TOXICOLOGICAL SCIENCES **110(1)**, 204–211 (2009)
doi:10.1093/toxsci/kfp077
Advance Access publication April 9, 2009

Differential Hepatic Effects of Perfluorobutyrate Mediated by Mouse and Human PPAR-α

Jennifer E. Foreman,* Shu-Ching Chang,† David J. Ehresman,† John L. Butenhoff,† Cherie R. Anderson,* Prajakta S. Palkar,* Boo-Hyon Kang,‡ Frank J. Gonzalez,§ and Jeffrey M. Peters*,[1]

*Department of Veterinary and Biomedical Sciences and The Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, Pennsylvania 16802; †Medical Department, 3M Company, St Paul, Minnesota 55144; ‡Non-Clinical Pathology Research Center, Medvill, Seoul, South Korea 153-801; and §Laboratory of Metabolism, National Cancer Institute, Bethesda, Maryland 20892

Received March 2, 2009; accepted April 3, 2009

Perfluorobutryate (PFBA) is a short chain perfluoroalkyl carboxylate that is structurally similar to perfluorooctanoate. Administration of PFBA can cause peroxisome proliferation, induction of peroxisomal fatty acid oxidation and hepatomegaly, suggesting that PFBA activates the nuclear receptor, peroxisome proliferator–activated receptor-α (PPAR-α). In this study, the role of PPAR-α in mediating the effects of PFBA was examined using PPAR-α null mice and a mouse line expressing the human PPAR-α in the absence of mouse PPAR-α (PPAR-α humanized mice). PFBA caused upregulation of known PPAR-α target genes that modulate lipid metabolism in wild-type and PPAR-α humanized mice, and this effect was not found in PPAR-α null mice. Increased liver weight and hepatocyte hypertrophy were also found in wild-type and humanized PPAR-α mice treated with PFBA, but not in PPAR-α null mice. Interestingly, hepatocyte focal necrosis with inflammatory cell infiltrate was only found in wild-type mice administered PFBA; this effect was markedly diminished in both PPAR-α null and PPAR-α humanized mice. Results from these studies demonstrate that PFBA can modulate gene expression and cause mild hepatomegaly and hepatocyte hypertrophy through a mechanism that requires PPAR-α and that these effects do not exhibit a species difference. In contrast, the PPAR-α–dependent increase in PFBA-induced hepatocyte focal necrosis with inflammatory cell infiltrate was mediated by the mouse PPAR-α but not the human PPAR-α. Collectively, these findings demonstrate that PFBA can activate both the mouse and human PPAR-α, but there is a species difference in the hepatotoxic response to this chemical.

Key Words: **PPARα; liver; PFBA; receptor.**

Perfluorobutryate (PFBA) is a perfluoroalkyl carboxylate that is structurally similar to perfluorooctanoate (PFOA). Perfluoralkyl carboxylates are manmade chemicals that have been found in environmental media and in biological samples from various wildlife species and humans (Chang *et al*., 2008; Houde *et al*., 2006; Lau *et al*., 2007). PFBA has been produced for industrial uses but can also arise from metabolism and/or abiotic transformation of fluorinated chemicals (D'Eon *et al*., 2006; Martin *et al*., 2006). Relatively low concentrations of PFBA (ng/l-μg/l) have been detected in precipitation, surface water, effluents and well water (Scott *et al*., 2006a,b; Skutlarek *et al*., 2006), and there is evidence that human exposure to PFBA may occur in occupational settings and through drinking water sources (Chang *et al*., 2008).

Although many studies have evaluated the biological effects of PFOA, considerably less is known about the effects of PFBA in biological systems. A recent report demonstrated that PFBA can activate peroxisome proliferator–activated receptor-α (PPAR-α) (Wolf *et al*., 2008), and previous studies have shown that PFBA can cause peroxisome proliferation and increased peroxisomal fatty acid oxidation (Ikeda *et al*., 1985; Just *et al*., 1989; Kozuka *et al*., 1991; Permadi *et al*., 1993), well-characterized biomarkers of PPAR-α activity. PPAR-α is a member of the nuclear hormone receptor superfamily, and is structurally similar to PPAR-γ and PPAR-β/δ (Burdick *et al*., 2006; Peraza *et al*., 2006; Peters *et al*., 2005, 2008). PPAR-α is best known as the molecular target of the fibrate class of lipid-lowering pharmaceuticals but can also be activated by a variety of structurally dissimilar chemicals including phthalate monoesters, PFOA, trichloroethylene and others (Bility *et al*., 2004; Maloney and Waxman, 1999; Peraza *et al*., 2006; Wolf *et al*., 2008). The central physiological role of PPAR-α is as a ligand activated transcription factor whose target genes encode enzymes and proteins involved in fatty acid transport and catabolism (Peters *et al*., 2005). However, chronic administration of PPAR-α agonists also causes peroxisome proliferation, hepatomegaly and liver cancer in rodent models through a mechanism that requires PPAR-α (Hays *et al*., 2005; Lee *et al*., 1995; Peters *et al*., 1997). Recent studies using a PPAR-α humanized transgenic mouse models have identified a species difference in the function of PPAR-α that likely explains why humans appear to be refractory to PPAR-α agonist-induced

[1] To whom correspondence should be addressed at Department of Veterinary and Biomedical Sciences and The Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA 16802. Fax: (814) 863-1696. E-mail: jmp21@psu.edu.

Published by Oxford University Press 2009.

liver cancer (Gonzalez and Shah, 2008; Peters, 2008). The present study examined the hypotheses that PPAR-α modulates the hepatic response to PFBA exposure and that there is a species difference in PPAR-α activities induced by PFBA between the mouse and human PPAR-α using a PPAR-α humanized transgenic mouse model.

MATERIALS AND METHODS

Animals and treatments. Male wild-type and PPAR-α null (Akiyama *et al*., 2001; Lee *et al*., 1995) and humanized PPAR-α (Cheung *et al*., 2004) on an Sv/129 genetic background were used for this study. The mice were 8–12 weeks of age and housed in a temperature controlled environment (25°C) with a 12-h light/dark cycle. The protocol was approved by The Pennsylvania State University Institutional Animal Care and Use Committee. Procedures involving mice were conducted in an International Association for Assessment and Accreditation of Laboratory Animal Care accredited facility. Fifty mice of each genotype were randomly divided into five treatment groups: vehicle control (water), PFBA (35, 175, or 350 mg/kg/day) or Wy-14,643 (50 mg/kg/day; used as a positive control for PPAR-α activation). Due to the random distribution of mice and the differences in age, which varied by 4 weeks, the average mouse weight in one group was significantly less than other groups (Table 1). PFBA, Wy-14,643 and water control were delivered via oral gavage (10 ml/kg body weight) once per day for 28 days. After 21 days, osmotic pumps containing 16 mg/ml bromodeoxyuridine (BrdU) were implanted to allow for labeling of hepatocytes during the last week of the experiment. Twenty-four hours after the last treatment, mice were euthanized by overexposure to carbon dioxide and blood and liver tissue was obtained. The liver was weighed and cut into sections and fixed in 10% phosphate buffered formalin (PBF) for histological analysis or snap frozen in liquid nitrogen for subsequent RNA isolation and analysis for PFBA concentration.

TABLE 1
Beginning and Ending Body Weights (BW) following Oral Dosing with PFBA or Wy-14,643 in Wild-Type (+/+), PPAR-α Null (−/−), and Humanized PPAR-α (hPPAR-α) Mice

	Treatment, mg/kg/day	Initial BW (g)	Final BW (g)	Change BW[a] (g)
(+/+)	Control	26.1 ± 5.0	26.1 ± 3.8	−0.1 ± 1.7
	PFBA, 35	25.3 ± 2.2	25.6 ± 2.8	0.3 ± 1.0
	PFBA, 175	24.5 ± 2.0	25.2 ± 2.0	0.6 ± 0.6
	PFBA, 350	24.7 ± 1.5	25.2 ± 1.7	0.4 ± 1.8
	Wy-14,643, 50	25.1 ± 2.0	25.4 ± 2.6	0.4 ± 1.3
(−/−)	Control	22.4 ± 3.7	23.3 ± 3.9	0.9 ± 0.9
	PFBA, 35	23.3 ± 1.6	24.7 ± 1.2	1.4 ± 1.0
	PFBA, 175	23.7 ± 2.7	24.7 ± 2.2	1.0 ± 0.9
	PFBA, 350	23.5 ± 2.9	24.3 ± 3.1	0.8 ± 1.0
	Wy-14,643, 50	25.1 ± 2.3	25.0 ± 2.2	−0.1 ± 0.9
hPPAR-α	Control	25.7 ± 5.5	23.7 ± 4.3	−2.0 ± 1.8
	PFBA, 35	26.8 ± 4.6	25.3 ± 3.8	−1.5 ± 2.4
	PFBA, 175	26.4 ± 3.3	24.9 ± 3.2	−1.5 ± 1.0
	PFBA, 350	19.2 ± 5.5**	19.4 ± 3.8**	0.2 ± 2.8
	Wy-14,643, 50	25.0 ± 2.7	23.5 ± 2.2	−1.5 ± 0.9

Note. **Significantly different at $p \leq 0.05$.

[a]The average difference between final and initial body weight. Values represent the mean ± SD.

Tissue and serum analysis of PFBA concentration. Serum was obtained from whole blood for analysis of serum PFBA concentration, which was performed as previously described (Chang *et al*., 2008). Similarly, a piece of whole liver was used to quantify the concentration of PFBA in liver using a previously described method (Chang *et al*., 2008).

Analysis of test compound concentrations in liver and serum. Serum (processed from whole blood) and liver were harvested during necropsy and stored frozen pending liquid chromatography-mass spectrometry/mass spectrometry analysis of test compound concentrations for PFBA (serum and liver) and Wy-14,643 (serum only). PFBA was determined using a previously described method (Chang *et al*., 2008). We were unable to find previously published methods for the determination of Wy-14,643 in serum and liver and believe that the following LC-MS/MS method is the first such method to be described. Serum and liver quantitations for Wy-14,643 were based on a liquid / liquid extraction at pH 7.4 using ethyl acetate. Liver samples were diluted one part tissue to four parts reagent grade water prior to homogenization. Samples were homogenized using an IKA T-25 homogenizer (Ultra Turrax, Wilmington, NC) at 20,000 rpm for 1 min. Liver samples were then sonicated for 30 min prior to sampling and subsequent extraction. Homogenized blank liver tissue from naïve rats and purchased newborn calf serum (Invitrogen-Gibco, Grand Island, NY) were used as matrix blanks for our standard curves and serum spiked controls. Dual ^{13}C PFOA was used as an internal standard and added to all tubes prior to extraction. Extractions were based on 100 µl of serum or liver homogenate to which 1.0 ml of phosphate buffer (pH = 7.4) and 100 µl of saturated ammonium sulfate were added. The tubes were vortex briefly and 10 ml of ethyl acetate was added. Tubes were mixed on a mechanical shaker for 1 h, centrifuged at 2500 × g for 5 min and the top layer (ethyl acetate) was transferred to an appropriately labeled, new, clean, polypropylene tube. The ethyl acetate was dried using a rotary evaporator mixer (LabConCo Rapid Evaporator, Kansas City, MO). Samples were taken up with 1500 µl of a solvent mixture containing 50% acetonitrile, 25% reagent grade water and 25% 0.2mM ammonium acetate. All tubes were vortexed well and the solvent mixture transferred to a polypropylene micro-vial, capped and placed on a high pressure liquid chromatograph (HPLC) mass spectrometer for analysis. The instrument used for analysis was the Sciex API 5000 mass spectrometer from Applied Biosystems/MDS-Sciex Instrument Corporation. We used Turbo Ion Spray (pneumatically assisted electrospray ionization) in negative ion mode. Separation of the compounds was completed on a Phenomenex Synergi, 4 µm Hydro-RP 80A, 150 × 3.0 mm internal diameter HPLC column. A gradient flow of 0.25 ml/min. with an initial mobile phase composition of 25% acetonitrile and 75% 2mM ammonium acetate solution made up in reagent grade water was used. All source parameters were optimized under these conditions according to manufacturer's guidelines. Transition ions were monitored as follows: for Wy-14,643, 322 amu → 278 amu; for dual ^{13}C-labeled PFOA internal standard, 415 → 370 amu. Quantitation was based on these transition ions. Peak areas were integrated and the ratio of the compound to the internal standard was calculated. The peak area ratio was plotted on the y-axis and the analyte concentration (ng/ml) was plotted on the x-axis. Curves are evaluated with either a linear regression or a quadratic equation model and reviewed for best fit. All standards are all weighted at $1/x$ for the regression analysis. Analytes below the lower limit of quantitation (LLQ) are reported as less than the LLQ. Our LLQ by default in this laboratory remains the lowest standard value that is included on the regression fit of the standard curve (usually our lowest standard employed unless otherwise noted). Serum matrix matched extracted standard curves were run from 5 to 1000 ng/ml where typical regression "R" values fell between 0.9993 and 0.9997. Between run, spiked quality control samples for target Wy-14,643 concentrations of 50 and 280 ng/ml (N = 14/concentration) gave average determinations of 51.8 and 277 ng/ml and coefficients of variation of 5.1 and 6.55%, respectively.

Histopathology. For histological analysis, liver sections were fixed in 10% PBF for 24 h and then transferred to containers with 70% ethanol. Fixed liver sections were embedded in paraffin and 3-µm sections obtained. Representative sections from each liver were stained with hematoxylin and eosin (H&E) and

examined by a pathologist using light microscopy. Hepatocyte hypertrophy and focal necrosis with inflammatory cell infiltration were scored based on incidence and severity with the following scoring system: grade 0 (normal); grade 1 (minimal); grade 2 (mild); grade 3 (moderate); and grade 4 (severe). Representative slides were used for immunohistochemical analysis of BrdU labeling to measure replicative DNA synthesis. BrdU staining was performed using the manufacturer's recommended procedures (Calbiochem, San Diego, CA). Five frames from representative slide from each group were scored for BrdU labeling at 400× magnification and are presented as the percentage of BrdU-positive cells.

Serum alanine aminotransferase assessment. Serum was obtained from mice and the concentration of l-alanine: 2-oxoglutarate aminotransferase (ALT) was measured using INFINITY125 ALT Reagent (Thermo Electron, Melbourne, Australia) according to the manufacturer's recommended procedure.

Quantitative real-time PCR analysis. Total RNA was isolated from snap-frozen liver samples of five animals from each group using Trizol following the manufacturer's recommended protocol (Invitrogen, Carlsbad, CA). The mRNAs encoding the known PPAR-α target genes, cytochrome P4504a10 (*Cyp4a10*) and acyl-CoA oxidase 1 (*Aco1*), were measured using quantitative real-time PCR (qPCR) analysis. The cDNA was generated using 2.5 μg total RNA with MultiScribe Reverse Transcriptase kit (Applied Biosystems, Foster City, CA). Primers were designed for real-time PCR using the Primer Express software (Applied Biosystems). The sequence and GenBank accession numbers for the forward and reverse primers used to quantify mRNAs were *Cyp4a10* (NM_010011) forward, 5′-TGCCCATGATCACACAGATGGAGT-3′ and reverse, 5′-TGAATGTGTCCACCTCAGCACGTA-3′; *Ccnd1* (NM_007631) forward, 5′-GAAGGAGATTGTGCCATCCATGCG-3′ and reverse, 5′-GGAAGACCTCCTCTTCGCACTTCT-3′; and *Aco1* (NM_015729) forward, 5′-TGCCTTTGTTGTCCCTATCCGTGA-3′ and reverse, 5′-TTACATA-CGTGCCGTCAGGCTTCA-3′. All mRNAs examined were normalized to the gene encoding glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*; BC083149) using the following primers: forward, 5′-GGTGGAGCCA-AAAGGGTCAT-3′ and reverse, 5'-GGTTCACACCCATCACAAACAT-3′. qPCR reactions were carried out using SYBR green PCR master mix (Finnzymes, Espoo, Finland) in the iCycler and detected using the MyiQ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The following conditions were used for PCR: 95°C for 15 s, 94°C for 10 s, 60°C for 30 s, and 72°C for 30 s and repeated for 45 cycles. The PCR included a no template control reaction to control for contamination and/or genomic amplification. All reactions had > 85% efficiency. Relative expression levels of mRNA were normalized to *Gapdh* and analyzed for statistical significance using ANOVA and *post hoc* testing.

Statistical analysis. Data were analyzed for statistical significance using analysis of variance and the Bonferroni post-test (Prism 5.0, GraphPad Software, Inc., San Diego, CA). Significant differences were determined when $p \leq 0.05$.

RESULTS

Effect of PFBA on Body and Liver Weights

The average initial and final body weight of the humanized PPAR-α group being treated with 350 mg/kg PFBA was significantly lower as compared with all groups (Table 1), and this was due to random assignment. The average change in body weight from the beginning to ending body weight was not different between any treatment group (Table 1). A dose-dependent increase in relative liver weight was observed following exposure to PFBA in wild-type mice and this effect was not found in similarly treated PPAR-α null mice (Fig. 1).

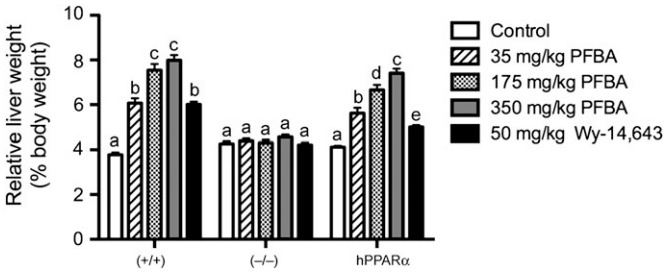

FIG. 1. Effect of PFBA on liver weight in wild-type (+/+), PPAR-α null (−/−) and humanized PPAR-α (hPPAR-α) mice. For a positive control, mice were treated with Wy-14,643. Values represent the mean ± SEM. Values with different letters are significantly different at $p \leq 0.05$.

A dose-dependent increase in relative liver weight was also observed in humanized PPAR-α mice after PFBA treatment, but the average increase in liver weight was significantly less as compared with similarly treated wild-type mice (Fig. 1). Consistent with previous studies (Cheung *et al.*, 2004; Morimura *et al.*, 2006), the increase in relative liver weight was significantly higher in the Wy-14,643–treated wild-type mice as compared with humanized PPAR-α mice (Fig. 1).

Liver tissue concentrations of PFBA are shown in Figure 2A. In wild-type mouse liver, the concentration of

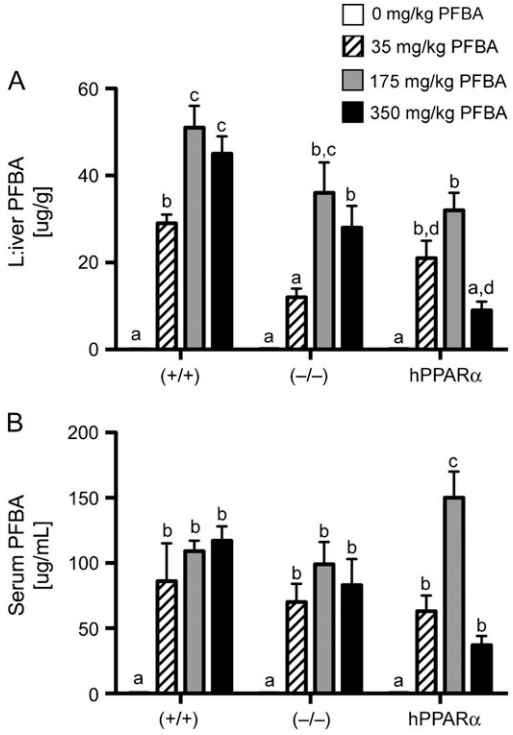

FIG. 2. Tissue concentration of PFBA in wild-type (+/+), PPAR-α null (−/−) and humanized PPAR-α (hPPAR-α) mice. PFBA concentration was determined as described in ''Materials and Methods.'' Values represent the mean ± SEM. Values with different letters are significantly different at $p \leq 0.05$.

PFBA increased in response to PFBA exposure, with the concentrations achieved with 175 and 350 mg/kg being higher as compared with the concentration observed following 35 mg/kg/day (Fig. 2A). In PPAR-α null mouse liver, the concentration of PFBA was significantly increased by exposure to 175 and 350 mg/kg/day (Fig. 2A). Although the hepatic concentration of PFBA was higher in PPAR-α null mice treated with 35 mg/kg/day, this increase was not significantly different compared with controls (Fig. 2A). In humanized PPAR-α mice, the concentration of PFBA in liver was increased relative to controls following treatment with 35 mg/kg/day or 175 mg/kg/day PFBA, but not in the mice dosed with 350 mg/kg/day (Fig. 2A). Between genotypes, the concentration of liver PFBA was lower in PPAR-α null mice as compared with both wild-type and humanized PPAR-α mice following exposure to 35 mg/kg/day PFBA (Fig. 2A). The concentration of PFBA in liver was lower in PPAR-α null and humanized PPAR-α mice as compared with wild-type mice treated with either 175 or 350 mg/kg/day PFBA (Fig. 2A).

Serum concentrations of PFBA by genotype are shown in Figure 2B. The concentration of PFBA in serum of wild-type and PPAR-α null mice was increased by PFBA but was not different between the three treatment levels (Fig. 2B). The concentration of PFBA in serum of humanized PPAR-α mice was increased by PFBA exposure, but was significantly higher in the mice treated with 175 mg/kg/day PFBA as compared with the other treatment groups (Fig. 2B). There were no differences in the concentration of serum PFBA in the PFBA-treated mice between genotypes, other than the higher level of serum PFBA in the humanized PPAR-α mice treated with 175 mg/kg/day PFBA (Fig. 2B).

PFBA Activates PPAR-α in Liver

qPCR was performed to examine the expression of PPAR-α target genes. Expression of mRNA encoding the PPAR-α target gene, *Cyp4a10*, was significantly increased in all of the PFBA treatment concentration groups and the Wy-14,643 group in wild-type mice as compared with control (Fig. 3A). In contrast, expression of *Cyp4a10* mRNA was not influenced by either PFBA or Wy-14,643 in either PPAR-α null mice or humanized PPAR-α mice (Fig. 3A). The expression of mRNA encoding *Aco* was increased by PFBA and Wy-14,643 in wild-type and humanized PPAR-α mice, and this effect was not found in similarly treated PPAR-α null mice (Fig. 3B).

Other mRNA Transcripts

No consistent changes in the expression of mRNA encoding *cyclin D1*, *c-myc*, *Cyp2b10*, or *Cyp3a11* were observed in any genotype with any treatment group (data not shown). The reason why no change was observed in these markers, which are typically increased by PPAR-α agonists, is uncertain.

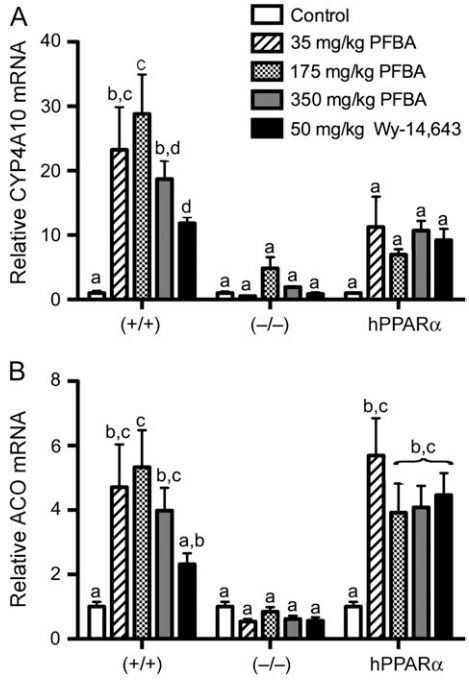

FIG. 3. PPAR-α-dependent regulation of gene expression by PFBA. Total RNA was isolated from the liver of wild-type (+/+), PPAR-α null (−/−), and humanized PPAR-α (hPPAR-α) mice and used for quantitative real-time PCR to quantify mRNA encoding (A) *Cyp 4a10* or (B) *Aco* as described in Materials and Methods. For a positive control, RNA from mice treated with Wy-14,643 was used. Values represent the mean ± SEM. Values with different letters are significantly different at $p \leq 0.05$.

Differential Effect of PFBA by Mouse and Human PPAR-α

Histopathological examination of liver sections revealed that PFBA caused mild to severe hepatocyte hypertrophy in wild-type and humanized PPAR-α mice, with the more severe hepatocyte hypertrophy being observed at the two higher doses (175 and 350 mg/kg/day) (Table 2). Similar changes in hepatocyte hypertrophy were observed in wild-type and humanized PPAR-α mice treated with Wy-14,643 (Table 2). Hepatocyte hypertrophy was not observed in PPAR-α null mice treated with either PFBA or Wy-14,643 (Table 2).

In wild-type mice, hepatic focal necrosis with inflammatory cell infiltration was observed in wild-type mice treated with 175 or 350 mg/kg/day PFBA (Table 3, Fig. 4). This phenotype was greatly diminished in incidence and severity in PPAR-α null and humanized PPAR-α mice. Minimal hepatic focal necrosis was observed in 1/10 and 2/10 PPAR-α null mice in the 175 and 350 mg/kg/day dose groups, respectively. Humanized PPAR-α mice treated with PFBA did not differ from PPAR-α null mice similarly treated with PFBA except that 1/10 humanized PPAR-α mice treated with 35 mg/kg/day PFBA had minimal hepatic focal necrosis. Minimal to mild hepatic focal necrosis with inflammatory cell infiltrate was observed in only 3/10 wild-type mice and 2/10 humanized PPAR-α mice following administration of Wy-14,643, but not in similarly treated PPAR-α null mice

TABLE 2
Incidence and Degree of Hepatocyte Hypertrophy following Exposure to PFBA in Wild-Type (+/+), PPAR-α Null (−/−), and Humanized PPAR-α (hPPAR-α) Mice

		Hepatocyte hypertrophy				
		Control		PFBA		Wy-14,643
		0 mg/kg	35 mg/kg	175 mg/kg	350 mg/kg	50 mg/kg
(+/+)	Minimal	0	0	0	0	0
	Mild	0	4	1	0	1
	Moderate	0	6	1	0	3
	Severe	0	0	8	10	6
	Total	0	10	10	10	10
(−/−)	Minimal	0	0	0	0	0
	Mild	0	0	0	0	0
	Moderate	0	0	0	0	0
	Severe	0	0	0	0	0
	Total	0	0	0	0	0
hPPAR-α	Minimal	0	0	0	0	0
	Mild	0	1	0	0	0
	Moderate	0	4	2	0	4
	Severe	0	5	8	10	6
	Total	0	10	10	10	10

Note. H&E stained liver sections were examined by a pathologist. The incidence and degree of hepatocyte hypertrophy were noted, ranging from minimal to severe. Representative sections from 10 independent mice were used for the analysis.

(Table 3). No significant differences in serum ALT were observed between any of the treatment groups (Table 4) and the average concentration of serum ALT for all groups was within the normal range reported in mice (Schnell *et al.*, 2002).

Centrilobular vacuolation was observed in PPAR-α null mice treated with PFBA (Fig. 4) and periportal vacuolation was found in humanized PPAR-α mice treated with PFBA (Fig. 4). These effects were not found in PFBA-treated wild-type mice.

Replicative DNA synthesis was increased in wild-type mice administered 35 mg/kg PFBA, but this effect was not found in any other genotype or experimental group (Table 5). The standard error in the 35 mg/kg/day PFBA wild-type group was quite large. Although relative BrdU labeling was modestly increased in wild-type mice treated with 175 and 350 mg/kg/day PFBA and Wy-14,643, these changes were not statistically significant. Similarly, relative BrdU labeling was lower in the PPAR-α null mice as compared with wild-type mice, but this difference was not statistically significant.

DISCUSSION

Results from these studies confirm that PFBA can activate PPAR-α as shown by increased expression of *Cyp4a10* and *Aco*. These findings are the first to demonstrate that these PFBA-dependent effects require a functional receptor as these changes do not occur in mice that lack expression of PPAR-α. Because these changes in gene expression were also noted in humanized PPAR-α mice, this also shows that the human PPAR-α mediates similar changes. This is consistent with previous work showing that lipid-lowering fibrates that are PPAR-α agonists cause changes in gene expression encoding targets that modulate lipid metabolism by activating either the mouse or human PPAR-α (Cheung *et al*. 2004). This is also consistent with the fact that PPAR-α agonists can lower serum lipids in both rodents and humans (Peraza *et al*. 2006; Peters *et al*. 2005). These observations demonstrate that PFBA can modulate expression of lipid metabolizing enzymes through both the mouse and human PPAR-α.

Administration of PFBA also caused a PPAR-α–dependent increase in average liver weight and hepatocyte hypertrophy because these changes were found in wild-type mice but not in similarly treated PPAR-α null mice. The relative increase in liver weight and hepatocyte hypertrophy was also observed in humanized PPAR-α mice. However, the increase in liver weight was less in Wy-14,643–treated humanized PPAR-α mice as compared with Wy-14,643–treated wild-type mice. A similar diminished response in liver weight following PFBA administration was also observed in the humanized mice treated with 175 mg PFBA/kg/day. Although the average liver weight was lower in the humanized PPAR-α mice as compared with wild-type mice treated with either 35 or 350 mg/kg/day PFBA, these differences were not statistically significant. Nevertheless, these findings are consistent with previous work showing that although Wy-14,643 and fenofibrate cause an increase in average liver weight in wild-type mice that is not found in PPAR-α null mice, a modest increase in liver weight is still found in the humanized PPAR-α mice (Cheung *et al*. 2004). However, it is worth noting that because the concentration of PFBA in the liver was lower in the humanized mice treated with 350 mg/kg/day PFBA, the difference in average liver weight may reflect this. The reason for the difference in liver PFBA cannot be determined from our study but could reflect PPAR-α–dependent changes in hepatic or renal organic anion transporters, which has been shown for PFOA (Cheng and Klaassen, 2008). Because PFBA caused a PPAR-α–dependent increase in hepatocyte hypertrophy that could not be distinguished between wild-type and humanized PPAR-α mice, this finding suggests that this effect is not differentially regulated by mouse and human PPAR-α. Collectively, these findings provide further evidence that PFBA can cause changes in liver weight through mechanisms that lead to hepatocyte hypertrophy through a PPAR-α–dependent mechanism that is somewhat conserved between mouse and human PPAR-α.

Results from these studies are also the first to demonstrate a potential species difference in the hepatic response to PFBA.

TABLE 3
Incidence and Hepatic Focal Necrosis following Exposure to PFBA in Wild-Type (+/+), PPAR-α Null (−/−), and Humanized PPAR-α (hPPAR-α) Mice

		Focal necrosis				
		Control		PFBA		Wy-14,643
		0 mg/kg	35 mg/kg	175 mg/kg	350 mg/kg	50 mg/kg
(+/+)	Minimal	0	0	2	0	1
	Mild	0	1	4	8	2
	Moderate	0	0	0	1	0
	Severe	0	0	0	0	0
	Total	0	1	6	9	3
(−/−)	Minimal	0	0	1	2	0
	Mild	0	0	0	0	0
	Moderate	0	0	0	0	0
	Severe	0	0	0	0	0
	Total	0	0	0	0	0
hPPAR-α	Minimal	0	1	1	2	2
	Mild	0	0	0	0	0
	Moderate	0	0	0	0	0
	Severe	0	0	0	0	0
	Total	0	1	1	2	2

Note. H&E stained liver sections were examined by a pathologist. The incidence and degree of hepatic focal necrosis were noted, ranging from minimal to severe. Representative sections from 10 independent mice were used for the analysis.

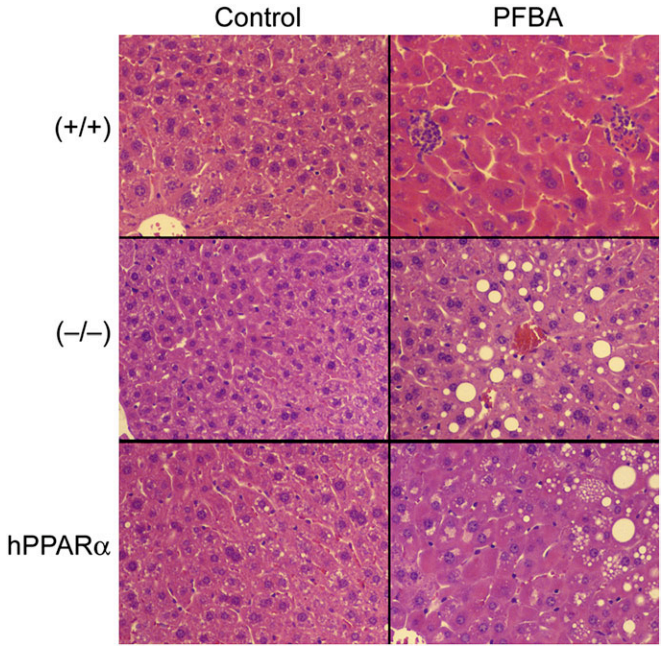

FIG. 4. Histopathology of liver following 2-week exposure to PFBA in wild-type (+/+), PPAR-α null (−/−), and humanized PPAR-α (hPPAR-α) mice. Representative photomicrographs of H&E-stained liver sections from control and PFBA-treated (350 mg/kg) mice. Focal necrosis was found in PFBA-treated (+/+) mice (upper right panel). Centrilobular vacuolation was found in PFBA-treated (−/−) mice (middle right panel) and periportal vacuolation was found in PFBA-treated hPPAR-α mice (lower right panel).

Focal necrosis with inflammatory cell infiltrate was observed in PFBA-treated wild-type mice, and this effect was not found in similarly treated PPAR-α null mice. This clearly demonstrates that the mouse PPAR-α is required for this effect, but the specific mechanism(s) underlying this effect cannot be determined from the present study. In contrast, incidence and severity of focal necrosis with inflammatory cell infiltrate was essentially similar in PPAR-α null mice and humanized PPAR-α mice. This suggests that the human PPAR-α does not mediate the same PFBA-induced hepatic response as the mouse PPAR-α. Because the average concentration of PFBA in liver was somewhat lower in the humanized PPAR-α mice, it remains possible that this phenotype could be observed if higher levels of PFBA were achieved in the liver. This also suggests that the human PPAR-α could mediate some biochemical or molecular change that causes lower liver concentrations of PFBA through an unidentified mechanism. Alternatively, it is also possible that the difference in body weight contributed to the observed difference in liver PFBA concentration. Delineating the specific molecular mechanisms that differentiate between these possibilities that underlie this apparent species difference in the hepatic response to PFBA mediated by PPAR-α cannot be determined from the present studies but should be explored in future studies.

Combined, these studies are the first to demonstrate that PPAR-α is required to mediate changes in hepatic function resulting from PFBA exposure including alterations in target

TABLE 4
Average Serum ALT Concentrations following Exposure to PFBA in Wild-Type (+/+), PPAR-α Null (−/−), and Humanized PPAR-α (hPPAR-α) Mice

	Treatment, mg/kg/day	*N*	Serum [ALT], U/l
(+/+)	Control	6	5.29 ± 3.38
	PFBA, 35	10	8.40 ± 6.03
	PFBA, 175	9	8.51 ± 9.15
	PFBA, 350	10	7.37 ± 4.95
	Wy-14,643, 50	10	9.77 ± 8.67
(−/−)	Control	10	7.39 ± 1.73
	PFBA, 35	10	8.53 ± 6.02
	PFBA, 175	10	17.40 ± 14.66
	PFBA, 350	10	20.28 ± 14.69
	Wy-14,643, 50	9	10.16 ± 3.30
hPPAR-α	Control	9	6.85 ± 4.02
	PFBA, 35	9	14.21 ± 8.05
	PFBA, 175	9	9.28 ± 6.25
	PFBA, 350	10	15.66 ± 18.58
	Wy-14,643, 50	10	10.54 ± 7.32

Note. Values represent the mean ± SD.

TABLE 5
Replicative DNA Synthesis following Exposure to PFBA in Wild-Type (+/+), PPAR-α Null (−/−), and Humanized PPAR-α (hPPAR-α) Mice

	Control		PFBA		Wy-14,643
	0 mg/kg	35 mg/kg	175 mg/kg	350 mg/kg	50 mg/kg
(+/+)	1.8 ± 0.6[a]	19.1 ± 11.7[b]	6.7 ± 3.0[a]	6.7 ± 1.9[a]	3.3 ± 1.9[a]
(−/−)	2.6 ± 2.0[a]	0.8 ± 0.3[a]	0.3 ± 0.1[a]	1.5 ± 1.0[a]	0.9 ± 0.1[a]
hPPAR-α	5.0 ± 1.2[a]	6.0 ± 3.3[a,b]	5.3 ± 3.8[a]	8.6 ± 4.1[a]	1.9 ± 0.2[a]

Note. Relative BrdU labeling is shown as the percentage of BrdU-positive cells as described in ''Material and Methods.'' Values represent the mean ± SEM. Values with different superscript letters are statistically significant at $p \leq 0.05$.

gene expression that modulates lipid metabolism and hepatomegaly. Additionally, the diminished PFBA-induced focal necrosis with inflammatory cell infiltrate in humanized PPAR-α mice suggests that there is a species difference in this hepatic response. Further studies are necessary to elucidate the mechanism(s) that cause this apparent species difference.

FUNDING

3M Company.

ACKNOWLEDGMENTS

We gratefully acknowledge Roberta Horner for technical assistance with these studies.

REFERENCES

Akiyama, T. E., Nicol, C. J., Fievet, C., Staels, B., Ward, J. M., Auwerx, J., Lee, S. S., Gonzalez, F. J., and Peters, J. M. (2001). Peroxisome proliferator-activated receptor-alpha regulates lipid homeostasis, but is not associated with obesity: studies with congenic mouse lines. *J. Biol. Chem.* **276,** 39088–39093.

Bility, M., Thompson, J. T., McKee, R. H., David, R. M., Butala, J. H., Vanden Heuvel, J. P., and Peters, J. M. (2004). Activation of mouse and human peroxisome proliferator-activated receptors (PPARs) by phthalate monoesters. *Toxicol. Sci.* **82,** 170–182.

Burdick, A. D., Kim, D. J., Peraza, M. A., Gonzalez, F. J., and Peters, J. M. (2006). The role of peroxisome proliferator-activated receptor-beta/delta in epithelial cell growth and differentiation. *Cell. Signal.* **18,** 9–20.

Chang, S. C., Das, K., Ehresman, D. J., Ellefson, M. E., Gorman, G. S., Hart, J. A., Noker, P. E., Tan, Y. M., Lieder, P. H., Lau, C., *et al*. (2008). Comparative pharmacokinetics of perfluorobutyrate in rats, mice, monkeys, and humans and relevance to human exposure via drinking water. *Toxicol. Sci.* **104,** 40–53.

Cheng, X., and Klaassen, C. D. (2008). Critical role of PPAR-alpha in perfluorooctanoic acid- and perfluorodecanoic acid-induced downregulation of Oatp uptake transporters in mouse livers. *Toxicol. Sci.* **106,** 37–45.

Cheung, C., Akiyama, T. E., Ward, J. M., Nicol, C. J., Feigenbaum, L., Vinson, C., and Gonzalez, F. J. (2004). Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor-a. *Cancer Res.* **64,** 3849–3854.

D'Eon, J. C., Hurley, M. D., Wallington, T. J., and Mabury, S. A. (2006). Atmospheric chemistry of N-methyl perfluorobutane sulfonamidoethanol, C4F9SO2N(CH3)CH2CH2OH: Kinetics and mechanism of reaction with OH. *Environ. Sci. Technol.* **40,** 1862–1868.

Gonzalez, F. J., and Shah, Y. M. (2008). PPARalpha: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. *Toxicology* **246,** 2–8.

Hays, T., Rusyn, I., Burns, A. M., Kennett, M. J., Ward, J. M., Gonzalez, F. J., and Peters, J. M. (2005). Role of peroxisome proliferator-activated receptor-{alpha} (PPAR{alpha}) in bezafibrate-induced hepatocarcinogenesis and cholestasis. *Carcinogenesis* **26,** 219–227.

Houde, M., Martin, J. W., Letcher, R. J., Solomon, K. R., and Muir, D. C. (2006). Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol* **40,** 3463–3473.

Ikeda, T., Aiba, K., Fukuda, K., and Tanaka, M. (1985). The induction of peroxisome proliferation in rat liver by perfluorinated fatty acids, metabolically inert derivatives of fatty acids. *J. Biochem.* **98,** 475–482.

Just, W. W., Gorgas, K., Hartl, F. U., Heinemann, P., Salzer, M., and Schimassek, H. (1989). Biochemical effects and zonal heterogeneity of peroxisome proliferation induced by perfluorocarboxylic acids in rat liver. *Hepatology* **9,** 570–581.

Kozuka, H., Watanabe, T., Horie, S., Yamada, J., Suga, T., and Ikeda, T. (1991). Characteristics of peroxisome proliferation: Co-induction of peroxisomal fatty acid oxidation-related enzymes with microsomal laurate hydroxylase. *Chem. Pharm. Bull.* **39,** 1267–1271.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., and Seed, J. (2007). Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol. Sci.* **99,** 366–394.

Lee, S. S., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L., Fernandez-Salguero, P. M., Westphal, H., and Gonzalez, F. J. (1995). Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **15,** 3012–3022.

Maloney, E. K., and Waxman, D. J. (1999). trans-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. *Toxicol. Appl. Pharmacol.* **161,** 209–218.

Martin, J. W., Ellis, D. A., Mabury, S. A., Hurley, M. D., and Wallington, T. J. (2006). Atmospheric chemistry of perfluoroalkanesulfonamides: kinetic and product studies of the OH radical and Cl atom initiated oxidation of N-ethyl perfluorobutanesulfonamide. *Environ. Sci. Technol.* **40,** 864–872.

Morimura, K., Cheung, C., Ward, J. M., Reddy, J. K., and Gonzalez, F. J. (2006). Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor α to Wy-14,643-induced liver tumorigenesis. *Carcinogenesis* **27,** 1074–1080.

Peraza, M. A., Burdick, A. D., Marin, H. E., Gonzalez, F. J., and Peters, J. M. (2006). The toxicology of ligands for peroxisome proliferator-activated receptors (PPAR). *Toxicol. Sci.* **90,** 269–295.

Permadi, H., Lundgren, B., Andersson, K., Sundberg, C., and DePierre, J. W. (1993). Effects of perfluoro fatty acids on peroxisome proliferation and mitochondrial size in mouse liver: dose and time factors and effect of chain length. *Xenobiotica* **23,** 761–770.

Peters, J. M. (2008). Mechanistic Evaluation of PPAR-mediated hepatocarcinogenesis: Are we there yet? *Toxicol. Sci.* **101,** 1–3.

Peters, J. M., Cattley, R. C., and Gonzalez, F. J. (1997). Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. *Carcinogenesis* **18,** 2029–2033.

Peters, J. M., Cheung, C., and Gonzalez, F. J. (2005). Peroxisome proliferator-activated receptor-alpha and liver cancer: where do we stand? *J. Mol. Med* **83,** 774–785.

Peters, J. M., Hollingshead, H. E., and Gonzalez, F. J. (2008). Role of peroxisome-proliferator-activated receptor beta/delta (PPARbeta/delta) in gastrointestinal tract function and disease. *Clin. Sci. (Lond.)* **115,** 107–127.

Schnell, M. A., Hardy, C., Hawley, M., Propert, K. J., and Wilson, J. M. (2002). Effect of blood collection technique in mice on clinical pathology parameters. *Hum. Gene Ther.* **13,** 155–161.

Scott, B. F., Moody, C. A., Spencer, C., Small, J. M., Muir, D. C., and Mabury, S. A. (2006a). Analysis for perfluorocarboxylic acids/anions in surface waters and precipitation using GC–MS and analysis of PFOA from large-volume samples. *Environ. Sci. Technol.* **40,** 6405–6410.

Scott, B. F., Spencer, C., Mabury, S. A., and Muir, D. C. (2006b). Poly and perfluorinated carboxylates in North American precipitation. *Environ. Sci. Technol.* **40,** 7167–7174.

Skutlarek, D., Exner, M., and Farber, H. (2006). Perfluorinated surfactants in surface and drinking waters. *Environ. Sci. Pollut. Res. Int.* **13,** 299–307.

Wolf, C. J., Takacs, M. L., Schmid, J. E., Lau, C., and Abbott, B. D. (2008). Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. *Toxicol. Sci.* **106,** 162–171.