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Comparative *in vitro* metabolism of short chain chlorinated paraffins (SCCPs) by human and chicken liver microsomes: First insight into heptachlorodecanes

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ABSTRACT

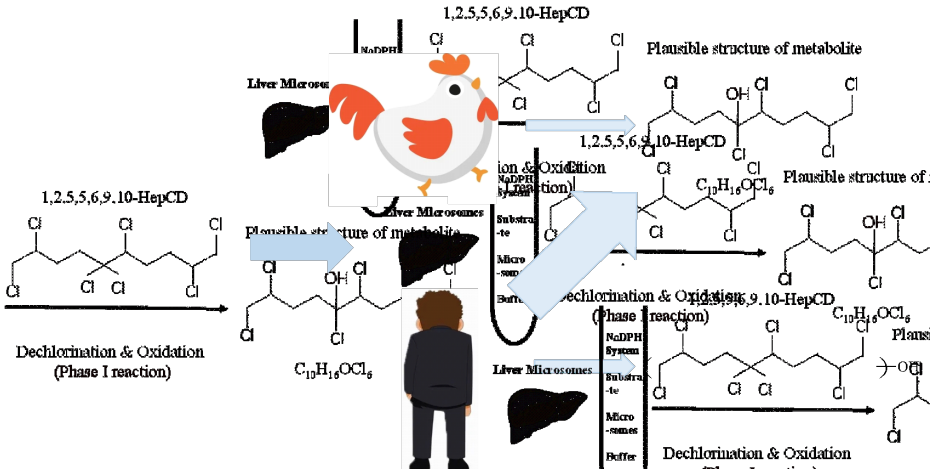
Short chain chlorinated paraffins (SCCPs) are emerging persistent organic pollutants of great concern due to their ubiquitous distribution in the environment. However, little information is available on the biotransformation of SCCPs in organisms. In this study, a chlorinated decane: 1, 2, 5, 5, 6, 9, 10-heptachlorodecanes (HeptaCDs) was subjected to *in vitro* metabolism by human and chicken liver microsomes at environmentally relevant concentration. Using ultra-performance liquid chromatography-Q-Exactive Orbitrap mass spectrometry, two metabolites: monohydroxylated hexachlorodecane (HO-HexCD) and monohydroxy heptachlorodecane (HO-HeptaCD) were detected in human liver microsomal assays, while only one metabolite (HO-HexCD) was identified in chicken liver microsomal assays. The formation of HO-HexCD was fitted to a Michaelis-Menten model for chicken liver microsomes with a V_{max} (maximum metabolic rate) value of 4.52 pmol/mg/min. Metabolic kinetic parameters could not be obtained for human liver microsomes as steady state conditions were not reached under our experimental conditions. Notwithstanding this, the observed average biotransformation rate of HeptaCDs was much faster for human liver microsomes than for chicken liver microsomes. Due to the lack of authentic standards for the identified metabolites, the detailed structure of each metabolite could not be confirmed due to the possibility of conformational isomers. This study provides first insights into the biotransformation of SCCPs, providing potential biomarkers and enhancing understanding of bioaccumulation studies.

Key words: SCCP, in vitro metabolism, liver microsome, Human, Chicken,

Highlights

- Biotransformation of SCCP was investigated by in vitro human and chicken liver microsomes
- Two metabolites: HO-HexCD and HO-HeptaCD were identified in liver microsomal assays.
- Biotransformation rate of HeptaCD was much faster for human than for chicken liver microsomes.
- The kinetics of HeptaCD metabolism by chicken liver microsomes were determined.

Graphical Abstract



1. Introduction

Short-chain chlorinated paraffins (SCCPs) are complex mixtures of chlorinated n-alkanes ranging from C_{10} to C_{13} with degrees of chlorination of 30-70% by mass (Bayen et al., 2006). They are extensively used as flame retardants and plasticizers in rubber compounds and polymers, additives in metal fluids, paints, sealants, and leather treatment agents, as well as in extreme pressure lubricants. SCCPs have raised wide concern due to their persistence in the environment, high potential for long-range transport, toxicity to organisms and bioaccumulation (van Mourik et al., 2016; Zhang et al., 2016; Li et al., 2016). They were listed under Annex A in Stockholm Convention in 2017, and thus face a global ban and elimination (UNEP, 2017).

SCCPs have been widely detected in abiotic media (Mourik et al., 2016), even in the Tibetan plateau (Wu et al., 2020) and polar regions (Tomy et al., 2000). They have also been found in aquatic and terrestrial biota (Reth et al., 2005; Houde et al., 2008; Luo et al., 2015), including humans (Xia et al., 2016; Li et al., 2017). Exposure to SCCPs can alter the intracellular redox status and cause significant metabolic disruption of human HepG2 cells (Geng et al., 2015) and may also disturb thyroid hormone homeostasis in rodents by constitutive androstane receptor (CAR)-implicated enhancement of hepatic thyroid hormone influx and degradation (Gong et al., 2018; Mourik et al., 2018; Wyatt et al., 1993).

Both trophic magnification and trophic dilution have been reported regarding SCCPs transfer in food chains (Luo et al., 2015; Li et al., 2019; Sun et al., 2017; Liu et al., 2020; Zeng et al., 2011). Differences in biotransformation of SCCPs in biota were suggested as a potential cause for variations in trophic magnification. Biotransformation is an important process for detoxification and elimination of xenobiotics and an understanding of it, is crucial for toxicity assessment. However,

93 little information is available on biotransformation of SCCPs in animals and humans.
94 Quantum chemical calculation has indicated that C_{10}-SCCPs with less Cl substitution
95 are susceptible to environmental degradation *via* nucleophilic substitution and
96 hydroxyl radical attack (Sun et al., 2016). Recently, dechlorination with subsequent
97 chlorine rearrangement of a hepta-chlorinated decane congener (1, 2, 5, 5, 6, 9, 10-
98 heptachlorodecanes) mediated by pumpkin seedlings (Li et al., 2017) and a
99 comprehensive metabolic molecular network of SCCP and MCCP in rice cell
100 suspension (Chen et al., 2020) were reported. To our knowledge, Darnerud et al (1982)
101 provided the first report of the degradation of ^{14}C-chlorododecanes to $^{14}CO_2$ in
102 C57BL mice. In this study, only the final product of metabolism, carbon dioxide, was
103 measured, with potential metabolites unidentified. By *in vivo* and *in vitro* exposure of
104 CPs to rat and liver microsomes, Dong et al.(2020) found that SCCPs were extremely
105 resistant to metabolism and mostly eliminated via biliary excretion. On the contrary,
106 He et al.(2021) recently reported that CPs can rapidly transform to OH-CPs, CO-CPs,
107 and COOH-CPs after incubating with human liver microsomes and shorter chain CPs
108 can be formed from longer chain CPs during biotransformation. Thus, greater
109 understanding of the metabolism of SCCPs in animals and humans is needed.

110 Liver is the major organ for metabolism, containing a variety of oxidative
111 enzymes. Cytochrome P450, oxidative enzymes in the liver, are crucial to metabolite
112 formation and metabolic activation (Girvan et al., 2016). *In vitro* metabolism using
113 liver microsomes is a valuable tool to provide information on the fate and
114 biotransformation of environmental pollutants to which humans are exposed (Van den
115 Eede et al., 2013), Oxidation or dechlorination of straight-chain paraffins may have
116 occurred in liver fractions with P450 enzymes (He et al., 2021). However, direct
117 determination of metabolites of SCCPs is challenging due to the complexity of SCCP

mixtures (Tomy et al., 1998), the lack of standards for individual congeners, and reliable quantification techniques.

The present study examines the biotransformation of one chlorinated decane (1, 2, 5, 5, 6, 9, 10-Heptachlorodecanes, HeptaCDs) by *in vitro* chicken and human liver microsomes (CLM and HLM). Non-target metabolite profiling was applied using ultra-performance liquid chromatography-Q-Exactive mass spectrometry and Compound Discoverer software as reported recently (Cuykx et al., 2018; Nguyen et al., 2017). The aim of the current study is to investigate the phase I metabolic rates and products of HeptaCDs in birds and humans. Results will improve understanding of the bioaccumulation, toxicology, and fate of SCCP in organisms.

2. Materials and methods

2.1 Chemicals and reagents

Human liver microsome pools, a 200-donor pool (mixed gender), were purchased from Xenotech LLC (USA), while chicken liver microsomes were purchased from PrimeTox Bio-pharma Technology Co. LTD (Wuhan, China). William's E. Medium (PH=7.4) was obtained from Gibco (United Kingdom). HepCD was purchased from Dr. Ehrenstorfer GmbH (Germany). $^{13}C_{12}$-α-hexabromocyclododecane (HBCDD) and d_{18}-α-HBCDD were obtained from Cambridge Isotope Laboratories (USA). Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (France). Acetonitrile (LC/MS grade), HPLC grade dichloromethane and methanol were purchased from Fisher Chemical (United Kingdom). Ultrapure water (18.2 MΩ was obtained from an Elga LabWater water purification instrument (France). Rapid NADPH system K5000 was purchased from Sekisui XenoTech (Kansas, KS, United States). Dosing solutions of HepCD were

prepared by solvent exchange to DMSO.

2.2 In vitro biotransformation assay

The incubation method was adapted, with slight modification, from previous studies.(Nguyen et al., 2017; Eede et al., 2015; Luo et al., 2017). The reaction mixture contained 100 μL William's E. Medium, 0.5 mg protein (human or chicken liver microsomes), and HeptaCDs at different concentrations (from 0.26-1.3 μM) in a total volume of 0.98 mL.The activities of cytochromes P450 of human and liver microsomes were quantitatively determined using 7-ethoxycoumarin O-deethylase (ECOD) kit (Jiang et al., 2017). They were 188±8 $pmol^{-1}$ mg $protein^{-1}$ and 11±2.9 $pmol^{-1}$ mg $protein^{-1}$, respectively, and fell within the acceptable range of the assay. The assay was pre-incubated in a shaking water bath at 37℃. The reaction was initiated by addition of 20 μL NADPH regenerating system (final concentration: 2.0 mM nicotinamide adenine dinucleotide phosphate, 10.0 mM glucose-6-phosphate and 2 units/mL glucose-6-phosphate dehydrogenase) to a total volume of 1 mL. The samples were then incubated at 37℃, 5% CO_2 and 98% relative humidity. The reaction was quenched using 1.0 mL ice-cold methanol after 60 min. Experiments were performed in triplicate. Blank and negative control experiments were also conducted. Three experimental blanks (solvent blank, heated-enzymatic blank, and NADPH-negative blank) were performed and analyzed alongside the sample batch for quality assurance (QA) and quality control (QC) purposes. The solvent blank contained only William's E Medium. The heated-enzymatic blank was same as those in the treatment with liver microsomes inactivated by heating above 80℃ for 10 min. The NADPH-negative blank did not include NADPH.

2.3 Extraction procedure

Due to the lack of isotopically-labelled standards for the studied HeptaCDs,

incubated samples were spiked with 20 ng of ^{13}C-α-HBCDD as an internal (surrogate) standard and extracted according to a previously reported method (Nguyen et al., 2017; Eede et al., 2015). Briefly, 3 mL dichloromethane and 100 μL acetonitrile (for protein precipitation) were added to each tube. Tubes were vortexed for 30 s and subsequently centrifuged for 5 min at 3500 rpm. The organic layer was transferred into a new tube. The extraction procedure was repeated twice. Combined extracts were evaporated to dryness under gentle nitrogen gas at room temperature, and then reconstituted in 200 μL acetonitrile containing 20 ng d_{18}-α-HBCDD, used as a recovery determination (syringe) standard, before instrumental analysis.

2.4 Instrument method

Instrumental analysis was performed using an ultra-performance liquid chromatography (UPLC)-Orbitrap-high-resolution mass spectrometry (HRMS) system (Thermo Fisher Scientific, Bremen, Germany). The system was composed of an UltiMate 3000 liquid chromatograph equipped with a HPG-3400RS dual pump, a WPS-3000 auto sampler, a TCC-3000 column oven and Q-Exactive Plus Orbitrap mass spectrometer with an electro-spray ionization source. Chromatographic separation was achieved using reversed phase chromatography on a Hypersil Gold analytical column (100 mm×2.1 mm, 1.9 μm, Thermo Fisher Scientific) kept at 45℃. The mobile phase consisted of 1 mM ammonium formate in water (A) and 0.5% formic acid in acetonitrile (B). The gradient began with (A/B) 95:5 (v/v), then ramped linearly to 0:100 over 5 min and returned to 95:5 over 2.3 min, followed by equilibration for 3min. The flow rate of the mobile phase was 0.4 mL/min and the sample injection volume was 5 μL. Samples were ionized in the negative mode as follows: sheath gas flow, 50 arbitrary units (AU); auxiliary gas flow, 5 AU; capillary temperature, 300℃; source heater temperature, 150℃; spray voltage, 2.5 kV; S-lens

radio frequency, 50 AU. HRMS data were acquired in full scan mode over the *m/z* range from 77 to 650 at a resolving power of 35,000 full width half maximum (FWHM) at *m/z* 200. The automatic gain control (AGC Target) was set at high dynamic range (5×10^5) and the maximum injection time was set to 100 ms.

2.5 Data analysis

The substrate and potential metabolites were identified based on retention time and expected *m/z* value of chromatographic peaks. Quantification of substrate was conducted based on a five-point standard calibration of HeptaCD with internal standard using Quan Browser 3.0 (Thermo Fisher Scientific, Bremen, Germany). The calibration curve was linear with R^2 = 0.996. The non-target metabolites were identified and semi-quantified with Compound Discoverer 3.0 software using predefined workflow inclusive of prerequisite parameters for quality control (Table SI-1 and Figure SI-1).

Non-linear regressions from the biotransformation of HeptaCD mediated by liver microsomes were plotted by Origin version 8.5 (OriginLab Corporation, MA, USA) and Enzyme Kinetics Model of SigmaPlot 13.0 (Systat Software Inc, USA). The details can be seen in the Support Information.

3. Results and discussion

3.1 Metabolites of HepCD in chicken and human liver microsomal assays.

The metabolism of HeptaCDs can be evidenced by the decreasing amount of HeptaCDs and the formation of new chemicals in the assays. No depletion of the substrates was observed in the negative control groups including heat inactivated blanks and NADPH-negative blanks. The recoveries of HeptaCDs in the control groups ranged from 101% to 110% corresponding to the nominal concentrations.

However, rapid depletion of HeptaCDs was observed in human liver microsomal incubation assays with the recoveries of HeptaCDs ranging between 48% and 55% of the respective exposure dose. By comparison, recoveries of HeptaCDs in the chicken liver microsomal assays ranged from 85% to 93% of exposure doses (Table 1). Evidently, human liver microsomes metabolize HeptaCDs faster than chicken liver microsomes. The species-specific enzymatic binding to the substrates, and other factors such as the different enzyme activities and metabolic capacities among different species under the specified conditions can explain this observed difference (Smith et al., 2007) .

At least two metabolites in the human liver microsomal assays and one metabolite in the chicken liver microsomal assays were positively identified by the Compound Discoverer 3.0 software via analyzing the obtained UPLC-Orbitrap MS chromatograms (Figures 1 and 2). These metabolic products were not detected in any of the blanks or the negative control groups, indicating the metabolite was NADPH-dependent and mediated by CPY 450 enzymes. As intended, the use of formic acid in the mobile phase has led to the formation of anionized formate adducts ($[M+HCOO]^-$ equivalent to M+45 mass units) of high intensity in the HESI ion source (Kruve et al., 2017). This formate ion adduct of the parent HeptaCDs was identified and confirmed in chemical standard injections at m/z = 426.88031 (Figure SI-2). The original HeptaCDs molecular ion peak at m/z = 381.89637 had much lower intensity than the formate adduct ion and could only be detected at higher concentrations. Therefore, formate adduct ions were monitored for both the parent HeptaCDs and its metabolites throughout this study.

The first metabolite was identified in both human liver microsomes and chicken liver

242 microsomes with an accurate mass of 408.91490. This compound was assigned by the
243 Compound Discoverer 3.0 software to $C_{11}H_{17}Cl_6O_3$ through both accurate mass and
244 isotope cluster distribution (Figure 1). After declustering the formate adduct $[HCOO]^-$
245 by the software, this metabolite was identified as monohydroxy-hexachlorodecane
246 (HO-HexCD，$C_{10}H_{16}Cl_6O$).

247 The second metabolite was only identified in human liver microsomes experiments
248 with an accurate mass of 442.87482. This chemical was assigned by the Compound
249 Discoverer 3.0 software to $C_{11}H_{16}Cl_7O_3$ through both accurate mass and isotope
250 cluster distribution (Figure 2). After the formate adduct was declustered, this
251 metabolite can be identified as monohydroxy-heptachlorodecanes (HO-HeptaCD，
252 $C_{10}H_{15}Cl_7O$). As can be seen in Figures 1 and 2, the match on both accurate masses
253 and isotope distribution for the two metabolites exceeded 90%, which confirms the
254 identity of the detected metabolites to a high confidence level.

255 The peak area of the second metabolite was about one tenth of that of the first
256 metabolite, which indicated that oxidative dechlorination rather than direct
257 hydroxylation is the major pathway of biotransformation of HeptaCDs in humans.
258 This is in accordance with previous studies which concluded that oxidative
259 dehalogenation can be catalyzed by CYP enzymes for substituted substrate (Kumar et
260 al., 2007).The second metabolite was not found in the chicken liver microsomal
261 assays, which may be related to the lower biotransformation efficiency.

262 The specific position of the hydroxyl group in the two metabolites cannot be
263 confirmed, due to the lack of reference standards. Regarding metabolite 1, it is highly
264 plausible that nucleophilic substitution reaction occurred on the carbon with double
265 chlorine substitution (5) since only one HO-HexCD peak was found in the

chromatograms. If the nucleophilic substitution reaction occurred in a carbon with single chlorine substitution (1, 2, 6, 9, 10), there would be at least three potential isomers. The direct hydroxylation may occur at carbon 6, which has higher reactivity than carbon 1, 2, 9, and 10 since it was in close proximity to more electrophilic carbon 5 with two chlorine atoms. These hypotheses need to be verified using pure chemical standards or other analytical methods (e.g. NMR analysis).

Knobloch et al (2021, 2022) reported mono- and di-hydroxylated metabolites of single-chain CP-mixtures by dehalogenase LinB from Sphingobium indicum. OH-CPs as well as CO-CPs (ketones), and COOH-CP were found in human liver microsomes incubation experiments conducted by He et al. (2021) In the present study, CO-CPs and COOH-CP were not found. This is likely because a single chemical rather than a mixture of CPs was used in the present study. Different levels and positioning of chlorination may lead to different metabolic pathways and products. No extracting was conducted in the study of He et al (2021), the supernatant of the complex after centrifugation was directly injected into the instrument. In the present study, only chemicals which can be extracted by DCM were recovered from the complex, some transformation products might still be in the complex. The assays were not conducted under the optimal conditions could also be a reason. The experiment conditions in the present study were adopted from previous studies (Nguyen et al., 2017; Eede et al., 2015; Luo et al., 2017) which deal with other chemicals not SCCPs. The optimal conditions were not obtained by conducting experiment with the heptachlorodecane substrate in the present study. This is a limitation of the present study. Li et al.(2017) observed dechlorination and chlorine rearrangement of HeptaCDs mediated by the whole pumpkin seedlings but did not isolate the oxidative dechlorination intermediate. In the present study, the chlorine

rearrangement of HeptaCDs was not recorded. This may reflect the difference in biotransformation of SCCP between plants and animals.

3.2 Kinetics of HepCD metabolism by human and chicken liver microsomes

In the present study, a series of incubations with different substrate concentrations (0.26, 0.39, 0.52, 0.65, and 1.3 μM for chicken liver microsomes and 0.26, 0.325, 0.39, 0.52, and 0.65 μM for human liver microsomes) were conducted (Liu et al., 2017). Accurate quantification of the HO-HexCD and the HO-HepCD is impossible in the absence of authentic standards. Therefore, the depletion rate of the substrates was considered as the production rate of the HO-HexCD. Nonlinear fitting regression analysis between the production rate of OH-HexCD and the concentration of HeptaCDs was then conducted to simulate the metabolic rate modeling (Michaelis-Menten, substrate-inhibition, and Hill) by SigmaPlot Enzyme Kinetics Module (Systat Software Inc, Richmond, CA). The best model was the one with the lowest standard deviation of residuals and Akaike Information Criterion corrected for small sample size (AICc).

The formation of HO-HexCD for chicken liver microsomal assays best fit a Michaelis-Menten model. The K_m was 0.57±0.15 μM and the V_{max} was 4.52±0.57 pM/ (mg protein/min). However, the formation of HO-HexCD for human liver microsomal assays did not show any signs of reaching a plateau that would indicate attainment of steady state. This was caused by the low substrate concentration used in the present study. Therefore, it did not fit any of the assessed enzyme kinetic models (Michaelis-Menten or Hill). On the other hand, the method used to calculate formation rate of OH-HexCD in the present study would also be a factor for the lack of fit. The depletion rate of HeptaCDs actually reflect the formation rate of all transformation products. Moreover, when we use the MS-signal intensity to reflect the

316 formation rate of transformation products. Same results were obtained (Figure SI-3).
317 This confirmed that the substrate concentration is too small to obtain the maxium
318 formation rate. The lack of fitting to the investigated kinetic models precluded the
319 estimation of metabolic kinetic parameters for HeptaCDs under the applied
320 experimental conditions. The formation rate of HO-HexCD was much faster in human
321 liver microsomal assays than in assays conducted using chicken liver microsomes,
322 although the metabolic kinetic parameters were not obtained (Figure 3).

323 Dehalogenation and hydroxylation are important biotransformation pathways for
324 halogenated organics such as polychlorinated biphenyl (PCBs) and polybrominated
325 diphenyl ethers (PBDEs) (Park et al., 2009; Wan et al., 2009). The hydroxyl
326 metabolites of PCBs and PBDEs are common more toxic than their parent compounds
327 (Ruel et al., 2019; Su et al., 2014). This could be also the case for SCCP. Therefore,
328 more studies on the toxicity of metabolites of SCCP are need.

329 4. Conclusions

330 This study firstly identified biotransformation products of CPs in birds and
331 humans. Our results indicated that biotransformation of a heptachlorinated decane
332 isomer by human liver microsomes was faster and produced more metabolites than
333 when chicken liver microsomes were exposed. This implies species-specific
334 metabolism of this and potentially other CPs. Notwithstanding this finding, there
335 remains a dearth of knowledge related to biotransformation of SCCPs. Since
336 hydroxylated and dechlorinated metabolites of CP congeners were identified in this
337 study, further studies should investigate the toxicity and phase II metabolic processes
338 of the identified SCCP metabolites. Also, biotransformation and biodegradation of
339 different SCCP congeners and mixtures in more species merit investigation in future
340 studies.

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The authors declare no competing financial interest.

References

Bayen, S., Obbard, J. P., Thomas, G. O., 2006. Chlorinated paraffins: A review of analysis and environmental occurrence. *Environ. Int. 32*, (7), 915-929.

Chen, W., Yu, M., Zhang, Q., Hou, X., Kong, W., Wei, L., Mao, X., Liu, J., Schnoor, J. L., Jiang, G.B., 2020. Metabolism of SCCPs and MCCPs in suspension rice cell based on paired mass distance (PMD) analysis. *Environ. Sci. Technol*, 54, 9990-9999.

Cuykx, M., Rodrigues, R. M., Laukens, K., Vanhaecke, T., Covaci, A., 2018. In vitro assessment of hepatotoxicity by metabolomics: a review. *Arch. Toxicol.* 92, 3007-3029.

Darnerud, P. O., Biessmann, A., Brandt, I., 1982. Metabolic fate of chlorinated

paraffins: Degree of chlorination of [1− 14 C]-chlorododecanes in relation to degradation and excretion in mice. *Arch. Toxicol. 50*, (3-4), 217-226.

Dong, Z. M., Li, D., Wan, Y., Sun, Y. B., Hu, J.Y., 2020. Physiologically based pharmacokinetic modeling for chlorinated paraffins in rats and humans: Importance of biliary excretion. *Environ. Sci. Technol.*54, 938-946.

Eede, N. V. D., Erratico, C., Exarchou, V., Maho, W., Neels, H., Covaci, A., 2015. In vitro biotransformation of tris(2-butoxyethyl) phosphate (TBOEP) in human liver and serum. *Toxicol. Appl. Pharmacol. 284*, 246-253.

Geng, N. B., Zhang, H. J., Zhang, B. Q., Wu, P., Wang, F. D., Yu, Z. K., Chen, J. P., 2015. Effects of short-chain chlorinated paraffins exposure on the viability and metabolism of human hepatoma HepG2 cells. *Environ. Sci. Technol. 49*, 3076-3083.

Girvan, H. M., Munro, A. W., 2016. Applications of microbial cytochrome P450 enzymes in biotechnology and synthetic biology. *Curr. Opin. Chem. Biol.* 31, 136-145.

Gong, Y., Zhang, H., Geng, N., Xing, L., Fan, J., Luo, Y., Song, X., Ren, X., Wang, F., Chen, J., 2018. Short-chain chlorinated paraffins (SCCPs) induced thyroid disruption by enhancement of hepatic thyroid hormone influx and degradation in male Sprague Dawley rats. *Sci. Total Environ.625*, 657-666.

He, C., Mourik, L., Tang, S.Y., Thai P., Wang X. Y., Brabdsna S.H., Leonards P. E.G., Thomas K.V., Mueller J F., 2021. In vitro biotransformation and evaluation of potential transformation products of chlorinated paraffins by high resolution accurate mass spectrometry. *J. Hazard. Mater.* 405, 124245

Houde, M., Muir, D. C., Tomy, G. T., Whittle, D. M., Teixeira, C., Moore, S., 2008. Bioaccumulation and trophic magnification of short-and medium-chain

chlorinated paraffins in food webs from Lake Ontario and Lake Michigan. *Environ. Sci. Technol. 42*, 3893-3899.

Jiang, P., Wang, J., Sheng, N., Wei, D., Dai, J., 2017. Effects of pentachlorophenol on the quail (Coturnix japonica) liver detoxification pathway. CHEMOSPHERE 177: 44–50.

Knobloch, M. C. ,Schinkel, L., Schilling, I., Kohler, H-P E., Lienemann, P., Bleiner, D., Heeb, N. V., 2021. Transformation of short-chain chlorinated paraffins by the bacterial haloalkane dehalogenase LinB – Formation of mono- and di-hydroxylated metabolites. Chemosphere, 262, 128288.

Knobloch, M.C., Mathis, F., Fleischmann, T., Kohler, H-P. E., Kern, S., Bleiner, D., Heeb, N.V., 2022. Enzymatic synthesis and formation kinetics of mono- and di-hydroxylated chlorinated paraffins with the bacterial dehalogenase LinB from Sphingobium indicum. Chemosphere, 291, 132939.

Kruve, A., Kaupmees, K., 2017. Adduct Formation in ESI/MS by Mobile Phase Additives. *J. Am. Soc. Mass Spectrom*. 28, 887–894.

Kumar, M. S., 2007. Biodegradation of chlorinated compounds—A review. *Crit. Rev. Environ. Sci. Technol.*37, 165-198.

Li, H. J., Bu, D., Fu, J. J., Gao, Y., Cong, Z. Y., Zhang, G. S., Wang, Y. W., Chen, X. F., Zhang, A. Q., Jiang, G. B., 2019. Trophic dilution of short-chain chlorinated paraffins in a plant-plateau pika-eagle food chain from the Tibetan Plateau. *Environ. Sci. Technol.* 53, 9472-9480.

Li, H. J., Fu, J. J., Zhang, A. Q., Zhang, Q. H., Wang, Y. W., 2016. Occurrence, bioaccumulation and long-range transport of short-chain chlorinated paraffins on the Fildes Peninsula at King George Island, Antarctica. *Environ. Int.* 94, 408-414.

Li, T., Wan, Y., Gao, S., Wang, B., Hu, J., 2017. High-throughput determination and

418 characterization of short-, medium-, and long-chain chlorinated paraffins in
419 human blood. *Environ. Sci. Technol. 51*, 3346-3354.
420 Li, Y., Hou, X., Yu, M., Zhou, Q., Liu, J., Schnoor, J. L., Jiang, G. B., 2017.
421 Dechlorination and chlorine rearrangement of 1,2,5,5,6,9,10-heptachlorodecane
422 mediated by the whole pumpkin seedlings. *Environ. Pollut.224*, 524-531.
423 Liu, Y., Luo, X. J., Zeng, Y. H., Wang, Q. Y., Tu, W.Q., Yang, C.Y., Mai B. X.,
424 2020.Trophic magnification of short- and medium-chain chlorinated paraffins in
425 terrestrial food webs and their bioamplification in insects and amphibians during
426 metamorphosis. *Environ. Sci. Technol.* 54, 9472-9480.
427 Luo, X.-J., Sun, Y.-X., Wu, J.-P., Chen, S.-J., Mai, B.-X., 2015. Short-chain
428 chlorinated paraffins in terrestrial bird species inhabiting an e-waste recycling
429 site in South China. *Environ. Pollut.198*, 41-46.

430 Luo, Y.-L., Luo, X.-J., Ye, M.X., Zeng, Y.H., Chen, S.J., Mai, B.X., 2017, Species-
431 specific and structure-dependent debromination of debromination of
432 polybrominated diphenyl ether in fish by in vitro gepatic metabolism. *Environ*
433 *Toxicol Chem.* 36, 2005-2011.

434 Nguyen, K. H., Abdallah, A. E., Moehring, T., Harrad, S., 2017. Biotransformation of
435 the flame retardant 1,2-dibromo-4-(1,2-dibromoethyl) cyclohexane (TBECH) in
436 vitro by human liver microsomes. *Environ. Sci. Technol. 51*, 10511-10518.
437 Park, J. S., Petreas,M., Cohn,B.A., Cirillo,P.M., Litvak, P. F., 2009. Hydroxylated
438 PCB metabolites (OH-PCBs) in archived serum from 1950-60s California
439 mothers: a pilot study.Environ Int. 35(6):937-42.
440 Reth, M., Zencak, Z., Oehme, M., 2005. First study of congener group patterns and
441 concentrations of short-and medium-chain chlorinated paraffins in fish from the
442 North and Baltic Sea. *Chemosphere*, *58*, 847-854

Ruel, M.V.M., Bos, A.F., Soechitram, S.D., Meijer, L, Sauer, P.J.J., Berghuis, S.A., 2019. Prenatal exposure to organohalogen compounds and children's mental and motor development at 18 and 30 months of age.Neuro Toxicol,72, 6-14.

Smith，T. L., Merry，S. T., Harris，D. L., Ford，J. J., Ike，J., Archibong，A. E., Ramesh A., 2007. Species-specific testicular and hepatic microsomal metabolism of banzo(a)pyrene, an ubiquitous toxicant and endocrine disruptor. *Toxicol. In Vitro.*21, 753-758.

Su,G.，Yu,H.，Lam, M.H.W.，Giesy,J.P.，Zhang, X.,2014. Mechanisms of toxicity of hydroxylated polybrominated diphenyl ethers (HO-PBDEs) determined by toxicogenomic analysis with a live cell array coupled with mutagenesis in escherichia coli. *Environ. Sci. Technol.* 2014, 48, 10, 5929–5937.

Sun, R. X., Luo, X. J., Tang, B., Chen, L. G., Liu, Y., Mai, B. X.,2017. Bioaccumulation of short chain chlorinated paraffins in a typical freshwater food web contaminated by e-waste in south china: Bioaccumulation factors, tissue distribution, and trophic transfer. *Environ. Pollut*. 222, 165-174.

Sun, Y., Pan, W., Lin, Y., Fu, J., Zhang, A., 2016. Chlorination pattern effect on thermodynamic parameters and environmental degradability for C10-SCCPs: Quantum chemical calculation based on virtual combinational library. *J. Environ. Sci. 39*, 184-197.

Tomy, G. T., Muir, D. C., Stern, G. A., Westmore, J. B., 2000. Levels of C10− C13 polychloro-n-alkanes in marine mammals from the Arctic and the St. Lawrence River estuary. *Environ. Sci. Technol. 34*, 1615-1619.

Tomy, G., Fisk, A., Westmore, J., Muir, D., 1998. Environmental chemistry and toxicology of polychlorinated n-alkanes. In *Reviews of environmental contamination and toxicology*, Springer, 53-128.

UNEP, 2017. Recommendation by the Persistent Organic Pollutants Review Committee to List Short-chain Chlorinated Paraffins in Annex A to the Convention and Draft Text of the Proposed Amendment. In UNEP, Ed. Geneva, Vol. UNEP/POPS/COP.8/14.

Van den Eede, N., Maho, W., Erratico, C., Neels, H., Covaci, A., 2013. First insights in the metabolism of phosphate flame retardants and plasticizers using human liver fractions. *Toxicol. Lett. 223*, 9-15.

van Mourik, L. M., Gaus, C., Leonards, P. E. G., de Boer, J., 2016. Chlorinated paraffins in the environment: A review on their production, fate, levels and trends between 2010 and 2015. *Chemosphere, 155*, 415-428.

van Mourik, L., van der Veen, I., Crum, S., de Boer, J., 2018. Developments and interlaboratory study of the analysis of short-chain chlorinated paraffins. *TrAC Trend Anal. Chem.* 102, 32-40.

Wan, Y., Wiseman, S., Chang, H., Zhang, X., Jones, P. D., Hecker, M., Kannan, K., Tanabe, S., Hu, J., Lam, Michael H. W., Giesy, J. P., 2009. Origin of Hydroxylated Brominated Diphenyl Ethers: Natural Compounds or Man-Made Flame Retardants? *Environ. Sci. Technol.* 43, 7536-7542.

Wu, J., Gao, W., Liang, Y., Fu, J.J., Shi, J.B., Lu, Y., Wang, Y.W., Jiang, G.B. ,2020. Short- and medium-chain chlorinated paraffins in multi-environmental matrices in the Tibetan Plateau environment of China: A regional scale study. *Environ. Int.* 140,105767.

Wyatt, I., Coutss, C., Elcombe, C., 1993. The effect of chlorinated paraffins on hepatic enzymes and thyroid hormones. *Toxicology 77*, (1-2), 81-90.

Xia, D., Gao, L., Zheng, M., Li, J., Zhang, L., Wu, Y., Tian, Q., Huang, H., Qiao, L., 2016. Human exposure to short-and medium-chain chlorinated paraffins via

493 mothers' milk in Chinese urban population. *Environ. Sci. Technol. 51*, 608-615.
494 Zeng, L. X., Wang, T., Wang, P., Liu, Q., Han, S. L., Yuan, B., Zhu, N. L., Wang, Y.
495 W., Jiang, G. B., 2011. Distribution and trophic transfer of short-chain
496 chlorinated paraffins in an aquatic ecosystem receiving effluents from a sewage
497 treatment plant. *Environ. Sci. Technol.* 45, 5529-5535.
498 Zhang, Q., Wang, J., Zhu, J., Liu, J., Zhang, J., Zhao, M.,2016. Assessment of the
499 endocrine-disrupting effects of short-chain chlorinated paraffins in in vitro
500 models. *Environ. Int. 94*, 43-50.