Biological effects of new-generation dialkyl phosphinate flame retardants and their hydrolysates in BALB/C mice

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Abstract
Aluminum methylcyclohexylphosphinate (AMHP), calcium methylcyclohexylphosphinate (CMHP), aluminum diethylphosphinate (ADEP), and aluminum methylethylphosphinate (AMEP) are organic dialkyl phosphinates (DPs) and emerging phosphorus-based flame retardants. The broad-spectrum DPs flame retardants occupy high-end industrial markets, but their ecologic risk has been reported rarely. By exposing male BALB/c mice to DPs and dialkyl phosphinic acids, we studied the toxic effects of these chemicals, and measured AMHP and methylcyclohexylphosphinic acid (MHPA) in blood and feces. We found that DPs and their main hydrolysates had mild toxicity in BALB/c mice. Exposure to 10 and 50 mg/kg/d of AMEP and ADEP caused mild hepatotoxicity in mice. Toxicity of CMHP was in the liver and kidneys. Toxicity of AMHP and its hydrolysate MHPA was low and affected the liver. These data suggest that AMHP has lower toxicity than the other DPs that we tested.

KEYWORDS
BALB/c mice, toxicity, dialkyl phosphinates, dialkyl phosphinic acid

1 | INTRODUCTION

The frequently used brominated flame retardants (BFRs) have been shown to have toxic effects on the nervous system,¹ immune system,²,³ liver,⁴,⁵ reproductive system,⁶ and endocrine system.⁷ With
gradual prohibition of certain BFRs by the Stockholm Convention on Persistent Organic Pollutants (2009), substitutes have been developed, including dialkyl phosphinate flame retardants (DPFRs).

DPFRs are new-generation flame retardants, but other organic phosphorus flame retardants (OPFRs) have been applied in varnishes, textiles, polyurethane, rubber, polyurethane foams, furniture, cotton, and electronic equipment for more than 150 years. In Europe, 85,376 tons of OPFRs were consumed in 2005. In China, total production of OPFRs was >70,000 tons in 2007 and the amount has been increasing each year since then.

OPFRs are additives mixed directly with polymer materials rather than bonded by chemical means, so they are released into the surrounding environment readily. Various concentrations of OPFRs have been detected in: indoor air samples in cities; the sediment of Lake Taihu in China; the air around the Great Lakes in northeastern North America; the Arctic and Antarctic. Abundance of OPFRs in the environment and their potential effect on human health and the environment has attracted considerable attention. In a recent study of human placenta, the concentrations of the nine most organophosphate esters detected are ranged from 34.4 to 862 ng/g lipid weight. Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and triphenyl phosphate (TPP) in indoor air samples have been postulated to be related to altered hormone levels and decreased quality of semen in males. TDCPP has been shown to be neurotoxic in vitro and can have severe effects upon embryogenesis in zebrafish. Experimental studies have shown that TCP is toxic to reproductive and nervous systems.

As a new generation of OPFRs, dialkyl phosphinates (DPs) occupy high-end industrial markets. In a field study carried out in a manufacturing plant, aluminum dialkyl phosphinates (ADPs) were detected in all samples of soil and sediment, and the highest concentration reached the milligram per kilogram level. Hydrolysates of ADPs, dialkyl phosphinic acids (DAPs), were detected in >90% of these samples.

A recent study reported that exposure to aluminum diethylphosphinate (AEEP) could affect the cumulative reproductive output and population growth rate of Daphnia magna. Also, the lethal concentration for 50% of the population (LC50) of Daphnia magna for AEEP at 21 days was 3.2 mg/L, which suggests moderate, chronic toxicity. However, the toxic effect of ADPs in mammals is not known.

To address this knowledge gap, we focused on four frequently used DPs: aluminum methylcyclohexylphosphinate (AMHP), calcium methylcyclohexylphosphinate (CMHP), aluminum methylcyclohexylphosphinate AMEP, and AEEP. Hydrolysates of AMHP, methylcyclohexylphosphinic acid (MHPA) were also studied. Using these DPs and DAPs, we performed toxicity experiments in male BALB/c mice. The outcome of this assessment could be the first scientific evidence showing if DPs are suitable alternative for BFRs.

8 | MATERIALS AND METHODS

2.1 | Chemicals

AMHP and CMHP (purity ≥95%) were synthesized by the Key Laboratory of Optoelectronic Chemical Materials and Devices of the Ministry of Education (Jianghan University, Wuhan, P. R. China) (supporting information Figure S1a,b). AMEP and AEEP (purity ≥95%) were purchased from Clariant International Ltd. (Basel, Switzerland) (supporting information Figure S1d,e). These four chemicals were dissolved in corn oil with an ultrasonic shaker to form stock solutions (0.5 and 2.5 mg/L). MHPA (purity ≥95%) was synthesized by the Key Laboratory of Optoelectronic Chemical Materials and Devices of the Ministry of Education (supporting information Figure S1c). MHPA was dissolved in physiologic (0.9%) saline to form stock solutions (0.5, 2.5, 5 mg/L). Stock solutions were stored in the dark. All chemicals were of analytical grade.

2.2 | Maintenance and treatment of animals

Animals were bred and used in accordance with guidelines set by the Ethics Committee on Animal Welfare of Jianghan University.

Four-week-old male BALB/c mice were purchased from Hubei Research Center of Laboratory Animals (Hubei, P. R. China). Mice were housed in cages in a room at 22–24°C and 40–60% humidity with a 12-h light-dark cycle and free access to food and tap water. Mice were allowed to acclimatize to their environment for 7 days before exposure to chemicals.

To handle the large amount of mice in chemical-exposure experiments, mice were divided randomly into three batches. Then, the first and second batches were divided into five groups of six mice.

The first batch comprised one corn-oil control group, and four experimental groups received (in mg/kg/d) 10 of AMHP, 50 of AMHP, 10 of CMHP, or 50 of CMHP. The second batch comprised one corn-oil control group, and four experimental groups received (in mg/kg/d) 10 of AMHP, 50 of AMHP, 10 of AEEP, 50 of AEEP, 10 of AEEP, or 50 of AEEP. The third batch contained 40 mice and was divided into four groups: one group received the negative control (physiologic saline), and three experimental groups received 10, 50, or 100 mg/kg/d of MHPA.

Treatment was through gavage each morning for 28 consecutive days. Volume of each stock solution of chemical used in the gavage was adjusted every 3 days according to body weight. During exposure, the body weight and food consumption of each mouse were recorded every 3 days.

2.3 | Collection of organs and sera

Mice were killed by decapitation on the morning of day-29. Animals were not permitted to consume food or water for the previous 12 h so that accurate measurement of serum biochemical indices could be undertaken. Trunk blood were extracted from the eye socket, collected into tubes, incubated for 2 h at room temperature, and centrifuged at 4000 rpm for 10 min at room temperature. Serum was kept at −80°C until assay. Cauda epididymises were placed into separate Petri dishes containing 8-mL modified Tyrode’s medium (MT6) for sperm count calculation. The heart, liver, spleen, lungs, kidneys, thymus glands, and testes were collected and weighed immediately. The Visceral Index (VI) was calculated as follows:

\[ VI = \left( \frac{\text{organ weight}}{\text{body weight}} \right) \times 100 \% \]
TABLE 1  Effects of AMHP and CMHP on the Visceral Index in organs in BALB/c mice after 28 days of exposure (n = 6)

| Group   | Control     | AMHP 10 mg/kg/d | AMHP 50 mg/kg/d | CMHP 10 mg/kg/d | CMHP 50 mg/kg/d |
|---------|-------------|-----------------|-----------------|-----------------|-----------------|
| Heart (%) | 0.58 ± 0.03 | 0.56 ± 0.03     | 0.55 ± 0.02     | 0.58 ± 0.02     | 0.55 ± 0.03     |
| Liver (%) | 4.18 ± 0.12 | 4.45 ± 0.08     | 4.26 ± 0.2      | 4.36 ± 0.16     | 4.47 ± 0.17     |
| Spleen (%)| 0.4 ± 0.03  | 0.49 ± 0.05*    | 0.34 ± 0.03     | 0.31 ± 0.01*    | 0.36 ± 0.02     |
| Lung (%)  | 0.68 ± 0.04 | 0.65 ± 0.05     | 0.57 ± 0.02*    | 0.64 ± 0.03     | 0.65 ± 0.03     |
| Kidney (%)| 1.48 ± 0.04 | 1.42 ± 0.11     | 1.44 ± 0.05     | 1.49 ± 0.04     | 1.47 ± 0.06     |
| Testis (%)| 0.76 ± 0.03 | 0.68 ± 0.03     | 0.73 ± 0.03     | 0.83 ± 0.04     | 0.83 ± 0.04     |
| Thymus gland (%) | 0.21 ± 0.03 | 0.22 ± 0.01     | 0.20 ± 0.02     | 0.22 ± 0.02     | 0.22 ± 0.02     |

*p < .05, **p < .01, in comparison with the control group, one-way ANOVA. Data are the mean ± SE.

A small piece of each organ was fixed in 10% formalin solution for further histology.

2.4 | Chemical measurements

Content of AMHP and MHPA in feces and serum was measured according to a validated method.24 Freeze-dried feces from five animals were pooled and randomized, and then 50 mg extracted using 1-mL NH3: water: methanol (20:10:70, vol/vol/vol). After vortex-mixing for 1 min, mixtures were centrifuged at 10 000 rpm for 10 min at 4°C. Supernatants were purified by hydrophilic/lipophilic balanced (HLB) and medium anion exchange (MAX) solid-phase extraction columns, eluted into 5-mL of 2% 2-furylmethanol and evaporated in a gentle stream of nitrogen at 40°C. Samples of feces and serum were analyzed by liquid chromatography-tandem mass spectrometry.

2.5 | Sperm counts

To extract sperm from seminiferous tubules, the cauda epididymis in the Petri dish was minced carefully into lengths of ≈1 mm with surgical scissors and incubated at 37°C for 40 min. Using aliquots of 10 μL out of a sperm solution of 8 mL, sperm counts were determined via a hemocytometer by counting the number of sperm in each of the four larger squares. A mean value of the four squares was used together with the mean value of the other cauda epididymis from the same mouse to calculate the mean amount. The final sperm count was determined as follows:

\[
\text{Sperm count} = (\text{mean amount}) \times 8 \times 10^4
\]

2.6 | Hormone measurements and analyses of biochemical indices

An AXSYM Automated Immunoassay Analyzer (Abbott Diagnostics, Abbott Park, IL, USA) was used to measure levels of estradiol and testosterone in serum using AxSYM Estradiol and AxSYM Testosterone kits (Abbott Laboratories, Abbott Park, IL, USA), respectively. An Aeriset Automated Analyzer (Abbott Diagnostics) was employed to measure levels of alanine transaminase (ALT), albumin (ALB), blood urea nitrogen (BUN), glucose (GLU), triglyceride (TG), cholesterol (CHOL), high-density lipoprotein-cholesterol (HDL-CHOL), and low-density lipoprotein-cholesterol (LDL-CHOL) in serum.

2.7 | Histology

Fixed organs were embedded in paraffin and sliced into sections (4-μm thick) by an Automatic Microtome (Leica Microsystems, Wetzlar, Germany). Sections were stained with hematoxylin and eosin (H&E) for observation under a BX41 Light Microscope (Olympus, Tokyo, Japan).

2.8 | Statistical analyses

Data are the mean ± SEM. Analysis of variance (one-way, ANOVA) followed by Tukey’s multiple range test was used to compare differences

TABLE 2  Effects of AMEP and ADEP on the Visceral Index of organs in BALB/c mice after 28 days of exposure (n = 6)

| Group   | Control     | AMEP 10 mg/kg/d | AMEP 50 mg/kg/d | ADEP 10 mg/kg/d | ADEP 50 mg/kg/d |
|---------|-------------|-----------------|-----------------|-----------------|-----------------|
| Heart (%) | 0.64 ± 0.05 | 0.66 ± 0.04     | 0.63 ± 0.04     | 0.58 ± 0.03     | 0.64 ± 0.04     |
| Liver (%) | 4.56 ± 0.17 | 4.57 ± 0.15     | 4.87 ± 0.26     | 4.62 ± 0.29     | 4.62 ± 0.25     |
| Spleen (%)| 0.38 ± 0.02 | 0.35 ± 0.02     | 0.33 ± 0.02     | 0.35 ± 0.03     | 0.25 ± 0.02**   |
| Lung (%)  | 0.77 ± 0.06 | 0.74 ± 0.05     | 0.82 ± 0.05     | 0.68 ± 0.03     | 0.67 ± 0.03     |
| Kidney (%)| 1.47 ± 0.06 | 1.55 ± 0.04     | 1.61 ± 0.06     | 1.36 ± 0.04     | 1.51 ± 0.05     |
| Testis (%)| 0.7 ± 0.03  | 0.74 ± 0.03     | 0.8 ± 0.03*     | 0.87 ± 0.03**   | 0.84 ± 0.04**   |
| Thymus gland (%) | 0.24 ± 0.03 | 0.25 ± 0.03     | 0.28 ± 0.04     | 0.29 ± 0.03     | 0.26 ± 0.03     |

*p < .05, **p < .01, in comparison with the control group, one-way ANOVA. Data are the mean ± SE.
between groups. Statistical analyses were carried out using SPSS v16.0 (IBM, Armonk, NY, USA). \( p \leq 0.05 \) was considered significant.

3 | RESULTS

3.1 | Chemical Measurements

To explore the fate of chemicals after exposure in mice, the presence of AMHP and MHPA was quantified. After exposure to AMHP at 50 mg/kg/d, 1.11 mg of AMHP was detected in feces and 0.003 \( \mu \)g was detected in serum. In the MHPA-exposure assay, 0.94, 2.10, and 4.97 mg of MHPA was detected in feces in 10, 50, and 100 mg/kg/d groups, and 0.003, 0.010, and 0.004 \( \mu \)g was detected in the sera of mice of these groups, respectively.

3.2 | DPs and DPA do not affect the general condition of mice

We monitored the physical and mental conditions of BALB/c mice. No obvious abnormalities were found in the behavior, gait, or climbing ability of mice in any group. Compared with the control group, exposure to the five chemicals showed no difference in body weight (supporting information Tables S1–S3).

3.3 | DPs affect the VI of the spleen and testes

The VI of the heart, liver, spleen, lungs, kidneys, testes, and thymus gland was studied. Exposure to AMHP at 10 mg/kg/d increased the VI of the spleen significantly \( (p < 0.05) \), whereas CMHP at 10 mg/kg/d decreased the VI of the spleen significantly \( (p < 0.05) \) (Table 1). Compared with the control group, mice in the 50 mg/kg/d groups of AMHP and CMHP showed a decreased VI of the spleen but the difference was not remarkable. ADEP exposure and exposure to a high dose of AMEP caused a dramatic increase in the VI of the testes that was significant \( (p < 0.01) \) (Table 2). The VI of the testes in the CMHP group showed an identical trend. Compared with the control group, DPA exposure showed no effect on the VI in experimental groups (supporting information Table S4).

3.4 | Exposure to high doses of AMEP and ADEP decrease sperm counts

The cauda epididymis was used to analyze sperm counts in mice. A dramatic decrease in sperm counts was found in the 50 mg/kg/d groups of AMEP and ADEP, and the differences were significant \( (p < 0.05) \) (Figure 1C). Compared with the control group, exposure to 10 mg/kg/d of AMHP, AMEP, ADEP, and CMHP elicited a mild decrease in sperm

![Figure 1](image-url)  
**FIGURE 1** Effects of DPs on sperm density in BALB/c mice after 28 days of exposure: (a) AMHP and CMHP; (b) AMEP and ADEP; (c) MHPA. \( *p < 0.05, **p < 0.01 \), in comparison with the control group, one-way ANOVA. Data are the mean ± SE. \( n \) = the number in the column.

| TABLE 3 | Effects of AMHP and CMHP on serum biochemical indices in BALB/c mice after 28 days of exposure (n = 6) |
|----------|---------------------------------------------------------------|
| Group    | Control | AMHP 10 mg/kg/d | AMHP 50 mg/kg/d | CMHP 10 mg/kg/d | CMHP 50 mg/kg/d |
| ALT (U/L) | 33.75 ± 2.63 | 36.5 ± 3.82 | 38.73 ± 6.04 | 34.09 ± 2.32 | 28.75 ± 1.88 |
| ALB (g/L) | 35.63 ± 0.54 | 31.78 ± 1.15* | 37.75 ± 1.22 | 36.77 ± 0.72 | 34.56 ± 0.83 |
| BUN (mmol/L) | 6.85 ± 0.25 | 7.1 ± 0.43 | 6.98 ± 0.55 | 8.32 ± 0.55* | 8.58 ± 0.52* |
| GLU (mmol/L) | 5.38 ± 0.41 | 7.36 ± 0.64** | 5.49 ± 0.33 | 6.38 ± 0.33* | 6.36 ± 0.21* |
| CHOL (mmol/L) | 2.17 ± 0.18 | 2.10 ± 0.18 | 2.52 ± 0.17 | 2.39 ± 0.14 | 2.29 ± 0.13 |
| TG (mmol/L) | 0.16 ± 0.03 | 0.13 ± 0.03 | 0.17 ± 0.03 | 0.48 ± 0.09** | 0.42 ± 0.07** |
| HDL-CHOL (mmol/L) | 1.41 ± 0.14 | 1.36 ± 0.11 | 1.62 ± 0.09 | 1.53 ± 0.07 | 1.46 ± 0.08 |
| LDL-CHOL (mmol/L) | 0.11 ± 0.02 | 0.12 ± 0.02 | 0.14 ± 0.01 | 0.08 ± 0.01 | 0.09 ± 0.01 |

*\( p < 0.05 \), **\( p < 0.01 \), in comparison with the control group, one-way ANOVA. Data are the mean ± SE.
result in abnormal biological indices in mice (Table 5). In the meanwhile, AMEP and ADEP exposure do not affect levels of gonadal hormones in serum (supporting information Figures S2–S4).

As revealed by serum biological indices, mice in CMHP groups showed severe metabolic disturbances. GLU level was increased significantly (p < .05) in both exposure groups. CMHP also showed a very significant increased effect (p < .01) upon TG level in exposure groups. BUN level was increased after CMHP exposure (p < .05) (Table 3). Exposure to other DPs caused fewer disturbances in metabolism. Exposure to AMHP at 10 mg/kg/d caused a large decrease in ALB level (p < .05), and very significant increase (p < .01) in GLU level. ALT levels in serum were increased significantly (p < .05) after ADEP exposure. Exposure to MHPA at 100 mg/kg/d had a very significant effect (p < .01) upon increases in BUN levels and decreases in LDL-CHOL levels (Table 4). In the meanwhile, AMEP and ADEP exposure do not result in abnormal biological indices in mice (Table 5).

### Table 4: Effects of MHPA on serum biochemical indices in BALB/c mice after 28 days of exposure (n = 8)

| Group          | Control  | MHPA 10 mg/kg/d | MHPA 50 mg/kg/d | MHPA 100 mg/kg/d |
|----------------|----------|-----------------|-----------------|------------------|
| ALT (U/L)      | 36.33 ± 4.36 | 38.57 ± 2.26    | 62.33 ± 25.60   | 37.00 ± 2.86     |
| ALB (g/L)      | 27.70 ± 0.52  | 27.31 ± 1.06    | 27.67 ± 0.46    | 27.97 ± 0.47     |
| BUN (mmol/L)   | 5.50 ± 0.20   | 6.31 ± 0.21     | 5.73 ± 0.25     | 6.43 ± 0.18**    |
| GLU (mmol/L)   | 3.07 ± 0.16   | 3.40 ± 0.46     | 3.30 ± 0.28     | 3.53 ± 0.50      |
| CHOL (mmol/L)  | 2.01 ± 0.11   | 2.10 ± 0.09     | 1.93 ± 0.10     | 1.87 ± 0.09      |
| TG (mmol/L)    | 0.42 ± 0.04   | 0.45 ± 0.07     | 0.34 ± 0.04     | 0.38 ± 0.04      |
| HDL-CHOL (mmol/L) | 1.54 ± 0.08 | 1.57 ± 0.08     | 1.41 ± 0.07     | 1.40 ± 0.06      |
| LDL-CHOL (mmol/L) | 0.13 ± 0.01 | 0.11 ± 0.01     | 0.13 ± 0.01     | 0.08 ± 0.01**    |

*3.5 DPs and DPA have effects upon various biological indices but no effects upon serum levels of gonadal hormones*

Measurements of levels of gonadal hormones showed that feeding of AMHP, CMHP, MHPA, AMEP, and ADEP to mice did not affect levels of gonadal hormones in serum (supporting information Figures S2–S4).

As a hydrophobic and oleophobic chemical, AMHP is present in BALB/c mouse urine. As revealed by renal pathologic analyses, AMHP is concentrated primarily in the liver and spleen. In the 10 mg/kg/d groups of AMHP and CMHP, liver cells were swollen and had “loose” cytoplasm (Figure 2B,D). Fatty degeneration of hepatocytes was detected after MHPA exposure (Figure 4F,G,H). Slight bleeding in renal medullae was observed in AMHP and CMHP groups at 50 mg/kg/d, and MHPA groups of 50 and 100 mg/kg/d, spotty necrosis were detected in the liver (Figures 2C,E; 3C,D; 5B,C,E). Increased numbers of macrophagocytes in the spleen were observed in AMHP and CMHP groups at 50 mg/kg/d, and MHPA group at 50 and 100 mg/kg/d (Figure 4H,L; 5G,H). Slight bleeding in renal medullae was detected after MHPA exposure (Figure 4F,G,H).

### Table 5: Effects of AMEP and ADEP on serum biochemical indices in BALB/c mice after 28 days of exposure (n = 6)

| Group          | Control  | AMEP 10 mg/kg/d | AMEP 50 mg/kg/d | ADEP 10 mg/kg/d | ADEP 50 mg/kg/d |
|----------------|----------|-----------------|-----------------|-----------------|-----------------|
| ALT (U/L)      | 34.83 ± 3.30 | 43.18 ± 3.52    | 43.6 ± 13.15    | 49.36 ± 14.88   | 54.08 ± 6.75*   |
| ALB (g/L)      | 36.91 ± 0.53 | 36.32 ± 0.86    | 38.77 ± 0.75    | 38.66 ± 0.72    | 38.78 ± 1.22    |
| BUN (mmol/L)   | 7.18 ± 0.25  | 6.69 ± 0.33     | 6.97 ± 0.26     | 6.77 ± 0.55     | 7.13 ± 0.31     |
| GLU (mmol/L)   | 5.74 ± 0.41  | 5.44 ± 0.33     | 5.96 ± 0.24     | 5.34 ± 0.66     | 6.18 ± 0.45     |
| CHOL (mmol/L)  | 2.69 ± 0.18  | 2.52 ± 0.21     | 2.53 ± 0.16     | 2.73 ± 0.23     | 2.38 ± 0.24     |
| TG (mmol/L)    | 0.26 ± 0.04  | 0.26 ± 0.04     | 0.24 ± 0.05     | 0.34 ± 0.08     | 0.39 ± 0.07     |
| HDL-CHOL (mmol/L) | 1.79 ± 0.11 | 1.68 ± 0.12     | 1.7 ± 0.10      | 1.73 ± 0.11     | 1.66 ± 0.11     |
| LDL-CHOL (mmol/L) | 0.17 ± 0.02 | 0.16 ± 0.02     | 0.13 ± 0.01     | 0.15 ± 0.01     | 0.17 ± 0.02     |

*p < .05, **p < .01, in comparison with the control group, one-way ANOVA. Data are the mean ± SE.

3.6 DPs and DPA cause mild toxicity in the liver, spleen, and kidneys of mice

Pathologic analyses of organs showed that the toxicity of DP and DPA was concentrated primarily in the liver and spleen. In the 10 mg/kg/d groups of AMHP and CMHP, liver cells were swollen and had “loose” cytoplasm (Figure 2B,D). Fatty degeneration of hepatocytes was observed in the ADEP group at 10 mg/kg/d (Figure 5D). In AMHP, CMHP and ADEP groups at 50 mg/kg/d, AMEP groups at 10 and 50 mg/kg/d, and MHPA groups of 50 and 100 mg/kg/d, spotty necrosis were detected in the liver (Figures 2C,E; 3C,D; 5B,C,E). Increased numbers of macrophagocytes in the spleen were observed in AMHP and CMHP groups at 50 mg/kg/d, and MHPA group at 50 and 100 mg/kg/d (Figure 4H,L; 5G,H). Slight bleeding in renal medullae was detected after MHPA exposure (Figure 4F,G,H).

### 4 DISCUSSION

We wished to provide important new data for novel DPFRs. We demonstrated, for the first time, that AMHP, CMHP, AMEP, ADEP, MHPA, and the hydrolysate of AMHP showed mild (but diverse) toxicity in male BALB/c mice.

As a hydrophobic and oleophobic chemical, AMHP is present mainly in soil and sediments. A field investigation carried out recently by our research team suggested that AMHP can be hydrolyzed into MHPA.
FIGURE 2  Histopathologic sections of mice liver, spleen, and testis after 28 days of exposure to AMHP and CMHP, H&E ×100. (a) Liver of control, which is normal; (b) liver of 10 mg/kg/d AMHP group, loose cytoplasm and cell enlargement (arrow); (c) liver of 50 mg/kg/d AMHP group, loose cytoplasm, cell enlargement (arrow), and spotty necrosis (triangle); (d) liver of 10 mg/kg/d CMHP group, loose cytoplasm and cell enlargement (arrow); (e) liver of 50 mg/kg/d CMHP group, loose cytoplasm, cell enlargement (arrow), and spotty necrosis (triangle); (f) Spleen of control, which is normal; (g) spleen of 10 mg/kg/d AMHP group, which is normal; (h) spleen of 50 mg/kg/d AMHP group, increased number of macrophagocytes (arrows); (i) spleen of 50 mg/kg/d CMHP group, which is normal; (j) spleen of 50 mg/kg/d CMHP group, increased numbers of macrophagocyte (arrows); (k) testis of control, which is normal; (l) testis of 10 mg/kg/d AMHP group, which is normal; (m) testis of 10 mg/kg/d CMHP group, which is normal; (n) testis of 50 mg/kg/d AMHP group, which is normal; (o) testis of 50 mg/kg/d CMHP group, which is normal. Scale = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 3  Histopathologic sections of mice liver, spleen and testis after 28 days of exposure to MHPA, H&E ×100. (a) Liver of control, which is normal; (b) liver of 10 mg/kg/d MHPA group, which is normal; (c) liver of 50 mg/kg/d MHPA group, spotty necrosis (triangle); (d) liver of 100 mg/kg/d MHPA group, spotty necrosis (triangle); (e) spleen of control, which is normal; (f) spleen of 10 mg/kg/d MHPA group, which is normal; (g) spleen of 50 mg/kg/d MHPA group, increased numbers of macrophagocytes (arrows); (h) spleen of 100 mg/kg/d MHPA group, which is normal; (i) testis of control, which is normal; (j) testis of 10 mg/kg/d MHPA group, which is normal; (k) testis of 50 mg/kg/d MHPA group, which is normal; (l) testis of 100 mg/kg/d MHPA group, which is normal. Scale = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]
weakly hydrophilic MHPA in the environment. Therefore, we used AMHP and MHPA in exposure experiments. After exposure to AMHP, we observed swollen hepatocytes and spotty necrosis in mice livers in 10 and 50 mg/kg/d groups, respectively. Taken together with the large decrease in serum levels of ALB, and dramatic increase in GLU level, AMHP clearly caused mild hepatotoxicity in BALB/c mice. Exposure to high-dose MHPA caused the same pathologic changes in the liver as after AMHP exposure, and the damage was dependent upon dose. Analyses of the spleen revealed that exposure to AMHP and MHPA increased the numbers of macrophagocytes. We speculated that

**FIGURE 4** Histopathologic sections of mice kidneys after 28 days of exposure to MHPA, H&E ×100. (a) Renal cortex of control, presents normal; (b) renal cortex of 10 mg/kg/d MHPA group, presents normal; (c) renal cortex of 50 mg/kg/d MHPA group, which is normal; (d) renal cortex of 100 mg/kg/d MHPA group, which is normal; (e) renal medulla of control, which is normal; (f) renal medulla of 10 mg/kg/d MHPA group, minor bleeding (arrows); (g) renal medulla of 50 mg/kg/d MHPA group, minor bleeding (arrows); (h) renal medulla of 100 mg/kg/d MHPA group, minor bleeding (arrows). Scale = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 5** Histopathologic sections of mice liver, spleen, and testis after 28 days of exposure to AMEP and ADEP, H&E ×100. (a) Liver of control, which is normal; (b) liver of 10 mg/kg/d AMEP group, spotty necrosis (triangle); (c) liver of 50 mg/kg/d AMEP group, spotty necrosis (triangle); (d) liver of 10 mg/kg/d ADEP group, fatty degeneration (arrow); (e) liver of 50 mg/kg/d ADEP group, spotty necrosis (triangle); (f) spleen of control, which is normal; (g) spleen of 10 mg/kg/d AMEP group, which is normal; (h) spleen of 50 mg/kg/d AMEP group, which is normal; (k) spleen of 10 mg/kg/d ADEP group, which is normal; (l) liver of 50 mg/kg/d ADEP group, which is normal; (m) testis of control, which is normal; (n) testis of 10 mg/kg/d AMEP group, which is normal; (o) testis of 50 mg/kg/d AMEP group, which is normal; (p) testis of 10 mg/kg/d ADEP group, which is normal; (s) testis of 50 mg/kg/d ADEP group, which is normal. Scale = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]
exposure to AMHP and MHPA may stimulate the immune system of mice. Furthermore, MHPA showed mild toxicity to the kidney that was not observed in mice exposed to AMHP. This observation could have been the result of the superior water solubility of MHPA. We explored the fate of these two chemicals after gavage in mice. As expected, most of the chemicals were discharged in feces, and only slight amounts were absorbed and present in the serum. Therefore, AMHP and its hydrolysate MHPA showed consistent, mild hepatotoxicity.

We used CMHP to understand the role of chemical structure upon the effects of exposure. With a different metal ion, Ca^{2+}, CMHP caused almost identical hepatotoxicity to AMHP. However, analyses of serum biological indices revealed an additional metabolic disturbance after CMHP exposure. The liver is the main organ for the metabolism of GLU, lipids, and proteins. Our results suggest that, though pathology showed the same extent of liver damage, the metabolic function of the liver was severely disturbed by CMHP. Moreover, serum levels of BUN were increased significantly after CMHP exposure, which suggests that CMHP can cause renal toxicity. Further research to ascertain if the chemical structure of CMHP is responsible for toxicity in mice should be carried out.

ADEP and AMEP are flame retardants under the commercial name of Exolit OP (Clariant International Ltd.) and have been used world-wide for decades. Their water solubility could result in toxicity in organisms. A recent study reported that ADEP had moderate toxicity in Daphnia magna, and caused growth and reproductive toxicity. In mice, however, ADEP and AMEP had different effects. Serum level of ALT is a sensitive indicator of liver damage. Compared with the control group, mice in AMEP and ADEP groups showed an increased level of ALT in serum. These results were in accordance with pathology, in which fatty degeneration and spotty necrosis in the liver was observed. However, the general condition, VI of the liver, and the metabolic system in these mice were not affected. Thus, AMEP and ADEP cause only mild hepatotoxicity in mice. ADEP exposure and exposure to a high dose of AMEP caused a dramatic increase in the VI of testes. Sperm counts in the cauda epididymis revealed a mild decrease in AMEP and ADEP groups at 10 mg/kg/d, and a dramatic decrease in AMEP and ADEP groups at 50 mg/kg/d. Levels of estradiol and testosterone in serum were not significantly different between the control group and experimental groups. Hence, AMEP and ADEP had some reproductive toxicity, and this damage may not be related directly to endocrine disruption.

5 CONCLUSIONS

The present study showed that DPs and their main hydrolysates had mild toxicity in BALB/c mice. AMEP and ADEP can cause mild toxicity in the liver and toxicity to the reproduction system. CMHP is toxic to the liver and kidneys. Toxicity of AMHP and its hydrolysate MHPA in mice is chronic, low, and affects the liver. Based on this research, AMHP seems to be a more suitable candidate as an environmentally friendly flame retardant than the other DPs that we tested.

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SUPPORTING INFORMATION
Additional Supporting Information may be found in the online version of this article.

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