Impact of nonalcoholic fatty liver disease on toxicokinetics of tetrachloroethylene in mice

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Supplemental methods

Analysis of PERC in tissues. Dynamic headspace gas chromatography/mass spectrometry (GC/MS) was used to analyze PERC in tissues according to the method of Cichocki et al (2016, in press). At necropsy, 20 µL of whole blood was immediately drawn and placed in a 40 mL amber headspace vial containing 1 mL of methanolic ethylbenzene (internal standard, 0.5 µM) and stored at -20^{o}C until used. Liver (25 mg) was homogenized in 1mL ice-cold methanolic ethylbenzene and the entire homogenate was transferred to a 40 mL amber headspace vial containing 4 mLs of water and quickly capped with a screw cap and PTFE-lined septa. 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 µm; 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. Ratios of peak areas of PERC and internal standards of tissue samples were used to quantitate PERC concentrations via interpolation of calibration curves generated by spiking known amounts of PERC into tissue homogenates.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of Trichloroacetate in Tissues. The analysis of TCA was modified from USEPA Method (EPA 815-B-03-002), as described by Cichocki et al (2016, in press). Briefly, liver tissue (100 mg) was added to tubes containing 1 mL of Chloroform:Methanol (1:1), 20 µL 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 µL 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% H_2SO_4 in methanol (esterifying reagent). For serum samples, 50 µL of serum was added directly to a vial containing internal standard and esterifying reagent. The vials were heated at 50^{o}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 µL under a steady gentle stream of N_2 gas. The final extract was placed into amber glass vials with 250 µL glass inserts, capped with screw-tops containing Teflon/Silicone-lined septa, and stored at -20^{o}C for less than one week prior to analysis via GC/MS. 2 µL was injected via an HP 7673 autosampler into an HP 6890 GC, with the injector maintained at 210^{o}C. Analytes were separated on a DB-5MS column (30m, 0.25mm, 0.5 um film thickness; Agilent 122-5536). The column flow was 1 mL/min. The initial GC temperature was 40^{o}C, which was held for 10 minutes. The temperature from ramped to 65^{o}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, methyl trichloroacetate and methyl 2-bromobutyrate eluted at 21.6 and 23.0 minutes, respectively. The single quadrupole MS (HP 5973) was maintained at 290^{o}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. Ratios of peak areas of TCA and internal standards of tissue

samples were used to quantitate TCA concentrations via interpolation of calibration curves generated by spiking known amounts of TCA into tissue homogenates.

Partition Coefficients. Blood:air (P_B) and liver:blood (P_L) partition coefficients were performed using a vial equilibration method (Morris and Cavanagh, 1986). Briefly, 100 µL of whole blood or tissue homogenate (25 mg liver/100 µL phosphate-buffered saline; PBS) was added to a 10 mL amber headspace vial with magnetic cap and PTFE-lined septa and placed on a CombiPAL Autosampler (CTC Analytics, Lake Elmo, MN). Additional vials contained no liquid or 100 µL of PBS. Methanolic PERC (1 µL) was injected through the septa using a gas-tight syringe to generate an air concentration of approximately 25 ppm. After 20 minutes on a 37^{o}C shaker, 1 mL of headspace air was injected into a Varian CP-3800 gas chromatograph (Agilent) from a 65^{o}C syringe. The DB-5MS column (30m, 0.250 mm narrowbore 0.25 um film; cat no. 122-5532; Agilent) was held at 35^{o}C for 0.5 minutes, ramped to 100^{o}C over 4 minutes, held for 0.1 minutes, ramped to 150^{o}C over 1 minute, then returned to 35^{o}C for a total run time of 7.2 minutes. With this method, PERC eluted at 3.2 minutes. PERC was detected by a Saturn 4000 mass spectrometer scanned for m/z of 164, 166, and 168 to monitor the chlorine isotope pattern. P_B and liver:air were determined by comparison to peak areas obtained from vials containing no liquid to those containing tissue assuming mass balance. P_L was determined based on the ratio of liver:air and P_B.

Supplementary Table 1: Correlation of transcript abundance with hepatic PERC or TCA levels[a]

						PERC AUC			TCA 4 HR		
Gene[b]	**Counts (LFD)**	**HFD FC**	**HFD FDR**	**MCD FC**	**MCD FDR**	**r[c]**	**p**	**q[d]**	**r[c]**	**p**	**q[d]**
cyp2c29	37974	-1.38	2.8E-09	-2.02	9.7E-23	-0.85	1.0E-04	3.5E-03	0.85	1.0E-04	3.4E-03
cyp27a1	9056	-0.42	1.4E-03	-0.73	5.2E-12	-0.85	1.0E-04	3.5E-03	0.85	1.0E-04	3.4E-03
cyp26c1	6	-1.01	NA	-1.19	9.4E-04	-0.82	4.0E-04	3.5E-03	0.80	5.0E-04	3.8E-03
cyp3a16	36	-1.90	1.9E-07	-1.61	3.9E-06	-0.82	4.0E-04	3.5E-03	0.81	5.0E-04	3.8E-03
cyp2c55	298	-2.13	1.2E-10	-2.13	1.1E-11	-0.81	5.0E-04	3.5E-03	0.80	6.0E-04	3.9E-03
cyp2c70	7623	-0.86	2.4E-02	-1.64	7.8E-09	-0.79	7.0E-04	4.4E-03	0.80	6.0E-04	4.0E-03
cyp3a59	595	-2.22	9.0E-18	-2.00	2.6E-16	-0.77	1.2E-03	6.0E-03	0.76	1.5E-03	7.5E-03
cyp3a11	84628	-2.68	4.9E-33	-1.88	2.1E-17	-0.77	1.2E-03	6.0E-03	0.76	1.6E-03	7.7E-03
cyp3a25	8244	-1.34	7.1E-11	-1.25	5.7E-11	-0.77	1.3E-03	6.4E-03	0.76	1.8E-03	8.1E-03
cyp2j9	161	-0.35	5.3E-01	-0.62	4.6E-02	-0.75	1.8E-03	8.1E-03	0.75	2.2E-03	9.1E-03
cyp2c53-ps	13	-1.00	NA	-1.46	3.1E-05	-0.75	2.0E-03	8.5E-03	0.75	2.1E-03	8.8E-03
cyp2c54	4885	-1.15	2.9E-04	-1.20	1.7E-05	-0.75	2.0E-03	8.5E-03	0.74	2.4E-03	9.5E-03
cyp2f2	25544	-0.43	2.1E-01	-0.59	9.6E-03	-0.73	2.9E-03	1.0E-02	0.73	2.8E-03	9.8E-03
cyp2b10	423	-1.84	5.9E-07	-1.87	1.3E-07	-0.73	3.3E-03	1.1E-02	0.72	3.9E-03	1.2E-02
cyp26a1	270	-1.48	1.2E-04	-1.45	6.2E-05	-0.72	3.9E-03	1.2E-02	0.71	4.6E-03	1.3E-02
cyp2c37	3996	-1.11	2.1E-04	-1.02	1.4E-04	-0.70	5.0E-03	1.4E-02	0.69	6.2E-03	1.6E-02
cyp2c50	11135	-0.81	7.0E-03	-0.72	4.8E-03	-0.61	2.2E-02	3.9E-02	0.59	2.5E-02	4.6E-02

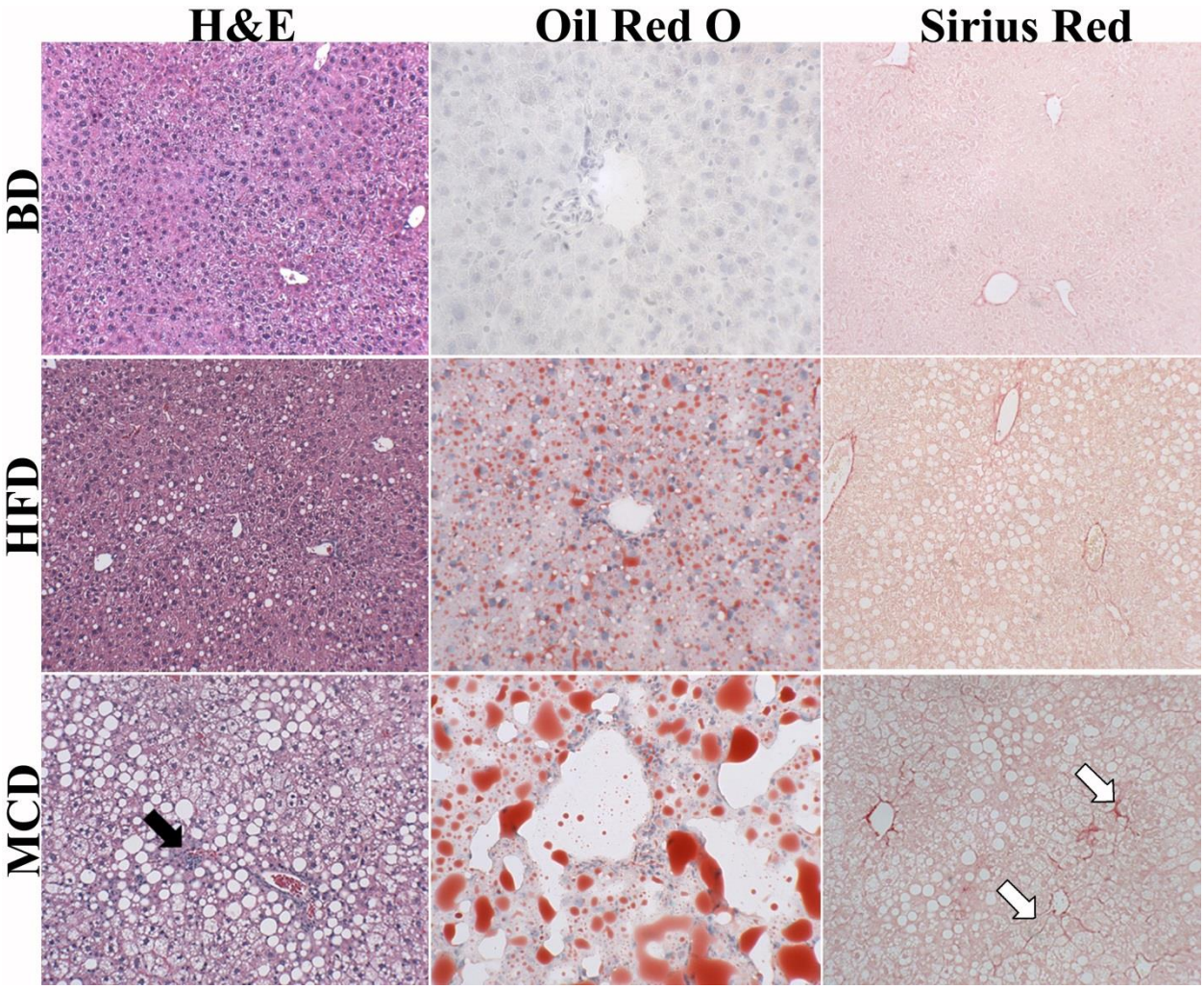
[a]AUCs were computed based on 0-36 hr kinetic data. Since each individual animal was only sampled at a single time point, AUC was calculated as a group (i.e. diet-specific) mean which was comprised of individual animals (n=5/group) euthanized at multiple time points (1-36 hrs post gavage). Mean TCA levels at 4 hours were calculated based on diet-specific group means.

[b] Individual transcript abundances (n=5 per diet group) were used for correlations.

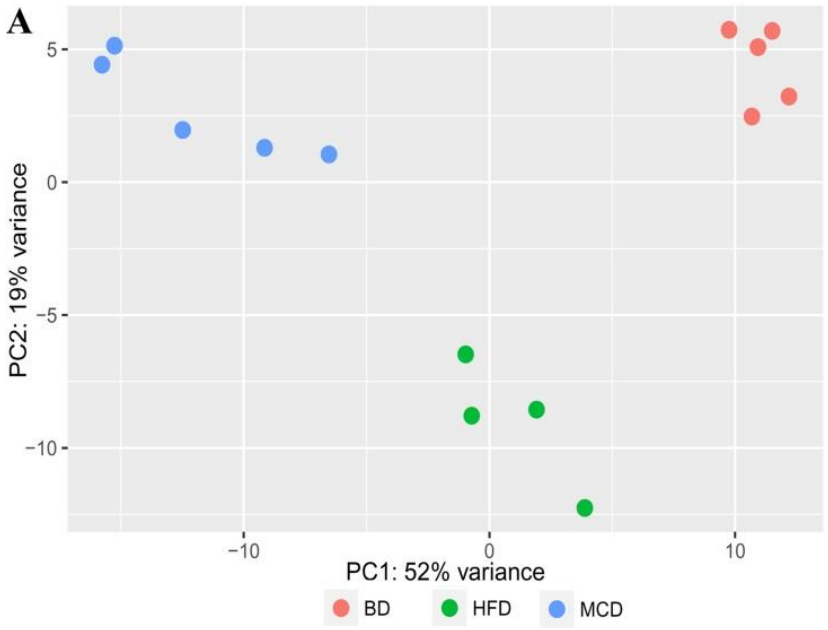
[c] Pearson's r correlation coefficient.

[d] q value represents the Benjamini and Hochberg (1995) corrected p-value.

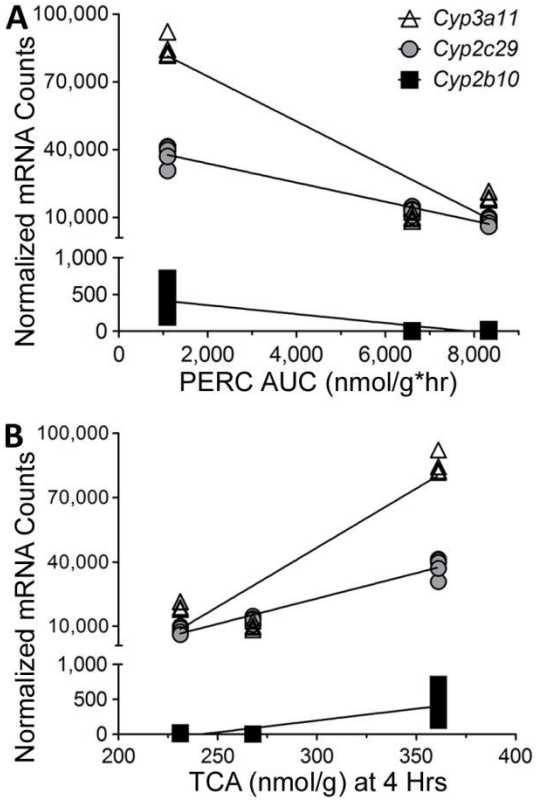
Supplemental Figure 1. Histopathology of liver tissue from mice treated with vehicle (24 hr time point) after administration of a LFD (top row), HFD (middle row), or MCD (bottom row) diet for 8 weeks. Hematoxylin & Eosin (H&E) staining (left column) revealed vacuolation in tissue from HFD and MCD-fed mice, with MCD mice also exhibiting evidence of inflammatory cell infiltration (filled arrow). Vacuolation was confirmed to be due to lipid accumulation, as evidenced by Oil Red O staining (middle column). Sirius Red staining revealed minimal collagen deposition only in MCD-fed animals only (open arrow, right column). All images were taken at 200X.



Supplemental Figure 2. Principal component analysis (PCA) of transcriptomic data from liver tissue of mice treated with vehicle (24 hr time point) after administration of a LFD (peach), HFD (green), or MCD (blue) diet for 8 weeks.



Supplemental Figure 3. Correlation analysis of transcript abundance (normalized mRNA counts) for *Cyp3a11* (open triangle), *Cyp2c29* (grey circle), and *Cyp2b10* (black square) and PERC AUC from 0-36 hours (A) or TCA concentration at 4 hours post gavage (B) in liver tissue of mice exposed to a single dose of PERC (300 mg/kg *i.g.*) after 8 weeks of LFD, HFD, or MCD diet.



References for Supplemental Information

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Morris JB and Cavanagh DG (1986) Deposition of ethanol and acetone vapors in the upper respiratory tract of the rat. *Fundam Appl Toxicol* 6:78-88.