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Tissue distribution, excretion, and the metabolic pathway of 2,2′,4,4′,5-penta-chlorinated diphenylsulfide (CDPS-99) in ICR mice

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ABSTRACT

The tissue distribution, excretion, and metabolic pathway of 2,2′,4,4′,5-penta-chlorinated diphenylsulfide (CDPS-99) in ICR mice were investigated after oral perfusion at 10 mg/kg body weight (b.w.). Biological samples were extracted and separated and, for the first time, were determined by a novel, sensitive, and specific GC–MS method under the full scan and selected ion monitoring (SIM) modes. The results showed that the concentrations of CDPS-99 in the liver, kidneys, and serum reached a maximum after a one-day exposure and that the CDPS-99 concentration in the liver was the highest (3.43 μg/g). The increase in the concentration of CDPS-99 in muscle, skin, and adipose tissue was slower, and the concentrations of CDPS-99 achieved their highest levels after 3 days of exposure. It was observed that the CDPS-99 concentration in adipose tissue was still very high (0.71 μg/g) after 21 days of exposure, which suggested that CDPS-99 was able to accumulate in adipose tissue. In addition, mouse feces accounted for approximately 75% of the total gavage dose, indicating that CDPS-99 was mainly excreted via mouse feces. Metabolism analysis demonstrated that there were three possible metabolic pathways of CDPS-99 in mice: dechlorination reactions with the formation of tetra-CDPS and hydroxylation and oxidation reactions with the formation of OH-CDPS-99 and chlorinated diphenylsulfone. The present study will help to develop a better understanding of mammalian metabolism of CDPS-99.

1. Introduction

Polychlorinated diphenyl sulfides (PCDPSs) are a series of sulfur-containing dioxin-liked compounds (DLCs) that contains one to ten chlorine substituents with a total of 209 possible congeners. PCDPSs and ploychlorinated diphenyl ethers (PCDEs) have similar chemical structures and physico-chemical properties (Fig. 1) [1]. PCDPSs have been applied in various fields, including high-temperature lubricants in gas turbines and steam machines, flame retardants, insulating media, drugs, and pesticides [2–4].

PCDPSs have been detected in various environmental samples. For example, Sinkkonen et al. reported that PCDPSs exist in fly ash from metal reclamation plants, and they also detected tri-CDPS isomers in pulp mill effluent samples as well as tri- and tetra-CDPSs in stack gases from a waste incinerator [5–7]. Schwarzbauer et al. [8] identified 4,4′-di-CDPS in sediment samples from the Elbe River. In a recent study, we investigated the levels and distribution of 21 types of PCDPSs in the Nanjing section of the Yangtze River, and the results suggested that a total of 19 PCDPSs were detected in the water and sediment samples [9].

Some studies have focused on the adverse effects of PCDPSs. Li et al. [10] and Zhang et al. [11] demonstrated that PCDPSs could induce hepatic oxidative stress in fish and mice. Kopponen et al. [12] found that 3,3′,4,4′-tetra-CDPS had CYP1A1-inducing potencies in mouse hepatoma cells. Recently, the aromatic hydrocarbon receptor (AhR) effect of 19 types of PCDPSs was also examined by our co-workers using the avian AHR1-luciferase report gene (LRG) assay, and the results showed that 2,4,4′,5-tetra-CDPS and 2,2′,3,3′,4,5,6-Hepta-CDPS elicited higher potencies compared to 2,3,7,8-TCDD, with potency values of 3.3×10^{-5} and 2×10^{-5}, respectively [13]. These data suggest that PCDPSs could cause damage to organisms in the environment and that researchers should pay more attention to them. Environmental pollutants are transformed upon entering organisms, and this metabolism process could influence the toxicity of the pollutants to the organisms. Many studies focusing on the distribution and metabolism of pol-

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lutants in organisms have been conducted. Knuden et al. [14] investigated the absorption, distribution, metabolism, and excretion of intravenously and orally administered tetrabromobisphenol A [2,3-dibromopropyl ether] in male Fischer-344 rats and found that the liver was the major site of disposition following oral or IV administration; Hakk et al. [15] studied the tissue distribution, excretion, and metabolism of 1,2,7,8-tetrachlorodibenzo-p-dioxin (1,2,7,8-TCDD) in rats, and the results suggested the non-toxic dioxin congener 1,2,7,8-TCDD interfered with thyroid hormone homeostasis via hydroxylation to the TTR-competitive binding species, 2-OH-1,3,7,8-TCDD. The tissue distribution, bioconcentration, metabolism, and biological effects of the macrolide antibiotic erythromycin (ERY) in fish using crucian carp have been reported; the resulting bioconcentration and metabolism of ERY can elicit negative biological effects [16]. Hakk et al. [17] determined the effect of the dose on 2,3,7,8-TCDD tissue distribution, metabolism, and elimination in CYP1A2 (-/-) knockout and C57BL/6N parental strains of mice, and their results showed that on percent-dose and concentration bases, the TCDD liver levels are significantly higher in C57BL/6N parental strain mice than in CYP1A2 (-/-) knockout mice. The tissue distribution of nuciferine in rats was studied with the liquid chromatography–tandem mass spectrometry method by Gu et al., and their results showed that nuciferine was distributed in brain, liver, and adipose tissues after intravenous administration [18]. However, there have been no studies that focused on the metabolism of a series of PCDPSs in organisms.

In the present study, the 2,2′,4,4′,5-penta-chlorinated diphenyl sulfide (CDPS-99) was selected as the test compound. The primary objectives of this study were to determine the distribution, excretion, and metabolism of CDPS-99 following oral administration to male ICR mice. The mice received 10 mg/kg CDPS-99 via the oral route, and the tissue distribution pattern and excretion path were investigated and the possible metabolites in mice feces and urine were identified. To the best of our knowledge, this is the first report of the tissue distribution, excretion, and metabolic pathway of 2,2′,4,4′,5-penta-chlorinated diphenylsulfide (CDPS-99) in ICR mice. The present study may help gain a better understanding of the mammalian metabolism of CDPS-99.

2. Materials and methods

2.1. Chemicals and materials

The structure of the synthesized compound was characterized by ^{1}H NMR and MS, and its purity (>99%) was determined in a GC–MS. AR grade silica gel (100–200 mesh) was supplied by Aladdin Industrial Corporation (Shanghai, China). N-methyl—N-(tert-butyldimethylsilyl) trifluoro-acetamide (MTBSTFA) was purchased from J&K Company (Shanghai, China). AR grade sulfuric acid, hydrochloric acid, anhydrous sodium sulfate, sodium chloride, sodium hydroxide, and potassium hydroxide were purchased from Nanjing Chemical Ltd. (Nanjing, China). Anhydrous sodium sulfate was baked at 450 °C for 4 h in a muffle furnace (BSX2-5-12TP, Shanghai Yiheng, China) and was stored in a glass desiccator before use. Pesticide analysis grade *n*-hexane and methanol were purchased from Merck (Darmstadt, Germany). Pesticide analysis grade dichloromethane and ethyl acetate were bought from Tedia, Ltd. (Ohio, USA). The materials used in the accelerated solvent extraction were obtained from Dionex Company (Sunnyvale, USA). Solid-phase extraction (SPE) columns packed with C18 sorbent (2 g, 12 mL) were purchased from Anpel Company (Shanghai, China). The water used was produced by a Milli-Q system (Millipore Company, USA). ^{13}C-labeled PCB-31 was purchased from Wellington Laboratories (Ontario, Canada). Laboratory glassware was soaked overnight in K_2CrO_7/H_2SO_4 solution, washed with tap water and redistilled water, baked at 250 °C for 12 h, and pre-rinsed with *n*-hexane before experiments. CDPS-99 was synthesized according to the method that was developed by our group [19].

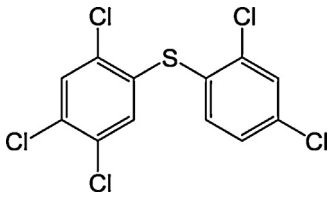

Fig. 1. Structural formula of 2,2′,4,4′,5-penta-chlorinated diphenylsulfide.

2.2. Animal treatments

Six-week old male mice (*Musmusculus*, ICR) were purchased from the Qinglongshan Animal Breeding Center (Nanjing, China). The mice were acclimated in the laboratory for one week prior to the experiments, housed in polypropylene mouse cages at a temperature of 25.0 ± 1.0 °C with a 12 h light/dark cycle and relative humidity of 50 ± 5%. The mice were provided with unrestricted rodent chow and clean water. In this project, all of the animal experiments were carried out according to the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals.

2.2.1. Dose selection

In the present study, 10 mg/kg body weight (b.w) CDPS-99 was selected as the gavage dose. The dose was selected for the following two reasons: because our previous study suggested that the LD_{50} of penta-CDPS in mice is greater than 1000 mg/kg b.w. [11] and 10 mg/kg b.w is thus approximately one hundredth of the LD_{50} value and would not alter the growth of the animals and because of a previous report by Komsta et al. [20] that investigated the distribution and metabolism of 2,2′,4,4′,5-pentachlorodiphenyl ether (CDE-99) in rats after a 10 mg/kg b.w. exposure. No mortality was observed during this exposure period.

2.2.2. Tissue distribution

A total of 24 healthy mice were randomly divided into 6 groups, and each group contained 4 mice. The mice received a single dose of 10 mg/kg CDPS-99 that was dissolved in corn oil via oral gavage (2 mL/100 g). The exposure period ranged from 0.5 day to 21 days. During the exposure period, food and water were provided ad libitum. The mice from the group 1 to 6 were sacrificed by cervical dislocation at 0.5, 1, 3, 7, 14, and 21 days. Serum, livers, kidneys, muscle, fat, and skin were collected and stored at -80 °C for subsequent analytical procedures.

2.2.3. Excretion and metabolism

Following the administration of CDPS-99 (10 mg/kg) by gavage (2 mL/100 kg), the animals were placed in stainless-steel metabolism cages for the collection of feces and urine. Each metabolism cage contained one mouse, and a total of ten mice were used. Both feces and urine were collected at 12, 24, 48, 72, and 96 h. The collected samples were stored at -20 °C for subsequent treatments.

2.3. Analysis methods

2.3.1. Extraction of CDPS-99 in mouse tissues, serum, feces, and urine

The collected tissues were freeze-dried by a freeze drier (FreezeZone, Labconco, USA) and then ground in a glass mortar. An accelerated solvent extractor (ASE-350, Dionex, USA) was used for