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# Polyfluorinated iodine alkanes regulated distinct breast cancer cell progression through binding with estrogen receptor alpha or beta isoforms☆

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ABSTRACT

Polyfluorinated iodine alkanes (PFIs) are a kind of emerging chemicals with endocrine disrupting effects. Based on the different binding preferences of PFIs to estrogen receptor alpha and beta isoforms (ERα and β), two representative PFIs, dodecafluoro-1,6-diiodohexane (PFHxDI) and tridecafluorohexyl iodide (PFHxI), were selected to evaluate their effects on the proliferation of two kinds of breast cancer cells with different ERα/β expression levels, MCF-7 and T47D. The cell viability assay showed PFHxDI could cause higher cellular toxicity than did PFHxI in both MCF-7 and T47D. MCF-7 with relatively higher ERα/β expression ratio was more vulnerable to the cytotoxic treatments of PFHxI and PFHxDI when compared with T47D cells with relatively lower ERα/β expression ratio. EdU incorporation and cell cycle analysis revealed that, similar to 17β-estrodiol ($E_2$), non-cytotoxic levels of PFHxDI could significantly promote the proliferation of MCF-7 by increasing cell population at S phase ($p < 0.01$), while T47D proliferation was not influenced by PFHxI exposure due to cell cycle arrest at G2/M phase. The cellular responses caused by estrogenic PFIs were dominantly mediated by their preferential binding affinities for ER isoforms, which would be helpful in the accurate assessment for their potential influences on the breast cancer progression.



## 1. Introduction

Polyfluorinated iodine alkanes (PFIs) are a kind of important intermediates in the synthesis of fluorotelomer alcohols (FTOHs) and other related polyfluoroalkyl substances (PFASs) (Ruan et al., 2010a). The improper processes of their production, storage, usage and disposal would cause inevitable environmental release and unintentional exposure (Wang et al., 2012). PFIs, including perfluorinated iodine alkanes (FIAs) and polyfluorinated telomer iodides (FTIs), have been detected in air and soil samples around a fluorochemical manufacturing plant in Shandong province, northern China (Ruan et al., 2010b). As a kind of emerging chemicals, their environmental pollution and health risks have been gaining increasing concerns (Jiang, 2016).

Considering the potential deleterious impacts on the environment and human health, the endocrine disrupting effects of emerging chemicals are now listed in tier 1 screening in US EPA (EPA, 2009), and being evaluated with high priority. As for PFIs, they have been reported to exert chemical structure-related estrogenic effects based on E-screen and MVLN transcriptional activity assays (Wang et al., 2012). As endocrine disrupting effects could be induced by the interference of chemicals in normal hormone biosynthesis, signaling, or metabolism (Shanle and Xu, 2011), the binding affinities of PFIs for estrogen receptor (ER) were further evaluated based on high throughput ER competitive binding assay to explain the mechanism of their estrogenic activities, and the

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results revealed that 6-carbon FIAs with different iodine substitutions would influence their binding preferences to ERα or β isoforms (Song et al., 2017). Wherein, dodecafluoro-1,6-diiodohexane (PFHxDI) was found to preferentially bind with ERα, and tridecafluorohexyl iodide (PFHxI) preferred to binding with ERβ (Song et al., 2017). It was thus interesting to investigate how these chemicals with different ER isoform binding preferences would regulate biological effects in target tissues.

Breast cancer, as one of the leading cancer forms in the world, especially for the developed world, has been well documented, and the mitogenic actions of estrogens are critical in their etiology and progression (Bardin et al., 2004). The use of combined estrogen and progestin hormone therapy (CHT) increased breast cancer risk according to the controlled trials of menopausal hormone therapy randomized by the Women's Health Initiative (WHI) (Li et al., 2014). 17β-estrodiol ($E_2$), as the predominant and the most biologically potent human estrogen, was reported to promote the progression of breast cancer cells *in vitro* (Hirsch et al., 2007; Okubo et al., 2001), and increase breast tumor volumes *in vivo* (Lee et al., 2014). These findings have indicated estrogens are closely correlated with breast cancer progression, thus bringing the public worries about the potential cancer risks of environmental chemicals with estrogenic activities.

Endocrine disrupting chemicals (EDCs) have been reported to be involved in the incidence of hormone-dependent diseases, such as breast, ovary and prostate cancers (Lecomte et al., 2017). For example, diethylstilbestrol ever use during pregnancy was associated with an overall 50% increase in subsequent risk of breast cancer compared with untreated groups (Malone, 1993). Bisphenol A (BPA) has been demonstrated to be a potential carcinogenic agent, contributing to the incidence of human breast cancer using meta-analyses of heterogeneous microarray sets (Jung et al., 2017). Similar to $E_2$, some EDCs with estrogenic activities, like parabens, octylphenol, triclosan, and some phytoestrogens, could promote the proliferation of breast cancer cells *in vitro* through regulation of ER signaling (Lee et al., 2014; Okubo et al., 2001; Pons et al., 2014). These studies have revealed the potential risks of environmental estrogenic compounds in cancer incidence, development, and progression. Nevertheless, how chemicals regulate the biological processes of cancer cells through their specific binding characteristics for ER isoforms remain to be elucidated.

ER isoforms (i.e. ERα and ERβ), exhibit different tissue distribution patterns, ligand selectivities, transcriptional properties, and biological functions (Henke et al., 2002). Approximately, 75% of breast cancers are ER positive (Milani et al., 2014), and exhibit estrogen-dependent growth and proliferative advantage (To et al., 2014). Different breast cancer cell types, like MCF-7 and T47D, could have different ERα/β ratios (Nadal-Serrano et al., 2013). The amino acid difference in the binding pocket contributes to ligand selectivity for ER isoforms (Shanle and Xu, 2011). Estrogenic compounds, like PFIs, may bind with these two ER isoforms with different affinities, thus exerting different regulatory effects on cancer cell growth, due to the opposite functions of ERα and β mediated pathways (Heldring et al., 2007). The research on how the estrogenic chemicals with different ER isoform binding affinities work on breast cancer cells with different ERα/β expression ratios would be important for the explanation of their cancer risks.

This study investigated two representative PFIs with different ER isoform binding affinities, PFHxI and PFHxDI, to reveal their effects on two types of breast cancer cells with different ERα/β expression ratios, MCF-7 and T47D. The cell viability, proliferation, and cell cycle distribution were thoroughly evaluated, and the findings gave important evidences on ER isoform-dependent breast cancer cell proliferation and progression regulated by emerging EDCs, like PFIs.

## 2. Materials and methods

### 2.1. Materials

The chemicals, including tridecafluorohexyl iodide (PFHxI, 98%) and dodecafluoro-1,6-diiodohexane (PFHxDI, 98%), were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). 17β-estradiol ($E_2$, 98%) and genistein (98%) were bought from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions were prepared by dissolving these chemicals in dimethyl sulfoxide (DMSO), and kept at 4 °C in darkness. The series of working solutions were freshly diluted with sterile cell exposure medium, and the final DMSO concentration was less than 0.5%.

The purified recombinant human ERα and ERβ proteins were obtained from OriGene Technologies Inc. (Rockville, MD, USA) and their aliquots were stored at −80 °C. The kits of HitHunter® ER Binding Assay and Cell-Light™ EdU In Vitro Imaging were purchased from DiscoveRx Corporation (Fremont, CA, USA) and Guangzhou Ribo Bio Co., Ltd (Guangzhou, China), respectively. The reagents for AlamarBlue assay and Muse™ Cell Cycle analysis were obtained from Promega Corporation (Madison, WI, USA) and Merck Millipore (USA), separately.

### 2.2. ER binding assay

The binding potencies of PFHxI and PFHxDI for ERα or β were evaluated according to the protocol of HitHunter® assay kit. Briefly, 2 μL of the tested chemical was added to 20 μL of ED-ES conjugate, and the mixture was incubated with 30 μL of 40 nM ERα or β at room temperature for 2 h in 96-well black plate. 20 μL of EA fragment and 20 μL of freshly prepared substrate reagent were finally transferred to each well of the plate. The luminescence intensities were monitored till the luminescence detection platform approached. The tested chemicals included 0.1 μM $E_2$, 1 μM genistein, 500 μM PFHxI, and 10 μM PFHxDI. The concentration of each chemical was selected when the maximal competitive ER binding affinity was achieved according to our previous study (Song et al., 2017). Competition percents of the tested chemicals for both ER isoforms were calculated as described in the previous study (Song et al., 2017).

### 2.3. Cell culture

MCF-7 cells (Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences) were cultured in DMEM (Hyclone, USA) containing 10% (v/v) fetal bovine serum (FBS, Gibco, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin. T47D (Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences) were cultured in RPMI-1640 medium (Hyclone, USA) containing 10% (v/v) FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1% Insulin-Transferrin-Selenium (ITS) (Gibco, USA). The incubations were kept in a humidified atmosphere of 5% $CO_2$ at 37 °C.

Before stimulation, MCF-7 and T47D cells were cultured in the steroid-free (SF) exposure medium, which were phenol red-free DMEM (Hyclone, USA) for MCF-7, and phenol red-free RPMI-1640 (Solarbio, China) for T47D, respectively, supplemented with 5% hormone-free FBS (Biological Industries, Israel), 100 U/mL penicillin, 100 μg/mL streptomycin, and 1% ITS (only for T47D). The incubation lasted for 24 h to minimize the basal hormonal influence on the cells to be tested.

### 2.4. Cell viability

The alamarBlue assay was used to evaluate the potential