Environmental Pollution 238 (2018) 421–430

Contents lists available at ScienceDirect

Environmental Pollution

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

Stereoselective bioaccumulation of chiral PCB 91 in earthworm and its metabolomic and lipidomic responses☆

Zeying He [a], Yuehua Wang [a], Yanwei Zhang [a], Haiyan Cheng [b], Xiaowei Liu [a, *]

[a] Key Laboratory for Environmental Factors Control of Agro-product Quality Safety, Ministry of Agriculture, Agro-Environmental Protection Institute, Ministry of Agriculture, Tianjin, 300191, PR China
[b] SCIEX, Analytical Instrument Trading Co., Ltd, Beijing, 100015, PR China

ARTICLE INFO

Article history:
Received 28 November 2017
Received in revised form
3 February 2018
Accepted 16 March 2018

Keywords:
PCB 91
Stereoselectivity
Metabolomics
Lipidomics
Bioaccumulation

ABSTRACT

Stereoselective bioaccumulation, elimination, metabolomic and lipidomic responses of earthworm *Eisenia fetida* exposed to chiral polychlorinated biphenyl (PCB) 91 in an earthworm-soil system were investigated. Preferential bioaccumulation of (−)-PCB 91 and elimination of (+)-PCB 91 were observed following 50 and 500 $\mu g/kg_{dwt}$ exposures. Enantiomer fraction (EF) values decreased over time during the uptake and elimination periods. Metabolomics and lipidomics techniques based on ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) revealed significant changes in 108 metabolites after earthworms exposure to (+)-, (−)-, and (±)-PCB 91, compared to control groups. Forty two of these metabolites were identified as amino acids, nucleosides, fatty acids, dicarboxylic acids, vitamins or others. Lysophospholipids including six lysophosphatidylcholines (LPC), six lysophosphatidylethanolamine (LPE), eight lysophosphatidylinositol (LPI) and five lysophosphatidylserine (LPS) were also differentially expressed between exposure and control groups. Alterations in the levels of metabolites and lipids indicated stereoselective effects of chiral PCB 91 on earthworm amino acid, energy, and nucleotide metabolism, neurodevelopment and gene expression. Overall, the effects of (+)-PCB 91 were more pronounced than that of (−)- and (±)-PCB 91.

1. Introduction

Polychlorinated biphenyls (PCBs) are an important class of ubiquitous and persistent organic pollutants, which have been widely used in various industrial and commercial applications. It was estimated that 500,000 tons of PCBs were manufactured worldwide (Kania-Korwel and Lehmler, 2016a). Although their production has been banned in many countries since the 1970s, these compounds remain important pollutants due to their persistence in the environment (Wu et al., 2014). Additionally, PCBs are inadvertently generated by certain industrial processes including electronic waste recycling (Zheng et al., 2016), and production of paint (Anezaki and Nakano, 2014) and adhesives (Anezaki and Nakano, 2015). Thus, PCBs are still persistent worldwide in air, soil, sediment, and biota samples, resulting in ongoing human exposure to these compounds (Kania-Korwel and Lehmler, 2016b; Zheng et al., 2015). Laboratory and epidemiological studies have demonstrated that PCB exposure is correlated with adverse health effects in humans, including cancer, neurological, and other non-cancer effects (Agency for Toxic Substances and Disease Registry, 2000; Robertson and Hansen, 2015).

Nineteen PCB congeners containing a chiral axis exist at ambient temperature as two stable rotational isomers due to the presence of three or four bulky ortho chlorine substituents (Kaiser, 1974). It was reported that chiral PCBs constitute 6% or 29,500 tons, of the total amount of PCB manufactured worldwide (Kania-Korwel and Lehmler, 2016a). Chiral PCBs were produced and released into the environment as racemates. However, they can display considerable atropisomeric enrichment in the environment (Lehmler et al., 2009). Stereoselective enrichment of chiral PCBs were observed in plants (Chen et al., 2014; Zhai et al., 2014), soils (Jamshidi et al., 2007), sediment (Ross et al., 2011), and animals (Kania-Korwel et al., 2012; Zheng et al., 2015). In addition, different PCB atropisomers display different toxicity (Kania-Korwel and Lehmler, 2016b). Available evidence indicates that exposure to

☆ This paper has been recommended for acceptance by Charles Wong.
* Corresponding author. Key Laboratory for Environmental Factors Control of Agro-product Quality Safety, Ministry of Agriculture, Agro-Environmental Protection Institute, Ministry of Agriculture, Tianjin, 300191, PR China.
E-mail address: hezeying222308@163.com (X. Liu).

https://doi.org/10.1016/j.envpol.2018.03.060
0269-7491/

stereoselective enriched chiral PCBs plays a poorly understood adverse role on wildlife, animals and, ultimately, on human health. Thus, chirality study of chiral PCBs in environment and biota is critical for better understanding the fundamental questions about stereoselective process involved in biological process.

Earthworms, a major part of soil fauna biomass, play a crucial role in transporting contaminants including PCBs from soil to organisms high up in the terrestrial food chain (Paul and Ghosh, 2011). Therefore, earthworms such as *E. fetida* have been widely used in bioavailability and toxicity tests as indicators of terrestrial contaminants. To demonstrate the behavior of PCBs in earthworms, the bioaccumulation, elimination, exposure route, and feeding activity of earthworms exposed to some PCB congeners have been investigated (Jager et al., 2003, 2005; Krauss et al., 2000; Wågman et al., 2001). The bioaccumulation patterns, uptake rate constants, elimination rate constants were reported in these studies. However, to the best of our knowledge, the chiral signatures and toxicokinetics of chiral PCBs, including PCB 91 atropisomers, in earthworms have not been investigated to date.

Environmental metabolomics have been widely used to better understand the response of earthworm to PCBs exposure. Metabolomics enable rapid screening of large quantities of metabolites under certain conditions to find interesting metabolic trends. And the metabolic changes can give insight into environmental toxicology and molecular mechanisms that underpin the interrelationships between an organism's normal function and its environment (Viant and Sommer, 2013). There is a rapid growth in utilization of metabolomics for pollutant toxicity study including earthworm exposure to PCBs. In recent years, this method has also benefited from the technical advances in both instrumentation including nuclear magnetic response (NMR), mass spectrometry (MS), and chemometrics based software. Simpson et al. investigated earthworm responses to PCBs exposure using NMR-based metabolomics (Åslund et al., 2011, 2012; McKelvie et al., 2011). However, no significant changes were observed except for adenosine triphosphate (ATP), although this might have been caused by the low sensitivity and limited applicability of NMR-based metabolomics, which are its major limitations (Ortmayr et al., 2016). Mass spectrometry, especially liquid chromatography tandem high resolution mass spectrometry like UPLC-QTOF, is a promising technique for metabolomics due to its high sensitivity, fast acquisition speed and accurate mass measurement. In recent years, lipidomics, a sub-field of metabolomics that aims to detect and quantify the presence of lipids in biological samples (Namasivayam et al., 2015), has thrived in omics study. Lipids play critical roles in biological processes and have been investigated in human disease and plant science (Afzal et al., 2016; Sokolowska et al.).

Atropisomeric behavior of PCB 91 (2,2′,3,4′,6-pentachlorobiphenyl) in some invertebrate and mammalian species has been investigated in previous studies. Stereoselective accumulation of PCB 91 was observed in adult zebrafish after racemic exposure and stereoselective oxidative stress was induced after exposure to (+)-, (−)- and racemic PCB 91 (Chai et al., 2016b). In addition, amino acids and other metabolites were stereoselectively regulated by PCB 91 atropisomers in zebrafish embryos and larva (Chai et al., 2016c). Atropisomeric enrichment of PCB 91 was observed in *Mysis relicta* (Warner and Wong, 2006) and rainbow trout (Buckman et al., 2006). Stereoselective enrichment or transformation of PCB 91 atropisomers were observed in rat CYP2B1 and rat liver microsomal incubations (Kania-Korwel et al., 2011; Lu et al., 2013). To further understand effects of chiral PCB 91 in earthworm, the present study investigated stereoselective bioaccumulation and elimination of these compounds in *E. fetida* in natural soil under the controlled condition. This study also evaluated the metabolic response of earthworm to sub-lethal (±)-PCB 91 and its two atropisomers, by applying UPLC-QTOF-based metabolomics and lipidomics to understand the mechanism of PCB-earthworm interaction. Results obtained here provide new insights into the comprehensive evaluation of the environmental risk of chiral PCBs to soil animals.

2. Materials and methods

2.1. Reagents

(±)-PCB 91 (99.9%) was purchased from Accustandard, Inc. (New Haven, CT, USA). The atropisomers of PCB 91 were separated and prepared on a Lux Cellulose-1 chiral column (250 × 4.6 mm, 5 μm, Phenomenex, Torrance, CA, USA) using a Shimadzu 20A series Ultra Fast Liquid Chromatography (UFLC, Shimadzu Corporation, Kyoto, Japan) with 100% n-hexane as the mobile phase at 1 mL/min. The prepared atropisomers were analyzed as described under "instrument" (see below), and atropisomeric purity was >99.5% for both (+)- and (−)-PCB 91. The elution orders of (+)- and (−)-PCB 91 were confirmed as (+)/(−) on both Lux Cellulose-1 and cyclosil-B chiral columns based on a previous study (Xu et al., 2015) and on our determination. Non-endogenous metabolites including L-tryptophan-d_5, d_3-malic acid, d_3-hexanoyl-carnitine and d_3-decanoyl-carnitine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Non-endogenous lipids including dLPC (16:0), dLPE (18:0), dPC (16:0/16:1) and dDAG (16:0/16:0) were provided by SCIEX (Foster City, CA, USA). Acetonitrile, n-hexane and cyclohexane were HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ). Water (electrical resistance of 18.2 MΩ cm) was purified in a Milli-Q (Millipore, Billerica, MA, USA) system. The ceramic homogenizer, dispersive solid phase extraction adsorbent (C18, Part No.:5982-5752), and sodium chloride were purchased from Agilent (Agilent Technologies, Lake forest, CA, USA).

2.2. Bioaccumulation and elimination tests

The soils (prepared and characterized as described in Supporting Information) were contaminated with (±)-PCB 91 at 50 and 500 $\mu g/kg_{dwt}$, and (±)-PCB 91 was added in steps to ensure that 250 g_{dwt} of soil was spiked homogeneously. First, 6.25 mL (±)-PCB 91 in acetone (2 and 20 mg/L) was slowly added to 50 g dry soil under continuous mixing for 5 min. Spiked soils were left in the fume hood overnight to evaporate the solvent. Contaminated soils were then thoroughly mixed with 200 g dry soil to obtain the final spiking levels of 50 and 500 $\mu g/kg_{dwt}$. Finally, contaminated soils were transferred to 500 mL glass jars and 90 g of tap water was added to each jar to restore the 36% water content. Control soils were prepared the same way as contaminated soils, using acetone instead of the PCB 91 solution.

Mature earthworms weighed between 200 and 300 mg were allowed to live in uncontaminated soil for one week to acclimate before transferring earthworms (5 g_{wwt}) to the glass jars containing contaminated soil. These jars were weighed, and the loss of water by evaporation was compensated by adding tap water every two days. The jars were placed in the dark, in an environmental chamber set at 20 °C.

For the bioaccumulation test, earthworms were collected after exposure to (±)-PCB 91 for 1, 3, 5, 7, 10, 14, 21, 29, and 35 days, rinsed in tap water, and allowed to depurate most of their gut contents on moist filter paper for 3 h. Water on the surface of earthworms was carefully dried using adsorbent paper, and earthworms were then frozen at −20 °C until use. Soil samples (5 g_{wwt}) taken from each jar were also frozen at −20 °C.

For the elimination test, earthworms in contaminated soil were transferred to uncontaminated soil at the 10th day of exposure,