

# Urinary Levels of Trichloroacetic Acid, a Disinfection By-Product in Chlorinated Drinking Water, in a Human Reference Population

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Trichloroacetic acid (TCAA), a known mouse liver carcinogen and a possible human carcinogen, is found in chlorinated drinking water. We measured TCAA in archived urine samples from a reference population of 402 adults using isotope-dilution high-performance liquid chromatography–tandem mass spectrometry. TCAA was detected in 76% of the samples examined at concentrations ranging from < 0.5 µg TCAA/L to more than 25 µg/L; the 90th percentile concentration was 23 µg/L (22 µg TCAA/g creatinine); and the geometric mean and median concentrations were 2.9 µg/L (2.6 µg/g creatinine) and 3.3 µg/L (3.2 µg/g creatinine), respectively. The frequency of detection of TCAA in urban areas was higher than in rural areas ( $p = 0.00007$ ), and sex and place of residence (i.e., urban vs. rural) were found to have a significant interaction in modulating the levels of TCAA ( $p = 0.012$ ). Urban residents had higher mean levels of TCAA (men, 5.3 µg/L, 3.8 µg/g creatinine; women, 2.9 µg/L, 2.8 µg/g creatinine) than did rural residents (men, 2.2 µg/L, 1.7 µg/g creatinine; women, 2.6 µg/L, 2.7 µg/g creatinine). The higher frequency of detection of TCAA in urban than in rural areas and higher levels of TCAA among urban than among rural residents may reflect the fact that urban residents use primarily chlorinated water from public water supplies, whereas those in rural areas are more likely to obtain water from private wells, which typically are not chlorinated. **Key words:** exposure, human, trichloroacetic acid, urine. *Environ Health Perspect* 111:151–154 (2003). [Online 28 October 2002] doi:10.1289/ehp.5644 available via <http://dx.doi.org/>

The chemical disinfection of drinking water to control microbial contaminants has been one of the most successful public health measures ever undertaken. However, in the 1970s, the chemical disinfection of drinking water was discovered to produce disinfection by-products (DBPs) (Rook 1974). Toxicologic studies on laboratory animals have found that some DBPs are carcinogenic and may have adverse reproductive outcomes (Boorman et al. 1999; Bull et al. 1995). When chlorine is used as the disinfecting agent, the volatile trihalomethanes (THMs) and the nonvolatile haloacetic acids (HAAs) are the most abundant groups of DBPs formed (Richardson 1998). Trichloroacetic acid (TCAA), one of the two major HAAs in water, is a mouse liver carcinogen; the U.S. Environmental Protection Agency (U.S. EPA) has classified TCAA as a possible human carcinogen (U.S. EPA 2001) and introduced regulations for the maximum contaminant levels of TCAA, among other DBPs, in drinking water.

Urinary TCAA has been proposed as a biomarker of chronic ingestion exposure to HAAs from chlorinated drinking water (Bader et al. 2001; Froese et al. 2002; Kim et al. 1999; Weisel et al. 1999). In addition, the levels of TCAA in urine (Bloemen et al. 2001; Fisher et al. 1998; Raaschou-Nielsen et al. 2001; Vartiainen et al. 1993) and in plasma (Ziglio 1981) have been used as a biomarker for occupational or unintentional exposure to trichloroethylene, (TCE), a compound that is partially metabolized to TCAA in humans.

No clear association has been established between human exposure to HAAs in drinking water and adverse health effects. However, further studies to investigate the human health relevance of exposure to HAAs are warranted because of their potential harmful effects on human health. We report here the levels of TCAA in urine from a reference sample of the noninstitutionalized U.S. adult population using a rapid and selective technique (Kuklenyik et al. 2002) as a tool for assessing the internal dose of HAAs.

## Materials and Methods

The urine samples analyzed for this study were selected from a nonrepresentative call-back cohort of those collected during the Third National Health and Nutrition Examination Survey (NHANES III), which was conducted from 1988 through 1994 (NCHS 1994). These samples have been stored securely at  $-70^{\circ}\text{C}$  since shipment to the Centers for Disease Control and Prevention (CDC), and have not been subjected to inadvertent thawing; under these conditions, the integrity of the specimens is maintained. The urine samples were collected at different times throughout the day and were not necessarily first-morning voids. Participants in our study included persons 20–59 years old (60% were < 50 years old; mean age  $\pm$  SD,  $42 \pm 12$  years), both sexes (50% men), and urban (70%) and rural residences. We used a cutoff of 100,000 inhabitants per county to distinguish rural from urban areas. This sampling

was designed not to be representative of the U.S. population but to serve as a reference range for this demographic group. The study was approved by the Institutional Review Board of the National Center for Health Statistics, CDC.

Before analysis, the NHANES III samples used for this study (stored at  $-70^{\circ}\text{C}$ ) were left to thaw overnight at  $5^{\circ}\text{C}$ . No chemical degradation of TCAA was detected in the quality-control samples and standards used for this study under these conditions. TCAA was measured using a method described previously (Kuklenyik et al. 2002), which involved the use of solid-phase extraction followed by the analysis of TCAA by isotope-dilution high-performance liquid chromatography–tandem mass spectrometry; with this method, specific fragment ions of TCAA and its  $^{13}\text{C}$ -labeled analog were monitored for quantification and confirmation. The limit of detection (LOD) for TCAA in a 1-mL urine sample was 0.50 µg TCAA/L.

The blood concentrations (in micrograms per liter) of 1,1,1-trichloroethane (TRI), TCE, and tetrachloroethylene [perchloroethylene (PERC)], measured by isotope-dilution purge-and-trap high-resolution mass spectrometry, have been reported elsewhere (Ashley et al. 1994). The LODs for TCE, PERC, and TRI were 0.01 µg/L, 0.03 µg/L, and 0.086 µg/L, respectively.

Statistical analyses of the data were carried out using SAS software (SAS Institute, Cary, NC, USA). Because the base-10 logarithm of the TCAA concentrations (log-transformed TCAA concentrations) were less skewed than were the nontransformed values, we used the log-transformed values in the analyses. Results are reported both as micrograms TCAA per liter of urine (µg/L) and micrograms TCAA per gram of urinary creatinine (µg/g creatinine). Creatinine adjustment was used to correct for urine dilution (Jackson

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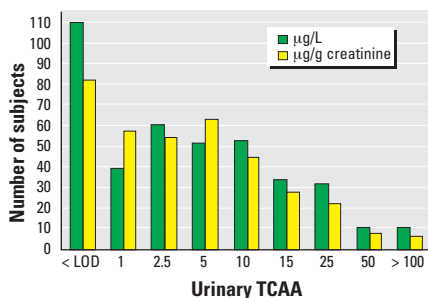
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1966); only those urine samples with creatinine values  $\geq 300$  mg/L were included in the analysis because those with values  $< 300$  mg/L are too dilute to provide results that can be interpreted correctly (Lauwerys and Hoet 1993).

We used an analysis of covariance model to study the effects of residence (rural vs. urban), sex (woman vs. man), age, and urinary creatinine on the urinary log-transformed TCAA concentration. The initial model included residence and sex as main effects, age and urinary creatinine as continuous covariates, and all possible two-way, three-way, and four-way interactions between and among the independent variables. We also determined the correlation between the urinary TCAA concentration and blood TCE, PERC, and TRI levels. Statistical significance was set at  $p < 0.05$ . TCAA levels less than the LOD were assigned a concentration equal to the LOD divided by the square root of two for the statistical analyses.

## Results and Discussion

We measured urinary concentrations of TCAA, a biomarker for ingestion exposure to chlorinated drinking water. We found that about three-quarters (76%) of the samples measured had detectable concentrations of TCAA (Figure 1); this frequent exposure to TCAA is not surprising because of the widespread use of chlorination of drinking water. Because of the TCAA's low dermal and inhalation absorption (Kim and Weisel 1998), this finding suggests that a substantial portion of this study population is exposed to TCAA from ingestion of chlorinated drinking water.



**Figure 1.** Distribution of TCAA in a human reference population consisting of 402 adults. LOD = 0.5 µg/L; geometric mean urinary TCAA concentration = 2.9 µg/L (2.6 µg/g creatinine); 25th, 50th (median), and 90th percentile concentrations = 0.6 µg/L (0.7 µg/g creatinine), 3.3 µg/L (3.2 µg/g creatinine), and 23 µg/L (22 µg/g creatinine), respectively. The creatinine-corrected concentrations exclude values with urine creatinine levels  $< 300$  mg/L ( $n = 367$ ). Urban residents had higher mean levels of TCAA [men, 5.3 µg/L (95% CI, 4.0–7.0), 3.8 µg/g creatinine (CI, 2.9–5.1); women, 2.9 µg/L (CI, 2.2–3.7), 2.8 µg/g creatinine (CI, 2.1–3.6)] than did rural residents [men, 2.2 µg/L (CI, 1.4–3.3), 1.7 µg/g creatinine (CI, 1.1–2.5); women, 2.6 µg/L (CI, 1.6–4.2), 2.7 µg/g creatinine (CI, 1.7–4.4)].

TCAA is also a metabolite of TRI, TCE, and PERC, commonly used industrial chemicals and groundwater contaminants (ATSDR 1995, 1996, 1997), and of chloral hydrate, a drug typically used in the elderly or the young for sedation and sleep induction and for the treatment of intractable status epilepticus (Humbert et al. 1994). TRI, TCE, and PERC also are found in many common household products [e.g., TRI: glue, paint, industrial degreasers, and aerosol sprays (ATSDR 1995); TCE: typewriter correction fluid, paint removers, adhesives, and spot removers (ATSDR 1997); PERC: water repellents, silicone lubricants, fabric finishers, spot removers, adhesives, and wood cleaners (ATSDR 1996)]. Therefore, we determined the correlation between the creatinine-corrected urinary concentration of TCAA and the blood concentrations of TCE, PERC, and TRI (Table 1). There was no significant correlation between the levels of urinary TCAA and blood PERC for the 84 samples for which we had both TCAA and PERC concentration values. These results were not totally unexpected because only 1–3% of the absorbed PERC is metabolized to TCAA by humans (ATSDR 1996). Furthermore, the excretion of TCAA after exposure to PERC may be delayed because of tight and extensive binding of TCAA to plasma proteins (ATSDR 1997; Muller et al. 1974; Sellers and Koch-Weser 1971). In contrast, although only 0.5–6% of the absorbed TRI is metabolized

to TCAA in humans (ATSDR 1995), we found a statistically significant correlation (Pearson correlation = 0.32,  $p = 0.0059$ ) between the levels of urinary creatinine-adjusted TCAA and blood TRI for the 73 samples for which we had TRI concentration data. This finding suggests that the urinary TCAA levels in those 73 people may be, at least in part, related to exposure to TRI. We also found a statistically significant relation (Pearson correlation = 0.43,  $p = 0.0001$ ) between the levels of urinary creatinine-corrected TCAA and blood TCE for 98 samples for which we had TCE concentration data; the sample with the highest level of TCE in blood also had the highest concentration of TCAA in urine. These findings suggest that the TCAA levels in those persons also may be associated with exposure to TCE, at least to a certain extent [an estimated 20–40% for the absorbed TCE is metabolized to TCAA in humans (ATSDR 1997)].

The results of the analysis of variance of urinary TCAA concentration and age, sex, residence, and urinary creatinine levels are shown in Table 2. We excluded from the analyses data from samples with creatinine levels below 300 mg/L because those urine specimens were too dilute (Lauwerys and Hoet 1993), and because the model parameter estimates changed significantly (~30%) if the data from those samples were included in the analyses (data not shown). Beginning with the initial model described above, we

**Table 1.** Correlation analysis of TCAA in urine and TCE, TRI, and PERC in blood.

Analyte	No.	Uncorrected TCAA <sup>a</sup>		Creatinine-corrected TCAA <sup>b</sup>	
		Pearson's <i>r</i>	<i>p</i> -Value	Pearson's <i>r</i>	<i>p</i> -Value
TCE <sup>c</sup>	98	0.36	0.0002	0.42	$< 0.0001$
TRI <sup>c</sup>	73	0.34	0.0035	0.32	0.0059
PERC <sup>c</sup>	84	0.19	0.083	0.18	0.097

Analyses were conducted using the log-transformed concentrations. Only samples with urine creatinine levels  $> 300$  mg/L were used for the analysis.

<sup>a</sup>Micrograms per liter of urine. <sup>b</sup>Micrograms per gram of creatinine. <sup>c</sup>Micrograms per liter of blood.

**Table 2.** Multivariate model<sup>a</sup> considering the relationship of measured TCAA<sup>b</sup> to independent predictor variables

Independent variables, specification	No.	<i>F</i> -value	GM <sup>c</sup>	<i>p</i> -Value
Residence				
Rural	113	7.5	2.4	0.0066
Urban	254		3.9	
Sex				
Women	172	0.01	3.0	0.92
Men	195		3.1	
Age, continuous	367	3.1	–0.011 <sup>d</sup>	0.078
Creatinine, continuous	367	3.3	–0.032 <sup>d</sup>	0.070
Age × creatinine, continuous	367	9.6	0.0011 <sup>d</sup>	0.0021
Residence × sex				
Rural women	52	6.4	3.0	0.012
Rural men	61		1.9	
Urban women	120		3.1	
Urban men	134		5.0	

<sup>a</sup>Each variable was adjusted for the others in the model. Only samples with urine creatinine levels  $> 300$  mg/L were used for the analysis. The  $r^2$  value for the model is 0.11. <sup>b</sup>Dependent variable is  $\log_{10}$ (TCAA) for model calculations. TCAA units are micrograms per liter of urine. <sup>c</sup>Model-calculated geometric mean. <sup>d</sup>Values shown are  $\beta$  [i.e., slope from the multivariate analysis of the regression of  $\log_{10}$ (TCAA) vs. the continuous independent variable].

arrived at a final model that included residence, sex, age, creatinine, residence-by-sex interaction, and age-by-creatinine interaction. We calculated model-adjusted geometric mean estimates of TCAA for sex and residence groups by using the mean values of the continuous covariates (i.e., age and creatinine). As we expected, men had a higher mean creatinine concentration (14 mmol/L, 1,554 mg/L) than did women (11 mmol/L, 1,225 mg/L).

The results of the analysis of covariance model suggested that the effect of age on urinary TCAA depended on the urinary creatinine level ( $p = 0.0021$ ). For every yearly increase in age, TCAA increased by 0.8% for the mean creatinine level for women and by 1.7% for the mean creatinine level for men; it is well known that creatinine clearance decreases with age (Kaplan and Pesce 1996). The model-estimated geometric mean TCAA concentration for a 40-year-old urban man with the mean creatinine value for men was 5.4  $\mu\text{g/L}$  (3.5  $\mu\text{g/g}$  creatinine); that for a 40-year-old rural man was 2.1  $\mu\text{g/L}$  (1.4  $\mu\text{g/g}$  creatinine). Likewise, the model-estimated geometric mean TCAA concentration for a 40-year-old urban woman with the mean creatinine value for women was 3.0  $\mu\text{g/L}$  (2.4  $\mu\text{g/g}$  creatinine), whereas that for a 40-year-old rural woman was 2.9  $\mu\text{g/L}$  (2.3  $\mu\text{g/g}$  creatinine).

The relation between TCAA concentration and place of residence varied with sex. This was confirmed by the regression analysis: The "sex  $\times$  residence term" was statistically significant ( $p = 0.012$ ) when added to the basic model, suggesting that the effect of residence on TCAA levels differs for men and women. The model-adjusted geometric mean of TCAA for urban men (5.0  $\mu\text{g/L}$ , 3.5  $\mu\text{g/g}$  creatinine) was higher than those for rural men (1.9  $\mu\text{g/L}$ , 1.4  $\mu\text{g/g}$  creatinine), urban women (3.1  $\mu\text{g/L}$ , 2.2  $\mu\text{g/g}$  creatinine), and rural women (3.0  $\mu\text{g/L}$ , 2.1  $\mu\text{g/g}$  creatinine). These values compare well with the observed values (Figure 1). The differences in the geometric mean TCAA levels were significant between rural men and urban men ( $p = 0.0001$ ), and urban men and urban women ( $p = 0.02$ ), but not between rural women and urban women or between rural men and rural women. The higher TCAA levels for urban men than for urban women, but lower levels for rural men than for rural women are difficult to explain with the available demographic data. We hypothesize that variables other than consumption of chlorinated drinking water, including personal habits (e.g., wearing dry-cleaned clothing, frequent use of chlorinated household products, diet), occupational exposure to TCAA or its precursors, differences in the metabolism and clearance of TCAA, or a combination of all of the above may also contribute to the effect of sex on the TCAA levels based on place of residence. For instance, we

speculate that the higher TCAA levels for rural women than rural men may be related, at least in part, to increased exposure at home while conducting household chores using chlorinated solvents that metabolize to TCAA; rural women are more likely to stay at home than are rural men, and rural men are not likely to use dry-cleaned clothes.

Interestingly, not only were the urinary TCAA levels higher in urban than in rural residents, but also the frequency of detection of TCAA for persons living in urban areas [81%; 95% confidence interval (CI), 76–86] was significantly higher ( $p = 0.00007$ ) than for those living in rural areas (62%; 95% CI, 53–71). In rural areas, drinking water is likely to come from private wells. The responsibility for ensuring a safe supply of private residential well water rests solely on the homeowner because this water is not monitored for quality by government agencies. Routine chlorination is unlikely to occur in private wells, except in isolated pockets in the United States, for two main reasons. First, groundwater sources, from which most private wells get their water, are not generally contaminated. Second, organic compounds that could be a precursor for THMs and other halogenated organic compounds do not generally occur in groundwater. A U.S. EPA survey representing more than 600 rural water supply systems indicated that for the surface water supplies, chloroform, one of the THMs, was found in 82% of the systems at a median level of 54  $\mu\text{g/L}$ ; in contrast, for the groundwater supplies, chloroform was detected in only 17% of the systems at a median value below 0.5  $\mu\text{g/L}$  (Wallace 1997). A recent report comparing the total HAA and THM concentrations suggests that the total HAA concentrations in finished drinking water probably are similar to the total concentration of THMs (Roberts et al. 2002). Therefore, the levels of HAAs, including TCAA, are presumably higher in surface water than in groundwater supplies. We postulate that the higher frequency of detection of TCAA among urban residents may be related to the potential for higher exposure to chlorinated drinking water in urban areas than in rural areas, in which residents may get their water from private wells or groundwater supplies.

In summary, this study provides the first broad-based reference range of human internal dose levels of TCAA. Despite sample population's nonrepresentativeness and relatively small size, which limit the applicability of the observed statistical associations, the data suggest the possibility of significant demographic variations in exposure to TCAA. Our findings provide both information about background human levels of TCAA and evidence of measurable exposure to HAAs, a major group of DBPs, in drinking water.

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