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Tributyl phosphate impairs the urea cycle and alters liver pathology and metabolism in mice after short-term exposure based on a metabonomics study

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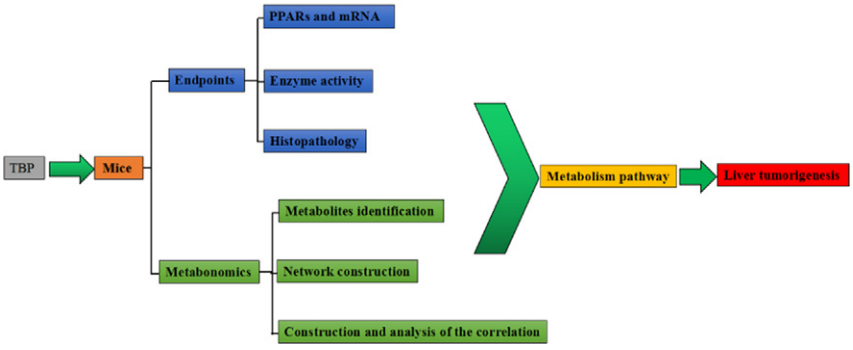
HIGHLIGHTS

- TBP could inhibit the synthesis of urea and influence the liver function.
- TBP is the activator for nuclear receptor CAR by reporter gene and RT-PCR test.
- TBP could induce mRNA expression of CYP*2b10*, but it inhibits the activity of *CYP3a11* and *CYP2b10* in liver.
- TBP may cause liver tumorigenesis disease.

GRAPHICAL ABSTRACT



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ABSTRACT

As a newly emerging environmental contaminant, tributyl phosphate (TBP) is of increasing concern because of the environmental problems it can cause. Studies have suggested that TBP induces hepatocellular adenomas and has malignant potential for hepatocellular carcinoma. However, the mechanisms of its adverse effects are unclear. In this study, metabonomic techniques were used to identify differential endogenous metabolites, draw network metabolic pathways and conduct network analysis to elucidate the underlying mechanisms involved in TBP induced pathological changes of the liver. The metabonomics study showed that TBP altered endogenous metabolites in the plasma and liver. The number of categories of endogenous metabolites with a VIP > 1 were 14 in plasma and 20 in liver. The results also showed that TBP impaired urea synthesis in the liver. In addition, results of both *in vitro* and *in vivo* experiments indicated that TBP activated nuclear receptor CAR and inhibited *CYP3a11* and *CYP2b10* activities in the liver of mice after short-term exposure. These effects may be the underlying causes leading to TBP induced hepatocellular adenomas. This study combined metabonomics and other technical methods to clarify the mechanism of TBP-induced liver tumorigenesis from a new perspective.

1. Introduction

Organophosphorus flame retardants (OPFRs) are widely used in textiles, building materials, electronics, chemicals and other industries as they possess good flame retardant properties, decrease corrosiveness of decomposition products and decrease production of toxic substances.

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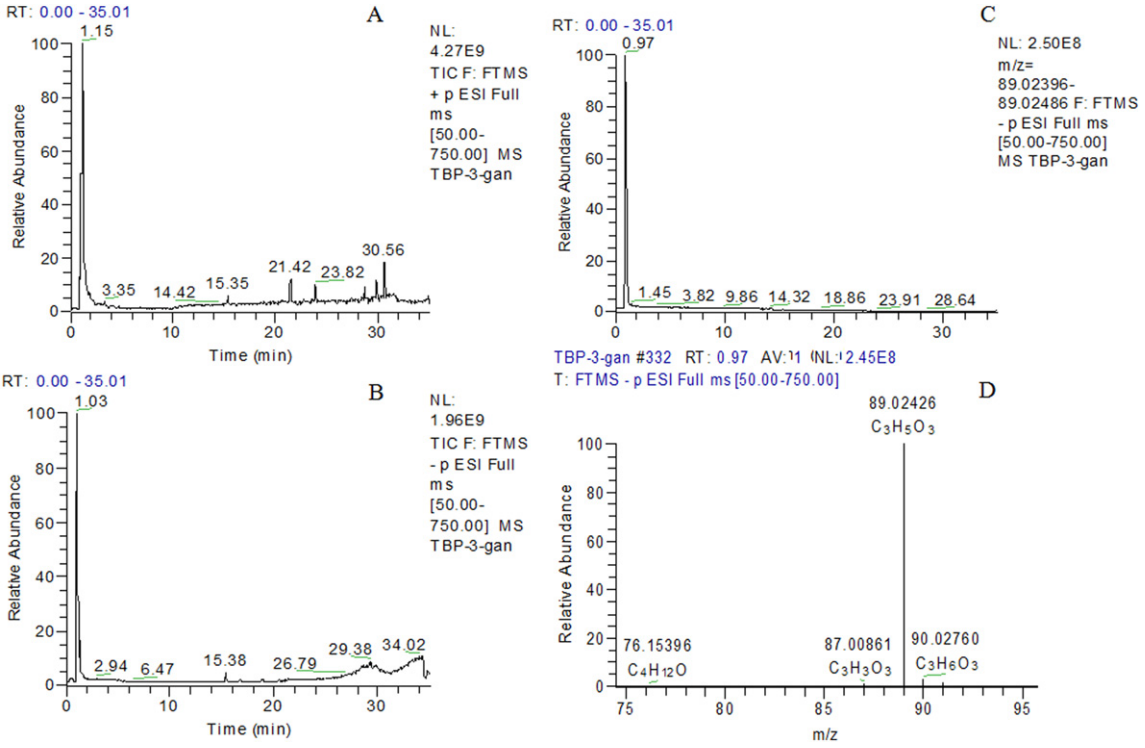

Fig. 1. Positive and negative ion full-scan chromatograms of representative plasma samples and metabolite identification. Total ion current chromatography (TIC) of samples derived from (A) positive or (B) negative ion scanning. (C) Accurate mass number of chromatographic peak rendering for lactic acid. (D) Chromatographic peak is lactic acid according to the precise mass number and the formula.

The global use of flame retardants in plastic additives is second only to that of plasticizers, indicating their widespread use in daily life. The total global demand of OPFRs has reached 1.5 million tons per year and will continue to grow by 4%–5% annually over the next few years (Segev et al., 2009). Tributyl phosphate (TBP) has a broad range of applications as an OPFR. It is the primary component of liquids in hydraulic machines and is also used as a defoamer for cement, a wetting agent for casein glue and a binder for pigments. Furthermore, TBP has become one of the most significant indoor air contaminants, being detectable in indoor dust (García et al., 2007). To limit occupational exposure in Finland, it is now stipulated that the TBP content in working environments must not exceed 5 mg/m^3 (Mäkinen et al., 2009). Fries et al. conducted a thorough investigation on environmental TBP in groundwater in Germany. They found groundwater levels of TBP in certain areas were higher than that in surface water indicating rainwater was leaching into aquifers (Fries and Wilhelm, 2001). Teo et al. reported that TBP was detectable in swimming pool water at concentrations ranging from 5 to 27 ng/L. A quantitative risk assessment revealed that the health risk of TBP in swimming pools was generally low, below commonly applied health risk benchmarks (Teo et al., 2016). Water pollution likely causes the secondary pollution of food and other processed products and, therefore, the emerging environmental problems caused by TBP have gradually attracted attention (Eggen et al., 2013; Calderón-Preciado et al., 2013).

Studies reported the LD_{50} value of orally administered TBP was between 2000 and 3000 mg/kg in rats (Dave and Lidman, 1978). Rats exposed to high doses of TBP developed bladder hyperplasia, papilloma and other toxicities. Zhao et al. found that TBP disrupted sphingolipid homeostasis and that sphingomyelin (SM) levels in the highest quartile of TBP were 153.6% higher than that in the lowest quartile. In addition, sphingosine 1-phosphate (S1P) levels in the highest quartile of TBP exposure were 36% lower than that in the lowest quartile (Zhao et al., 2016). An et al. reported TBP induced cytotoxicity in various cell lines at relatively high concentrations, as evidenced by decreased cell viability, reactive oxygen species (ROS) overproduction, induction of DNA lesions and increased lactate dehydrogenase leakage (An et al., 2016). Zhang et al. found that TBP was a possible thyroid hormone disruptor and had potential health and ecological risks (Zhang et al., 2016). Studies by Bruchajzer et al. reported that TBP was neurotoxic and caused disorders in fertility and/or fetal development in animal studies (Bruchajzer et al., 2015). These studies also showed that TBP had antagonistic activities toward androgen receptor (AR), glucocorticoid receptors (GR) and pregnane X receptor (PXR) (Kojima et al., 2013). Additionally, acute exposure of male Sprague-Dawley rats to TBP (15 mg/kg) resulted in tricarboxylic acid cycle energy metabolism disorders, as shown by metabonomics analysis in 24 h urine samples (Neerathilingam et al., 2010). A metabonomics study of chronic TBP exposure in rats showed that the metabolites most impacted were slightly different from those previously identified after acute exposure (Alam et al., 2012). Auletta et al. found that TBP at high doses induced hepatocellular adenomas in CD-1 mice and, at 24.1 mg/kg in male mice, TBP caused chronic toxicity without affecting the urinary bladder (Auletta et al., 1998).

The liver is an important organ for the biotransformation of chemical substances including enzymes involved in the metabolism of chemicals. Cytochrome P450 (CYP) is the major metabolic enzyme in the liver and is responsible for the biotransformation of exogenous and endogenous substances (SPatzenegger and Jaeger, 1995; Gunegerieh, 1991; Rendic and Di Carlo, 1997). Nuclear receptors widely expressed in the liver regulate biological effects, including gene expression levels of metabolic

Table 1
Primer sequences for RT-qPCR assay.

Gene	Primer	Length/bp
CYP3a11	F:5′-GTCAAACGCCTCTCCTTGCTG-3′	105
	R:5′-GGCTTGCCTTTCTTTGCCTTC-3′	105
CYP2b10	F:5′-TCAGGTGATCGGCTCACAC-3′	128
	R:5′-CATCCAGGAACTGGTCAGGA-3′	128
β-Actin	F:5′-GGCTGTATTCCCCTCCATCG-3′	154
	R:5′-CCAGTTGGTAACAATGCCATGT-3′	154