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Neonatal triphenyl phosphate and its metabolite diphenyl phosphate exposure induce sex- and dose-dependent metabolic disruptions in adult mice☆

Dezhen Wang [a, 1], Wentao Zhu [a, 1], Li Chen [a], Jin Yan [a], Miaomiao Teng [b], Zhiqiang Zhou [a, *]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, Department of Applied Chemistry, China Agricultural University, Beijing 100193, China
[b] Department of Applied Chemistry, China Agricultural University, Beijing 100193, China

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ABSTRACT

The widespread application of organophosphorous flame retardants (OPFRs) has led to considerable human exposure, with major concerns regarding their health risks. Herein, we investigate the effects of triphenyl phosphate (TPP), one of the most widely used OPFRs, and one of its main metabolite diphenyl phosphate (DPP) on the endocrine systems and metabolic profiles after neonatal exposure from postnatal days 1–10 at two dosages (2 and 200 μg per day). Both TPP and DPP had no negative effect on uterine weight, glucose tolerance, and estradiol. ^{1}H-NMR-based metabolomics revealed a sex-specific metabolic disturbance of TPP. Specifically, low dose of TPP altered the metabolic profiles of male mice while exerting no significant effects on female ones. Furthermore, a dose-dependent effect of TPP in male mice was observed, where a low toxicity dose up-regulated lipid-related metabolites, while a high toxicity dose down-regulated the pyruvate metabolism and TCA cycles. These results highlight the importance of carefully assessing the health impact of TPP on infants.

1. Introduction

Flame retardants are chemical additives in consumer products or construction materials that reduce the flammability of materials to meet fire safety standards (EFRA, 2007). Up until the mid-2000s, polybrominated diphenyl ethers were amongst the most common primary flame retardants used in textiles, plastics, foams and electronics (Kemmlein et al., 2003). However, concerns about their adverse health effects in human and wildlife led to a reduction of their use and subsequent discontinuation (UNEP, 2009; US-EPA, 2007). Consequently, alternative materials, such as organophosphorous flame retardants (OPFRs) were employed (van der Veen and de Boer, 2012). OPFRs are also used as additives rather than being chemically bonded and can therefore easily leach into the environment. Triphenyl phosphate (TPP), one of the most commonly used OPFRs is a frequently detected chemical in environmental samples, including indoor dust samples from homes, offices, and vehicle interiors (Hoffman et al., 2015b; Meeker and Stapleton, 2010; Stapleton et al., 2009; Wu et al., 2016) at relatively high levels of up to 1.8 mg/g (Meeker and Stapleton, 2010). TPP has also been detected in various aquatic environments, such as surface and drinking water (Benotti et al., 2009; Li et al., 2014; Rodil et al., 2012). TPP is hydrophobic, and has an affinity for sediment and soil, and has relative high bioconcentration factors (110–300) in fish (Hou et al., 2016). Numerous studies have also reported the detection of TPP and its main metabolite, diphenyl phosphate (DPP) in human samples (Hoffman et al., 2015b; Zhao et al., 2016), particularly of TPP in human breastmilk (Kim et al., 2014) and DPP in the urine of infants aged 2–18 months (Hoffman et al., 2015a). Monitoring data indicate that the general population may be exposed to TPP via hand-to mouth contact, dermal absorption, inhalation of ambient air and ingestion of food and drinking water (Betts, 2015; Rodil et al., 2012; Wu et al., 2016). Breastmilk has been supposed to be another important exposure source for infants (Kim et al., 2014). Thus, the widespread usage and exposure of TPP and DPP highlight the need for additional toxicity studies.

According to previous in vitro studies, TPP is an endocrine

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* Corresponding author. Yuanmingyuan west road 2, Beijing 100193, PR China.
E-mail address: zqzhou@cau.edu.cn (Z. Zhou).
[1] Dezhen Wang and Wentao Zhu contributed equally to this paper.

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disruptor (Kojima et al., 2013; Krivoshiev et al., 2016; Zhang et al., 2014). Indeed, a MCF-7 flow-cytometry proliferation assay revealed that TPP induced significant estrogenic activity (Krivoshiev et al., 2016). Similar results were obtained from reporter gene analyses, demonstrating that TPP is an estrogen receptor α agonist and an androgen receptor antagonist (Kojima et al., 2013; Zhang et al., 2014). High doses of TPP exposure (100 and 300 mg/kg body weight) in five weeks old male mice for 35 days led to histopathological damage and the decrease of testicular testosterone levels, indicating its potential reproductive toxicity (Chen et al., 2015). In vitro studies indicated that TPP could enhance adipogenic differentiation, glucose uptake and lipolysis via both endocrine and noradrenergic mechanisms (Cano-Sancho et al., 2017). TPP could also disrupt the homeostasis of thyroid hormone, demonstrated by in vitro studies in rat pituitary (GH3) and thyroid follicular (FRTL-5) cell lines (Kim et al., 2015). Zebrafish and medaka exposure demonstrated that TPP could induce neurotoxicity (Sun et al., 2016) and developmental toxicity (Isales et al., 2015).

Nevertheless, despite a growing number of studies having been conducted in the past decades to delineate the potential effects of TPP, knowledge regarding its health risks in early life exposure in rodents remains limited. In general, the early life stages are more sensitive to environmental pollutants, with their detrimental impacts in childhood often persisting until adulthood. Thus, the health effects of TPP exposure in the early life stages of infants and children should be carefully assessed.

The evaluation of the health effects of TPP in the early life stages requires the implementation of sensitive and comprehensive methods. Metabolomics, which has been widely used in environmental toxicology to determine biochemical changes and predict the mode of action of chemicals (Lankadurai et al., 2013), could provide a promising route to enhance our understanding of the health risks of TPP. Metabolomics has been used to highlight the effects of low doses of bisphenol A (0.025, 0.25, and 2.5 μg/kg body weight) by linking perinatal exposure to changes in global metabolism (Cabaton et al., 2013). Metabolomics has also been used to associate systemic metabolic dysfunction induced by 2,3,7,8-tetrachlorodibenzofuran with aryl hydrocarbon receptor activation (Zhang et al., 2015). Combined with appropriate traditional methods, such as q-PCR and western blotting, metabolic fingerprinting can detected slight changes in serum metabolite levels, while some of these changes may not appear apparent at the individual level. Thus, metabolomics is an ideal approach to evaluate the early life toxicity of TPP.

Herein, neonatal mice were subcutaneously injected on postnatal days 1–10 with TPP and DPP. Then we evaluated the metabolic changes in mice serum using a sensitive 1H-NMR-based metabolomics approach Moreover, a traditional uterotrophic bioassay was performed in immature female mice and rats to verify the estrogenic effects of TPP. All in all, ovarian histopathology, hormone levels (estradiol), uterine weight, and serum metabolomes were analyzed.

2. Materials and methods

2.1. Chemicals

TPP (99% purity) and DPP (99% purity) were purchased from J&K Scientific Ltd. (Beijing, China). The chemical structures of TPP and DPP are shown in Fig. 1A. Sodium 3-trimethylsilyl-2, -2, -3, -3-d4-propionate (TSP-d4) and D_2O (99.9% in D) were obtained from Aladdin (Shanghai, China).

2.2. Animals and treatment

Primigravida ICR mice were purchased from Peking University Health Science Center and were housed individually in plastic cages with a 12-h light/dark cycle at 22 °C with free access to water and food. After delivery, foster females were randomly assigned eight newborns to ensure the growth of pups. Both female and male pups were injected subcutaneously on postnatal days 1–10 with corn oil (control; CK), TPP (2 or 200 μg per day; TPP-L and TPP-H, respectively) or DPP (2 or 200 μg per day; DPP-L and DPP-H, respectively). We selected the low dosage (2 μg), because it was equivalent to a prenatal exposure dose to FM 550 (a mixture containing TPP) (Patisaul et al., 2013), and lower than a chronic toxicity dose of 2 mg/kg over a 15-week period in adult rat (Alam et al., 2012). TPP and DPP were primarily dissolved in corn oil at a concentration of 0.1 or 10 mg/ml. All treatments were delivered in a 0.02 ml corn oil solution. We administered TPP and DPP via subcutaneous injection to mimic both oral and dermal exposure, for a previous study showed that during the neonatal period, oral and non-oral administration give the same internal active dose in rodents because of the low liver enzyme activity in neonatal rodents (Taylor et al., 2008). The exposure period was restricted to postnatal day 1–10 to assess the health effects of TPP or DPP on infants. Pups were assigned to several experiments as outlined in Fig. 1B. All animal experiments were performed in accordance with the current Chinese legislation and were approved by the independent Animal Ethical committee of China Agricultural University with approval number CAU20170615-3.

2.3. Ovarian histology

To determine the effect of TPP and DPP exposure on the developing ovary, morphological evaluations were performed on ovaries at 19 days of age. Ovaries were collected and fixed in 10% neutral formalin and stained with hematoxylin and eosin according to standard laboratory procedure. Three sections of both ovaries from each mice (six replicates per group) were analyzed for the presence and number of multi-oocyte follicles (MOFs) as previously reported (Jefferson et al., 2009). The MOFs were determined because previous studies showed that exposure of fetal or newborn animals to endocrine disruptors and phytoestrogens, such as diethylstilbestrol (Iguchi et al., 1990), isoflavone (Takashima-Sasaki et al., 2006), genistein (Jefferson et al., 2002), and bisphenol A (Rodriguez et al., 2010) were associated with the increased MOFs numbers, and the increased MOFs numbers were thought to be generated by alterations of nest breakdown and/or follicle assembly.

2.4. Uterotrophic bioassay in immature mice or rats

Female ICR mice or SD rats (17-days-old) that had not undergone any previous treatment were subcutaneously injected once daily for three consecutive days with solutions of corn oil (control), 200 or 600 mg/kg TPP, 200 or 600 mg/kg DPP, or 100 μg/kg ethinyloestradiol as a positive control. Animals were terminated 24 h after the last treatment and the uteri were dissected and weighed.

2.5. Hormone analysis

Hormone analysis of estradiol levels was performed in serum collected at 12 weeks from all the treatment groups with an ELISA kit (Elabscience Biotechnology Co., Ltd) according to the manufacturers' instructions. Studies were performed with mice in the proestrus phase, determined by vaginal smears, and vaginal smears were performed before mice were killed. Mice in the proestrus phase were selected due to the sufficient numbers for all the experiments.