


**RESEARCH ARTICLE**

# Developmental exposure to the flame retardant, triphenyl phosphate, causes long-lasting neurobehavioral and neurochemical dysfunction

Andrew B. Hawkey<sup>1</sup> | Janequia Evans<sup>1</sup> | Zade R. Holloway<sup>1</sup> | Erica Pippen<sup>1</sup> | Olivia Jarrett<sup>1</sup> | Bruny Kenou<sup>1</sup> | Theodore A. Slotkin<sup>2</sup> | Frederic J. Seidler<sup>2</sup> | Edward D. Levin<sup>1,2</sup> 

<sup>1</sup>Department of Psychiatry and Behavioral Sciences, Duke University School of Medicine, Durham, North Carolina, USA

<sup>2</sup>Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, North Carolina, USA

**Correspondence**

Edward D. Levin, Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC 27710, USA.

Email: [edlevin@duke.edu](mailto:edlevin@duke.edu)

**Funding information**

National Institute of Environmental Health Sciences; Duke University, Grant/Award Number: ES010356

**Abstract**

**Background:** Human exposures to organophosphate flame retardants result from their use as additives in numerous consumer products. These agents are replacements for brominated flame retardants but have not yet faced similar scrutiny for developmental neurotoxicity. We examined a representative organophosphate flame retardant, triphenyl phosphate (TPP) and its potential effects on behavioral development and dopaminergic function.

**Methods:** Female Sprague–Dawley rats were given low doses of TPP (16 or 32 mg kg<sup>-1</sup> day<sup>-1</sup>) via subcutaneous osmotic minipumps, begun pre-conception and continued into the early postnatal period. Offspring were administered a battery of behavioral tests from adolescence into adulthood, and littermates were used to evaluate dopaminergic synaptic function.

**Results:** Offspring with TPP exposures showed increased latency to begin eating in the novelty-suppressed feeding test, impaired object recognition memory, impaired choice accuracy in the visual signal detection test, and sex-selective effects on locomotor activity in adolescence (males) but not adulthood. Male, but not female, offspring showed marked increases in dopamine utilization in the striatum, evidenced by an increase in the ratio of the primary dopamine metabolite (3,4-dihydroxyphenylacetic acid) relative to dopamine levels.

**Conclusions:** These results indicate that TPP has adverse effects that are similar in some respects to those of organophosphate pesticides, which were restricted because of their developmental neurotoxicity.

**KEYWORDS**

developmental neurotoxicity, dopamine, flame retardants, neurobehavioral dysfunction, triphenyl phosphate

## 1 | INTRODUCTION

Flame-retardant chemicals are commonly found in electronics, textiles, foams used in furniture, and various

building materials. Unfortunately, these additives are often mobile, allowing for human exposure through absorptive materials like household dust (Hoffman, Garantziotis, Birnbaum, & Stapleton, 2015; Percy et al., 2020;

Stapleton et al., 2008). Of potential concern, these compounds have been detected in samples from pregnant women (Bai et al., 2019; Castorina et al., 2017; Hoffman et al., 2017; Zhao, Chen, Gao, Shen, & Hu, 2017) and in breastmilk (Adgent, Hoffman, Goldman, Sjödin, & Daniels, 2014; Ma, Zhu, & Kannan, 2019), indicating that prenatal and infant exposures are common. As with other environmental toxicants, multiple classes of flame retardants have been introduced, phased out and replaced based on their potential toxicity, with older generations including polychlorinated biphenyls and polybrominated diphenyl ethers. However, their replacements, largely organophosphate flame retardants (OPFRs, e.g., triphenyl phosphate) were introduced as supposedly lower-risk alternatives but with relatively little toxicological data to support the idea that they represented an improvement from older classes. OPFRs may target nervous system development, as other OP have been shown to. Indeed, some OPFRs inhibit acetylcholinesterase (Kinugasa, Yamaguchi, Miyazaki, Okumura, & Yamamoto, 2001), the target for organophosphate insecticides, but the OPFRs also exhibit a range of other cellular and physiological effects that can affect development (see review Patisaul et al., 2021), including endocrine disruption, neuroinflammation, oxidative stress, shifts in neural/glial determination, differentiation and morphology, and disruption of neurotransmission.

Prenatal or neonatal exposure to OPFRs is associated with adverse developmental and cognitive effects in children, including hyperactivity, deficits in fine motor performance, language, and working memory, as well as reduced IQ and externalizing behaviors (Castorina et al., 2017; Doherty et al., 2019; Lipscomb et al., 2017). These human studies are supported by a range of behavioral toxicology studies in animals investigating OPFRs or OPFR-containing mixtures such as Firemaster-550. Studies with lower-vertebrate fish models, such as zebrafish, found both short- and long-term behavioral changes following embryonic individual OPFR exposures, including changes in larval motility, locomotor activity, anxiety-like diving, startle responses and predator avoidance (Dishaw, Hunter, Padnos, Padilla, & Stapleton, 2014; Glazer et al., 2018; Noyes, Haggard, Gonnerman, & Tanguay, 2015; Oliveri, Bailey, & Levin, 2015; Sun et al., 2016). Likewise, rodent studies have noted long-term affective and locomotor effects in rodent offspring exposed to OPFR mixtures during fetal or neonatal development (Baldwin et al., 2017; Walley et al., 2021; Wiersielis, Adams, Yasrebi, Conde, & Roepke, 2020; Witchey, Al Samara, Horman, Stapleton, & Patisaul, 2020), further indicating that the neurotoxic potential of OPFR is well conserved across vertebrate species. These findings raise the possibility that individual or mixed OPFRs may

impact developmental toxicity, similar to structurally similar organophosphate pesticides, a class of compounds that have been increasingly restricted because of those effects, which include behavioral dysfunction associated with structural and synaptic neurochemical disruption, with dopamine systems as a prominent target in rodent offspring (Hawkey et al., 2022; Karen, Li, Harp, Gillette, & Bloomquist, 2001; Slotkin et al., 2009; Slotkin & Seidler, 2007). Notably, another OPFR, tris (1,3-dichloropropyl) phosphate, which contains both the organophosphate moiety and halogens, has been shown to promote hyperdevelopment of the dopaminergic phenotype in vitro (Dishaw et al., 2011). What remains less clear is to what degree a simpler OPFR, devoid of halogen moieties, mimics the overall neurotoxic and behavioral profiles of known OP neurotoxicants and mixtures.

The present study was conducted to evaluate behavioral and dopaminergic consequences in offspring with maternal TPP exposure (0, 3.95 or 7.58 mg/day, corresponding to an initial dose of 16–32 mg kg<sup>-1</sup> day<sup>-1</sup>). TPP was selected as a representative OPFR based on its frequent detection in indoor environmental samples (Hartmann, Bürgi, & Giger, 2004; Mizouchi et al., 2015; Phillips et al., 2018; Zheng et al., 2017), and because of its inclusion in common OPFR mixtures like Firemaster 550 (FM550). TPP was delivered by osmotic minipump to maintain around-the-clock exposure, with treatment occupying the entire gestational period and into the second postnatal week. Offspring were then subjected to a battery of behavioral tests to evaluate cognitive, locomotor, and emotional function. In addition, we evaluated the impact on dopaminergic synaptic function, measuring dopamine levels and utilization (turnover). The latter was assessed by calculating the ratio of the chief dopamine metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) to dopamine (Slotkin et al., 2009). Dopamine is critical for cognition and emotional processing (Abraham, Neve, & Lattal, 2014; Puig, Rose, Schmidt, & Freund, 2014), as well as for a broader array of behavioral functions, and as noted earlier, it has been documented to be targeted by OP insecticides.

## 2 | METHODS

### 2.1 | Subjects and housing

Subjects consisted of Sprague Dawley rats (Charles Rivers Labs, Raleigh, NC, USA) and their offspring, which were bred in house. Animals were kept on a 12/12 reversed day:light cycle and provided with ad libitum access to food/water throughout breeding and early offspring development. Restricted feeding was used in three

behavioral tests among offspring to promote motivated feeding behaviors (see below). A total of 36 adult female Sprague Dawley breeder rats were used to generate offspring for testing (10–14 per condition, as needed based on mating success to generate 8+ litters). All offspring were housed in social housing (two to three rats per cage) with all members of a given cage representing either males or females of a given treatment condition. All surgical, husbandry and testing procedures were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee of Duke University. The animal protocol approval number is A253-19-11 and the facility is AAALAC approved.

## 2.2 | Minipump implantation, mating, and TPP exposure

A total of 36 adult female Sprague Dawley rats were surgically implanted with subcutaneous Alzet osmotic infusion pumps (2ML4; Durect Inc, Cupertino, CA, USA) under anesthesia (60 mg/kg ketamine; Covetrus, Dublin, OH, USA and 15 mg/kg dexdormitor; Dechra, Overland Park, KS, USA). TPP (Sigma Aldrich, St. Louis MO, USA, Lot#BCC88784) was dissolved in dimethyl sulfoxide (DMSO) and used to fill a single osmotic pump per animal for implantation. Solutions (100% DMSO + powdered TPP) were determined to provide doses of 0, 16, or 32 mg kg<sup>-1</sup> day<sup>-1</sup> of TPP, based on dam weight at the time of surgery. Control groups received identical pumps with 100% DMSO vehicle alone. The corresponding outputs of the osmotic minipumps were, on average, 0, 3.95, or 7.58 mg/day TPP. Rats were then allowed to recover for 72 hr prior to mating, and pumps remained in place until weaning (postnatal day PN21) of any resulting litters. Mating consisted of each female being housed with an untreated male rat for 5 days. These doses were selected based on preliminary experiments that established the tolerability during pregnancy, defined as falling below the threshold for disrupted fertility or for growth impairment in dams and offspring. These pumps are marketed as a 4-week delivery device, but they actually last 5 weeks (information supplied by the manufacturer). Consequently, in our study, TPP exposure lasted throughout pregnancy and up to 8–11 days into the neonatal stage, corresponding to human neurodevelopmental stages from conception into the third trimester (Semple, Blomgren, Gimlin, Ferrero, & Noble-Haeusslein, 2013). Dam weights increased during gestation, resulting in an average 25% reduction in dose rate by the time of birth, after which the dose rates rose back to nearly the original values

because of the weight loss associated with parturition. Throughout the gestation and preweaning period, maternal health and signs of distress were monitored daily through visual inspection, including lethargy, piloerection, discolored fur, and subcutaneous fluid buildup around the osmotic pumps. Handling was kept to a minimum needed to verify health, except in the case of fluid buildup, which was addressed by draining excess fluid and application of a topical cream (triple antibiotic cream).

At PN1 (the day of birth was designated as PN0), all offspring were weighed and litters were culled such that the number of remaining offspring did not exceed 10, and so that numbers of males and females were balanced, as available in each litter. At weaning, one randomly selected male and female were selected from each litter and were housed in same-sex groups for subsequent rearing and behavioral testing. All remaining siblings were retained for tissue collection for the neurochemical studies, which likewise utilized only one male and one female from each litter at each time point. Weaning was performed at 21 days of age, and rats selected for the behavioral study were group housed with same-sex, same-condition offspring (two to three rats per cage, based on pregnancy success) originating from other litters. These social housing groups were maintained for the duration of the study. Offspring used in the neurochemistry study were housed with same-sex littermates (two to three rats per cage, based on sex-balance within litter) and rats were removed at set ages for tissue collection. Behavioral testing began 1-week after weaning, whereas tissue samples for neurochemistry were obtained at PN60, PN100, and PN150.

## 2.3 | Behavioral testing of the offspring

The behavioral battery consisted of seven tests, described in detail (Table 1), with only one test being performed during any given week of development. Assessment began at 4 weeks of age and included tests of locomotor, affective, and cognitive function. All testing was performed during the dark phase of the reversed day/night cycle and under the low-level ambient light of the dark phase of the cycle (800–1700 hr). The same animals completed all behavioral tests sequentially. During the radial arm maze and attention task testing, rats were food restricted to maintain weight at 85% of free feeding levels, while allowing for developmentally appropriate weight gain (e.g., daily food was adjusted across the training and testing phases of each test). Appropriate levels were verified by consistent task engagement and weight gain, and

TABLE 1 Treatment and testing schedule for TPP exposure and behavioral assessment of their offspring

Maternal TPP exposure and behavioral testing of offspring							
Week	4	5	6	7	8–11	12–40	Post-OVSD
Test	Elevated Plus Maze	Figure-8 Maze	Novel Env. Suppressed Feeding	Novel Object Recognition	Radial Arm Maze	Operant Visual Signal Detection	Figure-8 Maze

Note: One test was performed in any given week, with testing beginning at 4 weeks of age.

daily food allotments were adjusted if needed to return the rats to the target restriction level.

### 2.3.1 | Week 4: Elevated plus maze

At 4 weeks of age, rats were tested in the elevated plus maze (Med Associates, St Albans, VT, USA). This test assessed open space aversion in these young animals, a characteristic that is associated with anxiety-like avoidance of space with an elevated risk of predation. Increased preference for enclosed spaces is generally interpreted as enhanced anxiety-like responses, while a greater open space preference is interpreted as reduced risk avoidance. The apparatus consisted of a central hub (11.5 × 11.5 cm) with four equally spaced arms extending from it (11.5 × 50 cm). Two of these arms were bordered with 15-cm high walls, while the other two were bordered by 2-cm railings. Each rat was allowed to freely explore the maze for one 5 min session. The two primary dependent variables were the time spent in the open arms of the maze and the number of center hub crossings (indicative of exploration and general locomotor activity).

### 2.3.2 | Week 5 and adulthood: Figure-8 locomotor activity test

At 5 weeks of age, offspring were tested for locomotor activity and locomotor habituation in the figure-8 maze. The figure-8 maze was an enclosed locomotor chamber (70 cm × 42 cm) made of sheet metal walls with a Plexiglas lid. This included a continuous 10 cm × 10 cm alley, which formed the shape of the number 8 with two side alleys extending from its center. Photobeams were placed at eight equally spaced locations throughout the maze to detect movement throughout the apparatus. Offspring were allowed to freely explore the maze for 1 hr. Locomotor activity and its attenuation across the 1 hr session (habituation) were measures as number of beam breaks per 5-min session block (×12 for the 1h session). The linear trend of locomotion reductions across the session represented the rate of habituation. This test was

repeated in adulthood, after completion of the signal detection task (see below).

### 2.3.3 | Week 6: Novelty-suppressed feeding

At 6 weeks of age, offspring were tested for fear/stress responsivity in the novel environment suppressed feeding test. This test leveraged the resistance of rats to freely eat food when in a novel, mildly stressful environment to detect the intensity of fear/stress-like responses (Roegge, Timofeeva, Seidler, Slotkin, & Levin, 2008). In this test, offspring rats were food restricted for 24 hr and then taken to a novel, bright room. Each rat was then individually placed into an empty (no bedding) plastic cage identical to its home cage with sat in the middle of the floor with no lid on top. Twelve rat chow pellets were placed in four rows of three pellets along the floor of the cage. This food was weighed prior to and immediately after a 10 min session, where rats were allowed to freely explore the cage and eat. The difference in food weight before versus after the session indicated food consumption. While the session was running, experimenters scored the eating behavior of each animal, including the total time spent eating, the number of bouts of eating, and the latency to begin eating. Eating was defined as visibly/audibly chewing the food and not unrelated investigation of the food such as niffing, holding, or carrying the food pellets.

### 2.3.4 | Week 7: Novel object recognition

At 7 weeks of age, offspring were tested for attention and memory in the novel object recognition test. This test represents basic memory processes in a low-motivational state, where behavior is not reinforced or punished by any consequence. Initially, offspring were acclimated to the testing apparatus, a 10 cm × 41 cm black plastic box, twice over 2 days (10 min each). To demonstrate familiarization and memory, offspring then completed two investigation sessions separated by 1 hr. During the first session (AA), two corners of the enclosure contained



randomly selected, identical objects to be investigated. These objects were made of glass, plastic, or ceramic and offspring were allowed to freely investigate them for 10 min. During the second (AB) session, one of the previously investigated objects was replaced with a novel object of similar size and material, but with distinct visual characteristics (shape, color, etc). Again, offspring were allowed to freely investigate the two objects for 10 min. Between testing sessions and between animals, the enclosure and objects were wiped with a 10% vinegar solution to remove latent odors. Collected videos of each investigation session were analyzed for the time spent investigating each object, defined as sniffing of the object. Climbing on or touching the objects, in the absence of sniffing, was excluded. Each 10-min session was then split into two 5 min blocks, to allow within-session changes in investigation due to familiarization to be assessed. Typically, time spent investigate with diminish over the course of the 10-min session, as, will preference for the novel object within the AB session. The primary dependent measures were the time spent investigating each object, the difference in investigation between the two co-presented objects (preference), and the change in each of these scores from the first to second 5 min block. Graphed data (Figure 3) represent the difference in investigation between the two co-presented objects (novel object investigation time – familiar object investigation time), interpreted as the magnitude of object discrimination or novelty preference.

### 2.3.5 | Weeks 8–11: Radial-Arm Maze

From weeks 8 to 11 of age, offspring were maintained on restricted feeding to facilitate food-motivated behavior and tested for spatial learning and memory in the 16-arm radial arm maze. This configuration of the test is designed to detect both short- and long-term memory function in one test, conducted over 12 sessions. The apparatus consisted of a central hub (50 cm diameter) with 16, 10 cm × 60 cm arms radiating out from it. Each arm ended with a food cup 2 cm from the end of the arm. To aid navigation throughout the maze, cardboard shapes were placed on the walls. Training began with 10 min acclimation sessions over 2 days, whereby each rat was placed in a round black cylinder in the middle of the central hub and allowed to eat pieces of sugar-coated cereal (Froot Loops®; Kellogg's Inc, Battle Creek, MI, USA). This was done to ensure that these rats would readily eat the food rewards during subsequent testing. For the remainder of training, the offspring were provided with one daily session, whereby they would explore the maze and retrieve any rewards from the ends of the maze arms.

During these sessions, 12 of the 16 arms had food hidden at the end, and 4 were left empty. The positions of baited/unbaited arms were randomized for each rat and kept consistent across the 12 training sessions. Sessions ran until the rat successfully entered all baited arms or when 10 min had elapsed, whichever came first. Arm entries were scored by an observer and split into correct responses and two types of errors. Any entry into an arm, which was never baited throughout training, was scored as a reference memory error. Any repeated entry into an arm, which was typically baited, was scored as a working memory error. Arm entry latency was calculated as the total time of the session divided by the total number of arm entries.

### 2.3.6 | Weeks 12–40: Operant visual attention task

Following the end of radial arm maze testing, offspring remained on restricted feeding, and completed training and testing in the operant signal detection, or visual attention, test (Holloway, 2020). This test measured the accuracy of choices made by the rats based on the presence or absence of a visual cue prior to that choice. All training and testing was conducted in a standard operant chamber equipped with two retractable levers, a visual cue light, and automated feeder which dispensed 20 mg sucrose pellets. Training began by using an autoshaping procedure (four sessions) to develop reliable lever processing responses and continued with training on two distinct trial types. On cued or “signal” trials, the cue light, placed immediately above the associated lever, was illuminated for 500 ms and turned off before both levers were presented. The position (left, right) of the light/associated lever was counterbalanced across rats and kept constant throughout training and testing. A lever press on the associated side resulted in the delivery of a sucrose pellet. On noncued or “blank” trials, the two levers were delivered without a prior light flash. On these trials, presses on the nonlight associated lever (on the opposite side) led to pellet delivery. On either trial type, if no response was made within 5 s, the trial ended and “no response” was recorded. Trial types were trained individually (50-trial sessions of cued-trials until 96% accuracy criterion reached [48/50 trials], then the same with blank trials). Following this, 100-trial sessions included both cued and blank trials, first sequentially (50 cued, 50 blank, completion criterion 99% accuracy) and then on a multiple schedule (mixed within session) (completion criterion 70% total accuracy). During this latter phase, the light position was transferred from being above the associated lever to a centered position at the top of the wall. On the

tertiary testing phase, accuracy and performance were recorded across a 240-trial session (120 presentations of each, randomized) and these data were used for the primary analysis. Performance criteria for data inclusion on the tertiary phase were a minimum of 70% average accuracy across the two trial types. Accuracy was measured as follows: correct responses during “signal” trials were recorded as hits and calculated as a percentage of all “signal” trials (% correct). Correct responses during “blank” trials were recorded as correct rejections and likewise calculated as a percentage of all “blank” trials. Data analysis focused on performance within this final phase of testing (light centered, 240 trial multiple schedule sessions), and on the percent correct responses for each trial type.

## 2.4 | Neurochemistry

Animals were euthanized via decapitation at PN60, PN100, and PN150, and the striatum (all ages) and frontal/parietal cortex (PN100, PN150) were dissected and frozen in liquid nitrogen, and then stored at  $-80^{\circ}\text{C}$  until utilized. Tissues were deproteinized by homogenization in 0.1 N perchloric acid containing 3,4-dihydroxybenzylamine (Sigma Chemical Co., St. Louis MO, USA) as an internal standard. Homogenates were sedimented at 26,000g for 10 min and supernatants were trace-enriched by alumina adsorption. Dopamine and DOPAC were separated by reverse-phase high performance liquid chromatography and quantitated by electrochemical detection (Seidler & Slotkin, 1981), standardized against external standards containing 3,4-dihydroxybenzylamine and known quantities of dopamine (Sigma) and DOPAC (Sigma); values were corrected for recovery of the internal standard. Transmitter utilization was then calculated by the DOPAC/dopamine ratio, so as to assess the proportion of dopamine released into the synapse (Slotkin et al., 2009). In the frontal/parietal cortex, we simultaneously measured the level of another catecholamine neurotransmitter, norepinephrine.

## 2.5 | Data analysis

All data were analyzed using analysis of variance (ANOVA), with factors of TPP treatment and sex (repeated measure within litter). As relevant to each test, additional within-subjects repeated measures were also included, such as time block, training session, or trial type. For neurochemistry, the test factors were TPP treatment, sex, age, and brain region (repeated measure); statistical tests on neurochemistry required log transformation because of heterogeneous variance between regions. For all tests, significance was assumed

at the level of  $p < .05$  (two-tailed). For interactions at  $p < .1$ , we also examined whether lower-order simple main effects were detectable (at  $p < .05$ ) after subdivision of the interactive variables (Snedecor & Cochran, 1967). The  $p < .1$  criterion for interaction terms was not used to assign significance to the effects but rather to identify interactive variables requiring subdivision for lower-order tests of the main effects of TPP, the variable of chief interest. Individual group comparisons were performed using Fisher's least significant difference test. Fertility rates, litter health, and growth are presented in the main body as are all significant findings within behavioral and neurochemical analysis (Table 2, Figure 1–5). Nonsignificant effects and summaries of outcomes by sex are presented in Tables S1–S4.

## 3 | RESULTS

### 3.1 | Clinical signs of fertility and neonatal health

During and following exposure, several key signs of clinical health were recorded and analyzed. Neither TPP treatment reduced fertility nor did they evoke changes in litter size, sex-ratio, body weights, or anogenital distance (Table 2).

### 3.2 | Elevated plus maze test of anxiety-like behavior

On the elevated plus maze, the primary outcomes were percent preference for the open arms of the maze and number of center crossings (Table S1). For open arm preference, there was an interaction of TPP exposure  $\times$  sex ( $p < .10$ ), which prompted follow-up tests of the simple main effects of TPP in each sex. Group comparisons with Fisher's LSD test showed that in females the high-dose group spent significantly more time in the open arms ( $p < .05$ ) than did the low-dose group but neither TPP group was significantly different from controls. No significant group differences were seen for males. No main effects of treatment or interactions of treatment with other variables were detected for center crossings.

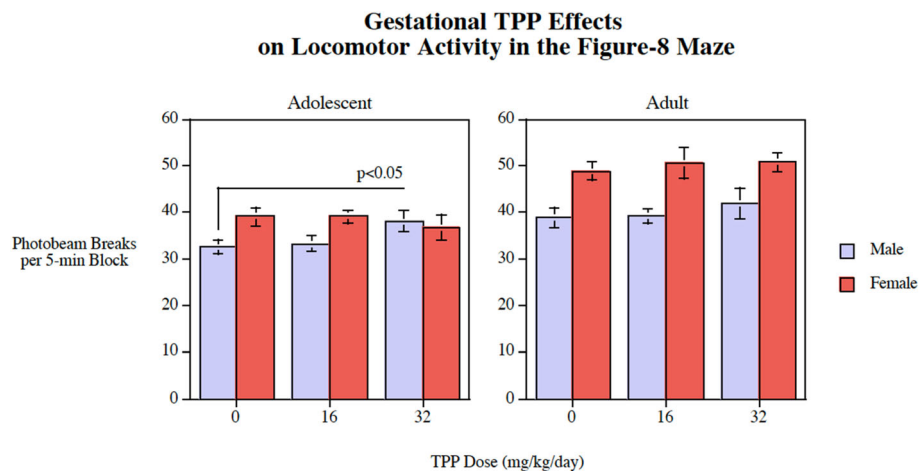
### 3.3 | Figure-8 apparatus locomotor activity

For the figure-8 maze, locomotor activity was assessed across time (5 min blocks) and at two ages (adolescence vs. adult). In adolescents, there was a TPP treatment  $\times$  sex interaction ( $p < .06$ ) that triggered tests

**TABLE 2** Developmental health measurements of conception success (% preg.), offspring weight gain, litter size sex ratio (mean  $\pm$  sem)

Developmental health measurements							
Litter information							
Treatment	% Preg.			Litter size			% male
Control	58%			10 $\pm$ 0.6			50 $\pm$ 3.8
TPP 16	90%			12 $\pm$ 1.0			48 $\pm$ 5.3
TPP 32	69%			9.4 $\pm$ 1.3			56 $\pm$ 6.9
Body weight (g) (by postnatal day)							
Males	1	2	4	7	10	17	21
Control	9.0 $\pm$ 0.3	10.6 $\pm$ 0.4	13.2 $\pm$ 0.5	18.6 $\pm$ 0.6	25.1 $\pm$ 0.8	42.2 $\pm$ 1.5	56.3 $\pm$ 5.7
TPP 16	8.2 $\pm$ 0.4	9.7 $\pm$ 0.6	13.3 $\pm$ 0.8	17.9 $\pm$ 0.9	24.6 $\pm$ 1.3	40.5 $\pm$ 1.3	59.7 $\pm$ 2.0
TPP 32	8.9 $\pm$ 0.3	10.3 $\pm$ 0.4	12.5 $\pm$ 0.4	16.7 $\pm$ 0.8	23.7 $\pm$ 0.6	38.8 $\pm$ 0.9	58.8 $\pm$ 3.0
Females	1	2	4	7	10	17	21
Control	8.6 $\pm$ 0.4	10.1 $\pm$ 0.4	12.6 $\pm$ 0.5	18.0 $\pm$ 0.6	24.1 $\pm$ 1.0	40.2 $\pm$ 1.6	53.9 $\pm$ 5.5
TPP 16	7.8 $\pm$ 0.4	9.3 $\pm$ 0.6	13.0 $\pm$ 0.9	17.7 $\pm$ 1.0	24.3 $\pm$ 1.3	39.7 $\pm$ 1.5	57.3 $\pm$ 2.1
TPP 32	8.5 $\pm$ 0.4	9.9 $\pm$ 0.3	12.1 $\pm$ 0.3	16.6 $\pm$ 0.6	23.4 $\pm$ 0.5	38.0 $\pm$ 1.1	57.3 $\pm$ 3.0

Note: No treatment effects were detected.



**FIGURE 1** Gestational TPP exposure effects on figure-8 maze locomotor activity in adolescent and adult rats, relative to vehicle exposed control rats (mean  $\pm$  sem). In adolescents, there was a TPP treatment  $\times$  sex interaction ( $F(2,32) = 3.10, p < .06$ ) that triggered tests of the simple main effects of TPP in males and females. There was a significant main effect of treatment in males,  $F(1,32) = 5.06, p < .05$  but not females ( $p > .05$ ). In adolescence, males from the 32 mg kg<sup>-1</sup> day<sup>-1</sup> group differed from control males on their average number of beam breaks per 5 min time block ( $p < .05$ ). No effects were detected among adult rats

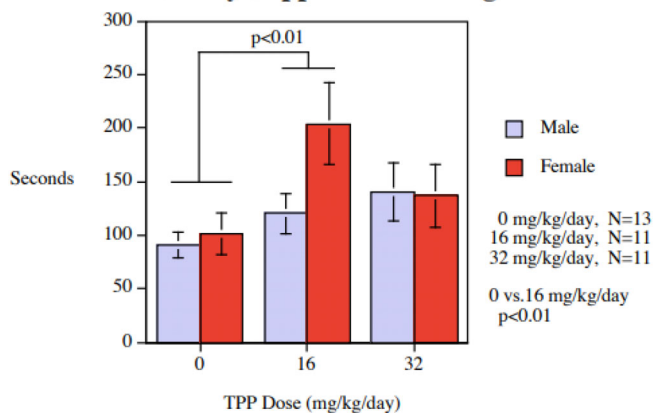
of the simple main effects of TPP in males and females (Figure 1). This showed that for the males 32 mg kg<sup>-1</sup> day<sup>-1</sup> of TPP during gestation caused a significant ( $p < .05$ ) increase in activity relative to controls. In adults, there were no TPP exposure-related effects, although there were significant main effects of sex ( $p < .05$ ), showing typical sex differences (female  $>$  male) and session block ( $p < .05$ ) where activity diminishes over time. To support interpretation of age-differences, a secondary analysis including age as a repeated-measures variable showed no overall age  $\times$  sex  $\times$  treatment or

age  $\times$  treatment interaction, indicating that the TPP effects detected above should not be interpreted as distinctly age-specific.

### 3.4 | Novelty-suppressed feeding test of fear response

For novel environment suppressed feeding, the primary outcomes were latency to begin feeding, time spent eating, number of eating bouts and amount of food eaten.

### Gestational TPP Effects on Response Latency in the Novelty Suppressed Feeding Test



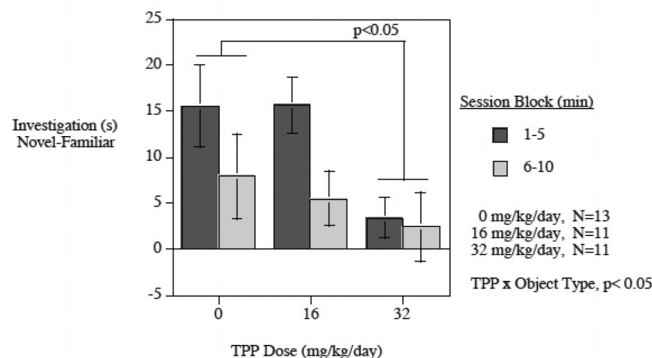
**FIGURE 2** Gestational TPP exposure effects on response latency (s) in the novelty suppressed feeding test, relative to vehicle exposed control rats (mean  $\pm$  sem). For latency to begin eating (square root of seconds), there was a main effect of TPP treatment,  $F(2,32) = 3.92$ ,  $p < .05$ , whereby offspring exposed to  $16 \text{ mg kg}^{-1} \text{ day}^{-1}$  waited longer to begin eating compared to controls,  $F(1,32) = 7.57$ ,  $p < .01$ . The  $16 \text{ mg kg}^{-1} \text{ day}^{-1}$  group differed from controls on the latency to begin eating during the test ( $p < .05$ )

For latency to begin eating, there was a main effect of TPP treatment ( $p < .05$ ), whereby offspring exposed to  $16 \text{ mg kg}^{-1} \text{ day}^{-1}$  waited longer to begin eating compared to controls ( $p < .05$ ) (Figure 2). The two TPP-treated groups did not significantly differ from one another on this outcome. No other measures showed significant effects of treatment or relevant interactions. Typical sex differences in latency to eat (male < female) were not significantly detected.

### 3.5 | Novel object recognition test of nonspatial memory

The novel object recognition test measured the difference in investigation between the novel and familiar object in the AB session, and the change in this score from the first to the second half of the session (first half–second half). A TPP  $\times$  object (familiar vs. novel) interaction ( $p < .05$ ) was detected which prompted follow-up with tests of the effects of TPP on novel preference. We found that the offspring exposed to  $32 \text{ mg kg}^{-1} \text{ day}^{-1}$  of TPP exhibited significantly less novel object preference compared to controls ( $p < .05$ ) (Figure 3) but not the  $16 \text{ mg kg}^{-1} \text{ day}^{-1}$  group. Unrelated to TPP treatment, there were also significant main effects of object (familiar vs. novel) ( $p < .05$ ) and 5-min time block of the session ( $p < .05$ ). Sex  $\times$  treatment interactions did not reach significance, although visible trends (TPP-related

### Gestational TPP Effects on Choice Accuracy in the Novel Object Recognition Test



**FIGURE 3** Gestational TPP exposure effects on novel object recognition, novel-familiar investigation (s), relative to vehicle exposed control rats (mean  $\pm$  sem). A treatment  $\times$  object interaction was observed,  $F(2, 23) = 4.26$ ,  $p < .05$ , and follow-up tests were performed on the net investigation difference between objects (novel-familiar) within each group. The  $32 \text{ mg kg}^{-1} \text{ day}^{-1}$  group differed from controls on their relative preference for the novel object ( $p < .05$ )

impairments in females appear more dramatic than males) may assist in interpretation, as provided in Table S2.

### 3.6 | 16-Arm radial maze test of spatial learning and memory

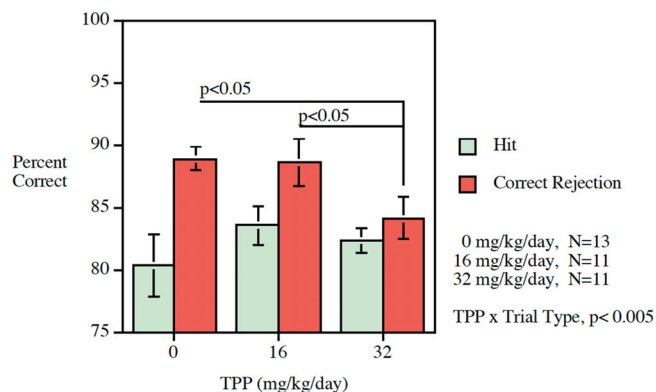
For the radial arm maze, the primary outcomes were the numbers of errors (working vs. reference memory) and response latency. With errors, there was a significant TPP  $\times$  error type interaction ( $p < .05$ ). However, after subdivision into the error types, we did not detect significant TPP-related effects on either working or reference memory (Table S3), nor was there a significant TPP effect on response latency. Typical sex differences (error rates, male < female) in task performance and accuracy were not significantly detected.

### 3.7 | Operant visual signal detection test

For the operant visual signal detection test, the primary outcomes were choice accuracy across two trial types (“signal” vs. “blank” trials). There was a significant interaction of TPP  $\times$  trial type ( $p < .05$ ). Follow-up tests of the simple main effects of TPP with each trial type showed a significant TPP effect on correct reject trials ( $p < .05$ ), with the TPP 32 group distinguishable from both the controls and the  $16 \text{ mg kg}^{-1} \text{ day}^{-1}$  group (Figure 4). A full sex  $\times$  treatment summary is provided in Table S4.



### Gestational TPP Effects on Choice Accuracy in the Signal Detection Attention Test



**FIGURE 4** Gestational TPP exposure effects on percent correct performance for hit and correct rejection trials in the visual signal detection attention test (mean  $\pm$  sem). A treatment  $\times$  trial type interaction was detected,  $F(2, 23) = 7.28$ ,  $p < .05$ , and follow-up tests were performed on each trial type separately. The 32 mg kg<sup>-1</sup> day<sup>-1</sup> group differed from both controls and the 16 mg kg<sup>-1</sup> day<sup>-1</sup> group in correct rejection accuracy (noncued trials) ( $p < .05$ ) but not hit accuracy (cued trials)

### 3.8 | Neurochemistry

Neurochemical analyses principally examined tissue levels of dopamine, DOPAC, and DOPAC/dopamine ratio in the striatum and frontal/parietal cortex of offspring animals, with additional assessment of norepinephrine in the frontal/parietal cortex (Figure 4). To enable ready visual comparison of all the different parameters, values are shown as the percent change from control, with the original values provided in Table 3. Effects of TPP exposure were specific to the striatum (Figure 4a), with no corresponding effects in the frontal/parietal cortex (Figure 4b). In the striatum, TPP effects were sex-selective, with males showing significant synaptic hyperactivity (elevated DOPAC and DOPAC/dopamine ratio), whereas females were unaffected.

## 4 | DISCUSSION

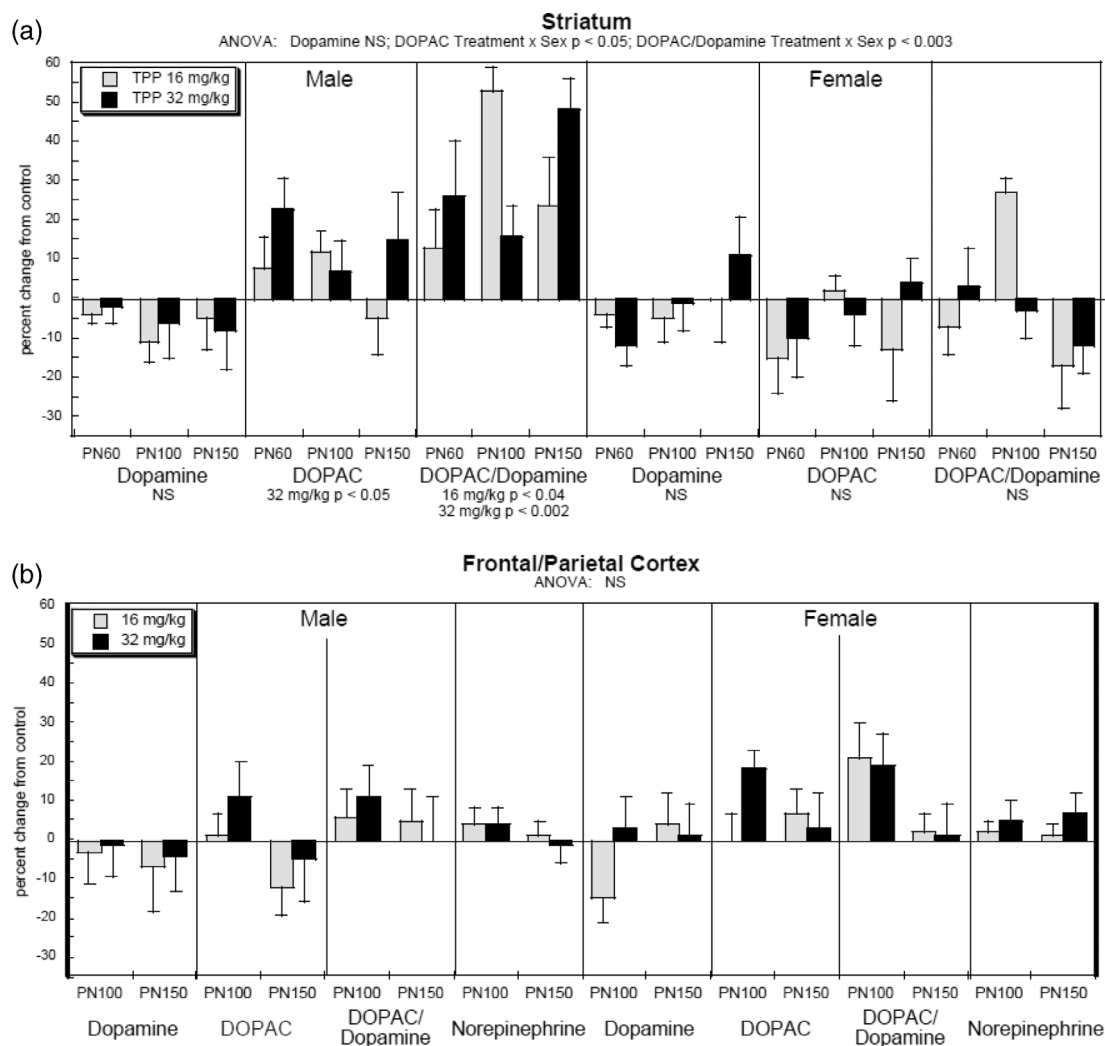
Our studies support the conclusion that TPP, a supposedly safer replacement for polybrominated flame retardants, is a neurobehavioral teratogen. Chronic maternal exposure to TPP during perinatal development evoked neurobehavioral and neurochemical anomalies, which persisted into adulthood. Importantly, we chose exposures that were devoid of effects on fertility, growth, or viability and thus would escape the definition of developmental toxicity as assessed simply by gross examination.

The affected behaviors included both affective and cognitive performance: male-specific hyperactivity in adolescence, enhanced fear-like avoidance of eating in a novel environment, reduced novel object recognition, and choice accuracy deficits in the visual signal detection test, associated with increased dopamine utilization of striatal tissue of males.

Maternal TPP exposure led to a specific cluster of behavioral symptoms, which complement prior animal studies and epidemiological associations between developmental OP exposure and cognitive/affective outcomes later in life. TPP notably targeted many of the same outcomes as other, better studied OP compounds, but often caused unique profiles of impairment within those outcomes. Sex-specific effects included male-driven increases in locomotor activity, and male-driven increases in adolescent locomotor activity. Superimposed on the sex-selective effects, we found effects shared by both sexes, including enhancements in novelty-induced suppression of eating initiation, an indication greater fear-like stress responses, and impaired novel object recognition, indicating interference with nonspatial memory processes under a low motivational state.

The four tests sensitive to early life exposure to TPP have been previously shown to be sensitive to similar exposures to other OP pesticides and flame-retardant mixtures (Chen et al., 2011; Chen, Zhang, Yuan, Li, & Li, 2014; Hawkey et al., 2020; Roegge et al., 2008), although the exact TPP-effects are partially distinct from the other OPs. For example, locomotor effects of other OPs include hyperactivity but not necessarily with the same sex selectivity. For example, prenatal diazinon exposure leads to female-, rather than male-, specific hyperactivity (Hawkey et al., 2020). In contrast, chlorpyrifos exposure on GD9-12 or PND 11-14 produced female-specific increases or general reductions in locomotor habituation, respectively (Icenogle et al., 2004; Levin et al., 2001; Roegge et al., 2008) with no main effect on activity levels. With respect to novelty-suppressed feeding, TPP appears to be distinguishable from diazinon, which reduces latency to feed in males (Roegge et al., 2008).

With respect to novel object recognition, developmental exposures to the OP insecticides, diazinon or malathion, produce impairments in novel object discrimination (Hawkey et al., 2020; N'Go et al., 2013; Win-Shwe, Nakajima, Ahmed, & Fujimaki, 2013). Prenatal exposure to chlorpyrifos, by contrast, was shown to enhance object investigation overall among males, without disrupting object discrimination (Levin et al., 2014). This suggests that some, but not all OP exposures may impair this outcome. With respect to the signal detection task, TPP appears unique from diazinon in producing



**FIGURE 5** Gestational TPP exposure effects on neurochemical assays in striatal (upper) and frontal–parietal cortical tissue (lower) (mean  $\pm$  sem). DOPAC analyses observed a sex  $\times$  tissue  $\times$  treatment interaction,  $F(1,41) = 4.1$ ,  $p < .05$ , whereby DOPAC was increased in the 32 mg kg<sup>-1</sup> day<sup>-1</sup> group in the male striatum. DOPAC/dopamine ratio also showed a sex  $\times$  tissue  $\times$  treatment interaction,  $F(2,65) = 8.0$ ,  $p < .0008$ , whereby this ratio was increased in the 16 and 32 mg kg<sup>-1</sup> day<sup>-1</sup> groups in the male striatum

choice impairments when no cue was presented (“blank” trials” or correct rejections). Prenatal diazinon exposure was found to impair choice accuracy regardless of trial type in middle-aged but not young adult rats (Hawkey et al., 2020, 2022). Overall, it is concluded that TPP, a representative OP flame retardant, shares the liability of developmental neurotoxicity of the OP insecticides, but that it also has a number of unique effects. Some of these differences may reflect kinetic and metabolic properties. For example, OP insecticides generate toxic oxon-forms through metabolism (Mutch & Williams, 2006; Poet, Wu, Kousba, & Timchalk, 2003), which are believed to contribute to the overall neurotoxicity of these compounds and their anti-cholinesterase properties (Betancourt & Carr, 2004). TPP, by contrast, is primarily metabolized into intermediate phenyl phosphates (e.g., hydroxylated

TPPs and diphenyl phosphate) (Martinson et al., 2020; Su, Crump, Letcher, & Kennedy, 2014), which may contribute to neurotoxicity in distinct ways.

With respect to TPP-alone versus TPP-containing mixtures, the present data suggest additional areas of agreement and potential differences. TPP-containing mixtures have been previously associated with changes in locomotor activity and/or open space avoidance (Baldwin et al., 2017; Walley et al., 2021; Wiersielis et al., 2020; Witchey et al., 2020). Similar to the current study, Patisaul and colleagues found male-specific FM550-induced hyperactivity among juvenile rats in the open field, while no similar effects were observed in adulthood (Baldwin et al., 2017; Witchey et al., 2020). In mice, Roepke and colleagues found that a mixture of TDCPP, TPP, and TCP led to female-specific effects in the open field test in

**TABLE 3** Full results of dopamine (striatum, upper; frontal–parietal cortex, lower) and norepinephrine (PFC) assays

TPP effects on dopamine (DA), DOPAC, and norepinephrine (NE)							
Region		Male			Female		
DA (ng/g)	Postnatal age (days)	Control	TPP (16 mg/kg)	TPP (32 mg/kg)	Control	TPP (16 mg/kg)	TPP (32 mg/kg)
Striatum	60	6,631 ± 268	6,379 ± 156	6,466 ± 250	6,619 ± 401	6,353 ± 181	5,792 ± 307
	100	7,514 ± 447	6,716 ± 406	7,040 ± 653	7,236 ± 489	6,906 ± 408	7,156 ± 536
	150	7,850 ± 780	7,495 ± 592	7,247 ± 763	7,381 ± 761	7,373 ± 814	8,193 ± 758
Frontal/parietal cortex	100	450 ± 28	437 ± 36	448 ± 34	437 ± 21	373 ± 27	451 ± 36
	150	884 ± 67	825 ± 97	846 ± 77	713 ± 54	740 ± 57	721 ± 55
DOPAC (ng/g)		Control	TPP (16 mg/kg)	TPP (32 mg/kg)	Control	TPP (16 mg/kg)	TPP (32 mg/kg)
Striatum	60	825 ± 49	895 ± 69	1,011 ± 115	958 ± 78	819 ± 84	863 ± 99
	100	598 ± 32	671 ± 32	641 ± 48	681 ± 45	693 ± 30	654 ± 56
	150	624 ± 116	594 ± 57	718 ± 74	693 ± 67	607 ± 93	720 ± 74
Frontal/parietal cortex	100	70 ± 2	71 ± 4	78 ± 6	65 ± 2	65 ± 5	77 ± 3
	150	132 ± 11	116 ± 9	126 ± 14	113 ± 8	121 ± 7	116 ± 10
% DOPAC/DA		Control	TPP (16 mg/kg)	TPP (32 mg/kg)	Control	TPP (16 mg/kg)	TPP (32 mg/kg)
Striatum	60	12.5 ± 0.8	14.1 ± 1.2	15.7 ± 1.7	14.3 ± 0.8	13.3 ± 1.1	14.8 ± 1.5
	100	8.0 ± 0.3	12.2 ± 0.5	9.3 ± 0.5	9.6 ± 0.6	12.2 ± 0.4	9.3 ± 0.7
	150	6.7 ± 0.5	8.3 ± 0.8	9.8 ± 0.5	9.5 ± 0.7	7.8 ± 1.0	8.4 ± 0.6
Frontal/parietal cortex	100	16.0 ± 0.9	17.0 ± 1.1	17.7 ± 1.2	15.0 ± 0.6	18.1 ± 1.4	17.9 ± 1.2
	150	15.2 ± 1.5	15.9 ± 1.2	15.2 ± 1.7	16.2 ± 1.1	16.6 ± 0.8	16.4 ± 1.3
NE (ng/g)		Control	TPP (16 mg/kg)	TPP (32 mg/kg)	Control	TPP (16 mg/kg)	TPP (32 mg/kg)
Frontal/parietal cortex	100	170 ± 5	176 ± 7	177 ± 7	168 ± 6	172 ± 5	176 ± 8
	150	288 ± 23	292 ± 13	287 ± 14	272 ± 17	275 ± 8	292 ± 14

Note: Data (mean ± SEM) obtained from 6 to 12 animals in each treatment group for each age and sex.

certain cases but not others (Wiersielis et al., 2020; Witchey et al., 2020). Additionally, each of these studies identified alterations in open space avoidance or similar anxiety-like outcomes, which were found to be spared in the elevated plus maze within the present study. Taken together, these similarities and differences may indicate that locomotor effects of OPFR mixtures may be partially attributable to TPP, while anxiolytic or anxiogenic effects may be more closely associated with other OPFR within those mixtures. It should also be noted that existing studies of TPP-containing mixtures have largely focused on locomotor and affective functions, and the present data emphasize that cognitive outcomes, such as recognition memory and attentional function, can be sensitive to OPFR effects as well, and should be included in future work in this area. While the present work complements prior rodent work, it should be acknowledged that the symptom cluster observed does not fully account for behavioral associations noted in human studies (e.g., fine motor impairments, working memory deficits), and as such, additional studies will be needed to fully model and

explain the bases of OPFR-induced behavioral dysfunction.

As with the OP insecticides, the behavioral anomalies were associated with lasting neurochemical changes in dopamine systems, characterized by increased dopamine utilization (turnover). However, we were unable to connect the neurochemical effects to specific behavioral outcomes, as the latter did not completely share the sex-selectivity seen for neurochemistry (males only). It would require an expanded battery of tests to determine whether there are specific links between increased dopamine utilization and specific behavioral defects. Importantly though, prior studies have shown that developmental exposure to OP pesticides likewise leads to increased dopamine utilization (Slotkin & Seidler, 2007), just as seen here for TPP. So, these effects may be common to OPs regardless of whether they are insecticides or flame retardants.

One notable limitation highlighted in this study pertains to the role of varying kinetics in the effects observed. The osmotic pumps used in this study model a

consistent, steady source of environmental exposure, which while theoretically “natural” does not provide a steady internal dose. The maternal dose per kg body weight was estimated to reduce by ~25% by the time of birth due to gestational weight gain. As a result, the kinetic profile during gestation provides greater levels of exposure during early stages of fetal development than during later stages. These factors may influence the variability of this dataset, as weight gain, and therefore relative dose, will necessarily vary across individuals within each maternal group. Additionally, lactational exposure during the neonatal period, equated with the third trimester of human brain development, has not been quantified so the offspring dose at this point cannot be precisely stated. The pump method is valuable insofar as it reduces the repeated handling and gestational stress associated with repeated injections or gavage, but this benefit is tempered somewhat by the lack of kinetic stability. Future studies in this area will need to provide a more comprehensive assessment of exposure kinetics using these pumps, and/or adopt osmotic pumps which can be externally programmed to provide a more precise and weight-appropriate output of the candidate toxin. Such future studies will be able to clarify which aspects of the present work may depend on the unique characteristics of this exposure method, and which are more representative across methods.

In summary, the current study identified multiple neurobehavioral and neurochemical consequences of perinatal TPP exposure at otherwise subtoxic exposures. The effects partially overlap those associated with OP pesticides while at the same time, and often target previously identified outcomes of interest; yet evidence suggests that the risks associated with TPP are distinct from other Ops or better aligned with some representative compounds than others. The developmental neurotoxicity of the OP pesticides and older generations of flame retardants (e.g., PBDEs) has been more rigorously assessed, and while these chemicals have been restricted or banned, studies like the current work emphasize that replacement compounds require a similar level of scrutiny. While there is ample evidence to support the conclusion that the continued use of OPs, whether insecticides or flame retardants, confers some risk of developmental neurotoxicity, there are distinctions among the various OP compounds, so that those risks are not identical across the broad array of OPs.

## ACKNOWLEDGMENTS

This research was supported by the Duke University Superfund Research Center ES010356. Theodore A. Slotkin and Frederic J. Seidler were responsible for the neurochemical measurements.

## CONFLICT OF INTEREST

Theodore A. Slotkin has received consultant income in the past 3 years from Edelson PC (Chicago, IL), Wicker Smith (Naples, FL), Gjording Fouser (Boise, ID), Thorsnes Bartolotta McGuire (San Diego, CA), Cracken Law (Dallas, TX), Matthews Law (Houston, TX), Segal Law (Charleston, WV), Armstrong Teasdale (St. Louis, MO), CNA Continental Casualty Company (Chicago, IL), The National Association of Attorneys General (Washington, DC) and the State of Arizona.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available to any interested. Please contact Dr. Edward Levin (edlevin@duke.edu).

## ORCID

Edward D. Levin  <https://orcid.org/0000-0002-5060-9602>

## REFERENCES

- Abraham, A. D., Neve, K. A., & Lattal, K. M. (2014). Dopamine and extinction: A convergence of theory with fear and reward circuitry. *Neurobiology of Learning and Memory*, *108*, 65–77.
- Adgent, M. A., Hoffman, K., Goldman, B. D., Sjödin, A., & Daniels, J. L. (2014). Brominated flame retardants in breast milk and behavioural and cognitive development at 36 months. *Paediatric and Perinatal Epidemiology*, *28*(1), 48–57.
- Bai, X. Y., Lu, S. Y., Xie, L., Zhang, B., Song, S. M., He, Y., ... Zhang, T. (2019). A pilot study of metabolites of organophosphorus flame retardants in paired maternal urine and amniotic fluid samples: Potential exposure risks of tributyl phosphate to pregnant women. *Environmental Science: Processes & Impacts*, *21*(1), 124–132.
- Baldwin, K. R., Phillips, A. L., Horman, B., Arambula, S. E., Rebuli, M. E., Stapleton, H. M., & Patisaul, H. B. (2017). Sex specific placental accumulation and behavioral effects of developmental Firemaster 550 exposure in Wistar rats. *Scientific Reports*, *7*(1), 1–13.
- Betancourt, A. M., & Carr, R. L. (2004). The effect of chlorpyrifos and chlorpyrifos-oxon on brain cholinesterase, muscarinic receptor binding, and neurotrophin levels in rats following early postnatal exposure. *Toxicological Sciences*, *77*(1), 63–71.
- Castorina, R., Bradman, A., Stapleton, H. M., Butt, C., Avery, D., Harley, K. G., ... Eskenazi, B. (2017). Current-use flame retardants: Maternal exposure and neurodevelopment in children of the CHAMACOS cohort. *Chemosphere*, *189*, 574–580.
- Castorina, R., Butt, C., Stapleton, H. M., Avery, D., Harley, K. G., Holland, N., ... Bradman, A. (2017). Flame retardants and their metabolites in the homes and urine of pregnant women residing in California (the CHAMACOS cohort). *Chemosphere*, *179*, 159–166.
- Chen, W. Q., Yuan, L., Xue, R., Li, Y. F., Su, R. B., Zhang, Y. Z., & Li, J. (2011). Repeated exposure to chlorpyrifos alters the performance of adolescent male rats in animal models of depression and anxiety. *Neurotoxicology*, *32*(4), 355–361.
- Chen, W. Q., Zhang, Y. Z., Yuan, L., Li, Y. F., & Li, J. (2014). Neurobehavioral evaluation of adolescent male rats following



- repeated exposure to chlorpyrifos. *Neuroscience Letters*, 570, 76–80.
- Dishaw, L. V., Hunter, D. L., Padnos, B., Padilla, S., & Stapleton, H. M. (2014). Developmental exposure to organophosphate flame retardants elicits overt toxicity and alters behavior in early life stage zebrafish (*Danio rerio*). *Toxicological Sciences*, 142(2), 445–454.
- Dishaw, L. V., Powers, C. M., Ryde, I. T., Roberts, S. C., Seidler, F. J., Slotkin, T. A., & Stapleton, H. M. (2011). Is the PentaBDE replacement, tris (1, 3-dichloro-2-propyl) phosphate (TDCPP), a developmental neurotoxicant? *Studies in PC12 cells. Toxicology and Applied Pharmacology*, 256(3), 281–289.
- Doherty, B. T., Hoffman, K., Keil, A. P., Engel, S. M., Stapleton, H. M., Goldman, B. D., ... Daniels, J. L. (2019). Prenatal exposure to organophosphate esters and cognitive development in young children in the pregnancy, infection, and nutrition study. *Environmental Research*, 169, 33–40.
- Glazer, L., Hawkey, A. B., Wells, C. N., Drastal, M., Odamah, K. A., Behl, M., & Levin, E. D. (2018). Developmental exposure to low concentrations of organophosphate flame retardants causes life-long behavioral alterations in zebrafish. *Toxicological Sciences*, 165(2), 487–498.
- Hartmann, P. C., Bürgi, D., & Giger, W. (2004). Organophosphate flame retardants and plasticizers in indoor air. *Chemosphere*, 57(8), 781–787.
- Hawkey, A., Pippen, E., White, H., Kim, J., Greengrove, E., Kenou, B., ... Levin, E. D. (2020). Gestational and perinatal exposure to diazinon causes long-lasting neurobehavioral consequences in the rat. *Toxicology*, 429, 152327.
- Hawkey, A. B., Pippen, E., Kenou, B., Holloway, Z., Slotkin, T. A., Seidler, F. J., & Levin, E. D. (2022). Persistent neurobehavioral and neurochemical anomalies in middle-aged rats after maternal diazinon exposure. *Toxicology*, 472, 153189.
- Hoffman, K., Garantziotis, S., Birnbaum, L. S., & Stapleton, H. M. (2015). Monitoring indoor exposure to organophosphate flame retardants: Hand wipes and house dust. *Environmental Health Perspectives*, 123(2), 160–165.
- Hoffman, K., Lorenzo, A., Butt, C. M., Adair, L., Herring, A. H., Stapleton, H. M., & Daniels, J. L. (2017). Predictors of urinary flame retardant concentration among pregnant women. *Environment International*, 98, 96–101.
- Holloway, Z., Koburov, R., Hawkey, A., & Levin, E. D. (2020). Measuring attention in rats with a visual detection task: Signal intensity vs. signal duration. *Pharmacology, Biochemistry and Behavior*, 199, 173069.
- Icenogle, L. M., Christopher, N. C., Blackwelder, W. P., Caldwell, D. P., Qiao, D., Seidler, F. J., ... Levin, E. D. (2004). Behavioral alterations in adolescent and adult rats caused by a brief subtoxic exposure to chlorpyrifos during neurulation. *Neurotoxicology and Teratology*, 26(1), 95–101.
- Karen, D. J., Li, W., Harp, P. R., Gillette, J. S., & Bloomquist, J. R. (2001). Striatal dopaminergic pathways as a target for the insecticides permethrin and chlorpyrifos. *Neurotoxicology*, 22, 811–817.
- Kinugasa, H., Yamaguchi, Y., Miyazaki, N., Okumura, M., & Yamamoto, Y. (2001). Evaluation of toxicity in the aquatic environment based on the inhibition of acetylcholinesterase. *Nippon Suisan Gakkaishi*, 67(4), 696–702.
- Levin, E. D., Addy, N., Nakajima, A., Christopher, N. C., Seidler, F. J., & Slotkin, T. A. (2001). Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. *Developmental Brain Research*, 130(1), 83–89.
- Levin, E. D., Cauley, M., Johnson, J. E., Cooper, E. M., Stapleton, H. M., Ferguson, P. L., ... Slotkin, T. A. (2014). Prenatal dexamethasone augments the neurobehavioral teratology of chlorpyrifos: Significance for maternal stress and preterm labor. *Neurotoxicology and Teratology*, 41, 35–42.
- Lipscomb, S. T., McClelland, M. M., MacDonald, M., Cardenas, A., Anderson, K. A., & Kile, M. L. (2017). Cross-sectional study of social behaviors in preschool children and exposure to flame retardants. *Environmental Health*, 16(1), 23.
- Ma, J., Zhu, H., & Kannan, K. (2019). Organophosphorus flame retardants and plasticizers in breast milk from the United States. *Environmental Science & Technology Letters*, 6(9), 525–531.
- Marteinson, S., Guigueno, M. F., Fernie, K. J., Head, J. A., Chu, S., & Letcher, R. J. (2020). Uptake, deposition, and metabolism of triphenyl phosphite in embryonated eggs and chicks of Japanese quail (*Coturnix japonica*). *Environmental Toxicology and Chemistry*, 39(3), 565–573.
- Mizouchi, S., Ichiba, M., Takigami, H., Kajiwara, N., Takamuku, T., Miyajima, T., ... Ueno, D. (2015). Exposure assessment of organophosphorus and organobromine flame retardants via indoor dust from elementary schools and domestic houses. *Chemosphere*, 123, 17–25.
- Mutch, E., & Williams, F. M. (2006). Diazinon, chlorpyrifos and parathion are metabolised by multiple cytochromes P450 in human liver. *Toxicology*, 224(1–2), 22–32.
- N'Go, P. K., Azzaoui, F. Z., Ahami, A. O. T., Soro, P. R., Najimi, M., & Chigr, F. (2013). Developmental effects of malathion exposure on locomotor activity and anxiety-like behavior in Wistar rat. *Health*, 5(3), 603–611.
- Noyes, P. D., Haggard, D. E., Gonnerman, G. D., & Tanguay, R. L. (2015). Advanced morphological—Behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. *Toxicological Sciences*, 145(1), 177–195.
- Oliveri, A. N., Bailey, J. M., & Levin, E. D. (2015). Developmental exposure to organophosphate flame retardants causes behavioral effects in larval and adult zebrafish. *Neurotoxicology and Teratology*, 52, 220–227.
- Patisaul, H. B., Behl, M., Birnbaum, L. S., Blum, A., Diamond, M. L., Rojello Fernández, S., ... Stapleton, H. M. (2021). Beyond cholinesterase inhibition: Developmental neurotoxicity of organophosphate Ester flame retardants and plasticizers. *Environmental Health Perspectives*, 129, 105001.
- Percy, Z., La Guardia, M. J., Xu, Y., Hale, R. C., Dietrich, K. N., Lanphear, B. P., ... Xie, C. (2020). Concentrations and loadings of organophosphate and replacement brominated flame retardants in house dust from the home study during the PBDE phase-out. *Chemosphere*, 239, 124701.
- Phillips, A. L., Hammel, S. C., Hoffman, K., Lorenzo, A. M., Chen, A., Webster, T. F., & Stapleton, H. M. (2018). Children's residential exposure to organophosphate ester flame retardants and plasticizers: Investigating exposure pathways in the TESIE study. *Environment International*, 116, 176–185.
- Poet, T. S., Wu, H., Kousba, A. A., & Timchalk, C. (2003). In vitro rat hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon. *Toxicological Sciences*, 72(2), 193–200.



- Puig, M. V., Rose, J., Schmidt, R., & Freund, N. (2014). Dopamine modulation of learning and memory in the prefrontal cortex: Insights from studies in primates, rodents, and birds. *Frontiers in Neural Circuits*, 8, 93. <https://doi.org/10.3389/fncir.2014.00093>
- Roegge, C. S., Timofeeva, O. A., Seidler, F. J., Slotkin, T. A., & Levin, E. D. (2008). Developmental diazinon neurotoxicity in rats: Later effects on emotional response. *Brain Research Bulletin*, 75(1), 166–172.
- Seidler, F. J., & Slotkin, T. A. (1981). Development of central control of norepinephrine turnover and release in the rat heart: Responses to tyramine, 2-deoxyglucose and hydralazine. *Neuroscience*, 6, 2081–2086.
- Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in Neurobiology*, 106, 1–16.
- Slotkin, T. A., & Seidler, F. J. (2007). Prenatal chlorpyrifos exposure elicits presynaptic serotonergic and dopaminergic hyperactivity at adolescence: Critical periods for regional and sex-selective effects. *Reproductive Toxicology*, 23(3), 421–427.
- Slotkin, T. A., Wrench, N., Ryde, I. T., Lassiter, T. L., Levin, E. D., & Seidler, F. J. (2009). Neonatal parathion exposure disrupts serotonin and dopamine synaptic function in rat brain regions: Modulation by a high-fat diet in adulthood. *Neurotoxicology and Teratology*, 31, 390–399.
- Snedecor, G. W., & Cochran, W. G. (1967). *Statistical methods*. Iowa State College Press, Ames, IA, USA.
- Stapleton, H. M., Allen, J. G., Kelly, S. M., Konstantinov, A., Klosterhaus, S., Watkins, D., ... Webster, T. F. (2008). Alternate and new brominated flame retardants detected in US house dust. *Environmental Science & Technology*, 42(18), 6910–6916.
- Su, G., Crump, D., Letcher, R. J., & Kennedy, S. W. (2014). Rapid in vitro metabolism of the flame retardant triphenyl phosphate and effects on cytotoxicity and mRNA expression in chicken embryonic hepatocytes. *Environmental Science & Technology*, 42(22), 13511–13519.
- Sun, L., Xu, W., Peng, T., Chen, H., Ren, L., Tan, H., ... Fu, Z. (2016). Developmental exposure of zebrafish larvae to organophosphate flame retardants causes neurotoxicity. *Neurotoxicology and Teratology*, 55, 16–22.
- Walley, S. N., Krumm, E. A., Yasrebi, A., Wiersielis, K. R., O'Leary, S., Tillery, T., & Roepke, T. A. (2021). Maternal organophosphate flame-retardant exposure alters offspring feeding, locomotor and exploratory behaviors in a sexually-dimorphic manner in mice. *Journal of Applied Toxicology*, 41(3), 442–457.
- Wiersielis, K. R., Adams, S., Yasrebi, A., Conde, K., & Roepke, T. A. (2020). Maternal exposure to organophosphate flame retardants alters locomotor and anxiety-like behavior in male and female adult offspring. *Hormones and Behavior*, 122, 104759.
- Win-Shwe, T. T., Nakajima, D., Ahmed, S., & Fujimaki, H. (2013). Impairment of novel object recognition in adulthood after neonatal exposure to diazinon. *Archives of Toxicology*, 87(4), 753–762.
- Witchey, S. K., Al Samara, L., Horman, B. M., Stapleton, H. M., & Patisaul, H. B. (2020). Perinatal exposure to FireMaster® 550 (FM550), brominated or organophosphate flame retardants produces sex and compound specific effects on adult Wistar rat socioemotional behavior. *Hormones and Behavior*, 126, 104853.
- Zhao, F., Chen, M., Gao, F., Shen, H., & Hu, J. (2017). Organophosphorus flame retardants in pregnant women and their transfer to chorionic villi. *Environmental Science & Technology*, 51(11), 6489–6497.
- Zheng, X., Qiao, L., Covaci, A., Sun, R., Guo, H., Zheng, J., ... Mai, B. (2017). Brominated and phosphate flame retardants (FRs) in indoor dust from different microenvironments: Implications for human exposure via dust ingestion and dermal contact. *Chemosphere*, 184(185–191), 185–191.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Hawkey, A. B., Evans, J., Holloway, Z. R., Phippen, E., Jarrett, O., Kenou, B., Slotkin, T. A., Seidler, F. J., & Levin, E. D. (2023). Developmental exposure to the flame retardant, triphenyl phosphate, causes long-lasting neurobehavioral and neurochemical dysfunction. *Birth Defects Research*, 115(3), 357–370. <https://doi.org/10.1002/bdr2.2125>