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Trihalomethane exposure and biomonitoring for the liver injury indicator, alanine aminotransferase, in the United States population (NHANES 1999–2006)

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Abstract

Exposure to trihalomethanes (or THMs: chloroform, bromoform, bromodichloromethane, and dibromochloromethane [DBCM]) formed via drinking water disinfection has been associated with adverse reproductive outcomes and cancers of the digestive or genitourinary organs. However, few studies have examined potential associations between THMs and liver injury in humans, even though experimental studies suggest that these agents exert hepatotoxic effects, particularly among obese individuals. This study examined participants in the National Health and Nutrition Examination Survey (1999–2006, N = 2781) to test the hypothesis that THMs are associated with liver injury as assessed by alanine aminotransferase (ALT) activity in circulation. Effect modification by body mass index (BMI) or alcohol consumption also was examined. Associations between blood THM concentrations and ALT activity were assessed using unconditional multiple logistic regression to calculate prevalence odds ratios (ORs) with 95% confidence intervals (CIs) for exposure among cases with elevated ALT activity (men: >40 IU/L, women: >30 IU/L) relative to those with normal ALT, after adjustment for variables that may confound the relationship between ALT and THMs. Compared to controls, cases were 1.35 times more likely (95% CI: 1.02, 1.79) to have circulating DBCM concentrations exceeding median values in the population. There was little evidence for effect modification by BMI, although the association varied by alcohol consumption. Among non-drinkers, cases were more likely than controls to be exposed to DBCM (OR: 3.30, 95% CI: 1.37–7.90), bromoform (OR: 2.88, 95% CI: 1.21–6.81), or brominated THMs

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(OR: 4.00, 95% CI: 1.31–12.1), but no association was observed among participants with low, or moderate to heavy alcohol consumption. Total THM levels exceeding benchmark exposure limits continue to be reported both in the United States and globally. Results from this study suggest a need for further characterization of ALT activity and possibly other hepatic or metabolic diseases in popu lations with elevated drinking water THM concentrations.

Keywords

Dibromochloromethane; Disinfection by-product; Metabolic syndrome; Non-alcoholic fatty liver disease

1. Introduction

The disinfection of public water supplies via chlorination remains a primary method for control of microbial contaminants in the United States (US) and worldwide. Despite the established benefit for controlling exposure to infectious agents, there is still a potential for adverse public health impacts that can result from exposure to residual contaminants that form during water disinfection. There are ~600–700 water disinfection by-products (DBPs) that are formed when naturally occurring organic matter interacts with halogens during treatment (Krasner, 2009; Nieuwenhuijsen et al., 2009). For many of these compounds, their toxic, mutagenic, or carcinogenic potential has not been fully characterized. However, the trihalomethanes (or THMs: chloroform, bromoform, bromodichloromethane [BDCM], and dibromochloromethane [DBCM]) are among the most abundant and thoroughly studied DBPs. Their formation in drinking water depends on various factors, including: concentrations of organic matter and residual halogens in the source water, residence time of disinfecting agents in the water, water temperature, season, and the size and configuration of the water distribution system (Krasner, 2009; Nieuwenhuijsen et al., 2009; Richardson et al., 2007; Rivera-Nunez et al., 2012). Human exposure to THMs depends not only on direct ingestion of drinking water, but also occurs due to volatilization of DBPs during showering, bathing, cooking, or to other behavioral or lifestyle factors (Richardson et al., 2007; Rivera-Nunez et al., 2012). THM exposure has been associated with adverse reproductive outcomes, as well as cancers of the digestive and genitourinary systems (Grellier et al., 2010; Nieuwenhuijsen et al., 2009; Rahman et al., 2010, 2014; Richardson et al., 2007; Villanueva et al., 2004). In the US, a maximum contaminant level (MCL) of 80 μg/L has been established for total trihalomethane (TTHM, the sum of chloroform, bromoform BDCM, and DBCM concentrations) in drinking water by the US Environmental Protection Agency (EPA) under the jurisdiction of the Safe Drinking Water Act. The World Health Organization (WHO) guideline for TTHM in drinking water is 100 μg/L (WHO, 2005). The biomonitoring equivalents for THMs (i.e., the chemical concentration in blood corresponding to the EPA reference dose [RfD], the daily oral dose to humans that is not likely to elicit adverse health effects during a lifetime) have been estimated as 230, 20, 80, 130 pg/mL for chloroform, BDCM, DBCM, and bromoform, respectively (Aylward et al., 2008; LaKind et al., 2010). Despite knowledge of health impacts and the establishment of benchmark concentrations for TTHM monitoring, concentrations in drinking water exceeding benchmark values still occur in the US and in various regions globally

(Fooladvand et al., 2011; Goslan et al., 2009; Mishra et al., 2014; Rivera-Nunez et al., 2012; Thacker et al., 2002; Tokmak et al., 2004; Uyak, 2006). In areas where monitoring is sporadic or absent, the impacts of exposure to TTHM above recommended levels is unknown.

Few studies have examined the potential relationship between THMs and liver injury in humans (WHO, 2005). One of the liver's primary functions is the metabolism of xenobiotic compounds in circulation, and processing of the absorbed contents of the gastrointestinal (GI) tract. Therefore, the liver is a primary target organ for human THM exposure, which is supported by toxicity studies in experimental animals especially mice (Das et al., 2013a, 2013b; Seth et al., 2013). Studies from rodent models have revealed the development of nonalcoholic steatohepatitis (NASH) as an underlying condition of obesity following chronic BDCM exposure and bioactivation primarily via cytochrome p450 (CYP) enzymatic activity in the liver (Seth et al., 2013; WHO, 2005). THM metabolism is mediated in part by CYP2E1, which is highly active in the liver (Tomasi et al., 1985). Hepatotoxicity likely occurs via pathways that induce oxidative stress due to biotransformation of THM to highly reactive dihalomethyl radicals that in turn cause lipid peroxidation and cytotoxicity via formation of stable hydroxynonenal adducts (Seth et al., 2013; Tomasi et al., 1985; WHO, 2005). Alanine aminotransferase (ALT) is one of several liver enzymes commonly used to screen for liver injury or hepatic disease in humans (Carobene et al., 2013). ALT is expressed primarily in hepatocytes and following liver injury its release from liver cells leads to elevated ALT activity in circulation (Liu et al., 2014). Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of clinical and pathological hepatic alterations ranging from elevated circulating ALT activity to fatty liver and steatohepatitis, which can further progress into more serious manifestations such as liver cirrhosis, cardiovascular disease, diabetes and hepatocellular carcinoma (Jeon et al., 2013; LaBrecque et al., 2014; Lazo et al., 2013; Liu et al., 2014; Luo et al., 2015; White et al., 2012). NAFLD is relatively common, with a prevalence of ~10–35% in the US, and an estimated global prevalence ranging from ~6% to 40% (median: ~20%) (Angulo, 2002; Clark et al., 2003; Ioannou et al., 2006; LaBrecque et al., 2014; Lazo et al., 2013; Liu et al., 2014; Pacifico et al., 2013; Vernon et al., 2011). NAFLD pathogenesis derives from liver injury, which is often assessed via measurement of ALT activity in circulation (Liu et al., 2014; Pacifico et al., 2013; Park et al., 2012). Commonly used cutpoints to define the upper limit of normal for ALT activity are >40 IU/L for men and >30 IU/L for women, although other cutpoints have been suggested and no clear consensus has been established (Liu et al., 2014; Pacifico et al., 2013; Park et al., 2012). Some of the more commonly observed risk factors for liver injury include: age, sex, ethnicity, smoking, viral hepatitis infection, hepatic cancer, metabolic disorders such as diabetes or pre-diabetes, and excessive consumption of alcoholic beverages or other hepatotoxic agents. In addition, obesity is considered a hepatic manifestation of metabolic syndrome that contributes to liver injury (Liu et al., 2014; Yun et al., 2011). Recent studies in experimental animals identified a relationship between chronic BDCM exposure (1 mmol/kg i.p., two doses per week for 4 weeks) and the development of nonalcoholic steatohepatitis (NASH); an effect that was exacerbated among obese mice (Seth et al., 2013). Because early stage liver disease is frequently asymptomatic, there is a need to identify modifiable risk factors that can be targeted for primary prevention. In an

effort to better understand the role of THM exposure in liver injury, the present study sought to extend the above observations by studying participants in the National Health and Nutrition Examination Survey (NHANES) between 1999 and 2006 to test the hypothesis that THM exposure is associated with liver injury as assessed by elevated ALT activity in circulation. Potential effect modification among those with or without elevated BMI or alcohol consumption also was examined.

2. Methods

NHANES is a serial cross-sectional study that combines interviews with detailed physical examinations to ascertain sociodemographic, dietary, medical, physiological and biochemical information on a nationally representative sample of the non-institutionalized United States population in 2-year increments (details of the study design and data collection methods are available at: http://www.cdc.gov/nchs/nhanes.htm). For this study, data were retrieved for those who participated in studies characterizing volatile organic chemicals (VOCs) during the 1999–2000, 2001–2002, 2003–2004, and 2005–2006 survey cycles. Participants younger than 21 years old or missing data for any of the four THMs or ALT activity were excluded. Participants with major risk factors for liver damage also were excluded, which included participants with: alcohol consumption greater than 5 drinks per day (Basra and Anand, 2011; O'Shea et al., 2010); a laboratory confirmed diagnosis of hepatitis B or C, or self-reported hepatic cancer; or high transferrin saturation levels (>60% for males, >50% for females). The final analytic dataset included 2781 individuals with complete exposure and outcome data (Fig. 1).

Peripheral blood samples were collected during the participant's scheduled medical examination. Among adults, up to 128 mL of blood was drawn by venipuncture and immediately processed in the NHANES mobile examination center for assay or storage (Zipf et al., 2013). Tap water samples from a bathtub or outside faucet of the participants' residence were collected within 46–76 h after the medical examination. THM concentrations in peripheral blood (pg/mL) or tap water samples (μg/L) were measured via solid-phase microextraction gas chromatography and mass spectrometry (Blount et al., 2006; Zipf et al., 2013). The NHANES program implements a comprehensive data quality assurance program to maintain the integrity of biospecimens, laboratory results, and other collected data; including secondary examinations of previously examined participants, use of blind split samples collected during practice sessions, and repeat testing of sample subsets. Blood concentrations for the brominated trihalomethanes (BTHM) were obtained by summing the concentrations of bromoform, BDCM, and DBCM; and total trihalomethane (TTHM) concentrations were obtained by summing chloroform and BTHM concentrations for each participant. Samples with values below the analytical limit of detection (LOD) were assigned the designated NHANES concentration (LOD divided by $\sqrt{2}$) (Zipf et al., 2013). Exposure variables were created by assigning individuals to tertiles of BTHM or TTHM. Because a relatively large proportion of observations were below the LOD for bromoform (55%), BDCM (22%), and DBCM (46%), exposure to these compounds was defined by categorizing individuals into three groups: <LOD; >LOD and below the median; or those at or above the median THM blood concentration.

Standardized procedures for characterizing ALT activity were developed for NHANES based on International Federation of Clinical Chemistry recommendations using the enzymatic rate method, which monitors the rate of change in absorbance at 340 nm as NADH is oxidized to NAD. The change in absorbance is directly proportional to the ALT activity in the sample over a fixed time interval. In NHANES 1999–2001, ALT assays were performed using a Hitachi Model 704 multichannel analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN), and from 2002 to 2006, assays were conducted using a Beckman Synchron LX20 analyzer. Both analyzers use the same biochemical reaction, and comparisons of the two systems indicated that there were no notable differences in the observed ALT activity values (Ioannou et al., 2006). The reportable range for serum ALT activity among NHANES participants was 4 to 400 IU/L during the study timeframe. Case status for elevated ALT activity in circulation was defined as men with >40 IU/L and women with values >30 IU/L (Liu et al., 2014; Pacifico et al., 2013; Park et al., 2012).

All statistical analyses were conducted using SAS version 9.4 statistical software (SAS Institute, Cary, NC). The NHANES study uses a clustered sample design to recruit participants. To obtain unbiased estimates of population parameters, NHANES design variables and 8-year weights were created for the analyses using standard methods (http://www.cdc.gov/nchs/tutorials/Nhanes/index_continuous.htm). The strata and cluster variables were included in all statistical analyses to account for the population stratification and cluster sampling procedures used during recruitment. Survey design procedures in SAS were used to generate descriptive statistics. Correlations between blood and tap water THM concentrations were performed by calculating Spearman rank correlation coefficients (r).

A manual backwards elimination procedure was used to screen and select covariates for inclusion in multivariable statistical analyses. Potential confounders or effect modifiers included: age, sex, race, education, marital status, family income, smoking status, BMI (low-to-normal: <25 kg/m^2, overweight: ≥25 to <30 kg/m^2; obese: ≥30 kg/m^2), a self-reported diagnosis of high blood pressure, any cancer, any circulatory disease, systolic and diastolic blood pressure (BP), total serum cholesterol, triglycerides, serum albumin, C-reactive protein (CRP), blood lead, mercury, and cadmium concentrations, self-reported alcohol consumption (defined as non-drinkers: no alcohol consumption within the last 12 months; mild consumption: average of ≤1 drink per day for women and an average of ≤2 drinks per day for men, over the last 12 months; and moderate/heavy consumption: average of >1 drink per day for women and >2 drinks per day for men, over the last 12 months), estimated intake of total calories, fats, carbohydrates, protein, and cholesterol; and diabetes (defined as normal: plasma glucose < 100 mg/dl with HbA1c ≤ 5.7% and no diabetes diagnosis; pre-diabetic: plasma glucose < 126 mg/dl or HbA1c between 5.8% and <6.5%, or diagnosis as pre-diabetic; or diabetic status: plasma glucose ≥ 126 mg/dl, or HbA1c ≥ 6.5%, or a diabetes diagnosis). Each variable was first evaluated via univariate logistic regression, and selected variables ($p \leq 0.15$) were included in a saturated model for further evaluation as potential confounders. Final models included variables that, when removed from the model, resulted in a 10% change in the odds ratio (OR) for the THM exposure variable, plus covariates that were pre-specified effect modifiers, or variables that were statistically significant ($p \leq 0.05$). To examine the relationship between THM exposures and ALT activity, unconditional fixed-effects multiple logistic regression analyses (PROC SURVEY LOGISTIC) were used

to calculate prevalence ORs with 95% confidence intervals (CIs), after adjusting for the effects of selected potential confounding factors. Separate models were used to evaluate the relationship between ALT and each THM variable (bromoform, BDCM, DBCM, BTHM, or TTHM). Alcohol consumption and BMI were forced into each model. In separate analyses, linear regression (PROC SURVEY REG) was used to evaluate the relationship between THM exposure and ALT activity using continuous, log-transformed values of ALT and each THM variable. Modification of the relationship between ALT activity and THM concentrations by either alcohol intake or BMI was evaluated by initially testing their interaction with each THM variable. Stratified logistic regression analyses were then used to characterize exposure-related effects among participants grouped among different levels of the modifying factor (alcohol consumption or BMI), after adjustment for the selected covariates.

3. Results

After eligibility criteria were applied, the average age of the study population was 40 ± 0.3 years, and it was comprised of approximately equal proportions of women (53%) and men (47%, Table 1). A majority (72%) of the participants were non-Hispanic Whites (non-Hispanic Black: 10%, Mexican American or Hispanic: 13%), and had some college education (58%, Table 1). The population was distributed about equally among categories of normal, overweight and obese participants. Approximately 10% did not consume alcohol, whereas 61% had mild and 29% had moderate to heavy alcohol consumption (>2 drinks/day for men, >1 drink/day for women, excluding n = 674 who consumed >5 drinks/day, Table 1, Fig. 1). The distribution of blood and tap water THM concentrations among study participants is presented in Table 2. Correlations between blood and tap water for individual THM compounds were moderate (BDCM r = 0.69, DBCM r = 0.71, bromoform r = 0.45, BTHM r = 0.64, TTHM r = 0.46, all $p < 0.001$). There were 127 participants with tap water TTHM concentrations that exceeded the MCL (80 μg/L), which corresponds to 5.6% of participants. Elevated ALT activity was observed among 12% of the study population.

The relationship between blood THM concentrations and odds of elevated ALT activity is presented in Table 3. Cases with elevated ALT were 35% more likely to have circulating DBCM concentrations that exceeded the median concentration of the study population relative to those without elevated ALT activity (OR: 1.35, 95% CI: 1.02, 1.79, Table 3). In ancillary analyses, correlations between the selected covariates were examined, and the results obtained after removal of variables with low to moderate correlation (self-reported high blood pressure, C-reactive protein; all correlation coefficients < 0.45) were unchanged from those obtained using full models (data not shown). When the analysis was performed with adjustment for only age, BMI, and alcohol consumption, the cases with elevated ALT had increased odds of exposure to DBCM (OR: 1.48, 95% CI: 1.12, 1.95), bromoform (OR: 1.44, 95% CI: 1.04, 1.99), and BTHM (OR: 1.48, 95%

CI: 1.05, 2.10) relative to those with normal ALT activity, whereas the association for TTHM was marginal (OR: 1.35, 95% CI: 0.97, 1.89), and no association was observed for BDCM (OR: 0.99, 95% CI: 0.67, 1.46). Parameter estimates of the linear relationship between THM concentrations and ALT activity that were evaluated using continuous, log-

transformed values of both the exposure and outcome are presented in Table 4. In adjusted models, a positive association between blood bromoform concentrations and ALT activity was suggested (parameter estimate: 0.019, p = 0.059), although this and the other effect estimates were not statistically significant (Table 4). Variance inflation factors for covariates in the regression analyses were all ~1.0–1.3. Scatter plots did not indicate a nonlinear relationship between ALT activity and each THM variable (data not shown).

When interactions between THMs and alcohol consumption were examined, evidence for effect modification was suggested for BDCM (interaction p-value: 0.061) and bromoform (interaction p-value: 0.080; all other interaction terms for THM versus alcohol consumption: p > 0.10). Table 5 presents the relationship between THM exposures and elevated ALT status after stratification by alcohol consumption. Among non-drinkers, cases with elevated ALT activity were ~3–4 times more likely to have higher circulating concentrations of DBCM (OR: 3.30, 95% CI: 1.37, 7.90), bromoform (OR: 2.88, 95% CI: 1.21, 6.81), or BTHM (OR: 4.00, 95% CI: 1.31, 12.1) relative to those with normal ALT. Among participants who consumed low, or moderate to heavy amounts of alcohol, there were no statistically significant associations between THM blood concentrations and elevated ALT case status (Table 5). The p-values for the interaction terms between THM levels and BMI were: 0.228 (BDCM), 0.503 (DBCM), 0.700 (Bromoform), 0.495 (BTHM), and 0.648 (TTHM), indicating no interaction. Models stratified by BMI were produced for exploratory purposes (Supplemental Table S1). Among obese participants, elevated THM exposure was 6% (BDCM) to 55% (bromoform) more likely among cases with elevated ALT relative to those with normal ALT, although none of the ORs were statistically significant (Table S1). Finally, there was no evidence for temporal trends in the relationship between blood THMs and ALT activity when interactions between THM exposure and survey year were evaluated (data not shown).

4. Discussion

Few studies have evaluated whether THM exposure is associated with liver damage in human populations. Elevated serum ALT activity is a valid biomarker of liver injury, and studies in experimental animals indicate that elevated THM exposure can induce hepatotoxicity and elevated ALT activity (Seth et al., 2013; WHO, 2005). Results from the current study indicate that NHANES participants enrolled between 1999 and 2006 who had elevated circulating ALT activity were 1.35 times more likely to be exposed to elevated DBCM concentrations; and those with high ALT and no alcohol consumption, suggestive of NAFLD, were 1.6 to 4.0 times more likely to have elevated exposure to THMs. The use of quantitative blood THM values in this study allowed for more direct comparisons with ALT activity than environmental measures (e.g., tap water or air concentrations), and reduced or eliminated uncertainties related to characterization of exposure among participants due to recall or exposure-related behaviors. The arithmetic mean concentration of blood DBCM was 5.2 ± 5.8 pg/mL in the group with elevated exposures, and corresponding mean tap water DBCM concentrations in that group were 9.7 ± 9.2 μg/L. These values are well below the estimated biomonitoring equivalent concentration in the blood that corresponds to the EPA reference dose (80 pg/mL) (Aylward et al., 2008; LaKind et al., 2010), as well as the EPA and WHO benchmark levels for drinking water quality (80 and 100 μg/L, respectively).

Correlations between THMs in the blood and tap water were moderate, consistent with previously published results (Miles et al., 2002; Rivera-Nunez et al., 2012). Overall, TTHM exposures in this sample were relatively low; only ~5% of the study population had household tap water TTHM levels that exceeded the MCL. These levels are consistent with previously published values among NHANES participants during the same time frame (Riederer et al., 2014).

This study had several noteworthy strengths, limitations and uncertainties. One uncertainty is that the results observed for DBCM were not consistent with those obtained for other THMs. In the reduced model with only three covariates (age, BMI, alcohol consumption), more associations with brominated THM were observed, although the possibility of confounding cannot be eliminated. When exposure and outcome were modeled as continuous variables, the effect estimates did not suggest a strong association with ALT activity, however the relatively large proportion of samples below the limit of detection (21–55% for brominated THMs, Table 2) may have reduced the likelihood of detecting an association. The relatively low THM exposures in this population may have contributed to the inconsistent results observed among the different THMs, and the lack of effect modification among those with an elevated BMI. This was somewhat unexpected since the proportion of elevated ALT among those with obesity (51.4%) was 50% greater than the proportion of obese participants with normal ALT (30.8%, Table 1). Also, note that the ORs among obese individuals tended to be elevated relative to those in the lower BMI categories (Table S1). By contrast, there was only an 11% difference between elevated and normal ALT activity among those who did not consume alcohol (Table 1). In experimental mice, BDCM-induced hepatotoxicity was accentuated in obese mice, although the effects occurred at exposures above the equivalent MCL concentration (Seth et al., 2013). Others have reported that human hepatocytic microsomal CYP2E1 protein expression tends to be reduced with progression of NAFLD (Fisher et al., 2009), which would tend to attenuate any effect of THMs on ALT activity in a time- or dose-dependent manner. Polymorphic variation in CYP2E1 activity also may have influenced THM metabolism and contributed to differential responsiveness among participants (Neafsey et al., 2009). The observation that elevated ALT activity was associated with the modest DBCM blood concentrations identified in the current study may suggest a relatively low threshold of hepatotoxicity to this compound relative to other THMs. However, the cross-sectional nature of the study design precludes the establishment of a cause–effect relationship, and additional research is needed to evaluate that possibility.

When the relationship between DBCM exposure and ALT activity was evaluated with stratification by alcohol consumption, an association was observed only among non-drinkers, and similar associations were noted for bromoform and BTHM (Table 5). The ORs among non-drinkers with elevated exposure to these agents were more than double those obtained for all subjects combined, and the ORs also were elevated among non-drinkers exposed to BDCM (OR: 1.59) or TTHM (OR: 1.97), although those risk estimates were not statistically significant. These findings suggest that non-drinkers may represent a sub-population that is more susceptible to THM exposure. However, the reason for such an association is uncertain. The conventional theory of THM hepatotoxicity is that reactive metabolites and oxidative stress are generated within the liver via CYP2E1 activity (Seth et

al., 2013; WHO, 2005). Alcohol (ethanol) is a substrate for CYP2E1, and alcohol exposure can induce CYP2E1 enzymatic activity. Thus, elevated ALT activity might have been predicted among participants with combined exposures to alcohol and THMs rather than the absence of an effect that was observed in these groups. One possible explanation for these results is that chronic alcohol consumption may have resulted in upregulation of antioxidant activity such as increased glutathione biosynthesis or other compensatory metabolic processes that had a neutralizing effect on THM biotransformation and toxicity. Other evidence suggests that CYP2E1 may lose its inducibility with chronic alcohol exposure due to the effects of reactive metabolites that are formed as a result of ethanol bioactivation by CYP2E1 (Knecht et al., 1990). For example, CYP2E1 inactivation has been observed with reactive metabolites that are formed from carbon tetrachloride metabolism primarily by binding to the active site of the enzyme (Dai and Cederbaum, 1995). Nitric oxide released in long term alcohol drinkers also can inhibit CYP2E1 activity (Gergel et al., 1997). If this is the case with THM exposures, then it may explain why an association between THM exposure and elevated ALT was observed only among non-drinkers. Prospective or experimental studies are needed to more comprehensively evaluate these possibilities.

Strengths of this study included the incorporation of a comprehensive data collection protocol with stringent quality control, and a robust, population-based sample with excellent generalizability. Although not all known or suspected risk factors were evaluated, exclusion or statistical adjustment was achieved for major predictors of elevated ALT activity. Inconsistencies among the results for each THM and a relatively narrow range of exposure limit the interpretation of the findings. Also, the cross-sectional nature of this study precludes an ability to infer causality. However, the results suggest a need for follow-up to evaluate the potential hepatotoxicity of brominated THMs using a design that characterizes cumulative dose, time course, or other key toxicological parameters. Studies are needed of THM-induced hepatotoxicity that target ALT activity in conjunction with other biochemical or molecular markers on the continuum from early liver injury to NAFLD, NASH, and other more serious chronic diseases. For example, studies using prospective measures of ALT in conjunction with more definitive diagnostic procedures, or biomarkers of oxidative stress, genetic or epigenetic susceptibility, CYP2E1 or other enzyme induction, or the contribution of other DBPs, should help elucidate these mechanisms. Because liver diseases such as NAFLD have relatively high global prevalence and in many cases are asymptomatic, routine screening for ALT and related biomarkers may serve as useful clinical tools for early detection and prevention (Pacifico et al., 2013). THM levels exceeding exposure guidelines have been reported both in the US and in various regions globally (Fooladvand et al., 2011; Goslan et al., 2009; Mishra et al., 2014; Rivera-Nunez et al., 2012; Thacker et al., 2002; Tokmak et al., 2004; Uyak, 2006). If an association between THMs and NAFLD is confirmed, then continued surveillance of drinking water quality and ALT biomonitoring can be targeted as public health strategies that can help prevent NAFLD and its related hepatic or metabolic diseases (Andra et al., 2014).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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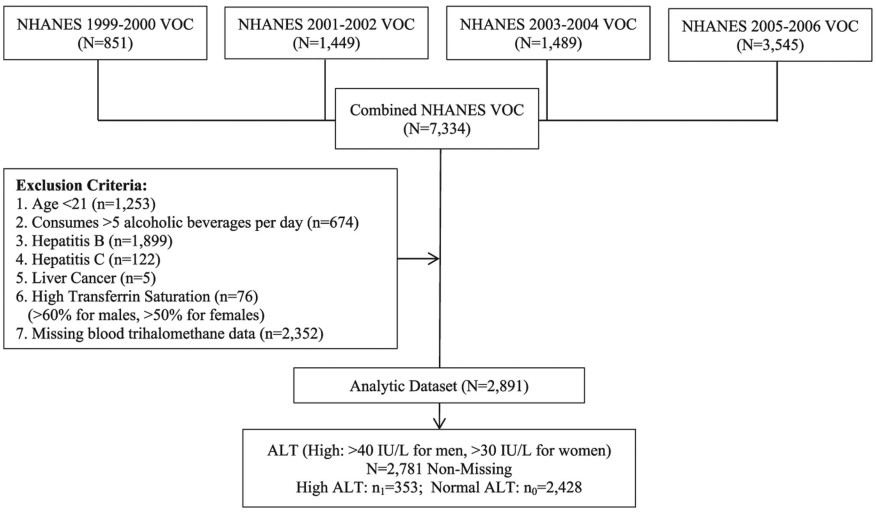

Fig. 1.

Schema for study population. Flow diagram of the study population size before and after applying eligibility criteria. Hepatitis B: positive laboratory test for hepatitis B surface antibody, core antibody or surface antigen. Hepatitis C: positive laboratory test for hepatitis C antibody. VOC: volatile organic compounds. ALT: alanine aminotransferase. NHANES: National Health and Nutrition Examination Survey.

Table 1

Characteristics of the study population, NHANES 1999-2006.

Variable	Study population (N = 2781)		High ALT[a] (n = 353)		Normal ALT (n = 2428)	
	n	Weighted % (95% CI)	n	Weighted % (95% CI)	n	Weighted % (95% CI)
Sex						
Male	1256	47 (45.2, 48.8)	181	54 (46, 61)	1075	46 (44, 48)
Female	1525	53 (51.2, 54.8)	172	46 (39, 54)	1353	54 (52, 56)
Age						
21 to 29 years	573	16.7 (14.8, 18.6)	71	15.6 (10.8, 20.4)	502	16.8 (14.7, 18.9)
30 to 39 years	598	23.4 (21.3, 25.6)	94	27.8 (22.8, 32.8)	504	22.8 (20.5, 25.2)
40 to 49 years	626	27.2 (24.6, 29.9)	95	30.4 (23.8, 37.1)	531	26.8 (23.9, 29.6)
50 to 59 years	475	21.4(19.1, 23.8)	58	20.2 (14.7, 25.7)	417	21.6 (19.1, 24.2)
60+ years	509	11.2 (8.2, 14.3)	35	6.0 (3.9, 8.2)	474	12.0 (8.5, 15.4)
Race						
Mexican American	603	8.3 (6.7, 9.9)	119	14.1 (10.7, 17.5)	484	7.5 (5.9, 9.0)
Other Hispanic	105	4.7 (3.2, 6.1)	23	8.7 (4.8, 12.6)	82	4.1 (2.7, 5.4)
Non-Hispanic White	1416	72 (68.6, 75.3)	142	63.6 (58.7, 68.5)	1274	73.2 (69.6, 76.7)
Non-Hispanic Black	569	10.5 (8, 13)	54	7.8 (4.9, 10.6)	515	10.9 (8.3, 13.5)
Other race or multi-racial	88	4.6 (3.4, 5.8)	15	5.9 (2.5, 9.2)	73	4.4 (3.2, 5.5)
Education						
<9th grade	313	5.5 (4.3, 6.6)	51	7.3 (4.5,10.1)	262	5.2 (3.9, 6.4)
9-11th grade	415	10.5 (9.3, 11.7)	53	11.1 (7.6, 14.6)	362	10.4 (9.1, 11.7)
High school diploma or GED	657	25.5 (23, 28)	83	24.5 (18.1, 30.9)	574	25.6 (23.1, 28.2)
Some college or post-high school training	823	32.4 (29.8, 34.9)	102	33.2 (26.5, 39.9)	721	32.3 (29.6, 34.9)
College graduate or above	570	26.2 (22.8, 29.6)	63	23.9 (17.3, 30.4)	507	26.5 (22.9, 30.1)
Marital status						
Married or living with partner	1814	68.4 (66, 70.8)	239	71.2 (64.6, 77.8)	1575	68.0 (65.6, 70.5)
Widowed, divorced, separated	497	16.4 (14.3, 18.5)	54	14.3 (10.1, 18.4)	443	16.7 (14.4, 19.0)
Never married	450	15.2 (13, 17.3)	58	14.5 (9.4, 19.7)	392	15.2 (13.1, 17.4)
Annual family income						
$0-19,999	670	18.2 (16.2, 20.2)	87	19.1 (14.6, 23.6)	583	18.0 (16.1, 20.0)
$20,000-34,999	523	16 (14.2, 17.7)	59	12.0 (8.7, 15.3)	464	16.5 (14.6, 18.4)
$35,000-64,999	736	30.1 (27.8, 32.4)	94	29.9 (23.4, 36.5)	642	30.1 (27.7, 32.6)
≥$65,000	740	35.8 (32.4, 39.1)	94	39.0 (29.9, 48.0)	646	35.3 (31.9, 38.7)
Smoking status						
Non-smoker	1512	51.8 (48.7, 54.8)	216	59.5 (53.1, 65.9)	1296	50.7 (47.7, 53.7)
Previous smoker	664	24.7 (22.1, 27.2)	71	22.76 (16.2, 29.3)	593	25.0 (22.4, 27.5)
Current smoker	604	23.5 (21.1, 26)	66	17.7 (12.6, 22.9)	538	24.4 (21.9, 26.8)
Alcohol consumption						
Non-drinker	356	10 (8.2, 11.7)	47	11.0 (6.88, 15.1)	309	9.8 (8.5, 11.6)

Variable	Study population (N = 2781)		High ALT[a] (n = 353)		Normal ALT (n = 2428)	
	n	Weighted % (95% CI)	n	Weighted % (95% CI)	n	Weighted % (95% CI)
Mild consumption[b]	1678	60.9 (58.4, 63.4)	212	60.7 (55.3, 66.1)	1466	60.9 (58.1, 63.8)
Moderate/heavy consumption[c]	747	29.1 (26.3, 32)	94	28.3 (22.7, 33.9)	653	29.3 (26.2, 32.3)
Body mass index						
<25 kg/m^2	841	33.6 (31.3, 35.8)	61	15.1 (10.9, 19.1)	780	36.2 (33.7, 38.3)
25 to <30 kg/m^2	918	33.1 (30.9, 35.4)	113	33.5 (26.9, 40.1)	805	33.1 (30.7, 35.3)
≥30 kg/m^2	980	33.3 (30.6, 36)	177	51.4 (44.5, 58.4)	803	30.8 (27.9, 33.6)
High blood pressure (self-reported)						
Yes	773	25.9 (23.6, 28.2)	107	34.3 (27.9, 40.7)	666	24.7 (22.3, 27.1)
No	1984	74.1 (71.8, 76.4)	240	65.7 (59.3, 72.1)	1744	75.3 (72.9, 77.7)
Diabetes status[d]						
Normal	1835	70.6 (68, 73.2)	210	62.5 (56.7, 68.3)	1625	71.7 (68.9, 74.5)
Pre-diabetic	632	20.9 (18.7, 23.2)	98	27.8 (21.9, 33.6)	534	20.0 (17.7, 22.1)
Diabetic	300	8.5 (7.3, 9.7)	42	9.7 (5.7, 13.8)	258	8.3 (6.9, 9.7)

Quantitative biological measures	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
Systolic blood pressure (mm Hg)	120 (119, 121)	124 (122, 126)	119.66 (118.4, 120.8)
Diastolic blood pressure (mm Hg)	72 (71, 73)	76 (75, 78)	71.4 (71, 72)
Total serum cholesterol (mg/dL)	203 (201, 204)	212 (206, 217)	201 (199, 203)
Albumin (g/L)	4.29 (4.27, 4.30)	4.33 (4.29, 4.37)	4.28 (4.26, 4.29)
C-reactive protein (mg/dL)	0.41 (0.37, 0.46)	0.52 (0.42, 0.62)	0.39 (0.35, 0.44)

ALT: alanine aminotransferase. CI: confidence interval. GED: general education development. NHANES: National Health and Nutrition Examination Survey.

[a] High ALT: >40 IU/L for men, > 30 IU/L for women.

[b] ≤2 drinks/day for men, ≤1 drink/day for women.

[c] >2 drinks/day for men, > 1 drink/day for women.

[d] Normal: plasma glucose < 100 mg/dL with HbA1c ≤ 5.7%, no diabetes diagnosis; pre-diabetic: plasma glucose < 126 mg/dLor HbA1c between 5.8% and <6.5%, or diagnosis as pre-diabetic; diabetic status: plasma glucose ≥ 126 mg/dL, or HbA1c ≥ 6.5%, or diabetes diagnosis.

Table 2

Distribution of trihalomethane concentrations in blood and tap water, NHANES 1999-2006 (N = 2781).

Trihalomethane	% below the LOD	Minimum	25th percentile	Median	75th percentile	Maximum
Blood concentrations (pg/mL)						
Bromoform	55	0.3	0.7	1.0	1.8	540
Chloroform	5	1.5	6.3	12.0	24.0	2000
BDCM	22	0.2	0.6	1.5	3.6	86
DBCM	46	0.1	0.4	0.4	1.7	81
BTHM[a]	21	0.7	2.1	3.6	7.6	550
TTHM[b]	3	2.6	9.9	18.1	33.8	2002
Tap water concentrations (μg/L)						
Bromoform	46	0.03	0.1	0.1	0.6	77
Chloroform	15	0.03	0.6	12	26	132
BDCM	15	0.03	0.5	4	8	52
DBCM	19	0.07	0.2	1	4	59
BTHM	14	0.18	1.5	6	13	124
TTHM	11	0.26	3.3	24	43	167

LOD: limit of detection. BDCM: bromodichloromethane. BTHM: total brominated trihalomethane. DBCM: dibromochloromethane. TTHM: total trihalomethane. The biomonitoring equivalents (the blood concentration corresponding to the EPA reference dose) have been estimated as 230, 20, 80, and 130 pg/mL for chloroform, BDCM, DBCM, and bromoform, respectively (Aylward et al., 2008; LaKind et al., 2010).

[a] Sum of bromoform, BDCM, and DBCM.

[b] Sum of BTHM and chloroform.

Table 3

Relationship between blood trihalomethane concentrations and elevated alanine aminotransferase activity, NHANES 1999-2006 (N = 2781).

THM exposure parameter	High ALT[a]	Normal ALT	Blood THM (pg/mL)[b]	Crude odds ratio	95% CI	Adjusted odds ratio[c]	95% CI
Bromodichloromethane							
Below LOD	75 (21%)	525 (22%)	0.4 ± 0.1	-ref-	-ref-	-ref-	-ref-
Below median	134 (38%)	953 (39%)	1.2 ± 0.5	0.88	(0.61, 1.28)	1.03	(0.72, 1.46)
Above median	144 (41%)	950 (39%)	6.1 ± 5.8	0.89	(0.61,1.31)	1.01	(0.67, 1.51)
Dibromochloromethane							
Below LOD	146 (41%)	1120 (46%)	0.4 ± 0.1	-ref-	-ref-	-ref-	-ref-
Below median	87 (25%)	656 (27%)	0.9 ± 0.3	0.82	(0.58, 1.16)	0.78	(0.54, 1.13)
Above median	120 (34%)	652 (27%)	5.2 ± 5.8	1.34	(1.02, 1.76)	1.35	(1.02, 1.79)
Bromoform							
Below LOD	185 (52%)	1320 (54%)	0.8 ± 0.2	-ref-	-ref-	-ref-	-ref-
Below median	69 (20%)	566 (23%)	1.2 ± 0.3	0.79	(0.62, 1.02)	0.75	(0.56, 0.97)
Above median	99 (28%)	542 (22%)	9.1 ± 29.2	1.39	(1.04, 1.86)	1.30	(0.91, 1.84)
Brominated trihalomethanes							
Tertile 1	98 (28%)	814 (34%)	1.9 ± 0.4	-ref-	-ref-	-ref-	-ref-
Tertile 2	111 (31%)	824 (34%)	3.8 ± 0.9	1.07	(0.76, 1.49)	1.04	(0.71, 1.51)
Tertile 3	144 (41%)	790 (33%)	17.2 ±26.9	1.38	(1.01, 1.89)	1.34	(0.93, 1.92)
Total trihalomethanes							
Tertile 1	101 (29%)	822 (34%)	7.4 ± 2.5	-ref-	-ref-	-ref-	-ref-
Tertile 2	122 (35%)	805 (33%)	17.9 ±4.0	1.29	(0.87, 1.89)	1.33	(0.92, 1.91)
Tertile 3	130 (37%)	801 (33%)	62.0 ± 88.9	1.25	(0.88, 1.76)	1.23	(0.87, 1.72)

ALT: alanine aminotransferase. CI: confidence interval. LOD: limit of detection. BDCM: bromodichloromethane. BTHM: total brominated trihalomethane. DBCM: dibromochloromethane. TTHM: total trihalomethane.

[a] High ALT activity >40 IU/L for men, >30 IU/L for women.

[b] Arithmetic mean ± standard deviation.

[c] Results for DBCM, bromoform, BTHM, and TTHM adjusted for the effects of: age, race, smoking, body mass index, alcohol consumption, self-reported high BP, diastolic BP, total cholesterol, and albumin. Results for BDCM adjusted for the same variables plus C-reactive protein.

Table 4

Relationship between blood trihalomethane concentrations and alanine aminotransferase activity, NHANES 1999-2006 (N = 2781).

THM exposure parameter[a]	Parameter estimate	p-Value	Adjusted parameter estimate[b]	p-Value
Bromodichloromethane	0.005	0.597	0.007	0.429
Dibromochloromethane	0.010	0.271	0.005	0.581
Bromoform	0.022	0.038	0.019	0.059
Brominated Trihalomethanes	0.012	0.220	0.010	0.332
Total Trihalomethanes	0.009	0.441	0.006	0.569

[a] Values for ALT activity and THM exposure were log-transformed.

[b] Results for DBCM, bromoform, BTHM, and TTHM were adjusted for the effects of: age, race, smoking, body mass index, alcohol consumption, self-reported high BP, diastolic BP, total cholesterol, and albumin. Results for BDCM were adjusted for the same variables plus C-reactive protein. ALT: alanine aminotransferase. CI: confidence interval. LOD: limit of detection. BDCM: bromodichloromethane. BTHM: brominated trihalomethane. DBCM: dibromochloromethane. TTHM: total trihalomethane.

Table 5

Relationship between blood trihalomethane concentrations and elevated alanine aminotransferase activity, stratified by alcohol consumption, NHANES 1999-2006 (N = 2781).

Exposure	Alcohol consumption	Corresponding tap water THM concentration (mean ± SD)	High ALT[a] n	Normal ALT n	Adjusted odds ratio[b]	95% CI
Bromodichloromethane						
Below LOD	Non-drinkers	1.0 ± 2.1	9 (19%)	48 (16%)	-ref-	-ref-
Below median		3.7 ± 3.7	15 (32%)	132 (43%)	0.49	(0.13, 1.69)
Above median		12.0 ± 9.5	23 (49%)	129 (42%)	1.59	(0.55, 4.50)
Below LOD	Mild consumption[c]	1.0 ± 1.8	47 (22%)	340 (23%)	-ref-	-ref-
Below median		4.8 ± 4.4	83 (39%)	556 (38%)	1.32	(0.90, 1.91)
Above median		11.0 ± 8.5	82 (39%)	570 (39%)	1.05	(0.65, 1.66)
Below LOD	Moderate/heavy consumption[d]	1.2 ± 2.2	19 (20%)	137 (21%)	-ref-	-ref-
Below median		3.9 ± 3.9	36 (38%)	265 (41%)	0.79	(0.34, 1.83)
Above median		9.9 ± 7.7	39 (42%)	251 (38%)	0.82	(0.31, 2.11)
Dibromochloromethane						
Below LOD	Non-drinkers	0.7 ± 1.0	17 (36%)	133 (43%)	-ref-	-ref-
Below median		2.1 ± 2.1	14 (30%)	88 (29%)	0.86	(0.33, 2.19)
Above median		10.8 ± 9.9	16 (34%)	88 (29%)	3.30	(1.37, 7.90)
Below LOD	Mild consumption	0.8 ± 1.2	92 (43%)	703 (48%)	-ref-	-ref-
Below median		2.5 ± 2.4	48 (23%)	369 (25%)	0.87	(0.51, 1.47)
Above median		10.3 ± 9.1	72 (34%)	394(27%)	1.37	(0.94, 2.00)
Below LOD	Moderate/heavy consumption	0.7 ± 1.1	37 (39%)	284 (43%)	-ref-	-ref-
Below median		2.0 ± 1.9	25 (27%)	199 (31%)	0.57	(0.29, 1.10)
Above median		7.9 ± 8.8	32 (34%)	170 (26%)	1.03	(0.56, 1.88)
Bromoform						
Below LOD	Non-drinkers	0.3 ± 0.9	24 (51%)	171 (55%)	-ref-	-ref-
Below median		1.3 ± 2.0	8 (17%)	66 (21%)	1.63	(0.54, 4.86)
Above median		3.2 ± 4.2	15 (32%)	72 (23%)	2.88	(1.21, 6.81)
Below LOD	Mild consumption	0.5 ± 2.8	118 (56%)	801 (55%)	-ref-	-ref-
Below median		0.9 ± 1.8	38 (18%)	344 (24%)	0.58	(0.38, 0.86)
Above median		3.8 ± 5.3	56 (26%)	321 (22%)	1.23	(0.78, 1.92)
Below LOD	Moderate/heavy consumption	0.3 ± 0.7	43 (46%)	348 (53%)	-ref-	-ref-
Below median		0.8 ± 1.9	23 (25%)	156 (24%)	0.88	(0.51, 1.50)
Above median		2.2 ± 3.0	28 (30%)	149 (23%)	1.09	(0.57, 2.07)
Brominated trihalomethanes						
Tertile 1	Non-drinkers	3.1 ± 4.0	10 (21%)	86 (28%)	-ref-	-ref-
Tertile 2		7.6 ± 6.0	15 (32%)	116 (38%)	1.26	(0.35, 4.53)
Tertile 3		23.3 ± 21.8	22 (47%)	107 (35%)	4.00	(1.31, 12.1)
Tertile 1	Mild consumption	3.7 ± 5.7	65 (31%)	521 (36%)	-ref-	-ref-

Exposure	Alcohol consumption	Corresponding tap water THM concentration (mean ± SD)	High ALT[a] n	Normal ALT n	Adjusted odds ratio[b]	95% CI
Tertile 2		8.8 ± 8.1	64 (30%)	478 (33%)	1.13	(0.73, 1.73)
Tertile 3		22.6 ± 19.8	83 (39%)	467 (32%)	1.29	(0.85, 1.93)
Tertile 1	Moderate/heavy consumption	2.7 ± 3.7	23 (25%)	207 (32%)	-ref-	-ref-
Tertile 2		7.7 ± 7.0	32 (34%)	230 (35%)	0.76	(0.32, 1.78)
Tertile 3		17.6 ± 17.7	39 (42%)	216 (33%)	0.97	(0.46, 2.05)
Total trihalomethanes						
Tertile 1	Non-drinkers	16.3 ± 22.3	13 (28%)	94 (30%)	-ref-	-ref-
Tertile 2		29.8 ± 25.7	17 (36%)	108 (35%)	1.65	(0.57, 4.71)
Tertile 3		39.1 ± 28.7	17 (36%)	107 (35%)	1.97	(0.66, 5.85)
Tertile 1	Mild consumption	14.3 ± 18.8	63 (30%)	504 (34%)	-ref-	-ref-
Tertile 2		32.3 ± 26.4	74 (35%)	477 (33%)	1.46	(0.90, 2.37)
Tertile 3		40.9 ± 27.3	75 (35%)	485 (33%)	1.17	(0.73, 1.85)
Tertile 1	Moderate/heavy consumption	14.6 ± 19.0	25 (27%)	224 (34%)	-ref-	-ref-
Tertile 2		27.7 ± 22.2	31 (33%)	220 (34%)	0.86	(0.43, 1.69)
Tertile 3		36.9 ± 26.2	38 (40%)	209 (32%)	1.16	(0.50, 2.68)

ALT: alanine aminotransferase. CI: confidence interval. LOD: limit of detection. BDCM: bromodichloromethane. BTHM: total brominated trihalomethane. DBCM: dibromochloromethane. TTHM: total trihalomethane.

[a] High ALT: >40 IU/L for men, >30 IU/L for women.

[b] Results for DBCM, bromoform, BTHM, and TTHM adjusted for the effects of: age, race, smoking, body mass index, self-reported high BP, diastolic BP, total cholesterol, and albumin. Results for BDCM adjusted for the same variables plus C-reactive protein.

[c] ≤2 drinks/day for men, ≤1 drink/day for women.

[d] >2 drinks/day for men, >1 drink/day for women.