Trichloroethylene (TRI) and tetrachloroethylene (TETRA) are solvents that have been widely used in a variety of industries, and both are widespread environmental contaminants. In order to provide a better basis for understanding their toxicokinetics at environmental exposures, seven human volunteers were exposed by inhalation to 1 ppm of TRI or TETRA for 6 h, with biological samples collected for analysis during exposure and up to 6-days postexposure. Concentrations of TRI, TETRA, free trichloroethanol (TCOH), total TCOH (free TCOH plus glucuronidated TCOH), and trichloroacetic acid (TCA) were determined in blood and urine; TRI and TETRA concentrations were measured in alveolar breath. Toxicokinetic time courses and empirical analyses of classical toxicokinetic parameters were compared with those reported in previous human volunteer studies, most of which involved exposures that were at least 10-fold higher. Qualitatively, TRI and TETRA toxicokinetics were consistent with previous human studies. Quantitatively, alveolar retention and clearance by exhalation were similar to those found previously but blood and urine data suggest a number of possible toxicokinetic differences. For TRI, data from the current study support lower apparent blood-air partition coefficients, greater apparent metabolic clearance, less TCA production, and greater glucuronidation of TCOH as compared to previous studies. For TETRA, the current data suggest TCA formation that is similar or slightly lower than that of previous studies. Variability and uncertainty in empirical estimates of total TETRA metabolism are substantial, with confidence intervals among different studies substantially overlapping. Relative contributions to observed differences from concentration-dependent toxicokinetics and individual and interoccasion variability remain to be determined.

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Certification: The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration.

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conjugation (Bull, 2000; Caldwell and Keshava, 2006; Lash and Parker, 2001; Lash et al., 2000b).

Simplified metabolism schemes for TRI and TETRA are shown in Figure 1. Briefly, as reviewed by Lash et al. (2000a) and Chiu et al. (2006), metabolism of TRI occurs through two main irreversible pathways: oxidation via the microsomal mixed-function oxidase system (i.e., cytochrome P450s) to chloral (metabolite 3) and/or TRI-oxide (metabolite 4) and conjugation with GSH by glutathione S-transferases to S-dichlorovinyl glutathione (DCVG, metabolite 5). For TRI oxidation, CYP2E1 is thought to be most important in vivo. Chloral, which forms chloral hydrate (metabolite 6) in the presence of water, is rapidly metabolized to TCOH (metabolite 7) (a reversible reaction) and TCA (metabolite 8). It is also known that TCOH is glucuronidated to form TCOH-glucuronide (TCOG) (metabolite 9), TCOG undergoes enterohepatic recirculation (excretion in bile with regeneration and reabsorption of TCOH from the gut), and that TCA and TCOG are excreted in urine. However, further other metabolisms of TCA and TCOH have not been well characterized, though DCA (metabolite 10) has been hypothesized to be among the metabolism products (Lash et al., 2000a). TRI-oxide can also form DCA, among other species (Cai and Guengerich, 1999). With respect to the conjugative pathway, DCVG is further processed to form the cysteine conjugate S-dichlorovinyl-L-cysteine (metabolite 11), which can either undergo (reversible) N-acetylation with excretion of mercapturates (metabolite 12) in urine (Bernauer et al., 1996; Birner et al., 1993) or bioactivation by beta-lyase or flavin-containing monoxygenases (FMO) to reactive species (Anders et al., 1988; Krause et al., 2003; Lash et al., 2003).

TETRA metabolism, while quantitatively less than TRI metabolism, is qualitatively similar in that TETRA is metabolized by oxidation by P450s (presumably also involving be CYP2E1) and by conjugation with GSH. For oxidation, it is hypothesized that an unstable epoxide intermediate TETRA-oxide (metabolite 13) is formed that has numerous possible fates, including oxalate dichloride (metabolite 14), trichloroacetyl chloride (metabolite 15), trichloroacetyl aminoethanol (metabolite 16), and perhaps chloral (Lash and Parker, 2001). Both trichloroacetyl chloride and chloral can lead to TCA formation, which is excreted in urine, although the pathway through trichloroacetyl chloride appears to predominate for TETRA. As with TRI, further metabolism of TETRA oxidation products has not been well characterized. GSH conjugation of

FIG. 1. Simplified TRI and TETRA metabolism schemes. 1, TRI; 2, TETRA; 3, chloral; 4, TRI-oxide; 5, DCVG; 6, chloral hydrate; 7, TCOH; 8, TCA; 9, TCOH glucuronide; 10, DCA; 11, dichlorovinyl cysteine; 12, N-acetyl dichlorovinyl cysteine; 13, TETRA-oxide; 14, oxalate dichloride; 15, trichloroacetyl chloride; 16, trichloroacetyl aminoethanol; 17, TCVG; 18, trichlorovinyl cysteine; 19, N-acetyl trichlorovinyl cysteine.
TETRA yields \( S \)-trichlorovinyl glutathione (TCVG) (metabolite 17), which, like DCVG, is further processed to a cysteine conjugate \( S \)-trichlorovinyl-L-cysteine (TCVC) (metabolite 18). TCVC then may undergo (reversible) N-acetylation with excretion of mercapturates in urine (metabolite 19) or bioactivation by beta-lyase or FMO to reactive species (Lash and Parker, 2001).

Previous human studies of TRI and TETRA toxicokinetics have been limited in two ways. First, with two exceptions, the data from human toxicokinetic studies have been at occupational exposure levels, typically greater than or equal to 40 ppm in air. As shown by Bois (2000), Bois et al. (1996), and Chiu and Bois (2006), extrapolation of such data to environmental exposure levels can introduce uncertainty spanning an order of magnitude or more. Second, the only studies including exposures < 40 ppm have collected very limited time-course data: TCA in blood and urine in Völkel et al. (1998) for TETRA exposures and alveolar air in Opdam (1989a,b) for TRI and TETRA exposures.

The experiments reported here study the human toxicokinetics of TRI, TETRA, and their major oxidative metabolites at 1-ppm exposures. This was the lowest exposure for which available analytic methods had sufficient sensitivity, and were an order of magnitude or more lower than all previous studies except Opdam (1989a,b). This paper first estimates empirical (classical) toxicokinetic parameters such as retention, peak and integrated concentrations, excretion, and recovery in human subjects exposed at 1 ppm. It then compares time courses and empirical toxicokinetic parameters with those from previously reported human studies, adjusted for exposure-concentration or cumulative exposure as appropriate.

### MATERIALS AND METHODS

#### Subjects

Seven adult male volunteers were recruited for this study. Prior to the study, they underwent a medical examination to check for absence of lung dysfunction or other disease and use of medicine. All volunteers were informed of the potential effects of the exposure to TRI and TETRA and of the purpose of this study before they gave written informed consent, and were free to withdraw from the study at any time. The study design had been reviewed and accepted by the Medical Ethical Committee of the Academic Medical Centre, Amsterdam, The Netherlands. Measured characteristics of the volunteers (labeled A to G) are included in Supplementary Data (age, height, weight, pulmonary ventilation, and percent body fat). The volunteers were asked not to consume alcohol during the first 24-h postexposure and not to have too vigorous physical activity during the first 3 postexposure days. After this, volunteers followed their normal daily life style.

#### Exposure Conditions and Experimental Design

The exposure was conducted in an open exposure chamber of \( 2 \times 2 \times 2 \) m with an air turnover rate of eight times per hour. The temperature was 22–25°C. Fresh air was distributed into the chamber through a perforated mixing box, resulting in a homogeneous concentration within the exposure chamber. A slight underpressure in the exposure chamber was generated in order to minimize leakage of solvent vapor into the surrounding laboratory. TRI vapor or TETRA vapor was generated by bubbling at 20°C a constant flow of medical air through the solvent in two glass bottles connected in series. The solvent vapor was supplied at the fresh-air side of the ventilation system.

Each exposure lasted 6 h. Before their entry into the chamber, the subjects were instructed to empty their bladder. They remained seated during the entire exposure time and did not smoke during exposure. At each of four separate occasions, subjects were exposed in two separate groups 2 days apart, as summarized in Table 1. Occasions were separated by 4 or 5 weeks, with TETRA exposures on the first two occasions and TRI exposures on the last two. Alveolar air, blood, and urine samples were also collected just prior to each exposure; except for TCA, all levels were at or below detection limits and consistent with no significant background exposure to TRI or TETRA. Preexposure TCA levels in blood and urine had mean \( \pm SD \) of 2 \( \pm 1 \) \( \mu g/l \) and 1 \( \pm 1 \) \( \mu g/l \), respectively, prior to TETRA exposures and <0.5 \( \mu g/l \) and 1 \( \pm 1 \) \( \mu g/l \), respectively, prior to TRI exposures.

Volunteer exposures and air sample analyses were carried out in the Academic Medical Center of Amsterdam; blood and urine samples were analyzed in INERIS (Verneuil-en-Halatte, France).

#### Sampling and Analysis

**Chamber air.** Inhalation chamber air samples were collected throughout the exposure period (at least 15 samples per 6-h period) by aspiration through a glass tube and analyzed as described below for alveolar air. TETRA concentrations in the exposure chamber were on average 1.01 ppm, with fluctuations during each experiment remaining within ± 0.35 ppm of the average. The time-weighted-average (TWA) exposure concentration for each occasion ranged from 0.96 to 1.14 ppm. TRI concentrations in the exposure chamber were on average 1.20 ppm. Except for one experiment in which the inlet valve was accidentally disconnected (resulting, over the course of about 90 min, in a drop in concentration and then an overshoot after the valve was reconnected), fluctuations during each experiment remained within ± 0.3 ppm of the average. The TWA exposure concentration for each occasion ranged from 1.13 to 1.26 ppm.

**Alveolar air sampling.** After 5 s of breath-holding the volunteers exhaled through a glass tube of 70 ml. At the end of breath expiration the glass tube was closed (so that only the last 70 ml [alveolar air] was collected) with a screw cap with a predrilled hole fitted with a Teflon liner and a silicon rubber. The alveolar air samples were collected in duplicate every 20 min during exposure and at shorter time intervals, especially just after exposure. From 6 h after exposure, alveolar air samples were collected for 6 days, three times a day, in duplicate. The TRI and TETRA concentrations in alveolar air samples were stable for at least 5 days at room temperature. Analysis of duplicate samples found coefficients of variation (CV) of 5–15%.

The concentration of TETRA in air was determined on a gas chromatograph (Carlo Erba HR 5300 Mega) equipped with a CP SIL 13 CB column (25 m, internal diameter [id] 0.25 mm) isothermal at 120°C and an electron capture

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detector (ECD) (63Ni). Calibration concentrations were made by evaporation of known amounts of TETRA in 200-l Tedlar airbags and with a standard generator, model 350 (Analytical Instruments Development Inc., Avondale, PA) equipped with capillary diffusion tubes at constant temperature. By means of dilution with an air flow, all desired concentrations over a certain range could be obtained. The injection volume (1 ml) of the samples was decreased (to 250 µl) toward the high concentrations in order to stay within the linear range of the ECD. The detection limit was 0.0005 ppm.

The concentrations of TRI in air were determined on a gas chromatograph (Hewlett Packard 5890 A) with an ECD (63Ni). Due to low TRI concentrations, these analyses were done with more sensitive equipment than were those for TETRA. The ECD was equipped with a CP SIIL 19 CB column (25 m, id 0.35 mm) isotherm at 65°C. Calibration concentrations were made by evaporation of known amounts of TRI in 200-l Tedlar airbags using the same techniques as for TETRA. The injection volume (1 ml) of the samples was decreased (to 250 µl) toward the high concentrations in order to stay within the linear range of the ECD. The detection limit was ≤ 0.0001 ppm.

Venous blood. Venous blood samples were collected in duplicate in 7-ml lithium-heparinized tubes four times during exposure (1, 2, 4, and 5.5 h after the beginning of exposure), at 6:10, 7, and 8 h after the beginning of exposure, and once daily for the 5 following days. Collected blood was then stored in triplicate (at least) in glass vials (2 ml each), which were immediately closed with a Teflon cap and stored at −20°C until analyzed.

TETRA and TCA were quantitated simultaneously in blood by automated headspace gas chromatography (HSGC) according to the method of Christensen et al. (1988). TCA was analyzed as volatile chloroform, which was produced by decarboxylation under heating. A Varian 3600 gas chromatograph with an ECD (63Ni) and data system GC STAR was used. Automatic headspace sampling was performed using the VARIAN Genesis sampling unit. The chromatograph was equipped with a SUPELCO 30 m by 0.53 mm SPB1 column with 5.0-μm film thickness. The temperature in the oven varied as follows: isotherm at 40°C for 15 min, then raised in 10°C/min increments to 250°C, and finally held at 250°C for 10 min. The carrier gas was nitrogen at 5 ml/min. The detector temperature was 300°C and the flow rate of make-up gas was of 30 ml/min.

For simultaneous determination of TETRA and TCA, 2 ml of blood was introduced into a 9-ml glass vial containing 2 ml of saturated diammonium sulfate solution (60 g for 100 ml of water) and 20 µl of the internal standard (bromoform). The glass vials were closed with a Teflon line rubber septum and incubated at 90°C for 80 min before HSGC analysis. The injection headspace gas volume was 1 ml. The quantitation was based on a calibration curve built using known concentrations, and performed over the range 0.5–10 µg/l, with a detection limit of 0.5 µg/l.

The sensitive HSGC method using automated sampling, capillary gas chromatography, and electron capture detection proposed by Christensen et al. (1988) was used for simultaneous determination of TRI, TCA, TCOH, and the glucuronic acid conjugate of TCOH (TCOG) produced after TRI exposures. TCA was analyzed as volatile chloroform, which was produced by decarboxylation under heating. Actual amounts of TCOH reported are free (TCOH) only and total (TCOH and TCOG combined), with TCOG hydrolyzed to TCOH. Other materials and techniques were the same as presented above for TETRA exposures.

Urine. Every spontaneous urinary micturition was collected on the day of exposure and the following day; for the next 5 days, two spontaneous micturitions were collected daily (typically the first in the morning and the last in the evening). Urine micturitions were collected in 700-ml bottles (at home), stored at 0–6°C (refrigerator), and carried to the laboratory. For each micturition, total weight of urine, pH, and density were measured. Collected urines samples were then stored in small glass containers closed with a top inside of which an aluminum sheet was inserted. These urine samples were frozen and stored at −20°C until analyzed.

The analytical conditions for the quantitation of metabolites in urine were the same as those described for venous blood except for sample preparation: 5 ml of urine was introduced into a 9-ml glass vial with 20 µl of the internal standard (bromoform). Quantitation was also performed over the range 0.5–10 µg/l; the detection limit was 1 µg/l.

Toxicokinetic Calculations and Comparisons

All calculations were performed in either the R statistical package version 1.9.0 or Microsoft Excel version 2003.

Data were first analyzed to calculate empirical toxicokinetic measurements. Time points where duplicate measurements were taken were averaged before analysis. For each individual and exposure occasion, area-under-the-curve (AUC) determinations were calculated at each time point and the maximum concentration for all metabolites analyzed were used for the calculation of the area under the curve. The maximum concentration for each metabolite was used for the calculation of the area under the curve.

Cumulative urinary excretion of free TCOH, total TCOH, and TCA was calculated by integrating estimates of the rate of urinary excretion for each metabolite. Specifically, the amount of metabolite excreted in a particular micturition was calculated by the formula $\Delta Q = C \times M/I$, where $C$ is the concentration in the sample, $M$ is the urine mass, and $D$ is the urine density. About 13% of the total TCOH and 60% of the TCA urine measurements from TRI exposures were saturated (no urine measurements from TETRA exposures were saturated). Unfortunately, because of the length of time between when the measurements were done and the analysis of the data, the remaining samples have since been destroyed. While dropping saturated measurements of total TCOH would have had a minimal effect on estimated urinary recovery, dropping saturated measurements of TCA would have left very sparse data for some individuals. Therefore, it was decided to use all saturated measurements without adjustment, understanding that this may underestimate urinary recovery, particularly for TCA. Values below the detection limit were treated as zero. The rates of excretion were calculated as $\Delta Q/\Delta t$, where $\Delta t$ is the time between the current micturition (being analyzed) and the previous one, and this rate was assumed to be constant during that time interval. For the first 2 days, when in general all spontaneous urine micturitions were collected, these rates could be calculated for all time intervals. For the remaining period of follow-up, they could be calculated only for the period between consecutively recorded micturitions. The rates of excretion for other time periods (i.e., when times of the current or previous micturition were missing) were estimated by linear interpolation. The resulting estimate for the urinary excretion rate as a function of time was integrated numerically to estimate cumulative excretion as a function of time.

One-way analysis of variance (ANOVA) was conducted on selected toxicokinetic measures (normalized by the occasion-specific exposure concentration where appropriate) to assess the relative contributions of interindividual and other sources (experimental, interoccasion) of variance. Because of the low number of occasions (one or two per individual), the power of this test is low; at 10% significance, < 50% power to detect equal contributions of interindividual and other variance.

The results of these data analyses were used to derive the following toxicokinetic parameters for comparison to other studies: alveolar retention ($R_{alv}$), lung clearance (LC), fraction of intake exhaled ($F_{exh}$), apparent metabolic clearance (CLmet), and estimated blood-air partition coefficient (PBA). A detailed description of the equations used in these derivations is provided in Supplementary Data. All calculations were performed separately for each individual and occasion.

The following previous human toxicokinetic studies of inhaled TRI and TETRA were used for comparison: Fisher et al. (1998), Kimmerle and Eben.
Variability in empirical toxicokinetic parameters was observed among different individuals and occasions, with the largest CV in free and total TCOH concentrations (see Supplemental Tables S-2 and S-4). Results of ANOVA indicate significant (p value < 0.1) interindividual differences in the peak TRI alveolar air concentration, the terminal half-life in alveolar air, the peak and AUC during exposure of TRI in venous blood, and the peak and AUC of total TCOH in venous blood. However, as noted in Methods, the ANOVA test for this dataset has low power to distinguish interindividual variance from other sources of variance.

Comparing TRI Toxicokinetics across Studies

TRI retention, LC, and postexposure disposition by exhalation were within the ranges of variability seen across previous studies, as shown in Table 2 (individual results for the current study are in Supplemental Table S-5). Using the literature-derived in vitro value for PB, the implied CLmet of 1.0 l/min was also consistent with previous studies. However, PBest estimated using the ratio of measured venous blood to air concentrations of 4.4 ± 0.6 during exposure was substantially lower than the mean ± SD of 9.4 ± 1.8 (range, 6.5–12.1) from pooling five published in vitro studies (Fiserova-Bergerova et al., 1984; Fisher et al., 1998; Gargas et al., 1989; Sato and Nakajima, 1979; Sato et al., 1977). Note that the postexposure estimates, while higher than the during exposure estimate, are more uncertain because they are dominated by scatter in the venous blood data due to the relatively sparse collection and low concentrations. In addition, although this estimate is based on an assumed ratio Qalv/Qcard = 1, increasing that ratio to 1.4 would only increase PBest during exposure to 5.5 ± 0.7. The PBs derived from the comparison studies were generally consistent with, though a little higher than, the in vitro value. The CLmet derived using PBest was about two-fold greater than previous studies, and was greater than a typical hepatic blood flow rate of 1.3 l/min (26% of cardiac output of 5 l/min).

Blood time courses of TRI, TCA, total TCOH, and free TCOH during the exposure period are compared in Figure 2. Concentrations of TRI in venous blood, adjusted for exposure concentration (upper left panel Fig. 2), were found to be on average two- to 11-fold less than in previous studies. Average ratios of TRI venous blood to inhaled air concentrations at 4 h were 0.61, 1.2 to 1.3 in Kimmeler and Eben (1973), 3.9 in Monster et al. (1976), and 3.4 to 7.0 in Fisher et al. (1998). Note that it is not clear from the published study if the value from Kimmeler and Eben (1973) was taken just prior or just after the end of exposure, during which blood concentrations would be expected to be rapidly declining. At 6 h, the ratio was 0.75 here and 2.5 in Laparé et al. (1995). Exposure-adjusted concentrations of TCA in venous blood (upper right panel Fig. 2) were lower by two- to four-fold as compared to previous studies. At 4 h, the ratio of TCA venous blood to TRI inhaled air concentrations averaged 2.0 here (not including one outlier measurement at 15.9),
whereas previous studies found average ratios of 4.6 to 6.5 (Fisher et al., 1998) and 4.3 to 7.1 (Monster et al., 1976). By contrast total TCOH concentrations adjusted for exposure concentration were completely consistent with those previously reported, as shown in the bottom left panel of Figure 2. Taken relative to TRI blood concentrations, concentrations of both TCA and total TCOH were at least two-fold higher in this study than previously reported. However, as mentioned previously, a substantial amount of circulating total TCOH in the subjects examined here appears to be as TCOG, in contrast to Fisher et al. (1998), who reported TCOG levels to be below detection limits. Therefore, free TCOH concentrations, shown in the bottom right panel of Figure 2, were lower on an exposure-adjusted basis than those found previously (but were still higher by about two-fold when taken relative to TRI blood concentrations).

As shown in Figure 3, the overall kinetics of TCA were qualitatively consistent with those in previous studies: the formation of TCA was delayed, with peaks generally occurring during the second or third day after exposure, and a half-life longer than could be reliably measured empirically given the short collection period. In the individuals who were studied on multiple occasions, TCA levels had returned to background levels at 1 month after exposure, which is consistent with previously reported half-lives of 70–100 h (Monster et al., 1976, 1979; Paykoc and Powell, 1945). However, the venous blood concentrations of TCA adjusted for cumulative exposure were lower in the subjects here than in those from previous studies. Table 4 compares peak concentrations of TCA across studies, showing lower peak concentrations by at least two-fold. The excretion of TCA in urine also was less than previous studies, shown in the left panel of Figure 4. However, many measurements of urinary TCA following TRI exposures were saturated, and may have underestimated the actual concentrations those times.

Urinary excretion of total TCOH, shown in the right panel of Figure 4, was overall quite consistent with previous studies, with the exception of that reported by Laparé et al. (1995), which was quite low by comparison. However, Laparé et al. (1995) only reported TCOH excretion at 24 h, so overall cumulative excretion cannot be compared.

Total recovery was slightly less than that in Monster et al. (1976), reflecting the smaller amount of TCOH and TCA recovered in urine but slightly more appearing in exhaled air. In both studies, a substantial fraction of intake (30–40%) was unaccounted for.

**TETRA Toxicokinetics at 1 ppm**

During and following TETRA exposures, TETRA was readily detected in alveolar air and venous blood, and TCA was found in venous blood and urine (see Supplemental Figs. S-9 to S-12 for individual data). Peak levels of TETRA in venous blood and air occurred near the end of exposure and declined thereafter but not as rapidly as for TRI. This was to be expected, given the higher lipid solubility of TETRA, which has a reported human fat-air partition coefficient of 1450 (Gearhart et al., 1993), versus that of TRI, reported to range from 583 to 674 (Fiserova-Bergerova et al., 1977). Blood levels of TCA peaked well after the end of exposure, and were still substantially above background at the end of the reporting period (~5 days). As with TRI, TCA venous blood levels returned to background after 1 month (the time between exposure occasions).

Recovery was primarily through exhaled air, with a small amount (< 1% of intake) of TCA undergoing urinary excretion. Total recovery of exhaled TETRA and urinary TCA at the end of the reporting period accounted for 64–94% of intake, with an additional 0–10% estimated to be exhaled as TETRA after the reporting period. The remaining TCA to be excreted in urine could not be calculated confidently due to its long half-life.

Variability in toxicokinetic parameters was observed among different individuals and occasions, with the largest CV in TCA...
Results of ANOVA indicate significant ($p$ value < 0.1) interindividual differences in AUC during exposure of TETRA in venous blood, the peak and AUC of TCA in venous blood, and the amount of TCA in recovered urine. However, as noted in Methods, the ANOVA test for this dataset has low power to distinguish interindividual variance from other sources of variance.

Comparing TETRA Toxicokinetics across Studies

TETRA retention, LC, and postexposure disposition by exhalation were within the ranges of variability seen across previous studies, as shown in Table 3 (individual results for the current study are in Supplemental Table S-6). Although $\text{CL}_{\text{met}}$ differed by up to five-fold, the range of variability was quite high. PB estimated by TETRA venous blood and air concentrations during exposure of 9.4 ± 1.4 were consistent with the mean ± SD of 11.4 ± 2.0 (ranges not reported) from pooling three in vitro studies (Gargas et al., 1989; Gearhart et al., 1993; Sato et al., 1977). Using either estimate of PB yields similar estimates of $\text{CL}_{\text{met}}$. Estimates of PB based on postexposure data tended to be higher than in vitro values, but as was noted with TRI, these values are more uncertain because they are dominated by scatter in the venous blood data due to the relatively sparse collection and low concentrations.

Blood time courses of TETRA and TCA during the exposure period are compared in Figure 5. Concentrations of TETRA in venous blood, adjusted for exposure concentration ($\mu$M in blood/$\mu$M in inhaled air) (left panel Fig. 5), were found to be on average about two-fold less than Monster et al. (1979). Exposure-adjusted concentrations of TCA in venous blood (right panel Fig. 5) were consistent with previous studies, with values very similar to those in Monster et al. (1979).
et al. (1979) and within the range of variability of those from Volkel et al. (1998).

As shown in Figure 6, the overall kinetics of TCA post-exposure were qualitatively consistent with those in previous studies of both TRI and TETRA. The postexposure venous blood concentrations of TCA, adjusted for cumulative exposure, were consistent with previous studies. As shown in Table 4, peak concentrations of TCA adjusted for cumulative exposure were consistent across studies. As shown in Figure 7, however, the excretion of TCA in urine was four- to five-fold less than that from Monster et al. (1979) and two- to three-fold less than that from Volkel et al. (1998). Note that unlike with TRI, no urinary TCA measurements from TETRA exposures were saturated.

Total recovery was slightly less than that in Monster et al. (1979), primarily reflecting the smaller amount appearing in exhaled air but consistent with the previously observed range of variability. However, because recovery is primarily through exhalation, it is sensitive to uncertainties in minute-volume (MV) in the postexposure period. For instance, increasing the postexposure MV to 10 l/min (probably an overestimate since subjects were asked to limit their activity for 3-days postexposure) would increase the average total recovery to > 100%.

DISCUSSION
Sources of Variance

For both the TRI and TETRA experiments, the extent of interoccasion variability was high, so that interindividual differences could only be statistically separated for a few toxicokinetic measures. High interoccasion variability could explain why, in the Fisher et al. (1998) study of TRI, several of the subjects exposed to both 50 and 100 ppm did not exhibit dose-related increases in TCA excretion. Significant interoccasion variation on top of interindividual variability further complicates the determination of whether differences between the current study and previous results are related to exposure concentration. It also raises questions as to how to extrapolate results from short-term exposures of a small group of volunteers to chronic,

FIG. 3. Comparison mean ± SD venous blood concentrations of TCA following TRI exposure, adjusted for cumulative exposure (μM in blood/(μM·h) in inhaled air). Thick and thin solid indicate the mean ± SD over all individuals and occasions in the current study. Due to small variations in collection time, data from the current study were binned to the nearest hour (t < 24 h) or nearest 6 h (t > 24 h) prior to taking the mean and SD for clarity of display, with horizontal error bar showing the range of times included in each data point. Other studies and symbols: Fisher et al. (1998) (× = females 50 ppm, ◊ = males 50 ppm, ▽ = females 100 ppm, ◐ = males 100 ppm); Monster et al. (1976) (● = 70 ppm, ▲ = 140 ppm).

FIG. 4. Comparison of mean ± SD cumulative urinary excretion of TCA and total TCOH from different TRI studies. The amount excreted is adjusted for cumulative exposure (μmol in urine/(μM·h)) in inhaled air. Thick and thin solid lines indicate the mean ± SD over all individuals and occasions in the current study. Due to small variations in collection time, data from the current study were binned to the nearest hour (t < 24 h) or nearest 6 h (t > 24 h) prior to taking the mean and SD for clarity of display, with horizontal error bar showing the range of times included in each data point. Other studies and symbols: Laparé et al. (1995) (□); Kimmerle and Eben (1973) (○ = females, △ = males); Fisher et al. (1998) (× = females 50 ppm, ◊ = males 50 ppm, ▽ = females 100 ppm, ◐ = males 100 ppm); Monster et al. (1976) (● = 70 ppm, ▲ = 140 ppm).
long-term exposures in a diverse population. Using a limited number of subjects (even after pooling across studies) that are relatively homogeneous in terms of age, health status, and ethnic background may lead to underestimation of true population variability. However, if interoccasion variability is high and not accounted for, the inferred interindividual variability could be overestimated.

**TRI and its Oxidative Metabolites**

Although TRI alveolar air concentrations relative to exposure appear consistent with previous studies, as measured by retention and recovery postexposure, exposure-adjusted TRI venous blood concentrations appear to be lower. Our empirical analysis suggests two (not mutually exclusive) hypotheses to explain the low TRI blood concentrations: an anomalously low PB and a large extraction of TRI by tissues during exposure, either through metabolism or storage.

With respect to PB, the result may simply be due to interindividual variation—Fisher et al. (1998) measured TRI PB in 13 subjects and obtained values ranging from 6.47 to 12.1, so the values of 4.4 ± 0.6 derived above may not be entirely unrealistic. Unfortunately, no blood samples remain with which to validate this hypothesis. It should be noted, however, that neither a wash-in/wash-out effect nor pulmonary metabolism can explain the anomalously low values, and either would in fact further decrease estimates of PB. (See Supplementary Data for relevant equations. In the first case, the effective value of $Q_{alv}$ would be reduced $[Q_{alv} \rightarrow Q_{alv}/(1 + H)$, where $H$ is the “resistance” term], so that the right side of equation (S-16) is reduced. For metabolism in the transitional airways, both the value of $C_{inh}$ in the alveolae decreases and that of $C_{alv}$ exhaled

![Graph](image-url)

**FIG. 5.** Comparison of mean ± SD venous blood concentrations of TETRA and TCA, during TETRA exposure across studies. All blood concentrations are adjusted for exposure concentration ($\mu$M in blood/µM in inhaled air). Thick and thin solid lines indicate the mean ± SD over all individuals and occasions in the current study. Due to small variations in collection time, data from the current study were binned to the nearest half-hour prior to taking the mean and SD for clarity of display, with horizontal error bar showing the range of times included in each data point. Other studies and symbols: Vökel et al. (1998) ($\square$ = 10 ppm, $\triangle$ = 40 ppm); Monster et al. (1979) (● = 70 ppm, ▲ = 140 ppm).
would be reduced. For metabolism in the gas exchange region, an additional metabolism term would be added to the right side of equation (S-14), leading to a negative term on the right side of equation (S-16).

Another possibility is that the venous blood drawn had concentrations lower than that in actual mixed venous blood. A˚strand (1983) noted that in individuals exposed for a total of 2 h at increasing activity levels venous blood samples from a cubital vein reflected the concentrations in specific “resting muscles,” adipose tissue, and skin and were lower than those in mixed venous blood. However, the exposures in the current study were 6 h long at a constant (resting) activity level, so tissues other than adipose would be expected to have reached equilibrium by that time. Thus, A˚strand’s observation appears unlikely to explain the discrepancy. Moreover, there is little reason to suspect that such a bias would be substantially greater for TRI than for TETRA, as would be needed to explain the data here. Finally, experimental error cannot be ruled out.

The estimate of apparent TRI metabolic clearance implies a blood clearance rate greater than typical hepatic blood flow. That points to the possibility of extra-hepatic metabolism, as suggested for the lung and kidney (Clewell et al., 2000; Green, 2000; Lash et al., 1999a). It is also possible that these individuals had larger than average hepatic blood flow. U.S. Environmental Protection Agency (U.S. EPA) (2006) reported 59 hepatic blood flow measurements ranging from 0.6 to 3.8 l/min (geometric mean 1.3 l/min and geometric SD 1.6).

Both venous blood and urine data suggest lower TCA formation from TRI than in previous studies. Blood TCA levels on a cumulative exposure-adjusted basis were more than three-fold lower than in previous studies. Though relative urinary excretion of TCA was three- to 10-fold lower, this may be due in part to saturation of many urinary TCA measurements. Again, interindividual variance cannot be ruled out, as in the experiments of Fisher et al. (1998).

### FIG. 6
Comparison mean ± SD venous blood concentrations of TCA following TETRA exposure, adjusted for cumulative exposure (µM in blood/ (µM-h) in inhaled air). Thick and thin solid indicate the mean ± SD over all individuals and occasions in the current study. Due to small variations in collection time, data from the current study were binned to the nearest hour (t < 24 h) or nearest 6 h (t > 24 h) prior to taking the mean and SD for clarity of display, with horizontal error bar showing the range of times included in each data point. Other studies and symbols: Völkel et al. (1998) (□ = 10 ppm, △ = 40 ppm); Monster et al. (1979) (● = 70 ppm, ▲ = 140 ppm).

### FIG. 7
Comparison of mean ± SD cumulative urinary excretion of TCA across TETRA studies. The amount excreted is adjusted for cumulative exposure (µmol in urine/(µM-h) in inhaled air). Thick and thin solid indicate the mean ± SD over all individuals and occasions in the current study. Other studies and symbols: Völkel et al. (1998) (□ = 10 ppm, △ = 20 ppm, △ = 40 ppm); Monster et al. (1979) (● = 70 ppm, ▲ = 140 ppm).

### TABLE 4
Comparison of TCA Peak Venous Blood Concentrations

<table>
<thead>
<tr>
<th>Study</th>
<th>Subgroup</th>
<th>TCA Cmax&lt;sub&gt;ven&lt;/sub&gt;/ (C&lt;sub&gt;inh&lt;/sub&gt; × t&lt;sub&gt;exp&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRI</td>
<td>Current study&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.29 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>Fisher et al. (1998)</td>
<td>F 50 ppm 3.77 ± 1.23</td>
</tr>
<tr>
<td></td>
<td>M 50 ppm</td>
<td>4.27 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>F 100 ppm</td>
<td>3.57 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>M 100 ppm</td>
<td>3.36 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>Monster et al. (1976)</td>
<td>70 ppm 5.46 ± 1.05</td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>3.94 ± 0.96</td>
</tr>
<tr>
<td>TETRA</td>
<td>Current study&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Monster et al. (1979)</td>
<td>70 ppm 0.20 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>0.19 ± 0.03</td>
</tr>
</tbody>
</table>

Note. C<sub>inh</sub> = inhaled air concentration; F = Females; M = Males; t<sub>exp</sub> = exposure duration.

<sup>a</sup>Outlier B-1 not included (if included, mean ± SD is 1.43 ± 0.54).

<sup>b</sup>Outliers D-1 and F-1 not included (if included, mean ± SD would be 0.41 ± 0.35).
Total TCOH in venous blood and urine were consistent with those observed in previous studies, even though a substantial fraction of TCOH in venous blood was found to be glucuronidated. This suggests that urinary excretion of TCOG is the rate-limiting step in total TCOH + TCOG clearance—otherwise, the increased concentration of TCOG should have increased its rate of elimination in urine. It is notable that for 1-ppm exposures the decrease in the relative amount of free TCOH in venous blood is similar to the smaller relative amount of TCA in venous blood. This is consistent with the hypothesis that TCA formation is predominantly a result of “back-conversion” of free TCOH (Fisher et al., 1998; Marshall and Owens, 1954), with increased glucuronidation reducing free TCOH. Moreover, Fisher et al. (1998) reported the need to model TCOH glucuronidation using Michaelis-Menten, rather than linear, kinetics in analyzing data from human volunteers exposed to 50 and 100 ppm TRI. This would suggest that the increased glucuronidation of TCOH observed here is possibly due to partial saturation at higher exposures.

Consistent with previous studies, total recovery of TRI in air, and of oxidative metabolites in urine, account for only 60–70% of intake. Thus, all the available human studies suggest that other pathways of elimination or metabolism of TRI (e.g., GSH conjugation or through TRI-oxide), TCA, or TCOH, are active. In mice and rats, fecal elimination has been found to account for up to 17 and 9% of oral doses of TRI, respectively (Prout et al., 1985). However (Bartonicek, 1962) reported that fecal excretion of TCA and total TCOH in humans was about 10-fold less than urinary excretion. With respect to oxidative metabolism, a number of other products of TRI oxidation other than TCA and TCOH have been identified in rodents and humans, including CO2, N-(hydroxyacetyl)-aminoethanol, monochloroacetic acid, and (possibly) DCA (Lash et al., 2000a). CO2, perhaps indicative of TRI-oxide formation, has been found account for up to 1–10% of oral doses of TRI in rats and mice (Prout et al., 1985) but no studies of this metabolite are available in humans. Human urinary excretion of monochloroacetic acid from TRI exposure has been reported in one study, and was found to be five-fold lower than excretion of TCA (Soucek and Vlachova, 1960). With respect to GSH conjugation, excretion of urinary mercapturates, downstream detoxification products of GSH conjugation, does not appear to be a significant contributor to recovery (Bernauer et al., 1996; Birner et al., 1993; Bloemen et al., 2001). However, as noted by Lash et al. (2000a), data on these detoxification products do not capture the total flux through the GSH pathway if there is significant bioactivation to reactive species that do not appear in urine. Peak blood concentrations of DCVG have been found to be similar to those of oxidative metabolites TCA and TCOH (Lash et al., 1999b). Similarly, in vitro clearance rates of TRI GSH conjugation in isolated hepatocytes are comparable to those of TRI oxidation in hepatocytes prepared using similar methods (Lash et al., 1999a; Lipscomb et al., 1998), with high variability (> two-fold) among samples in both cases. Interestingly, these same data suggest the Km for GSH conjugation may be lower than that for oxidation: Lipscomb et al. (1998) reported a Km of 195 ppm in headspace for oxidation, while Figure 1 of Lash et al. (1999a) is suggestive of a Km of 50–100 ppm in headspace for GSH conjugation. Lash et al. (1999a) also reported that in vitro GSH conjugation rates measured in subcellular fractions (liver and kidney cytosol and microsomes) were much greater in humans than in rodents (humans > mice > rats). However, Green et al. (1997) reported GSH conjugation rates in human liver cytosol 30-fold lower than that of Lash et al. (1999a) and with a different species dependence (mice > rats > humans), discrepancies that have yet to be resolved.

**TETRA and TCA**

Compared to TRI, the TETRA experiments show only modest differences from previous studies. Blood levels of TETRA, relative to exposure, are about two-fold lower, but given the large variability observed in the larger TRI database, this difference may be consistent with interindividual variability. Moreover, the estimated PBext is consistent with the range from in vitro studies. Average recovery by exhalation was calculated to be 82%, implying that about 18% was metabolized. While higher than estimates from previous studies, the uncertainty and variability in these calculations are substantial, with confidence intervals among different studies substantially overlapping. Venous blood and urine TCA data suggest similar, or perhaps slightly lower, TCA formation from TETRA than that observed in previous studies. Because of the substantial uncertainty in recovery, these data are not highly informative as to the fraction of TETRA metabolism leading to TCA.

The kinetics of TCA following TETRA exposure seem to suggest similar blood concentrations across studies (Fig. 6) but less urinary excretion in the current study (Fig. 7) than Monster et al. (1979) and (marginally) Völk et al. (1998). One can speculate the existence of a high affinity, low capacity metabolic pathway for TCA metabolism to account for this difference. Alternatively, Covington et al. (2006), in their PBTK analysis of TETRA, suggested that the TCA blood concentration levels from Monster et al. (1979) were inconsistent with the one-compartment kinetics of TCA as derived from PBTK analyses of TRI (Clewell et al., 2000), and therefore not reliable. However, the many metabolites of TRI complicate this inference, particularly since TCA from TRI appears to be predominantly derived from “back-conversion” of TCOH (Fisher et al., 1998). For this reason, the consistency or inconsistency of TCA kinetics across TRI and TETRA studies should be better investigated using PBTK models.

**Summary and Next Steps**

In sum, extensive toxicokinetic data were collected in human subjects exposed to 1 ppm of TRI and TETRA in controlled experiments on multiple occasions. Qualitatively, TRI and TETRA toxicokinetics were consistent with previous human
studies, most of which were at > 10-fold higher exposures. Quantitatively, for TRI these data provide evidence for lower PB, greater metabolic clearance, less TCA formation and excretion, and greater TCOH glucuronidation as compared to previous studies. For TETRA, the case for toxicokinetic differences is weaker. Although these data are suggestive of greater metabolism to species other than TCA, variability and uncertainty in estimating TETRA total metabolism are substantial in both the current and previous studies. For both TRI and TETRA, the relative contributions of interindividual variance, interoccasion variance, and dose dependence to these differences cannot be determined with confidence from an empirical analysis of the data. Additionally, contributions to variability from analytical errors are possible, as a variety of methods were used among the different studies (e.g., colorimetric, HSGC, GC-ECD) that have not to our knowledge been directly compared.

The next step would logically be the development of a PBTK model-based analysis of the data, which would better account for the simultaneous, time-dependent effects of tissue uptake, metabolism via multiple pathways, and excretion via exhaled breath and urine. Through incorporation of a rigorous statistical framework, such an analysis would provide individual and population parameter estimates for improved comparisons with previous studies, and would characterize the uncertainty and variability in predictions relevant for risk assessment. We are currently conducting such an analysis used a Bayesian population approach (Chiu et al., in preparation), though we are willing to make the data available to interested readers as well. A further step would be to develop, to the extent feasible, a combined statistical analysis of these data and those at higher exposures in order to quantitatively assess the possible range of contributions from dose dependence and interindividual or interoccasion variability. In order to better separate these different effects, future toxicokinetic studies in human volunteers should consider directly measuring PB (e.g., in vitro) and including multiple exposure levels along with multiple occasions at the same exposure level. Finally, because of the complex toxicokinetics of TRI, it may also be beneficial to collect toxicokinetic data on the metabolites themselves (e.g., after iv or oral dosing of chloral hydrate and/or TCA) in the same individuals.

SUPPLEMENTARY DATA

Supplemental data included with the manuscript consist of the following detailed methods and results: (1) measured characteristics of study subjects; (2) tables containing empirical toxicokinetic measurements from the current study (individual- and occasion-specific results, summary statistics, and ANOVA results); (3) equations for the derived toxicokinetic measures used for comparison among studies, and tables containing individual- and occasion-specific results; (4) methods for comparing blood time courses, urinary excretion, and total recovery among studies; (5) a table summarizing the exposure levels and toxicokinetic measurements reported in the comparison studies; and (6) figures showing individual- and occasion-specific breath concentrations, blood concentrations, and urinary excretion from the current study.

Supplementary data are available online at http://toxsci.oxfordjournals.org/

Appendix: Abbreviations and Symbols used in Manuscript and Supplementary data

\[
A_{exh,t1-t2} = \text{amount exhaled from time } t1 \text{ to } t2 \\
A_{met} = \text{amount metabolized} \\
AUC_{x,t1-t2} = \text{area under the concentration curve of } x (alv = \text{alveolar air}, art = \text{arterial blood}, inh = \text{inhaled air}, ven = \text{venous blood}) \text{ from time } t1 \text{ to } t2 \\
C_{alv} = \text{alveolar air concentration} \\
C_{art} = \text{arterial blood concentration} \\
C_{exh} = \text{exhaled air concentration} \\
C_{inh} = \text{inhaled air concentration} \\
C_{ven} = \text{venous blood concentration} \\
CV = \text{coefficient of variation (SD/mean)} \\
CYP2E1 = \text{cytochrome P450, family 2, subfamily E, polypeptide 1} \\
DCA = \text{dichloroacetic acid} \\
DCVG = S-\text{dichlorovinyl glutathione} \\
F_{exh,t1-t2} = \text{fraction of intake exhaled from time } t1 \text{ to } t2 \\
FMO = \text{flavin-containing monoxygenases} \\
GSH = \text{glutathione} \\
HSGC = \text{headspace gas chromatography} \\
inf = \text{infinity} \\
LC = \text{lung clearance} \\
MV = \text{minute-volume} \\
PB = \text{blood-air partition coefficient} \\
PB_{met} = \text{blood-air partition coefficient estimated from blood and alveolar or exhaled air data} \\
PBTK = \text{physiologically based toxicokinetic} \\
Q_{alv} = \text{alveolar ventilation rate} \\
Q_{card} = \text{cardiac output} \\
R_{alv} = \text{alveolar retention} \\
t_{1/2} = \text{half-life} \\
TCA = \text{trichloroacetic acid} \\
TCOG = \text{trichloroethanol glucuronide} \\
TCOH = \text{trichloroethanol (free)} \\
TCVC = S-\text{trichlorovinyl-L-cysteine} \\
TCVG = S-\text{trichlorovinyl glutathione} \\
TETRA = \text{tetrachloroethylene} \\
t_{exp} = \text{exposure duration} \\
t_{last} = \text{time point of last measurement} \\
t_{max} = \text{time point of maximum concentration} \\
total TCOH = TCOH + TCOG \\
TRI = \text{trichloroethylene}
\]

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