ToxSci Advance Access published July 30, 2015

OXFORD

SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 2015, 1–11

doi: 10.1093/toxsci/kfv134
Advance Access Publication Date: July 1, 2015

The Contribution of Peroxisome Proliferator-Activated Receptor Alpha to the Relationship Between Toxicokinetics and Toxicodynamics of Trichloroethylene

Hong Sik Yoo*,[1], Joseph A. Cichocki†,[1], Sungkyoon Kim‡, Abhishek Venkatratnam*,†, Yasuhiro Iwata†, Oksana Kosyk*, Wanda Bodnar*, Stephen Sweet§, Anthony Knap§, Terry Wade§, Jerry Campbell¶, Harvey J. Clewell¶, Stepan B. Melnyk||, Weihsueh A. Chiu† and Ivan Rusyn†,[2]

*Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, North Carolina; †Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, Texas, USA; ‡Graduate School of Public Health, Seoul National University, Seoul, Republic of Korea; §Geochemical and Environmental Research Group, Texas A&M University, College Station, Texas; ¶The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina; and ||Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA.

[1]These authors contributed equally to this study.
[2]To whom correspondence should be addressed at Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, Texas, USA. E-mail: irusyn@cvm.tamu.edu

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

ABSTRACT

Exposure to the ubiquitous environmental contaminant trichloroethylene (TCE) is associated with cancer and non-cancer toxicity in both humans and rodents. Peroxisome proliferator-activated receptor-alpha (PPARα) is thought to be playing a role in liver toxicity in rodents through activation of the receptor by the TCE metabolite trichloroacetic acid (TCA). However, most studies using genetically altered mice have not assessed the potential for PPARα to alter TCE toxicokinetics, which may lead to differences in TCA internal doses and hence confound inferences as to the role of PPARα in TCE toxicity. To address this gap, male and female wild type (129S1/SvImJ), *Ppar*α-null, and humanized *PPAR*α (h*PPAR*α) mice were exposed intragastrically to 400 mg/kg TCE in single-dose (2, 5 and 12 h) and repeat-dose (5 days/week, 4 weeks) studies. Interestingly, following either a single- or repeat-dose exposure to TCE, levels of TCA in liver and kidney were lower in *Ppar*α-null and h*PPAR*α mice as compared with those in wild type mice. Levels of trichloroethanol (TCOH) were similar in all strains. TCE-exposed male mice consistently had higher levels of TCA and TCOH in all tissues compared with females. Additionally, in both single- and repeat-dose studies, a similar degree of induction of PPARα-responsive genes was observed in liver and kidney of h*PPAR*α and wild type mice, despite the difference in hepatic and renal TCA levels. Additional sex- and strain-dependent effects were observed in the liver, including hepatocyte proliferation and oxidative stress, which were not dependent on TCA or TCOH levels. These data demonstrate that PPARα status affects the levels of the putative PPARα agonist TCA following TCE exposure. Therefore, interpretations of studies using *Ppar*α-null and h*PPAR*α mice need to

© The Author 2015. Published by Oxford University Press on behalf of the Society of Toxicology.
All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com

consider the potential contribution of genotype-dependent toxicokinetics to observed differences in toxicity, rather than attributing such differences only to receptor-mediated toxicodynamic effects.

Key words: PPAR; trichloroethylene; liver

Trichloroethylene (TCE) is classified as a human carcinogen based on convincing evidence for a positive association with (1) renal-cell carcinoma in humans and (2) tumors of multiple sites in mice and rats of both sexes (Guha *et al.*, 2012). The epidemiological evidence for the association between TCE exposure and liver cancer in humans is limited, even though liver is a well-established target organ in mice. Activation of peroxisome proliferator-activated receptor alpha (PPARα) is one of the mechanisms thought to be involved in the pathogenesis of liver cancer in mice exposed to TCE. In humans, the role of PPARα remains as a contentious issue in hazard assessment of TCE and other agents (Corton *et al.*, 2014; Keshava and Caldwell, 2006).

The absence of functional PPARα completely abolished the hepatocarcinogenic response from the prototypical and highly potent ligand WY-14 643 in the mouse (Peters *et al.*, 1997). Mice expressing human *PPARα* (h*PPARα*) also have diminished hepatotoxic or hepatocarcinogenic responses when exposed to the peroxisome proliferators fenofibrate (Cheung *et al.*, 2004) or WY-14 643 (Morimura *et al.*, 2006). A number of hypotheses have been proposed to link PPARα and liver carcinogenesis through alterations in cell proliferation and apoptosis (Peters, 2008; Peters *et al.*, 2012). At the same time, in a mouse model of constitutive activation of this nuclear receptor in liver, cell proliferation but not liver cancer were reported, which suggests that ligand activation and recruitment of co-effector proteins may also play an important role (Yang *et al.*, 2007). Inter-individual and inter-species differences in genomic sequence, expression patterns and signaling cascades of PPARα have been reported, further compounding the challenge of assessing the relative role of this mechanism in carcinogenesis (Rusyn and Corton, 2012). Additional mechanisms may also be operational in the pathogenesis of environmental chemicals that are weak or nonselective agonists of PPARα (Ito *et al.*, 2012; Ren *et al.*, 2010; Wood *et al.*, 2014).

TCE is a relatively weak activator of either human or murine PPARα, but TCE metabolites tri- and di-chloro acetic acids (TCA and DCA) were found to be more potent activators (Maloney and Waxman, 1999; Zhou and Waxman, 1998). TCE-induced peroxisome proliferation response in mouse liver and kidney is thought to be mediated exclusively by TCA and DCA (Corton, 2008; Rusyn *et al.*, 2014). Moreover; TCE metabolism to TCA and DCA is not thought to involve PPARα-inducible cytochrome P450 enzymes (Lash *et al.*, 2014). Several studies observed abrogated toxic effects (eg, increased peroxisomal volume and peroxisomal enzyme activity) of TCE in *Pparα*-null mice (Laughter *et al.*, 2004; Nakajima *et al.*, 2000; Ramdhan *et al.*, 2010). In addition, in mouse exposed to TCE, strain-specific tissue levels of TCA and DCA have been shown to be highly correlated with PPARα activation in liver (Yoo *et al.*, 2015a) and kidney (Yoo *et al.*, 2015b).

Taken together, these studies suggest a simple toxicokinetic-toxicodynamic adverse outcome pathway whereby: (1) TCE is metabolized to TCA and DCA in the liver; (2) these metabolites activate PPARα in the liver (where they are formed *in situ*) and kidney (where they are transported for urinary excretion); and (3) activation of PPARα leads to a cascade of hepatocellular responses that may contribute to TCE-associated hepatocarcinogenesis in mice. However, a study of TCE inhalation in wild type, *Pparα*-null, and h*PPARα* mice (Ramdhan *et al.*, 2010) showed genotype-dependent differences in levels of urinary TCA and trichloroethanol (TCOH), suggesting that PPARα status may actually affect TCE toxicokinetics. This in turn may affect the interpretation of previous studies which exposed *Pparα*-null and h*PPARα* mice to TCE, since differences in responses may not be due solely to differences in activation of PPARα (or lack thereof) but also in the production of metabolites. To test the hypothesis that PPARα status affects TCE toxicokinetics, we measured TCE and its metabolites in serum, liver, and kidney in wild type, *Ppara*-null, and h*PPARα* mice exposed to TCE acutely and sub-chronically by oral gavage. Additionally, to assess the relative contributions of toxicokinetics or toxicodynamics to TCE-induced hepatic and renal toxicity, we measured hepatic and renal levels of PPARα-responsive genes as well as biochemical markers of toxicity. Our results demonstrate that PPARα status affects TCE toxicokinetics in the liver and kidney. Such alternations in toxicokinetics may contribute to genotype-dependent differences in toxic responses in mouse liver and kidney.

MATERIALS AND METHODS

Animals and treatments.. Male and female mice from 3 different genotypes were used. Wild type (129S1/SvImJ) and *Ppara*-null (129S4/SvJae-*Ppara*^{tm1Gonz}/J) mice of 9–10 weeks of age were purchased from the Jackson Laboratory (Bar Harbor, Maine), and humanized-*PPARa* (h*PPARα*) mice on an Sv/129 genetic background (Cheung *et al.*, 2004) were provided by Dr Frank Gonzalez (Laboratory of Metabolism, National Cancer Institute). All mice were housed in polycarbonate cages on Sani-Chips (P.J. Murphy Forest Products Corp., Montville, New Jersey) irradiated hardwood bedding. Animals were fed an NTP-2000 (Zeigler Brothers, Inc., Gardners, Pennsylvania) wafer diet and water *ad libitum* on a 12-h light-dark cycle. All studies were approved by the UNC Institutional Animal Care and Use Committee.

Two study designs were utilized in this work. First, we performed a sub-chronic study where TCE (400 mg/kg/day, in 5% Alkamuls EL-620 in saline) was administered by gavage to male and female mice from the 3 different genotypes for 4 weeks (5 days/week). Mice were also given drinking water containing 0.2 g/l of 5-bromo-2′-deoxyuridine (BrdU) for 72 h prior to sacrifice. Blood, liver, kidney, and a section of a duodenum were collected 5 h after the last TCE treatment in order to evaluate levels of TCE metabolites in mouse tissues and cell proliferation in the liver and kidney. This time point was selected based on a toxicokinetics study of TCE metabolism in the mouse to represent a time window when all metabolites are close to their peak levels (Kim *et al.*, 2009b). Second, we conducted a toxicokinetic study where wild type, *Ppara*-null, and h*PPARα* mice received a single dose (400 mg/kg) of TCE in 5% Alkamuls EL-620 in saline by gavage and sacrificed 2, 5, and 12 h after TCE treatment followed by the collection of liver, kidney, and blood. Blood was drawn from vena cava and centrifuged to prepare serum using Z-gel tubes (Sarstedt, Germany) according to the manufacturer's

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

instructions. In both studies, body and organ weights were recorded. Liver, kidney, and duodenum sections were fixed in neutral buffered formalin for 24 h, and the remainder of the liver and kidney tissues were frozen in liquid nitrogen. All serum and tissue samples were stored at −80°C until analyzed.

Quantification of TCE.. Prior to extraction, liver (100 mg) and kidney (30 mg) samples were homogenized with 2 volumes of deionized water (w/v) using a finger pestle. Tissue homogenate (200 μl) were transferred to autosampler vials containing 200 μl of ammonium sulfate solution, and then 1 μl of internal standard (TCE-deuterated) were added using a microsyringe. The vials were vortexed for 30 s and placed into the autosampler for analysis. The analyses were carried out on an Agilent 7890 gas chromatograph (GC) coupled with a 5975C mass selective detector. The GC was equipped with a 0.75 mm i.d. Solid Phase Microextraction (SPME) liner. Separation of the analytes was obtained on DB-5MS column (Phenomenex, 30 m × 0.25 mm i.d., 0.25 μm film thickness) using helium as a carrier gas (flow rate, 1 ml/min). The GC injection port and interface transfer line were maintained at 200 and 280°C, respectively. During the fiber desorption process, the splitless mode of injection was operated. After 2 min, the split vent valve opened to sweep any residual vapors from the liner. The oven temperature was initially held at 35°C for 3 min, and then increased to 70°C at 10°C/min. The mass spectrometer was operated positive electron ionization mode with electron energy of 71 eV. Quantitation of TCE was performed using selected-ion monitoring mode by measuring the signal for m/z 130 (131 for TCE-d). GC-SPME was performed using a 100 μm polydimethylsiloxane fiber mounted on a Combi-Pal system autosampler. Fibers were conditioned at 200°C for 30 min prior to use. Sample vials were preheated in the agitator for 5 min before analysis, and the SPME fiber was then exposed to the headspace by piercing the septum with the needle of the fiber assembly. After extraction for 15 min at 30°C under agitation, the fiber was withdrawn into the needle and immediately desorbed at 200°C for 2 min into the GC injection port.

Quantification of TCE metabolites. The levels of TCA in liver and kidney tissues were determined using HPLC-ESI-MS/MS as detailed elsewhere (Kim *et al.*, 2009a) with slight modifications as follows. Two milliliter Eppendorf Safe Lock Tubes containing one stainless steel ball each with 300 μl of chloroform and 60 μl of water were incubated on dry ice for 10 min in a cooling block. Liver (100 mg) or kidney (50 mg) tissue was then incised from frozen tissue samples and placed into each of these tubes. Internal standard (trifluoroacetic acid, 40 nmol/ml) was added to make 100 μl of final aqueous volume. The tubes were then homogenized at 30 Hz for 3 min using TissueLyser (Qiagen, Valencia, California). After homogenization, the tubes were centrifuged at 14 000 ×g for 30 min at 4°C. Aqueous liver or kidney extract was transferred to a centrifugal filter unit (Amicon Ultra 0.5, Millipore, Masschusetts) and centrifuged at 14 000×g for 60 min at 4°C. After centrifugation, the filtrate was transferred to a glass vial containing 300 μl vial insert and was stored at −80°C until injection to HPLC-ESI-MS/MS with an Aquity UPLC system (Waters, Milford, Masschusetts) coupled to a TSQ Quantum Ultra triple-quadrupole mass analyzer (Thermo Fisher Scientific, Waltham, Masschusetts) using a heat-assisted electrospray ionization source. A YMC ODS-AQ analytic column (150 × 2 mm, 3 μm; Waters, Milford, Masschusetts) was used in an isocratic mode with mobile phase (1 mM ammonium citrate in the mixture of 70% acetonitrile and 30% water). The lower limit of quantification (LLOQ) in this study for TCA was 8 nmol/g in liver and 15 nmol/g in kidney.

The method of Song and Ho (2003) was used for quantitation of total TCOH (free TCOH plus TCOH-glucuronide) in liver, kidney, and serum, with minor modifications. Briefly, liver (30 mg), kidney (30 mg), or serum (50 μl) was homogenized in 500 μl of sodium acetate buffer (pH 4.6) with 1000 units of β-glucuronidase (Sigma [G0751], St. Louis, Missouri) using Tissuelyser (Qiagen) for 1 min, followed by overnight incubation at 37°C. After centrifugation at 14 000×g for 5 min, the supernatant was transferred to a new tube, then mixed with 20 μl internal standard (DCA, 10 mM in methanol) and 550 μl of water/0.1 M sulfuric acid/methanol (6:5:1). The mixture was heated at 70°C for 20 min. After cooling to room temperature, 2.5 ml hexane was added, the mixture vortexed for 10 min and centrifuged at 2500×g for 2 min. The upper layer was concentrated under a stream of N_2 to <20 μl and used for GC-MS analysis as detailed in (Song and Ho, 2003). The LLOQ was 5 nmol/g in liver.

Determination of triglyceride content in liver.. Triglycerides were extracted by homogenizing 20 mg of frozen liver tissue in 500 μl of isopropyl alcohol, and 4 μl of the extract was used in subsequent analysis. The level of triglycerides was determined by using L-type Triglyceride-M Assay Kit (Wako Chemicals, Richmond, Virginia) according to the manufacturer's instructions.

Quantification of glutathione, cysteine, and nicotinamide adenine dinucleotide phosphate redox status.. The concentrations of free reduced (GSH) and oxidized glutathione (GSSG) and cellular methylation biomarkers, S-adenosyl-L-methionine (SAM) and S-adenosyl-L-homocysteine (SAH) were determined as measures of redox/metabolic status in liver and kidney by using the high performance liquid chromatography (HPLC) with colorimetric electrochemical detection (HPLC-ED) system (MCM, Inc., Tokyo, Japan). The methodological details for the detection of GSH and GSSG (Melnyk *et al.*, 1999), SAM and SAH (Melnyk *et al.*, 2000) by HPLC have been described previously. NADPH/nicotinamide adenine dinucleotide phosphate (NADP)+ ratio in liver was measured using a NADP/NADPH Quantification Kit (Sigma, St. Louis, Missouri) according to the manufacturer's instructions.

Gene Expression Analysis by Real-Time PCR

Total RNA was isolated from liver and kidney samples using an RNeasy kit (Qiagen) according to the manufacturer's instructions. RNA concentration and quality were determined using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, Delaware) and Agilent 2000 Bioanalyser, respectively. Total RNA was reverse transcribed using random primers and the high capacity complementary DNA archive kit (Applied Biosystems, Foster City, California) according to the manufacturer's protocol. The following gene expression assays (Applied Biosystems) were used for quantitative real-time PCR: peroxisome proliferator–activated receptor alpha (*Ppara*, Mm00440939_m1); palmitoyl acyl-Coenzyme A oxidase 1 (*Acox1*, Mm01246831_m1); cytochrome P450, family 4, subfamily a, polypeptide 10 (*Cyp4a10*, Mm01188913_g1); and beta glucuronidase (*Gusb*, Mm00446953_m1). Reactions were performed in a 96-well plate, and all samples were plated in duplicate using LightCycler 480 instrument (Roche Applied Science, Indianapolis, Indiana). The cycle threshold (Ct) for each sample was determined from the linear region of the amplification plot. The ΔCt values for all genes relative to the control gene *Gusb* were determined. The ΔΔCt were calculated using

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

treated group means relative to strain-matched control group means. Fold change data were calculated from the 2^-ΔΔCt values (Livak and Schmittgen, 2001).

Determination of hepatocyte and proximal tubule cell proliferation.. Deparaffinized and rehydrated liver and kidney sections from the sub-chronic study were immersed in 4 N HCl and subsequently pepsin solution (Dako, Carpinteria, California) for antigen retrieval and then incubated in peroxidase blocking reagent (Dako). Dako EnVision System HRP kit was used for the detection of BrdU-incorporated nuclei (monoclonal anti-bromodeoxyuridine antibody, Dako, 1:200 dilution). Data for liver tissues were presented as a fraction of BrdU staining-positive nuclei in the centrilobular region (no fewer than 1000 nuclei counted per liver section). Data for kidney tissues were presented as a fraction of BrdU staining-positive nuclei in the tubular epithelium of the renal cortex (no fewer than 1000 nuclei counted per kidney section).

Determination of KIM-1 expression in kidney.. Detection of KIM-1 was accomplished by modifying a published method (Humphreys *et al.*, 2011). Formalin-fixed and paraffin-embedded kidney sections were deparaffinized and rehydrated. Antigens were retrieved by 4N HCl and pepsin solution (Dako) afterward. After peroxidase blocking, immunohistochemical detection was conducted using Dako Liquid DAB Substrate Chromogen System with primary anti KIM-1 antibody (2 μg/ml in PBS) (R&D Systems, Minneapolis, Minnesota) and secondary goat IgG HRP-conjugated Antibody (1:100 in PBS) (R&D Systems). The proportion of positive-stained proximal tubules in outer medulla was determined under light microscopy. Data were presented as a fraction of proximal renal tubules staining positive for KIM-1 (no fewer than 200 proximal renal tubules counted per kidney section).

Statistical analysis.. Toxicokinetic data from the single dose study were fit using nonlinear 2-phase exponential association and statistical analysis was performed via repeated measures (strain and time) ANOVA. For all other end points, ANOVA with Newman-Keul's *post hoc* test was performed. For all statistical tests, a p-value of $<.05$ was required for statistical significance.

RESULTS

Concentration-Time Profiles of TCE Metabolism Through Oxidative Pathway in Wild Type, Ppara-Null and hPPARα Mice

First, levels of TCE were measured in liver and kidney of male and female wild type, *Ppara*-null and hPPARα mice at 2, 5, or 12 h following a single intragastric dose of 400 mg/kg. Relatively low, but detectable, levels of TCE (Fig. 1) were found in both tissues and sexes across strains, consistent with rapid metabolism (Lash *et al.*, 2014). Generally, in both tissues, levels of TCE were highest (5–15 nmol/g tissue) at 2–5 h postdosing; TCE was essentially cleared from these tissues 12 h after administration. In liver of male mice of all 3 strains, no differences in TCE concentration-time profiles were observed; however, in kidney of male *Ppara*-null mice we found a significantly lower amount of TCE at 2 h after dosing, as compared with wild type mice. In female mice, liver levels of TCE in wild type and *Ppara*-null mice were greater than in hPPARα mice at 2 h; at 12 h, levels of TCE in liver of wild type mice were also different from those in *Ppara*-null

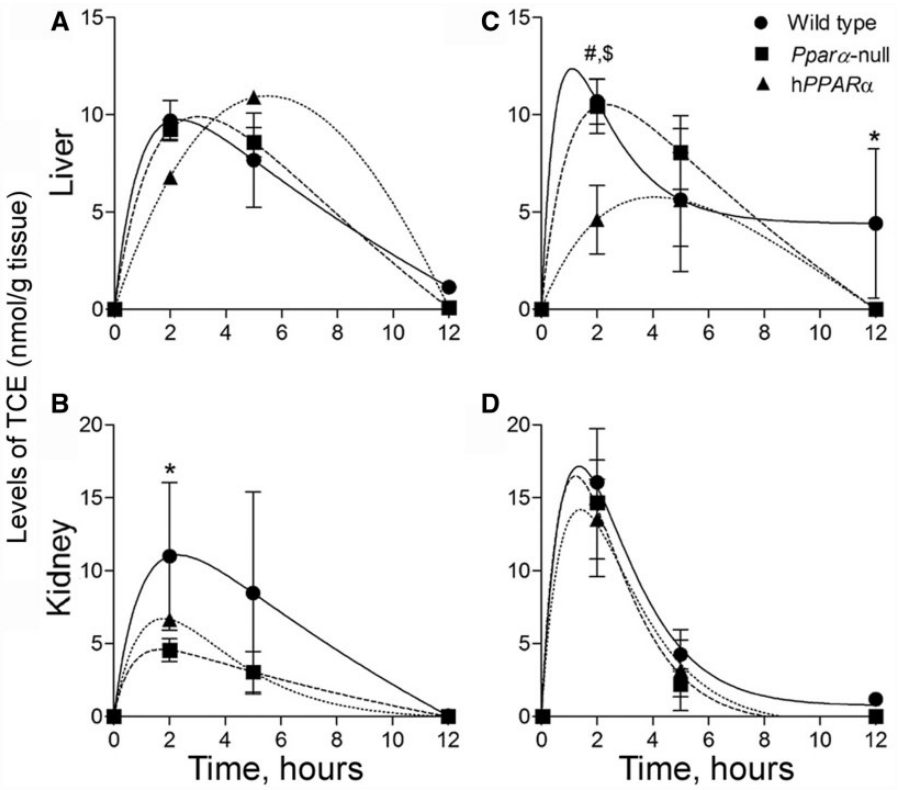

FIG. 1. Kinetics of TCE in **(A)** liver and **(B)** kidney of male mice, and in **(C)** liver and **(D)** kidney of female mice following a single dose of TCE (400 mg/kg *i.g.*). The data shown are mean ± SD, $n = 3$ animals per group. Symbols indicate significant ($p < .05$) differences between the values at each time point between strains as follows: wild type versus *Pparα*-null (*), wild type versus hPPARα (#), and *Pparα*-null versus hPPARα ($) mice.

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

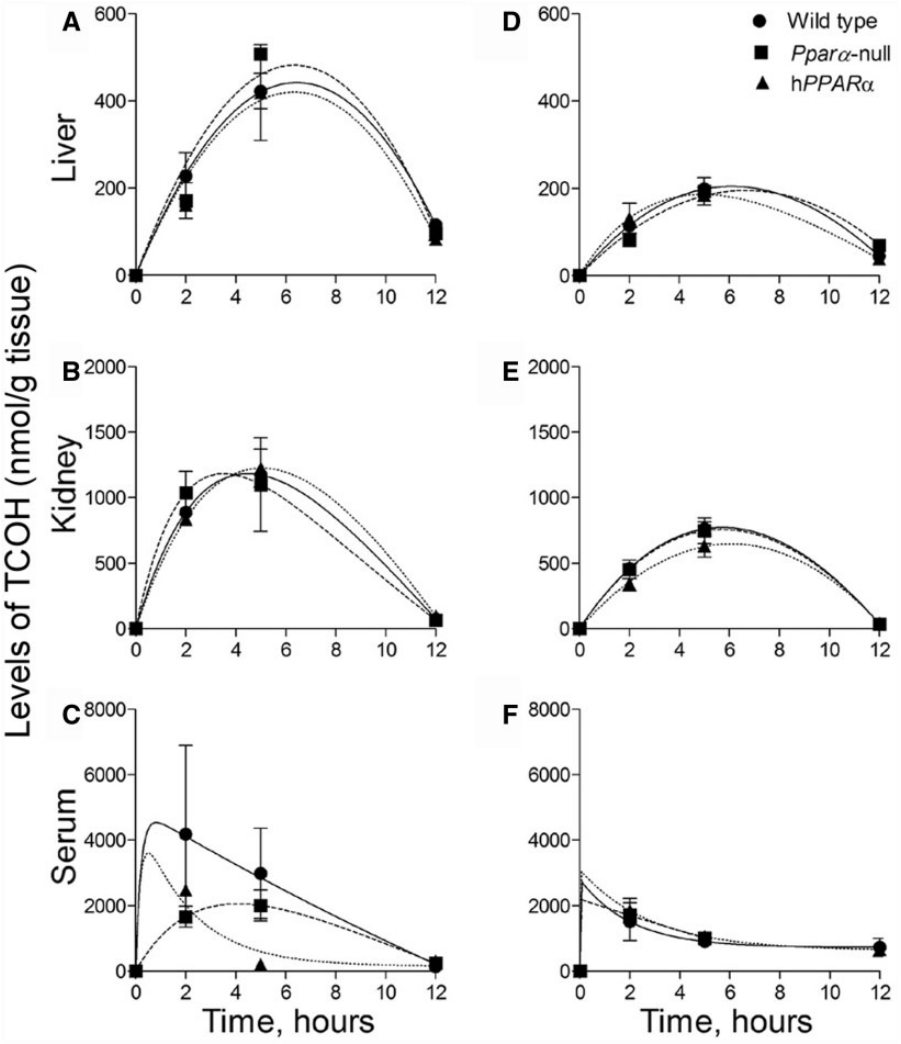

FIG. 2. Kinetics of TCOH in **(A)** liver, **(B)** kidney, and **(C)** serum of male mice, and in **(D)** liver, **(E)** kidney, and **(F)** serum of female mice following a single dose of TCE (400 mg/kg *i.g.*). The data shown are mean ± SD, $n = 3$ animals per group. No significant differences between groups were observed.

mice. No inter-strain differences in concentration-time profiles of TCE in kidney were observed in female mice.

Second, we evaluated concentration-time profiles of TCOH. Of the 3 tissues examined, the highest levels of TCOH were found in serum in both male and female mice of all strains (Fig. 2). TCOH levels in liver and kidney were 40- to 100-fold higher than TCE levels in these tissues, respectively. TCOH levels in serum were 2- to 5-fold higher than those in liver and kidney. There were sex differences in concentration-time profiles of TCOH such that in female mice, the amounts of TCOH were about one half of those in male mice. No strain differences were observed in the levels of TCOH in liver, kidney, or serum, except for male h*PPARα* mice at 5 h after dosing.

Third, concentration-time profiles of TCA, another abundant oxidative metabolite of TCE, were assessed. Differences in TCA levels in liver and kidney were found between sexes and strains (Fig. 3). In male wild type mice, levels of TCA in liver were significantly greater than those in other strains. In kidney of male mice, TCA levels in *Ppara*-null mice were significantly different from those in wild type at all time points examined, and those in h*PPARα* mice at 2 and 5 h. No difference in concentration-time profiles in serum of male mice was observed among 3 strains. In females, the levels of TCA were about 2- to 4-fold lower than in male mice. Similar to the findings in male mice, levels of TCA in liver of wild type mice were significantly greater than those in other strains.

Levels of TCE Metabolites Through Oxidative Pathway in Wild Type, Pparα-Null and hPPARα Mice Following Subchronic (4 Weeks) Treatment

To determine the effect of repeat administration of TCE on metabolite profiles through oxidative pathway in multiple target tissues, male and female mice of wild type, P*parα*-null and h*PPARα* strains were dosed with 400 mg TCE/kg (*i.g.*) for 5 days/week for 4 weeks. Liver, kidney, and serum were collected 5 h after the final dose of TCE to enable comparisons with concentration-time profiles conducted following a single TCE dose. Following sub-chronic exposure, TCE levels in liver and kidney (Fig. 4A and B) were 2- to 5-fold lower than those after a single dose (Fig. 1) and were not different among sexes or strains. However, levels of TCOH and TCA after sub-chronic exposure to TCE (Fig. 4C–H) were consistent with those after acute TCE treatment (Figs. 2 and 3, respectively), and their levels in male mice were considerably higher than in female mice. Overall, levels of TCOH were highest in serum, followed by kidney, then liver; levels of TCOH were 30-fold higher in serum and 10-fold higher in

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

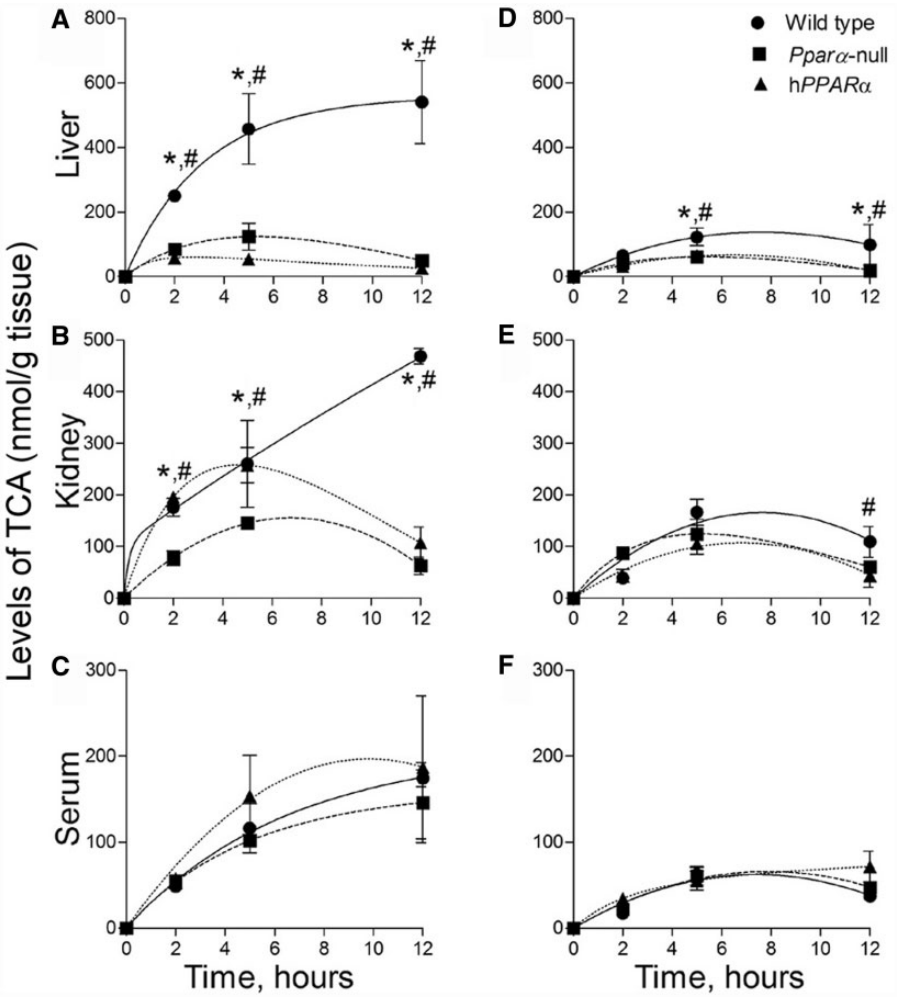

FIG. 3. Kinetics of TCA in (A) liver, (B) kidney, and (C) serum of male mice, and in (D) liver, (E) kidney, and (F) serum of female mice following a single dose of TCE (400 mg/kg *i.g.*). The data shown are mean ± SD, $n = 3$ animals per group. Symbols indicate significant ($p < .05$) differences between the values at each time point between strains as follows: wild type versus *Pparα*-null (*), and wild type versus h*PPARα* (#) mice.

kidney compared with levels of TCA in these tissues, but hepatic levels of these 2 metabolites were similar. Of all compounds measured, the only significant differences were observed among strains in liver levels of TCA, whereby the greatest amounts of TCA were found in wild type mice of both sexes (Fig. 4F).

We also compared strain- and sex-specific differences in oxidative metabolism of TCE (ie, levels of TCOH and TCA in 3 tissues examined) in the single dose study and a sub-chronic study (Fig. 5). To enable this comparison, we calculated the total amount of oxidative metabolites formed (in serum, liver, and kidney) from TCE at each time point. There was no effect of repeat TCE exposure on oxidative metabolite levels. Total level of oxidative TCE metabolites was about 3-fold lower in females compared with males in all 3 strains. Even though levels of TCA in liver of wild type mice were different from those in *Ppara*-null and h*PPARα* mice (Figs. 3 and 4F–H), when total oxidative metabolism of TCE is considered, no significant strain differences were evident. However, the lack of differences in total oxidative metabolite levels is due to dominating effect of TCOH which was not different among strains (Figs. 2 and 4C–E). This is illustrated by the significant differences in ratios of TCA to TCOH (Fig. 5E and F).

Strain (Wild Type, Pparα-Null, and hPPARα Mice)- and Sex-Specific Effects of TCE on Liver and Kidney Toxicity

Because of the longer duration of TCE exposure (4 weeks) in a sub-chronic study, as compared with an acute study (12 h), most toxicity phenotypes were evaluated in the liver and kidney tissues from the sub-chronic study. In liver, we examined liver to body weight ratios, cell proliferation index, triglyceride levels, levels of glutathione, s-adenosyl methionine and homocysteine, NADPH/NADP+ ratios, and liver histology (Supplementary Figs. S1 and S2). The most notable findings were a significant increase in liver/body weight ratios of male wild type and *Pparα*-null mice treated with TCE (Supplementary Fig. S1A) and an associated decrease in GSH/GSSG ratio indicative of oxidative stress (Supplementary Fig. S1D). In addition, in male wild type mice exposed to TCE, liver triglycerides and NADP/NADP+ ratio were significantly higher (Supplementary Fig. S1C and S1F). Histopathological assessment revealed slight centrilobular necrosis in male wild type mice treated with TCE for 4 weeks (Supplementary Fig. 2). In addition, hepatic steatosis was evident in vehicle-treated P*parα*-null mice, an effect that was diminished upon exposure to TCE for 4 weeks. In the kidney, no notable effects of TCE or strain-/sex-differences were found (Supplementary Fig. S3).

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

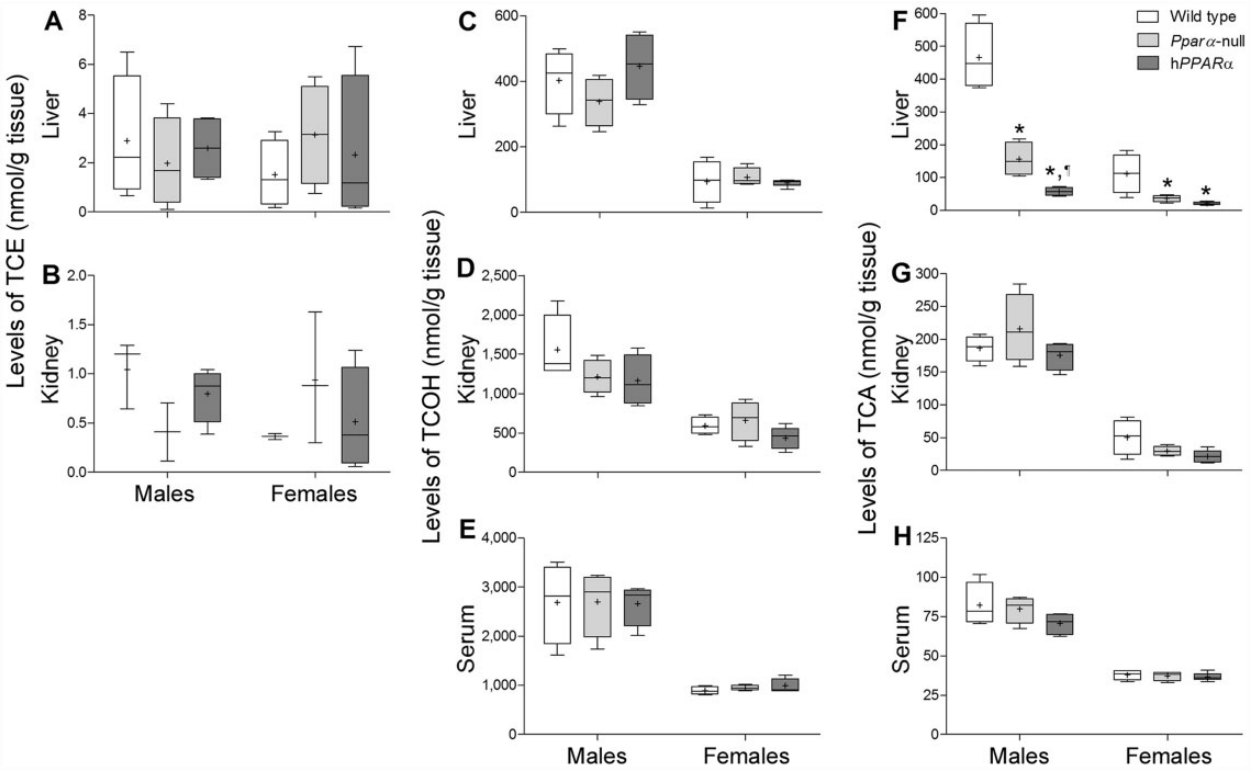

FIG. 4. Levels of TCE and its metabolites 5 h following the final dose of 400 mg/kg/d (*i.g.*) for 4 wk. Levels of TCE in **(A)** liver and **(B)** kidney. Levels of TCOH in **(C)** liver, **(D)** kidney, and **(E)** serum. Levels of TCA in **(F)** liver, **(G)** kidney, and **(H)** serum following a single dose of TCE (400 mg/kg *i.g.*). Box and whisker plots are shown (+, mean; line, median; box, inter-quartile range; whiskers, min to max). When box is shown, 4 animals per group were available. Otherwise, there were 3 animals per group. Symbols indicate significant ($p < .05$) differences as compared with wild type (*) or *Pparα*-null (¶) mice.

Next, we examined whether liver levels of Cyp2e1, a major putative enzyme responsible for the oxidative metabolism of TCE, vary across wild type, P*parα*-null and h*PPARα* mice, or are affected by sub-chronic treatment with TCE. Protein levels of Cyp2e1 were measured in the livers of animals treated with 400 mg/kg TCE for 4 weeks and we found no strain-, sex-, or treatment-associated differences (Fig. 6). However, TCE treatment resulted in a significant increase in liver and kidney expression of the PPARα-responsive genes *Cyp4a10* (Fig. 7) and *Acox1* (Supplementary Fig. S4) in both wild type and h*PPARα* mice. Induction of *Cyp4a10* was most pronounced and of similar magnitude (following either acute or sub-chronic treatment) in male wild type and h*PPARα*, but not *Ppara*-null mice, albeit liver induction was much greater than that in the kidney. In females, these responses were muted with respect to the magnitude of the effect, but the patterns of response were very similar (ie, elevated in the wild type and h*PPARα*, but not *Ppara*-null mice) to those in male mice. Effects on expression of *Acox1* were similar (Supplementary Fig. S4), but not identical. For instance, induction of *Acox1* was only observed in wild type male liver and female liver and kidney following a single dose of TCE. Following sub-chronic exposure to TCE, *Acox1* induction was observed only in wild type and h*PPARα* mouse liver.

DISCUSSION

Association between exposure to TCE and PPARα signaling in the liver of rodents is well-established (Corton *et al.*, 2014; Klaunig *et al.*, 2003; Rusyn *et al.*, 2014). Indeed, TCE oxidative pathway metabolites, TCA and DCA, are capable of activating mouse PPARα as evidenced *by in vitro* receptor activation assays (Issemann and Green, 1990; Zhou and Waxman, 1998) and an *in vivo* mouse study (Laughter *et al.*, 2004). Likewise, an *in vitro* transactivation study has demonstrated that human PPARα is activated by either TCA or DCA, while TCE is relatively inactive (Maloney and Waxman, 1999).

The importance of PPARα in TCE-induced hepatotoxicity was previously examined in *Ppara*-null (Laughter *et al.*, 2004; Nakajima *et al.*, 2000) and h*PPARα* mice (Ramdhan *et al.*, 2010). These studies consistently found that PPARα-mediated signaling and morphological events, such as induction of peroxisomal and other genes, were affected by TCE in wild type and h*PPARα* mice, but not in *Ppara*-null mice. Our recent studies that examined the linkages between TCE metabolism and toxic effects in liver and kidney in a multi-strain mouse population model (Yoo *et al.*, 2015a,b) found a significant positive correlation between levels of TCA and induction of PPARα-responsive genes. These and other studies conform to the simple hypothesis that differences in hepatotoxic responses across wild type, h*PPARα* mice, and *Ppara*-null mice exposed to TCE are due to differences in activation PPARα (or lack thereof) by TCE metabolites. However, most studies of TCE effects in genetically-modified PPARα mouse models did not examine the metabolism of TCE. Only Ramdhan *et al.* (2010) reported that urinary levels of TCA were significantly lower in *Pparα*-null as compared with wild type mice. This suggests that diminished toxicity of TCE in h*PPARα* mice and *Ppara*-null mice may be due, at least in part, to a lower internal dose of the active metabolite TCA, and not solely due to diminished receptor-related responses.

This study extends the findings of Ramdhan *et al.* (2010) to include tissue-specific levels of TCE metabolites, elucidates the potential role of PPARα in the relationship between

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

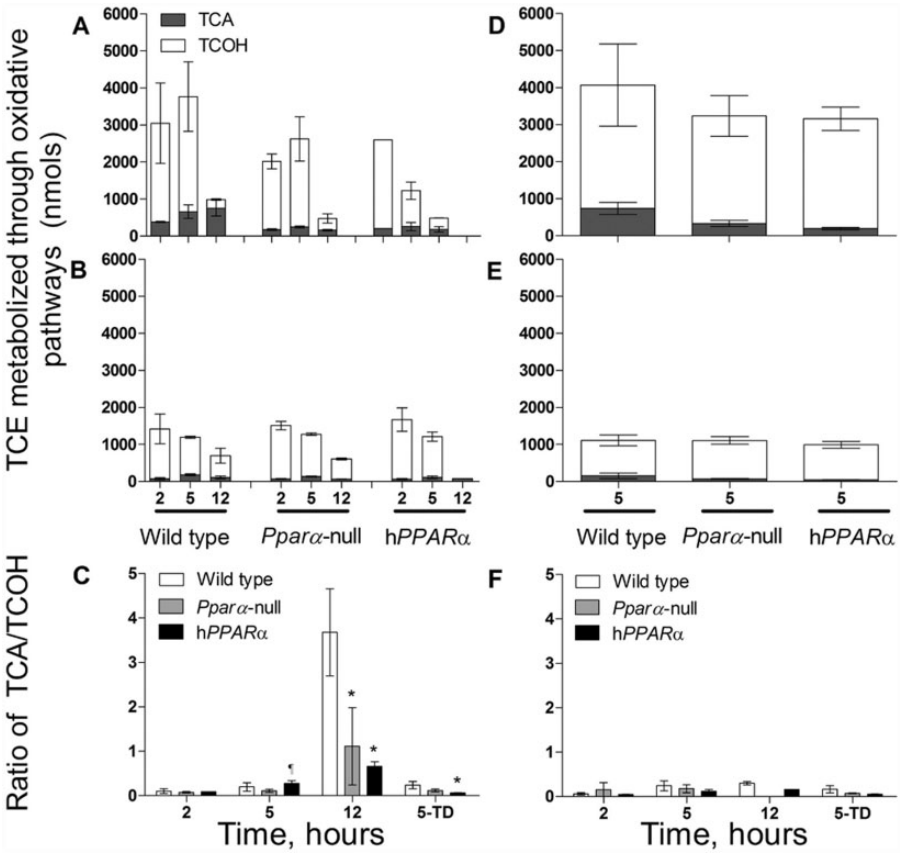

FIG. 5. Amount of TCE metabolized through oxidative pathways to TCA and TCOH from single- and repeat-dose studies following a 400 mg/kg (*i.g.*) dose. Top row shows data from male mice, middle row shows data from female mice. Panels A, B, D, and E display total (liver, kidney, and serum) amounts of TCA (shaded areas) and TCOH (white areas) formed at different time points in the single-dose study (A and B) and at 5 h after dosing in a repeat-dose study (panels D and E). The data for 3 mouse strains are grouped as indicated in the x-axis legend. Panels C and F show the ratio of total TCA to TCOH formed at each time point and strain (as indicated by the insets) in male (C) and female (F) mice. Data are mean ± SD, $n = 3$/group. Symbols indicate significant ($p < .05$) differences as compared with wild type (*) or *Pparα*-null (¶) mice.

toxicokinetics and toxicodynamics of TCE in mice, and specifically highlights the role of PPARα in metabolism of TCE. Our current findings in orally treated mice are similar to those of Ramdhan *et al.* (2010) who performed inhalation exposure to TCE, in that we observed differences in TCE metabolism to TCA, but not TCOH, among wild type, *Pparα*-null, and h*PPARα* mice. Because our study used a different route of exposure and examined different durations of exposure and multiple tissues, this consistency increases the confidence in our results. Together these 2 studies conclusively show that PPARα status does play a role in TCE metabolism. However, our study also found a consistent (with respect to time, sex, and tissue) difference in TCA levels between wild type and *hPPARα* mice, an effect not observed in urinary TCA levels after TCE inhalation (Ramdhan *et al.*, 2010).

Additionally, we found that wild type mice have higher levels of TCA in their livers and kidneys, as compared with *Pparα*-null and h*PPARα* mice. This suggests that PPARα may contribute to the cellular metabolic capacity of TCE through oxidative pathways. Formation of TCA and other oxidative metabolites is thought to occur primarily through CYP2E1 (Lash *et al.*, 2014; Nakajima *et al.*, 1992). The role of Cyp2e1 in TCE metabolism was directly challenged in *Cyp2e1*-null mouse studies (Kim and Ghanayem, 2006; Ramdhan *et al.*, 2008). However, no differences in levels of Cyp2e1 were observed in this study or in Ramdhan *et al.* (2010). It was reported that TCE metabolism to TCA and TCOH is 2- to 4-fold lower in *Cyp2e1*-null mice, as compared with wild type animals, but is not completely abrogated. This suggests that other P450 enzymes, such as mouse Cyp1a1/2 (Nakajima *et al.*, 1993) and Cyp2f2 (Forkert *et al.*, 2005), may also play a role in TCE metabolism.

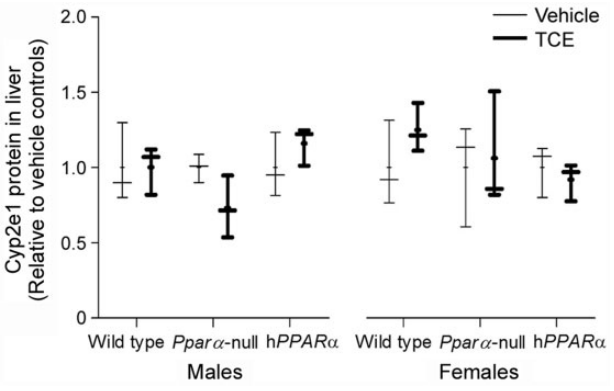

FIG. 6. Protein levels of CYP2E1 in mice treated with 400 mg/kg (*i.g.*) TCE for 4 weeks. Protein expression was normalized to vehicle-treated group of the same sex and strain. No strain- or sex-dependent differences were observed in basal protein levels. Box and whisker plots are shown (+, mean; line, median; box, inter-quartile range; whiskers, min to max). When box is shown, 4 animals per group were available. Otherwise, there were 3 animals per group. There were no significant differences among groups.

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

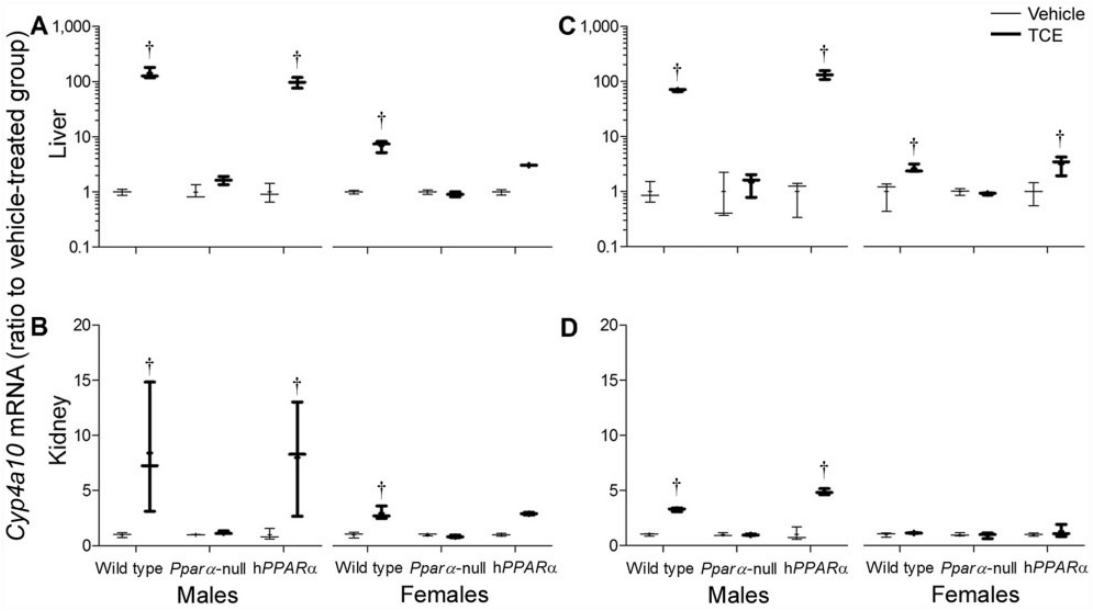

FIG. 7. Expression of *Cyp4a10* mRNA following administration of 400 mg/kg (*i.g.*) TCE. Expression in **(A)** liver and **(B)** kidney of mice treated with a single dose of TCE. Expression of *Cyp4a10* mRNA in **(C)** liver and **(D)** kidney of mice treated with repeat-dose of TCE (5 days/week, 4 weeks). Box and whisker plots are shown (+, mean; line, median; box, inter-quartile range; whiskers, min to max). When box is shown, 4 animals per group were available. Otherwise, there were 3 animals per group. Symbol (†) denotes a significant difference ($p < .05$) compared with vehicle-treated group within same strain and sex.

TCE also undergoes metabolism via conjugative pathways, particularly with GSH, which are mediated by glutathione s-transferases (Rusyn *et al.* 2014). Although TCE-GSH conjugates have not been examined with respect to their potential binding to PPARα, they are very low abundance metabolites (4–5 orders of magnitude lower than levels of TCA) (Kim *et al.*, 2009b; Yoo *et al.*, 2015a,b) and are thus not likely contributing to TCE-associated PPARα activation. It is also possible that modulating basal PPARα levels increases the flux of TCA from the tissues to the excreta, which would explain the decreased TCA levels in *Pparα*-null and h*PPARα* mice; however, the effect would have to be male-specific. Thus, the mechanism of how PPARα status may alter the metabolism of TCE remains unclear and further examination is warranted.

It is interesting, however, that despite major differences in TCA levels in liver and kidney among strains, a similar magnitude of induction of PPARα-responsive genes (eg, *Cyp4a10*) was observed in both wild type and h*PPARα* mice. Although levels of hepatic and renal *Acox1* induction were generally higher in wild type compared with h*PPARα* mice, the differences were not as dramatic as one would have anticipated given the nearly 10-fold difference in liver and kidney TCA levels between these 2 strains. Previous reports have shown that basal nuclear levels of PPARα in the livers of h*PPARα* mice is about 10-fold greater than levels of PPARα in the livers of wild type mice (Ramdhan *et al.*, 2010). Additionally, the inducibility of PPARα is similar in h*PPARα* and wild type mice (Cheung *et al.*, 2004). Therefore, the difference in PPARα protein levels between h*PPARα* and wild type mice may be contributing to the observed concordance in the downstream effects on gene expression, in spite of discordance in TCA levels. In addition, we note that tissue levels of TCA levels alone may not be indicative of the potential to induce PPARα signaling events, because the total amount of oxidative metabolites was not different among strains.

It is also noteworthy that the association between PPARα genotype and TCE-associated hepatomegaly is controversial and our work provides independent observations that strengthen one side of the argument. Specifically, Nakajima *et al.* (2000) and Ramdhan *et al.* (2010) (750 mg/kg/day of TCE [*i.g.*] for 2 weeks and 2000 ppm of TCE [equivalent to 1600 mg/kg/day] for 7 days [8 h/day], respectively), reported the increase in liver to body weight ratio in both wild type and *Ppara*-null mice. A third study (Laughter *et al.*, 2004), in which 1500 mg/kg/d of TCE was administered (*i.g.*) to mice for 3 weeks, reported that liver enlargement was observed in wild type and *Pparα*-null mice; however, statistical significance was only reached in wild type mice. In the present study, increased liver to body weight ratio was observed in both male wild type and *Pparα*-null mice exposed to TCE (400 mg/kg/day for 4 weeks). Thus, the liver to body weight ratio data presented here are consistent with Nakajima *et al.* (2000) and Ramdhan *et al.* (2010), and not with Laughter *et al.* (2004). The reasons for these discrepancies are unknown but may be reflective of inter-laboratory variability, such as differences in diet.

Nakajima *et al.* (2000) also reported significant sex differences in TCE-induced PPARα activation, where male mice were more sensitive to PPARα induction, and had higher basal levels of PPARα compared with female mice. However, the authors also reported that TCE-induced peroxisome proliferation was similar in both males and females, suggesting that PPARα is not the only contributor to TCE-induced peroxisome proliferation (Nakajima *et al.*, 2000). Although male mice are thought to be more sensitive to hepatic induction of PPARα compared with female mice following TCE administration, and a higher percentage of male mice develop liver tumors after chronic TCE exposure, both sexes are sensitive to hepatocellular carcinoma and adenoma development after exposure to TCE (National Toxicology Program, 1990). Moreover, while TCA induces peroxisome proliferation in both rats and mice, TCA has been shown to be tumorigenic in mice, but not rats (DeAngelo *et al.*, 1997, 2008). Furthermore, TCE-induced mouse liver tumors have a different pattern of H-ras mutation frequency and/or c-jun immunoreactivity from those induced by TCA alone or other peroxisome proliferators (Bull *et al.*, 2002; Fox *et al.*, 1990).

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

In this study, the degree of *Cyp4a10* and *Acox1* induction was higher in males compared with females. In the liver, there was an approximate 10-fold increase of *Cyp4a10* in male wild type and h*PPARα* mice compared with strain-matched female mice. This was observed in both single and repeat dose studies; thus, these results are consistent with findings of Nakajima *et al.* (2000). This is also consistent with the observation of increased TCA levels in the livers and kidneys of male mice compared with female mice. However, the amount of TCA in the liver or kidney was at most 5-fold higher in males compared with females. This is in agreement with the existence of a nonlinear relationship between tissue levels of TCA and PPARα activation (Yoo *et al.*, 2015a,b).

In summary, these results conclusively demonstrate that altering PPARα leads to changes in the toxicokinetics of TCE metabolites. Specifically, hepatic and renal levels of TCA are significantly higher in wild type mice compared with *Pparα*-null and h*PPARα* mice after a single- or repeat-dose TCE exposure. A mechanistic basis for these differences remains to be elucidated. These effects are not likely to be due to changes in the production of TCA, as our results show that neither hepatic CYP2E1 expression nor serum levels of TCA differed among the 3 genotypes. Interestingly, despite the differences in TCA levels, activation of PPARα as measured by *Cyp4a10* induction was similar in wild type and h*PPARα* mice, possibly due to differences in basal PPARα levels. TCE exposure at the level and duration of our experiment did not elicit much in the way of liver or kidney toxicity beyond hepatomegaly, even in wild type mice, so the relative roles of toxicokinetic and toxicodynamic factors affected by PPARα status in TCE toxicity remains unclear and requires further study. Future studies of TCE, and other compounds where one or more metabolites is the active toxic moiety, should take into account the possible interactions between genotype and toxicokinetics by simultaneously measuring internal markers of both toxicity and metabolite levels, even in the absence of an *a priori* hypothesis for how such an interaction may occur. Otherwise, studies comparing wild type, knockout or transgenic animals may incorrectly attribute observed differences in toxicity exclusively to receptor-mediated toxicodynamic factors, thereby ignoring possible toxicokinetic factors that affect internal dose.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

FUNDING

The funding for this work was provided, in part, by grants from the National Institutes of Health (P42 ES005948, P30 ES010126) and the U. S.Environmental Protection Agency (STAR RD83561201).

REFERENCES

Bull, R. J., Orner, G. A., Cheng, R. S., Stillwell, L., Stauber, A. J., Sasser, L. B., Lingohr, M. K., and Thrall, B. D. (2002). Contribution of dichloroacetate and trichloroacetate to liver tumor induction in mice by trichloroethylene. *Toxicol. Appl. Pharmacol.* **182**, 55–65.

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 alpha. *Cancer Res.* **64**, 3849–3854.

Corton, J. C. (2008). Evaluation of the role of peroxisome proliferator-activated receptor alpha (PPARalpha) in mouse liver tumor induction by trichloroethylene and metabolites. *Crit. Rev. Toxicol.* **38**, 857–875.

Corton, J. C., Cunningham, M. L., Hummer, B. T., Lau, C., Meek, B., Peters, J. M., Popp, J. A., Rhomberg, L., Seed, J., and Klaunig, J. E. (2014). Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARalpha) as a case study. *Crit. Rev. Toxicol.* **44**, 1–49.

DeAngelo, A. B., Daniel, F. B., Most, B. M., and Olson, G. R. (1997). Failure of monochloroacetic acid and trichloroacetic acid administered in the drinking water to produce liver cancer in male F344/N rats. *J. Toxicol. Environ. Health* **52**, 425–445.

DeAngelo, A. B., Daniel, F. B., Wong, D. M., and George, M. H. (2008). The induction of hepatocellular neoplasia by trichloroacetic acid administered in the drinking water of the male B6C3F1 mouse. *J. Toxicol. Environ. Health A* **71**, 1056–1068.

Forkert, P. G., Baldwin, R. M., Millen, B., Lash, L. H., Putt, D. A., Shultz, M. A., and Collins, K. S. (2005). Pulmonary bioactivation of trichloroethylene to chloral hydrate: relative contributions of CYP2E1, CYP2F, and CYP2B1. *Drug Metab. Dispos.* **33**, 1429–1437.

Fox, T. R., Schumann, A. M., Watanabe, P. G., Yano, B. L., Maher, V. M., and McCormick, J. J. (1990). Mutational analysis of the H-ras oncogene in spontaneous C57BL/6 x C3H/He mouse liver tumors and tumors induced with genotoxic and nongenotoxic hepatocarcinogens. *Cancer Res.* **50**, 4014–4019.

Guha, N., Loomis, D., Grosse, Y., Lauby-Secretan, B., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Baan, R., Mattock, H., Straif, K., and International Agency for Research on Cancer Monograph Working Group. (2012). Carcinogenicity of trichloroethylene, tetrachloroethylene, some other chlorinated solvents, and their metabolites. *Lancet Oncol.* **13**, 1192–1193.

Humphreys, B. D., Czerniak, S., DiRocco, D. P., Hasnain, W., Cheema, R., and Bonventre, J. V. (2011). Repair of injured proximal tubule does not involve specialized progenitors. *Proc. Natl. Acad. Sci USA* **108**, 9226–9231.

Issemann, I., and Green, S. (1990). Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**, 645–650.

Ito, Y., Nakamura, T., Yanagiba, Y., Ramdhan, D. H., Yamagishi, N., Naito, H., Kamijima, M., Gonzalez, F. J., and Nakajima, T. (2012). Plasticizers may activate human hepatic peroxisome proliferator-activated receptor alpha less than that of a mouse but may activate constitutive androstane receptor in liver. *PPAR Res.* **2012**, 201284.

Keshava, N., and Caldwell, J. C. (2006). Key issues in the role of peroxisome proliferator-activated receptor agonism and cell signaling in trichloroethylene toxicity. *Environ. Health Perspect.* **114**, 1464–1470.

Kim, D., and Ghanayem, B. I. (2006). Comparative metabolism and disposition of trichloroethylene in Cyp2e1-/-and wild-type mice. *Drug Metab. Dispos.* **34**, 2020–2027.

Kim, S., Collins, L. B., Boysen, G., Swenberg, J. A., Gold, A., Ball, L. M., Bradford, B. U., and Rusyn, I. (2009a). Liquid chromatography electrospray ionization tandem mass spectrometry analysis method for simultaneous detection of trichloroacetic acid, dichloroacetic acid, S-(1,2-dichlorovinyl)glutathione and S-(1,2-dichlorovinyl)-L-cysteine. *Toxicology* **262**, 230–238.

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015

Kim, S., Kim, D., Pollack, G. M., Collins, L. B., and Rusyn, I. (2009b). Pharmacokinetic analysis of trichloroethylene metabolism in male B6C3F1 mice: Formation and disposition of trichloroacetic acid, dichloroacetic acid, S-(1,2-dichlorovinyl)glutathione and S-(1,2-dichlorovinyl)-L-cysteine. *Toxicol. Appl. Pharmacol.* **238**, 90–99.

Klaunig, J. E., Babich, M. A., Baetcke, K. P., Cook, J. C., Corton, J. C., David, R. M., DeLuca, J. G., Lai, D. Y., McKee, R. H., Peters, J. M., *et al.* (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance. *Crit. Rev. Toxicol.* **33**, 655–780.

Lash, L. H., Chiu, W. A., Guyton, K. Z., and Rusyn, I. (2014). Trichloroethylene biotransformation and its role in mutagenicity, carcinogenicity and target organ toxicity. *Mutat. Res. Rev. Mutat. Res.* **762**, 22–36.

Laughter, A. R., Dunn, C. S., Swanson, C. L., Howroyd, P., Cattley, R. C., and Corton, J. C. (2004). Role of the peroxisome proliferator-activated receptor alpha (PPARalpha) in responses to trichloroethylene and metabolites, trichloroacetate and dichloroacetate in mouse liver. *Toxicology* **203**, 83–98.

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402–408.

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.

Melnyk, S., Pogribna, M., Pogribny, I., Hine, R. J., and James, S. J. (1999). A new HPLC method for the simultaneous determination of oxidized and reduced plasma aminothiols using coulometric electrochemical detection. *J. Nutr. Biochem.* **10**, 490–497.

Melnyk, S., Pogribna, M., Pogribny, I. P., Yi, P., and James, S. J. (2000). Measurement of plasma and intracellular S-adenosylmethionine and S-adenosylhomocysteine utilizing coulometric electrochemical detection: alterations with plasma homocysteine and pyridoxal 5'-phosphate concentrations. *Clin. Chem.* **46**, 265–272.

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 alpha to Wy-14,643-induced liver tumorigenesis. *Carcinogenesis* **27**, 1074–1080.

Nakajima, T., Kamijo, Y., Usuda, N., Liang, Y., Fukushima, Y., Kametani, K., Gonzalez, F. J., and Aoyama, T. (2000). Sex-dependent regulation of hepatic peroxisome proliferation in mice by trichloroethylene via peroxisome proliferator-activated receptor alpha (PPARalpha). *Carcinogenesis* **21**, 677–682.

Nakajima, T., Wang, R. S., Elovaara, E., Park, S. S., Gelboin, H. V., and Vainio, H. (1992). A comparative study on the contribution of cytochrome P450 isozymes to metabolism of benzene, toluene and trichloroethylene in rat liver. *Biochem. Pharmacol.* **43**, 251–257.

Nakajima, T., Wang, R. S., Elovaara, E., Park, S. S., Gelboin, H. V., and Vainio, H. (1993). Cytochrome P450-related differences between rats and mice in the metabolism of benzene, toluene and trichloroethylene in liver microsomes. *Biochem. Pharmacol.* **45**, 1079–1085.

National Toxicology Program. (1990). Carcinogenesis Studies of Trichloroethylene (Without Epichlorohydrin) (CAS No. 79-01-6) in F344/N Rats and B6C3F1 Mice (Gavage Studies). *Natl. Toxicol. Program Tech. Rep. Ser.* **243**, 1–174.

Peters, J. M. (2008). Mechanistic evaluation of PPARa-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., Shah, Y. M., and Gonzalez, F. J. (2012). The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention. *Nat. Rev. Cancer* **12**, 181–195.

Ramdhan, D. H., Kamijima, M., Yamada, N., Ito, Y., Yanagiba, Y., Nakamura, D., Okamura, A., Ichihara, G., Aoyama, T., Gonzalez, F. J., and Nakajima, T. (2008). Molecular mechanism of trichloroethylene-induced hepatotoxicity mediated by CYP2E1. *Toxicol. Appl. Pharmacol.* **231**, 300–307.

Ramdhan, D. H., Komijima, M., Wang, D., Ito, Y., Naito, H., Yanagiba, Y., Hayashi, Y., Tanaka, N., Aoyama, T., Gonzalez, F. J., *et al.* (2010). Differential response to trichloroethylene-induced hepatosteatosis in wild-type and PPARa-humanized Mice. *Environ. Health Perspect.* **118**, 1557–1563.

Ren, H., Aleksunes, L. M., Wood, C., Vallanat, B., George, M. H., Klaassen, C. D., and Corton, J. C. (2010). Characterization of peroxisome proliferator-activated receptor alpha–independent effects of PPARalpha activators in the rodent liver: di-(2-ethylhexyl) phthalate also activates the constitutive-activated receptor. *Toxicol. Sci.* **113**, 45–59.

Rusyn, I., Chiu, W. A., Lash, L. H., Kromhout, H., Hansen, J., and Guyton, K. Z. (2014). Trichloroethylene: Mechanistic, epidemiologic and other supporting evidence of carcinogenic hazard. *Pharmacol. Ther.* **141**, 55–68.

Rusyn, I., and Corton, J. C. (2012). Mechanistic considerations for human relevance of cancer hazard of di(2-ethylhexyl) phthalate. *Mutat. Res.* **750**, 141–158.

Song, J. Z., and Ho, J. W. (2003). Simultaneous detection of trichloroethylene alcohol and acetate in rat urine by gas chromatography-mass spectrometry. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **789**, 303–309.

Wood, C. E., Jokinen, M. P., Johnson, C. L., Olson, G. R., Hester, S., George, M., Chorley, B. N., Carswell, G., Carter, J. H., Wood, C. R., *et al.* (2014). Comparative time course profiles of phthalate stereoisomers in mice. *Toxicol. Sci.* **139**, 21–34.

Yoo, H. S., Bradford, B. U., Kosyk, O., Shymonyak, S., Uehara, T., Collins, L. B., Bodnar, W. M., Ball, L. M., Gold, A., and Rusyn, I. (2015a). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Liver effects. *J. Toxicol. Environ. Health A* **78**, 15–31.

Yang, Q., Ito, S., Gonzalez, F. J. (2007). Hepatocyte-restricted constitutive activation of PPAR alpha induces hepatoproliferation but not hepatocarcinogenesis. *Carcinogenesis* **28**, 1171–1177.

Yoo, H. S., Bradford, B. U., Kosyk, O., Uehara, T., Shymonyak, S., Collins, L. B., Bodnar, W. M., Ball, L. M., Gold, A., and Rusyn, I. (2015b). Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: Kidney effects. *J. Toxicol. Environ. Health A* **78**, 32–49.

Zhou, Y. C., and Waxman, D. J. (1998). Activation of peroxisome proliferator-activated receptors by chlorinated hydrocarbons and endogenous steroids. *Environ. Health Perspect.* **106**(Suppl 4), 983–988.

Downloaded from http://toxsci.oxfordjournals.org/ at CIIT Library on August 14, 2015