Impacts of di(2-ethylhexyl) terephthalate on multi-generational fitness of Caenorhabditis elegans via lipid metabolism and neural regulation

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Abstract

Di(2-ethylhexyl) terephthalate (DEHTP) is a substitutive plasticizer with various industrial applications. However, its wide occurrence in environmental matrices and human tissues is urging concerns on its toxicities. Presently, the effects of DEHTP on the fitness of *Caenorhabditis elegans* were explored with a consecutive exposure from F1 to F4 generations. The fitness was represented by reproduction, lifespan and behavior. Effects of DEHTP on reproduction showed an oscillation pattern with alteration from stimulation to inhibition and backwards. Influences of DEHTP on reproduction and lifespan showed trade-off relationships. Regarding behavior, DEHTP inhibited satiety quiescence duration, body bending and head swing, while stimulated reverse and Omega turns. At the biochemical level, DEHTP disturbed the lipid metabolites and lipid metabolism enzymes. Moreover, positive correlations were found between the effects on reproduction and with those on fatty acid synthase (FAS) and acyl-CoA (FA-CoA). DEHTP also disturbed the neural regulations including neural transmitters and the expressions of related genes. Out of expectation, the effects on neural regulations were positively correlated with those on lipid metabolism, but not with locomotion behavior. Hierarchical clustering analysis (HCA) results also indicated oscillatory changes underlying multi-generational effects of DEHTP, e.g., serotonin was more connected with other neural regulations than other indicators in F1 and F3, while it was more connected with behavior than others in F2 and F4.

1. Introduction

Phthalates, e.g., di-2-ethylhexyl phthalate (DEHP), are widely used as plasticizers and they were detected in environments and also human tissues with various health impacts (Ma et al., 2022; Wang et al., 2022b; Zhao et al., 2022). Di(2-ethylhexyl) terephthalate (DEHTP), as a substitutive plasticizer, is increasingly used in toys, food packing materials and even medical devices (Bernard et al., 2023; Lessmann et al., 2019). Consequently, DEHTP and its metabolites were detected in environmental matrices such as sediments at µg/kg levels (Bulbul et al., 2022) and also in human urine samples at µg/L levels (Bernard et al., 2023; Lessmann et al., 2019; Schwedler et al., 2020). Accordingly, the effects of DEHTP on living organisms need further investigation to fully demonstrate its hazards.

Reproductive toxicities of phthalates had been widely reported on both humans and model organisms (Sedha et al., 2021; Sree et al., 2023). Notably, the reproduction processes involve embryo and germline development to connect adjacent generations (Wang et al., 2023). Accordingly, the long-term influences of phthalates earned special attentions. On one hand, the long-term influences of DEHP (one traditional phthalate) were recently explored with particular attentions on the hazards over generations. For example, parental or maternal exposure to DEHP resulted in obesogenic outcomes in offspring of drosophila (Chen et al., 2019) and behavior disorder and reproductive dysfunction in mice (Quinnies et al., 2015; Rattan et al., 2018).

Moreover, the disturbances of DEHP on liver functions lasted over three generations in mice (Wen et al., 2020). On the other hand, the substitutive DEHTP can transfer over generations (Pacyga et al., 2023). Both the reproductive and generational effects demonstrated the impacts of plasticizers on the fitness which is important in sustaining ecological stability (Yue et al., 2021). Accordingly, the long-term effects of DEHTP are urging comprehensive studies and mechanism exploration.

Lipid metabolism provides energy for reproduction that connects generations. As expected, lipid metabolism disorder was employed to explain the multigenerational reproductive toxicities of environmental pollutants such as ionic liquids (Zhang et al., 2022). It was reported that phthalates disturbed lipid metabolism in *Daphnia magna,* with impacts on reproduction, development and lifespan (Seyourm and Pradhan, 2019) and they also impacted lipid metabolism in the dolphin (Xie et al., 2023). Therefore, DEHTP is assumed to provoke long-term exposure over generations, including lipid metabolism disorder. However, such an assumption needs support by empirical evidences.

Neural signals, e.g., dopamine (DA), acetylcholine (ACh), acetylcholinesterase (AChE), γ-aminobutyric acid (GABA) and serotonin (5-HT), are also essential for the behavior and reproduction (Liu et al., 2013). Moreover, it is also well connected with changes in lipid metabolism (Luo et al., 2022; Magnan et al., 2015). The stimulation on DA and inhibition on 5-HT reduced fat accumulation and metabolic dysfunction (Ali and Mirza, 2021; Crane et al., 2015). Meanwhile, the disturbance of antibiotics on the neural signals significantly inhibited satiety to induce fat accumulation (Luo et al., 2022). Lipid metabolism signals can also trigger neural regulations (Hamilton et al., 2015; Magnan et al., 2015). Therefore, investigations combining both neural signals and lipid metabolism are expected to provide more information regarding the toxicity mechanisms of DEHTP.

The purpose of the present study was to analyze the multi-generational toxicities of DEHTP on *Caenorhabditis elegans* . Moreover, lipid metabolism and neural regulations were explored to explain the multi-generational toxicities. We hypothesized that the multigenerational effects of DEHTP involved with both lipid metabolism and neural regulations. The findings not only confirmed such hypothesis and also showed oscillatory patterns in the effects over generations. The environmental hazards of plasticizer substitutes are indeed of concern, and further investigation is still needed to explore both impacts and underlying mechanisms.

2. Materials and Methods

2.1. Chemicals

Di(2-ethylhexyl) terephthalate (DEHTP, CAS: 6422-86-2, Purity > 98%) was purchased from Adamas (Swiss). The stock solutions were prepared with 1% dimethyl sulfoxide (DMSO, Amresco, USA) in sterile K-medium (0.051 M NaCl and 0.032 M KCl) (Yu et al., 2022). The stock solutions were maintained at 4 °C until use.

2.2. Nematode preparation

Escherichia coli (*E. coli*) OP50 was cultured in lysogeny broth (LB) medium at 37 ºC and served as the food for subsequent nematode culture. *Caenorhabditis elegans* (*C. elegans* , wild-type N2) nematodes were cultured at 20 ºC on nematode growth medium (NGM) agar. Gravid hermaphrodite nematodes were lyzed by fresh Clorox solution (1% NaClO and 0.5 mol/L NaOH), and the eggs were resistant to such lysis and therefore were age-synchronized for subsequent experiments (Li et al., 2019).

2.3. Multi-generational experiment and measurement on lifespan and reproduction

In preliminary experiments, DEHTP at 8.0 mg/L provoked lethality smaller than 5% (LC5). Accordingly, the nominal exposure concentrations were arranged as 0.0008, 0.008, 0.08, 0.8, and 8.0 mg/L, three of which represented realistic µg/L levels (Bulbul et al., 2022). Briefly, warm NGM were mixed with DEHTP stock solutions or sterile K-medium solutions containing 1% DMSO (as the control) with a volume ration of 99:1 to reach the nominal concentrations. Then, they were aliquoted into Petri dishes. After cooling down to form agar, they received the same amounts of *E. coli* OP50 suspensions with the same bacterial density. Then, they were kept overnight for mild evaporation to form a bacterial lawn. At last, the age-synchronization eggs were added on the NGM agars to start the first-generation exposure (marked as F1) (Li et al., 2019; Yu et al., 2017; Yue et al., 2021). Each experimental group including the control had at least ten replicates of NGM agars.

On the 3^{rd} day of the F1 exposure, in each experimental group the nematodes were collected and randomly allocated to four experimental manipulations: (1) 48 adults were transferred onto 48 NGM agars with continuous treatment of DEHTP or K-medium solutions to measure lifespan and reproduction where disappeared nematodes were not calculated (Liu et al., 2020; Yu et al., 2017); (2) approximately 1000 adults were transferred onto clean NGM agars for behavior measurement; (3) 200 adults were transferred onto new NGM agars with continuous DEHTP or K-medium treatment to reproduce eggs for 24 h. Then, the adults were discarded, and the age-synchronized eggs started next generation exposure (i.e., F2) (Yue et al., 2021); (4) the remaining nematodes in each group were collected to 20 centrifuge tubes and stored at -80 ^{O}C until assayed. The procedure was repeated to finish a multi-generational exposure with 4 consecutive generations from F1 to F4.

2.4. Behavior measurement

Briefly, nematodes were captured for videos by dissecting microscope (XTL-BM-9TD, Shanghai BM optical instruments manufacture co., Ltd) equipped with an industrial digital camera (UCMOS05100KPA). The head swing, body bending frequency, reversal movement and Omega turns in videos were counted for each individual for one minute (Yu et al., 2011). A slow-playing mode was employed to facilitate the counting and recording of the pharyngeal pumping times within 10 s (Luo et al., 2022). The satiety quiescence referred to the time when nematodes did not move for more than 5 s within 1 min, and the satiety quiescence duration was also recorded (Luo et al., 2022). Each group was consisted of at least 6 replicates.

2.5. Biochemical assays

Briefly, nematodes were homogenized followed by a centrifugation at 4 ^{O}C, and the supernatants were aliquoted for subsequent assays. Regarding the neural signals, the contents of 5-HT, DA, ACh and GABA and the activities of AChE were determined using the commercial enzyme-linked immune-sorbent assay (ELISA) kits (Shanghai Ketao Biotechnology Co. LTD) according to the instructions (Liu et al., 2020; Yue et al., 2021). Regarding the lipid metabolism, the contents of triacylglycerol (TG), non-esterified fatty acid (NEFA) and the metabolites acetyl-CoA (ACA) and acyl-CoA (FA-CoA) that combine both lipogenesis and lipolysis were measured. In addition, the activities of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and glycerol-3-phosphateacyl transferases (GPAT), which facilitate lipogenesis, and lipase, acyl-CoA synthetase (ACS) and carnitine palmitoyl transferase (CPT), were analyzed. Finally, adipose triglyceride lipase (ATGL) that facilitates lipolysis was also measured. Measurements were conducted with the ELISA method (Zhang et al., 2022). Briefly, the ELISA kits provided 96-well plates, substrates, buffer and standard enzyme solutions. Sufficient substrates were added with a series of enzyme solutions (diluted with the buffer) whose concentrations were known, and the results were used to establish standard curves which were used to calculate the results of the samples. In each group, three were at least six replicates with 500 nematodes in each to ensure the methodological accuracy (Zhang et al., 2017). The biomass among samples was balanced by the normalization where each biochemical was represented as its proportion against the total protein (TP) in each sample. Each group was composed of at least three replicates.

2.6. Gene expression analysis

Genes including *ser-1* , *mod-1* , *ser-6* , *acs-4* ,*dop-3* and *cpt-1* are directly related with neural signals and lipid metabolism (Lemieux and Ashrafi, 2016; Srinivasan et al., 2008; Watts, 2009). Their expression levels were determined with real-time polymerase chain reaction (RT-PCR) (Li et al., 2020). Briefly, TRIzol reagents were used to extract the total RNA which was further synthesized to cDNA for subsequent RT-PCR with SRBR Premix Ex Taq (Takara Bio Inc., Japan) in FTC-3000 (Funglyn Biotech Inc., Canada). The expression levels of chosen genes were quantified by the $2^{-\Delta\Delta CT}$ method with *gpd-2* as the endogenous reference gene. The primers are listed in Table S1 in Supporting Information.

2.7. Data presentation and statistical analysis

Briefly, all data were presented as fold-change against the concurrent control in each generation (Liu et al., 2020; Yue et al., 2021). The values in the concurrent control in each indicator were normalized to 1.0. Therefore, fold-change values lower than 1.0 indicate inhibition and those higher than 1.0 indicate stimulation. All data passed normality test and went through two-way ANOVA with a Tukey *post hoc* test at $a = 0.05$ ($p < 0.05$) to determine significant differences among treatments (control and two concentrations) and among generations. Parallel analysis presented the individual data of each sample in each indicator with a purpose to check the changing patterns between indicators in corresponding samples. At the same time, the mean values that were calculated from the individual data of each sample went through Spearman's rank correlation and hierarchical clustering analysis (HCA) to further analyze connections among the indicators (Yue et al., 2021).

3. Results

3.1. Multi-generational effects on fitness

Reproductive influences represent the first aspect in fitness evaluation. The effects of DEHTP on the reproduction are shown in Figure 1(A). Collectively, DEHTP caused oscillatory effects over generations. For example, at 0.8 mg/L, DEHTP showed stimulation in F1, inhibition in F2 and stimulation again in F3, and the stimulation was much less in F4. Generally, the stimulations were commonly greater in F3 than in F1, and the inhibitions were usually less severe in F4 than in F2. Moreover, the results of reproduction even showed a regular oscillation pattern, with stimulation in F1 and F3 and inhibition in F2 and F4.

(Figure 1 around here)

The influences on the lifespan represent the second aspect in fitness evaluation. The effects on lifespan (Figure 1(B)) also showed alterations between inhibition and stimulation, but there were no particular concentration- or generation-dependent patterns. Notably, the stimulation of DEHTP on reproduction (Figure 1(A)) was accompanied with inhibition of lifespan in F1 and F3 at most concentrations (Figure 1(B)). Meanwhile, the inhibition on reproduction in F2 and F4 was accompanied with the stimulation on lifespan in the corresponding generations. The opposite relationship between effects on reproduction and those on lifespan showed potential trade-off relationships. Further parallel analysis was performed with the data of reproduction and lifespan in each individual. Results showed that the individual with the greatest stimulation on reproduction showed the greatest inhibition on lifespan, and that with the greatest inhibition on reproduction showed stimulation on lifespan (Figure 1(C)). Moreover, such relationship covered 65% of the total cases, and confirmed the trade-off relationships.

The impacts on the behavior represent the third aspect in fitness evaluation. Nematodes have more quiescence duration at satiety with reverse and Omega turns or no locomotion. In F1, DEHTP inhibited the satiety quiescence duration at 0.8 and 8.0 mg/L (Figure 2(A) and Figure S1). The shorter satiety quiescence duration should spare more time for foraging via body bending and head swing. Out of expectation, the effects of DEHTP in F1 showed inhibitions on body bending at 8.0 mg/L (Figure 2(A) and Figure S2), with stimulations on reverse and Omega turns. In other generations, the effects showed similarities, i.e., inhibition on the satiety quiescence duration and body bending, and stimulation on reverse and Omega turns (Figure 2(B-D)). Such similarities were supported by Spearman's correlation analysis where the satiety quiescence duration was positively correlated with body bending and head swing but negatively correlated with reverse locomotion (Figure 2(E)). Collectively, the results showed that DEHTP dispatched less time in foraging but more time in backward movement. Moreover, DEHTP also inhibited the pharyngeal pumping (Figure S2), which further aggravated the lack of food supply.

(Figure 2 around here)

3.2. Multi-generational effects on lipid metabolism

The multigenerational effects of DEHTP on the lipid metabolites, including TG, NEFA, ACA and FA-CoA, are shown in Figure S2. In F1, at 0.8 mg/L DEHTP inhibited TG, ACA, lipase, ATGL, ACS, CPT and ACC but stimulated NEFA, FA-CoA, GPAT and FAS. In F2 to F4, the effects showed alterations between inhibition and stimulation without specific tendencies or relationships (Figure S2).

Notably, there were opposite effects on TG and NEFA, with 60% of the cases where the stimulations on TG were usually accompanied with inhibitions on NEFA and vice versa (Figure 3(A)). The opposite effects were also found between lipase and ATGL (40% of the total cases), and also between the effects on ATGL and GPAT (55% of the total cases) (Figure 3(B)).

The Spearman's correlation analysis demonstrated the connection between the metabolites and the enzymes (Figure 3(C)). The significant stimulations of DEHTP on ACA were accompanied with positively correlated stimulation on a series of enzymes in ACA metabolism (ACS, CPT and ACC) and also in the balance between TG and NEFA (ATGL and GPAT). Combining the apical and biochemical effects, there were positive correlations between the effects on reproduction and with those on FAS and FA-CoA, and negative ones between the influences on behavior and those on FA-CoA, ACA and GPAT (Figure 3(E)).

3.3. Multi-generational effects on neural regulation

In F1, at 0.8 mg/L DEHTP stimulated 5-HT, DA, AChE and GABA and inhibited ACH, with down-regulations on the expressions of *ser-1* ,*ser-6* , *mod-1* and *dop-3* (Figure S3). In F2 to F4, DEHTP stimulated 5-HT, DA, AChE, ACH, GABA, with both up- and down-regulations on the gene expressions. Moreover, DEHTP caused mostly stimulations of 5-HT, DA, AChE and GABA, while it provoked mostly inhibition of ACH. The results showed the opposite effects on AChE and ACH, where stimulations on AChE with inhibition on ACH (and vice versa) covered 55% of the total cases (Figure 4). Moreover, the effects of DEHTP on 5-HT and DA showed positive Spearman's correlation with each other (0.5534, $p < 0.05$). However, there were no correlations between the effects on neural regulations and those on locomotion (Figure 4).

3.4. Overall multi-generational influences of DEHTP combining various aspects

Further analysis combined the aforementioned aspects to explore the underlying connections. The correlation analysis demonstrated positive correlations (i) between the effects on 5-HT and those on GPAT and ACS, (ii) between DA and GPAT, FAS and FA-CoA, (iii) between ACH and ATGL, GPAT, ACS, FAS and ACA, (iv) between AChE and NEFA, and (v) between GABA and NEFA (Figure 4).

The HCA further assessed the potential strategies in the nematode response to DEHTP over generations (Figure 5). The indicators in F1 had shorter distance (with the longest distance < 2 in x axis) than those in F2-F4. Such results implied closer connections among neural regulation, lipid metabolism, reproduction and locomotion in F1 than subsequent generations. Notably, 5-HT was connected firstly with other neural indicators and secondly with behavior and lipid metabolism in F1, while it was connected firstly with behavior and later with other neural indicators. Interestingly, 5-HT showed closer connection with other neural indicators in F3 again, which was similar to the results in F1, while it had later connection in F4 which was similar to those in F2.

4. Results and Discussion

4.1. Multi-generational influences of DEHTP on fitness

The fitness evaluation was performed in reproduction, lifespan and behavior. In the aspect of reproduction, the oscillation between stimulation and inhibition over generations was also reported in the multi-generational effects of ionic liquids and antibiotics (Zhang and Feng, 2022; Zheng et al., 2022). On one hand, the alteration from inhibition in earlier generation (e.g., in F2) to stimulation in later one (F3) showed generation-dependent hormesis, which was also observed in the reproductive effects of antibiotics (Zheng et al., 2022), ionic liquids (Shi et al., 2021; Zhang and Feng, 2022) on *C. elegans* and those of graphene oxide on *Acheta domesticus* (Dziewiecka et al., 2020). Hormesis over generations (also referred as trans-hormesis) were found in various organisms (Agathokleous et al., 2022; Costantini, 2022). On the other hand, the alteration in effects across generations indicated that the organisms actively adjusted fetal response to the maternal challenges, which is essential to ensure the fitness or adaption to sustain the population survival (Yue et al., 2021). Notably, the responses in the offspring did not always match the stresses that the offspring actually experience. From an ecological perspective, pre-conditioning hormesis can improve resilience to subsequent exposure (Costantini et al., 2010; Costantini et al., 2014). From a health perspective, it may even strengthen therapeutic benefit (Dhawan et al., 2020). However, pre-conditioning hormesis is costly under the mismatch between the actual environment in offspring and the predicted environment by parents. Therefore, the long-term effects of DEHTP deserve ecological concerns.

The trade-off effects between lifespan and reproduction by DEHTP were also observed in the toxicities of other pollutants, e.g., ionic liquids (Shi et al., 2021; Zhang and Feng, 2022) and antibiotics (Yu et al., 2017). The trade-off relationship showed a common strategy to balance energy investment in adapting environments for better fitness. On one hand, more reproduction at the expense of lifespan is essential to the population's survival and continuation (Yu et al., 2017). On the other hand, lower reproduction with an extended lifespan aids individual organisms to escape from environmental stress. Both aspects were observed in the effects of DEHTP but in different generations. Again, the trade-off effects over generations also supported the active adjustment of organisms to environmental stresses. The results collectively indicated that phthalate might potentially disturb adaptation and evolutionary changes.

In the aspect of behavior, the results demonstrated that DEHTP caused avoidant behavior in nematodes, which allows the individuals to escape from environmental stresses. Notably, the influences of DEHTP on reproduction, lifespan, growth and behavior were all observable at 0.8 mg/L and even lower concentrations that are environmental realistic (e.g., 0.07-6.1 mg/kg as the arithmetic mean values in dust) (Nagorka et al., 2011). The results also brought up an assumption that DEHTP resulted in caloric restriction by less foraging behavior and less efficient food uptake. The caloric restriction decreased the overall available energy, which subsequently resulted in energy allocation among life traits according to the energy budget theory (Matyja et al., 2020; Yu et al., 2018). The energy allocation also influenced the trade-off relationships over generations (Wang et al., 2022a). Moreover, the caloric restriction is important in the evolutionary responses with more resilient to other stressors, and therefore partially explained the pre-conditioning hormesis (Speakman, 2020). Therefore, further studies on the caloric restriction would explain the aforementioned trade-off relationships between reproduction and lifespan, and also the generational effects.

4.2. Lipid metabolism underlying the multi-generational effects

Lipid metabolism plays crucial roles in supporting reproduction and growth and regulating lifespan (Hansen et al., 2013). It is known that TG is hydrolyzed to NEFA by lipase and ATGL, and NEFA can be synthesized back to TG by GPAT (Figure 3(D)) (Pradhan et al., 2018; Srinivasan et al., 2008). Therefore, the opposite effects on the metabolites (between TG and NEFA) and enzymes (between lipase and ATGL, between ATGL and GPAT) demonstrated that DEHTP disturbed the overall lipid metabolism with potential biased directions. Moreover, NEFA can form FA-CoA by ACS, and FA-CoA can react to ACA by CPT, while ACA can re-form NEFA by ACC and FAS (Pradhan et al., 2018; Srinivasan et al., 2008). The correlation between ACA and ACS (and also CPT and ACC) and between ATGL and GPAT demonstrated that DEHTP significantly promoted the overall lipid metabolism. Especially, such promotion occurred at environmental levels of DEHTP (e.g., 0.008 mg/L), showing the potential of DEHTP as an environmental obesogen. Combining the apical and biochemical effects, the positive correlations supported the connection between energy supply and reproduction. Meanwhile, the negative correlations indicated that the energy supply was balanced and even allocated with preference towards reproduction instead of locomotion behavior.

Notably, there were significant mismatches between lipid metabolism and the apical responses (i.e., locomotion). Such mismatch can be resulted from other energy consuming responses. It was reported that oxidative stress and antioxidant investment are known to cost energy and may directly involve trade-offs with other life traits (e.g., growth) (Yu et al., 2018). The influences of DEHTP on oxidative stress and antioxidant responses had been demonstrated in the human body (Huang et al., 2020). The mismatch urged further studies on the energy budget and also multi-omic analysis (e.g., lipidomic and transcriptomic) to reveal the essential connections among various responses.

4.3. Neural regulation underlying the multi-generational effects

The opposite effects between AChE and ACH supported the function of AChE in hydrolyzing ACH (Behl et al., 2023). The up-regulated expressions of*ser-1* (encoding G-protein coupled receptors family 1 profile domain-containing protein), *ser-6* , *mod-1* (encoding serotonin-gated chloride channel) and *dop-3* (encoding dopamine receptor 3) are involved in the increases of 5-HT and GABA transductions (McDonald et al., 2006; Srinivasan et al., 2008). However, the effects of DEHTP on their expressions were not consistent with those on 5-HT or GABA. The lack of such consistence suggests that more regulations were involved in impacts on the neural regulation other than *ser-1* ,*ser-6* , *mod-1* and *dop-3* . The neuro-toxicities of DEHTP showed some similarities to those of DEHP on nematodes and fishes (Huang et al., 2022a; Huang et al., 2022b), which raises more concerns about the toxicities of the plasticizer substitutes.

It is well known that neural signals regulate behavior. Both 5-HT and DA support the nematode locomotion and feeding (Cermak et al., 2020; Srinivasan et al., 2008), and DA is essential in avoidance responses of nematodes (Chou et al., 2022). However, the lack of correlations between the effects on neural regulations and those on locomotion demonstrated that there was more complicated regulation between the biochemical neural signals and the apical locomotion.

4.4. Combined analysis on the multi-generational influences of DEHTP

Neural signals not only regulate behavior, but also connect with lipid metabolism and reproduction (Luo et al., 2022; Magnan et al., 2015). The positive correlations between biochemicals in the neural regulation and those in lipid metabolism supports the neural regulation on lipid metabolism (Ali and Mirza, 2021; Crane et al., 2015). The oscillatory changes in the HCA results demonstrated the active responses of organisms to the multi-generational signals. Such multigenerational responses can involve epigenetic regulations (e.g., DNA methylations), which were observed in the disturbances of DEHP on liver in mice over three generations (Wen et al., 2020). Notably, the 5-HT results in HCA demonstrated an oscillatory pattern over generations, which could be related with the oscillation between stimulatory and inhibitory apical effects of DEHTP over generations. Future studies should consider both transcriptomic and epigenomic analysis to further explore mechanism.

5. Conclusion

In summary, DEHTP showed a regular oscillation pattern in the multigenerational reproductive toxicities, with stimulation in F1 and F3 generations and inhibition in F2 and F4 ones. The influences of DEHTP on reproduction and lifespan showed trade-off relationships. Further analysis demonstrated the involvement of lipid metabolism and neural regulation underlying the multi-generational effects. HCA results pointed out the oscillatory connection between 5-HT and other neural regulation in F1 and F3 and between 5-HT and other indicators in F2 and F4. The overall oscillatory effects of DEHTP over generations need further transcriptomic and epigenomic analysis to explore the underlying mechanisms.

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Conflict of interest

The authors declare no conflict of interest associated with this publication to disclose.

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