Differential toxicity of water versus gavage exposure to trichloroethylene in rats.

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7 pages; 5 figures ; 1 table

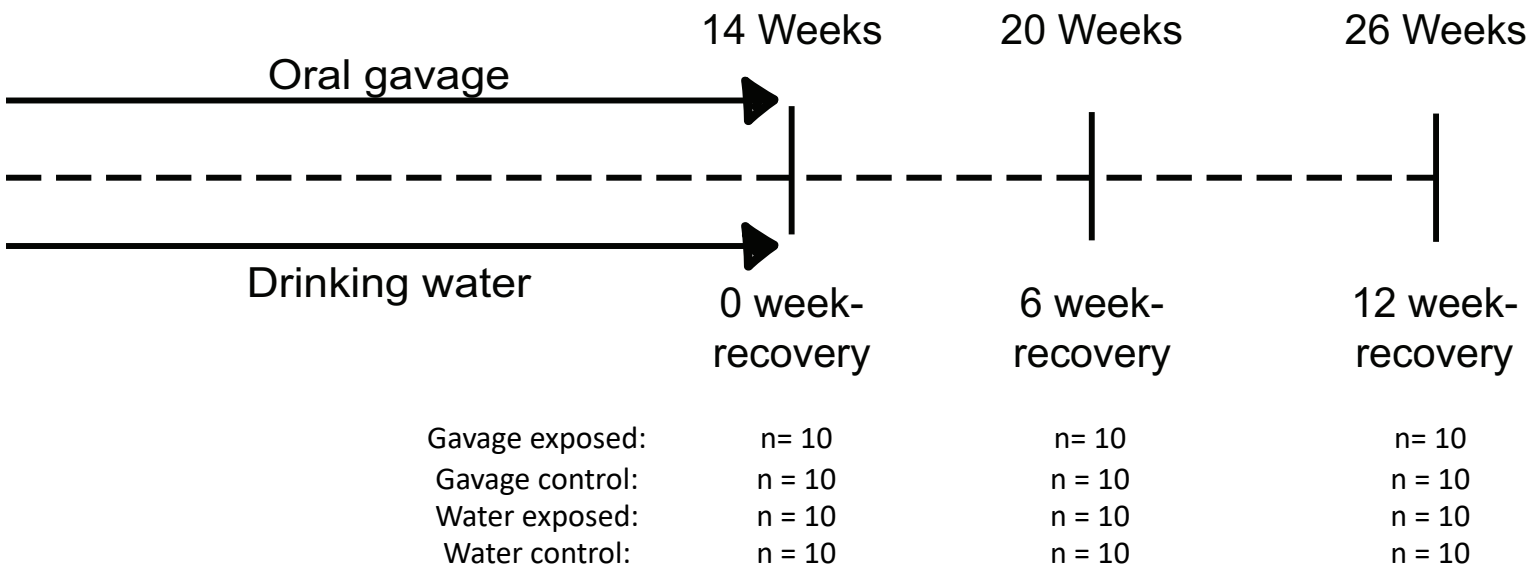

Figure S1. Exposure paradigm illustrating duration of exposure, length of recovery after cessation of exposure for each time point, and the number of samples in each group at each time point.

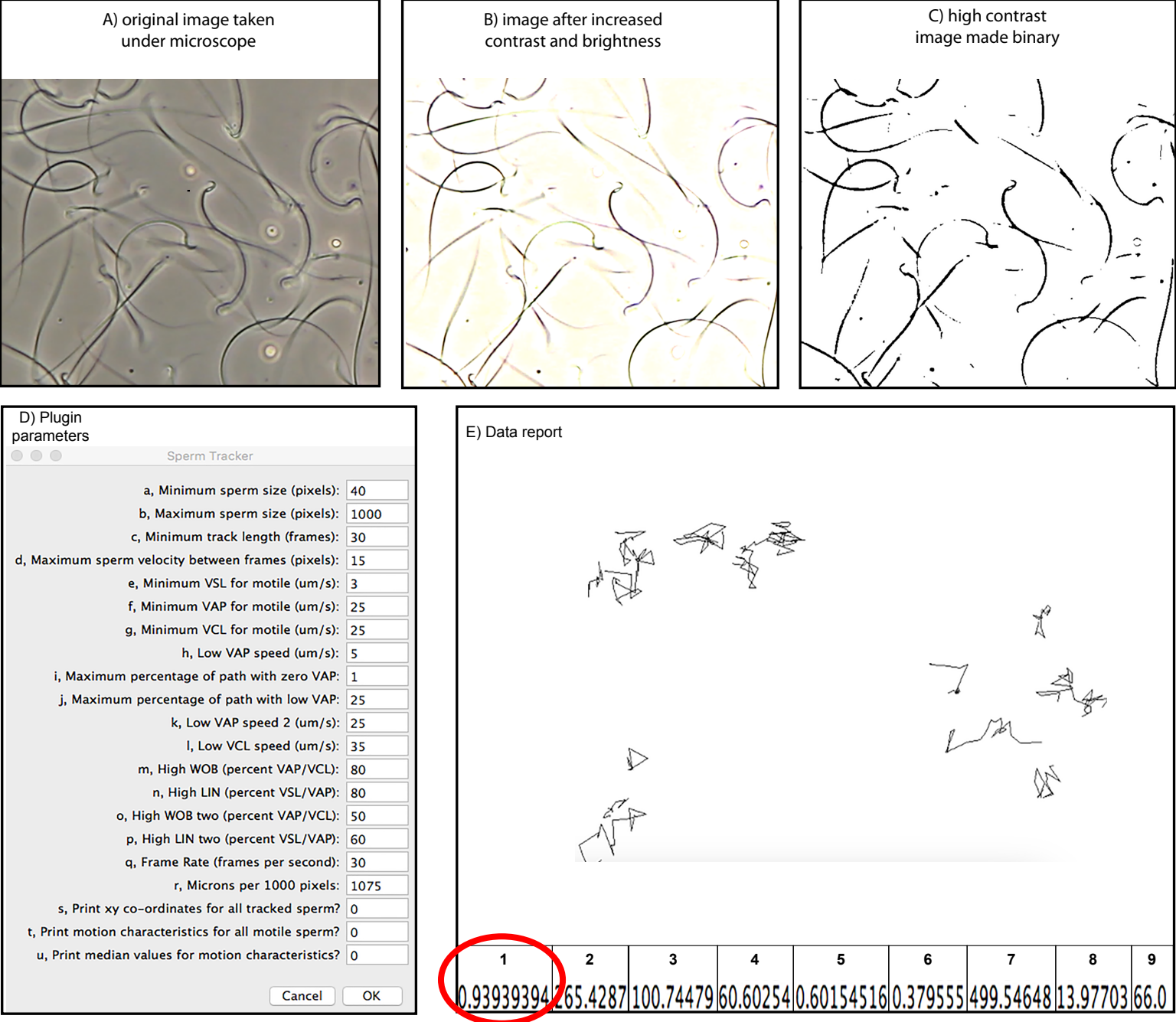

Figure S2. ImageJ processing and automated sperm motility analysis A) Raw image taken from series of images rendered from video. B) the contrast and brightness were increased in these images to make the background nearly white and sperm nearly black. C) the images are "made binary" so the sperm are black against the white background. This is to accommodate the 8-bit grayscale requirement for the CASA-like plugin https://imagej.nih.gov/ij/plugins/casa.html. D) Launching the plugin pulls up a parameter box as imaged here. The values shown are optimized for rat sperm. E) Screen shot of the output returned from the plugin analysis. The values are the outputs reported by the casa-like plugin. Value 1 is the percent motility, value 2 is the velocity curvilinear, value 3 is the velocity average path, value 4 is the velocity straight line, value 5 is the linearity, value 6 is the wobble, value 7 is the progression, value 8 is the beat cross frequency, and value 9 is the number of sperm tracked.

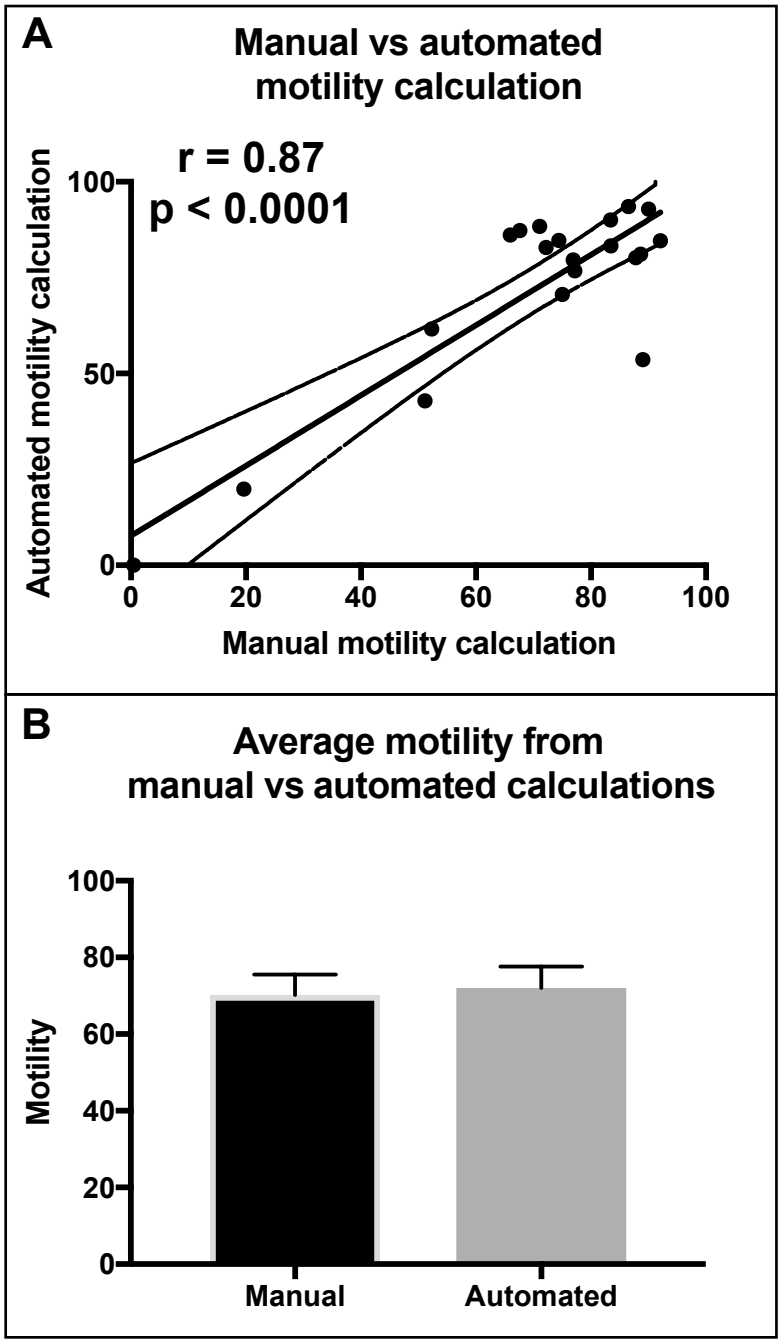

Figure S3. For a subset of control rats, both manual counting of motile/total sperm as a percentage, and automated calculation using the described method was performed. A) The automated count plotted against the manual count for each rat. A linear regression model was run on these values, a significant correlation ($p < 0.0001$) was found with correlation coefficient (r) of 0.87. B) The mean ± SEM of the automated compared manual counts were statistically the same (paired students t-test $p > 0.05$).

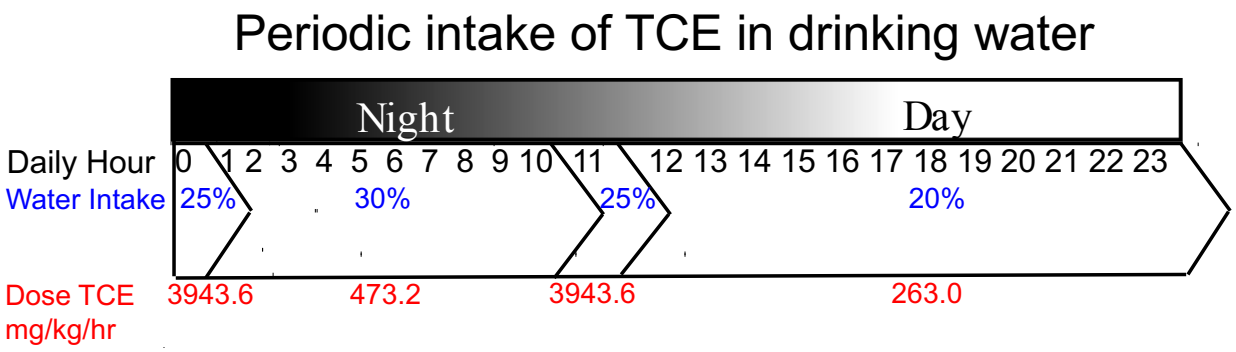

Figure S4. Intermittent water drinking of laboratory rats was used to extrapolate dosing paradigm of drinking water TCE exposure based on diurnal drinking patterns of laboratory rats observed on an hourly basis. The drinking distribution includes 50% of the total water intake is within the hour after lights out and one hour before lights on and total night water consumption is 80% of total intake per day. Water intake was broken down into four dosing periods, and the percentage of total drinking water exposure is shown here in blue, and the hourly dose (per day exposure) is reported in red. These hourly doses of TCE were used as input parameters for the PBPK modeling. This periodic intake resulted in a 15-fold change in the minimal hourly exposure dose to the maximal hourly exposure dose.

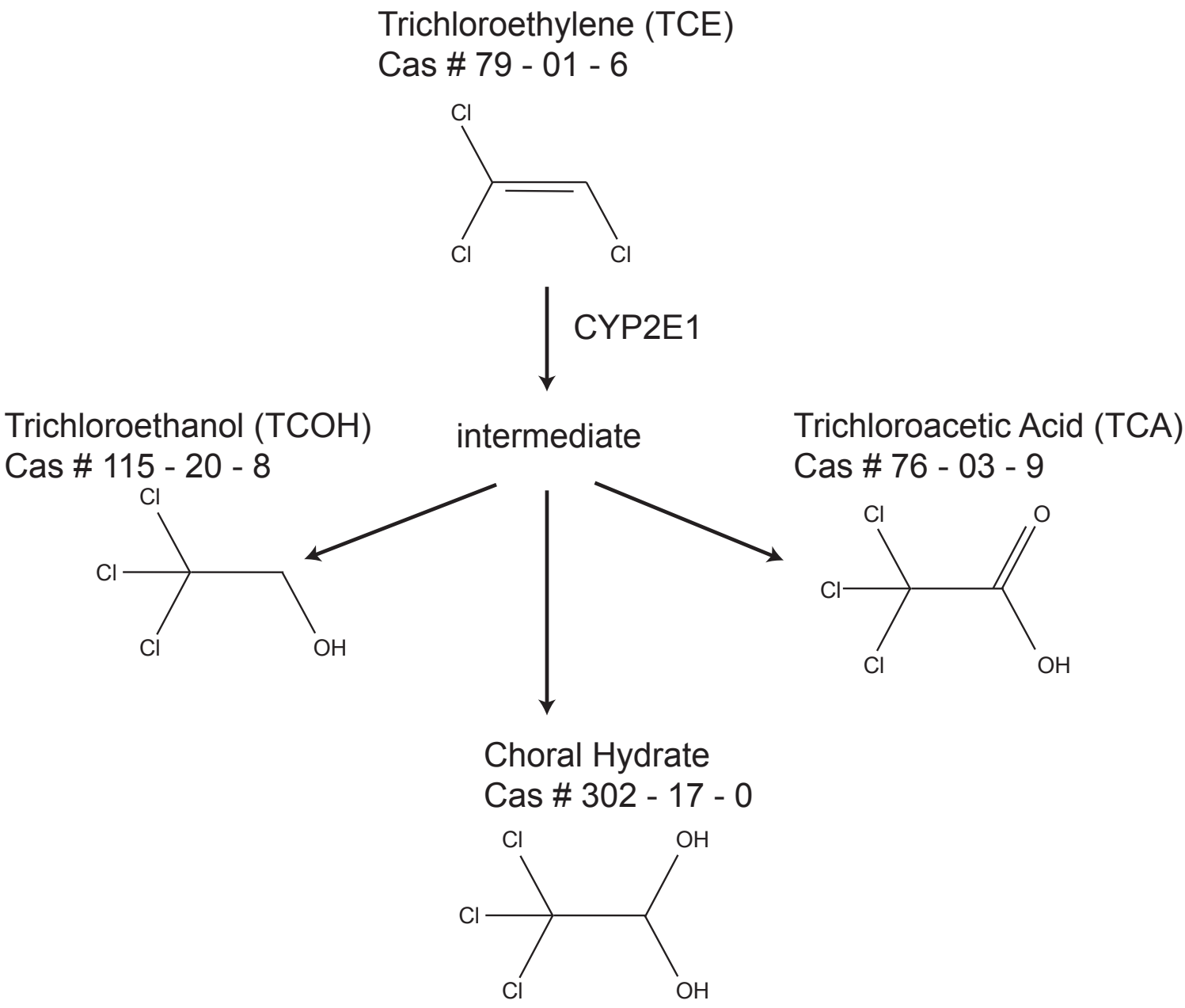

Figure S5. Chemical drawings, CAS numbers and simplified metabolic flow chart for TCE, TCA, TCOH and Choral Hydrate.

Chemical	Blood TCE	Blood TCOH	Blood TCA	Plasma TCA
C_{max} (mg/L) (median (5%, 95%))				
Drinking water	68.22 (39.55, 111.4)	9.24 (0.24,190.46)	39.08 (7.68, 122.91)	50.32 (9.3, 157.3)
Gavage	128.4 (22.91, 339.69)	8.25 (0.229, 157.8)	32.52 (5.9, 112.38)	42.67 (7.46, 140.28)
AUC (mg-hr/L) at 12 weeks of recovery (median (5%, 95%))				
Drinking water	20693 (7753, 54969)	20990 (723.73, 392550)	160290 (31830, 533160)	86695 (14723, 342398)
Gavage	45350 (15166, 102773)	9027 (288.5, 225696)	67673 (10147, 250940)	36890 (4769, 167475)

Table S1. Model-derived blood Cmax and AUC values for TCE, TCOH and TCA for each route of exposure, gavage or drinking water.