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Supplemental Material

Characterization of Variability in Toxicokinetics and Toxicodynamics of Tetrachloroethylene Using the Collaborative Cross Mouse Population

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Supplemental Methods

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of PERC in Tissues.

Tissue levels of PERC were assessed via dynamic headspace GCMS. Liver or kidney (25 mg) was added to a tube containing 5 stainless steel beads and 1 mL of 5 μ M methanolic ethylbenzene (internal standard). Tissues were homogenized using the Omni Bead Ruptor, were quickly uncapped, and the entire content (including beads) quickly removed and added to 40 mL amber glass vials containing 4 mLs of water, after which the vials were quickly capped with PTFE-lined septa. All samples were run on the same day as homogenization. The purge and trap system (Teledyne Tekmar Atomx) was set to purge the system with helium for 15 mins, followed by adsorption of analytes onto the trap, and finally thermal desorption into a Thermo Trace Ultra gas chromatograph. Analytes were separated on a DB-1 column (60m X 0.25m, 1.0 μ m; Agilent 122-1063; Agilent, Santa Clara, CA) and detected via a Thermo DSQ II single quadrupole mass spectrometer operated in full-scan mode. Under these conditions, PERC and ethylbenzene eluted at 17.2 and 18.5 minutes, respectively. Quantitation of PERC and ethylbenzene were based on the ions of m/z 129 and 91, respectively. The ratios of peak areas of PERC to internal standard of tissue samples were used to quantitate PERC concentrations via interpolation of calibration curves generated by spiking known amounts of PERC into tissue homogenates. The assay was linear from 6 to 2,000 nmol PERC per gram of tissue.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of Haloacetic Acids (TCA and DCA) in Tissues.

The analysis of TCA and DCA was modified from USEPA Method (EPA 815-B-03-002). Briefly, liver (100 mg) or kidney (50 mg) were added to tubes containing 1 mL of Chloroform:Methanol (1:1), 20 μ L of 2-bromobutyric acid (550 nmol/mL; internal standard), and 5 stainless steel beads. The tissues were homogenized using the Omni Bead Ruptor and allowed to sit at RT for 5 minutes before the addition of 200 μ L of LCMS-grade water. The tubes were then centrifuged (15,000g, 10 mins, RT) and the top (aqueous) layer was removed and added to 8-mL amber glass vials containing 1.5 mL of 10% H₂SO₄ in methanol (esterifying reagent). For serum samples, 50 μ L of serum was added directly to a vial containing internal standard and esterifying reagent. The vials were heated at 50°C for 2 hrs and then allowed to cool to RT. 2 mLs of methyl-tert butyl ether (MtBE) were added to the vials, the vials were vortexed, and then 3 mLs of sodium sulfate (150g/L) were added. After vortexing, the layers were allowed to separate for 2-3 minutes, and then the organic layer was removed and added to 3 mLs of sodium bicarbonate (saturated aqueous solution). After vortexing, the layers were again allowed to separate for 2-3 minutes, and then the organic layer was removed and reduced in volume to less than 50 μ L under a steady gentle stream of N₂ gas. The final extract was placed into amber glass vials with 250 μ L glass inserts, capped with screw-tops containing Teflon/Silicone-lined septa, and stored at -20°C for less than one week prior to analysis via GC/MS. 2 μ L was injected via an HP 7673 autosampler into an HP 6890 GC, with the injector maintained at 210°C. Analytes were separated on a DB-5MS column (30m, 0.25mm, 0.5 μ m film thickness; Agilent 122-5536, Santa Clara, CA). The column flow was 1 mL/min. The initial GC temperature was 40°C, which was held for 10 minutes. The temperature from ramped to 65°C over the next 10 minutes, then to 85 degrees over 2 minutes, and finally to 205 degrees over 6 minutes with a

final run time of 28 minutes. Under these conditions, trichloroacetate and methyl 2-bromobutyrate eluted at 21.6 and 23.0 minutes, respectively. The single quadrupole MS (HP 5973) was maintained at 290°C and operated in splitless mode. Ions with m/z 59, 83, 85, 117, 119, 121, 132, and 151-154 were scanned at 0.75 cycles/sec. The assay was linear from 4-1,000 nmol/g tissue. The ratios of peak areas of TCA to internal standard of tissue samples were used to quantitate TCA concentrations via interpolation of calibration curves generated by spiking known amounts of TCA into tissue homogenates. The assay was linear from 4-2,000 nmol TCA per gram of tissue.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR).

Liver left lobe and kidney samples were pulverized in liquid nitrogen using CellCrusher™ (Cell Crusher, Cork, Ireland). 15-20 mg of pulverized tissue was used for isolation of RNA using miRNeasy kits (Qiagen, Valencia, CA) according to manufacturer's protocol. RNA concentration was determined by NanoDrop C-1000 (Nanodrop, Wilmington, DE) and RNA integrity was determined by BioAnalyzer (Agilent, Santa Clara, CA). cDNA was synthesized using the high-capacity cDNA archival kit (Thermo Fisher Scientific, Waltham, MA) from 2 µg of RNA. qRT-PCR was performed using 2 µL of cDNA (25 ng/µL) in a 20 µL total reaction volume using Taqman® probes (*Acox1*, Mm01246831_m1; *Cyp4a10*, Mm02601690_gH) on a Roche Light Cycler 480 (Roche, Indianapolis, IN) according to manufacturer's recommendations. Expression of target probe was normalized to *Gusb* (Mm00446953_m1) expression. Importantly, for two of these probes, SNPs are located under the probe sequence (*Cyp4a10*, rs8248795; *Gusb*, rs36830782). While all 8 CC founders have the same allele at rs8248795 (*Cyp4a10*), CAST/EiJ mice do have an A->G polymorphism at rs36830782 (*Gusb*). However, in our analyses, the coefficient of variation was 2.37% (44 strains, n=88 reactions in duplicate, 176 total wells). Thus, we acknowledge the potential for probe hybridization to influence our results, although we anticipate its overall effect to be negligible, as shown by our C_T values.

Western Blotting for CYP2E1.

Total protein was isolated from 15-20 mg of pulverized left liver lobe and kidney using the T-PER Tissue Protein Extraction Kit (Thermo Fisher Scientific) and Halt Protease Inhibitor Single-Use Cocktail (100X; Thermo Fisher Scientific) according to the manufacturer's protocol. Protein content was measured using Qubit (Thermo Fisher Scientific). Each gel contained two reference samples to account for inter-gel variability. 45 µg of protein was loaded onto pre-cast 10% acrylamide/bisacrylamide gels (BioRad, Hercules, CA), electrophoresed, transferred to PVDF membranes (TurboBlot, BioRad), blocked for 2 hr at RT (Odyssey Blocking Buffer, LI-COR, Lincoln, NE), and incubated with rabbit anti-CYP2E1 antibody (Cat No. Lot No. Abcam, Cambridge, MA) overnight at 4°C. After washing, membranes were incubated with goat anti rabbit HRP-conjugated secondary antibody for 1 hr at RT. After washing, chemiluminescence was used as a detection method of bands using a C-digit blot scanner (LI-COR) according to manufacturer's protocol. To ensure equal protein loading, membranes were stripped (Odyssey Stripping Buffer, LI-COR) and re-blotted with goat anti-β-actin for 1 hr at RT. The same protocol was used for detection of β-actin as for CYP2E1. After detection of β-actin, amido black staining of membranes was employed as additional confirmation of equal protein loading.

Supplemental Tables

Table S1: Descriptive Statistics for PERC AUCs

Tissue	GM^a	GSD	Max^a	Min^a	n	Uncertainty Factor^b
Liver	6,007	1.65	12,772	1,535	26	3.19
Kidney	5,070	1.74	16,802	1,264	34	3.62

^a unit is nmols/g*hr

^bUncertainty factor is defined by $GSD^{2.3263}$, where 2.3263 is the corresponding z-score for 1% of a normal distribution

GM, geometric mean; GSD, geometric standard deviation; max/min, maximum and minimum values; n, number of strains in dataset

Table S2: Descriptive Statistics for TCA AUCs

Tissue	GM^a	GSD	Max^a	Min^a	n	Uncertainty Factor^b
Liver	13,608	1.64	44,953	5,831	31	3.16
Kidney	8,108	1.43	16,427	3,987	30	2.31
Serum	29,467	1.37	51,734	14,648	27	2.07
Brain	3,259	1.53	6,519	1,562	9	2.71
Lung	9,706	1.39	18,753	5,721	8	2.16
Fat	808	1.78	1,514	282	8	3.81

^a units are nmols/g*hr

^b Uncertainty factor is defined by $GSD^{2.3263}$, where 2.3263 is the corresponding z-score for the 1% quantile of a normal distribution

GM, geometric mean; GSD, geometric standard deviation; max/min, maximum and minimum values; n, number of strains in dataset

Supplemental Figures

Figure S1. Representative photomicrographs of histopathological evaluation of liver sections from mice treated with vehicle (top row) or 1,000 mg/kg PERC (bottom row). Some strains, such as CC021/Unc, show no PERC-associated hepatotoxicity. The most common finding among PERC-exposed animals was hepatic vacuolation (indicative of hepatosteatorosis). For example, CC015/Unc and CC004/TauUnc exhibited mid-zonal or diffuse hepatosteatorosis, respectively, after PERC exposure. H&E, 200X; inset, 600X.

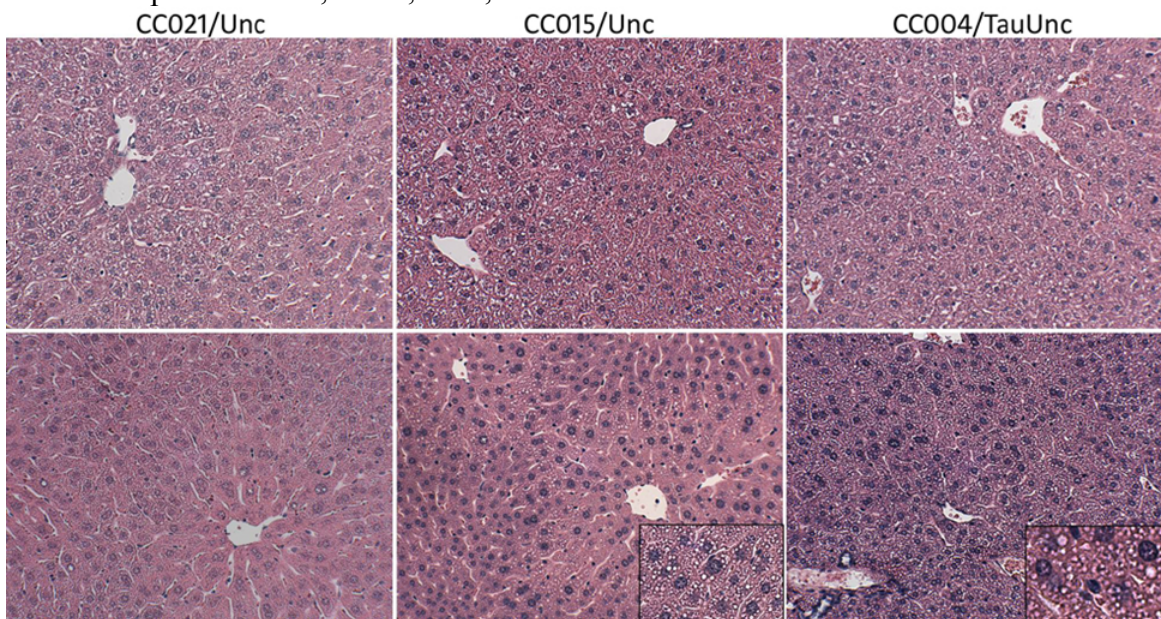


Figure S2. Concentration-time profiles of TCA in various mouse tissues from 9 strains of the Collaborative Cross. Tissues are colored as indicated by figure legend.

