Activation of Mouse and Human Peroxisome Proliferator–Activated Receptor Alpha by Perfluoroalkyl Acids of Different Functional Groups and Chain Lengths

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Perfluoroalkyl acids (PFAAs) are synthetic chemicals with a carbon backbone saturated with fluorine and a charged moiety on one end, making them both lipophobic and hydrophobic, and very stable. These properties lend them to widespread consumer and industrial applications as surfactants, flame retardants, water repellants, and oil repellants on food packaging (Kissa, 2001; Renner, 2001). These properties also make them persistent in the environment. PFAAs have been found globally, in air, water, soil, and house dust (Boulanger et al., 2005; Emmett et al., 2006; Giesy et al., 2001; Hansen et al., 2002; Harada et al., 2006; So et al., 2004; Shoeib et al., 2005). PFAAs have also been found in liver, fat, and serum of wildlife everywhere from the polar regions to industrialized areas (DeSilva and Mabury, 2006; Giesy and Kannan, 2001; Giesy et al., 2001; Taniyasu et al., 2003), in human serum and blood around the world (Butenhoff et al., 2004; Calafat et al., 2006, 2007; Kannan et al., 2004; Kuklenyik et al., 2004; Olsen et al., 2003; 2007b), in breast milk (Karrman et al., 2007; Kuklenyik et al., 2004; Volkel et al., 2008), and in umbilical cord blood (Apelberg et al., 2007). These PFAAs include not only the well-studied perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) but also others such as perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorohexane sulfonate (PFHxS), perfluorobutanoic acid (PFBA), and perfluorobutane sulfonate (PFBS). These chemicals are slow to clear from the body of most animals, with a half-life estimated at days or months, and as long as years in humans for PFOA, PFOS, and PFHxS (Olsen et al., 2005; 2007a).

Perfluoroalkyl acids (PFAAs) are surfactants used in consumer products and persist in the environment. Some PFAAs elicit adverse effects on rodent development and survival. PFAAs can activate peroxisome proliferator–activated receptor alpha (PPARα) and may act via PPARα to produce some of their effects. This study evaluated the ability of numerous PFAAs to induce mouse and human PPARα activity in a transiently transfected COS-1 cell assay. COS-1 cells were transfected with either a mouse or human PPARα receptor-luciferase reporter plasmid. After 24 h, cells were exposed to either negative controls (water or dimethyl sulfoxide, 0.1%); positive controls (WY-14643, PPAR agonist); perfluorooctanoic acid or perfluorononanoic acid at 0.5–100μM; perfluorobutanoic acid, perfluorohexanoic acid, perfluorohexane sulfonate, or perfluorodecanoic acid (PFDA) at 5–100μM; or perfluorobutane sulfonate or perfluorooctane sulfonate at 1–250μM. After 24 h of exposure, luciferase activity from the plasmid was measured. Each PFAA activated both mouse and human PPARα in a concentration-dependent fashion, except PFDA with human PPARα. Activation of PPARα by PFAA carboxylates was positively correlated with carbon chain length, up to C9. PPARα activity was higher in response to carboxylates compared to sulfonates. Activation of mouse PPARα was generally higher compared to that of human PPARα. We conclude that, in general, (1) PFAAs of increasing carbon backbone chain lengths induce increasing activity of the mouse and human PPARα with a few exceptions, (2) PFAA carboxylates are stronger activators of mouse and human PPARα than PFAA sulfonates, and (3) in most cases, the mouse PPARα appears to be more sensitive to PFAAs than the human PPARα in this model.

Key Words: perfluoroalkyl acids; PFAA; peroxisome proliferator–activated receptor alpha; PPARα; PFOS; PFOA; PFNA; PFBA; transient transfection assay; COS-1 cells.

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induces adenomas of the Leydig cells and pancreatic acinar cells in rodents (Biegel et al., 2001; Cook et al., 1992; Goldenthal, 1978). Other PFAAs (perfluorohexanoic acid [PFHxA], perfluorohexanoic acid, and PFNA) were found to induce hepatomegaly as well (Kudo et al., 2006). Developmental effects of PFOA and PFOS include full litter resorptions, low birth weight, neonatal mortality, delayed eye opening, and stunted mammary gland development in the offspring of mice, rats, and/or rabbits (Case et al., 2001; Lau et al., 2003; 2006; Luebker et al., 2005; Thibodeaux et al., 2003; White et al., 2007). Recently, levels of PFOS and PFOA in human cord blood were linked to low birth weights in infants (Apelberg et al., 2007), although in another study (Fei et al., 2007), there was no association between maternal PFAA levels and low birth weight.

One mechanism of action by which PFAA induces these effects may include activation of peroxisome proliferator–activated receptor (PPAR) α. PPARs are a class of ligand-activated transcription factors of the steroid/thyroid nuclear hormone receptor superfamily (Dreyer et al., 1992). They are involved in many cell processes including energy metabolism, cell differentiation, and lipid homeostasis and are expressed in many organs and species, as early as the developing embryo (Finkelstein et al., 1992; Biegel et al., 2001; Cook et al., 1992; Goldenthal, 1978). There are three isoforms of PPAR, α, β/δ, and γ. PPARα is primarily involved in lipid homeostasis, fatty acid catabolism, peroxisome proliferation, and inflammation (Escher and Wahli, 2000; Gonzalez et al., 1998). It has been postulated that PFAAs can induce hepatomegaly and liver tumors in rodents by activating PPARα. PPARα induction of peroxisome proliferation is associated with non-genetic hepatocarcinogenesis (Klaunig et al., 2003). PFAAs are capable of activating PPARα in vitro (Maloney and Waxman, 1999; Shipley et al., 2004; Vanden Heuvel et al., 2006), inducing peroxisomal enzymes in male rats (PFOA, PFNA, PFDA; Kudo et al., 2000), inhibiting peroxisomal beta-oxidation in female rats (PFDA; Borges et al., 1993), and inducing peroxisome proliferation in rats and mice (Berthiaume and Wallace, 2002; Ikeda et al., 1985; 3M Company, 2003). Consistent with PPARα-mediated responses, PFOA is immunosuppressive in the mouse (Yang et al., 2000) and anti-inflammatory in the rat (Griesbacher et al., 2008). In addition, deletion of PPARα in knockout mice prevented the postnatal lethality produced by gestational PFOA exposure (Abbott et al., 2007). It is unclear whether peroxisome proliferation is a mechanism of toxicity in humans. The involvement of PPARα in inducing other toxic effects in humans remains to be determined.

Transiently transfected cell models using COS-1 and 3T3-L1 cells have been used to determine the ability of PFAAs to bind PPARα (Bility et al., 2004; Intrasukski et al., 1998; Maloney and Waxman, 1999; Shipley et al., 2004; Takacs and Abbott, 2007; Vanden Heuvel et al., 2006). These PPARα-transfected cell models have been used to compare relative activity in response to PFAAs. Takacs and Abbott (2007) used COS-1 cells transiently transfected with a PPARα, β/δ, or γ plasmid to evaluate the potential for PFOA and PFOS to activate the mouse and human PPAR isoforms. In the current study, we extend the investigation to evaluate the ability of PFAAs with different carbon chain lengths, including perfluorooalkyl carboxylates (PFBA, PFHxA, PFOA, PFNA, and PFDA) and sulfonates (PFBS, PFHxS, and PFOS) to activate the mouse and human PPAR isoforms most likely involved in mediating some toxic effects of PFAAs, the PPARα.

### MATERIALS AND METHODS

**Chemicals.** WY-14643 (4-chloro-6-(2,3-xylidine)-pyrimidinylthioacetic acid) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO). PFOS (potassium salt; purity > 98%), PFHxA (purity > 97%), and PFOA (ammonium salt; purity > 98%) were purchased from Fluka Chemical (Steinheim, Switzerland). PFNA (purity 97%) and PFDA (purity 98%) were purchased from Aldrich (St Louis, MO). PFBA (ammonium salt; 28.9% solution in distilled water), PFBS (potassium salt; purity 98.2%, linearity 99.98%), and PFHxS (potassium salt; purity 98.6%) were a gift from 3M Company (St Paul, MN). WY-14643 was dissolved in DMSO to make a 25mM stock solution. PFAAs were dissolved in deionized distilled water (PICUPore Hydro Services and Supplies, Inc., Durham, NC) to make dilutions, except PFDA, which was dissolved in DMSO. PFOS, poorly soluble, was dissolved in boiling water to make stock and cooled to room temperature before adding to dose solution. Chemical solutions were prepared fresh on the day of treatment.

**Plasmids.** The origin, preparation, and use of mouse and human PPARα plasmids (a gift from Dr Jeffrey M. Peters and Dr John F. Vanden Heuvel, Penn State University, PA) are described previously in Takacs and Abbott (2007). Briefly, the plasmids contain a construct of the ligand-binding domain (LBD) of mouse or human PPARα fused to the DNA-binding domain of Gal4 under the control of an SV-40 promoter and a construct of an UAS-firefly luciferase reporter under the control of a Gal4 DNA response element.

**Cell culture and transactivation assay.** Cell culture methods were performed as previously described (Takacs and Abbott, 2007). Briefly, COS-1 cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, Grand Island, NY) with 10% fetal bovine serum (Gibco) and antibiotic (0.2 mg/ml streptomycin and 100 U/ml penicillin; Gibco). Cells were plated at a density of 10^5 cells per 100 μl DMEM well in 96-well plates. After 24 h, cells were transfected with the plasmid (1 μg/μl) in 10-μl serum-free DMEM using FuGENE 6 transfection reagent (Roche Diagnostics Corporation, Indianapolis, IN). Transfection solutions were prepared in triplicate, one tube per plate, so that each plate was a separate assay. DMEM containing serum (100 μl) was added after 3 h of incubation with the transfection reagents. The medium was aspirated and replaced with 100-μl serum-free DMEM containing test chemicals 24 h after transfection. Cells were treated with WY-14643 (positive control; 10μM), DMSO (control vehicle for WY-14643; 0.1%), deionized distilled water (negative control vehicle for the PFAAs; 0.1%), or PFDA in the following concentrations: PFOA or PFNA at 0.5–100μM; PFBA, PFDA, PFHxA, or PFHxS at 5–100μM; or PFBS or PFOS at 1–250μM. Solutions of the PFAAs were prepared fresh on the day of treatment. Each compound was tested in four or eight wells per concentration per plate in three plates per assay in at least two assays to provide 24–48 replicates for each concentration of test compound. Twenty-four hours after treatment, cells were rinsed with Dulbecco’s phosphate-buffered saline, lysed with reporter lysis buffer (cat# E3971 or E1531; Promega, Madison, WI), and luciferase activity was measured in relative luciferase units (RLUs) within an hour using the Luciferase reporter.
Viability and transfection control assays. Tests for cytotoxicity were performed as described previously in Takacs and Abbott (2007). Briefly, a fluorescence detection CellTiter-Blue cell viability kit (Promega) was used to test the cell viability for each compound at each concentration in one plate per compound. Transfection efficiency for the PPARα plasmids using the secreted alkaline phosphatase concentration assay was described and tested previously in our laboratory (Takacs and Abbott, 2007).

Statistics and calculations. Data were analyzed using SAS for Windows V9.1. All data values were log10 transformed before analysis. Outliers were defined by calculating residuals within a fixed effects linear model (SAS Proc GLM) that accounted for dose group and plate, and residuals greater than 2.6 (roughly equivalent to using a two-sided p value of 0.01) were identified as outliers. The data with outliers removed were analyzed by compound and species with mixed effects linear models (SAS Proc Mixed) using restricted maximum likelihood estimation, with dose group as a fixed effect and experiment and plate (nested within experiment) as random effects. Differences between each dose group and the control group were tested using Dunnett’s test, and this was used to identify the lowest observed effects concentration (LOEC). The responses across the dose range were tested with regression analyses, providing slope and intercept estimates for each compound by species. Goodness of fit for each model was estimated by calculating an R² value for each model using SAS type III sums of squares. The difference in response between species for each compound was tested as the difference in slope between mouse and human, using a similar regression model that allowed a separate intercept and slope for each species.

To allow direct comparison of responses between PFAA compounds, a measurement was devised called the C20max, described below. Responses were set on the same scale by defining the highest log-transformed RLU obtained in all assays (1.11 RLU produced by PFDA in the mouse plasmid) as the overall maximal possible response. The range of responses was set from 0 to 1.0. Thus, if 1.11 RLU = 1.0 (or 100% response), then 20% of the maximal response would be 0.22 RLU. With this relative response scale and the regression formula for each slope, it is possible to calculate a C20max or concentration at which each PFAA compound is predicted to produce 20% of the overall maximal response. For example, shown here mathematically and illustrated in Figure 1 for PFBA, a linear fit of the slope gives the regression formula Y = aX + b, in which we set Y at 20% of maximal RLU or 0.22, a = the slope (0.0043 for mouse PFBA in this example), and b = the intercept which is set to 0. Thus, 0.22 = 0.0043X + 0 and X (the C20max) = 51 μM.

RESULTS

The positive control, WY-14643, its negative vehicle control (DMSO), and a negative vehicle control for the PFAAs were run concurrently on every plate along with each PFAA compound tested. PFAAs were tested at nine different concentrations per plate. The positive control elicited a significant increase in activity over its vehicle control for every assay, at p < 0.0001, indicating proper performance of the assay. Except PFDA when tested with the human PPARα plasmid, each PFAA elicited a significant dose-dependent response compared to the vehicle control, indicating activation of each PPARα by the PFAA. PFDA did not activate the human PPARα. The lowest concentration of the PFAA that produced an effect, or the LOEC, and the next lower concentration tested which did not produce an effect, or the no observable effects concentration (NOEC), are shown in Table 1. The NOEC and the LOEC were obtained by comparing the luciferase activity produced by the PFAA at each concentration to the activity in the vehicle control. A lower LOEC or ppm indicates that a smaller concentration of the PFAA is able to elicit a significant response. The longer carbon chain carboxylates, PFOA and PFNA (mouse and human) and PFDA (mouse), produced effects at low ppm concentrations and were more active in the assay than the shorter chain carboxylates or the sulfonates.

Overall response across concentrations was examined for each compound, and a curve was fit to the data that best characterized the dose-response. The dose-response for most compounds was linear, although PFOA and PFNA produced a biphasic curve that was linear in the 0–30 μM range and plateaued at higher concentrations. A generalized comparison of the responsiveness of PPARα to the PFAAs can be made by plotting these curves together (Fig. 2). In general, the sulfonates were not as active as the carboxylates in either species, and the carboxylates were more active in the mouse PPARα than in the human. In Figure 3, the data are plotted by compound and species. This figure illustrates that, for most PFAA, the compounds elicited higher PPARα activity with the mouse plasmid compared to the human. The slope of the response to PFHxS was not different between the two species, and with PFBS, activity was higher with the human plasmid compared to the mouse. The slopes, p values, and r² of the slopes are shown in Table 2, along with the statistical comparison.
between mouse and human. Except in the case of PFDA, which was inactive with the human plasmid, p values of the regression were significant, indicating a significant dose-response for these compounds in each species.

In order to more directly compare responses, the outcomes were adjusted to the same scale using a percentage of the maximal response. The concentration of the PFAA that produces 20% of the maximal response was extrapolated from the regression.
formula and slope for each analysis and is referred to as the C_{20}\text{max} (Table 3, Fig. 1). PFNA (C9) and PFOA (C8) were the most potent, eliciting significant PPARα activity at the lowest concentrations of all the PFAAs (mouse, 5 and 6 μM, respectively; human, 11 and 16 μM, respectively) followed by PFDA in mouse. Activity of the plasmids decreased with decreasing chain length of the carboxylate. Sulfonates were found to elicit activity in either species at higher concentrations than the carboxylates. PFBS, the sulfonate with the shortest chain length, induced the least activity of any of the PFAAs tested.

FIG. 3. Linear regression plots showing the dose-response activity of mouse and human PPARα induced by various PFAA compounds, shown by compound and in order of increasing carbon chain length for the perfluoroalkyl acids in (A), (C), (E), (G), and (H), and for the sulfonates in (B), (D), and (F). PPARα plasmid of mouse or human was transiently transfected into COS-1 cells, and cells were exposed to each PFAA in serum-free DMEM for 24 h at concentrations shown, in four or eight replicate wells per concentration, in three replicate plates per assay, and in at least two assays. Dashed lines and open symbols represent mouse plasmid and solid lines and solid symbols represent human plasmid. m, mouse; h, human.
DISCUSSION

This study was undertaken based on the finding that PFOA and PFOS can activate PPARα in vitro (Intrasukri et al., 1998; Maloney and Waxman, 1999; Takacs and Abbott, 2007; Vanden Heuvel et al., 2006) and affect PPARα-mediated responses (Berthiaume and Wallace, 2002; Ikeda et al., 1985; Kudo et al., 2000; Wilson et al., 1995). It is important to know whether other PFAA activate PPARα as well to explore the possible role of the receptor in their mechanism of action as well. This study is the first to examine the ability of multiple PFAAs (PFBA, PFBS, PFHxA, PFHxS, PFOA, PFOS, PFNA, and PFDA) as a class to activate PPARα in one transiently transfected COS-1 cell model. This study demonstrates that many perfluoroalkyl carboxylates and sulfonates activate the mouse and human PPARα in a transiently transfected COS-1 cell model and that the magnitude of the response largely depends on the chain length of the carbon backbone and the functional group.

In general, activation of PPARα increased with increasing chain length of the carboxylate, up to C9. In the mouse PPARα, the LOEC for the four-carbon (C4) PFBA was 40 μM, indicating a relatively weak agonist. The LOEC for PFHxA (C6) was 20 μM, and the longer chained PFOA (C8), PFNA (C9) and PFDA (C10) had LOECs ranging from 1 to 5 μM, indicating stronger agonist activity. Likewise, in the human PPARα, the LOEC for PFBA was 40 μM, the LOEC for PFHxA and PFOA was 10 μM, and the LOEC for PFNA was 5 μM. Unexpectedly, PFDA was inactive. This comparison is more discernable when examining the data on a relative scale using the C20max values for these PFAAs. PFBA had the lowest relative activity, indicated by the highest C20max within species (51 μM, mouse; 75 μM, human), and PFNA had the highest relative activity, indicated by the lowest C20max (5 μM, mouse; 11 μM, human). This effect of chain length on activation of PPARα is consistent with reports of increased hepatomegaly and peroxisomal beta-oxidation by PFAA with increasing chain length (Kudo et al., 2006) or increased PPARα activity with increasing chain length of phthalate compounds, also PPARα ligands (Bility et al., 2004; Lampe et al., 2003). The association between PFAA chain length and receptor activity may be a characteristic of the PPARα LBD. Interestingly, in the current study, the PFAA acid with the longest carbon chain length, PFDA, did not induce activity from the human PPARα at any concentration. This may indicate a difference in the size or conformation of the binding site of the mouse and human PPARα LBD. The relatively large molecule of the C10 compound may be too large and make it stearically impractical to properly bind to the site of the human PPARα LBD in its closed, active conformation, leading to little or no activity, whereas C10 may tightly bind the mouse LBD due to sequence differences at the site that allows a larger conformation and activate the receptor.

The present study also demonstrated that the sulfonates, C4, C6, and C8, induced lower activity in both mouse and human

Table 2
Regression Analysis of Dose-Response Activation by PFAA of Transfected Human and Mouse PPARα in COS-1 Cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mouse</th>
<th>Human</th>
<th>Mouse versus human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope of regression line</td>
<td>Regression p value</td>
<td>R²</td>
</tr>
<tr>
<td>PFBA</td>
<td>0.0043</td>
<td>&lt; 0.0001</td>
<td>0.71</td>
</tr>
<tr>
<td>PFHxA</td>
<td>0.0059</td>
<td>&lt; 0.0001</td>
<td>0.48</td>
</tr>
<tr>
<td>PFOA</td>
<td>0.0354</td>
<td>&lt; 0.0001</td>
<td>0.85</td>
</tr>
<tr>
<td>PFNA</td>
<td>0.0411</td>
<td>&lt; 0.0001</td>
<td>0.82</td>
</tr>
<tr>
<td>PFDA</td>
<td>0.0111</td>
<td>&lt; 0.0001</td>
<td>0.87</td>
</tr>
<tr>
<td>PFBS</td>
<td>0.0007</td>
<td>&lt; 0.0001</td>
<td>0.81</td>
</tr>
<tr>
<td>PFHxS</td>
<td>0.0029</td>
<td>&lt; 0.0001</td>
<td>0.84</td>
</tr>
<tr>
<td>PFOS</td>
<td>0.0024</td>
<td>&lt; 0.0001</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Note. ns, not significant.

*For concentrations 0–20 μM.

Table 3
Relative Responses of PPARα to PFAAs in transiently transfected COS-1 cells, Measured by C20max

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mouse C20max (μM)</th>
<th>Human C20max (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFNA</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>PFOA</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>PFDA</td>
<td>20</td>
<td>na</td>
</tr>
<tr>
<td>PFHxA</td>
<td>38</td>
<td>47</td>
</tr>
<tr>
<td>PFBA</td>
<td>51</td>
<td>75</td>
</tr>
<tr>
<td>PFHxS</td>
<td>76</td>
<td>81</td>
</tr>
<tr>
<td>PFOS</td>
<td>94</td>
<td>262*</td>
</tr>
<tr>
<td>PFBS</td>
<td>317*</td>
<td>206</td>
</tr>
</tbody>
</table>

Note. na, not active; compounds are arranged in order of decreasing potency.

*C20max, predicted concentration at which compound elicits 20% of the overall maximal response.

*Value exceeds highest concentration actually tested (250 μM).
PPAR\(\alpha\) compared to the carboxylates. This confirms and adds to our previous report that PFOS elicited lower activity than PFOA (Takacs and Abbott, 2007) and is consistent with reports from other laboratories (Maloney and Waxman, 1999; Shipley et al., 2004; Vanden Heuvel et al., 2006).

In addition, the activation response of the mouse PPAR\(\alpha\) was higher than that of the human for all PFAAs at each concentration when compared by regression analysis across the dose-response, with two exceptions; PFHxS induced identical responses in each species, and PFBS induced higher activation of human PPAR\(\alpha\) than of mouse. Slopes of the dose-response curves with mouse PPAR\(\alpha\) were higher and roughly twice that obtained with human PPAR\(\alpha\) in many cases (PFNA, PFBA, and PFOA). Higher sensitivity in the mouse PPAR\(\alpha\) compared to the human PPAR\(\alpha\) in response to other PPAR\(\alpha\) ligands (Bility et al., 2004) or to PFOA or PFOS (Maloney and Waxman, 1999; Takacs and Abbott, 2007; Vanden Heuvel et al., 2006) has been demonstrated previously. The sequence of LBD of the PPAR\(\alpha\) differs between mouse and human, and it has been postulated that this difference and the differential gene expression between human and rodent PPAR\(\alpha\) may be responsible for the difference in response to PFAAs or other ligands in the assay (Bility et al., 2004).

The present study confirms work by others who have shown activation of mouse and human PPAR\(\alpha\) by PFOS (Shipley et al., 2004) and PFOA (Intrasukrit et al., 1998; Maloney and Waxman, 1999; Vanden Heuvel et al., 2006). Previous studies and ours report similar LOECs and concentration ranges for activation of PPAR\(\alpha\) by PFOA and PFOS. In COS-1 cells, Maloney and Waxman (1999) reported activation of mouse and human PPAR\(\alpha\) by PFOA beginning at concentrations ranging from 0.5 to 1\(\mu\)M, and in 3T3-L1 cells, Vanden Heuvel et al. (2006) reported activation by PFOA beginning at 50\(\mu\)M for both mouse and human. PFOS was reported to activate mouse PPAR\(\alpha\) in COS-1 cells at 8\(\mu\)M (Shipley et al., 2004). In our study, the LOEC of PFOA were 1 and 10\(\mu\)M, and for PFOS, the LOEC were 90 and 30\(\mu\)M, for mouse and human PPAR\(\alpha\), respectively. Differences between their previously reported LOECs and ours may be influenced by the model used, that is, variations in the plasmid construct (plasmid sequences, one or two-plasmid transfection model), cell type utilized, and other possible differences in the protocol. Regardless, it is noteworthy that our results are within ranges reported in previous studies by other investigators.

A previous study in our laboratory examined the ability of PFOA and PFOS to activate PPAR\(\alpha\), \(\beta/\delta\), and \(\gamma\) plasmids in transfected COS-1 cells (Takacs and Abbott, 2007). That study demonstrated that PFOA activated the mouse and human PPAR\(\alpha\) and PFOS activated the mouse PPAR\(\alpha\) but not the human. In the present study, we report lower LOECs for both PFOA and PFOS in both species and were able to detect activation of both mouse and human PPAR\(\alpha\) by PFOS. We report an LOEC for PFOA of 1 and 10\(\mu\)M for mouse and human, respectively, whereas Takacs and Abbott (2007) reported LOECs of 10 and 30\(\mu\)M, respectively. In addition, PFOS activated the human PPAR\(\alpha\) at concentrations of 30\(\mu\)M and higher, whereas PFOS did not induce activation in the previous study. This increased sensitivity may be attributable to using a higher number of replicates of each concentration on each plate, more plates per assay, and more assays per compound relative to the previous study.

The concentrations of PFAAs that activated PPAR\(\alpha\) in this study are similar to the serum levels of these PFAAs found in experimental rodents displaying toxicological effects. Gestational exposure of rats and mice to PFOA and PFOS has been shown to induce full litter resorption, delay eye opening and preputial separation, inhibit mammary gland and lung development, increase liver weight, and result in postnatal mortality of pups (Grasty et al., 2003; Lau et al., 2006; White et al., 2007). Mice exposed to PFOA at doses that were associated with reduced survival of their offspring (dosed on gestational day [GD] 1–17 at 5 mg/kg/day) had serum levels on postnatal day 22 of 37 ppm for dams and 22–25 ppm for pups (Wolf et al., 2007). After exposure to PFOS from GD1 to 17 at 10 or 20 mg/kg/day, maternal mouse serum levels on GD18 were 179 and 261 ppm (Lau et al., 2007). These concentrations are higher than the range of concentrations of PFOA and PFOS that activated the PPAR\(\alpha\) in our culture assay (LOEC: PFOA 0.43 and 4.3 ppm and PFOS 48 and 16 ppm, for mouse and human, respectively), although the correlation between these in vivo and in vitro values remains to be determined. It is also notable that concentrations of PFAAs that activated the PPAR\(\alpha\) in this study approach the levels of PFAAs found in the environment and in human tissues. PFHxS was measured in house dust at 0.4 ppm (Shoeib et al., 2005), compared to 4.38 ppm of PFHxS that activated human PPAR\(\alpha\) in the current study. PFOA elicited significant PPAR\(\alpha\) activity in mouse in our study at 0.43 ppm or 430 ng/ml, ~10 times the level found in the serum of some human populations (14–56 ng/ml, Kannan et al., 2004). Other PFAA (PFBA, PFBS, PFNA, PFHxA, and PFHxS) that activated the human PPAR\(\alpha\) in this study were found in human sera around the world (e.g., PFHxS at 28 ppb, Karrman et al., 2007; PFDA at 0.64 ppb and PFNA at 3.8 ppb, Kuklenyik et al., 2004; reviewed by Lau et al., 2007). For comparison, in our study, the LOEC for human plasmid for PFHxS was 4.38 ppm, for PFDA was > 51 ppm, and for PFNA was 2.3 ppm. Some of these compounds are currently being tested in laboratory animals in vivo for possible developmental toxicity.

Activation of PPAR\(\alpha\) may be associated with the developmental or hepatic toxicity mentioned above. PPAR\(\alpha\) is found in various organs in the developing fetus (Brassart and Wahli, 1998). Abbott et al. (2007) demonstrated in knockout mice that PPAR\(\alpha\) was necessary for the induction of postnatal lethality after exposure to PFOA. While it is impossible by this study to determine whether PPAR\(\alpha\) activation is involved in developmental toxicity, the overlap of PPAR\(\alpha\) activation with developmental toxicity at similar concentration ranges alerts us
to the possibility that PPARα may be involved in developmental effects of PFAAs. Activation of PPARα may be a useful parameter by which to compare PFAA activity and may be an indicator of PFAA toxicity.

Presented herein is a comprehensive study on the activation of PPARα in vitro by various perfluorinated compounds. The transactivational assay developed by Takacs and Abbott (2007) proved to be a good model for screening PFAAs for PPARα activation. The highlight of this study is the ability to effectively compare various PFAAs of different carbon chain lengths and functional groups across mouse and human species on PPARα activity. It is important to note that activity in this model only evaluates the potential for a compound to interact with the PPAR LBD and activate the reporter and is not necessarily predictive of the chemical’s ability to produce a toxicological response in vivo. It is also possible that in an in vivo setting, PFAAs could produce biological responses that are independent of the PPAR receptors. However, this culture model was very useful for making comparisons between PFAAs and between mouse and human PPAR activity.

We have demonstrated that (1) all PFAA tested (PFBS, PFBA, PFHxS, PFHxA, PFOA, PFOS, PFNA, and PFDA), except for PFDA with human plasmid, activated the PPARα in mouse and human plasmids, (2) PFAA induced higher activation of PPARα as their carbon backbone chain length increased, (3) the carboxylates induced higher activation compared to the sulfonates, and (4) activity of PPARα by PFAAs was usually higher in the mouse PPARα compared to the human. This information enables investigators and risk assessors to identify PFAAs that require more attention or to select PFAAs for industrial and commercial use that are of less risk to health and safety.

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