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

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Abstract

Trichloroethylene (TCE) is a persistent environmental contaminant that causes male reproductive toxicity. We investigated whether transient increases in TCE exposure modulated male reproductive toxicity by exposing rats via daily oral to repeated gavage exposures (1000 mg/kg/day) and through drinking water (0.6% TCE) for 14 weeks. The gavage route resulted in reversible reduction of epididymis weight, and reduced body weight that persisted for up to 12-weeks after cessation of exposure. Physiologically-based pharmacokinetic modeling predicted that the gavage route results in higher C_{max} and AUC exposure of TCE compared to drinking water exposure, explaining the observed differences in toxicity between dosing regimens.

Keywords

Trichloroethylene; Physiologically based pharmacokinetic modeling; male reproductive toxicity

1. INTRODUCTION

Trichloroethylene (TCE) is a common volatile environmental contaminant that the population can be exposed to via vapor intrusion. Air concentration correlated with blood levels in residents of impacted buildings (Archer et al., 2015). Real-time TCE monitoring shows 1000-fold fluctuations in exposure to TCE in indoor air (Holton et al., 2013). Transient environmental exposures may result in higher peak or total blood concentrations,

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CONFLICTS OF INTEREST

Kim Boekelheide and Susan J. Hall own stock in a small start-up biotechnology company (Semma Therapeutics) developing a treatment for type 1 diabetes. David Klein has been employed by the FDA for the past 18 months, on unrelated work. The authors have no additional financial interests.

depending on the pharmacokinetics of TCE or its metabolites. Physiologically based pharmacokinetic (PBPK) modeling for TCE is highly refined due to the large amount of data available for rat, mouse and human (Chiu et al., 2009). PBPK modeling has discerned the different toxicokinetics of oral vs. inhalational TCE exposures (Evans et al., 2009), and is well suited to predict differential toxicokinetics of TCE and its metabolites depending on temporal variation in external exposure.

Previous animal models have failed to consider the temporal variability in TCE exposure. Testicular, epididymis, and sperm toxicities have been found in rat models of exposure to TCE (Kumar et al., 2001; DeTeaux et al., 2003, 2004). Epidemiological studies of exposed workers correlated TCE exposure with poor pregnancy outcomes (Lindholm et al., 1991; Sallmen et al., 1998). In the present study, we investigated the extent to which continuous versus intermittent exposures modulated TCE-induced male reproductive toxicity.

2. MATERIALS AND METHODS

2.1. Animals.

Two- month old male Fisher CDF (Strain code 002, Charles River Laboratories, Raleigh, NC) rats were housed in humidity- and temperature-controlled rooms, maintained on a 12 hour light/ dark cycle with food (Purina Rodent Diet 5010, PharmaServ, Inc., Framingham, MA) and water *ad libitum*. All procedures were performed in accordance with the National Research Council's Guide for Care and Use of Laboratory Animals, and approved by Brown University's Institutional Animal Care and Use Committee (IACUC).

2.2. Experimental design.

Rats (n = 120) were randomly divided into four groups: drinking water control, drinking water exposed, oral gavage control and oral gavage exposed. Drinking water exposed group was given 0.6% TCE (Sigma- Aldrich, cat # 251402-1L) dissolved in 3% Tween20 water and compared to a vehicle control. Gavage exposed rats were administered 1,000 mg/kg TCE dissolved in corn oil (2 ml/kg) 5 times a week and compared to a vehicle control. Exposure for all four groups continued for 14 weeks. At 0, 6, and 12 weeks after cessation of exposure (Figure S1), one third of each treatment group was euthanized and body weights, testis weights and epididymis weights were collected. A pilot study demonstrated that 0.6% TCE did not significantly alter water intake, and led to a similar total exposure amount between gavage and drinking water (84 g/kg total water exposure, 70 g/kg total gavage exposure).

2.3. Sperm motility.

The vas deferens were collected and placed in prewarmed (37°C) 1% BSA-PBS media and incubated for 5 minutes at 37°C to allow for the sperm release. After 5 minutes, 10 μL of solution was place on a headed 37°C hemocytometer and recorded (Nikon Rebel T3) on a Ziess Axiovert 35 microscope (Germany). The videos were analyzed by an in-house automated sperm motility analysis program (Figure S2). There was high correlation (r = 0.87) between the computer-generated values and manually-generated motility values (Figure S3).

2.4. PBPK modeling of TCE toxicokinetics.

The PBPK model used a Bayesian population approach based on Markov chain Monte Carlo (MCMC) simulation to estimate variability (Chiu et al., 2009). Posterior population mean distributions were used to predict the median and 95% confidence intervals for each exposure paradigm. Modeling the drinking water exposure was based on the average weight of the rats at the beginning of the experiment, and the average water consumption per rat. The drinking habits of rats in a 12-hr light/ dark cycle has been previously described (Spiteri, 1982) (Figure S4). Chemical structures, a simplified metabolic flow chart (Figure S5), and model-derived raw values for blood C_{max} and AUC of TCE, and metabolites (Table S1) are reported in the supplemental information.

2.5. Statistics:

Body weight, weight gain, testis weight, epididymis weight and motility data were analyzed for the mean ± standard error of the mean (SEM) (Graphpad Prism; La Jolla, California). One-way ANOVAs were performed individually at each time point (0-weeks recovery, 6-weeks recovery, 12-weeks recovery) across the four treatment groups, using Sidak's post-hoc for multiple comparisons with significance at $p < 0.05$.

3. RESULTS

TCE caused a significant decrease in body weight, and reduced weight gain in rats exposed through both water and gavage, at 0-weeks recovery. Only the gavage TCE-treated rats had lower body weights at 6-weeks recovery, with significantly less weight gain at 12-weeks compared to controls (Table 1). TCE exposure did not significantly alter testis weight at any time point by either route of exposure (Table 1). Rats had significantly lower epididymis weight at 0-weeks by oral gavage only, that reversed following a 6-week recovery period (Table 1). Since sperm motility is known to be highly variable, a combined "treated" group was compared to a combined "control" group to account for the high variation. There was a significant decrease ($p = 0.004$) in motility in the treated compared to control group at 0-weeks recovery when the data was analyzed in this way.

PBPK modeling compared peak and total concentrations between the two exposure routes for TCE. The model predicted the maximum concentration in the blood (C_{max}) of TCE would be two-fold higher after gavage exposure compared to water exposure; the C_{max} of TCA and TCOH were predicted to be similar in the two dosing paradigms (Table 2). The total blood concentrations of TCE were predicted by the AUC at 12 weeks of recovery with gavage 2-fold higher for TCE then drinking water exposure, while drinking water was 2-fold higher for metabolites than gavage exposure (Table 2).

4. DISCUSSION

The gavage-dosing paradigm used here experimentally replicated transient increases in vapor intrusion (Holton et al., 2013), and were compared to continuous TCE water exposure. Despite similar cumulative administered doses of TCE between the two regimens, the PBPK model predictions showed higher TCE concentrations in the blood after gavage exposure, and greater metabolite concentrations in the blood after drinking water exposure (Table 2).

Under linear metabolism kinetics, the AUCs of parent and metabolites depend on the cumulative external exposure (Chiu and White, 2006). However, saturable first-pass metabolism is the predominant process for pre-systemic elimination of TCE after oral exposure (Liu et al., 2009). More rapid input of TCE into the liver results in more pronounced saturation of metabolism, resulting in less efficient first-pass elimination (Lee et al., 1996). Thus, the higher AUC and C_{max} of TCE in the blood after gavage exposure is due to non-linear metabolism, with saturation kinetics.

After gavage exposure, epididymis weight and sperm motility were reduced but returned to control levels 6-weeks after cessation of TCE exposure, yet body weight suppression was prolonged for at least 12 weeks (Table 1). The circulating TCE levels were higher after gavage exposure than water exposure (Table 2). These results indicate that gavage-associated elevated circulating TCE elicited toxic effects. Local bioactivation of TCE occurs in organs that contain CPY2E1 (Lash et al., 2000). Testis and epididymis have less CYP2E1 than either the liver or kidney (DeTeaux et al., 2003), providing a plausible mechanistic explanation for the differential systemic and reproductive toxicity. In summary, gavage-exposed rats showed greater toxicity than water-exposed rats which may be of concern in high, transient exposure scenarios of TCE vapor intrusion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Body weight, weight gain, organ weight and sperm motility after TCE exposure as a function of route of exposure and recovery.

TCE toxicity measured via body weight, weight gain, testis weight, epididymis weight and sperm motility for 0- 6- and 12- week recovery time points and water and gavage TCE exposure routes. Weights of left and right organs are combined (mean ± SEM). Significant measures are bolded and italicized.

Weeks Recovery	Route	Treatment	Body weight	Weight gain	Testis weight	Epididymis weight	Motility
0	water	Control	309 ±4.91	99.5 ±2.67	2.97 ±0.05	0.94 ± 0.03	83.5% ± 2.78
		TCE	***284 ± 5.65*** **	***73.1 ± 2.82*** *	2.99 ±0.04	0.95 ±0.03	***69.2% ± 5.71*** †
	gavage	Control	304 ± 3.83	90.6 ± 1.73	2.99 ±0.04	0.92 ± 0.02	83.6% ± 3.46
		TCE	***270 ± 3.92*** ***	***65.1 ±4.78*** **	3.00 ±0.04	***0.84 ± 0.02*** *	***70.9% ± 4.03*** †
6	water	Control	346 ± 7.95	132 ±6.46	3.19 ±0.05	0.99 ±0.03	83.3% ± 3.66
		TCE	337 ±5.13	123 ±3.83	3.16 ±0.04	1.00 ±0.03	75.3% ± 6.88
	gavage	Control	326 ± 4.88	112 ±1.87	3.06 ± 0.04	0.94 ± 0.02	81.2% ±4.75
		TCE	***302 ±3.11*** *	97 ± 6.52	3.13 ±0.03	0.95 ± 0.02	87.8% ±2.19
12	water	Control	366 ± 8.46	146 ±5.52	3.18 ±0.05	1.02 ±0.02	82.5% ± 5.24
		TCE	369 ± 9.74	144 ±4.58	3.27 ±0.05	1.03 ±0.03	87.6% ± 2.28
	gavage	Control	367 ± 7.38	142 ±3.81	3.28 ±0.04	1.04 ±0.03	81.4% ±5.33
		TCE	346 ± 8.05	***121.5 ± 4.90*** **	3.33 ±0.04	1.06 ±0.03	77.1% ±5.65

* $p<0.05$,

** $p<0.01$,

*** $p<0.001$ when exposure groups were tested by ANOVA at each timepoint.

† $p < 0.01$ when tested by t-test between combined “control” and “treated” TCE exposure groups at 0-weeks.

Table 2.

C_{max} and AUC ratio of TCE, TCA and TCOH in drinking water exposure/ oil gavage exposure.

Results from a Bayesian population PBPK model based on the given experimental design and MCMC simulation. The maximum concentration (C_{max}) and area under the curve (AUC) after 12 weeks of recovery (total exposure for entire experiment) are expressed as a ratio of the predicted concentrations after drinking water exposure divided by the concentrations after gavage exposure. Ratios less than one show concentrations are greater after gavage exposure, ratios greater than one show concentrations are greater after drinking water exposure.

Parameter	Chemicals	Ratio drinking water/ oil gavage (median (5%, 95%))
C_{max}	Blood TCE	0.53 (1.73, 0.33)
	Blood TCOH	1.12 (1.04, 1.21)
	Blood TCA	1.2 (1.3, 1.1)
	Plasma TCA	1.18 (1.25, 1.12)
AUC 12 weeks after exposure	Art.blood TCE	0.502 (0.24, 0.83)
	Blood TCOH	2.28 (0.83, 6.36)
	Plasma TCA	2.39 (1.19, 5.4)
	Plasma TCAfree	2.35 (1.26, 4.98)