Verification of In Vivo Estrogenic Activity for Four Polyfluoroalkyl Substances (PFAS) Identified as Estrogen Receptor Agonists in High-Throughput Screening

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Introduction

- Per- and polyfluoroalkyl substances (PFAS) are a large class of fluorinated organic chemicals of concern due to their broad occurrence and persistence in humans and the environment and potential health effects.
- In response to these concerns, over 140 PFAS were screened for 81 different transcription factor activities in two multi-factorial transactivation assays. Over 40 distinct PFAS structures including perfluorooctanoic acid (PFOA) and many less studied PFAS showed activity against the estrogen receptor (ER).
- Most PFAS compounds screened were partial agonists with maximum efficacy less than that of 17β-estradiol (E2), with 1H,1H,8H,8H-Perfluorooctane-1,8-diol (FC8-diol), 1H,1H,10H,10H-Perfluorodecane-1,10-diol (FC10-diol), and 1H,1H,8H,8H-perfluoro-3,6-dioxaoctane-1,8-diol (PFDOD) identified as notable exceptions that displayed full agonist activity.

Objective:

To evaluate whether the ER agonist activity detected through *in vitro* high-throughput screening would translate into estrogen-dependent effects in fish *in vivo*.

Methods

- Four ER-active and one ER-inactive PFAS were selected based on activity in several *in vitro* assays (e.g., ATG_ERa_TRANS_UP, ATG_ERE_cis_UP, ACEA_ER [T47D proliferation]) to further evaluate estrogenic *in vivo* effects in fish.
- Adult male fathead minnows (*Pimephales promelas*) were exposed for four days to:
  - Perfluorooctanoic acid (PFOA)
  - 1H,1H,8H,8H-Perfluorooctane-1,8-diol (FC8-diol)
  - 1H,1H,10H,10H-Perfluorodecane-1,10-diol (FC10-diol)
  - 1H,1H,8H,8H-perfluoro-3,6-dioxaoctane-1,8-diol (FC8-DOD)
  - Hexafluoropropylene oxide dimer acid (HFPO-DA, commonly called GenX) - negative control for ER response
- Each in vivo exposure included a positive control, 17β-estradiol (E2, 70-140 ng/L), for estrogen-dependent effects.
- Five concentrations, with the maximum target concentration set at either 50x (FC8-diol, FC10-diol) or 5x (PFDOD and PFOA) the 50% activity concentration (AC50) of the *in vitro* assays were tested. The maximum target concentration of HFPO-DA was set to 20X the AC50 for PPARα activity in the Attagene assay.
- Liver tissues were collected and expression of four genes known to be modulated by estrogen exposure (Feswick et al., 2017) were evaluated by quantitative real-time polymerase chain reaction (QPCR):
  - Up-regulated:
    - *Vitellogenin (vtg)*
    - *Estrogen receptor-α (esr1)*
  - Down-regulated:
    - *Apolipoprotein Eb (apoeb)*
    - *Insulin-like growth factor 1 (igf1)*

Analytical Chemistry

- Water samples were collected daily for analytical verification of PFAS compounds and positive control (E2).
- Liquid Chromatography-Mass Spectrometry (LC-MS; Agilent 6410)

Statistical Analysis

- Analyzed using a one-way ANOVA, followed by Duncan's or Dunn's post-hoc test using Statistica software
- Differences considered significant at $p < 0.05$

Exposure Details	
Fish species	*Pimephales promelas*
Tanks per treatment	2
Fish per tank	6 males
Exposure duration	4 days
Samples collected	Plasma (vtg, PFAS residues) Liver (QPCR) Gonad (GSI) Tubercle scores Carcass (PFAS residues)

Results

Gene Expression: *vtg* *esr1* *apoeb* *igf1*

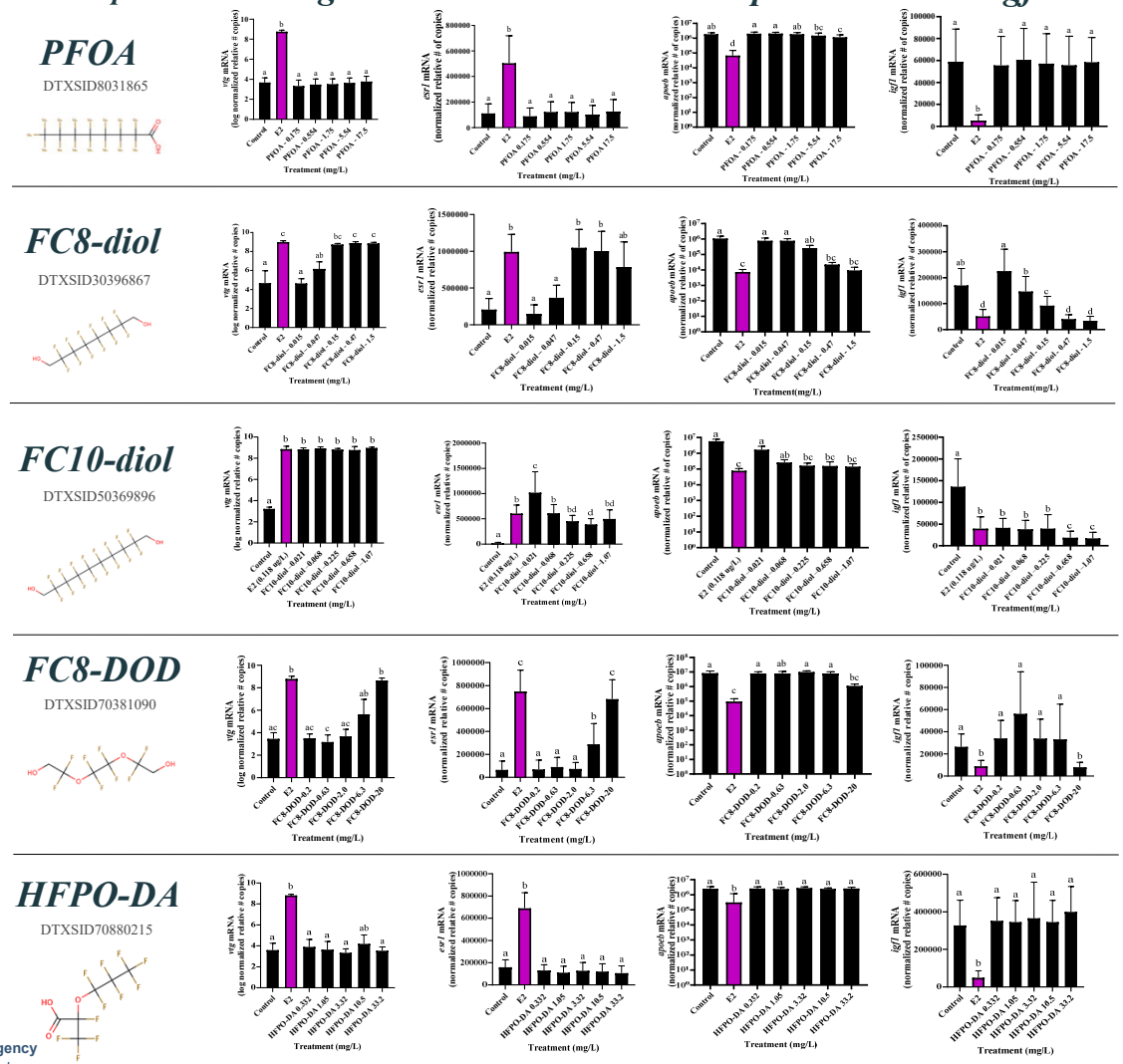

Figure 1. Relative abundance of estrogen-modulated gene transcripts (*vtg, esr1, apoeb, igf1*) in livers of male fathead minnows exposed for 4 d to five PFASs (PFOA, FC8-diol, FC10-diol, FC8-DOD, and HFPO-DA). Purple bars = E2 positive control (70-140 ng/L). Different letters indicate statistically significant differences between all treatment groups within a study ($p<0.05$).

Potencies

Table 1. *In vitro* potencies of five PFAS compounds relative to ER activation in the T47D cell proliferation assay and Attagene (ATG) ERa-trans and ERE-cis assays, as well as *in vivo* lowest observed effect concentrations (LOEC) for gene expression data.

Compound	T47D prolif	ATG mean AC50 (μM)	Rel to FC8-diol (ATG only)	Mean AC50 equiv mg/L	*In vivo* LOEC (mg/L)
FC8-diol	0.2955	0.079	1	0.0286	0.15
FC10-diol	0.1974	0.15	2.00	0.0912	0.021
PFOA	Not active	8.25	110	3.42	≥ 17.5
FC8-DOD	22.25	13.2	166	3.98	6.3
HFPO-DA	Not active	Not active	N/A	N/A	> 33

Discussion

- *In vitro* results were predictive of *in vivo* estrogenic activity.
- Of the four ER-active PFAS tested, FC8-diol and FC10-diol were the most potent compounds both *in vitro* and *in vivo*.
  - 0.15-1.5 mg FC8-diol/L caused induction of *vtg* and *esr1* expression and suppression of *igf1* and *apoeb* at magnitudes similar to those elicited by the E2 positive control.
  - All concentrations tested for FC10-diol resulted in significant up-regulation of *vtg* and *esr1* compared to the controls. Significant down-regulation of *apoeb* and *igf1* was observed at concentrations ≥ 0.021-0.225 mg/L.
- PFOA and FC8-DOD were the least potent *in vitro* as well as *in vivo*.
  - PFOA did not elicit impacts on gene expression consistent with those observed for the positive control (E2). In vivo estrogenic activity not definitive at concentrations tested (up to 17.5 mg/L).
  - FC8-DOD elicited gene expression effects consistent with the E2 positive control only at the highest concentration, 20 mg/L.
- Consistent with the *in vitro* ER data, HFPO-DA did not elicit any ER-modulated *in vivo* effects.
- The use and ecological relevance of FC8-diol, FC10-diol, and FC8-DOD are unknown.

Conclusions:

- These data suggest that at least some novel PFAS are able to elicit effects *in vivo* consistent with exposure to a steroidal estrogen.
- These results demonstrate that additional information regarding the use of these compounds is needed to better characterize the potential ecological risks associated with their use. Further evaluation is needed to understand the potential for environmental release and if environmental exposures could reach concentrations sufficient to elicit adverse effects associated with estrogenic endocrine disruption.

Acknowledgements/References

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Mention of products or trade names does not indicate endorsement by the U.S. federal government. The contents of this poster do not necessarily reflect U.S. EPA policy.

References

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