PPAR CALUX BIOASSAYS FOR SINGLE COMPOUNDS AND COMPLEX MIXTURES OF PERFLUOROALKYL ACIDS (PFAAs)

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Introduction

Perfluoroalkyl acids (PFAAs), a group of man-made compounds widely used as surfactants, lubricants, polymers, and firefighting foams, are wide-spread in the environment, wildlife, and in humans. Toxicity of PFAAs in mammals is related to immunotoxicity, hepatotoxicity, developmental toxicity and reproductive toxicity. PFAAs are suspected endocrine disrupting compounds (EDCs). In a previous report, we investigate the endocrine disrupting activities of several PFAAs on various human steroid hormone receptors by means of the ERα, ERβ, AR, PR and GR CALUX bioassays, both agonistic as well as antagonistic[1]. These U2-OS-based CALUX bioassays measure the activities on the androgen receptor, estrogen receptors alpha and beta, progesterone receptor, and glucocorticoid receptor. The study showed that the suspected endocrine disrupting activity, if any, of PFAAs did not act via direct interference with human steroid hormone receptors. In the present study, an expanded series of PFAAs were evaluated on their ability to affect peroxisome proliferator-receptors PPARα and PPARγ using the PPARα and PPARγ CALUX bioassays. These receptors are thought to regulate physiological processes that influence e.g. lipid homeostasis, peroxisome proliferation, adipogenisis and reproduction[2,3]. The results from this study clearly indicate that some PFAAs activate the PPARα receptor whereas others are activators of the PPARγ receptor or both. We will also report results from several real world samples containing complex mixtures of PFAAs in comparison to chemical analysis by using REP values of both PPAR CALUX bioassays.

Materials and Methods

Compounds
The PFAAs tested are given in Table 1., GW7674 (PPARα CALUX) and Rosiglitazone (PPARγ CALUX) were used as positive controls. All PFAAs were tested in a concentration range of $3.0*10^{-7}$ M to $1.0*10^{-3}$ M.

CALUX bioassays
PPARα and PPARγ CALUX cells were plated in 96 well plates with phenol red free DF medium supplemented with 5% dextran coated charcoal stripped FCS (DCC-FCS) at a volume of 100 µl per well. After 24 h, medium was removed from the cells and 200 µl of DCC-FCS medium containing the compound(s) of interest (dissolved in DMSO), final dilution 1:1000) was added. After 24 hours the medium was removed, cells were lysed in 30 µl Triton-lysis buffer and measured for luciferase activity using a Berthold luminometer for 0.1 min/well. In addition to the PPARα and PPARγ CALUX, all compounds were also tested on the GR CALUX bioassay for their ability to activate the glucocorticoid receptor and the cytotoxicity CALUX to determine the potency of the compounds to cause cell-death in the CALUX bioassays.

Data analysis
Analysis of peroxisome proliferator-receptors mediated luciferase activity in PPARα and PPARγ CALUX cells was performed as followed. Luciferase activity per well was measured as relative light units (RLUs). Fold induction was calculated by dividing the mean value of light units from exposed and non-exposed (solvent control) wells. For evaluation of data, only concentrations were taken into account that did not cause cytotoxicity as determined using the cyotoxicity CALUX bioassay.

Table 1 List and source of PFAAs tested

Product name	Supplier	Batch	CAS	Purity	Powder/Fluid	Mw (g/mol)
Perfluoropentanoic acid	Aldrich	06316KH	2706-90-3	97	fluid	264.1
Perfluoroundecanoic acid	Aldrich	00107DJ	2058-94-8	95	powder	564.1
Perfluorododecanoic acid	Aldrich	03727PH	307-55-1	95	powder	614.1
Perfluorotetradecanoic acid	Aldrich	MKBC9206	376-06-7	97	powder	714.1
Perfluorononanoic acid	Aldrich	07726JH	375-95-1	97	powder	464.1
Perfluorodecanoic acid	Aldrich	01603DH	335-76-2	98	powder	514.1
Perfluoroheptanoic acid	Aldrich	04804DJ	375-85-9	99	powder	364.1
Perfluorobutyric acid	Aldrich	S86465-319	375-22-4	98	fluid	214.0
Nonafluoro-1-butanesulfonic acid	Aldrich	24318BB	29420-49-3	98	powder	338.2
Perfluorooctanoic acid	Aldrich	11419LE	335-67-1	96	powder	414.1
Tridecafluorohexane-1-sulfonic acid potassium salt	Aldrich	1394969	3871-99-6	98	powder	438.2
Heptadecafluorooctanesulfonic acid potassium salt	Aldrich	1424328	2795-39-3	98	powder	538.2
Heptadecafluorooctanesulfonic acid	Fluka	46139/1 11200	1763-23-1	40?	fluid	500.1
Perfluorotridecanoic acid	Aldrich	03025BD	72629-94-8	97	powder	654.1
Undecafluorohexanoic acid	Fluka	1372006 60908012	307-24-4	97	fluid	314.1

Table 2 Qualitative representation of the activity of PFAAs tested in the PPARα, PPARγ and GR CALUX bioassay. A compound is indicated as positive in case the maximum observed induction was more than 1.5-times the background induction.

PFOA	PPARα CALUX	PPARγ CALUX	GR CALUX
Perfluoropentanoic acid	+	-	-
Perfluoroundecanoic acid	-	+	-
Perfluorododecanoic acid	-	+	-
Perfluorotetradecanoic acid	+	+	-
Perfluorononanoic acid	+	-	-
Perfluorododecanoic acid	-	-	-
Perfluoroheptanoic acid	+	-	-
Perfluorobutyric acid	+	-	-
Nonafluoro-1-butanesulfonic acid	+	+	-
Perfluorooctanoic acid	+	+	-
Tridecafluorohexane-1-sulfonic acid	+	+	-
Heptadecafluorooctanesulfonic acid	+	+	-
Heptadecafluorooctanesulfonic acid	+	+	-
Perfluorotridecanoic acid	-	+	-
Undecafluorohexanoic acid	-	-	-

Results and Discussion

Although the mechanism through which PFAAs produce toxicity remains unclear, it has been postulated that activation of the PPARα and PPARγ receptors may be a factor[3,4]. In both laboratory animals and in vitro models, the potential toxicity of PFAAs have been demonstrated. Here, the potential of PFAAs to activate the PPARα and PPARγ receptor in the human U2-OS-based CALUX bioassays were studied. Ten out of the 15 PFAAs tested showed to be activators of the PPARα receptor. Only perfluoroundecanoic acid, perfluorododecanoic acid, perfluorododecanoic acid, perfluorotridecanoic acid and undecafluorohexanoic acid could not induce the PPARα CALUX bioassay at the concentrations evaluated.
Nine of the PFAAs tested showed activation of the PPARγ receptor in the PPARγ CALUX bioassay. Only undecafluorohexanoic acid showed no activity in either one of the PPAR CALUX bioassays tested. By using the dose-response curves below, we will also evaluate REP values for the here tested PFAAs and will apply them on the chemical data of real samples representing a complex mixture of PFAAs.

Although the suspected endocrine disrupting activity of PFAAs does not act via direct interference with human steroid hormone receptors as shown in our previous study[1], the present study clearly shows that various PFAAs directly activate either one or both the PPARα and PPARγ receptor in the human U2-OS-based CALUX bioassays. Therefore, the panel of PPAR CALUX bioassays may well contribute to further study and understand the mechanism of toxicity of PFAAs in the environment, wildlife and humans.

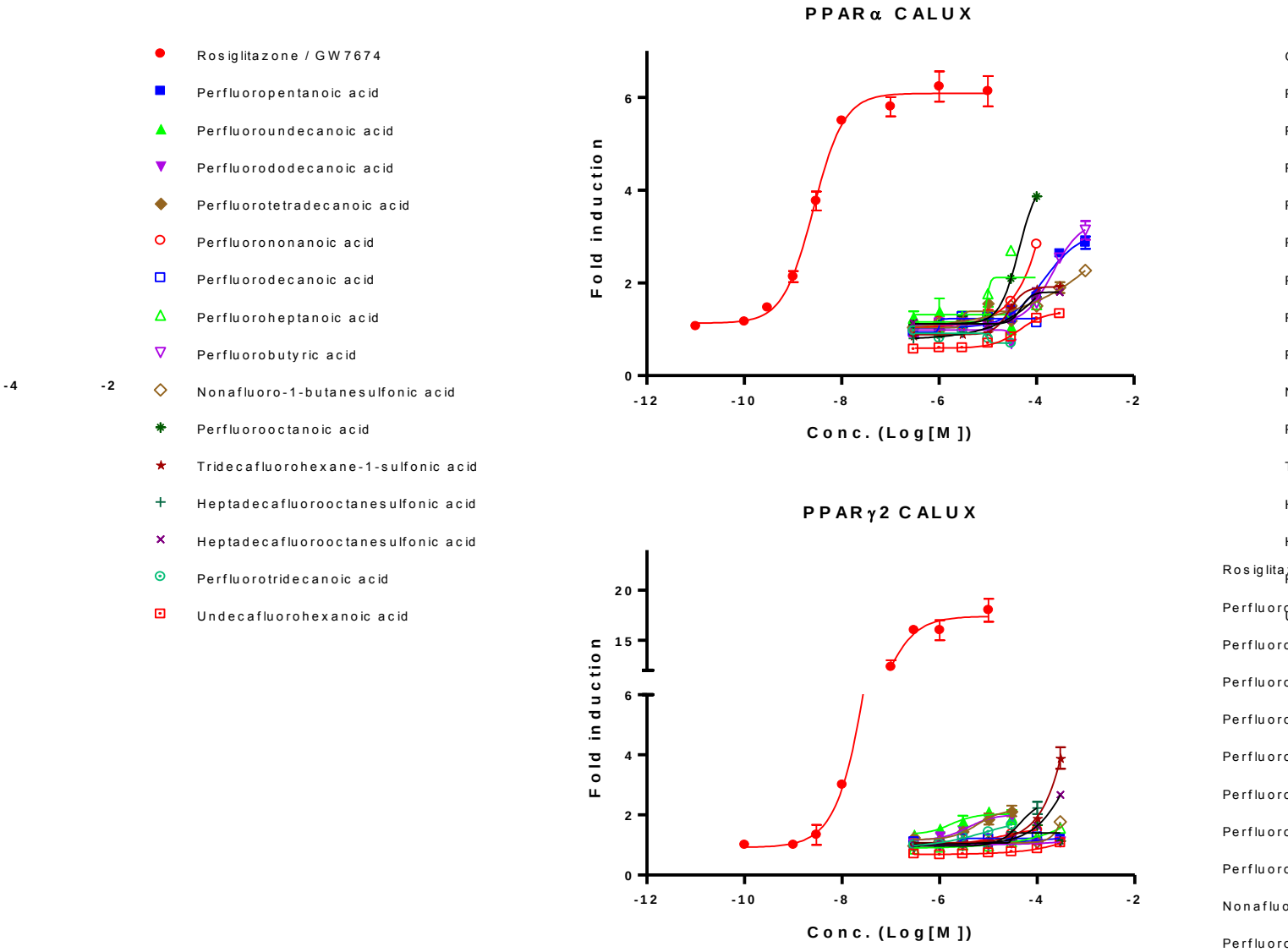

Figure 1. Induction of PPARα (top) and PPARγ (bottom) CALUX following 24-hours of exposure to a wide concentration range of PFAAs. As positive controls, GW7674 (PPARα CALUX) and Rosiglitazone (PPARγ CALUX) were used. Only concentrations of PFAAs are shown that did not cause cytotoxicity as determined using the cytotoxicity CALUX.

References

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