Perfluorooctyl Iodide Stimulates Steroidogenesis in H295R Cells via a Cyclic Adenosine Monophosphate Signaling Pathway

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S Supporting Information

ABSTRACT: Perfluorinated iodine alkanes (PFIs) are used widely in the organic fluorine industry. Increased production of PFIs has caused environmental health concerns. To evaluate the potential endocrine-disrupting effect of PFIs, we investigated the effects of perfluorooctyl iodide (PFOI) on steroidogenesis in human adrenocortical carcinoma cells (H295R). Levels of aldosterone, cortisol, 17 β -estradiol, and testosterone were measured in H295R culture medium upon treatment with perfluorooctanoic acid (PFOA) and PFIs. Expression of 10 steroidogenic genes (*StAR*, *HMGR*, *CYP11A1*, *3 β HSD2*, *17 β HSD*, *CYP17*, *CYP21*, *CYP11B1*, *CYP11B2*, and *CYP19*) was measured by real-time polymerase chain reaction. Levels of cyclic adenosine monophosphate (cAMP) and adenylate cyclase (AC) activity were measured to understand the underlying mechanism of steroidogenic perturbations. Levels of production of aldosterone, cortisol, and 17 β -estradiol were elevated significantly, and the level of testosterone generation decreased upon treatment with 100 μ M PFOI. Similar to the effect induced by forskolin (AC activator), expression of all 10 genes involved in the synthesis of steroid hormones was upregulated significantly upon exposure to 100 μ M PFOI. PFOA had no effect on steroid hormone production or steroidogenic gene expression even though it is highly structurally similar with PFOI. Therefore, the terminal -CF₂I group in PFOI could be a critical factor for mediation of steroidogenesis. PFOI increased AC activity and cAMP levels in H295R cells, which implied an underlying mechanism for the disturbance of steroidogenesis. These data suggest that PFOI may act as an AC activator, thereby stimulating steroidogenesis by activating a cAMP signaling pathway.

INTRODUCTION

Perfluorinated chemicals (PFCs) are used widely in industry as surfactants, water-resistant materials, and food packaging because of their unique surfactant properties and chemical stability. The environmental health risk of these man-made chemicals is based on their long-term production and ubiquitous distribution in the environment and wildlife.^{1–4}

PFIs are important intermediates in the synthesis of various types of fluorinated products and other fluorinated intermediates, such as fluorotelomer alcohols (FTOHs). As a result of high-energy consumption and side reactions in electrochemical fluorination processes, production of perfluorinated raw materials is based almost entirely upon telomerization reactions.⁵ Because of the increasing market demand for fluoride products, production of FTOHs has reached 12×10^6 kg/year, and the estimated annual production of PFIs is ≈ 4000 t.⁶

FTOHs are widely distributed throughout the North American troposphere with mean concentrations ranging from 11 to 165 pg/m³.⁷ Numerous studies have shown that biodegradation and oxidation of FTOHs can contribute to the

environmental burden and worldwide distribution of PFCs, such as perfluorocarboxylic acids.^{8–10} PFIs have also been detected in the soil and atmosphere near an industrial facility that makes fluorinated chemicals in Shandong Province (northern China).^{11,12}

Production of perfluorooctyl iodide [PFOI (Figure 1)] has increased in recent years because of its great commercial value. Studies have shown that the concentration of PFOI is relatively higher in the air and soil than the concentrations of the other derivatives of PFIs.¹¹ In addition, degradation of PFIs can cause formation of other PFCs, thereby resulting in environmental behaviors similar to those of FTOHs. Release of PFIs may increase the environmental burden of PFCs.

Recent studies have shown the endocrine-disrupting effects of FTOHs and PFIs in various *in vitro* or *in vivo* models. FTOHs and PFIs stimulate the proliferation of MCF-7 cells and increase the level of expression of the estrogen-responsive gene TFF1.¹³

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Figure 1. Structures of PFOI and PFOA.

The yeast two-hybrid assay and reporter gene assay have further demonstrated the estrogenic effects of these chemicals.^{14,15} Furthermore, FTOHs and PFI have been shown to elicit expression of the estrogen-responsive gene vitellogenin through activation of estrogen receptors in male medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*).^{16–18} Perfluorooctanoic acid (PFOA) has been shown to induce vitellogenin expression in the liver and disturb the development of gonads and reproduction of the female rare minnow (*Gobiocypris rarus*).¹⁹

An *in vitro* assay system based on human adrenocortical carcinoma cells (H295R) has been developed for screening the endocrine-disrupting effects of toxicants.^{20–22} H295R cells maintain the physiologic characteristics of undifferentiated fetal adrenal cells and can produce mineralocorticoids, glucocorticoids, and sex hormones *in vitro*.²³ H295R cells express all the genes involved in steroidogenesis.²⁴ The measurement methods of the genes involved in steroidogenesis and hormone production have been optimized by several laboratories and standardized by the Environmental Protection Agency. Evaluation of the endocrine-disrupting effects of persistent organic pollutants has been conducted using different systems.

The risk of exposure to chemicals of the endocrine and reproductive systems has been the focus of much research. Bisphenol A, polybrominated diphenyl ethers, and their metabolites disturb hormone production by affecting the genes in the steroidogenic pathway and exhibit endocrine-disrupting effects in H295R cells.^{25–27} Exposure to perfluorooctanesulfonic acid and PFOA does not significantly change hormone production or expression of steroidogenic genes. However, the estrogenic compounds 8:2 fluorotelomer alcohol and 6:2 fluorotelomer alcohol decrease the levels of hormone production and expression of steroidogenic genes by reducing cellular levels of cyclic adenosine monophosphate (cAMP) and inhibiting adenylate cyclase (AC) activity.²⁸ Such studies have suggested the importance of nonreceptor pathways in the regulation of the endocrine system. Also, this *in vitro*-based method has been confirmed for the study of the effects of endocrine disruption and validated by the Organization for Economic Co-operation and Development to replace testis explant assays using rodents.^{21,29}

Here, we studied the effects of PFOI on expression of all steroidogenic genes and production of four hormones to ascertain the modulation of steroidogenesis and endocrine-disrupting effects of PFOI. To investigate the potential toxicity mechanisms of steroidogenesis, cAMP levels and AC activity were examined to elucidate the steroidogenic pathway.

MATERIALS AND METHODS

Chemicals. PFOI (purity, 98%) was obtained from Alfa Aesar (Ward Hill, MA). Forskolin was purchased from Sigma-Aldrich (St. Louis, MO). PFOA (purity, 98%) was obtained from Fluka (Buchs, Switzerland). PFOI and forskolin were dissolved in ethanol as 100 mM stock solutions and stored at -20°C .

Cell Culture and Exposure. The H295R human adrenocortical carcinoma line was obtained from American Type Culture Collection (ATCC CRL-2128, ATCC, Manassas, VA). Cells were maintained in

Dulbecco's modified Eagle's medium (DMEM/F-12, Hyclone, Logan, UT) containing 1% insulin-transferrin-selenium-G (Gibco, Grand Island, NY), 1% penicillin-streptomycin (Gibco), and 2.5% Nu-Serum (BD Bioscience, Bedford, MA).

Cells were cultured in 100 mm culture dishes in a humidified atmosphere of 5% CO_2 at 37°C . Cells were used between passages 5 and 10. The exposure medium consisted of phenol red-free DMEM/F-12 medium (Hyclone) supplemented with 1% insulin-transferrin-selenium-G, 1% penicillin-streptomycin, and 2.5% charcoal-stripped fetal bovine serum (Hyclone). Cells were seeded in culture plates, starved in exposure medium for 24 h to minimize the disturbance of steroid hormones in the culture medium, and then treated with test chemicals. Cytotoxicities of 5, 25, 50, 100, and 125 μM PFOA and PFOI were tested with a Cell Viability MTS kit (Promega, Madison, WI) following manufacturer's instructions. This method is based on reduction of a MTS tetrazolium compound by viable cells to generate a colored formazan product that is soluble in cell culture medium. The formazan dye produced by viable cells was quantified by measuring the absorbance at 490 nm. For each well of the 96-well assay plate, 20 μL of the MTS solution was added to 100 μL of culture medium. After incubation for 4 h in a humidified atmosphere of 5% CO_2 at 37°C , the absorbance at 490 nm was measured using a 96-well plate reader. Three replicate wells were used in each experiment. On the basis of the results of cytotoxicity, 25 and 100 μM PFOI and PFOA were used in subsequent exposure assays.

Hormone Measurement. Cells (1×10^6 cells/well) were seeded in six-well plates with 2.5 mL of culture medium in each well. After exposure for 48 h, the medium was collected for detection of steroid hormones. Aldosterone, cortisol, testosterone, and 17β -estradiol (E2) were measured using radioimmunoassay kits (Beijing North Institute of Biological Technology, Beijing, China) according to the manufacturer's instructions and measured using a liquid scintillation counter (XH6080, Xian Nuclear Instruments, Xian, China). Three replicate wells were used in each experiment. Limits of detection were 50 pg/mL, 10 ng/mL, 0.1 ng/mL, and 10 pg/mL for aldosterone, cortisol, testosterone, and E2, respectively. Interassay coefficients of variation were $<10\%$.

RNA Isolation and Real-Time Polymerase Chain Reaction (PCR). Cells were rinsed with phosphate-buffered saline. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Absorbance readings at 260 and 280 nm of total RNA were taken using a Nanodrop-2000 spectrophotometer (Thermo Scientific, Waltham, MA). The RNA concentration was determined by the reading at 260 nm. A 260 nm to 280 nm ratio for RNA samples of >1.8 was qualified for cDNA synthesis. Total RNA (2 μg) combined with 0.5 μg of Oligo (dT)15 in nuclease-free water in a final volume of 15 μL was employed. Reaction mixtures were denatured at 70°C for 5 min and cooled on ice immediately. Then, 1.5 μL of dNTP Mix (Promega), 0.5 μL of RNase Inhibitor (Thermo Scientific), 5 μL of M-MLV RT Buffer, 1 μL of MMLV Reverse Transcriptase (Promega), and 2 μL of nuclease-free water were added. Reaction mixtures were incubated at 42°C for 60 min and then reactions terminated at 87°C for 1 min in a PCR machine. The final cDNA solution was diluted 5-fold with DNase/RNase-free water (Gibco). Quantitative PCR was undertaken with a Stratagene MX3005 thermal cycler (Stratagene, La Jolla, CA). PCR reaction mixtures (25 μL) contained 12.5 μL of GoTaq Green Master Mix (Promega), 2 μL of diluted cDNA, and 0.2 μM sense/antisense primers. Primer sequences for genes in this study have been described previously (Table S2 of the Supporting Information).²¹ The thermal cycle was 2 min at 95°C followed by 45 cycles of 15 s at 95°C , 30 s at 59°C , and 30 s at 72°C . Quantification of expression of target genes was based on a comparative cycle threshold (Ct) value. Expression

of each target gene was normalized to its reference gene *prophobinogen deaminase* (PBGD). The fold change of target genes was analyzed using the $2^{-\Delta\Delta C_t}$ method.³⁰ Analyses of melting curves and agarose gel electrophoresis were used to confirm that the correct PCR products were present.

Quantification of Cellular cAMP. Cells were seeded in the six-well plates at a density of 1×10^6 cells/well and cultured for 24 h. Then, they were exposed for 24 h to 25 and 100 μ M PFOI and PFOA, respectively. Cells were collected to measure cAMP content using a cAMP ELISA kit (Cayman Chemical Co., Ann Arbor, MI) in accordance with the manufacturer's instructions. Cell samples and cAMP standards were acetylated in these processes. The absorbance was measured at 410 nm by a microplate reader (Varioskan Flash, Thermo Scientific). The cAMP content was calculated by comparison with a standard curve. Three replicate wells were used in each experiment.

AC Activity. H295R cells (3×10^6 cells/well) were seeded in 60 mm culture dishes and cultured for 24 h. After exposure for 24 h, preparations of cell membranes and measurement of AC activity were undertaken following a method described previously.³¹ Cells were homogenized in 1 mL of suspension buffer [20 mM HEPES, 0.25 mM sucrose, 1 mM EDTA, 5 mM benzamidine, and a protease inhibitor cocktail tablet from Roche (Basel, Switzerland)]. Cell membranes were isolated by two centrifugation steps (1000g for 5 min at 4 °C and 4000g for 20 min at 4 °C), and the protein content was determined using the Bradford assay.³² Then, 30 μ g of membrane protein was added to reaction buffer [50 mM Tris-HCl (pH 7.4), 5.0 mM MgCl₂, 0.5 mM EDTA, 1 mM ATP, 0.1 mM GTP, 0.2 IU pyruvate kinase, 0.1 unit of myokinase, and 2.5 mM phosphoenolpyruvate] and incubated at 37 °C for 15 min. Converted cAMP content denoted AC activity and was determined by the ELISA kit. Three replicate wells were used in each experiment.

Statistical Analyses. All statistical analyses were undertaken using Sigma Plot version 10.0 (Systat, San Jose, CA). Results are means $\pm$ the standard deviation. One-way analysis of variance (ANOVA) and Tukey's test were employed to assess the significance of mean differences. $p < 0.05$ was considered significant.

RESULTS

Viability of H295R Cells. Exposure of H295R cells to PFOI (<100 μ M) and PFOA (<100 μ M) did not cause significant toxicity within the concentration ranges tested ($p > 0.05$). However, 125 μ M PFOI and PFOA induced significant toxicity in H295R cells [$p < 0.05$ (Figure S1 of the Supporting Information)]. No obvious morphological alterations were observed in the cells treated with PFOI or PFOA at levels lower than 100 μ M upon microscopic examination.

Effects on the Production of Steroid Hormones. PFOI (25 μ M) and PFOA (100 μ M) did not change the production of any steroid hormone (Figure 2). Upon treatment with 20 μ M forskolin, levels of production of aldosterone, cortisol, E₂, and testosterone were increased significantly by 3.4-, 2.64-, 2.91-, and 2.07-fold, respectively. Exposure to 100 μ M PFOI increased the levels of production of aldosterone, cortisol, and E₂ by 2.3-, 1.73-, and 2.16-fold, respectively, but decreased the level of production of testosterone by 2.1-fold, in H295R cells.

Effects on Expression of Steroidogenic Genes. Ten genes involved in steroidogenesis were evaluated by real-time PCR. Exposure to 100 μ M PFOI and 20 μ M forskolin altered gene expression in a consistent manner (Figure 3). Expression of *StAR*, *CYP11A1*, *3 β HSD2*, *CYP17*, *CYP21*, *CYP11B1*, *CYP11B2*, and *CYP19* was significantly upregulated by 100 μ M PFOI (changes of 2.43-, 4.62-, 5.91-, 12.2-, 6.84-, 5.62-, 5.24-, and 8.71-fold, respectively) and 20 μ M forskolin (2.93-, 4.62-, 4.46-, 14.2-, 12.5-, 3.62-, 5.24-, and 5.12-fold, respectively). Such fold changes were comparable. Exposure to 100 μ M PFOI upregulated expression of *HMGR* and *17 β HSD* by 1.83- and 2.39-fold,

Figure 2. Effects of test chemicals on production of steroid hormones in H295R cells: (A) aldosterone, (B) cortisol, (C) estradiol, and (D) testosterone. Results are means $\pm$ SD of three replicate samples. Each sample was measured twice in one representative experiment. Compared with the control, * $p < 0.05$ and ** $p < 0.01$. ANOVA and Tukey's multiple-range test were used to assess the significance of mean differences.

respectively, but forskolin did not alter expression of these genes. Exposure to 25 μ M PFOI increased the level of expression of *CYP11B2* and *CYP17* by 1.42- and 1.64-fold, respectively. There were no significant effects on expression of steroidogenic genes upon exposure to 100 μ M PFOA ($p > 0.05$).

Effects on the Activity of cAMP and AC. Forskolin is used to increase the cellular level of cAMP by direct activation of AC in cells. The second-messenger cAMP has a pivotal role in diverse cellular functions. To investigate the mechanism of stimulation on steroidogenesis caused by PFOI, we assessed cAMP content and AC activity in H295R cells upon treatment with PFOI, PFOA, and forskolin.

Exposure to 20 μ M forskolin increased AC activity and cAMP levels dramatically by 2.72- and 3.75-fold, respectively, compared with the control (Figure 4). Exposure to 100 μ M PFOI increased cAMP content (2.1-fold) and induced AC activity (1.7-fold) significantly. Exposure to PFOA and PFOI at 20 μ M did not change cAMP content, AC activity, expression of steroidogenic genes, or hormone production.

Figure 3. Effects of test chemicals on mRNA expression of 10 steroidogenic genes (*StAR*, *HMGR*, *CYP11A*, *3βHSD2*, *17βHSD*, *CYP17*, *CYP21*, *CYP11B1*, *CYP11B2*, and *CYP19*) in H295R cells. (A) Cells were exposed to 25 or 100 μM PFOI for 48 h. (B) Cells were exposed to 100 μM PFOA or 20 μM forskolin for 48 h. Results are means $\pm$ SD of three replicate samples. Each sample was measured thrice in one representative experiment. Compared with the control, * $p < 0.05$ and ** $p < 0.01$. ANOVA and Tukey's multiple range test were used to assess the significance of mean differences.

Figure 4. Effects of test chemicals on (A) cellular cAMP content and (B) AC activity in H295R cells. Results are means $\pm$ SD of three replicate samples in one representative experiment. Compared with the control, * $p < 0.05$ and ** $p < 0.01$. ANOVA and Tukey's multiple range-test were used to assess the significance of mean differences.

DISCUSSION

In this study, significant stimulation of steroidogenesis was observed in H295R cells exposed to PFOI, as demonstrated by an increased level of production of steroid hormones. Mechanisms of activation involved upregulation of expression of steroidogenic genes, increases in cellular levels of cAMP, and AC activity.

Each steroidogenic gene controls one or more steps of steroid synthesis. Regulation of expression of these genes eventually determines hormone production. Upregulation of expression of steroidogenic genes suggests that PFOI would probably affect

the synthesis of sex steroid hormones. Levels of production of aldosterone, cortisol, and E2 were increased significantly upon treatment with 100 μM PFOI, but the level of testosterone production was decreased 2-fold. Exposure to 25 μM forskolin stimulated production of all four hormones significantly.

These results suggested that PFOI would probably interfere with steroidogenic processes, thereby stimulating production of aldosterone, cortisol, and E2. To understand the potential mechanisms of PFOI upon hormone production, we measured the transcription of steroidogenic genes.

PFOI induced expression of all steroidogenic genes significantly. In general, transcription levels of *StAR* and *HMGR* mRNA are not altered markedly.^{33,34} The *HMGR* enzyme catalyzes the rate-limiting reaction of cholesterol synthesis, so PFOI could be used to determine the efficiency of steroidogenesis by monitoring of this initial step. *CYP11A*, *CYP11B1*, *CYP11B2*, *CYP17*, and *CYP21* enzymes catalyze the synthesis of cortisol and aldosterone, starting with cleavage of cholesterol. *CYP11A* is responsible for the conversion of cholesterol to pregnenolone. Then, progesterone is catalyzed sequentially by *CYP21*, *CYP11B1*, and *CYP11B2* to produce aldosterone, and cortisol is converted from 17 α -OH-progesterone by *CYP21* and *CYP11B1*. The final rate-limiting step of the synthesis of cortisol and aldosterone is regulated by *CYP11B1* and *CYP11B2*, respectively, and upregulation of expression of *CYP11B1* or *CYP11B2* corresponds with an increase in the level of production of cortical hormones.^{35–37}

Likewise, some contaminants, such as bromophenol, bromobiphenyls, and bromodibenzo-*p*-dioxin, induce significant expression of *CYP11B2*.^{38,39} Among these steroidogenic genes, expression of *CYP11B2* and *3βHSD2* was upregulated dramatically by PFOI and forskolin (*CYP11B2* expression was upregulated by ~12-fold). Likewise, expression of *CYP11A*, *CYP11B1*, *CYP17*, and *CYP21* was upregulated upon PFOI treatment. An increasing level of expression of these genes may result in an increased level of production of cortisol and aldosterone. Alteration of expression of these hormones may change physiologic functions such as the homeostasis of blood pressure, obesity regulation, and the synthesis of carbohydrates and proteins *in vivo*.⁴⁰ These results suggest that PFOI may exhibit endocrine-disrupting effects by altering the production of cortical hormones.

3βHSD2 is responsible for the oxidation and isomerization of 5-ene-3 β -hydroxy steroids to 4-ene-3-ketosteroids as an intermediate step in the synthesis of estrogens, androgens, aldosterone, and cortisol.⁴¹ PFOI could activate the synthesis of sex hormones by promoting upregulation of expression of *3βHSD2*, *CYP17*, and *17βHSD*, which control the intermediate step of steroidogenesis. Our studies also suggested that induction of transcription of *CYP17* and *CYP19* would probably affect the synthesis of sex hormones and change production of E2 and testosterone. Exposure to PFOI and forskolin upregulated *CYP19* expression, so E2 production was increased significantly. However, forskolin increased testosterone production, whereas PFOI decreased it by 2-fold. Regulation of *17βHSD* expression by forskolin and PFOI was also quite different. Such discrepancies in gene expression could be attributed to the basic chemical characteristics and different signaling pathways they activate in H295R cells. The *CYP19* enzyme catalyzes the conversion of androgens to estrogens, which is involved in behavior, reproduction, and development *in vivo*. Hence, alteration of *CYP19* expression by chemicals may result in endocrine-disrupting effects.⁴²

To further understand the endocrine-disrupting effects of PFOI, cAMP levels and AC activity were studied to assess the potential mechanism of action. The cAMP/protein kinase A (PKA) signaling cascade has an important role in regulation of steroidogenesis. AC activation induces cAMP production, which activates a PKA signaling pathway. Synthesis of steroid hormones is mediated by cellular levels of cAMP. High levels of cellular cAMP suggest stimulation of steroidogenesis in H295R cells.⁴³ Steroidogenic genes such as *StAR*, *CYP11A*, *CYP11B*, *CYP17*, and *CYP21* are dependent upon cAMP. During steroidogenesis, transport of cholesterol to the inner mitochondrial membrane is the rate-limiting step, and *StAR* controls this step.^{44,45} Forskolin is an activator of the cAMP/PKA pathway and inducer of steroidogenesis. In H295R cells, *StAR* expression is upregulated by trophic hormones, forskolin, or cAMP analogues through a cAMP second-messenger pathway.⁴⁶ cAMP is an important second messenger for the synthesis of trophic hormone-stimulated steroids. In the study presented here, PFOI increased cAMP levels by ~2-fold and forskolin increased cAMP levels significantly by 2-fold. Stimulation of steroidogenesis by forskolin occurs via AC activation. Then, increasing intracellular levels of cAMP result in PKA activation. Thus, PKA stimulates steroidogenesis and expression of steroidogenic genes.

Expression of *StAR* protein is regulated by cAMP/PKA-dependent mechanisms. However, the cAMP response element is not located in the *StAR* promoter region, suggesting that other transcription factors, such as SF-1, AP-1, C/EBP, and DAX-1, are involved in the regulation of cAMP responsiveness and *StAR* expression.⁴⁷ However, in the study presented here, PFOA did not change *StAR* expression, cAMP levels, or AC activity. Hence, the -CF₂I functional group may play an important part in stimulatory effects. We could conclude that PFOI activates AC, increases cAMP levels, and upregulates *StAR* expression and eventually stimulates steroidogenesis in H295R cells.

This study demonstrates that modulation of steroidogenic genes by PFOI could interfere further with production of steroid hormones and induce endocrine-disrupting effects. Expression of steroidogenic genes was altered significantly by PFOI, but measurement of the protein content and activities of these steroidogenic enzymes clearly elucidated the toxicity of perfluorinated chemicals.

CONCLUSIONS

We investigated the effects of PFOI on steroidogenesis and the potential mechanism of action. PFOI could stimulate hormone production in H295R cells. These effects are probably mediated by activation of steroidogenic genes via increasing cellular levels of cAMP. AC activation by PFOI appears to be the mechanism for increasing cAMP levels. Further *in vivo* studies are needed to assess the potential health risks of PFOI.

ASSOCIATED CONTENT

Supporting Information

Endocrine-disrupting effects of PFOI (schematic) (Table S1), primer sequences of steroidogenic genes used in this study (Table S2), and cytotoxicities of PFOA and PFOI (Figure S1). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/tx5004563.

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Author Contributions

C.W. and T.R. performed the experiments. Q.Z., J.L., and B.H. designed the projects and revised the manuscript. C.W. prepared the manuscript. G.J. directed the project.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

PFI, perfluorinated iodine alkanes; PFOI, perfluorooctyl iodide; H295R, human adrenocortical carcinoma; PFOA, perfluorooctanoic acid; PFCs, perfluorinated chemicals; FTOH, fluorotelomer alcohols; cAMP, cyclic adenosine monophosphate; AC, adenylate cyclase; E2, 17 β -estradiol; PKA, protein kinase A; CYP11A, side-chain cleavage enzyme; CYP11B2, aldosterone synthetase; CYP17, steroid 17 α -hydroxylase/17,20-lyase; CYP19, aromatase; CYP21, steroid 1-hydroxylase; 3 β HSD2, 3 β -hydroxysteroid dehydrogenase isomerase; 17 β HSD, 17 β -hydroxysteroid dehydrogenase; *StAR*, steroidogenic acute regulatory protein; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt

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