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Short-chain chlorinated paraffins (SCCPs) induced thyroid disruption by enhancement of hepatic thyroid hormone influx and degradation in male Sprague Dawley rats

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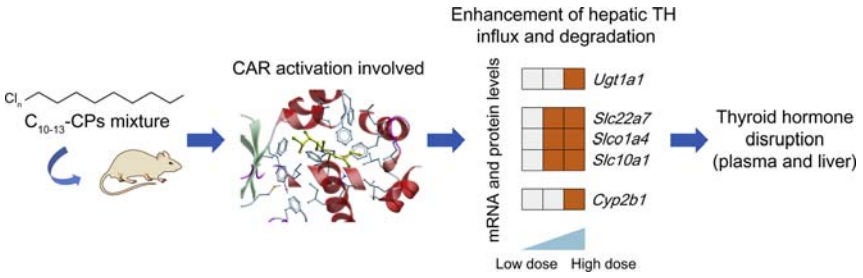
HIGHLIGHTS

- Oral SCCPs exposure induced hypothyroid state in male rats.
- SCCPs exerted no direct effects on the thyroid gland tissue.
- SCCPs induced hepatic TH uptake and degradation gene mRNA and protein levels.
- SCCPs showed activity against CAR according to molecular docking.

GRAPHICAL ABSTRACT



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ABSTRACT

Short-chain chlorinated paraffins (SCCPs) are known to disturb thyroid hormone (TH) homeostasis in rodents. However, the mechanism remains to be fully characterized. In this study, male Sprague Dawley rats received SCCPs (0, 1, 10, or 100 mg/kg/day) via gavage once a day for consecutive 28 days. Plasma and hepatic TH concentrations, thyrocyte structure, as well as thyroid and hepatic mRNA and protein levels of genes associated with TH homeostasis were examined. Moreover, we performed molecular docking to predict interactions between constitutive androstane receptor (CAR), a key regulator in xenobiotic-induced TH metabolism, with different SCCP molecules. Exposure to SCCPs significantly decreased the circulating free thyroxine (T_4) and triiodothyronine (T_3) levels, but increased thyroid-stimulating hormone (TSH) levels by a feedback mechanism. Decreased hepatic T_4 and increased hepatic T_3 levels were also seen after 100 mg/kg/day SCCPs exposure. SCCPs didn't show any significant effects on the expression of thyroid TH synthesis genes or thyrocyte structure. However, stimulation effects were observed for mRNA and protein levels of hepatic uridine diphosphoglucuronosyl transferase (UGT) 1A1 and organic anion transporter 2, suggesting an accelerated TH metabolism in rat liver. The increased cytochrome P450 2B1 but not 1A1 mRNA and protein levels indicated that the CAR signaling was activated by SCCPs exposure. According to docking analysis, SCCPs form hydrophobic interactions with CAR and the binding affinity shows dependency on chlorine content. Overall, our data showed that CAR implicated enhancement of hepatic TH influx and degradation could be the main cause for SCCPs induced TH deficiency in male rats.

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1. Introduction

Short-chain chlorinated paraffins (SCCPs) are a group of chlorinated *n*-alkanes with 10–13 carbon atoms and an average chlorine content of 30%–70% by mass (De Boer et al., 2010). They are persistent in the environment (Gao et al., 2012), bioaccumulative in wildlife and humans (Xia et al., 2017; Zeng et al., 2015) and has been newly listed as persistent organic pollutants (POPs) by the Stockholm Convention (United Nations Environment Program (UNEP), 2017). As a constituent part of chlorinated paraffins (CPs), SCCPs are used in a wide range of industrial applications, such as flame retardants, plasticisers, sealants, adhesives, and additives (Zeng et al., 2011). In the past two decades, the accumulative production volume of SCCPs may have exceeded 3 million tons (Xu et al., 2014). An assessment of the available data indicates that SCCPs are of low acute toxicity in animals. The no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) for rats were deduced to be 10 and 100 mg/kg/day, respectively (United Nations Environment Program (UNEP), 2017). However, the chronic and sub-chronic effects of SCCPs have not yet been sufficiently characterized (Wang et al., 2013), which is important considering their large production volume and high environmental levels.

SCCPs have been considered to be thyroid-disrupting chemicals (TDCs). Long-term exposure to SCCPs was found to cause mild histological changes in the thyroids of rodents (Serrone et al., 1987). Additionally, high-dose SCCP (1000 mg/kg/day for 14 days) exposure also depressed plasma thyroxine (T_4) levels by increasing T_4 glucuronidation via hepatic microsomal uridine diphosphoglucuronosyl transferase (UGT) and thereby enhanced biliary clearance of T_4 in rats and mice (Wyatt et al., 1993). However, it is still unclear whether the increased clearance of thyroid hormone (TH) alone is responsible for the decrease in circulating TH by SCCP treatment. The mode of action by which SCCPs induced thyroid disruption remains to be fully determined.

The precise regulation of the TH balance and action includes a complex interaction of physiologic processes. Environmental pollutants have been claimed to impair TH homeostasis via a multitude of mechanisms (Boas et al., 2006; Boas et al., 2009). The up-regulation of hepatic TH glucuronidation is a well-known mode of action resulting in TH depression by chlorinated chemicals (Hallgren and Darnerud, 2002; Paul et al., 2010; Seo et al., 1995). In addition, thyroid TH synthesis, circulating and cellular TH transport, and hepatic deiodination also have effects on TH homeostasis (Szabo et al., 2009). Recently, Liu et al. (2016) found that C_{10}-SCCPs (i.e., $C_{10}H_{18}Cl_4$, $C_{10}H_{16}Cl_6$, and $C_{10}H_{15}Cl_7$) could decrease the expression of transthyretin (TTR, encoded by *Ttr*), type II deiodinase (ID2, encoded by *Dio2*), and type III deiodinase (ID3, encoded by *Dio3*) genes in a dose-dependent manner in zebrafish (*Danio rerio*) larvae. These results suggested that other mechanisms are implicated in SCCP-triggered thyroid disruption.

In an effort to reveal key events responsible for SCCP-induced thyroid disruption, our present study first examined the effects of oral SCCPs administration (1, 10, and 100 mg/kg/day) on plasma and hepatic TH homeostasis and thyroid morphology in adult male Sprague Dawley rats. The mRNA and protein levels of genes related to TH synthesis, regulation, transport, metabolism, and degradation were determined by quantitative polymerase chain reaction (qPCR) and enzyme-linked immune-sorbent assay (ELISA). Moreover, we performed molecular docking studies to predict the interactions between constitutive androstane receptor (CAR), a key regulator in xenobiotic-induced TH metabolism, and different CP molecules.

2. Materials and methods

2.1. Chemicals

A standard mixture of C_{10-13}-CPs (mass ratio of C_{10}-CPs: C_{11}-CPs: C_{12}-CPs: C_{13}-CPs = 1: 1: 1: 1; chlorine content of 56.5% by mass) for the exposure experiment was produced by the chlorination of *n*-alkane according to the method described by Tomy et al. (2000). The production details, corresponding chromatogram, and congener group abundance profile were shown in Supporting Information (SI).

2.2. Animal exposure and sampling protocols

A total of 36 approximately 5-week-old male Sprague Dawley rats, weighing 339–407 g, were used for this experiment. The rats were housed in stainless steel cages (L 32.0 cm × W 28.0 cm × H 20.0 cm; one animal per cage) at a temperature of 25 ± 3 °C and a 12-h light/dark cycle. Food and water were provided ad libitum. Following 5 days of acclimatization, the rats were randomly assigned into four groups ($N = 9$ for each) and administered C_{10-13}-CPs mixture in corn oil by daily gavage at doses of 0, 1, 10, or 100 mg/kg body weight. The middle dose (10 mg/kg/day) is the NOAEL, whereas the highest dose (100 mg/kg/day) is the LOAEL for rats. The control group received corn oil alone, and their final intake of corn oil was 5 mL/kg body weight.

Male rats were sacrificed after diethyl ether anesthesia at 28 days of exposure. All surgeries were performed under anesthesia, and all efforts were undertaken to minimize suffering in the surgical procedure. Blood was collected by the abdominal aortic method. After centrifugation (1500 ×*g*, 15 min), plasma was frozen in liquid nitrogen for the detection of plasma TH. The liver and thyroid tissues were meticulously removed during necropsy, rinsed, weighed, and stored in RNase-free tubes at −80 °C until RNA extraction and qPCR analysis. The remaining isolated livers were homogenized in 9 volumes (w/v) of cold phosphate buffer saline (PBS, pH 7.4) on ice. Then, the supernatant was prepared using centrifugation at 1500 ×*g* for 15 min at 4 °C for the quantification of hepatic TH levels and protein expressions of genes associated with TH homeostasis. Meantime, the remaining isolated thyroid from control and 100 mg/kg/day group were used for histological examination.

2.3. Plasma and hepatic thyroid hormone measurements

Plasma total (T) T_4 and T_3, physiologically relevant free (F) T_4 and T_3, and thyroid-stimulating hormone (TSH) were measured by double-antibody sandwich ELISA kits obtained from Nanjing Jiancheng Biocompany (Nanjing, China) according to the manufacturer's instructions. The detection limits were 0.5 μg/L for TT_4, 0.05 μg/L for TT_3, 0.5 ng/L for FT_4, 0.3 ng/L for FT_3, and 0.05 mIU/L for TSH, respectively, derived from the lowest standards of these kits. The inter- and intra-assay coefficients of variation for all of the above hormones were controlled under 8% and 10%. In addition, hepatic T_4 and T_3 levels were also measured in liver homogenates by using the same ELISA kits. The specificities of these kits for tissue TH determination were evaluated in a preliminary experiment using serial diluted samples.

2.4. Thyroid and liver gene expression analysis

Relative qPCR quantification was conducted in accordance with established rules (Udvardi et al., 2008). Briefly, total RNA was isolated from thyroid and liver using the Takara RNAiso plus reagent (Takara, Tokyo, Japan) following the manufacturer's protocol. RNA quality and concentration were determined by spectrophotometric analysis (Agilent 2100 Bioanalyzer, Agilent Technologies, Palo Alto, CA) and 1% agarose gel electrophoresis. Only samples with a 260/280 nm ratio of 1.9–2.0 were processed further. Equal amounts of RNA (500 ng) were reverse-transcribed into cDNA with the iScript cDNA Synthesis kit (Bio-Rad, Richmond, CA). Specific primers (SI; Table S1) were designed based on sequence data from the GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Primers were placed at the junction between 2 exons and were synthesized by Sangon biotech (Shanghai, China). qPCR analyses were performed on the Light Cycler 480 PCR System (Roche Diagnostics, Mannheim, Germany) using a FastStart Universal SYBR Green Master kit (Roche Applied Science, Mannheim, Germany). The geNorm analysis of 8 candidate housekeeping genes (SI; Table S2 and S3)