Science of the Total Environment 622–623 (2018) 214–221

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Developmental and metabolic responses of zebrafish (*Danio rerio*) embryos and larvae to short-chain chlorinated paraffins (SCCPs) exposure

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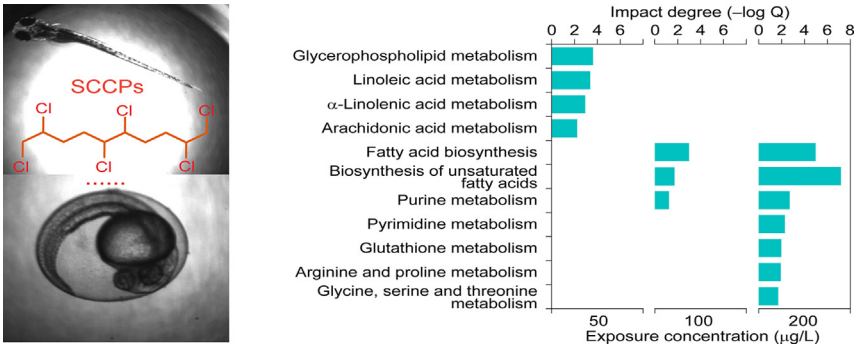
HIGHLIGHTS

- Effects of SCCPs on development and metabolism of zebrafish were evaluated.
- SCCPs were highly toxic to zebrafish larvae, with 13-day LC_{50} value of 34.4 μg/L.
- Low-dose SCCPs slightly disturbed the overall metabolism in zebrafish embryos.
- SCCPs mainly disturbed metabolisms of glycerophospholipids, fatty acids and purine.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 October 2017
Received in revised form 26 November 2017
Accepted 26 November 2017
Available online xxxx

Editor: Jay Gan

Keywords:
Short-chain chlorinated paraffins
Zebrafish embryo/larvae
Metabolomics
Developmental toxicity

ABSTRACT

Short-chain chlorinated paraffins (SCCPs) are highly toxic to aquatic organisms, but their toxicity is yet not well characterized. In this study, the developmental toxicity of SCCPs to zebrafish embryos/larvae was evaluated, and a metabolomics approach was adopted to explore the impact of SCCPs exposure on the metabolism in zebrafish embryos. Exposure to SCCPs at concentrations of 1–200 μg/L did not produce an observable effect on the hatching rate and morphological deformities of zebrafish embryos/larvae. However, the survival rate of zebrafish larvae in SCCPs exposure groups decreased in a concentration-dependent manner. The 13-day 50% lethal concentration (LC_{50}) value of SCCPs was calculated to be 34.4 μg/L. Exposure to SCCPs induced a significant change of overall metabolism, even at environmentally relevant concentrations (1–5 μg/L). The most relevant pathways affected by SCCPs exposure were *glycerophospholipid metabolism*, *fatty acid metabolism* and *purine metabolism*. Exposure to SCCPs at concentrations of 1–5 μg/L had begun to accelerate the β-oxidation of unsaturated fatty acids and very long chain fatty acids, and affect the transformation of guanine to xanthine in the pathway of purine metabolism. Furthermore, when the exposure concentrations of SCCPs were increased to 50–200 μg/L, the levels of phospholipids and amino acids were significantly raised; whereas the levels of fatty acids, carnitines and inosine were significantly decreased. In view of the significant effect on metabolism, the sub-chronic and chronic toxicity of SCCPs to fish should be concerned.

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http://dx.doi.org/10.1016/j.scitotenv.2017.11.304
0048-9697/

1. Introduction

Short-chain chlorinated paraffins (SCCPs) are a large and complex family of chlorinated *n*-alkanes with carbon chain lengths of 10–13 and chlorine content of 30%–70% (mass weight) (Barber et al., 2005). They are widely applied in metal-working fluids, paints, sealants, adhesives, leather softener, and as flame retardants and plasticizers for plastics and textiles (Fiedler, 2010). In view of their properties of environmental persistence, long-range transport potential, bioaccumulation potential and high toxicity to aquatic organisms (UNEP, 2009), SCCPs have been listed as a new group of POPs by the Convention's POP Review Committee (POPRC) of Stockholm Convention (UNEP, 2017).

SCCPs were ubiquitously found in the aquatic ecosystem, and they could be accumulated in aquatic organisms (UNEP, 2009; van Mourik et al., 2016). The concentrations of total SCCPs generally ranged from 4 to 1700 ng/L in natural surface water (Nicholls et al., 2001; Castells et al., 2004; Zeng et al., 2011; Ma et al., 2014a), and 10 to 4000 ng/g wet weight in fishes and aquatic invertebrates (Houde et al., 2008; Yuan et al., 2012; Ma et al., 2014a; Ma et al., 2014b; Wei et al., 2016). Previous studies have indicated that SCCPs are highly toxic to aquatic invertebrates (UNEP, 2009). The no observed effect concentration (NOEC) of SCCPs for daphnids (*Daphnia magna*) were determined to be 5 μg/L based on a 21-day chronic exposure (Thompson and Madeley, 1983a), and 28-day chronic NOEC of SCCPs for the mysid shrimp (*Mysidopsis bahia*) were determined to be 7.3 μg/L (Thompson and Madeley, 1983b). Fish embryos and larvae were also found to be sensitive to SCCPs exposure. The acute toxicity of SCCPs to Japanese Medaka (*Oryzias latipes*) is narcosis, and the lowest observed effect concentration (LOEC) in embryo-larval assays varied from 55 μg/L to 460 μg/L (Fisk et al., 1999).

SCCPs could cause sub-chronic toxicity in the kidney (Warnasuriya et al., 2010), liver (Cooley et al., 2001) and thyroid (Wyatt et al., 1993) on laboratory animals, and induce developmental malformation of *Xenopus laevis* frog embryos (Burýšková et al., 2006). In addition, several previous studies indicated that SCCPs could act as endocrine disruptor (Wyatt et al., 1993; Cooley et al., 2001; Liu et al., 2016; Zhang et al., 2016). SCCPs exposure could affect gene expression in the hypothalamic-pituitary-thyroid (HPT) axis and level of thyroid hormone in zebrafish larvae (Liu et al., 2016). A study with H295R cells indicated that SCCPs not only exerted potential endocrine-disrupting effects through nuclear receptors but also disrupted the production of steroid hormones *via* non-receptor-mediated mechanisms (Zhang et al., 2016). Moreover, the aliphatic structure could also make SCCPs act as PPAR-α activator to induce the peroxisome proliferation (Warnasuriya et al., 2010). These endocrine-disrupting effects together with PPAR-α activation imply the possible adverse effects of SCCPs at the environmentally-relevant concentrations on aquatic organisms.

Metabolomics is a powerful tool of understanding metabolic regulation by systematically detecting low-molecule metabolites present in biological samples (Chen et al., 2013). It can provide new sights for how mechanistic biochemistry relates to the phenotypic state of an organism, because metabolites act as direct signatures of biochemical activity (Patti et al., 2012). As a crucial "omics" science in system biology, metabolomics has been extensively used for drug safety evaluation, disease diagnosis and toxicity assessment (Xu et al., 2015). In this study, the metabolomics strategy was adopted to investigate the aquatic toxicity of SCCPs. The embryos and larvae of zebrafish (*Danio rerio*) were adopted as the animal model. Zebrafish is an attractive aquatic model for chemical toxicity assessment, and its embryos and larvae is more sensitive to environmental stress compared with adult. The developmental toxicity was first evaluated, and then a pseudotargeted metabolomics approach was adopted to explore the impact of SCCPs exposure on the metabolism in zebrafish embryos. The acquired results are looked forward to offering a better knowledge about the aquatic toxicity of SCCPs for fish, and to providing new evidence and clues concerning the toxicological mechanisms of SCCPs from a metabolomics perspective.

2. Methods and materials

2.1. Chemicals and reagents

SCCP mixtures with different carbon chain lengths (mass ratio, C_{10}-CPs:C_{11}-CPs:C_{12}-CPs:C_{13}-CPs = 1:1:1:1) were respectively synthesized by chlorination of the *n*-alkanes according to the method described by Tomy et al. (2000). The chlorine content of test SCCP mixtures was determined to be 56.5%. The corresponding chromatogram and congener group abundance profile are shown in Fig. S1 (Supplementary data). Acetonitrile and Methanol (LC-MS grade) were obtained from Merck (Germany). Formic acid (HPLC-grade) was obtained from TCI (Japan). Ammonium bicarbonate (HPLC-grade) was purchased from J&K (China). Dimethyl sulfoxide (ACS grade) was obtained from AMRESCO (USA). Six internal standards (L-phenylalanine-d5, octanoyl (8,8,8-D3)-L-carnitine, 1-lauroyl-2-hydroxy-*sn*-glycero-3-phosphocholine, 1,2-diheptadecanoyl-*sn*-glycero-3-phosphoethanolamine, hendecanoic acid and nonadecanoic acid) were purchased from Sigma-Aldrich (Sigma Chemical Co, USA). Stock solution of SCCPs was dissolved in dimethyl sulfoxide at a concentration of 10 mg/mL. For static exposure, solutions were diluted in rearing water with a 0.02% final dimethyl sulfoxide concentration.

2.2. Zebrafish maintenance and embryo collection

Wild-type AB-strain zebrafish (*Danio rerio*) were obtained from Safety Evaluation Center, Shenyang Research Institute of Chemical Industry. Adult male and female fish were maintained separately at 27 ± 0.5 °C in holding tanks with aerated water for 18 d with a photoperiod 12/12 h, and dissolved oxygen concentration of at least 80% of the air saturation value before toxicity test. They were fed two to three times per day with live brine shrimp. Before spawning, male and female adult fish (male/female ratio: 2/1) were placed separately by isolation boards in spawning boxes overnight. In the following morning, spawning and fertilization started after the light was turned on and the isolation boards were removed. Fertilized and normally developed embryos were selected from the spawning boxes using a dissecting microscope and transferred to clean rearing water using plastic disposable pipets to rinse them. All of the rearing water was pretreated using Aqua Pro Pure Water System (USA).

2.3. Exposure experiments

The toxicity test of zebrafish embryos/larvae was conducted according to the OECD 210 guideline (OECD, 2013). Zebrafish embryos were exposed to the SCCPs mixture (mass ratio, C_{10}-CPs:C_{11}-CPs:C_{12}-CPs:C_{13}-CPs = 1:1:1:1; 56.5% Cl) with varying concentrations (1, 5, 10, 50, 100 and 200 μg/L) in rearing water. The final dimethyl sulfoxide content in rearing water was adjusted to be 0.02% (v/v), and solvent control group was handled with only 0.02% dimethyl sulfoxide. The exposure concentrations of 1 and 5 μg/L are comparable with SCCPs concentrations in surface water (concentrations: 4 to 1700 ng/L) (Nicholls et al., 2001; Castells et al., 2004; Ma et al., 2014a) and polluted water (concentrations: 4200–4700 ng/L) (Zeng et al., 2011), respectively. At about 2 h of post-fertilization (hpf), embryos were randomly distributed in beakers containing 100 mL of exposure solution. There were 6 replicates for each treatment. Before 120 hpf, the rearing water was renewed every 24 h. After 120 hpf, the rearing water was renewed twice per day, and larvae were fed twice per day.

During the period of experiment, dead embryos/larvae were immediately removed. Criteria for mortality were defined as coagulation, missing heartbeat, failure to develop somite and a non-detached tail. The hatching and malformation of embryos/larvae were checked daily.