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Ethanol-Induced Increase in the Metabolic Clearance of 1,1,1-Trichloroethane in Human Volunteers

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This study evaluated the effect of moderate doses of ethanol over a short period of time on the toxicokinetics of an organic solvent, 1,1,1-trichloroethane. A group of 10 moderate drinkers were recruited and exposed via inhalation for 2 h to a low concentration of 1,1,1-trichloroethane (175 ppm) on two separate occasions. Subjects were administered ethanol (0.35 g/kg body weight) on each of the 7 days preceding one of the exposures. Blood and urine samples were collected during and following each exposure, with blood analyzed for 1,1,1-trichloroethane and urine analyzed for the metabolites of 1,1,1-trichloroethane: trichloroethanol and trichloroacetic acid. Prior ethanol consumption resulted in a significant increase in apparent metabolic clearance of 1,1,1-trichloroethane (mean increase = 25.4%). The results of this study demonstrate that ethanol consumption over time can affect the rate at which an organic solvent is cleared through metabolism in humans. For chemicals with toxic metabolic products, this inductive effect of ethanol consumption on the rate of biotransformation could be potentially harmful to exposed individuals. Metabolic clearance of compounds with high hepatic extraction may not be affected by enzyme induction as it is likely that these compounds are essentially completely metabolized while passing through the liver.

Key Words: **1,1,1-trichloroethane; methyl chloroform; ethanol; enzyme induction; biotransformation.**

Ethanol consumption is a potentially important factor affecting the toxicokinetics of organic solvents, in part because it is commonly consumed in relatively large quantities (one 5-oz glass of wine contains approximately 14 g of ethanol). In humans, the biotransformation of ethanol is mediated primarily by alcohol dehydrogenase, producing acetaldehyde. Further oxidation of acetaldehyde is then catalyzed by the enzyme aldehyde dehydrogenase to form acetic acid. A minor fraction (10–20%) of ingested ethanol is biotransformed by a microsomal ethanol-oxidizing system, with cytochrome P450 2E1 (CYP2E1) as the major enzyme involved (Agarwal, 2001; Holford, 1987). It has been found that, depending on its timing, consumption of ethanol can either induce or inhibit CYP2E1 activity. Immediately following ethanol consumption, CYP2E1 activity toward other substrates has been shown to decrease in humans owing to competitive inhibition (Loizou and Cocker, 2001). However, if the ethanol is administered daily over time, CYP2E1 activity increases due to enzyme induction which may involve both transcriptional and posttranslational mechanisms (McCarver *et al.*, 1998; Oneta *et al.*, 2002). This is an important observation as many chemicals cause their health effects as a result of biotransformation to a toxic intermediate product. This is true for several compounds whose biotransformation is mediated by CYP2E1, including acetaminophen, benzene, carbon tetrachloride, vinyl chloride, dimethylnitrosamine, chloroform, and styrene (Guengerich and Shimada, 1991, 1998).

In terms of workplace exposures to solvents, the effect of induction by ethanol of CYP2E1 activity may be of greater concern than the effect of inhibition. In some cases, workers may consume alcoholic beverages at lunch, which could result in the inhibition of solvent biotransformation. However, it is far more common for adults to drink one to two bottles of beer or glasses of wine with dinner or in the evening before retiring, potentially resulting in the induction of CYP2E1 activity (Harford *et al.*, 1992). Two groups have attempted to evaluate the effect of ethanol on solvent biotransformation by conducting controlled human exposures to an inhaled solvent following one or more days of administered ethanol (Hjelm *et al.*, 1994; Tardif *et al.*, 1994). However, a consistent inductive effect of ethanol was not observed in either study. Hjelm *et al.* (1994) found a statistically significant increase in the mass of the metabolite hippuric acid excreted following exposures to toluene when the exposure was preceded by ethanol ingestion, but only while subjects were on a low carbohydrate diet. Tardif *et al.* (1994) found a significant decrease in blood concentration of *m*-xylene following ethanol administration, but the effect was observed only at high exposure concentrations of *m*-xylene (400 ppm).

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In these studies, ethanol was administered (ethanol dose > 0.5 g/kg/day) for only 1 or 2 days prior to exposure. Therefore, it is possible that at the doses given, insufficient time was allowed for enzyme induction. In contrast, Sato *et al.* (1981) found that a single, very large dose of ethanol (4 g/kg) administered to rats resulted in an increase in the rate of biotransformation of several different hydrocarbons 16 h after the administration. In the same study, no effect of ethanol on solvent biotransformation was observed following a single ethanol dose of 2 g/kg. Therefore, the amount of ethanol given in the Tardif and Hjelm studies may have been too small to significantly affect CYP2E1 levels over such a short period of time.

The more likely reason for the inconsistency of the findings is the choice of solvents used for the exposures. Most compounds whose intrinsic metabolic clearance (V_{max}/K_m) is greater than the hepatic blood flow (Q_h) have a high hepatic extraction ratio (E_h), i.e., E_h approaches the maximum of 1.0 (Andersen, 1982). The intrinsic metabolic clearances of both *m*-xylene and toluene are greater than the average hepatic blood flow (Tardif *et al.*, 1993); thus, they undergo nearly complete extraction as they pass through the liver, even in the noninduced state. For high extraction compounds, enzyme induction would not be expected to result in a detectable increase in hepatic clearance or total body clearance if liver metabolism is the major route of elimination. The goal of this study was to investigate experimentally the effects of moderate doses of ethanol (0.35 g/kg) on the biotransformation of a solvent in human volunteers. A chemical with low intrinsic clearance (1,1,1-trichloroethane) was delivered at a low but realistic concentration, with exposure preceded by an ethanol-dosing regimen that extended over a longer period than has been used in previous studies.

The majority of absorbed 1,1,1-trichloroethane (> 91%) is excreted unchanged in exhaled breath, with a small percentage of the absorbed dose (5–6%) biotransformed via CYP2E1 to form trichloroethanol (TCEOH), which is excreted in urine. Some of the TCEOH is further oxidized to a secondary metabolite, trichloroacetic acid (TCA), which is also excreted in urine (Nolan *et al.*, 1984). 1,1,1-Trichloroethane has been identified as an ozone-depleting substance, and as such, its production is being phased out (den Elzen *et al.*, 1992). However, due to large volume usage in the 1970's and 1980's, 1,1,1-trichloroethane is frequently encountered at hazardous waste sites and is a common groundwater pollutant (ATSDR, 1995). Its use in this study is relevant primarily as a model for other chemicals with similar toxicokinetic properties.

MATERIALS AND METHODS

Human Subjects

Subjects were male and female social drinkers, defined as individuals who normally consume between 3 and 14 drinks (e.g., bottle of beer or small glass of wine) per week. Five males and five females between the ages of 22 and 57 participated in this study (Table 1). Each subject provided informed written consent, and all recruitment, exposure, and sampling protocols were approved by the Institutional Review Board of the University of Washington. Prior to enrolling, each potential subject was asked to fill out a medical and work history questionnaire seeking information on medications, health status, exposures to solvents, alcohol use and history of alcohol abuse, and diet. Individuals who reported a history of respiratory, heart, liver, kidney, or nervous system illnesses were excluded from participation. Liver function tests were performed on blood samples collected from each subject prior to their enrollment in the study to screen for preexisting liver disease or injury. The tests included serum bilirubin concentration, as well as the serum activity of liver enzymes: aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, gamma glutamyl transpeptidase, and alkaline phosphatase. Individuals currently taking medications known to alter CYP2E1 activity, such as chlorzoxazone, tamoxifen, acetaminophen, and isoniazid, were excluded. Women of childbearing potential were encouraged to maintain effective contraceptive methods, and each had a negative result for human chorionic gonadotropin in blood on the evening before each solvent exposure.

Any potential subject who was routinely exposed to paints, paint thinners, gasoline, varnishes, glues, dry cleaning solvents, or industrial cleaning products was excluded from the study. All these products contain solvents that may alter CYP2E1 activity. A variety of different foods have been found to affect CYP2E1 activity, specifically garlic, onions, leeks, watercress, and broccoli (Brady *et al.*, 1991; Kall *et al.*, 1996; Kim and Wilkinson, 1996). Subjects were asked to keep their diet consistent from week to week and to try to avoid these foods while participating in the study. Individuals who reported taking garlic supplements were excluded from participation.

Subject Schedule of Activities

A typical study subject's schedule is presented in Figure 1. Each subject served as his or her own control and was therefore exposed to 1,1,1-trichloroethane on two separate occasions, with one exposure preceded by 7 days of daily ethanol administration and the other exposure preceded by no ethanol. The time between active participation phases, defined as the 18-day period shown in Figure 1, varied between 2 and 8 weeks, depending on the schedules of the investigators and subjects. The order of exposure (ethanol preceding the first solvent exposure or the second solvent exposure) was counterbalanced among the subjects.

Ethanol Administration

At approximately the same time on each of the seven evenings preceding one of the two solvent exposures for each subject, moderate doses of ethanol were given. Subjects were asked to fast for 4 h before ethanol administration to reduce variability in uptake and in blood alcohol concentration (BAC) after

TABLE 1
Gender, Age, Body Weight, Height, and Body Mass Index for Each Subject

Subject	Gender	Age	Body weight (kg)	Height (m)	Body mass index (kg/m^2)
1	Male	57	85.0	1.86	24.6
2	Male	23	82.0	1.80	25.3
3	Male	37	126.8	1.94	33.7
4	Male	22	68.0	1.84	20.1
5	Male	28	96.4	1.88	27.3
6	Female	26	59.0	1.69	20.7
7	Female	41	70.7	1.73	23.6
8	Female	25	55.7	1.73	18.6
9	Female	45	61.0	1.72	20.6
10	Female	36	74.0	1.76	23.9

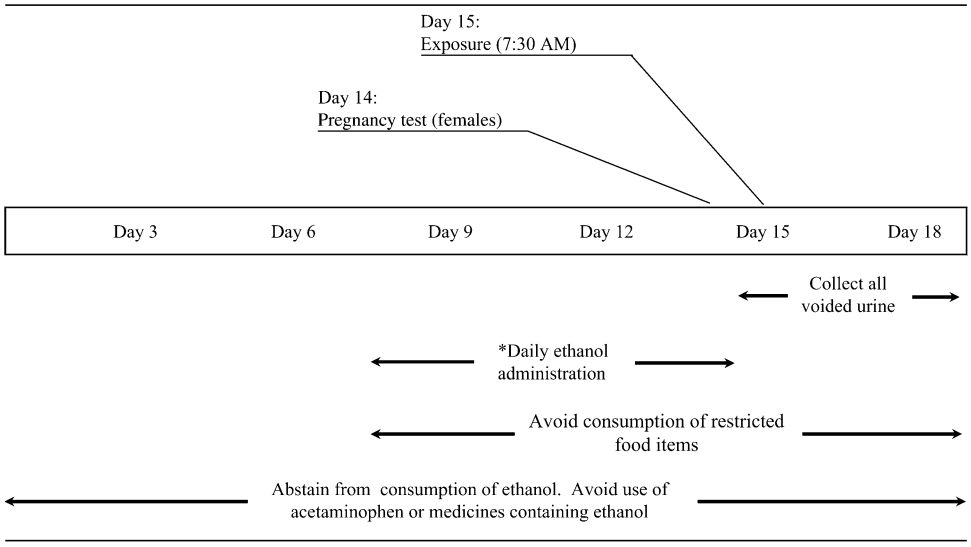

FIG. 1. Typical schedule of a study subject prior to and following each exposure. This schedule was repeated for each subject following a period of 2–8 weeks during which subjects were not actively participating in the study.

ingestion. Doses of 0.35 g/kg were used, which was not expected to yield blood ethanol concentrations exceeding 0.05 g/100 ml blood. Beverages were prepared using 100 proof vodka diluted with orange juice to an ethanol concentration of between 8 and 14% by volume (total volume approximately 375 ml). Subjects ingested the ethanol over a period of 50 min. Subjects then rinsed their mouths and waited 10 min to ensure there was no residual ethanol in the mouth or throat to allow for an accurate assessment of the BAC using an alveolar sample. After a deep breath, subjects held their breath for 10 s to ensure equilibration between alveolar air and arterial blood, and then exhaled completely into a long (3 m), heated Teflon tube. Approximately 0.5 l of exhaled breath was then withdrawn from the proximal end of the tube. The concentration of ethanol in this sample was then determined using a calibrated gas chromatograph (GC), equipped with a 1-ml gas sample loop. The GC was calibrated with standard air concentrations of ethanol created using a Dynacalibrator from VICI Metronics, Inc. (Poulsbo, WA). The analytical response for ethanol in breath was linear with a dynamic range of 1.0–500 mg/m^3. To estimate the BAC, the blood-air partition coefficient (K_{ba}) for ethanol was assumed to be 2100 (Jones, 1983). The BACs for each subject are presented in Table 2.

Controlled Solvent Exposures

Reagent-grade 1,1,1-trichloroethane was obtained from Aldrich Chemical (Milwaukee, WI) with a chemical purity of > 99%. Subjects inhaled air containing 175 ppm 1,1,1-trichloroethane, which is one-half the American Conference of Governmental Industrial Hygienists Threshold Limit Value for a period of 2 h. The inhaled mass was therefore equal to 1/8 the level of exposure permitted for workers who are exposed 8 h/day for the duration of their working life (ACGIH, 2004). Exposure concentrations of 1,1,1-trichloroethane were generated with a motor-driven syringe filled with liquid solvent (Morgan *et al.*, 1993). The solvent was passed through a heated fitting, vaporized, and diluted with medical-grade air. The mixture was directed past a mouthpiece at flow rates in excess of each subject's maximum inhalation rate. Nose clips were used to prevent subjects from breathing room air. The concentration of 1,1,1-trichloroethane directly upstream of the mouthpiece was continuously recorded using a photoionization detector. Pulmonary ventilation rate was also continuously recorded using a capillary pneumotachometer. The product of the average ventilation rate (l/min) and the average exposure concentration (mg/l) multiplied by the duration of exposure yielded the total inhaled dose for each subject. The end-tidal CO_2 concentrations were measured continuously using an infrared monitor (Model CO2SMO, Novametrix, Wallingford, CT) to verify that the subjects were maintaining normal respiration.

Sample Collection

Blood. Blood was drawn using a cannula (saline lock) inserted into a forearm vein. During the exposure and for 1 h following the exposure, samples were collected every 20 min using vacutainers containing potassium oxalate. From 1 to 6 h postexposure, samples were collected hourly by the same method. Blood samples were refrigerated at 10°C until they were prepared for analysis on the same day.

Urine. Subjects collected all voided urine for approximately 70 h following exposure, using a separate container (500 ml Nalgene) for each void. A urine sample was also collected 5–15 min before the start of the exposure. The date and time of each void was recorded, along with the date and time of the previous void. Subjects were asked to note if a urine sample was

TABLE 2
Mean Estimated BAC for Each Subject Over the 7 Days of Administered Ethanol ($n = 7$ for each subject)

	BAC (g/dl)	
Subject	Mean (SD)	Min–Max
1	0.034 (0.004)	0.029–0.036
2	0.038 (0.008)	0.027–0.048
3	0.035 (0.007)	0.028–0.046
4	0.031 (0.007)	0.028–0.044
5	0.037 (0.005)	0.031–0.043
6	0.040 (0.004)	0.034–0.048
7	0.039 (0.003)	0.035–0.044
8	0.040 (0.007)	0.032–0.048
9	0.046 (0.004)	0.041–0.049
10	0.042 (0.003)	0.039–0.048

not collected or if any urine was spilled during collection. Samples were refrigerated until analysis.

Sample Analysis

A detailed description of the analytical methods used for the determination of 1,1,1-trichloroethane in blood along with TCEOH and TCA in urine has been published recently (Johns *et al.*, 2005). These methods are summarized below.

1,1,1-Trichloroethane in blood. Blood samples were prepared within 2 h of collecting the last sample from each solvent exposure. One-milliliter aliquots of blood were placed in 20-ml headspace vials with Teflon septa and spiked with an internal standard ($^2H_3$-1,1,1-trichloroethane). Samples were assayed for 1,1,1-trichloroethane using dynamic headspace collection and analysis with gas chromatography/mass spectrometry (GC/MS). The response for 1,1,1-trichloroethane in blood was linear between concentrations of 0.9 ng/ml and 2.2 μg/ml with a limit of quantitation (LOQ) of 0.8 ng/ml.

TCEOH and TCA in urine. TCA in urine was also analyzed using dynamic headspace coupled with GC/MS. TCA was converted to the more volatile methyl trichloroacetate using deionized water, concentrated sulfuric acid, and methanol (6:5:1 by volume). This derivatizing reagent (600 μl) was added to 300-μl aliquots of urine, which had been spiked with butyric acid as an internal standard. The butyric acid was also converted to its methyl ester and analyzed as methyl butyrate. Prior to analysis, samples were briefly vortexed and allowed to equilibrate for 2 h. Dynamic headspace collection and analysis were performed using the same parameters used for 1,1,1-trichloroethane in blood. The response for TCA in urine was linear between concentrations of 0.01 and 7.0 μg/ml with an LOQ of 0.009 μg/ml.

An assay was developed for the analysis of TCEOH in urine using organic extraction with methylene chloride. *In vivo*, much of the TCEOH formed is conjugated as TCEOH-glucuronide (urochloralic acid). To analyze total TCEOH in urine (free plus conjugated), this conjugate was hydrolyzed using β-glucuronidase (Sigma, *Helix pomatia*, type H-1, St Louis, MO). Total TCEOH was extracted with methylene chloride and analyzed using GC/MS. The response for TCEOH in urine was linear between concentrations of 0.1 and 15.5 μg/ml with an LOQ of 0.06 μg/ml.

Statistical Analysis

Using the statistical software Stata (Stata Corp, College Station, TX), the area under the blood concentration–time curve (AUC) for 1,1,1-trichloroethane was calculated using the trapezoidal rule. Apparent metabolic clearance of 1,1,1-trichloroethane was then calculated by dividing the total mass of metabolite excreted by the AUC for 1,1,1-trichloroethane, assuming that renal clearance represents the sole route of excretion for both TCA and free and conjugated TCEOH. A paired *t* test was used to assess the within-subject difference in apparent metabolic clearance between exposures preceded by ethanol versus exposures preceded by no ethanol. Paired *t* tests were also used to evaluate the effect of ethanol consumption on the AUC for 1,1,1-trichloroethane and the total mass of metabolite excreted.

Modeled Steady-State Relationship

The findings in this study and their comparison to those of Tardif *et al.* were described in approximate terms using a physiologically based compartmental model as developed by Andersen (1982). Assuming that the liver is the most important site of biotransformation for each solvent, the biotransformation rate ($\dot{m}$) is given by

$$\dot{m} = Q_h(C_{in} - C_{out}), \tag{1}$$

where Q_h is the perfusion rate for the liver (approximately 0.25 cardiac output, 1.5 l/min at rest), C_{in} is the concentration of solvent in blood entering the liver (portal vein and hepatic artery), and C_{out} is the concentration of solvent in blood exiting the liver (hepatic vein).

Based on this relationship, any effect of induction on biotransformation rate can be estimated if the corresponding blood concentrations can be predicted. Andersen (1982) has shown that these concentrations are related at steady state by integrating the Michaelis-Menten equation for the liver:

$$(C_{in} - C_{out}) = \frac{V_{max}}{Q_h} + K_m \ln \frac{C_{out}}{C_{in}}, \tag{2}$$

where V_{max} = maximum rate of biotransformation and K_m = Michaelis-Menten constant.

In Equation 2, induction would be reflected by an increase in the value of V_{max}. This equation cannot be solved explicitly for either blood concentrations, but once reasonable values are chosen for the three parameters (V_{max}, Q_h, and K_m), the equation can be solved by iteration. Since arterial blood concentration also depends on the concentration of inhaled solvent, a third relationship is needed to complete the model predictions:

$$\frac{C_{arterial}}{C_{inhaled}} = N_{eff} = \frac{N}{1 + E_t(Q_t/\dot{V}_{alv})N}, \tag{3}$$

where N_{eff} = steady-state ratio of solvent concentrations in arterial blood and inhaled air, N = equilibrium blood-air partition coefficient for the solvent, Q_t = cardiac output, $\dot{V}_{alv}$ = alveolar ventilation rate, and E_t = systemic extraction ratio.

The systemic extraction ratio is based on the hepatic extraction ratio (E_h), defined by

$$E_h = \frac{C_{in} - C_{out}}{C_{in}}. \tag{4}$$

The systemic extraction ratio is equal to $0.25E_h$ based on the assumption that hepatic perfusion is one-fourth of cardiac output and all biotransformation occurs in the liver.

A simultaneous solution of Equations 1–4 allows a comparison of the biotransformation rates of the two solvents, at the inhaled concentrations used in the two studies, before and after treatment with ethanol. For xylene, the values of the critical parameters were based on rat data, with V_{max} scaled allometrically to human body weight (Loizou *et al.*, 1999; Pierce *et al.*, 1996; Tardif *et al.*, 1993): V_{max} = 180 mg/h (native state), V_{max} = 270 mg/h (induced state); K_m = 0.2 mg/l; and N = 30.

For 1,1,1-trichloroethane, the following parameters were used (Reitz *et al.*, 1988): V_{max} = 9 mg/h (native state), V_{max} = 13.5 mg/h (induced state); K_m = 5.75 mg/l; and N = 2.53.

Resting values of 350 l/h for cardiac output and 315 l/h for alveolar ventilation were used (Gurtner *et al.*, 1975; West *et al.*, 1974). For modeling the effect of induction, V_{max} was increased 1.5-fold based on a recent study using chlorzoxazone to determine the activity of CYP2E1 in rats with and without pretreatment with moderate doses of ethanol (Howard *et al.*, 2001).

RESULTS

1,1,1-Trichloroethane in Blood

The concentration of 1,1,1-trichloroethane in blood quickly reached a plateau, with the mean concentration at 20 min equal to 85% of the mean concentration at the end of the exposure (2 h). 1,1,1-Trichloroethane concentrations in blood during and following exposures with and without prior ethanol consumption are presented in Figure 2. It is apparent that the blood concentrations were at steady state after approximately 1 h of exposure. Differences in pulmonary ventilation rate following the two exposures for a given individual could potentially affect the AUC of 1,1,1-trichloroethane in blood as the majority of 1,1,1-trichloroethane is excreted unchanged in exhaled breath. However, subjects remained seated in the laboratory for several hours following exposure; therefore, within-subject

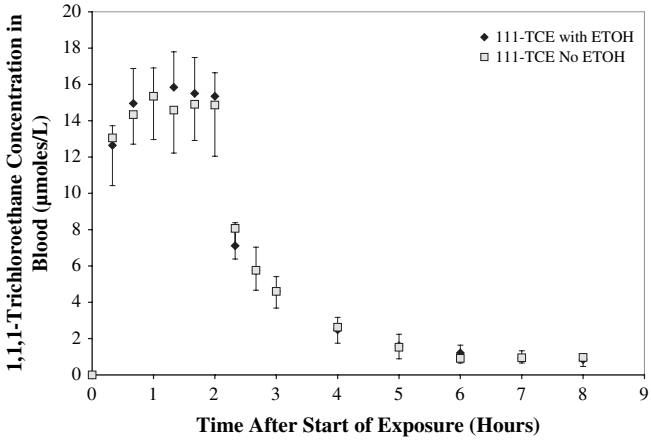

FIG. 2. 1,1,1-Trichloroethane concentration in blood during and following exposures with and without prior ethanol consumption. Data represent mean concentrations from all 10 subjects (error bars = 1 SD).

variability in postexposure ventilation rate was assumed to be small. Procedural differences occurred for subjects 3, 6, and 7 between their exposures with and without previous ethanol consumption, resulting in clear differences in blood concentrations between the two exposures for those subjects. On the day of exposure without prior ethanol consumption for subject 3, technical problems resulted in the inhaled concentration being reduced by approximately 40% from the target value. During the exposure without prior ethanol for subject 6 and during the exposure with prior ethanol for subject 7, the seal around the mouthpiece was not maintained throughout the exposure, resulting in a lower inhaled dose. As shown in Table 3, there was no significant difference in solvent AUC between exposures with and without prior ethanol (paired t test: $p = 0.34$). The mean difference in AUC without prior ethanol consumption relative to the AUC with prior ethanol consumption was − 3.5% (a 3.5% decrease), with a range of − 58.4 to 85.9%. When the three subjects with explainable differences in AUC are removed from the analysis, the mean difference in AUC with no prior ethanol consumption relative to the AUC with prior ethanol consumption is − 1.8% with a range of − 15.8 to 14.7% (paired t test: $p = 0.72$). The relationship between AUC and inhaled dose was assessed using Pearson's correlation coefficient ($r = 0.85$, $p < 0.001$). These data are presented in Figure 3.

TCEOH and TCA in Urine

TCEOH was excreted very rapidly following exposure, with the majority of the total TCEOH excreted in urine (~ 75%) recovered within 24 h after the start of exposure. Of the total metabolite excreted, 10–20% was recovered as TCA. The excretion rates of both metabolites displayed first-order kinetics, with linear semilog plots for times after the peak in excretion rate (~ 3 h for TCEOH, ~ 40 h for TCA). Missing data, including missed samples and samples below LOQ, were estimated by interpolation or extrapolation of semilog plots of excretion rate versus time. The fraction of the total mass of metabolite excreted which was estimated from interpolated/extrapolated data ranged from 0 to 15%, with a mean of 6%. Figure 4 displays the cumulative mass of both metabolites excreted over 70 h postexposure. Urine samples were not collected at predetermined times; therefore, in order to incorporate the data from all 10 subjects, the cumulative mass excreted at specified times postexposure was determined for each subject by interpolation. The total mass of urinary metabolites excreted over 70 h and the apparent metabolic clearance of 1,1,1-trichloroethane (Cl_m) following each exposure are presented in Table 3.

TABLE 3
AUC for 1,1,1-Trichloroethane, Total Micromoles of Metabolites Excreted (70 h postexposure), and Apparent Metabolic Clearance (total metabolite excreted/AUC of 1,1,1-trichloroethane) with and without Prior Ethanol Consumption

	AUC of 1,1,1-trichloroethane (µmol min/ml)		Total metabolite excreted (µmol)		Apparent Metabolic Clearance (ml/min)	
Subject	ETOH	No ETOH	ETOH	No ETOH	ETOH	No ETOH
1	2.94	2.80	50.63	40.82	17.24	14.59
2	2.71	3.05	47.86	39.51	17.68	12.94
3	3.41	1.75	87.22	36.38	25.55	20.74
4	2.60	2.19	46.39	43.60	17.81	19.87
5	2.19	2.14	59.04	45.82	26.90	21.45
6	1.37	0.57	25.35	10.04	18.48	17.71
7	0.78	1.45	35.42	39.99	45.26	27.53
8	2.40	2.12	51.60	38.41	21.52	18.09
9	2.58	2.96	56.49	51.77	21.88	17.52
10	2.64	2.50	39.19	25.02	14.84	10.02
Mean (standard deviation)	2.36 (0.76)	2.15 (0.76)	49.92 (16.56)	37.14 (11.75)	22.72 (8.79)	18.05 (4.89)
Mean difference	0.21		12.78		4.67	
Paired t test (p value)	0.34		0.023		0.018	

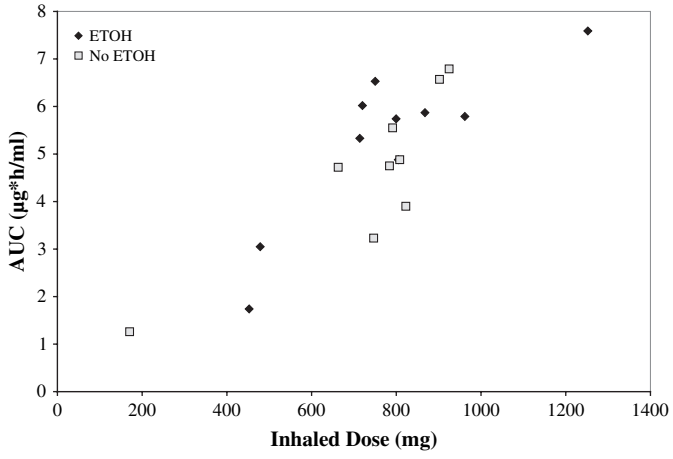

FIG. 3. Correlation between the AUC for 1,1,1-trichloroethane in blood and inhaled dose of 1,1,1-trichloroethane. With prior ethanol consumption: $r = 0.85$, $p = 0.002$. Without prior ethanol consumption: $r = 0.86$, $p = 0.003$.

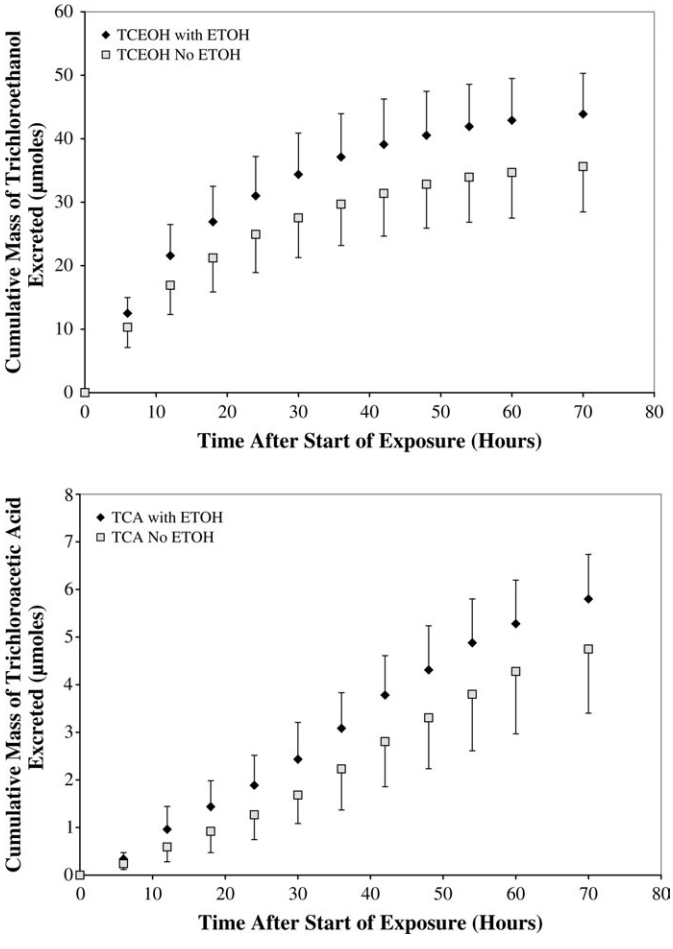

FIG. 4. Cumulative mass of TCEOH (upper) and TCA (lower) excreted over 70 h postexposure for exposures with and without prior ethanol consumption. Data represent mean interpolated values at specified times from all 10 subjects (error bars = 1 SD).

Comparison of control and ethanol treatments. The mean difference in apparent metabolic clearance of 1,1,1-trichloroethane following exposures with versus without prior ethanol consumption is shown in Table 3. These data, expressed in terms of percent increase in apparent metabolic clearance with prior ethanol versus without prior ethanol, are presented in Figure 5 (mean increase of 25.4%; paired t test, $p = 0.004$). With the exception of subjects 4 and 6, an increase of at least 18% was found in apparent metabolic clearance of 1,1,1-trichloroethane following exposures with prior ethanol.

Steady-State Model

Table 4 contains detailed results for the calculations used in the Andersen model, showing the values predicted for blood concentrations and rates of biotransformation for *m*-xylene (Tardif study) and 1,1,1-trichloroethane (present study). These were obtained from the solution of Equations 1–4 for the six conditions described. The model assumption of steady state is reasonable since in both the present study and that of Tardif, blood concentrations reached steady-state values early in the exposure. The model equations were solved at inhaled *m*-xylene concentrations of 100 and 400 ppm, the levels given by Tardif *et al.* in their study. Figure 6 shows the predicted steady-state relationship between inhaled and arterial solvent concentrations. In this and subsequent plots, C_{max} is defined as V_{max}/Q_h to simplify the legends. As pointed out by Andersen (1982), this ratio is the maximum concentration difference sustainable across the liver. In the native state (without induction) $C_{max} \approx 10K_m$ for xylene. The solid line in Figure 6 corresponds to the hypothetical condition of no biotransformation, where at steady state, arterial blood reaches equilibrium with the inhaled air. The slope of this line is equal to the blood-air partition coefficient. The two broken lines depict the relationship when biotransformation is normal and when it is elevated owing to induction. The vertical distance from the equilibrium line to either broken lines is proportional to the biotransformation rate

$$C_{\text{arterial}[0]} - C_{\text{arterial}} = \frac{\dot{m}N}{\dot{V}_{\text{alv}}}, \tag{5}$$

where $C_{\text{arterial}[0]}$ refers to the arterial blood concentration in the hypothetical condition of no biotransformation.

When the inhaled xylene concentration is 100 ppm (0.43 mg/l), the curves in Figure 6 for the native and induced states are nearly superimposed, which is consistent with the notion that hepatic clearance is limited by perfusion to the liver. In this circumstance, increasing the enzyme activity in the liver yields no measurable increase in metabolic clearance. At 400 ppm (1.72 mg/l) inhaled xylene concentration, the biotransformation rate approaches saturation, and metabolic clearance is limited to some degree by biochemical reaction in the liver, so induction yields a measurable increase in metabolic clearance. For our assigned values of the model parameters, metabolic clearance is predicted to increase with induction by 40% at 400 ppm.

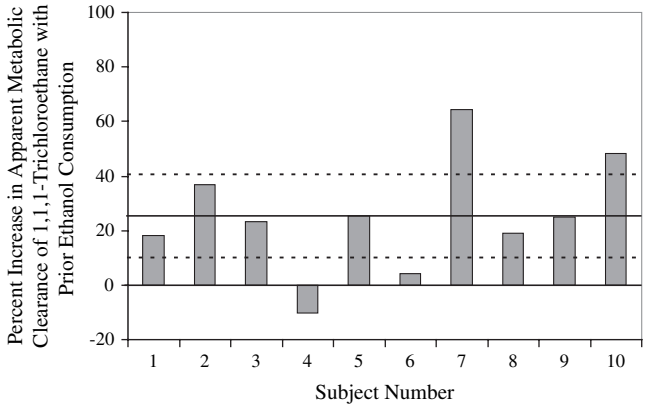

FIG. 5. Percent increase in apparent metabolic clearance of 1,1,1-trichloroethane with prior ethanol consumption for each subject. Mean increase shown (bold line) along with 95% CIs for the mean (dashed line).

The predicted dependence of the hepatic extraction ratio E_h on the arterial concentration is shown in Figure 7 for xylene. At arterial concentrations up to 2.0 mg/l, hepatic extraction is complete ($E_h = 1.0$), and no apparent effect of induction is predicted. With higher inhaled and arterial concentrations of xylene, the divergence of the curves between the native and induced states is evident, consistent with metabolic saturation and a transition to capacity-limited metabolism and a consequent measurable effect of induction on biotransformation rate. These predictions are in qualitative agreement with the results of Tardif *et al.*

For 1,1,1-trichloroethane, the modeled relationship between arterial and inhaled concentrations reveals a small predicted effect of induction (< 1% decrease in arterial concentration at steady state). Since metabolism is a minor route of elimination for this compound, this is not surprising. This change in steady-state clearance of parent compound from blood is not likely detectable. Figure 8 shows the predicted behavior of E_h with arterial concentration. The hepatic extraction ratio is low, but a clear elevation in E_h is predicted upon induction: at the arterial concentrations resulting from exposure to 175 ppm, E_h is predicted to increase by 42%. The observed production rate of metabolites in this study increased by a mean of 25%, similar to the predictions of the steady-state model.

TABLE 4
Model-Predicted Kinetic Outcomes at Steady State for Xylene and 1,1,1-Trichloroethane

Xylene (K_m = 0.2 mg/l)	$C_{inhaled}$ = 100 ppm (0.43 mg/l)	$C_{inhaled}$ = 400 ppm (1.72 mg/l)
Native state	(C_{max}/K_m = 10)	
N_{eff}	3.1	19
$C_{arterial}$	1.32 mg/l	33 mg/l
E_h	0.97	0.062
E_t	0.242	0.0155
$C_{hepatic\ vein}$	0.029 mg/l	31 mg/l
Biotransformation rate	116 mg/h	182 mg/h
Induced state	(C_{max}/K_m = 15)	
N_{eff}	3	14.2
$C_{arterial}$	1.29 mg/l	24.3 mg/l
E_h	1.00	0.126
E_t	0.25	0.031
$C_{hepatic\ vein}$	< 0.0001 mg/l	21.3 mg/l
Biotransformation rate	116 mg/h	270 mg/h
1,1,1-Trichloroethane (K_m = 6.0 mg/l)	$C_{inhaled}$ = 175 ppm (1.0 mg/l)	
Native state	(C_{max}/K_m = 0.017)	
N_{eff}	2.48	
$C_{arterial}$	2.48 mg/l	
E_h	0.012	
E_t	0.0029	
$C_{hepatic\ vein}$	2.45 mg/l	
Biotransformation rate	2.7 mg/h	
Induced state	(C_{max}/K_m = 0.025)	
N_{eff}	2.47	
$C_{arterial}$	2.47 mg/l	
E_h	0.017	
E_t	0.0043	
$C_{hepatic\ vein}$	2.43 mg/l	
Biotransformation rate	3.8 mg/h	

DISCUSSION

Understanding the effect of ethanol consumption on the biotransformation of solvents is important for workers exposed to a range of industrial solvents. When the parent compound itself is toxic, an inductive effect of ethanol consumption on solvent biotransformation might be considered beneficial. However, in many instances, the metabolites of a chemical are more toxic than the chemical itself. In such cases, an increase in the rate of biotransformation of a given chemical could have significant health implications for exposed individuals. Even brief increases in acute exposures to certain chemical metabolites can increase an individual's risk of acute or chronic illness or injury (Klotz and Ammon, 1998).

To our knowledge, this is only the third published study to assess the inductive effect of ethanol consumption on solvent biotransformation in a controlled setting. There are not likely many chemical solvents used today with lower intrinsic clearance than 1,1,1-trichloroethane. Therefore, a finding of no effect of ethanol on the rate of biotransformation of 1,1,1-trichloroethane would have provided evidence that moderate consumption of ethanol over time is unlikely to influence the rate of biotransformation of any chemical solvent in the body resulting from low-level exposures. In contrast, empirical evidence is presented here that moderate levels of ethanol

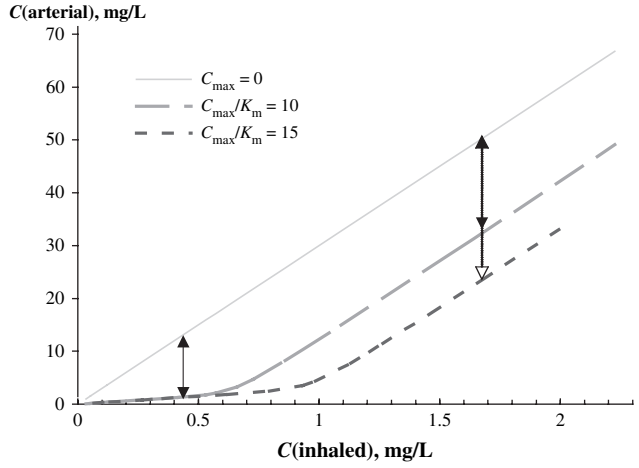

FIG. 6. Modeled relationship between arterial blood and inhaled concentrations for xylene at steady state. The unbroken line shows the result with complete inhibition of biotransformation; the two broken lines show the prediction for native state ($C_{max}/K_m = 10$) and ethanol-induced ($C_{max}/K_m = 15$) biotransformation. The vertical arrows show the effect of biotransformation at two inhaled concentrations: 100 (0.43 mg/l) and 400 ppm (1.72 mg/l); the vertical distance is proportional to the biotransformation rate. See text for details.

consumption can affect the rate of biotransformation of a solvent in humans.

This analysis suggests that for substrates whose metabolic clearance is perfusion limited in the native state, induction by realistic doses of ethanol will result in very small changes in the production of metabolites. As seen for xylene, this will be the case at air concentrations of 100 ppm or less. At higher air concentrations, biotransformation of xylene becomes saturated, and metabolic clearance shifts from being perfusion rate

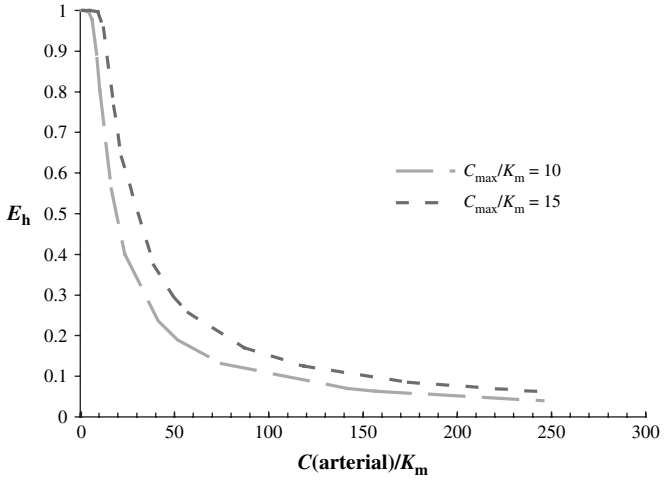

FIG. 7. Predicted hepatic extraction ratio E_h for xylene at steady state. Native state and induced state are compared. At 100 ppm inhaled concentration, $C_{arterial}/K_m = 6.6$ in the native state and 6.4 in the induced state. There is no measurable difference in E_h at this level of exposure. At 400 ppm inhaled concentration, $C_{arterial}/K_m = 165$ in the native state and 122 in the induced state, and a measurable difference is predicted.

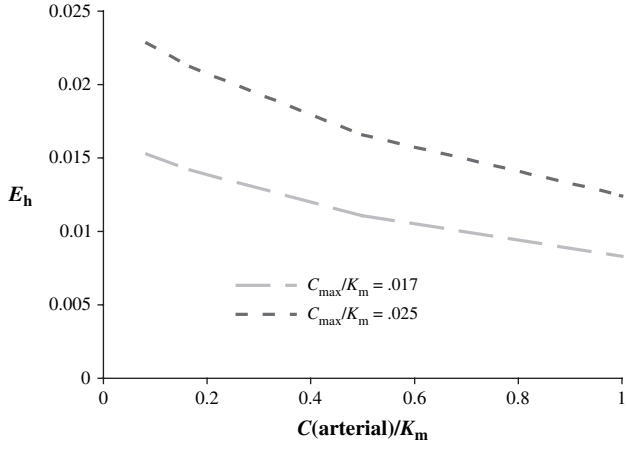

FIG. 8. Predicted hepatic extraction ratio E_h for 1,1,1-trichloroethane at steady state. Native and induced states are compared. At 175 ppm inhaled concentration, $C_{arterial}/K_m = 0.41$ in the native state and 0.42 in the induced state. There is a substantial difference in hepatic extraction ratio, leading to a measurable difference in formation rate of metabolites.

limited to capacity limited; as a result, the effects of metabolic induction become apparent. In contrast, for substrates whose metabolic clearance rates are capacity limited in the native state, realistic doses of ethanol should yield significant increases in production of metabolites. 1,1,1-Trichloroethane appears to meet this condition in humans, at inhaled concentrations of 175 ppm or greater and most likely at lower concentrations as well.

The increase in metabolic clearance of a solvent following regular ethanol consumption as observed in the current study (25%) could have significant implications for an individual's risk of toxicity. For 3 of the 10 subjects, this increase was greater than 37%. For a metabolite whose toxicity exhibits a clear dose-response relationship, any change in its production would directly affect the risk of an adverse health outcome for a given individual. For metabolites exhibiting a threshold, increasing their rate of formation would elevate the effective dose, potentially to a level above this threshold. The increase in the rate of biotransformation with prior ethanol consumption may also increase the between-individual variation in metabolite formation. However, it remains to be seen whether the magnitude of this change is sufficient to affect the ability to assess exposure and risk within a population. A number of factors may affect the interindividual variation in activity of CYP2E1, including drug use, diet, ethanol consumption, obesity, fasting, and genetic polymorphisms (McCarver *et al.*, 1998; Oneta *et al.*, 2002). In the current study, the interindividual variation in apparent metabolic clearance for exposures with and without prior ethanol consumption (expressed as the coefficient of variation) was 38.7 and 27.1%, respectively. Another recent study with human subjects observed interindividual coefficients of variation in noninduced CYP2E1 activities (assessed using the rate of biotransformation of administered chlorzoxazone) of approximately 40% (Ernstgard

et al., 2004). While confirming data are needed, the added variability arising from ethanol consumption may have an important impact on the interpretation of available measures (biomarkers) used in assessing exposure or risk in a population.

In summary, although limited by a relatively small sample size, this study found that moderate doses of ethanol over 1 week significantly increase the rate of biotransformation of 1,1,1-trichloroethane after a single exposure to the solvent in human volunteers. We may reasonably expect that chemicals that are substrates of CYP2E1 and that have toxicokinetic properties comparable to those of 1,1,1-trichloroethane would be affected by ethanol in a similar fashion. Carbon tetrachloride and *n*-hexane, with low to medium hepatic extraction ratios, are two such chemicals. The metabolisms of carbon tetrachloride, with an estimated intrinsic clearance of 50.8 l/h (Paustenbach *et al.*, 1988), and *n*-hexane, with an estimated intrinsic clearance of 81 l/h (Ali and Tardif, 1999; Hamelin *et al.*, 2005), are likely slow enough to be affected by ethanol consumption at relatively low exposure concentrations. This would certainly be of concern, as the metabolites of these chemicals are more toxic than the parent compounds (Recknagel *et al.*, 1974; Iba *et al.*, 2000).

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