Preliminary results from 2-week toxicity studies in B6C3F1/N mice with replacement organophosphate flame retardants

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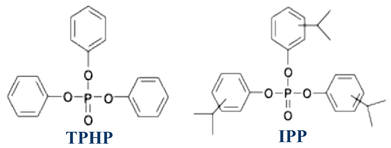
Introduction



USE

- Triphenyl phosphate (TPHP) and isopropyl phosphate (IPP) emerged as replacement organophosphate flame retardants (FR) following the phase out of known toxic FR, polybrominated diphenyl ethers (PBDEs) and organohalogens. TPHP is a single isomer whereas IPP is a mixture consisting of isomers having a TPHP backbone with isopropyl substitutions on the phenyl rings.
- Used in FR mixtures as an additive to treat foam-based furniture, baby products, electronics as well as a myriad other common items.

HUMAN EXPOSURE

- Due to TPHP and IPP readily leaching from products, both are prevalent pollutants found in indoor dust, indoor/outdoor air, aquatic biota and food.
- Humans are exposed via a combination of oral, inhalation, and dermal routes.
- Higher levels of exposure reported in firefighters and children.

TOXICITY

- Majority of TPHP and IPP research have focused on toxicological profiling and proper risk assessment studies have yet to be conducted.
- Reported oral LD50 values show a wide range up to in excess of 20,000 mg/kg in rats and in excess of 3000 mg/kg in mice (reviewed in ATSDR, 2012 and IPSC, 1991).

CONCERN

TPHP and IPP are structurally similar to organophosphates pesticides that are known to disrupt cholinergic pathways

HYPOTHESIS

Continuous exposure to TPHP and IPP will cause systemic toxicity and alter cholinesterase activity in mice.

Experimental Design

Model/species: B6C3F1/N mice (n= 5/sex/dose group)

Age: 36-42 days

Doses: 0, 1875, 3750, 7500, 15,000 and 30,000 ppm TPHP or IPP

Exposure route: Dosed feed (NTP-2000)

Duration:14 days

In Life Endpoints: clinical observations (2x/day), body weight (SD1, SD8 & SD15), food consumption (weekly males and twice weekly females)

Terminal Endpoints: blood collected retro-orbitally, gross necropsy (organ body weights for liver, thymus, kidneys, testes, epididymides, ovaries, heart, and lungs) and examination of blood and brain cholinesterase activity

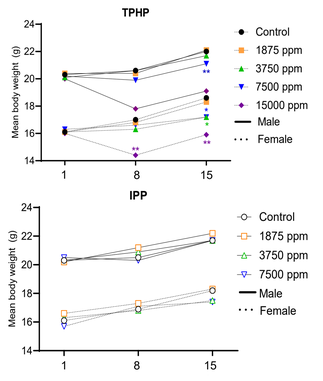
SUMMARY OF KEY FINDINGS

- **Removal of 30,000 ppm TPHP and ≥15,000 ppm IPP groups on SD4 due to clinical signs of toxicity.**
- **Body weight was significantly reduced in 15,000 ppm TPHP males and ≥3750 ppm TPHP females. No body weight changes observed in IPP exposure groups.**
- **Relative liver weights were 12-20% and 15-37% higher than controls in TPHP and IPP exposed animals.**
- **Relative thymus weights were ~50% lower in TPHP 15,000 ppm males and females and 32% lower in 7500 ppm IPP males.**
- **Blood and brain cholinesterase levels decreased in a dose-dependent manner in TPHP and IPP exposed animals.**

Conclusions

- Significant decreases in AChE and BChE observed in blood and brains of males and females across all TPHP and IPP exposure groups.
- Increased liver weights were observed following continuous exposure to ≥3750 TPHP and IPP groups (also 1875 ppm IPP males). The underlying cause of the increased liver weight was not examined in this study and will need to be followed up in future work.
- Although few effects of exposure were noted for body weight, clinical observations, or organ weights in the lowest exposure group (1875 ppm TPHP or IPP) large decreases in cholinesterase activity were observed. This highlights the importance of including clinical chemistry to short term studies.
- Future work will include lower doses to determine a point of departure for cholinesterase activity for both TPHP and IPP.

1. Body Weight



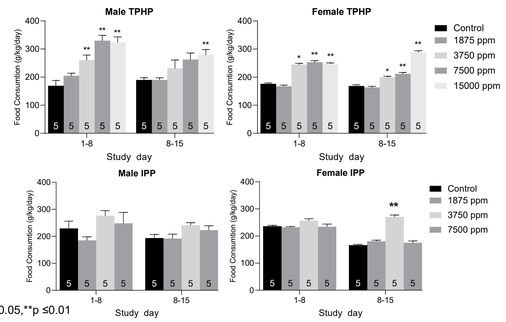
Body weight significantly reduced in 15,000 ppm TPHP males and ≥3750 ppm TPHP females.

No body weight changes observed in IPP exposure groups.

30,000 ppm TPHP and ≥15,000 IPP groups removed on SD4 due to toxicity

*p≤0.05,**p ≤0.01

2. Food/Test Article Consumption



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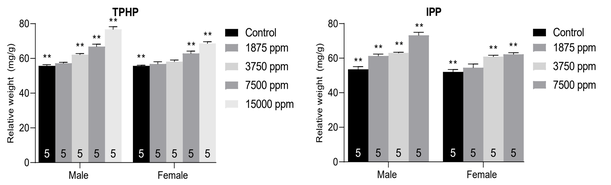
The mean daily TPHP intake was calculated to be 367, 921, 2205, and 4459 mg/kg/day in males and 309, 829, 1740, and 3964 mg/kg/day in females. For IPP, the mean daily intake was calculated to be 337, 969, and 1753 mg/kg/day for males and 385, 988, and 1551 mg/kg/day for females.

3. Relative Organ Weight

Relative liver weights 12-20% and 15-37% higher than controls in TPHP and IPP exposed animals.

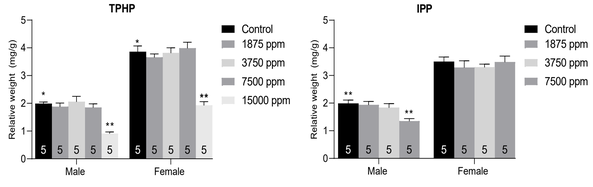
Relative thymus weights ~50% lower in TPHP 15,000 ppm males and females and 32% lower in 7500 ppm IPP males.

Liver Weight



TPHP	0		1875		3750		7500		15000	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Heart	8.37 ± 0.2	5.81 ± 0.2*	8.56 ± 0.2	6.87 ± 0.4*	8.20 ± 0.3	5.80 ± 0.3	7.74 ± 0.2	5.35 ± 0.2	8.18 ± 0.2	5.16 ± 0.2
L. Kidney	8.10 ± 0.2	6.23 ± 0.2	8.12 ± 0.2	6.55 ± 0.2	7.92 ± 0.2	6.14 ± 0.17	7.37 ± 0.3	6.05 ± 0.2	7.75 ± 0.2	6.16 ± 0.2
R. Kidney	8.37 ± 0.2	6.66 ± 0.1	8.56 ± 0.2	7.10 ± 0.3	8.20 ± 0.28	6.72 ± 0.25	7.74 ± 0.2	6.27 ± 0.1	8.18 ± 0.2	7.16 ± 0.3
Lung	10.08 ± 1.1	8.95 ± 0.7	9.90 ± 0.6	8.97 ± 0.4	9.08 ± 0.61	9.12 ± 1	10.85 ± 1.1	7.80 ± 0.5	9.07 ± 0.7	8.28 ± 0.4

Thymus Weight

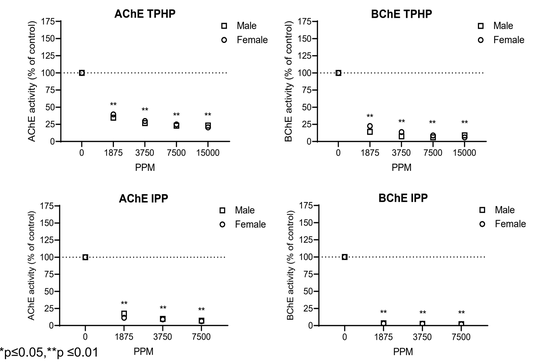


IPP	0		1875		3750		7500	
	Male	Female	Male	Female	Male	Female	Male	Female
Heart	5.80 ± 0.1	5.72 ± 0.1**	6.30 ± 0.2	5.57 ± 0.3	5.62 ± 0.4	5.36 ± 0.1	6.09 ± 0.4	5.15 ± 0.1
L. Kidney	8.38 ± 0.2**	6.70 ± 0.2	8.36 ± 0.1	6.55 ± 0.2	7.93 ± 0.1*	6.39 ± 0.2	7.55 ± 0.1**	6.20 ± 0.2
R. Kidney	8.83 ± 0.2	6.80 ± 0.2	8.64 ± 0.20	6.87 ± 0.4	8.39 ± 0.2	6.95 ± 0.2	8.19 ± 0.1	6.77 ± 0.2
Lung	11.12 ± 0.7	8.58 ± 0.3	9.16 ± 0.5	8.48 ± 0.7	9.29 ± 0.7	8.92 ± 0.5	10.60 ± 0.4	7.79 ± 0.1

*p≤0.05,**p ≤0.01

4. Blood Cholinesterase Activity

Blood AChE and BChE were reduced to 20-39% and 6-22% of controls in TPHP exposed males and females. In IPP males and females, blood AChE and BChE were 6-17% and 2-3% of controls, respectively.



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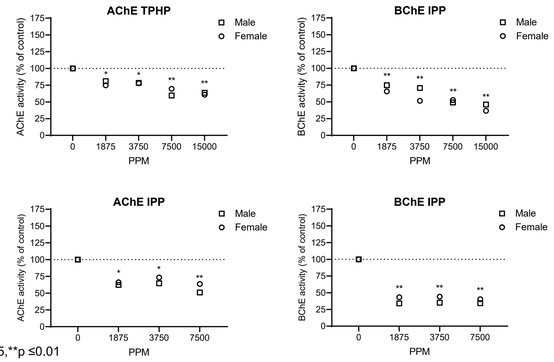
5. Brain Cholinesterase Activity

Brain AChE and BChE were reduced to 60-81% and 36-74% of controls in TPHP exposed males and females. In IPP males and females, brain AChE and BChE were 51-73% and 30-44% of controls, respectively.



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