Chemosphere 193 (2018) 978–988

Contents lists available at ScienceDirect

Chemosphere

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

Effect of 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47) and its metabolites on cell viability, oxidative stress, and apoptosis of HepG2

Shaoyu Tang [a, b], Hao Liu [a], Hua Yin [a, *], Xintong Liu [a], Hui Peng [c], Guining Lu [a], Zhi Dang [a], Chang He [b]

[a] Key Laboratory of Ministry of Education on Pollution Control and Ecosystem Restoration in Industry Clusters, Guangdong Provincial Engineering and Technology Research Center for Environmental Risk Prevention and Emergency Disposal, School of Environment and Energy, South China University of Technology, Guangzhou 510006, Guangdong, China

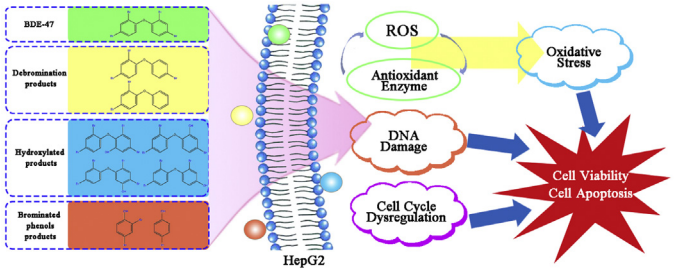
[b] Queensland Alliance for Environmental Health Science (QAEHS), Formerly National Research Centre for Environmental Toxicology (ENTOX), The University of Queensland, Brisbane, Australia

[c] Department of Chemistry, Jinan University, Guangzhou 510632, Guangdong, China

HIGHLIGHTS

- Toxicity of BDE-47 and its degradation products were compared in HepG2 cells.
- BDE-47 and all the metabolites induced oxidative stress and DNA damage.
- Cells were arrested in G2/M phase after exposure to BDE-47 and its metabolites.
- Brominated phenol products posed the highest cell apoptosis among all products.
- Pretreating with low dose of BDE-47, BDE-28, and BDE-7 adapt cells to high dose.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 September 2017
Received in revised form
17 November 2017
Accepted 20 November 2017
Available online 23 November 2017

Handling Editor: David Volz

Keywords:
BDE-47
Metabolites
Cell apoptosis
Cell cycle
Low concentration adaptation effect

ABSTRACT

2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47), an extensively used brominated flame retardant (BFR), is frequently detected in biotic environments. To date, studies have reported that BDE-47 induces hepatotoxicity, reproductive toxicity, and neurotoxicity in vitro. However, little is known regarding BDE-47 metabolites-mediated cell toxicity in relevant human cell models. The cytotoxic effects of BDE-47 and its eight metabolites on hepatoblastoma cell line-HepG2 cells were investigated in this study. We found that BDE-47 and all its metabolites inhibited cell viability in both a dose- and time-dependent manner. For BDE-47 and its debromination products (BDE-28 and BDE-7), they had less severe effects on cell viability when the cells were pretreated with lower dose of the same compound, however, no significant difference was observed in control, suggesting that low concentrations have an adaptation effect on HepG2 cells. BDE-47 and its metabolites also induce changes in ROS generation, SOD and GSH activity, cell cycle regulation, DNA damage and cell apoptosis, indicating that the toxicity mechanisms of BDE-47 and its degradation products are mediated by oxidative stress, DNA damage and cell cycle dysregulation. Moreover, brominated phenol products (2,4-DBP and 4-BP) posed the highest toxic effects on HepG2, followed by hydroxylated products (6-OH-BDE-47, 5-OH-BDE-47, 2-OH-BDE-28, and 4-OH-BDE-17), and

* Corresponding author.
E-mail address: huayin@scut.edu.cn (H. Yin).

https://doi.org/10.1016/j.chemosphere.2017.11.107
0045-6535/© 2017 Elsevier Ltd. All rights reserved.

BDE-47 and its debromination products were comparatively less toxic to HepG2 cells. Taken together, these results demonstrate the hepatotoxic potential of BDE-47 and its metabolites.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are low cost brominated flame retardants (BFRs) that are extensively used as additives in consumer products such as cables, textiles, and various electronics (Xiong et al., 2016; Cruz et al., 2015). Due to their widespread use and long half-life, high concentrations of PBDEs have accumulated in the air, water, soil, sediment and biotic environment (Zhang et al., 2013). The accumulation of PBDEs has raised great concerns about their potentially adverse effects on human health (Xiong et al., 2015). Multiple studies have demonstrated that high PBDE levels were implicated in hepatotoxicity, endocrine disruption, reproductive toxicity and neurotoxicity (Hoffman et al., 2012; Evandri et al., 2003).

One of the most abundant PBDEs in the abiotic and biotic environments is 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) (He et al., 2016; Thornton et al., 2016). Toxicity assessment studies of BDE-47 have revealed that this PBDE can modulate nuclear hormone receptor activity, negatively impact reproductive outcomes, and exhibit neurotoxic properties. A study by Song et al. (2016) found that BDE-47 alters thyroid hormone levels and related gene transcription in manila clams. Additionally, BDE-47 functions as a neurotoxin in mouse cerebellar granule neurons by increasing DNA damage, oxidative stress, and promoting cell apoptosis (Costa et al., 2015a,b).

Under the influence of environmental factors, such as UV rays from the sun and biotransformation in vivo, some contaminants can be transformed to other products, contributing to secondary pollution in the environment (Li et al., 2016). For example, UV irradiation produces six less-brominated BDEs (BDE-28, BDE-17, BDE-15, BDE-8, BDE-3, and BDE-1) and two brominated phenols (4-bromophenol (4-BP) and 2,4-dibromophenol (2,4-DBP)) from BDE-47 (Wang et al., 2013). In addition to synthetic BDE-47, its hydroxylated (OH−) forms, 6-OH-BDE-47 and 5-OH-BDE-47, have been suggested to be natural metabolic products and have been detected in a wide variety of freshwater and marine organisms (Wiseman et al., 2011; Yogui et al., 2009; Valters et al., 2005). There is evidence that some of these metabolic products may be more toxic than the mother compounds. For example, 4,4′-thiodiphenol (TDP) as one of BPA derivatives exhibits more potent estrogenic activity than BPA does (Lei et al., 2017). Moreover, the BDE-47 hydroxylated metabolite 6-OH-BDE-47 has been shown to have a greater impact on swimming behavior of zebrafish (Macaulay et al., 2015), and is more potent in disturbing Ca^{2+} homeostasis and neurotransmitter release than the parent compound BDE-47 (Dingemans et al., 2008). Despite the potentially harmful effects of BDE-47 metabolic products, a general assessment and comparison of the toxicity of BDE-47 and its degradation products has not been conducted.

Human hepatoblastoma cell line-HepG2 is a widespread model used to assess PBDEs toxicity (Hu et al., 2014; Liu et al., 2015; Souza et al., 2016). To determine and compare the toxicity of BDE-47 and its degradation products, we examined the cytotoxic effects of BDE-47 and its eight metabolites (BDE-28, BDE-7, 6-OH-BDE-47, 5-OH-BDE-47, 2-OH-BDE-28, 4-OH-BDE-17, 2,4-DBP and 4-BP) on HepG2 cells. Further understanding of the toxicity of PBDEs degradation products would provide insight into the risks exposed to those PBDEs metabolites.

2. Materials and methods

2.1. Chemicals

BDE-47, BDE-28, BDE-7, 6-OH-BDE-47, 5-OH-BDE-47, 2-OH-BDE-28, 4-OH-BDE-17, 2,4-DBP and 4-BP were purchased from Accu Standard (Inc. USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco (USA). All other reagents of analytical grade were obtained from Guangzhou Chemical Reagent Factory, China. All other consumables for cells were purchased from Guangzhou Jetway Biotech Co., Ltd, unless otherwise stated.

2.2. Cell culture

HepG2 cells were cultured in DMEM containing 10% fetal bovine serum (FBS, PAN Biotech), 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C and 5% CO_2. After 2–3 days, the media were discarded, and the cells were rinsed with phosphate buffered solution (PBS) three times, trypsinized and transferred to clean centrifuge tubes and centrifuged at 800 *g* for 5 min. Harvested cells were then counted with a red blood count plate.

2.3. BDE exposure

A stock solution of BDE-47 and its degradation products (10 mM) were prepared in DMSO, and diluted with DMEM to various concentrations (2, 10, and 50 μM) for exposure experiments. Harvested cells were plated in 96 or 6 well plate at a seeding density of 3×10^4 cells/mL. After culturing for 24 h, the media was replaced with fresh DMEM with different concentrations of BDEs. In the three concentration cases (2, 10, and 50 μM), the DMSO volume were 0.02%, 0.1%, and 0.5% of the total volume, respectively. Cells were relatively exposed to 0.02%, 0.1%, and 0.5% DMSO in the control group as well.

2.4. Cell viability assays

Cell viability was assessed using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide, according to the MTT assay (Stockert et al., 2012). HepG2 cells were seeded at a density of 3×10^4 cells/mL and exposed to BDE-47 or one of its metabolites for 24, 48, and 72 h at final concentrations of 2, 10, and 50 μM, respectively. Following incubation with BDE-47 or BDE-47 metabolites, the cells were incubated in a 50 μL MTT solution for 4 h. The media were subsequently discarded and cells were dissolved in 150 μL DMSO. Final absorbance was determined at 570 nm in a microplate reader. The percentage of viable cells was determined by calculating the ratio of final absorbance of treated cells to that of untreated ones.

2.5. Reactive oxygen species (ROS) generation

ROS generation was determined using DCFH-DA, a ROS indicator that produces fluorescent DCF in the presence of intracellular oxygen. After treatment with BDE-47 or a BDE-47 metabolite, HepG2

Download English Version:

https://daneshyari.com/en/article/8852692

Download Persian Version:

https://daneshyari.com/article/8852692

Daneshyari.com