mehdi, M.M., Solanki, P., Singh, P., 2021. Oxidative stress, antioxidants, hormesis and calorie restriction: the current perspective in the biology of aging. Arch. Gerontol. Geriatr. 95, 104413.

Meng, Q.-Y., Kang, C.-Q., Dang, W., Melvin, S.D., Lu, H.-L., 2023. Minor metabolomic disturbances induced by glyphosate-isopropylammonium exposure at environmentally relevant concentrations in an aquatic turtle, *Pelodiscus sinensis*. Aquat. Toxicol. 256, 106415.

Meng, X.-J., Li, F., Wang, X.-Q., Ji, C.-L., Wu, H.-F., 2020. Physiological and biochemistry responses of graphene combined with triphenyl phosphate (TPP) to *Mytilus galloprovincialis*. Chin. Sci. Bull. 65, 1599–1609.

Øverli, Ø., Winberg, S., Pottinger, T.G., 2005. Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout-a review. Integr. Comp. Biol. 45, 463–474.

Precup, G., Vodnar, D.C., 2019. Gut *Prevotella* as a possible biomarker of diet and its eubiotic versus dysbiotic roles: a comprehensive literature review. Brit. J. Nutr. 122, 131–140.

Ramesh, M., Angitha, S., Haritha, S., Poopal, R.K., Ren, Z., Umamaheswari, S., 2020. Organophosphorus flame retardant induced hepatotoxicity and brain AChE inhibition on zebrafish (*Danio rerio*). Neurotoxicol. Teratol. 82, 106919.

Sala, B., Balasch, A., Eljarrat, E., Cardona, L., 2021. First study on the presence of plastic additives in loggerhead sea turtles (*Caretta caretta*) from the mediterranean sea. Environ. Pollut. 283, 117108.

Scanlan, L.D., Loguinov, A.V., Teng, Q., Antczak, P., Dailey, K.P., Nowinski, D.T., Kornbluh, J., Lin, X.-X., Lachenauer, E., Arai, A., Douglas, N.K., Falciani, F., Stapleton, H.M., Vulpe, C.D., 2015. Gene transcription, metabolite and lipid profiling in eco-indicator *Daphnia magna* indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. Environ. Sci. Technol. 49, 7400–7410.

Shi, Q.-P., Tsui, M.M.P., Hu, C.-Y., Lam, J.C.W., Zhou, B.S., Chen, L.-G., 2019. Acute exposure to triphenyl phosphate (TPhP) disturbs ocular development and muscular organization in zebrafish larvae. Ecotoxicol. Environ. Saf. 179, 119–126.

Sun, Z.-J., Ma, W.-Q., Tang, X.-X., Zhang, X., Yang, Y.-Y., Zhang, X.-X., 2022. Toxicity of triphenyl phosphate toward the marine rotifer *Brachionus plicatilis*: changes in key life-history traits, rotifer-algae population dynamics and the metabolomic response. Ecotoxicol. Environ. Saf. 241, 113731.

Sutton, R., Chen, D., Sun, J., Greig, D.-J., Wu, Y., 2019. Characterization of brominated, chlorinated, and phosphate flame retardants in San Francisco Bay, an urban estuary. Sci. Total Environ. 652, 212–223.

Tan, X.-X., Luo, X.-J., Zheng, X.-B., Li, Z.-R., Sun, R.-X., Mai, B.-X., 2016. Distribution of organophosphorus flame retardants in sediments from the Pearl River Delta in South China. Sci. Total Environ. 544, 77–84.

Tripathi, A., Debelius, J., Brenner, D.A., Karin, M., Loomba, R., Schnabl, B., Knight, R., 2018. The gut-liver axis and the intersection with the microbiome. Nat. Rev. Gastroenterol. Hepatol. 15, 397–411.

Umamaheswari, S., Karthika, P., Suvenitha, K., Kadirvelu, K., Ramesh, M., 2021. Dose-dependent molecular responses of *Labeo rohita* to triphenyl phosphate. Chem. Res. Toxicol. 34, 2500–2511.

Wang, C., Sun, G.-X., Li, S.-S., Li, X., Liu, Y., 2018. Intestinal microbiota of healthy and unhealthy Atlantic salmon *Salmo salar* L. in a recirculating aquaculture system. J. Ocean. Limnol. 36, 414–426.

Wang, Y., Sha, W.-L., Zhang, C., Li, J.-Y., Wang, C., Liu, C.-C., Chen, J.-F., Zhang, W.-L., Song, Y.-H., Wang, R.-J., Gao, P.-K., 2022. Toxic effect of triphenyl phosphate (TPhP) on *Cyprinus carpio* and the intestinal microbial community response. Chemosphere 299, 134463.

Wiegand, J., Avila-Barnard, S., Nemarugommula, C., Lyons, D., Zhang, S., Stapleton, H. M., Volz, D.C., 2023. Triphenyl phosphate-induced pericardial edema in zebrafish embryos is dependent on the ionic strength of exposure media. Environ. Int. 172, 107757.

Wiertsema, S.P., van Bergenhenegouwen, J., Garssen, J., Knippels, L.M.J., 2021. The interplay between the gut microbiome and the immune system in the context of infectious diseases throughout life and the role of nutrition in optimizing treatment strategies. Nutrients 13, 886.

Xing, L.-Q., Zhang, Q., Sun, X., Zhu, H.-X., Zhang, S.-H., Xu, H.-Z., 2018. Occurrence, distribution and risk assessment of organophosphate esters in surface water and sediment from a shallow freshwater lake, China. Sci. Total Environ. 636, 632–640.
Yan, S.-H., Wu, H.-M., Qin, J.-H., Zha, J.-M., Wang, Z.-J., 2017. Halogen-free organophosphorus flame retardants caused oxidative stress and multixenobiotic resistance in Asian freshwater clams (*Corbicula fluminea*). Environ. Pollut. 225, 559–568.
Yao, C., Yang, H.-P., Li, Y., 2021. A review on organophosphate flame retardants in the environment: occurrence, accumulation, metabolism and toxicity. Sci. Total Environ. 795, 148837.
Yuan, S.-L., Li, H., Dang, Y., Liu, C.-S., 2018. Effects of triphenyl phosphate on growth, reproduction and transcription of genes of *Daphnia magna*. Aquat. Toxicol. 195, 58–66.
Zhang, L.-M., Nichols, R.G., Patterson, A.D., 2017. The aryl hydrocarbon receptor as a moderator of host-microbiota communication. Curr. Opin. Toxicol. 2, 30–35.
Zhang, Q., Wu, R.-T., Zheng, S.-K., Luo, C.-Y., Huang, W.-L., Shi, X.-L., Wu, K.-S., 2024. Exposure of male adult zebrafish (*Danio rerio*) to triphenyl phosphate (TPhP) induces eye development disorders and disrupts neurotransmitter system-mediated abnormal locomotor behavior in larval offspring. J. Hazard. Mater. 465, 133332.
Zhang, Y., Zheng, X.-B., Wei, L.-F., Sun, R.-X., Guo, H.-Y., Liu, X.-Y., Liu, S.-Y., Li, Y., Mai, B.-X., 2018. The distribution and accumulation of phosphate flame retardants (PFRs) in water environment. Sci. Total Environ. 630, 164–170.
Zhang, Y.-T., Chen, R.-N., Wang, F.-P., Huang, Z.-K., He, S.-Q., Chen, J.-M., Mu, J.-L., 2022. Potential involvement of the microbiota-gut-brain axis in the neurotoxicity of triphenyl phosphate (TPhP) in the marine medaka (*Oryzias melastigma*) larvae. Sci. Total Environ. 817, 152945.
Zheng, Z.-P., Wang, B.-H., 2021. The gut-liver axis in health and disease: the role of gut microbiota-derived signals in liver injury and regeneration. Front. Immunol. 12, 775526.
Zhou, C.-P., Lin, H.-Z., Huang, Z., Wang, J., Wang, Y., Yu, W., 2020. Effects of dietary leucine levels on intestinal antioxidant status and immune response for juvenile golden pompano (*Trachinotus ovatus*) involved in Nrf2 and NF-κB signaling pathway. Fish. Shellfish Immunol. 107A, 336–345.