Sub-chronic Exposure to Tris(1,3-dichloro-propyl) Phosphate Induces Hepatotoxicity in Rats

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Abstract. Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), one of the most widely used organophosphorus flame retardants (OPFRs) is frequently detected in various environment and biota. Thus, its potential risk on wildlife and human health should be concerned. It has been implicated to induce hepatotoxicity, but the toxicological mechanism still remains unclear. Thus, Sprague Dawley (SD) male rats were administrated TDCIPP at 125, 250, or 500 mg/kg/d for 8 weeks in the present study. And hepatic histopathology and enzyme activities in serum and livers were analyzed to determine the molecular mechanisms of hepatotoxicity induced by TDCIPP. The histopathology showed the hepatocytes of rats exposed to TDCIPP were damaged, and the necrosis was more severe in 500 mg/kg/d TDCIPP-exposed group. And the decrease of transaminases in serum and livers were observed after TDCIPP exposure for 8 weeks, which indicated that severe hepatotoxicity was induced by TDCIPP. The abnormalities of oxidative stress makers and inflammation factors also indicated that TDCIPP caused dysfunction of oxidative system and severe inflammation response in livers of rats. Collectively, the results in our study demonstrated that TDCIPP induced hepatic oxidative stress and inflammatory response, and caused hepatotoxicity in rats.

Keywords: tris(1,3-dichloro-2-propyl) phosphate; hepatotoxicity; autophagy; oxidative stress; inflammation.

1. Introduction

As the phasing out of polybrominated diphenyl ethers (PBDEs), the production and application of OPFRs have thus been increasing in recent years. OPFRs are not chemically bonded to products and can leach into various environments easily. TDCIPP has been used as the main replacement of PBDEs and constantly detected in indoor air, water, dust and biota. Furthermore, TDCIPP and its metabolite have been detected in human breast milk, urine, and seminal fluid. Although the usage of TDCIPP is increasing rapidly, there is limited toxicological information available for TDCIPP. Recent studies showed that TDCIPP could adversely affect the nervous system in zebrafish and induce neurotoxicity in cultured cells. Based on several animal studies, TDCIPP is regarded as a probable human carcinogen. It has also been reported that TDCIPP could pervert endocrine system. Some studies indicated that exposure to TDCIPP could alter the genes involved in growth and the thyroid hormone (TH) pathway in avian hepatocytes. It has been suggested that TDCIPP exposure could also disturb the expression of hepatic genes involved in inflammation significantly in zebrafish. In chicken embryos, studies showed that hepatic genes containing immune response and xenobiotic may be changed after TDCIPP exposure. The lack of reports about hepatotoxicity in mammals is
surprising since the studies above demonstrated that liver is a critical target for TDCIPP toxicity. Consequently, in the present study, the hepatic histopathology and enzyme activities in serum and livers were analyzed in SD rats to obtain reliable risk assessment and explore underlying molecular mechanisms of TDCIPP-induced hepatotoxicity.

2. Materials and Methods

2.1. TDCIPP Treatment of Rats
TDCIPP (CAS#13674-87-8; >95% purity) was purchased from Tokyo Chemical Industry (Tokyo, Japan) and primarily dissolved in extra virgin olive oil (Abril, Spain). SD rats were from Beijing Vital River Laboratory Animal Technology and raised under standard animal housing condition (12-hr light/dark cycle, 20 ± 2 °C, and 50 ± 5% humidity). After acclimation for 2 weeks, 48 male rats were then divided into 4 groups. The rats in TDCIPP-exposed groups were administered TDCIPP in olive oil at dose of 125, 250, and 500 mg/kg/d for 8 weeks. The control group was exposed to the same volume olive oil per day. Plasma sample from each group were collected by retro-orbital bleeding to detect hepatic function related biochemical indicators at the end of 2nd, 4th, 6th, and 8th week. After TDCIPP exposure for 8 weeks, 3 rats from each group were sacrificed. The liver tissues were excised for histopathology and TEM analysis. All experimental procedures were conducted in accordance with Chinese legislation and approved by Animal Ethical committee of Nankai University.

2.2. Histopathological and TEM Analysis
The liver tissues were fixed in 10% buffered formalin for no less than 24 h. Fixed samples were embedded in paraffin wax, sectioned to 5 μm and then stained with hematoxylin and eosin staining (H&E staining). The sections were examined by brightfield microscope (IX71, Olympus, Japan). For TEM, liver tissues were quickly excised and then fixed in 2.5% glutaraldehyde for 48 h, followed by osmium tetroxide at 4 °C. Samples were then sectioned ultrathin which were analyzed using TEM (H-7650, Hitachi, Japan).

2.3. Biochemical Analysis
The levels of ALT, AST, GSH, SOD, IL-1β and TNF-α in hepatic homogenate were detected using commercial kits according to the manufacturers’ instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). And the activities of ALT and AST in serum were analyzed with an automatic biochemical analyzer (MD-2880, U.S.A).

2.4. Statistical Analysis
Data were analyzed using one-way ANOVA with Tukey's test. Differences were accepted as significant when P-value < 0.05 compared with control. SPSS 21.0 was used for statistical analyses. All experiments were repeated with no less than 3 biological replicates.

3. Results and Discussion

3.1. Validation of Hepatotoxicity by Histological and TEM Examination
To explore detailed hepatic damages, focal inflammation and hydropic degeneration were examined by examining 30 images from four hepatic sections for three rats in each group. H&E staining of liver slices revealed no evident abnormal pathological in the livers of control group (Fig.1a). Focal inflammation were both detected in the livers of all TDCIPP-exposed rats, with mild to moderate hydropic degeneration observed in 250 and 500 mg/kg/d TDCIPP-exposed rats (Fig.1b, 1c and 1d). In addition, the hepatic ultrastructural analysis was evaluated using TEM. No abnormality in hepatocellular organelle morphology was detected in the livers of control group (Fig.1e). Nuclear condensation was observed in the livers of 500 mg/kg/d TDCIPP-exposed group compared with control, which is a sign of apoptosis. And the results also showed increasing autophagy formation in the livers of 500 mg/kg/d TDCIPP-exposed rats (Fig.1f, 1g and 1h). Taken together, histopathological examination and TEM analysis confirmed that TDCIPP exposure can cause pathology damages and
hepatocellular injuries of rats.

![Figure 1](image)

**Figure 1.** a–d, H&E stained hepatocytes. (a) control group; (b) 125 mg/kg/d TDCIPP-exposed group; (c) 250 mg/kg/d TDCIPP-exposed group; (d) 500 mg/kg/d TDCIPP-exposed group. e–h, TEM ultrastructure of rats hepatocytes. (e) control group; (f-h) 500 mg/kg/d TDCIPP-exposed group.

### 3.2. Enzymatic Activity Analysis

In serum, the activities of ALT and AST were frequently considered to be the clinical and laboratory biomarker of hepatotoxicity. The activities of ALT (Fig.2a) and AST (Fig.2b) in serum were measured every two week during TDCIPP administration for 8 weeks. The results showed that no statistically differences were observed in ALT and AST activities between TDCIPP-exposed groups and control group from 2nd week to 4th week. At the end of 6th week, the levels of ALT and AST in 500 mg/kg/d TDCIPP-exposed rats were significantly decreased compared with control. And after TDCIPP exposure for 8 weeks, the activities of ALT and AST in serum of TDCIPP-exposed groups were found to be significantly lower than control group. We further determined the levels of ALT (Fig.2c) and AST (Fig.2d) in livers of rats after TDCIPP exposure for 8 weeks. The levels of ALT in livers of TDCIPP-exposed groups were statistically decreased compared with control. And the activities of AST in livers of TDCIPP-exposed groups were lower than that of control and the data showed a statistically decreased in 250 and 500 mg/kg/d group. Elevated levels of ALT and AST in serum and livers are correlated with hepatotoxicity, while reductions of ALT and AST have been demonstrated in some hepatic disease.[14] Consequently, the data above indicated that a potential liver injury was induced by TDCIPP.
3.3. Biochemical Indicators Involved in Oxidative Stress and Inflammation

To further confirm the TDCIPP-induced hepatotoxicity in rats at molecular level, the activities of GSH, SOD, IL-1β and TNF-α in livers of rats were determined. In TDCIPP-exposed groups, the levels of GSH (Fig.3a) were significantly decreased dose-dependently compared with control. The levels of SOD (Fig.3b) were statistically decreased in the livers of 500 mg/kg/d TDCIPP-exposed rats while there was no significantly changes in any other TDCIPP-exposed groups. The results revealed that oxidative damages in the livers of rats can be induced by TDCIPP. The data indicated that the level of IL-1β (Fig.3c) can be increased after TDCIPP exposure and it was significantly increased in 500 mg/kg/d TDCIPP-exposed group. The levels of TNF-α (Fig.3d) were increased in the livers of TDCIPP-exposed groups dose-dependently. And the activities of TNF-α were statistically increased in 250 and 500 mg/kg/d TDCIPP-exposed groups. The results above verified that TDCIPP induced inflammation response in the livers of rats. Recent studies also indicated that TDCIPP exposure can cause inflammation response and change inflammatory genes in zebrafish and chicken embryos. [12-13]
Figure 3. (a-b) Biochemical indicators of oxidative stress: (a) GSH and (b) SOD; (c-d) Biochemical indicators of inflammation: (c) IL-1β and (d) TNF-α in the livers of rats. **p<0.01, *p<0.05

4. Conclusions
In the present study, hepatotoxicity induced by TDCIPP was evaluated due to its increasing detection in environment and potential risk on human health. The observation of hepatic pathology damages, hepatocellular injury, abnormality of transaminase, oxidative stress and inflammation indicated the hepatotoxicity in rats can be induced by TDCIPP. And our study further added the toxic data of TDCIPP in mammals.

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