BIOTRANSFORMATION OF TRIS (1-CHLORO-2-PROPYL) PHOSPHATE (TCIPP) AND TRIS (P-TERT-BUTYLPHENYL) PHOSPHATE (TTBPP) IN HUMAN LIVER MICROSOMES

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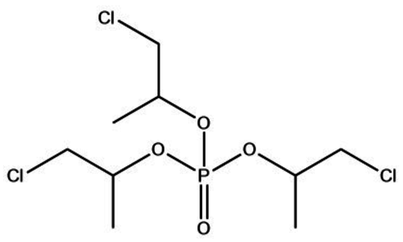
INTRODUCTION

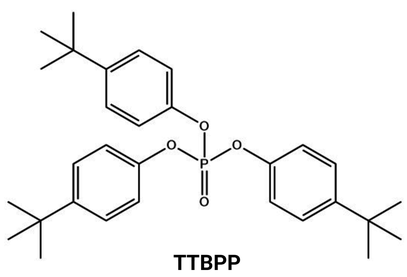
- **Organophosphate flame retardants (OPFRs)** are increasingly used in Europe since the ban on brominated flame retardants.
- **Tris(1-chloro-2-propyl) phosphate (TCIPP)** is one of the major OPFRs in the indoor and outdoor environment [1].
- Adverse health effects of TCIPP are not fully known yet [1].
- **Tris(p-tert-butylphenyl) phosphate (TTBPP)** is an novel OPFR which has been detected in fish, indoor dust and air [2-5].

- **Bis(1-chloro-2-propyl) hydrogen phosphate (BCIPP)** and 1-hydroxy-2-propyl **bis(1-chloro-2-propyl) phosphate (BCIPHIPP)** have already been identified as TCIPP metabolites in urine [1].
- **No metabolites** for TTBPP have been reported yet.

To gain more insight into the metabolites of TCIPP and TTBPP, Phase I and Phase II *in vitro* biotransformation of these OPFRs was investigated using **human liver microsomes (HLM)** and **human liver cytosol (HLCYT)**.

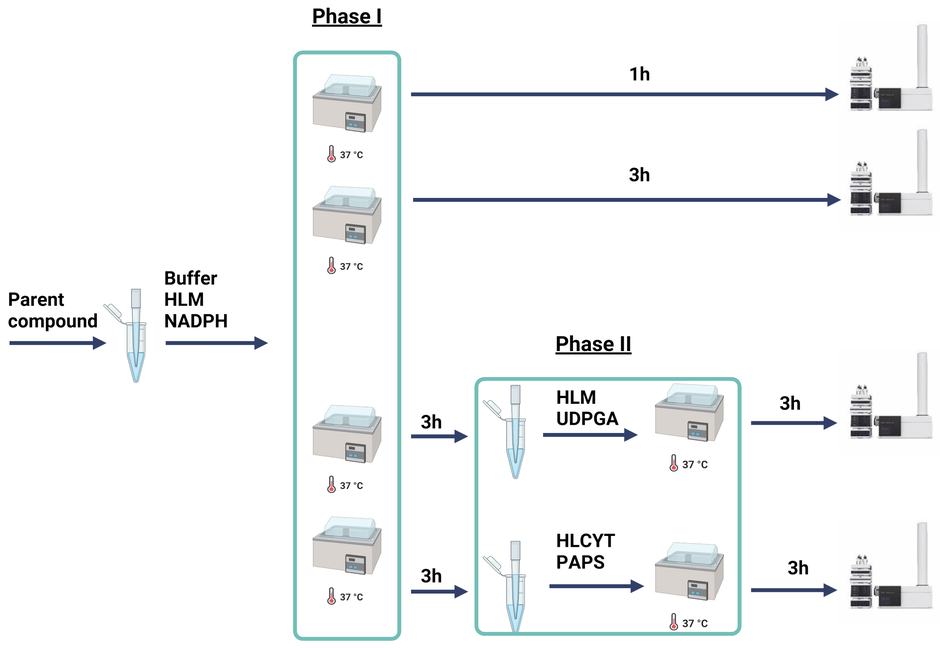


TCIPP



TTBPP

METHODS



Analytical analysis with Agilent 6530 LC-QTOF-MS
Column: Phenomenex Kinetex Biphenyl 100x2.1mm, 2.6 µm
Acquisition mode: Auto MS/MS and target MS/MS **Ionisation mode:** ESI+ and ESI-
Mobile phase ESI+:
A: H_2O + 0.1% formic acid **B:** MeOH + 0.1% formic acid
Mobile phase ESI-:
A: H_2O/MeOH (98:2) + 5 mM NH_4Ac **B:** MeOH/H_2O (98:2) + 5 mM NH_4Ac

RESULTS AND DISCUSSION

In vitro biotransformation of TCIPP

- BCIPP, BCIPHIPP, 1-carboxyethyl bis(1-chloro-2-propyl) phosphate (TCIPP-M1) and 1-chloro-3-hydroxypropan-2-yl bis(1-chloropropan-2-yl) phosphate (TCIPP-M3) were confirmed as human metabolites of TCIPP.
- Bis(1-chloropropan-2-yl) (1-oxopropan-2-yl) phosphate (TCIPP-M2), a metabolite that has not been reported before, was identified as intermediate product.
- No phase II metabolites (glucuronides or sulfates) were identified.
- Based on semiquantification, BCIPHIPP was the major metabolite formed, while BCIPP was formed to the least extent after 3h.
- Further research is needed to confirm *in vivo* presence of these metabolites in urine.

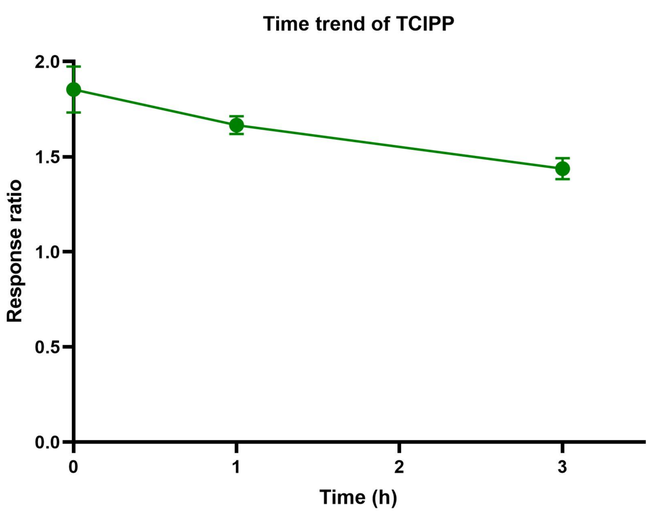


Figure 1. Time trend of TCIPP.

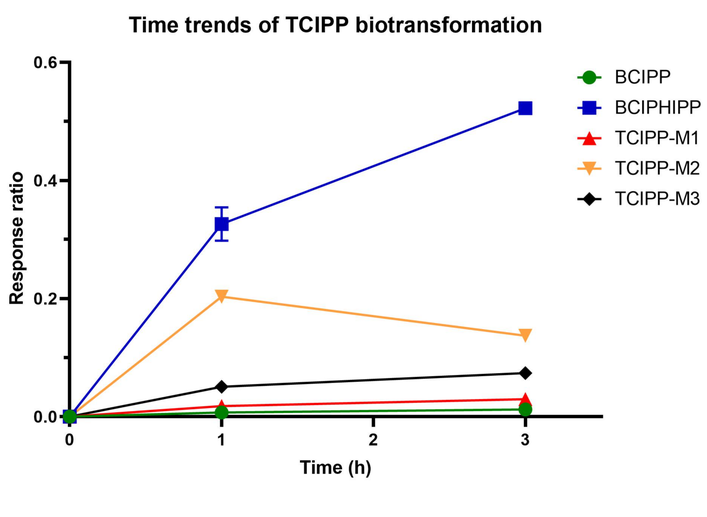


Figure 2. Time trends of formation of TCIPP metabolites.

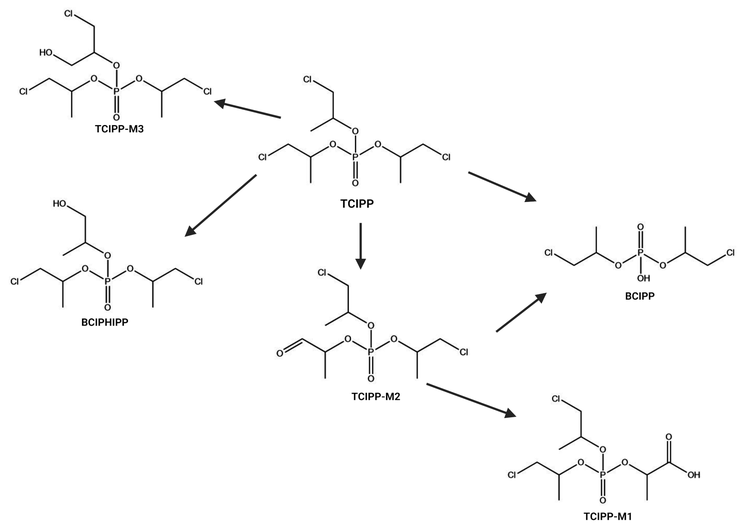


Figure 3. Proposed biotransformation pathway of TCIPP.

In vitro biotransformation of TTBPP

- Tris(4-(1-hydroxy-2-methylpropan-2-yl)phenyl) phosphate (TTBPP-M1) was identified as metabolite.
- No hydrolysis products were detected as metabolites.
- Due to low formation yields further investigation into the biotransformation of TTBPP is required.

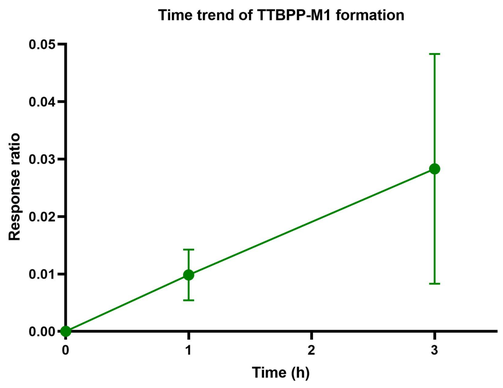


Figure 4. Biotransformation of TTBPP.

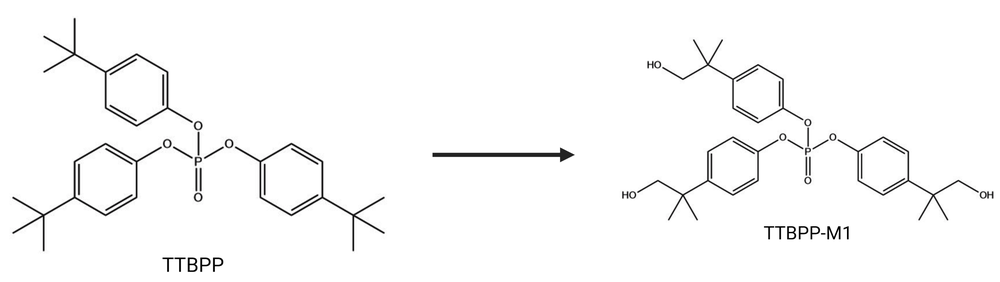


Figure 5. Biotransformation of TTBPP.

CONCLUSION

This research shed a new light on the *in vitro* biotransformation of TCIPP and TTBPP.

- For TCIPP, **four metabolites** were found. In addition, **one intermediate product** was detected. This intermediate product has not been reported before.

- For TTBPP, **one hydroxylation metabolite** was identified. To our knowledge this is the first time a metabolite of TTBPP is reported.

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