# Estrogen-like properties of fluorotelomer alcohols as revealed by MCF-7 breast cancer cell proliferation .

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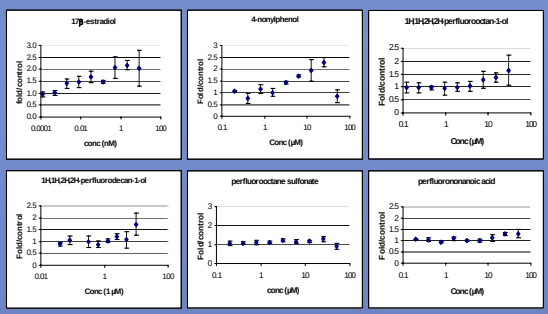
## INTRODUCTION

**During past years, major research efforts in environmental health sciences have been devoted to the development of easy to perform and reliable *in vitro* bio-assays. The need for latter assays is very urgent, since the number of chemicals that needs toxicological screening is enormous. *In vitro* cell based assays have already shown their usefulness for studying, for instance, endocrine disrupting chemicals. MCF-7 breast cancer cells are used for the detection of estrogen-like compounds. This project started with the well known 'E-screen assay', in which chemicals are tested for their capacity to re-induce the proliferation of growth arrested breast cancer cells (in estrogen-free growth medium). We analyzed diverse perfluorinated compounds, such as perfluorosulfonate (PFOS), perfluorononanoic acid (PFNA), perfluorooctanoic acid (PFOA), and the fluorotelomer alcohols 1H,1H,2H,2H-perfluorooctan-1-ol (6:2 FTOH) and 1H,1H,2H,2H-perfluoro-decan-1-ol (8:2 FTOH)**

## METHODOLOGY

### 1. The E-screen assay

**The proliferation inducing capacity of chemicals was tested using the E-screen assay according to Payne et al. (2000), with minor modifications to the protocol. MCF-7 cells were incubated in phenol red-free DMEM supplemented with estrogen-free bovine serum with 17-β estradiol or test compounds during 6 days. Incubation with 0.1% DMSO alone was performed as control. After 6 days, cell numbers were determined by the CyQuant assay to measure cell proliferation. The proliferative effect is expressed as fold over control (= the ratio between the cell yield obtained with the test chemical and with the hormone-free control). Results are expressed as means +/- S.D. of three replicates for each data point Cells were then exposed to chemicals during 6 days.**

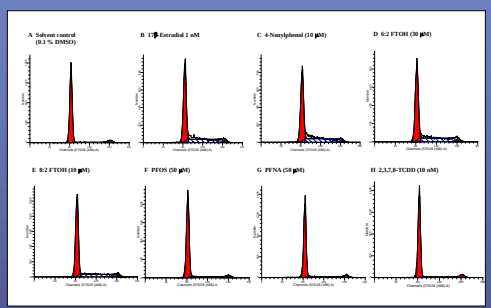



### 2. Flow cytometry analysis of cell cycle

**Flow cytometric analysis of cell cycle distribution and apoptosis was performed with a LSRII flow cytometer with a 488-nm argon-ion laser (Becton Dickinson, San Jose, CA). PI fluorescence was collected at BP 575/25 nm (FL2, red fluorescence channel) in the linear mode. For each measurement, data from 10,000 single cell events were collected, while cell aggregates and doublets were gated out in the two parameter histograms of pulse height to pulse width of PI fluorescence. Cell cycle histograms were analysed using ModFit LT 3.0 software packages (Variety Software House, Topsham, ME)**

| | G1/G0 phase (%) | S phase (%) | G2/M phase (%) |
|---|---|---|---|
| **Treatments** | | | |
| **Solvent control (0.1 %DMSO)** | **90.43 +/- 1.01** | **6.00 +/- 1.00** | **3.57 +/- 1.64** |
| **Estradiol 1 nM** | **63.14 +/- 1.61** | **34.84 +/- 2.48** | **2.03 +/- 0.88** |
| **4-Nonylphenol 10 µM** | **63.71 +/- 1.86** | **34.64 +/- 0.47** | **1.64 +/- 1.41** |
| **6:2 FTOH 30 µM** | **66.98 +/- 4.09** | **30.83 +/- 3.23** | **2.19 +/- 0.87** |
| **8:2 FTOH 10 µM** | **68.53 +/- 1.48** | **29.36 +/- 1.78** | **2.11 +/- 0.49** |
| **PFOS 50 µM** | **85.63 +/- 0.94** | **10.49 +/- 0.71** | **3.87 +/- 0.73** |
| **PFNA 50 µM** | **85.53 +/- 1.64** | **9.89 +/- 1.53** | **4.57 +/- 0.42** |
| **PFOA 50 µM** | **83.57 +/- 1.04** | **9.17 +/- 0.57** | **6.83 +/- 0.59** |

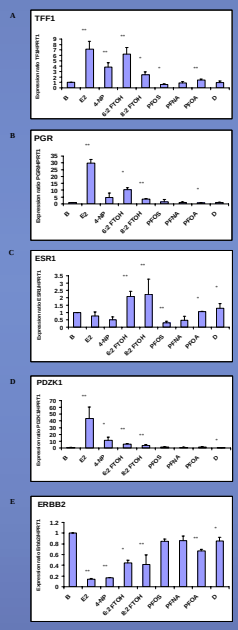
**Data are mean values of three measurements per treatment. During all measurements, Cv values of the G0/G1 peak were below 3.6. (n=3)**




**DNA content histograms showing the effects of perfluorinated compounds on cell cycle distribution**

### 3. Analysis of expression of estradiol-responsive genes by real-time PCR.

**MCF-7 cells were treated with 0.1 % DMSO (B = blanc) 1 nM 17β-estradiol (E2), 10 µM 4-nonylphenol (4-NP), 30 µM 6:2 FTOH, 10 µM 8:2 FTOH, 50 µM PFOS, 50 µM PFNA, 50 µM PFOA or 10 nM 2,3,7,7-TCDD (D). Upon exposure to the test compounds during 48 hours, mRNA levels of (A) trefoil factor 1 *TFF1*, (B) Progesterone receptor *PGR*, (C) estrogen receptor-alpha *ESR1*, (D) *PDZK1* and of (E) *ERBB2* were measured by real time-PCR and normalized using hypoxanthine phosphotransferase 1 *HPRT1* as internal control. We used the Roche Lightcycler to perform real-time PCR analyses. Results are means from 3 replicate measurements and are expressed as fold relative to 0.1% DMSO. * p < 0.05; ** P < or = 0.001**




## CONCLUSIONS

**Fluorotelomer alcohols were characterized as estrogen-like chemicals *in vitro*, by a combination of the E-screen assay, flow cytometric analysis of the cell cycle and gene expression analysis of estrogen-responsive genes. The structural similarities of these compounds and 4-nonylphenol, the reference xeno-estrogen during this study, offer a possible explanation why these new compounds may act as ligands for the estrogen receptor. The characterization of fluorotelomer alcohols as new xeno-estrogens during this study demonstrates the need to carefully monitor their environmental distribution and to investigate further the effects of perfluorinated compounds on biota.**

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