Replacement organophosphate flame retardants suggest short-term reproductive and developmental toxicity in Sprague Dawley rats

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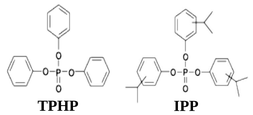
Introduction

HUMAN EXPOSURE

- Triphenyl phosphate (TPHP) and isopropyl phosphate (IPP) are found in indoor dust, indoor/outdoor air, aquatic biota and food.



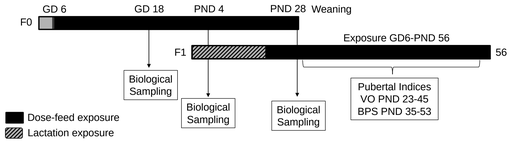
TOXICITY

- Data suggests reproductive and developmental toxicity and neurodevelopmental effects in exposed populations.
- Evidence of developmental and neurodevelopmental toxicity in *in vitro* and alternate animal models.
- NTP has previously evaluated TPHP and IPP in in vitro and benchmark concentration to compare minimal risk levels. https://sandbox.ntp.niehs.nih.gov/neurotox/integrative/

STUDY RATIONALE AND OBJECTIVES

- Hand-to-mouth is a common route of exposure (oral). Several studies have reported children have higher organophosphate flame retardant exposure compared to adults (risk).
- Systematic studies to assess the effects of these compounds on the development and maturation of the F1 pups are lacking.
- In support of on-going regulatory actions for TPHP and IPP, NTP conducted the following studies to:
  1. Evaluate potential developmental toxicity following pre- and postnatal oral exposure to either TPHP and IPP, probing the limits of toxicity.
  2. Assess exposure effects (15,000 ppm TPHP and 10,000 ppm IPP) on cholinesterase activity, thyroid hormones and brain inflammation (using marker translocator protein 23 kDa (TSPO).
  3. Determine maternal transfer in utero and PND4.

EXPERIMENTAL DESIGN



GD = gestational day PND = postnatal day

Model/species: Pregnant Harlan Sprague Dawley female rats (n= 6-7/dose group)

Doses: 0; 1000; 3000; 10,000; 15,000; 30,000 ppm TPHP or IPP

Exposure route/Duration: Dosed feed (NIH-07) from GD6-PND 28 (dams), PND 28-56 (offspring)

Endpoints: Clinical observations, body weight, litter parameters, pubertal attributes and biological sampling to examine cholinesterase activity, maternal thyroid hormone levels, and maternal transfer

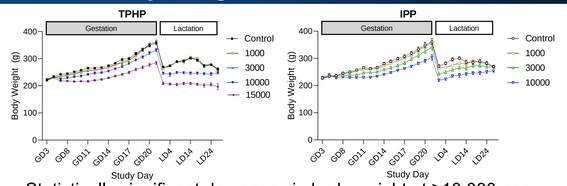
SUMMARY OF KEY FINDINGS

- **Maternal toxicity occurred at ≥10,000 ppm TPHP and ≥3,000 ppm IPP.**
- **Pubertal delays observed across all TPHP exposure groups and ≥3,000 ppm IPP.**
- **Blood and brain cholinesterase levels decreased in a dose-dependent manner in TPHP and IPP exposed animals.**
- **Maternal transfer of TPHP and IPP components are evident in the 1000 ppm group.**
- **Overall, developmental exposure effects observed at 1000 ppm in both TPHP and IPP groups.**
- **Data Tables: https://doi.org/10.22427/NTP-DATA-002-00600-0003-0000-1**

Treatment Effects across groups

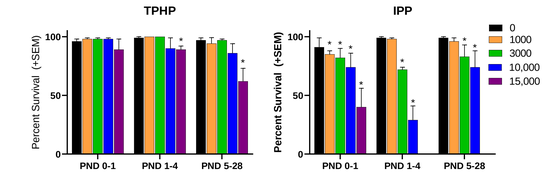
	Dam		Male Offspring		Female Offspring	
Treatment	TPHP	IPP	TPHP	IPP	TPHP	IPP
F0 Body weight	↓ ≥10,000	↓ ≥10,000	↓ ≥10,000	↓ ≥3,000	↓ ≥10,000	↓ ≥3,000
F0 Food consumption	↑ ≥10,000 (ges &lac)	↑ 10,000 (gestation) ↓ ≥1,000 (lactation)	N/A	N/A	N/A	N/A
F0 Liver weight	↑ ≥3,000	↑ 10,000	N/A	N/A	N/A	N/A
F0 Brain weight	No Effect	↑ 10,000	↓ ≥10,000	↓ 10,000	↓ ≥10,000	↓ 10,000
Reproductive Performance/ Litter Viability	↓ ≥10,000	↓ ≥1,000	N/A	N/A	N/A	N/A
Pubertal Indices	N/A	N/A	↑ delay ≥1,000	↑delay 3,000	↑ delay ≥3,000	No Effect
Blood AChE/BChE	↓ ≥3,000	↓ ≥1,000	↓ ≥10,000	↓ ≥1,000	↓ ≥10,000	↓ ≥1,000
Brain AChE/BChE	N/A	N/A	↓ ≥1,000 AChE ↓ ≥3000 BChE	↓ ≥1,000 AChE ↓ ≥3000 BChE	↓ ≥3,000	↓ ≥1,000 AChE ↓ ≥1,000 BChE
Thyroid Hormones	↓ ≥10,000	No Effect	N/A	N/A	N/A	N/A
TSPO	No Effect	No Effect	N/A	↑ dorsal brain regions	N/A	No Effect

1. Dam Body Weight



Statistically significant decreases in body weight at ≥10,000 ppm TPHP and 3,000 ppm IPP compared to controls ($p < 0.05$)

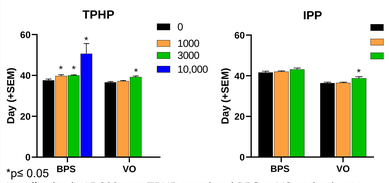
2. Litter Survival



PND= postnatal day; *p<0.05

Survival rate decreased at ≥15,000 TPHP ppm and 1,000 IPP ppm

3.Pubertal Indices

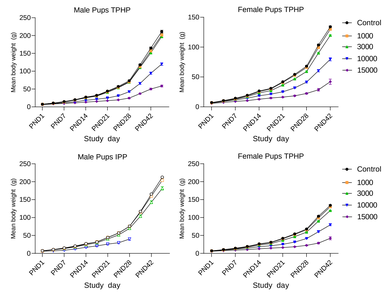


*p≤ 0.05

Delays in BPS in males at ≥1000 ppm and in VO in females at ≥ 3000 ppm TPHP. At 3000 ppm IPP, VO delayed in females.

No offspring in 15,000 ppm TPHP completed BPS or VO and only one 10,000 ppm TPHP female completed during time of examination.

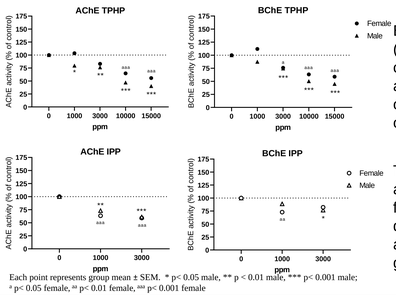
4. Offspring Body Weight



At ≥3000 ppm TPHP body weight decreases observed in male and female offspring.

At ≥1000 ppm IPP body weight decreases observed in male and female offspring.

5. Offspring Brain Cholinesterase Activity

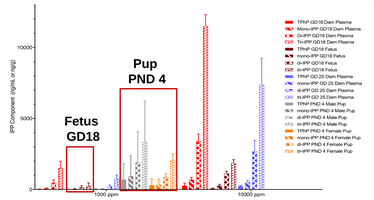


Brain acetyl cholinesterase (AChE) and butyl cholinesterase(BChE) activity in male and female offspring exposed to TPHP or IPP.

The AChE and BChE activity in brain collected from pups decreased in a dose-dependent manner across all TPHP and IPP groups.

Each point represents group mean ± SEM. * p< 0.05 male, ** p < 0.01 male, *** p< 0.001 male; [a] p< 0.05 female, [aa] p< 0.01 female, [aaa] p< 0.001 female

6. Maternal Transfer



- Maternal transfer low at GD18 and higher at PND 4.
- Circulating plasma levels increase with dose.
- TPHP and IPP components were found in PND 28 offspring brains (data not shown).

These data have not yet been evaluated in the NTP data/peer review process and therefore should be regarded as preliminary at this time.