**The in Vitro Estrogenic Activities of Polyfluorinated Iodine Alkanes**

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**Background:** Polyfluorinated iodine alkanes (PFIs) are important intermediates in the synthesis of organic fluoride products. Recently, PFIs have been detected in fluoropolymers as residual raw materials, as well as in the ambient environment.

**Objectives:** High production volumes and potential environmental releases of PFIs might become a concern, but the exposure risk and toxicity of these chemicals are still unclear. In this study, we investigated the potential estrogenic effects of PFIs.

**Methods:** We studied the estrogenic effects of fluorinated iodine alkanes (FIAs), fluorinated telomer iodides (FTIs), and fluorinated diiodine alkanes (FDIs) using the E-screen and MV LN assays and the evaluation of estrogen-responsive genes in MCF-7 cells.

**Results:** FIAs have an iodine atom at one end of the fluorinated carbon chain. 1-Iodoperfluorohexane (PFHxI) and 1-iodoperfluoroctane (PFOI) promoted the proliferation of MCF-7 cells, induced luciferase activity in MV LN cells, and up-regulated the expression of TFF1 and EGR3. In these assays, other FIAs gave negative responses. FDIs have an iodine atom at each end of the perfluorinated carbon chain, and all the FDIs showed estrogenic effects. The estrogenic potencies of FIAs and FDIs correlate well with the carbon chain length of the chemicals. The optimum chain length for estrogenic effects is six carbons, and then eight and four carbons. All FTIs have a single iodine atom at the end of a partially fluorinated carbon chain. None of the FTIs showed estrogenic effects in the tests.

**Conclusions:** The estrogenic effects of PFIs are dependent on the structural features of iodine substitution and chain length. This research will be helpful in further understanding the estrogenic effects of perfluorinated compounds.

**Key Words:** endocrine disruptor, estrogenic effects, in vitro assay, perfluorinated chemicals, polyfluorinated iodine alkanes. *Environ Health Perspect* 120:119–125 (2012). http://dx.doi.org/10.1289/ehp.1103773 [Online 11 October 2011]

Perfluorinated chemicals (PFCs) have a broad range of applications in the manufacture of various industrial and commercial products, such as fluoropolymers, surfactants, emulsifiers, and nonstick coatings. PFCs have been of considerable scientific and public concern because some of them are environmentally persistent, bioaccumulative, and widely detected in humans, wildlife, and the environment (Giesy and Kannan 2001; Olsen et al. 2007).

Because of environmental concerns, the 3M company voluntarily phased out electrochemical fluorination-based fluorochemicals in 2001 (Dupont 2005). Consequently, the current production of fluorinated polymers and surfactant is mostly based on telomerization processes (Lehmler 2005). Polyfluorinated iodine alkanes (PFIs) are organic iodides composed of a fluorinated carbon backbone terminated by iodine substitution (Table 1) and are important intermediates in the synthesis of various fluorinated chemicals (Brace 1999; Prevedouros et al. 2006). In the telomerization process, PFIs are used to synthesize fluorotelomer alcohols (FTOHs) and other related PFCs. In turn, FTOHs are intermediates in the production of surfactants and fluoropolymers, and these volatile compounds have been detected in the atmosphere around the world (Ellis et al. 2004). The annual production of FTOHs increased to $11-13 \times 10^3$ metric tons in 2002 (Ellis et al. 2003). The annual world production of PFIs has been estimated to exceed 4,000 metric tons (Organisation for Economic Co-operation and Development 2004), and the increasing demand of fluorotelomer products might increase the risk of emission of volatile PFIs to the environment (Ruan et al. 2010a, 2010b). Fluorinated iodine alkanes (FIAs) and fluorinated telomer iodides (FTIs) have been detected in air and soil samples around a fluorochemical manufacturing plant in Shandong province in northern China (Ruan et al. 2010a). Residual FIAs and FTIs could also be incorporated into FTOH containing raw materials and fluorotelomer-based products during manufacturing, 1-Iodoperfluoroctane (PFOI) and 6:2 FII have been detected in fluorotelomer raw materials and selected fluorotelomer-based products, such as urethane polymer and phosphate surfactant (Larsen et al. 2006). Furthermore, unreacted residual FTOH has also been found in commercial and industrial products and could be released to the ambient environment as well (Dinglasan-Panlilio and Mabury 2006; Larsen et al. 2006). Likewise, residual PFIs in fluorinated polymers and surfactants can be released into the environment and degrade to other persistent PFCs. Abiotic or biotic transformation of FTIs could contribute to the environmental burden of FTOHs and perfluorocarboxylic acids (PFCAs) (Young et al. 2008). There is therefore a potential risk for release of PFIs to the environment due to direct emission during manufacturing and indirect emission from some fluorinated products.

Increasing evidence has shown that some PFCs may have endocrine-disrupting potency. Some PFCs can disturb the thyroid system and neuroendocrine function, activate both peroxisome proliferator-activated receptors and estrogen receptors (ERs), and induce developmental toxicity in rodents (Lau et al. 2004).

The estrogenic effects of some PFCs have been studied in many aspects. For example, Maras et al. (2006) demonstrated the estrogen-like properties of FTOHs in MCF-7 cells. Using yeast two-hybrid assays, Ishibashi et al. (2007, 2008) demonstrated that FTOHs can activate the male medaka (Oryzias latipes) and human ER. Liu et al. (2007) reported that vitellogenin expression was induced by perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and FTOHs in primary cultured tilapia hepatocytes, and they suggested that estrogenic effects may be mediated through the ER pathway. FTOHs also induced vitellogenin in male medaka fish through the activation of ER, whereas PFOS and PFOA did not (Ishibashi et al. 2008).

Little information is currently available regarding the estrogenic effects of PFIs. In the present study, we used three *in vitro* bioassays—E-screen assay, MV LN assay, and evaluation of an estrogen-responsive gene—to comprehensively evaluate the estrogenic potencies of PFIs. The structural features responsible for estrogenic effects were identified by the alternations in potency derived from specific structural changes.

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Materials and Methods

Chemicals. The chemical structures of tested compounds are shown in Table 1. We purchased 1-iodoperfluorobutane (PFBI; 98% pure), 1-iodoperfluorohexane (PFHxI; 99% pure), 1-iodoperfluorodecanes (PFODI; 97% pure), 1-iodoperfluorododecanes (PFDDi; 97% pure), 6:2 FTI (96% pure), 8:2 FTI (96% pure), hexadecafluoro-1,8-diiodooctane (PFDOI; 98% pure), 1H-perfluoroctane (PFOC; 99% pure), 1-bromoperfluorooctane (PFOB; 99% pure), 1-iodohexane (98% pure), and 4-hydroxytamoxifen (OHT; 98% pure) from Sigma Chemical Company (St. Louis, MO, USA); PFOI (98% pure), 4:2 FTI (95% pure), 10:2 FTI (95% pure), and PFOA (98% pure) from Fluka (Buchs, Switzerland); and octadecafluoro-1,4-diiodohexane (PFBDI; 97% pure), dodecafluoro-1,6-diiodohexane (PFHxDI; 97% pure), and 17β-estradiol (E2; 99% pure) from Alfa Aesar (Ward Hill, MA, USA). We dissolved all the PFLs, PFOB, E2, and 1-iodohexane in ethanol. PFOA and PFOC were dissolved in dimethyl sulfoxide as 100 mM stock solutions. All stock solutions were stored at –20°C.

Cell culture. Human MCF-7 BUS breast adenocarcinoma cells and MVLN cells were cultured in 100-mm culture dishes in a humidified atmosphere of 5% CO2 at 37°C. Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM)/F-12 (Hyclone, Logan, UT, USA) containing 10% fetal bovine serum, 100 U/mL streptomycin-penicillin, 2 mM l-glutamine, and 1% insulin-transferrin-selenium supplement (all from Gibco, Grand Island, NY, USA).

E-screen assay. MCF-7 BUS cells were kindly provided by A.M. Soto and C. Sonnenschein (Tufts University School of Medicine, Boston, MA, USA). In response to ERα agonists, the mitotic effect leads to the proliferation of MCF-7 BUS cells. We performed the E-screen assay following a method modified from the protocol by Soto et al. (1995). Cells were trypsinized and plated into the interior 60 wells of 96-well plates at the density of 3,000 cells/well. Before each experiment, cells were starved in steroid-free (SF) medium for 48 hr to minimize the basal hormonal activity during assays. SF medium consisted of phenol red-free DMEM/F-12 (Hyclone) supplemented with 5% dextran-charcoal-free DMEM/F-12 (Hyclone) containing 10% fetal bovine serum, 100 U/mL streptomycin-penicillin, and 2 mM l-glutamine. Cells were treated with serial dilutions of test chemicals (from 1 nM to 100 μM) in SF medium; a concentration range of 0.01–200 pM E2 was used as the positive control. We used a WST-1 proliferation kit (Roche Diagnostics, Mannheim, Germany) to assess proliferation after 6 days of exposure according to the kit instructions. The WST-1 assay is based on the enzymatic cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases present in viable cells. The absorbance of the WST-1 solution was detected by a microplate reader (Varioskan Flash, Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm, with the reference wave length at 690 nm. The cell proliferation effect was calculated from the solvent control (0.1% ethanol)-corrected absorbance and expressed as the percentage of maximal absorbance of the positive control. Three replicates were used in each experiment.

MV LN assay. The MVLN cell line was kindly provided by J.P. Giesy (Michigan State University, East Lansing, MI, USA). This cell line was stably transfected with the luciferase reporter gene and estrogen-responsive element derived from the Xenopus vitellogenin A2 gene. ERα agonists can induce the production of luciferase in MVLN cells (Pons et al. 1990). Cells were seeded in the interior 60 wells of a 96-well ViewPlate (Packard Instrument Company, Boston, MA, USA) at a density of 7 × 104 cells/well, starved in SF medium for 48 hr, and exposed to test compounds for 2 days. A concentration range of 0.5 pM–1 nM E2 was used as a positive control, whereas the exposure concentration range of test chemicals was 0.1–100 μM. Luciferase activity was measured with the LucLite kit (Packard Instruments) according to the manufacturer’s protocol. We measured luminescence by microplate reader (Varioskan Flash) and integrated the luminescence signal for 10 sec. Total protein content was measured by the Bradford assay (Tiangen, Beijing, China) to normalize luminescent units. The results are given as relative luminescent units per microgram protein. The maximal induction of positive control (corrected for solvent control, 0.1–0.2% ethanol) was set as 100%, and the responses of other chemicals were converted to a percentage of the maximum level. Three replicates were used in each experiment. The cytotoxicity of tested chemicals was examined by WST-1 kit in parallel and routinely observed under microscope to identify the exposure concentration range.

RNA isolation and reverse transcription polymerase chain reaction (RT-PCR). MCF-7 BUS cells were seeded in six-well plates at the density of 1.0 × 106 cells per well, starved in SF medium for 48 hr, and exposed to test compounds for 48 hr. First, cells were rinsed twice with cold phosphate-buffered saline, and total RNA was isolated using Trizol reagent (Invitrogen Inc., Carlsbad, CA, USA) following the manufacturer’s protocol. The 260 nm and 280 nm absorbance reading of total RNA was performed using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). The concentration of
RNA was quantified by the reading at 260 nm. The 260:280 nm ratios were between 1.8 and 2.0, which indicates that the extracted RNA was sufficiently pure.

We used a two-step quantitative RT-PCR to quantify gene expression. Total RNA was converted to cDNA using M-MLV (Mooney murine leukemia virus) reverse transcriptase (Promega, Madison, WI, USA) with oligo dT(15), following the manufacturer’s instructions. The final cDNA solution was diluted five times with DNase/RNase-free water (Gibco). Quantitative PCR was performed with a Stratagene MX3005 thermal cycler (Stratagene, La Jolla, CA, USA). PCR reaction mixtures (25 μL) contained 12.5 μL GoTaq Green Master Mix (Promega, Madison, WI, USA), 2 μL diluted cDNA, and 0.2 μM sense/antisense primers. The thermal cycle was 5 min at 95°C, followed by 45 cycles of 15 sec at 95°C, 30 sec at 55°C, and 30 sec at 72°C. The primer sequences of early growth response protein 3 (EGR3) were derived from Terasaka et al. (2004). We designed the primers of internal gene \( \beta\)-actin and trefoil factor 1 (TFF1, NM_001101), 5´-AGGTCTTTGCG GATGTCCAC-3´ (forward) and 5´-GTGGATCTGCT GTGTTTGAATG-3´ (reverse); for \( \beta\)-actin (NM_001101), 5´-AGGTCTTTGCG GATGTCCAC-3´ (forward) and 5´-GTGGATCTGCT GTGTTTGAATG-3´ (reverse); and for TFF1 (NM_003225), 5´-AGAAGGCC GTGCTGAGGTGC-3´ (forward) and 5´-GCAAATAG GGCCTGTCTT-3´ (reverse). The quantification of target gene expression was based on a comparative cycle threshold (Ct) value. We normalized the expression level of each target gene to its reference gene \( \beta\)-actin. The fold change of the target gene expression was based on a comparative cycle threshold (Ct) value.

**Results**

**Stimulation of MCF-7 cell proliferation.** We used the E-screen assay to investigate the estrogenic activities of 12 PFIs in MCF-7 cells. FIAs are monoiodinated fluorinated alkyls with even-numbered chains that have 4–12 carbons. PFBI [4 carbons in its alkyl chain (C-4)], PFDFI [C-10], and PFDOI (C-12) did not show proliferation effects within the concentration ranges, whereas PFHxI (C-6) and PFFO (C-8) produced full concentration–response curves compared with E2 (Figure 1A); EC50 values were 0.63 μM and 1.15 μM for PFHxI and PFFO, respectively (Table 2). The proliferation effects appeared to be dependent on the chain length of FIAs. Fluorinated diiodine alkyls (FDIAs) have even-numbered chains with 4 to 8 carbons. All FDIAs produced full concentration–response curves in the E-screen assay (Figure 1B). The relative proliferation effects were in the following order: PFHxDI (C-6) > PFODI (C-8) > PFBDI (C-4). Likewise, proliferation potency also seems to be related to the specific carbon chain length of FDIAs. The EC50 values of PFBDI (1.45 μM), PFHxDI (7.5 nM), and PFODI (43.3 nM) were much lower than those of the corresponding FIAs with the same chain length. The order of their relative proliferation potencies was PFHxDI > PFODI > PFHxl.

**Figure 1.** Concentration–response proliferation effects of FIAs (A) and FDIAs (B) in the E-screen assay. The relative proliferation effects are expressed as mean ± SD of triplicate measurements in one representative experiment.

**Table 2.** Maximum induction and effective concentrations of tested chemicals based on the E-screen and MVN assays.

| Chemical | Maximum induction (%) | \( EC_{50} \) | Relative potency |
|----------|-----------------------|--------------|----------------|
| E2       | 100                   | 3.12 pM      | 1              |
| PFBI     | ND                    | —            | —              |
| PFHxI    | 99                    | 0.63 μM      | 4.9 × 10⁻⁶      |
| PFDOI    | 101                   | 1.15 μM      | 2.7 × 10⁻⁶      |
| PFDFI    | ND                    | —            | —              |
| PFPODI   | ND                    | —            | —              |
| 4:2 FFI  | ND                    | —            | —              |
| 6:2 FFI  | ND                    | —            | —              |
| 8:2 FFI  | ND                    | —            | —              |
| 10:2 FFI | ND                    | —            | —              |
| PFHxDI   | 97                    | 1.45 μM      | 2.2 × 10⁻⁶      |
| PFODI    | 100                   | 7.5 nM       | 4.2 × 10⁻⁶      |
| PFDOI    | 105                   | 43.3 nM      | 7.2 × 10⁻⁵      |
| PFPOI    | ND                    | —            | —              |
| PFPO     | ND                    | —            | —              |
| 1-Iodohexane | ND | — | — |

EC50 values were calculated from this nonlinear regression model. EC20 were calculated as

\[ EC_{50} = \left[ \frac{x}{(100 - x)} \right] \left[ \frac{1}{\text{Hill slope}} \right] \times EC_{50}, \]

where \( x \) is 20% of the maximum effects, and the Hill slope and EC50 were calculated from Equation 1.

All statistical analyses were performed using Sigma Plot (version 10.0; Systat Software Inc., San Jose, CA, USA).

### Data Availability

- The data and materials generated during the current study are available from the corresponding author upon reasonable request.
- The raw data used to support the findings of this study are available from the corresponding author upon reasonable request.

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**Conflict of Interest**

- The authors declare that they have no competing interests.

**References**

- Livak, K. J., & Schmittgen, T. D. (2001). \( \Delta \Delta C_t \) analysis of realtime quantitative PCR data. \textit{Methods}, 25(4), 402–408.
- Terasaka, K., Orita, Y., Fujii, N., & Aso, Y. (2004). \( \beta\)-actin and trefoil factor 1 expression in synthroid and coffee-sensitive thyroid carcinoma. \textit{Endocrine Journal}, 51(3), 135–140.

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> PFOI > PFBDI. These compounds are thus considered to behave like xenoestrogens in the E-screen assay.

**Transactivation in MVLN cells.** The MVLN assay has been widely used to study ER activity of test compounds (Freyberger and Schmuck 2005). In the present study, we used the MVLN assay to further investigate ER activity and estrogenic potency of PFIs. Before the MVLN assay, we tested the cytotoxic effects of each compound using the WST-1 assay. Exposure of MVLN cells to FIAs, FTIs, or FDIAs did not produce significant cytotoxicity within the concentration ranges, and no cytotoxic effects were observed by microscopic examination (data not shown).

Because these compounds did not show maximum induction compared with E2, relative potency based on the EC20 would be more reliable than that derived from the EC50 (Villeneuve et al. 2000). The estrogenic effects of PFIs revealed by the MVLN assay were in accordance with the results of the E-screen assay. As shown in Figure 2A, the induction of luciferase activity by PFBI, PFDI, and PFDIol were at the basal level (< 5%), whereas PFHxI and PFOI induced luciferase activity in a dose-related manner. PFHxI (EC20 = 14.1 μM) showed higher estrogenic activity than did PFOI (EC20 = 20.4 μM) in MVLN cells, with maximum induction values of 47% and 25%, respectively (Table 2). Luciferase activity induced by FDIAs seems to be related to the specific carbon chain length (Figure 2B). PFHxDI (EC20 = 0.38 μM) showed stronger estrogenic potency than did PFODI (EC20 = 1.07 μM) and PFBDI (EC20 = 13.8 μM), with the maximum induction values of 73%, 38%, and 21%, respectively.

Because the difference of EC50 or EC20 values among PFHxI, PFOI, and PFBDI were small, we compared the estrogenic potency with the maximum induction value in an MVLN assay. The order of estrogenic potency was PFHxDI > PFHxI > PFODI > PFOI > PFBDI, which is comparable to results from the E-screen assay. Similarly, FDIAs possessed stronger estrogenic potency than did FIAs in the MVLN assay (PFBDI > PFBI; PFHxDI > PFHxI; PFODI > PFOI), which indicated that iodine substitution at the end of a fluorinated chain may enhance the estrogenic potency of FIAs. The optimum chain length for estrogenic activity was six carbons for FIAs and for FDIAs in both of these estrogen-screening assays.

**Comparison of PFCS with similar structures.** FTIs are partially fluorinated alkyl iodides, which are produced by the ethylation of PFAs in telomerization processes. Compared with FIAs and FDIAs, FTIs with various chain lengths did not show estrogenic effects in the E-screen or MVLN assays within the tested concentration ranges (0.01–200 μM). We used a nonfluorinated hydrocarbon, 1-iodohexane (C-6), as the control to study the effects of fluorination on estrogenic effects. Three eight-carbon PFCS—PFOA, PFOC, and PFBO—that contain no iodine substitution on the carbon chain were used as comparisons to study the effects of iodine substitution on estrogenic effects. As we suspected, 1-iodo octane, PFOA, and PFBO showed negative results in the estrogen-screening assays (Table 2). These results further emphasize that a perfluorinated alkyl chain and iodine substitution are important structural features for the estrogenic effects of PFIs.

**Coexposure effect of OHT with FIAs in MVLN assay.** We used OHT, a strong estrogen antagonist in the mammary gland, to block the ER in the MVLN assay. OHT was coexposed with PFHxI, PFOI, PFBDI, PFHxDI, or PFODI. We used the highest induction concentrations obtained from MVLN assay in the coexposure experiments and the gene expression assay. As shown in Figure 3, coexposure of OHT with the tested chemicals resulted in marked reduction of luciferase activity, which further confirmed that these xenoestrogens can activate the ER.

**Expression of estrogen-responsive genes.** After MCF-7 cells were exposed to a series of PFCs for 48 hr, the expression levels of two estrogen-responsive genes (EGR3 and TFF1) were analyzed by real-time PCR. The TFF1 gene is involved in cell proliferation and also serves as a biomarker gene responding to estrogens (Brown et al. 1984; Jorgensen et al. 2000). As one of the ER-mediated estrogen-inducing genes, EGR3 belongs to the early growth response family and plays an important role in the estrogen-dependent induction of the immune evasion system (Inoue et al. 2004). The expression levels of EGR3 and TFF1 are up-regulated by natural and synthetic estrogens in MCF-7 cells (Terasaka et al. 2004). PFHxI, PFOI, PFBDI, PFHxDI, and PFODI, which showed estrogenic effects in the E-screen and MVLN assays, significantly up-regulated the estrogen-responsive genes by 4.4-, 2.7-, 2.7-, 5.7-, 8.5-, and 7.7-fold for TFF1 and by 2.4-, 3.6-, 2.4-,
Discussion

The endocrine-disrupting effects elicited by industrial chemicals have been of extensive concern (Colborn et al. 1993). Exposure to xenoestrogens may lower sperm count and male fertility and increase the incidence of breast and testicular cancer in humans (Toppappi et al. 1996). Most of the adverse effects of these compounds are thought to be mediated through ER activation. Although the environmental behaviors of PFIs are not known, these volatile and high-production-volume chemicals could be released into the ambient environment during production, storage, and transport. The atmospheric oxidation of PFIs may contribute to the increased levels of other PFCs in the environment. Studies of the potential toxicities of PFIs are therefore needed for health risk evaluation. In this study, we investigated the estrogenic effects of PFIs by the E-screen and MVLN assays and the expression of estrogen-responsive genes. Our results showed that PFHxI, PFOS, PBFDI, PFFxDI, and PFDoI exerted estrogenic effects through activation of the ER. The relative estrogenic potencies obtained from the E-screen and MVLN assays are both related to the specific carbon chain length of FIAs and FDIs. The optimum chain length for estrogenic effects is six carbons, and iodine substitution on the perfluorinated chain was crucial for the estrogenic effects. Those potent compounds were able to fully stimulate cell proliferation of MCF-7 cells, but this was not the case for the induction of reporter gene expression in MVLN cells. This discrepancy might be due to the difference of initial seeding density, exposure time, and sensitivity between the two assays. The expression of the estrogen-responsive gene by these PFIs further confirmed the results. The estrogenic potencies of FIAs were higher than that of the FDIs, indicating that the increasing number of iodine substitutions on FIAs renders the chemical more potent in inducing estrogenic activity. PFHxDI (C-6), with two iodine substitutions (one at each end) of the perfluorinated chain, showed the highest potency among the PFIs.

Considerable evidence has indicated that chain length determines the biological effect of PFCs (Hu et al. 2002; Liao et al. 2009; Upham et al. 1998). Bioconcentration and bioaccumulation of PFCs are related to the length of the fluorinated chain in different species (Martin et al. 2003). Cytotoxic end points of PFCs such as in vitro cytotoxic effects, the alteration of cell membrane potential, and cytosolic pH are directly related to perfluorinated chain length (Kleszczynski and Sklanadowski 2009). The inhibition of perfluorinated fatty acids on gap junction intercellular communication also depends on chain length; shorter PFCs, including perfluorobutanesulfonate and perfluorohexanesulfonate, did not show effects, whereas PFOS significantly inhibits gap junction intercellular communication (Hu et al. 2002; Upham et al. 1998). The interference of PFCs on cultured rat hippocampal neurons was also related to the carbon chain length and functional groups (Liao et al. 2009). Our findings suggest that FDIs and some of the FIAs exert estrogen effects through the activation of ER. Because the solubility of nonpolar FIAs in culture media decreased with increasing chain length, the lack of estrogenic effects for PFDI and PFDoI might be attributed, in part, to decreased solubility and bioavailability of long-chain FIAs.

We used the nonfluorinated organic iodide 1-iodohexane to study the effect of fluorination on estrogenic effects compared with PFHxI. In the screening assays, 1-iodohexane did not exert estrogenic effects, indicating that fluorination is an important structural feature for estrogenic activity. The hydrophobic property of the fluorinated chain imparts the proteinophilic and lipophilic property of PFCs and results in the interaction of PFCs with multiple biological molecular targets in various species. PFOA did not show proliferation effects in MCF-7 cells, as previously reported by Maras et al. (2006). In the present study, we found that PFOA, PFOB, and PFOC also lack estrogenic effects. By comparing the structure–activity relationship between these PFCs, we propose that the iodine substitution is a key attribute for the estrogenic effect. The estrogenic effect was also lower for moniodoiodinated fluorinated alkanes than for diiodoiodinated fluorinated alkanes, which further supports our assumption.

FTOHs exert estrogenic activity in MCF-7 cells and aquatic organisms (Ishibashi et al. 2008; Maras et al. 2006). FTOHs behave as estrogens because of the similarity of their chemical structure and properties to other xenoestrogens, such as 4-nonylphenol. In the telomerization processes, fluorotelomer iodides

**Figure 4.** Effects of tested chemicals on mRNA expression of estrogen-responsive genes TFF1 (pS2) and EGR3 in MCF-7 cells. (A) Cells exposed to 0.1% ethanol (control), 50 μM PFBI, 50 μM PFHxI, 50 μM PFBI, 40 μM PFDoI, 40 μM PFODI, 20 μM PFHxDI, 40 μM PFODI, or 100 μM E2 for 48 hr. (B) Cells exposed to 0.1% ethanol, 50 μM PFDA, 50 μM PFOC, 50 μM PFOB, 50 μM 1-iodohexane, 50 μM 4:2 FTI, 50 μM 6:2 FTI, 50 μM 8:2 FTI, 40 μM 8:2 FTI, 40 μM 10:2 FTI, or 100 μM E2 for 48 hr. Results are expressed as the mean ± SD of triplicate measurements in one representative experiment.

*p < 0.05, and **p < 0.01, compared with the control. ANOVA and Tukey’s multiple range test were used to assess the significance of mean differences.

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are oxidized to produce FTOHs. Compared with FTOHs, none of the FTIs induced cell proliferation, which indicated that the hydroxyl group is more important for the estrogenic effects than is iodine substitution in partially fluorinated chemicals. Some of the PFIs activated the ER and induced luciferase activity in MVLN cells. However, it is questionable whether these PFIs are able to directly bind to and activate the ER. Structural features such as a phenol ring and a hydrophobic group attached para to the hydroxyl group are essential for the estrogenic effects (Blair et al. 2006; Laws et al. 2006; Suzuki and Shapiro 2007). Furthermore, hydroxylated analogs of polybrominated diphenyl ethers and polychlorinated biphenyls have been shown to exert estrogenic effects (Bergeron et al. 1994; Fielden et al. 1997; Meerts et al. 2001). Therefore, it may be reasonable to expect that hydroxylated forms of FIAs and FDIAs could also be estrogenic.

Compared with PFOI, both PFOB and PFOC showed no estrogenic activity. It is likely that bond strength also determines their reactivity. The strength of the bonds is C–F (467 kJ/mol) > C–H (453 kJ/mol) > C–Br (290 kJ/mol) > C–I (228 kJ/mol). Among the four halogens, fluorine is the most electrono- negative and iodine the least. The polarization of the C–I bond is lower than that of the C–H bond and the other carbon–halogen bonds. Because iodine is a good leaving atom and because of the chemical reactivity of the C–I bond, it would be easier for PFIs to be converted to their hydroxylated analogs during the exposure studies. Oxidation of FIAs can result in the formation of PFCAs (Lehmler 2005). In this reaction, C,Fn+2OH is thought to have been formed by the cleavage of C–I bonds in FIAs and addition of OH (Yamamoto et al. 2007). We hypothesize that C,Fn+2OH is hydrolyzed to C,Fn+1OH in the culture media or inside the cells, and the degradation products or the metabolites of PFIs are the possible targets for ER, thereby exerting estrogenic activity. However, the underlying mechanisms for the estrogenic effects of PFIs have not been clearly established, and further studies are warranted to characterize possible catabolites of PFIs which might also exhibit estrogenic activity.

The main functions of hormones are to maintain homeostasis and regulate reproduction and development. Exposure to endocrine-disrupting chemicals may cause adverse effects to the organs and glands that secrete hormones, further resulting in endocrine toxicity such as impaired reproduction and development. PFIs are volatile chemicals and have been detected around fluoroc hemical manufacturing areas (Ruan et al. 2010a). As important precursors for the synthesis of organic fluoride products, PFIs could be incorporated into fluorotelomer raw materials and fluorotelomer-based products as residues (Larsen et al. 2006). Occupational and indoor environments might be exposure risk zones, and inhalation could be a possible exposure route.

Conclusion

Some PFIs could act on ERs and potentially cause detrimental effects on reproductive and developmental systems. To our knowledge, this is the first study to find estrogenic activity of PFIs using three in vitro methods. Considering the current large and increasing production volume of telomerization-based PFCs, more extensive studies should be conducted on the environmental distribution and toxicological effects of PFIs.

References

Blair JM, Crews D, Mcchlavan JH. 1994. PCBs as environmental estrogens—turdle sex determination as a biomarker of environmental contamination. Environ Health Perspect 102:710–711.

Blair RM, Fang H, Branham WS, Hass BS, Dial SL, Moland CL, Brown AMC, Jeltsch JM, Roberts M, Chambon P. 1984. Estrogenic activity in MVLN cells. However, it is questionable whether these PFIs are able to directly bind to and activate the ER. Structural features such as a phenol ring and a hydrophobic group attached para to the hydroxyl group are essential for the estrogenic effects (Blair et al. 2006; Laws et al. 2006; Suzuki and Shapiro 2007). Furthermore, hydroxylated analogs of polybrominated diphenyl ethers and polychlorinated biphenyls have been shown to exert estrogenic effects (Bergeron et al. 1994; Fielden et al. 1997; Meerts et al. 2001). Therefore, it may be reasonable to expect that hydroxylated forms of FIAs and FDIAs could also be estrogenic.

Compared with PFOI, both PFOB and PFOC showed no estrogenic activity. It is likely that bond strength also determines their reactivity. The strength of the bonds is C–F (467 kJ/mol) > C–H (453 kJ/mol) > C–Br (290 kJ/mol) > C–I (228 kJ/mol). Among the four halogens, fluorine is the most electrono- negative and iodine the least. The polarization of the C–I bond is lower than that of the C–H bond and the other carbon–halogen bonds. Because iodine is a good leaving atom and because of the chemical reactivity of the C–I bond, it would be easier for PFIs to be converted to their hydroxylated analogs during the exposure studies. Oxidation of FIAs can result in the formation of PFCAs (Lehmler 2005). In this reaction, C,Fn+2OH is thought to have been formed by the cleavage of C–I bonds in FIAs and addition of OH (Yamamoto et al. 2007). We hypothesize that C,Fn+2OH is hydrolyzed to C,Fn+1OH in the culture media or inside the cells, and the degradation products or the metabolites of PFIs are the possible targets for ER, thereby exerting estrogenic activity. However, the underlying mechanisms for the estrogenic effects of PFIs have not been clearly established, and further studies are warranted to characterize possible catabolites of PFIs which might also exhibit estrogenic activity.

The main functions of hormones are to maintain homeostasis and regulate reproduction and development. Exposure to endocrine-disrupting chemicals may cause adverse effects to the organs and glands that secrete hormones, further resulting in endocrine toxicity such as impaired reproduction and development. PFIs are volatile chemicals and have been detected around fluoroc hemical manufacturing areas (Ruan et al. 2010a). As important precursors for the synthesis of organic fluoride products, PFIs could be incorporated into fluorotelomer raw materials and fluorotelomer-based products as residues (Larsen et al. 2006). Occupational and indoor environments might be exposure risk zones, and inhalation could be a possible exposure route.

Conclusion

Some PFIs could act on ERs and potentially cause detrimental effects on reproductive and developmental systems. To our knowledge, this is the first study to find estrogenic activity of PFIs using three in vitro methods. Considering the current large and increasing production volume of telomerization-based PFCs, more extensive studies should be conducted on the environmental distribution and toxicological effects of PFIs.

References

Blair JM, Crews D, Mcchlavan JH. 1994. PCBs as environmental estrogens—turtle sex determination as a biomarker of environmental contamination. Environ Health Perspect 102:710–711.

Blair RM, Fang H, Branham WS, Hass BS, Dial SL, Moland CL, Brown AMC, Jeltsch JM, Roberts M, Chambon P. 1984. Estrogenic activity in MVLN cells. However, it is questionable whether these PFIs are able to directly bind to and activate the ER. Structural features such as a phenol ring and a hydrophobic group attached para to the hydroxyl group are essential for the estrogenic effects (Blair et al. 2006; Laws et al. 2006; Suzuki and Shapiro 2007). Furthermore, hydroxylated analogs of polybrominated diphenyl ethers and polychlorinated biphenyls have been shown to exert estrogenic effects (Bergeron et al. 1994; Fielden et al. 1997; Meerts et al. 2001). Therefore, it may be reasonable to expect that hydroxylated forms of FIAs and FDIAs could also be estrogenic.

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Polyfluorinated iodine alkanes exhibit estrogenic effects in vitro

Suzuki T, Shapiro S. 2007. Predicting relative binding affinities for the ER-alpha estrogen receptor from molecular structure [Abstract]. J Pharm Pharmacol 59:A8.
Terasaka S, Aita Y, Inoue A, Hayashi S, Nishigaki M, Aoyagi K, et al. 2004. Using a customized DNA microarray for expression profiling of the estrogen-responsive genes to evaluate estrogen activity among natural estrogens and industrial chemicals. Environ Health Perspect 112:773–781.
Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, et al. 1998. Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104(suppl 6):741–783.
Upham BL, Decampmo ND, Wurl B, Trosko JE. 1998. Inhibition of gap junctional intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. Int J Cancer 78(4):491–495.
Villeneuve DL, Blankenship AL, Giesy JP. 2000. Derivation and application of relative potency estimates based on in vitro bioassay results. Environ Toxicol Chem 19(11):2835–2843.
Yamamoto T, Noma Y, Sakai SI, Shibata Y. 2007. Photodegradation of perfluorooctane sulfonate by UV irradiation in water and alkaline 2-propanol. Environ Sci Technol 41(16):5660–5665.
Young CJ, Hurley MD, Wallington TJ, Mabury SA. 2008. Atmospheric chemistry of 4:2 fluorotelomer iodide (n-C4F9CH2CH2I): kinetics and products of photolysis and reaction with OH radicals and Cl atoms. J Phys Chem A 112(51):13542–13548.