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Division of Health Studies

A CASE-CONTROL STUDY OF NEURAL TUBE DEFECTS AND DRINKING WATER CONTAMINANTS

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ATLANTA, GEORGIA

A CASE-CONTROL STUDY OF NEURAL TUBE DEFECTS AND DRINKING WATER CONTAMINANTS

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EXECUTIVE SUMMARY

A statewide case control study of neural tube defects (NTDs) and certain drinking water contaminants was conducted in New Jersey. Cases and controls were population based for the years 1993-1994. Exposures were estimated both from public monitoring records concurrent with the fourth week of gestation and from tap water sampling at participants' residences one year later. There was general consistency between the exposure estimates and findings using these two sources of data.

We found elevated odds ratios (ORs) of 1.5 to 2.1 for the association of NTDs with total trihalomethanes (THMs). Most of the ORs were not statistically significant at the 95% confidence interval level, except for analyses limited to isolated defects and limited to subjects whose residence histories at time of conception were known. Associations of almost equal strength were also found for chlorine residual in tap water and for surface water source (i.e., regardless of THM concentration estimates). Other major groups of disinfection by-products (haloacetic acids, haloacetonitriles) did not show clear relationships to NTDs. Nitrates were not observed to be associated with NTDs. There were too few instances of chlorinated solvent contamination to assess the relationship of NTDs to those exposures. There was little confounding factors assessed through birth certificates and interviews of participating mothers. Other risk factors for NTDs in this study as derived from birth certificates and interviews were generally consistent with those seen in former studies of neural tube defects. Biological monitoring conducted on a subset of participant mothers indicated that exhaled breath after showering and urinary trichloracetic acid may be useful as exposure markers, particularly if beverage ingestion and other pertinent behaviors are known. Cases and controls were not distinguishable by these markers.

Strengths of the study included the statewide study base, use of objective data for exposure estimation, concurrent ascertainment and rapid contact of most subjects, and supplementation of monitoring data with sampling of index residences. However, inferences which can be drawn from the study are limited by the possibility of exposure misclassifications, loss of some incident cases due to prenatal diagnoses, possible participation bias, and low statistical power for some exposures.

The results on surface water and trihalomethanes are generally consistent with previous findings in New Jersey, and cannot distinguish between effects of THMs or other characteristics of chlorination or surface water. Previous study results of associations with nitrates over 2 ppm were not corroborated. There is also an intriguing suggestion from the data that disinfection by-product associations with NTDs were concentrated in the subset of subjects whose mothers reported lack of multivitamin or folate supplements before pregnancy. Such a possibility is consistent with biochemically-based hypotheses regarding interaction of chloroform and folate, and could lead to specific applications of this research for prevention if this observation is corroborated by follow-up investigations here or elsewhere.

Although a causal relationship between disinfection by-products cannot be inferred on the basis of the current observations, the study adds to the weight of evidence that disinfection by-

products may contribute to certain birth defects and suggests careful consideration of using current and emerging water treatment technologies designed to minimize exposure while ensuring protection from microbial contamination. Since the critical time for neural tube closure is during the first month of pregnancy and inhalation is a major route of THM exposure, no advisories regarding drinking water substitution or point of use treatment for pregnant women are applicable at this time. Continued assurance by all authorities of monitoring and assurance of strict drinking water quality in accordance with current State drinking water quality standards is urged; the successes of such efforts since 1985 are responsible for the scarcity of public water contamination by chlorinated solvents in New Jersey. Nutrition and vitamin supplementation assuring sufficient folate and other B vitamins prior to conception and early prenatal care are advised as the best preventive actions for neural tube defects.

A CASE-CONTROL STUDY OF NEURAL TUBE DEFECTS AND DRINKING WATER CONTAMINANTS

INTRODUCTION

Neural tube defects (NTDs) characterized by incomplete closure of the spinal column and/or cranium, comprise one of the most prevalent birth defects. As defined here they include anencephaly, spina bifida and encephalocele. These defects, when compatible with post-natal survival, often involve lifelong disability as well as great suffering and medical expense for the affected individuals and their families. NTDs have been extensively studied, but the underlying etiologies and attendant opportunities for prevention have been elusive, although recent identification of folate deficiency as a risk factor can result in major decreases of incidence. Since closure of the neural tube occurs around the fourth week of gestation *(1,2)* maternal factors contributing are likely to operate before the time that pregnancy has been verified or even perceived. Therefore, it is important to focus on preconception and periconceptual exposures and behaviors when seeking maternal risk factors for NTDs.

This study followed up on previous epidemiologic investigations of public drinking water and adverse reproductive outcomes, including neural tube defects conducted by the New Jersey Department of Health and Senior Services (NJDHSS, formerly Department of Health) under a cooperative agreement with CDC, funded by ATSDR. The earlier project included a birth-certificate based cross-sectional study of selected birth defects in one region of New Jersey complemented by a case-control telephone interview study *(3-5)*. The current study built upon the research methods developed under the former project. It also utilized prompt ascertainment of cases and controls after birth, in-home interviews, tap water sampling in current residences during the same season of the year as the critical time for neural tube development, and assays of other major classes of disinfection by-products in addition to trihalomethanes (THMs). This study was statewide and focused on only one type of health outcome, expanding its statistical power to test a few specific hypotheses.

HYPOTHESES

This case-control study was designed to test the hypotheses of an association of neural tube defects with maternal exposure during early gestation to drinking water chlorination by-products (THMs and other by-products), nitrates, and the chlorinated solvents trichloroethylene, perchloroethylene, dichloroethylenes, and carbon tetrachloride. These hypotheses were based upon the positive associations observed in the former studies in New Jersey.

RECENT TRENDS IN NEURAL TUBE DEFECT INCIDENCE AND PREVALENCE

Worldwide and U.S. prevalences of neural tube defects have been consistently declining during the past several decades *(6,7)* and U.S. patterns have been consistent with these trends. A

major contributor to the decreasing prevalence, especially for anencephaly, has been the growing and widespread use of prenatal detection, particularly first trimester sonography *(8-13)*.

PREVIOUSLY REPORTED RISK FACTORS FOR NEURAL TUBE DEFECTS

Despite decades of research on neural tube defects, their major causes remain obscure. A combination of inherited and diverse environmental factors appear to interplay in their etiology.

1. Sociodemographic

Traditional risk factors for NTDs include low socioeconomic status *(7)*. In the previous New Jersey regression analyses, NTDs were found to be correlated with low maternal education, low income, inadequate housing and inadequate prenatal care *(14,15)*

2. Race and Ethnicity

Wide variation of NTDs among different nations and ethnic groups has been well documented. In addition to people of Celtic descent, many Hispanic populations monitored, including those of Mexico and South America (especially Chile) are higher than most other nations for which systematic data are available *(6,7,16,17)*.

3. Dietary Factors

In recent years, folate deficiency during early pregnancy became established as a strong risk factor for neural tube defects *(18-20)*.

4. Seasonality of Conception

The peak months reported for conception of NTDs have varied among investigations conducted in different geographic areas. A predominance of studies in which season was specified indicated a tendency for NTD births to be conceived in the spring or summer *(7,21-23)*. Such observations are consistent with a role for environmental contaminants which tend to peak in warmer months. While not all the pertinent reports reviewed found statistically significant patterns, underlying phenomena involving seasonal changes appeared to be worthy of special attention in the current study because of the potential influence of season on environmental factors such as water quality in temperate regions such as New Jersey. In particular, concentrations of disinfection by-products in New Jersey drinking water tend to peak during the months of July through September.

5. Parental Smoking

Parental smoking has been associated with NTDs in only a few studies *(7,24)*.

6. Medical Conditions of Mother

Certain medical conditions during early pregnancy and their treatments, including diabetes, epilepsy, high fevers, and influenza have been previously identified as risk factors *(7)*. The earlier New Jersey studies observed consistent findings relating to high fevers *(4)*.

7. Other Maternal Factors

Extremes of maternal age, high parity, history of stillbirths, and family history of NTDs have been identified as risk factors in previous reports *(7)*.

8. Occupational Exposures

(a) Paternal.—Numerous reports regarding paternal occupational associations with NTD *(7)* have suggested associations with various occupations and industries, particularly for painters, metal workers, farmers, electrical workers, and woodworkers *(25-27)*. However, a recent report *(28)* did not corroborate the earlier observations except for carpenters and woodworkers. Other investigators *(29)* found anencephaly to be associated with solvent exposures among painters and their data also suggested a pesticide association. Children of agricultural workers, including gardeners and groundsmen likely to handle 2,4,5-T, were observed to have increased risk ratios of spina bifida *(30)*. Hypotheses linking spina bifida to 2,4,5-T exposure in Southeast Asia have also been proposed *(31)*.

(b) Maternal.—Maternal occupational exposures were recently investigated in relation to spina bifida *(32)*. Agriculture workers had higher odds ratios (ORs) for these defects, but the associations did not seem to be related either to pesticides or any other specific exposures; women employed in cleaning also had elevated rates, but neither pesticide nor disinfectant exposure were seen. Increased spina bifida and anencephaly were previously observed in offspring of nurses *(26)*. Others reported that CNS defects were associated with exposure of mothers to solvents during the first trimester *(25)* and more congenital defects (primarily spina bifida) among children of women exposed to metallic mercury through their work in dental surgery *(33)*.

9. Environmental Exposures in the Community Setting

Few specific environmental exposures have been found to be associated with NTDs, and few drinking water investigations of these defects were conducted until the former New Jersey study *(3-5)*.

(a) Drinking Water.—Most previous drinking water studies of NTDs focused on inorganic contaminants. Nitrates were found to be positively associated with NTDs in an older Australian study *(34)* but other studies have not corroborated these findings *(35,36)*. Lack of water hardness had previously been suggested as a possible etiologic factor in NTDs but studies in Canada which supported this notion *(37)*. It was also suggested *(38)* that hard water may mitigate contributors to anencephaly etiology by other factors, such as deficiency of a trace element. An investigation in

Canada *(39)* found no association of NTDs with hardness or nitrate concentrations. A recent review has suggested hat arsenic exposures could be a risk factor *(40)*. Others *(36)* noted elevated ORs lacking statistical significance for associations of NTDs but associated with residence in single family dwellings, "climatic stagnation", and private well water.

(b) Radiation.—Radiation exposure has been documented as a risk factor *(16)*. Variations in NTD incidences in Turkey and other countries of Eastern Europe were noted after the Chernobyl accident, but their association with radiation has not been established *(41)*.

(c) Hazardous Waste Sites.—Recent work on neural tube defects and other birth defects relating to residence in proximity to hazardous waste sites has suggested associations with exposure to chemical toxins from these sites. Reports from California *(42)* found elevated odds ratios of NTDs for "potential" or "definite" exposure to hazardous chemicals from Superfund sites. Specific media, certain chemicals or chemical classes, and defined distance limits from sites also yielded elevated ORs, albeit with confidence intervals including 1.0.

PREVIOUS NEW JERSEY STUDIES OF WATER CONTAMINANTS AND NEURAL TUBE DEFECTS

Before the previous New Jersey studies, there had been no reported investigations of the reproductive effects of common drinking water contaminants at the relatively low levels commonly found in public drinking water systems. It was important to follow up the leads which were generated in order to provide more reliable guidance on possibly preventable risk factors for neural tube defects and other adverse reproductive outcomes. The current study was therefore designed to either provide corroborating evidence or to countervail the earlier findings. The current study has been conducted in a subsequent birth population, a larger geographic area (statewide), includes private wells in addition to public water systems, and can account for more detailed information on alternate routes of exposure and of alternate sources of exposure for the chemicals being investigated. However, contaminants typical of groundwater (including common chlorinated solvents) now have lower concentrations in most public groundwater systems in New Jersey than they did when the previous study was initiated, in part because the current State concentration limits, monitoring requirements, and enforcement program have become stricter and more effective *(43,44)*. In contrast, the levels and regulations for the typical surface water contaminants, especially THMs, have not substantially changed since the initiation of the previous NJDHSS studies. In fact, there has been a tendency for contaminated public groundwater sources to be substituted by surface water sources.

The two related New Jersey studies focused on seventy five towns among four counties in the State where the study population was served primarily by public water systems. The New Jersey Birth Defects Registry (BDR) and Fetal Death Registry, together with individual birth certificates were utilized to ascertain the birth defects series and other adverse pregnancy outcomes such as low birthweight for the years 1985-1988. Infant death certificates were also searched and did not identify any further cases. The drinking water parameters which were analyzed were total trihalomethanes, the concentrations of individual and aggregated volatile organics, nitrates, and the type of drinking water source (groundwater only, surface water only, or mixture of the two). Estimates of exposure

for each month of the study period were constructed from sampling data obtained from New Jersey Department of Environmental Protection (DEP) and verified by the water companies. However, these exposure estimates required numerous assumptions about residency history and utilization of water in the home. The first of the two individual-based studies performed used vital records data for the entire study population but no interviews. All unaffected births constituted the controls. Some information on some potentially confounding factors could be obtained via the birth certificates. This cross-sectional study included over 80,000 subjects and therefore had extensive statistical power. In contrast, a concurrent and related study utilized a case-control design to sample the study population and collected more detailed information on each subject through phone interviews with mothers of the subjects. The participation rates were 63% for the 68 cases and 55% for the 271 controls.

Based upon an analysis including and not including the mothers who were ascertained but not interviewed, selection bias appeared to influence the observed associations of neural tube defects with trihalomethanes and nitrates. Consequently, rapid ascertainment and rapid scheduling of data collection in the current, follow-up study have been emphasized in the current report. In both former studies there were uncertainties regarding the relative exposures via ingestion, dermal, and inhalation routes and the present investigation was designed to evaluate these distinctions.

METHODS

The methods of this study were designed to utilize and extend those developed in the earlier New Jersey investigations and also to address some of their limitations.

DEFINITION AND ASCERTAINMENT OF SUBJECTS

1. Study Base

All New Jersey births during the two year period of 1993 and 1994 comprised the study base. About 125,000 births per year occurred.

2. New Jersey Birth Defects Registry and Fetal Death Certificates Registry

The New Jersey Birth Defects Registry (BDR) became population-based in 1985. It uses a modified passive ascertainment system. All maternity hospitals in the State are required to report to the Department. Registry staff conduct annual inspections of each hospital for completeness and accuracy of data, and they follow up on all reports received which need additional information or clarification of data submitted *(14,45)*. The Fetal Death Registry, located in the Center for Health Statistics, receives reports of stillbirths of 20 or more weeks gestation.

3. Definition and Identification of Cases

During the data collection phase of the study, staff of the New Jersey Birth Defects Registry (BDR) regularly extracted pertinent information on new registrations of neural tube defects, and study staff received these reports within a few days. Some cases of anencephaly were identified through the Fetal Deaths Registry. Certificates of fetal and infant deaths were retrieved through regular scans of reports arriving at the Center for Health Statistics. A previous comparison of rates of selected groupings of congenital anomalies drawn from several other birth defects monitoring programs in the U.S. with the BDR indicated that ascertainment of neural tube defects has been relatively complete, probably due to the more active use of the fetal death and infant death surveillance in conjunction with the registrations of live births with congenital anomalies *(14)*.

All BDR registrations among births in the study base and received by the BDR during the period of fieldwork were included. Cases were assigned diagnostic codes according to diagnoses as they appeared on the birth defects registration or on the fetal death certificate. In addition, each case was categorized as to "isolated" (single) or "multiple" defect, using the criteria of CDC *(46)*. Table 1 shows the cases according to the above categories. In all instances in which the primary records were ambiguous as to whether a birth was affected by an NTD or whether the defect was single or multiple, the attending physician's office was contacted in order to obtain clarification. In several instances, medical records were sent to CDC for review by a dysmorphologist.

4. Number and Selection of Controls

The sampling was conducted so as to yield approximately twice the number of controls as cases in order to maximize statistical power given existing personnel resources. Because birth certificate rolls for the state were incomplete until more than a year after the control births needed to be identified, the following procedure was used for each month during the study period. For the month two years prior to the pertinent month, all New Jersey birth records were sorted by hospital and date. A series of random numbers was generated based upon the total number of births in the month. For the births corresponding to a sufficient quantity of random numbers, we recorded the corresponding hospital, birth date, and order of appearance in the list within that date. During each month during the study period, the same hospitals were contacted to ascertain the current month's births which corresponded to births two years earlier with respect to date and order on the birth log. The names, addresses, and telephones of the control newborns and their mothers were then queried from those hospitals. Enough potential control births were identified to yield about eight controls per month for the study period. Most controls were identified during the month following their birth. Term births identified by the hospital staff as less than 2500 grams and children with other birth defects were excluded from the control series because these categories were observed in the previous New Jersey study to be associated with THMs and other specific water contaminants comprising the hypothetical risk factors for this study.

5. Contact of Subjects' Mothers

Letters and telephone calls were used to approach mothers of ascertained subjects. Written and oral contact in Spanish was utilized as needed. Every attempt was made to obtain full participation, including emphasis on the value of the household tap water tests results. Partial participation was accepted where the mother did not consent to either the full interview or to all of the environmental measurements in the home. A potential participant without a listed telephone number and who did not respond to our repeated letters was considered a refusal if she signed for a certified letter or if we had knowledge that she was familiar (such as via family members) with our efforts to obtain her participation. Refusal conversion was attempted by follow-up letters three or more months after the original refusal in all instances in which it was reasonable to believe that there could be a change of decision to not participate.

TIMING OF CONTACT AND ENVIRONMENTAL ASSESSMENT

This study was designed to minimize the loss of study subjects due to inability to contact their mothers, particularly since difficulties in locating subjects' mothers constituted the single greatest source of subject loss (especially for controls) in the previous case-control study.

The design and procedures for implementation of this study reflect the issue of seasonality in NTDs and the critical period of neural tube development, i.e., the 4th week of gestation. We attempted to minimize errors of environmental estimates of exposure during pregnancy for variables which are sensitive to seasonal variation (such as chlorination by-products and other contaminants of surface water) by selecting the same time of year for these measurements as the critical period of embryonic development for the birth defects in question. For term births, the seventeenth week (i.e., four months) after the due date was the preferred time for the residential visit for sampling and interviewing, for both cases and controls, because it represents the time of year at which neural tube development took place in utero.

In addition, ethical considerations required a lapse of several months before contacting a family who had faced a traumatic health crisis such as birth of a child with a major congenital anomaly. Consequently, we did not contact these families until at least three months after the birth, regardless of the date of receipt of the registration. For cases, the physician's advice was sought prior to contact. There were no cases lost due to inability to contact the subject's mother on the basis of medical contra-indication.

During 1993 and 1994 the majority of neural tube defect registration forms were received by the BDR within four weeks of birth. Therefore most case families were able to be contacted within three months after birth, and we were able to schedule and to visit current households during the fourth month after birth for most participants (see Appendix B).

For cases of anencephaly ascertained through fetal death records, the lag before notification of study staff was somewhat longer. However, for premature births, particularly anencephalics, there

was more time available between delivery and the target date for home visits. When visits could not be accomplished within four months of the stillbirth, they were scheduled as soon as feasible. In some instances, tap water sampling was conducted two years (instead of one year) after the critical time in order to obtain samples during the appropriate season.

WATER QUALITY DATA AND ENVIRONMENTAL MEASUREMENTS

1. Sources, Monitoring, and Quality of Drinking Water in New Jersey

About 90% of the New Jersey population is served by public drinking water; approximately 30% of these by public wells, 35% by surface water, and 25% by systems which use a mixture of these two, either simultaneously or seasonally. Typical contaminants of surface water are chlorination by-products, such as THMs, formed through the chemical interaction of chlorine and naturally occurring humic substances derived from vegetable matter. Contaminated wells are typified by solvents and other substances, such as trichloroethylene, perchloroethylene, and carbon tetrachloride which cannot evaporate from groundwater; on the other hand, the humic substances are rarely present in quantities permitting formation of significant quantities of chlorination by-products in groundwater.

(a) New Jersey "A-280" Drinking Water Database.—Beginning in 1985, the NJ Safe Drinking Water Act of 1984, known as the "A-280" law, required semi-annual testing of all public water systems for designated organic compounds including the following volatile organics: trichloroethylene, perchloroethylene, carbon tetrachloride, dichloroethylenes, dichloroethane, trichloroethane, and benzene. The schedule of testing was changed during 1993 in conformance with new U. S. Environmental Protection Agency rules *(47)* which decreases the frequency of mandated testing for systems which initially test "clean" and which have characteristics consistent with very low risk of contamination. Until that time, public water companies were required to sample their distribution systems twice annually for the designated chemicals and NJDEP-approved laboratories use standard QA/QC procedures to perform the sample analyses. The DEP drinking water database identifies the water company, date of the sample, the type of sample (raw water, point of entry into system, or a distribution sample), the contaminants found, and their concentrations. When contamination is detected, in order to determine whether use of a supply needs to be halted, DEP typically requires additional sampling. Some systems with contamination problems have been sampled monthly.

(b) Trihalomethane Database.—New Jersey public drinking water systems that serve at least 10,000 people are required to test quarterly for trihalomethanes in their distribution system (i.e., after chlorination). There is virtually complete compliance. Most of the excluded systems rely on groundwater sources that are relatively free of the organic material necessary for the creation of THMs. Consequently, concentrations of total THMs in those systems are usually below 5 ppb.

(c) Nitrate Database.—Nitrate concentrations were previously recorded in DEP databases from triennial sampling for public drinking water supplies. Like VOCs, as of January 1993, the

USEPA mandated a nitrate monitoring strategy which decreased the frequency of nitrate monitoring for systems with characteristics consistent with low risk of contamination *(47)*.

(d) Federal and State Standards.—For the pertinent drinking water contaminants, current standards are shown in Table 2. The vast majority of public drinking water systems have been in compliance with these standards for solvents since before 1990. However, solvents have frequently been found in private wells, particularly in certain counties where extensive contaminated wellfields have been documented. Conversely, exceedences for disinfection by-products are common among surface water systems. The current maximum contaminant level (MCL) for total trihalomethanes, 100 ppb, is not health-based and is currently proposed for revision by USEPA such that total THM MCL would be reduced to 80 ppb. As of early 1997, there has been no further action on the proposed change.

2. Retrieval of DEP Data for Public Systems

For each ascertained study subject, information on the pertinent water company's distribution system and monitoring results approximating the critical period for neural tube closure and also one year later were retrieved from DEP files. Available information on specific address and estimated date of conception were utilized for this process. Discussions were held with water company staff in order to select the most appropriate monitoring sample for the specific addresses. In some instances, infrequent sampling meant that exposure over a considerable period of time was estimated by a single sample. Nitrate and VOC data were incomplete after the first birth year of the study due to two factors: (a) the changes in regularity of monitoring data collection for nitrates and the paucity of detectable VOCs in any public drinking water sampling, as evidenced during the first year of such data collection; (b) an evaluation of the first year led to a decision to use resources more efficiently by focusing only on regular THM data retrieval from monitoring data, since data from any systems contaminated with solvents could be retrieved, if necessary, at a later date. (See Appendix A for further description on how the exposure estimates were conducted.)

3. Retrieval of Private Well Data from DEP and Local Health Departments

Pertinent information was sought from local officials for subjects deriving water from private wells and from DEP private well contamination files, where available. The participants were asked the block and lot number for their residence in order to facilitate retrieval of this information.

4. Tap Water Sampling from Residences

(a) Chemical Assays Conducted for All Participants.—Drinking water samples were collected from the tap. Samples were analyzed for trihalomethanes, total and free chlorine, other volatile organics regulated under the NJ A-280 legislation (including TCE, PCE, carbon tetrachloride, benzene, the DCEs, trichloroethane, dichloroethane) and nitrates. Lead and mercury were also analyzed, principally as an education and prevention service for the participating mothers.

(b) Other Disinfection By-Products.—In counties and municipalities which had the potential to use surface water, other disinfection byproducts were also assayed. After the second month of field work, haloacetonitriles were assayed, and for the last fourteen months of data collection, haloacetic acids were also assayed. The USEPA protocols utilized by the NJDHSS laboratory for the assays listed above were methods 524.2, 551, 552, and 353.1 *(48-51)*. The tap water samples were generally collected immediately after the interview. Standard procedures were used for collecting, preserving and transporting samples *(51)*.

(c) Neighborhood Facility Surrogates.—In some instances, the relevant tap water samples could not be collected at the index residence. However, if the index residence during the critical period was known, surrogate sampling was conducted in the immediate neighborhood. Tap water samples at surrogate locations near the index residence were drawn under these circumstances: (1) if the subject's family had moved since the target sampling period and we could not gain access to the index address; (2) tap water sampling could not be conducted in the home but residency history was obtained from the mother by telephone; (3) the subject's mother did not participate at all but pertinent residential history was obtained through another reliable source, such as the obstetrician. The surrogate locations were usually public buildings such as fire stations or police stations. Surrogate locations were ultimately utilized for 25.5% of sampling, (21% and 28% for cases and controls, respectively).

5. Naturally-Occurring Radiation

Observations in New Jersey and elsewhere during the past decade have documented extensive exposure of the population to geologically-derived radiation. The component which comprises indoor exposure to radon and its short-lived decay products is characterized by individual variation among and within neighborhoods. Crude surrogate measures of such radiation were made in each home visited via microR readings for background gamma radiation. These readings could be accomplished immediately and without laboratory costs or the necessity of return visit or mailing of a detector.

INTERVIEWS OF SUBJECTS' MOTHERS

1. Interview Content

The interview questionnaire was adapted from the instrument used for the previous NJDHSS telephone interview study conducted here *(4)* with input from ATSDR, CDC and numerous other researchers conducting similar investigations. It was structured to document known risk factors for neural tube defects and it queried behaviors and exposures for the three months before conception and for the first trimester of the index pregnancy. It included details about the number and outcome of pregnancies, medical conditions and treatments, parental occupational exposures, and the home environment. It also included details about water usage which were designed to facilitate more accurate estimation of ingestion, inhalation, and dermal exposures than reliance only on tapwater or system water sampling. Specific questions designed for this study included those about swimming pool use; consumption of beverages made with tapwater; description, including location, operation,

and purpose of any water filters used in the home; location and type of water used at workplaces for women who worked outside the home; specific medications believed to be risk factors for NTDs, consumption of breakfast cereals which provided the recommended daily intake of folate and other vitamins of interest to NTDs; family history of NTDs, prenatal diagnostics, and ethnic origin of both parents.

2. Interview Procedures

The interviews were conducted by staff members of the NJDHSS who were trained with the questionnaire. The interviewers were not cognizant of the public water sampling results related to the subjects' households, but they knew whether the source of water might be surface or mixed. The same person generally scheduled and conducted the home visit. The interviewers could not avoid knowing the case or control status of the subjects. Before commencing questions, a form with the dates defining the timeframes of interest for the particular pregnancy were presented visually and discussed with the participant to maximize accuracy of responses regarding occurrences before and after estimated dates of conception one year earlier. The interviews took approximately 70 minutes to administer (unless there had been a very large number of previous pregnancies).

While most of the interviews were complete and were conducted in the respondent's home, some potential respondents did not consent to a home visit or the full interview, or could not be visited for some other reason. In such instances, a telephone interview, abbreviated interview, or both were conducted instead. Telephone interviews were utilized for about 10% of interviews, with similar proportions for cases and controls. Abbreviated interviews were utilized for 16% and 12% of cases and controls, respectively (see Appendix B).

For participating mothers who spoke only Spanish, another staff member served as interpreter in scheduling appointments and conducting abbreviated or full interviews. In instances when the potential participant spoke neither English nor Spanish, attempts were made to enlist the assistance of native speakers from Department staff or from friends, relatives, or health care providers of these participants.

AVOIDANCE OF BIASES

The following procedures were implemented or attempted in order to minimize biases in data collection.

1. Investigator Bias

To minimize investigator bias, the study protocol separated the basic data collection relating to exposure from knowledge of outcome. As described above, the interviewers necessarily knew the case or control status of the subjects, but were not aware of the degree of contamination of the water system. They also necessarily found out in the course of the interview whether the drinking water

source was a private well. In order to avoid the expense of assaying disinfection by-products other than THMs from areas served entirely by groundwater, the field staff were aware of such regions of the State.

The exposure assignments of each subject, on the basis of laboratory analyses of tap water and retrieval of DEP monitoring data, were made without knowledge of the case or control status of the subject. The laboratory personnel who analyzed the tap water samples had no knowledge of either the case/control status or the addresses of the subjects. Follow-up counseling and education were provided without knowledge as to the case or control status of the subject.

2. Recall Bias

Differential recall regarding pregnancy history and pertinent exposures by cases and controls can influence results of a retrospective investigation. The design of this study included the following elements in order to reduce such possibilities to the greatest extent feasible: (a) for both categories of participants, the interviews were planned for one year after the period of interest for exposures in order to minimize elapsed time since the first trimester and in order to query behaviors and exposures during the same season as the time period of interest; (b) in order not to focus the attention of participating mothers on the primary hypotheses of the study, they were not specified in the recruitment process, and the questionnaire dealt with many subjects in addition to drinking water, and the tap water sampling included metals such as lead and mercury which were not hypothesized as potential risk factors for neural tube defects.

3. Participation Bias

Monetary incentives could not be offered to potential participants under the rules of the funding authority. However, contacts with potential subject households emphasized the value of tap water analyses, and many participants were motivated by the tests for lead in water. Concerted efforts were made to recruit the largest proportion possible of mothers of ascertained subjects. Abbreviated interviews and/or telephone interviews were offered where necessary. Birth certificates for all subjects were obtained in order to enable key analyses to include all ascertained subjects, regardless of participation. (These data on potential participation bias are presented in the Discussion chapter.)

4. Selection Bias

Although an attempt was made to evaluate the demographic characteristics of incident cases which were detected prenatally and aborted, it was determined that systematic characterization of such potential cases was not possible in New Jersey during the period of the study. Comparable data from California were recently evaluated *(13)*.

QUALITY ASSURANCE/QUALITY CONTROL

1. Pilot Interviews and Tap Water Sampling

Pilot exercises were conducted whereby the NJDHSS research team conducted interviews and acquired water samples from non-subject volunteer residences. In addition, a series of trip blanks were analyzed concurrently with study samples, particularly during the early months of field work and sporadically during the rest of the two years of tap water sampling. (see Appendix B for a table presenting the number and timing of trip blanks collected and analyzed for quality assurance).

2. Tap Aerators

The standard protocol for tap water sampling of volatiles includes removal of aerators from faucets in order to decrease likelihood of headspace bubbles in sample vials. In many residences it was too difficult to remove aerators. Eleven tap water samples were drawn with the aerator both on and off in order to ascertain whether the presence of the aerator appreciably and consistently affected the outcome. For nine of these sample pairs, there was no substantial difference in the result. The other two diverged in opposite directions. In the case control analyses, therefore, the aerated sample result was used for instances in which two samples were collected. Not only did aerated faucets represent the vast majority of samples, but they also represent the normal condition of tap water utilized by residents.

PROTECTION OF SUBJECTS

Appropriate procedures for assurance of confidentiality were instituted by the research staff, consistent with standard protocols of the Department. Follow-up counseling and education regarding sampling results were offered by study staff. In several instances, repeat water samples were drawn by the study staff at the residence or by DEP at a nearby location (without knowledge of the index address or other personal information) as a result of exceedence of state or federal standards, but only the initial readings were used for exposure estimates using tap water.

STATISTICAL ANALYSIS

1. Unadjusted Tests of Association of Neural Tube Defects with Exposures Studied

This case control study was analyzed using odds ratios as a measure of strength of associations found between the exposures of interest and NTDs. Stratified 2 x K tests (exact method) and logistic regression analyses were conducted using the statistical package EGRET *(52)*. Statistical significance was evaluated using 95% confidence intervals around the ORs. Chemical exposures were grouped into categories based upon the underlying distribution of exposures in the controls and according to previously or commonly used concentration increments.

2. Adjusted Analyses

Potential risk factors from birth certificates and interview responses were selected for exploration according to previously published observations of associations with NTDs or according to systematic explorations of the birth certificate and questionnaire data for factors which were predictive of case or control status in this study. Any such factors which were related to the outcome with odds ratios greater than 1.5 or less than 0.67 were individually tested with the exposure of interest. If the adjusted odds ratio for the association between water contaminant exposure and NTDs ~~did not vary~~ varied by at least 10% from that of the unadjusted OR then the additional factor was retained *(53)*. For the purpose of comparing adjusted and unadjusted ORs, the unadjusted ORs were based on those subjects for whom data on the factor in question was not missing in the adjusted analysis. The analysis strategy called for such factors to be added one at a time, in order of strength in affecting the OR of the exposure of interest, until the resulting OR did not change by 10% or more.

Potential effect modification was also examined for important NTD risk factors in this study; we examined the probability value of the common odds of stratified 2 x K tables.

BIOLOGICAL MONITORING PILOT STUDY

A subset of participants was selected for a biological monitoring investigation of disinfection by-products. This component of the overall study was conducted by collaborating scientists at the University of Medicine and Dentistry of New Jersey (UMDNJ)/Environmental and Occupational Health Studies Institute (EOHSI) Division of Exposure Assessment.

The available resources permitted the target number for the subset to be set at 50. The subset was designed to include approximately equal numbers from the following categories based upon residential tap water sampling results for THMs: cases with lower THM levels, cases with higher levels, controls with lower levels, and controls with higher levels. During additional home visits for collecting exhaled breath and urine samples, volatile organics were also collected from indoor air and tap water. An honorarium of $50 was offered as an incentive for the additional cooperation. Less than half of the previous participants were initially selected for eligibility in the biomonitoring portion of the study, and only half of those had not since moved to a new address and also agreed to participate. Appendix C presents the biological monitoring module in its entirety, together with a description of the selection criteria and procedures.

RESULTS

ASCERTAINMENT OF SUBJECTS

The numbers of cases and controls ascertained were 112 and 248 respectively. The rate of NTDs among New Jersey births from these two years was thus approximately 0.45/1,000. This is consistent with recent national trends *(7)*. Table 3 shows the proportions who were interviewed,

sampled, or lost due to refusal or other reasons. The response was greater than for the previous New Jersey case control study of birth defects and drinking water, especially for the controls (previously 55%, currently 66%). Table 4 illustrates the distribution of cases and controls according to demographic and related factors available from birth certificates. Late prenatal care inception (after the first trimester) was analyzed alone, rather than in combination with number of prenatal visits as is sometimes used as an algorithm for adequate prenatal care *(54)*. The reason for that decision for this study is that NTDs were frequently diagnosed prenatally resulting in a greatly increased number of medical visits related directly to case status. We were able to obtain samples of tap water in the same season as the critical period for 76% of subjects for whom such samples were obtained, with similar distributions for cases and controls (see Appendix B).

DISTRIBUTION OF DRINKING WATER CONTAMINANTS AMONG POPULATION-BASED CONTROLS

Tables 5a through 5e present the distributions for the 1993-1994 population-based control sample of all New Jersey births for the contaminants of interest.

1. Trihalomethanes

The THM concentrations (Table 5a) tended to be lower than those in 1985-1988 New Jersey study of 75 towns which had mean and median total THM concentrations of 38.1 and 46.2 ppb respectively *(3)*. Water concentration tertiles were constructed based upon the (rounded) distribution of total THMs for the first trimester public monitoring data of the controls. For the remainder of the analyses, 5 and 40 ppb were used for all tertile cutpoints for trihalomethanes, irrespective of sources of estimate or subject series. Private wells (for which there were no public monitoring data) were assigned to the lowest tertile, i.e., referents for subsequent odds ratio analyses. The presumption of low trihalomethane concentrations among private wells was consistent with the internal data collected by tap water sampling; all 18 private well samples we collected indicated total THM levels below 3 ppb (most nondetected). The distributions of the four individual THMs are also shown in Table 5a.

2. Other Major Classes of Disinfection By-Products

Tables 5b and 5c present the distribution of individual and total haloacetic acids and haloacetonitriles in the control subjects who were sampled at the tap; these comprised only those subjects utilizing surface or mixed water sources, so the population-based distribution cannot be inferred from these data without taking into account the proportion of control subjects on groundwater sources (which are presumed to have very low concentrations of these by-products). No population-based public monitoring data for these compounds are yet available. See Appendix A for a plot of the total trihalomethanes against total haloacetic acids for the control subjects which were sampled for the latter, illustrating that there can be wide divergence between concentrations of these two chemical classes but that their abundance tends to be approximately equivalent.

3. Nitrates

Distribution of total nitrates in the control population are presented in Table 5d for the sampled tap water and the public monitoring data. The latter, however, was not required to be collected annually after 1993 because of triennial sampling up to 1993 and because of changes in federal and state regulations exempting many systems from testing. As discussed below, the tap water results are more complete and reliable for nitrate exposures and were utilized for all odds ratio analyses.

4. Volatile Organic Compounds (VOCs) Including Solvents

There was very little contamination with the "A-280" volatile organic solvents in the tap water or public monitoring samples in this study. This observation is in accordance with the dramatic improvement of New Jersey public drinking water which has been documented since the implementation of the A-280 regulatory program in 1985 *(43,44)*. Table 5e illustrates that few control water supplies had contamination with any individual VOC of 1 ppb or more as indicated by tap water sampling results a year after the first trimester.

ASSOCIATIONS OF NEURAL TUBE DEFECTS WITH DRINKING WATER SOURCE AND CONTAMINANT CLASSES

In the sections below, association of neural tube defects with drinking water characteristics are presented for various series of subject subsets based upon the information which was available for each set and upon the clinical criteria used to define cases. All analyses were categorical. For trihalomethanes, two categorical schemes were used for the basic analyses: tertiles derived from the distribution of contaminants in study controls, and the 20 ppb increments which were used in the previous New Jersey study *(5)*.

1. Odds Ratios of Public Monitoring Data Concurrent with First Trimester

(a) All subjects.—For the full series of study subjects, estimating exposure via public monitoring data at the critical time for neural tube closure (Table 6) showed ORs of 1.5 for surface water source, $\geq$ 1.6 for each category of 20 ppb THM concentrations increment over 40 ppb (compared with < 20 ppb), and 1.6 for the highest THM tertile compared with the lowest tertile. Only birth certificate data could be used to assess possible confounding, since interviews were not completed on the entire subject series. None of the data on potential risk factors which were available from birth certificates changed the OR by 10% or more, and there was no statistically significant effect modification evidenced by examining individual strata. Therefore, only unadjusted results are presented. The middle tertile had an OR of 0.6. The 95% confidence intervals (CI) included 1.0.

Although the foregoing analysis was able to include all ascertained subjects, two limitations should be noted: for 23% of these subjects, the identity of the actual residence during the critical time period was not known. In addition, the location of the monitoring sample might not have represented

adequately the actual index residence. Therefore, a number of further analyses were conducted to test whether the above observations would be corroborated by samples obtained from the index addresses themselves or, where index addresses were known but there was no access, from neighborhood facilities used as surrogates.

(b) Subjects with Known Residency Histories During the Critical Time Period.—When the analyses were restricted to subjects for whom the residency during gestation was known, the odds ratios for THM tertiles increased slightly, with the OR for the highest tertile = 1.7 (Table 7). Surface water ORs similarly increased marginally to OR = 1.6. Again, 95% CIs included 1.0.

(c) Isolated Defects.—Investigators at CDC *(46)* have recommended limiting some epidemiological analyses to a clinically restricted case series. Approximately equal percentages of all cases, those sampled for tap water and those interviewed, consisted of "isolated" neural tube defects in which there were no unrelated malformations. When analysis of all ascertained subjects was restricted to isolated defects (Table 8) an OR of 1.7 was found for the highest THM tertile, slightly higher than that found for all cases. The ORs for subjects both with known residency histories and also restricted to cases with central nervous system and other associated defects increased the odds ratio to 2.1 with a 95% CI of 1.1 to 4.0 (Table 9). For all of the above observations, adjusting by data obtained from birth certificates did not alter the odds ratios by 10% or more, compared with the unadjusted observations on those subjects for whom information on the adjusted factors were not missing, and there was no significant effect modification.

(d) Specific Anatomical Defects.—Analyses also explored odds ratios for spina bifida alone (within the isolated defects series); anencephaly and encephalocele did not comprise large enough series of cases for analyses. Isolated spina bifida had ORs of 1.6 for the highest THM tertile, i.e., slightly weaker than for all NTDs. The decreased number of cases produced wider CIs.

(e) Specific Trihalomethanes.—The four trihalomethanes were also examined individually. Total THMs in New Jersey are comprised principally of chloroform because of relatively low bromine levels in the water, in turn driven by regional geological characteristics. Chloroform and the next most abundant THM, bromodichloromethane (BDCM) appeared to drive the total THM observations (Table 11). A combination of the three THMs containing bromine was examined because of the toxicological data suggesting higher potential toxicity of these compounds *(55)* and the observations were very similar to those for chloroform, the only THM not containing bromine.

(f) Season of Subjects' Conception.—No strong seasonal pattern was seen for the month of conception of cases in this study and, as described above, control births were approximately evenly distributed throughout the year.

(G) Effect of Including Key Demographic Factors and Prenatal Care Onset in the Analysis Models.—Tables 12 and 13 indicate the odds ratios when stratifying simultaneously for mother's education, hispanic ethnicity, African American, and late prenatal care. Although none of these factors were confounders according to the criterion of 10% or greater change in the ORs, illustrating the

effect of this combination of factors, albeit interrelated, could be important in evaluating the main results, particularly since there are different distributions of these factors in participants and nonparticipants among cases and controls. Tables 12 and 13 illustrate that including these factors in combination do not appreciably change the dimensions of the ORs corresponding to Tables 6 and 9 above, although the confidence intervals became wider because of missing values.

(h) Other Sensitivity Analyses.—Controlling for VOC (i.e., solvent) contamination had no effect on the odds ratios. In addition, the above patterns were not changed when subjects born out of state were excluded, or when subjects not sampled in the target season were excluded. An urban-rural index was constructed from U.S. census data on New Jersey municipalities; controlling for the urban and rural character of the municipality of residence did not alter the results.

(i) Collapse of THM Exposure into Two Categories.—Since there was similarity among the odds ratios for THM exposures less than 40 ppb, it is interesting to note the effect of combining the THM exposure groups into categories above and below this concentration. The resultiing odds ratio for 40 ppb and greater in public monitoring data, compared to all exposures below that level, is 1.9 (95% CI = 1.2-3.1) for all subjects (compared to 1.6, CI 0.9-2.7 in Table 6). For subjects with known first trimester residences and excluding multiple defects, the corresponding OR is 2.2, CI = 1.3-3.9 (compared to 2.1, CI 1.1-4.0 in Table 9).

2. Odds Ratios Based on Tap Water Sampling One Year after First Trimester

It was of interest to test the consistency of findings using different sources of estimates for drinking water contaminant exposure. For those subjects with known residence location during the first month of gestation, we conducted parallel analyses to those above using exposure estimates based on tap water sampling one year after the critical time for neural tube closure. The tap water testing conducted from participants' residences also enabled us to relate THM concentrations to total chlorine and other disinfection by-products (data not available from the public monitoring data).

(a) All Subjects Sampled.—The surface water ORs and THM tertile results are similar to the public monitoring data and to each other (Table 14). The OR for more than 0.5 ppm total chlorine in tap water was 1.5, i.e., similar to the unadjusted OR for the highest tertile for total THMs. ORs for THM increments were elevated above 40 ppb except for the highest category (80+ ppb) for which the OR was decreased (but for which there were only three cases). Adjusting for onset of prenatal care after the first trimester increased the THM ORs by more than 10%; therefore ORs stratified for late onset of prenatal care are shown for this and subsequent tables on the tap water sampling results.

(b) Isolated Defects.—As with exposure estimates from public monitoring, when cases with multiple defects were excluded (Table 15) the observations were somewhat stronger (highest tertile OR = 1.7 unadjusted and 1.9 adjusted).

(c) Subjects Sampled at Index Residence Only.—Table 16 presents the analogous observations restricted to subjects for whom the index home was sampled (i.e., excluding surrogate

neighborhood facilities). The observed ORs increased slightly, but 95% CIs still included 1.0, due in part to the loss of subjects under this restriction.

3. Specific Routes of Exposure to Trihalomethanes

The data elicited via the interview questionnaire concerning home use of tap water, including use of household filters, beverage ingestion, and bathing/showering were utilized to explore the possibility of identifying more specific routes of exposure which might be associated with observed odds ratios for contaminant concentrations in water.

(a) Estimates of Removal of THMs by Water Filters.—In most residences using kitchen sink water filters, tap water samples both with and without the filters were collected for the volatile organic scan (EPA Method 524.2). Twelve such pairs were analyzed; most showed a substantial decrease in the concentration of organics by use of the filter. The median proportional decrease (four-fold) was applied to the ingestion pathway estimate for households with regularly maintained tap water filters for which a filtered sample was not collected. The unfiltered sample results were used to estimate inhalation pathway exposures.

(b) Ingested Trihalomethanes.—Since detailed histories were elicited from interviewed subjects on the quantity of hot and cold beverages ingested during the period of interest, an analysis was conducted of trihalomethanes estimated to have been ingested, based upon these interviews and upon the tap water sampling results at the index residences. For this series of subjects, the quantity of total THMs ingested by mothers was not associated with NTDs, using control-derived tertiles of micrograms per day ingested, calculated on the basis of number and size of beverages made from unboiled unfiltered tap water (OR for highest tertile: 1.1). There was similarly no association with case status of the amount of total tap water or cold tap water ingested, irrespective of THM concentrations.

(c) Noningestion Exposure to Trihalomethanes.—Since trihalomethanes are volatile organics for which exposure has been widely modeled to occur through inhalation and dermal routes to an equal or greater extent than via ingestion, the interviews elicited information on frequency and length of bathing/showering during the critical time periods. A composite categorical variable combining higher THM and higher bathing time categories increased the apparent ORs; however, the higher tertiles of time spent bathing were themselves related to NTDs, regardless of THM levels. Reported water temperature was explored as an explanatory variable, since fever and hyperthermia are known risk factors for NTDs, but these interview responses did not influence the relationship of bathing duration to NTDs.

4. Other Disinfection By-Products

(a) Haloacetic Acids (HAAs) comprise the most abundant group of disinfection by-products in addition to trihalomethanes. As described in the Methods chapter above, they were collected during the second of the two years of fieldwork, but there is no comparable data from public monitoring in

New Jersey. Since HAAs are not volatile, the exposure metrics were the water concentrations (ppb) in the tap water samples drawn from index residences one year after the critical period and also the estimated quantities ingested in micrograms per day (ug/d), using interview data to derive the quantities ingested.

The odds ratios for water concentrations of haloacetic acids (categorized by tertiles in controls) did not show elevated ORs suggesting association with NTDs (Table 17). The estimated ingested quantities had ORs of 1.7 and 1.2 for the second and third tertiles, respectively. Wide confidence intervals resulted in part from the reduced sample sizes due to the restriction of these data to the second year of fieldwork. None of the potential risk factors identified through the birth certificates or questionnaire substantially altered the HAA observations.

(b) Haloacetonitriles (HANs), another major category of volatile disinfection by-products, occurred at much lower concentrations than HAAs. Assays were initiated during the third month of fieldwork. The second and third tertiles of HAN concentrations tap water had ORs of 1.3 with wide confidence intervals (Table 18).

5. Nitrates

Tap water samples were selected as a more reliable exposure estimate than the public monitoring data for nitrates for the following three reasons: (1) Private well concentrations could be estimated; in contrast to disinfection by-products, it is not possible to assume that private wells entailed the lowest category of nitrate concentrations; (2) Most public water systems were required to sample only every three years (compared to quarterly for THMs); (3) Beginning in 1993, points of entry of the water system, rather than distribution samples, were required to be collected, and in some instances less frequently.

For the 271 individuals for whom tap water samples of nitrates were collected, unadjusted odds ratios did not show any association with NTDs, using both arbitrary concentration cutpoints (1, 2, and 4 ppm) and tertiles rounded from distribution of controls (Tables 19). When the model included late onset of prenatal care and tertiles of trihalomethanes, marginal increases of the odds ratios were seen.

6. Volatile Organic Compounds (VOCs)

Odds ratios were not calculated for exposure to solvents and other volatile organic compounds typical of groundwater contamination because the number of subjects with appreciable exposures were too small. Table 20 presents a tabulation of the observations by NTD outcome status.

7. Gamma Radiation

Gamma radiation levels were not associated with NTD outcome. The OR for living area gamma readings of 10 or more microR per hour was 1.2.

RISK FACTORS QUERIED IN INTERVIEW

Data elicited through the interview of subjects' mothers were examined regarding the potential confounding of the observed odds ratios of drinking water contaminants (especially trihalomethanes) and neural tube defects. Particular attention was given to previously identified risk factors for NTDs. Table 21 presents the factors identified in the current data set which had ORs greater than 1.5 or less than 0.67 (regardless of precision of the OR as estimated by 95% CIs). Risk factors with previously established importance based on former epidemiologic or toxicologic reports and standard sociodemographic variables are also presented. Binary variables were constructed for most of these factors in order to facilitate their exploration via logistic regression and stratified 2 x K tables with regard to their effect on the main hypotheses of the study.

Table 22 presents unadjusted and adjusted ORs for total THM concentrations based upon public monitoring estimates for the subset of subjects for whom interview data were collected. Three factors altered the OR by just over 10%, (compared to unadjusted OR for the same series of subjects, i.e., those for which information on the factors in question were not missing): pesticide exposure of mother (increased the OR), asthma or allergy before conception (increased the OR) and employment outside the home during the year before birth (decreased the OR). Models which included all three of these factors gave odds ratios virtually identical to those without adjustment. (Mother's employment outside the home also appeared to decrease odds ratios for the non-interviewed mothers, but occupational data were missing on 30% of the birth certificates of nonparticipants.)

Effect modification of THM exposure by other risk factors was explored and was found only for lack of daily prenatal multivitamin or folate ingestion before pregnancy (Table 23). That is, a strong THM effect was seen for those participants who reported lack of supplements of these critical vitamins. Controlling for ethnicity, prenatal care, and maternal education did not remove the effect of prenatal vitamins on THM exposure. No other risk factors for NTDs reached statistical significance for effect modification (as seen via the probability level for common odds using 2 x K exact methods for odds ratios and confidence intervals *(52)*. However, several other factors, including Hispanic ethnicity and tobacco usage, exhibited similar patterns suggesting that statistical effect modification might have been established had the total numbers of subjects or the proportion of subjects with those factors been higher.

BIOLOGICAL MONITORING PILOT

Detailed observations from the biomonitoring pilot portion of the study are found in Appendix C. For the samples collected after showering, there was overall correlation of trihalomethanes in tap water and exhaled breath. However, background exhaled breath had very low concentrations of THMs and no correlation with water or air concentrations were detected. Participants who collected breath samples immediately after showering showed stronger correlations. Water concentrations of trichloroacetic acid (TCAA), but not dichloroacetic acid (DCAA), were associated with urinary excretion of that compound. Such observations are consistent with the rapid metabolism of DCAA. Use of available information about household exposures and quantities of

unfiltered tap water ingested strengthened the correlations with TCAA. No differences could be detected between cases and controls regarding the degree to which biological indices of exposure were correlated with environmental monitoring in this abbreviated series (n = 49) of subjects.

DISCUSSION

INTERPRETATION OF WEIGHT OF EVIDENCE REGARDING ASSOCIATION OF CHLORINATED AND/OR SURFACE WATER WITH NEURAL TUBE DEFECTS

This study has added to the weight of evidence that THMs or some other feature or component of surface water may be risk factors for neural tube defects. No clear evidence of an association has been demonstrated between any specific water contaminants and neural tube defects; restrictions on interpretation of our observations include the relatively wide confidence intervals around the odds ratios and the study design limitations discussed below. Although the odds ratios for THMs were the strongest findings of the study, NTDs were also associated with surface water source itself and with total chlorine residual in tap water.

The association between disinfection by-products and neural tube defects is strengthened by numerous sensitivity analyses addressing possible misclassifications of exposure and outcome: (1) restricting analyses of public monitoring data concurrent with the first trimester only to those for whom the address during the periconceptual period was known eliminated misclassifications which might have arisen from those subjects who moved between conception and birth; (2) restricting analyses of tap water sampling one year after the first trimester to those who were sampled at the index address only, rather than neighborhood facilities, eliminated some potential misclassification; (3) restricting analyses to cases with "isolated" NTDs, thereby excluding "multiple" defects which included birth defects not associated with NTDs *(46)*. For all the above sensitivity analyses, statistical power was lost due to the smaller number of subjects. In most of these instances, however, the ORs increased enough to maintain or to narrow the 95% CIs. Additionally, when THM exposure was treated as a binary variable with cutpoint at 40 ppb, all ORs approached or exceeded 2.0 with confidence intervals excluding 1.0.

CONSISTENCY OF TRIHALOMETHANE BY-PRODUCT FINDINGS WITH PREVIOUS NEW JERSEY DRINKING WATER STUDIES

Generally consistent observations with the earlier New Jersey observations on trihalomethanes were found in the current study, but there are distinctive findings regarding magnitude and precision of the odds ratios and the exposures for which elevated ORs were detected.

In the previous New Jersey cross-sectional study *(3,5)* statistically significant ORs of about 3.0 were found for THM concentrations exceeding 80 ppb, and elevated ORs with very wide CIs were seen above 20 ppb. The pattern appeared stronger for isolated NTDs than for multiple defects. As reviewed in the Introduction above, the number of cases in those analyses was only 56, and the

number of controls exceeded 50,000. In the former New Jersey case control study *(4)* corresponding ORs were higher, the CIs were for the most part wider, and the relative strength of observations for isolated vs multiple observations were similar. Because of the high non-participation rate of controls (45%) the results were considered less reliable than for the cross-sectional analysis.

In the current study, the ORs of 1.6-2.1 were found for NTDs and total trihalomethanes in public water greater than 40 ppb, but confidence intervals included 1.0 except when the more stringent restrictions were imposed on both exposure (residence at conception known) and case definition (isolated defect) or when exposure was assessed as a binary category with a 40 ppb cutpoint.

TOXICOLOGICAL PLAUSIBILITY OF TERATOGENICITY OF TRIHALOMETHANES

The genotoxicity and lipophilic properties, facilitating placental transport of some of these compounds or their metabolic by-products, provide plausibility that these substances could theoretically be human teratogens.

1. Whole Animal Toxicity and Teratogenicity Studies of Disinfection By-Products

The toxicological literature on reproductive effects of disinfection by-products is quite limited. Recent general toxicity studies of laboratory animals have suggested synergy between chloroform and haloacetic acids *(56)*. Carcinogenicity studies of the individual trihalomethanes and other by-products have been used as the basis for USEPA regulation and proposed revised regulations of these compounds *(47)*. Several teratological investigations of haloacetic acids and haloacetonitriles via drinking water indicated that heart malformations were formed, although the vehicle appeared to influence the lowest effective dose. Central nervous system defects were not reported *(57-60)*. Pertinent whole animal bioassays for these by-products utilizing comparable dosages have not been conducted to date.

2. Interaction of Water Contaminants with Folate Utilization

Chen and Sever *(61)* recently suggested a possible mechanism whereby certain chemicals, including chloroform, could plausibly contribute to neural tube defect formation. Folate functions as a methyl (and methylene and formate) donor in the syntheses of the amino acid methionine (and three DNA bases). Methionine is formed via the methylation of homocysteine, which has been found at elevated concentrations in the blood of mothers of NTD babies *(62,63)* and it has been proposed that abundant folate can function to overcome a genetically-determined deficits in methionine synthase activity. Deficient methionine and excess homocysteine in laboratory animals are also related to embryotoxicity and/or development of NTDs *(64)*. Vitamin B-12 (cobalamin) is a cofactor for the key reaction catalyzed by the enzyme methionine synthase. Work with microorganisms and laboratory mammals *(65)* has indicated that methionine synthesis can be blocked by chloroform and carbon tetrachloride (one of the chlorinated solvents found to be associated with NTDs in the earlier New Jersey study).

OBSERVATIONS AND TOXICOLOGICAL DATA ON OTHER DISINFECTION BY-PRODUCTS

We did not observe a clear association of NTDs with disinfection by-products other than THMs. The toxicological literature suggests that some of the scarcer HAAs may be neuroteratogens in mammals; in vitro experiments on mouse embryos have tested the type and relative potency of teratogenicity for specific haloacetic acids *(66)*. Neural tube defects were found to be one of the most sensitive effects. The brominated HAAs were the most potent. The monobrominated species, MBAA, had activities over three orders of magnitude greater than DCAA or TCAA. Our data only allowed limited exploration of consistency with these observations, since MBAAs were found at only very low concentrations in the water tested (median of those 89 water samples with detected levels was 0.47 ppb, with only 2 samples above 1 ppb). Within these limited data, there was no evidence of stronger associations of neural tube defects with MBAA than with total haloacetic acids.

SPECIFIC EXPOSURE ROUTES TO DISINFECTION BY-PRODUCTS

As described in the Results chapter narrative and tables, the observations relating to the ingestion vs other routes of THM exposures did not enable us to draw any strong conclusions about the relative importance of specific exposure pathways in determining the association of NTDs with THM concentrations in drinking water. However, the stronger associations of NTDs with THM water concentrations, compared with the associations based upon estimated THM quantities ingested, are consistent with previous modeling and measurements predicting that noningestion routes provide greater biological doses to these chemicals than ingestion *(67-69)*.

CONSISTENCY OF FINDINGS ON OTHER DRINKING WATER CONTAMINANTS

As reviewed in the Introduction, earlier New Jersey studies by Bove et al. *(3-5)* also observed associations of neural tube defects with several chlorinated solvents and with nitrate exposures in drinking water.

1. Nitrates

In the current study, no associations of NTDs with nitrates in tap water were found. No subjects were estimated to be exposed to levels exceeding the standard of 10 ppm for which a recent report from China *(70)* found a relationship with NTDs.

2. VOCs

It was not possible to test the hypotheses of association with solvents because of the low number of exposed subjects.

BIOLOGICAL MONITORING PILOT

The two main correlations observed (i.e., between individual THMs in tap water and exhaled breath after showering and between trichloroacetic acid (TCAA) in tap water and urine) suggest specific biological exposure markers that may be fruitful for development as investigation of potential health effects of disinfection by-products continue. The improvement in the correlations with incorporation of beverage ingestion data from the 48-hour questionnaire indicates that collecting such information is important for maximizing the utility of biological monitoring for exposure estimation in this context. The lack of correlation of dichloroacetic acid (DCAA) for tap water and urine is consistent with recent reports on the physiological half-life of this compound (Appendix C) The lack of difference observed between cases and controls do not point to physiological differences between the case and control series. It should be noted that the number of subjects in this pilot was very limited *(49)* and that they were not representative of the population-based cases and controls but were selected according to categories of previous tap water concentration results.

OBSERVATIONS OF THIS STUDY WITH REGARD TO OTHER PREVIOUSLY IDENTIFIED RISK FACTORS FOR NEURAL TUBE DEFECTS

The questionnaire utilized in the participant interviews was designed to detect important factors which might be confounders of odds ratios for drinking water contaminants. The study size and design does not permit rigorous evaluation of such risk factors. Consistent with observations in other recent environmental studies of birth defects in New Jersey and elsewhere *(5,42)* few of these factors appeared to be confounders. Although other risk factors were not the focus for the current investigation, it is of interest to review the associations of previously-identified risk factors for NTDs with case status in this study (see Table 21).

1. Gender

An excess of female cases has generally been observed for NTDs, but sex ratios have varied for isolated vs non-isolated defects and with gestational age *(71)*. The cases in this study were 51% female, i.e., consistent with these observations.

2. Socioeconomic Status

It has repeatedly been noted *(7,24,37)* that NTD cases tended to occur to lower income mothers, although others have not seen a relationship of NTD prevalence with social class *(72-74)*. We observed a relationship of reported cases with lower education and lower income. This tendency has probably been strengthened in recent years by differential availability and usage of prenatal diagnosis according to socioeconomic status, thus determining which incident cases come to term *(13)*.

3. Race and Ethnicity

In the United States, NTDs are also seen at higher rates in whites than blacks *(7,74,72)*. In our series, there was an over-representation of Hispanics and under-representation of African Americans among the cases, consistent with other reports.

4. Previous Pregnancy History

A history of stillbirth, or childhood mortality has been known to be a risk factor for NTDs *(7,37)* but conflicting findings have been reported concerning miscarriage as a risk factor *(76)*. Our cases had higher rates of previous stillbirth or child mortality than our control series. Mothers of cases in this study were also more apt to have had at least three prior live births (Table 21).

5. Medical Conditions

Maternal fever, influenza, and certain medications frequently taken for these symptoms have been previously linked to increased NTD rates *(77)*. Flu, fever, decongestants, and antihistamines were each found to be related to case status in our series. Because of the overlap in these medical conditions and remedies, and based upon these earlier findings the term selected for consideration in the final model was influenza accompanied by fever for which medications were taken. One unexpected observation in the present series was the association of neural tube defects with asthma or allergy in the mother (Table 21). There were insufficient cases of diabetes or seizures in our series to analyze for consistency with previous findings.

6. Folate Intake

Our study included items on the vitamin supplements taken by participant mothers, specifically querying frequency of use of prenatal multivitamins or folate supplement during the three months before conception and during the first trimester. (Dietary folate intake was queried only via commercial cereals which are designed to provide 100% of the recommended daily intake of folate; the composite variable of adequate folate intake included those responses). Given the vast and consistent literature confirming the efficacy of prenatal folate supplements in preventing NTDs *(18,19,78)*, it was surprising that a higher proportion of cases than controls reported taking daily multivitamins before becoming pregnant. Since it happened that the official CDC recommendations regarding prenatal folate for prevention of NTD incidence was announced very close to the time of the earliest conceptions for the study subjects (i.e., 1992), we considered the possibility of selective recall bias among case mothers regarding folate intake. Throughout the two year observation period, case mothers showed increasing rates of affirmative responses regarding preconceptual intake of multivitamins/folate, but control mothers did not show such a trend (Table 24). While in no way proving recall or response bias, Table 24 is suggestive of one or both of these. Nevertheless, in light of the above discussion of possible interaction of folate (or vitamin B12) deficiency with chloroform and certain other drinking water contaminants previously associated with NTDs, it is intriguing that strong effect modification was observed between reported preconceptual vitamin supplements and

THM exposure; that is, the association of NTDs with THMs was increased to 2.6 (95% CI 1.15-6.0) in the subjects whose mothers replied that they had not taken folate or multivitamin supplements before pregnancy (Table 23). Observations by others regarding subgroups for which folates did not appear to be protective (Hispanic and college-educated women) may also be pertinent.

7. Occupational and Other Environmental Chemical Exposures

In light of the previous findings of maternal and paternal occupational association with NTDs (see Introduction) we particularly examined potential occupational exposures to pesticides, paints, and solvents. As indicated in the Results chapter (see Table 21), residential pesticide exposures were related to NTDs, but occupational exposures to paint and solvents were not. These observations are extremely limited given the small number of parents exposed and the lack of quantitative or documented exposure data: i.e., the interview questions could not be used to adequately assess these factors, and were included, with the rest of the interview items, in order to assess the degree and type of confounding of the main hypotheses of the study. Indeed, pesticide exposures reported by the subjects' mothers were seen to have a borderline confounding effect on the odds ratios for the subset of subjects on whom interview data were available.

SALIENT STRENGTHS OF THIS STUDY

• Concurrent ascertainment and rapid contact of subjects decreased the numbers of subjects lost to follow-up.

• The population-based control series, with birth certificates retrieved for all, enable the referents of the study to be the entire non-case birth population of the State.

• The exposures of interest consisted of objective, verifiable data and were not subject to recall bias on the part of the respondents (subjects' mothers).

• Investigator bias was minimized by ensuring that the researcher responsible for retrieval and selection of historical data was not familiar with the case vs control status of the subjects.

• The supplementation of monitoring data concurrent with the critical time for neural tube closure with the tap water sampling at the index residences enabled us to evaluate consistency of exposure by more than one source of concentration estimate.

• The current tap water samples were obtained, for the most part, in the same season, usually the same month, as the critical time period one year earlier, thus enabling seasonal fluctuations of disinfection by-products to be controlled for.

• Results utilizing the public monitoring data concurrent with timeframe of interest for NTDs and the residential tap water sample results one year later were in general agreement, thereby

strengthening our impression of validity of the exposure estimates. Some of these features represent improvements on the methodology of the previous New Jersey case control study *(4,5)*.

• The detailed questionnaire data enabled us to evaluate potential risk factors and indicated that the observed associations are unlikely to be due to confounding by the factors queried.

EVALUATION OF SALIENT STUDY LIMITATIONS

1. Exposure Misclassification Potential

Given that tap water quality of the subjects' mothers at or before the first month of pregnancy is the exposure of interest in this study, there were several sources of potential misclassification. Estimates from public monitoring records may not have been representative of the time or location of tap water quality at all index residences. Although the State monitoring records were carefully evaluated for each subject and the water utilities were consulted by telephone in most instances and/or by letter where needed, it would have been preferable to personally visit plants of complex systems. The large number of New Jersey water companies involved precluded such procedures.

For nonparticipants, the birth address used as a surrogate for early pregnancy address may not have been accurate. Among interviewed parents, approximately equal proportions of cases (22%) and controls (23%) moved between the first month of pregnancy and birth. Those proportions were generally consistent with other recent studies *(80)*. Furthermore, some address changes may be within the same water system or between systems with similar water quality.

2. Loss of Cases Via Prenatal Diagnosis

In recent years, increasing use of prenatal diagnosis and elective pregnancy termination has increased the proportion of incident cases of NTDs which do not result in live births or in stillbirths after 20 weeks of gestation. This phenomenon has been reviewed and its implications for epidemiological studies of risk factors for NTDs have recently been investigated in California *(13)*. Not surprisingly, their cases which were detected in utero and aborted were not representative of the cases which came to term or were stillborn; mothers of electively terminated cases were more likely to be white, of higher education, higher income, and have earlier inception of prenatal care. Aborted cases comprised a higher proportion of ascertained anencephaly than other NTDs. Forty percent of all cases were terminated. Similarly, in a recent evaluation of the role of prenatal diagnosis on all NTDs in metropolitan Atlanta *(12)* the number of terminated pregnancies with NTDs was 28 during a two-year period, while the number of affected births was 59. Terminated cases were found disproportionately among whites. Two out of the four syndrome cases were terminated. Isolated cases were somewhat more frequently terminated (32%) compared with multiple defects (27%).

Increased use of prenatal diagnosis and elective abortion also appears to have influenced the numbers and distribution of ascertained cases in this study. The prevalence rate of NTD births during the two years of the study (112/250,000 or about 0.4 per 1,000 births) indicates a recent decline in

prevalence analogous to that seen in much of the U.S. and the world *(81)*. In particular, the proportion of anencephalics in our case series (17%) was much lower than the 33.7% observed from 1985 to 1987 as part of an earlier birth defect surveillance project from this Department *(14)*. Abundant anecdotal information in New Jersey indicate that early prenatal diagnosis of anencephaly and pregnancy termination are responsible for a major part of this decrease. The analysis of spina bifida alone (Table 10) was conducted in part to observe if anencephalics not ascertained could have greatly skewed the results; the associations with spina bifida alone were slightly lower than for the entire case series, so this source of selection bias appears more likely to bias toward than away from the null hypotheses. Furthermore, lack of confounding by the factors shown *(13)* to be associated with prenatal diagnosis (e.g. inception of prenatal care, education) should address a major portion of this potential selection bias.

3. Representativeness of Participants

We cannot quantitatively assess selection bias introduced by incomplete participation. Nevertheless, from birth certificate data retrieved on all ascertained cases and controls, the representativeness of participants according to birth certificate data was explored (Table 25). The characteristics of those subjects' mothers who refused or could not be located tended to be the same as those who did not have prenatal diagnosis in the recent California study *(13)*. Although the odds ratios for the full series of subjects was not confounded by these characteristics, controlling for late prenatal care increased the odds ratios for the association of NTDs with trihalomethanes assayed in tap water about one year after conception. The essential agreement of the strength of association of the highest THM tertile for the subjects whose mothers participated and for the full series of subjects suggests that we did not have serious participation bias.

CONCLUSION

It is clearly necessary to ensure adequate disinfection of drinking water in order to protect the public from a myriad of infectious disease. During the past two decades, epidemiological and toxicological data have indicated carcinogenicity and teratogenicity of some disinfection by-products, particularly trihalomethanes and some haloacetic acids, suggesting that it is also appropriate to utilize source protection and disinfection practices which minimize chronic hazards to the public from these and other by-products. Evaluation of the weight of evidence and identification of further information needs regarding reproductive outcomes are essential parts of the overall process of working toward optimum water treatment technologies and appropriate exposure limitations *(82)*. It is also important to recognize that alternative treatments to traditional chlorination involve formation of unintended by-products which may be toxic as well, and that the relative advantages and disadvantages of modifying or substituting technologies need to be carefully considered.

From the point of view of public health, investigations of disinfection by-products which might be reproductive toxins are important for ensuring the maximum feasible protection that can be accomplished. In refining water treatment and disinfection technologies and regulation, the potential

hazard of even low concentrations of THMs should be carefully considered. Since the observed associations with trihalomethanes were not strong (ORs generally between 1.5 and 2.0) even if these results are verified by future work, major prevention of these birth defects in the population can be accomplished by controlling the even stronger risk factors for NTDs, e.g. folate deficiency and inadequate prenatal care. For example, estimates of the population attributable risk of the highest tertile of trihalomethane exposure (i.e., over 40 ppb) suggested by the results of this study range between about 13% (for odds ratio of 1.5) and about 23% (odds ratio of 2.0) while attributable risks of from 50% to 72% (have been proposed for inadequate folate consumption *(18,20)*

It must be emphasized to the public, particularly women who may be pregnant and their health care providers and advocates, that the New Jersey studies do not establish THMs as teratogens or suggest changes in drinking water behavior. The critical time of NTD closures occurs before most pregnancies have been verified or even perceived. Therefore, we are not advising use of bottled water or other alternative water sources as long as the public water is in compliance with current standards. It is noteworthy that associations in this study were found for concentrations of trihalomethanes above about 40 parts per billion, less than half of the current federal and state standard. While bottled water is required to meet drinking water standards, there is no guarantee that concentrations of contaminants would be lower than those in public drinking water.

The results found here need to be verified by analogous work elsewhere, e.g. other States and/or countries. For that purpose, we recommend that (a) cross-sectional design utilizing birth certificate data on potential risk factors and public monitoring records concurrent with the first trimester be emphasized, (b) public monitoring records on chlorine residuals be utilized as additional predictors of disinfection by-product exposure *(83)* and (c) data on other disinfection by-products be retrieved in addition to trihalomethanes. In particular, identification of the surface water characteristics producing the current observations need to be elucidated. The exciting findings emerging from current physiological and toxicological work elsewhere regarding the mechanism by which folate supplements can prevent NTDs also suggest pursuit of the role of genetic polymorphisms and biochemical interactions in driving susceptibility to drinking water contaminants. Although extremely tentative, the observations here on trihalomethanes and vitamin supplements are consistent with the biological plausibility of an interaction of folate deficiency with exposure to certain environmental chemicals. Most importantly, women who may become pregnant are urged to ensure that they maintain recommended diet and vitamin supplements to ensure protection against deficiencies of B vitamins during the earliest periods of pregnancy, and they are urged to begin prenatal care early in pregnancy.

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TABLES

Table 1.—Diagnostic categories and sources of cases (N = 112).

Defect	Number	Percent
Spina bifida only	76	68%
Anencephaly only	19	17%
Encephalocele only	8	7%
Combination of 2 or more	7	8%
Isolated versus Multiple Defect		
Isolated: NTD and allied only	97	87%
Multiple: other non-associated defects present	15	13%
Reporting Source		
Birth Defects Registry	104	93%
Fetal Death Certificates	8	7%

Table 4.—Distribution of cases and controls according to birth certificate data on sociodemographic and other pregnancy history factors.*

	Cases		Controls	
	N	%	N	%
Total	**112**		**248**	
Age of Mother:				
<20	16	14	29	12
20 - 29	54	48	121	49
30+	42	38	98	40
Race of Mother:				
White	85	76	180	73
Black	18	16	57	23
Other	5	4	11	4
Hispanic Ethnicity of Mother:				
Non-Hispanic	72	64	208	84
Hispanic	35	31	40	16
Education of Mother:		24		
≤11 years	27	40	49	20
12 years	38	13	80	32
13 - 15 years	14	17	51	21
≥16 years	19		59	24
Parity (including subjects):				
1 child	39	35	121	49
2 - 3 children	66	59	122	49
≥ 4 children	1	1	4	2
Month Prenatal Care Began:				
1st	13	12	48	19
2nd - 3rd	51	46	144	58
4th - 5th	11	10	19	8
>5th (including none)	13	12	20	8
Number of Prenatal Visits (Total):				
≤5 (including none)	29	26	24	10
6 - 9	19	17	45	18
10 - 11	20	18	47	19
12 - 13	13	12	52	21
14 - 15	10	9	42	17
≥16	5	4	17	7
Number of Gestational Weeks:				
≤37	51	46	34	14
38 - 41	53	47	206	83
≥42	3	3	7	3

* Because of missing data, categories may not total 100%.

Table 5a.—Distribution of trihalomethanes among controls (ppb) N = 224 public monitoring; N = 181 tap water samples (includes private wells).

Total Trihalomethanes	**Median**	**Mean**	**Std. Dev.**	**Max**
Public Monitoring	23.5	33.8	32.8	134.8
Tap Water Sampling	18.0	26.6	30.5	151.5
Chloroform (CF)				
Public Monitoring	15.8	24.9	27.2	122.0
Tap Water Sampling	10.0	19.3	25.1	130.0
Bromodichloromethane (BDCM)				
Public Monitoring	4.9	6.3	6.4	30.3
Tap Water Sampling	4.0	5.1	5.8	28.0
Dibromochloromethane (DBCM)				
Public Monitoring	1.0	1.7	1.6	9.4
Tap Water Sampling	1.0	1.4	1.4	7.0
Bromoform (BF)				
Public Monitoring	0.5	0.9	1.3	13.6
Tap Water Sampling	0.5	0.7	1.4	7.0

Table 5b.—Distribution of haloacetic acids (HAA) among controls for whom tap water was sampled (ppb) N = 108.

	Median	Mean	Std. Dev.	Max
Total HAA	25.7	32.3	31.6	152.6
MBAA	0.3	0.4	0.2	1.4
DBAA	0.5	0.6	0.4	2.1
MCAA	1.3	1.4	1.3	6.0
BCAA	1.8	2.0	1.6	6.8
DCAA	12.7	15.4	15.0	65.2
TCAA	7.4	12.5	15.5	78.8

M = mono D = di T = tri B = bromo C = chloro

Assays commenced during the 11th month of the two years of fieldwork. Sampling for these compounds was conducted in localities which had the potential for using surface or mixed water sources. Therefore, this distribution is not representative of the underlying control population.

Table 5c.—Distribution of haloacetonitriles (HAN) among controls with tap water sampled (ppb) N = 136.

	Median	Mean	Std. Dev.	Max
Total HAN	2.1	2.8	2.6	15.1
DBAN	0.2	0.3	0.3	1.3
DCAN	1.6	2.0	2.2	12.6
TCAN	0.0	0.0	0.1	0.5
BCAN	0.4	0.5	0.4	2.0

M = mono D = di T = tri B = bromo C = chloro

Assays commenced during the third month of two years of fieldwork. Sampling for these compounds was conducted in localities which had the potential of using surface or mixed sources, and this distribution is therefore not representative of the underlying control population.

Table 5d.—Distribution of nitrates and nitrites among controls (ppm)* N = 168 public monitoring; N = 178 tap water samples (includes private wells).

Nitrates	**Median**	**Mean**	**Std. Dev.**	**Max**
Public Monitoring	0.80	1.2	1.4	8.5
Tap Water Sampling	0.75	1.2	1.5	9.6
Nitrites				
Tap Water Sampling	0.003	0.013	0.058	0.068
Nitrates + Nitrites				
Tap Water Sampling	0.76	1.2	1.5	9.6

*Nitrite levels were not generally available from public monitoring data base.

Table 5e.—Distribution of "A280" VOC concentrations (ppb) in tap water samples by case/control status (N = 271).

Contaminant	Cases (N = 90)	Controls (N = 181)
PCE		
<1	88	178
≥1	2	3
TCE		
<1	89	179
≥1	1	2
1,1,1-TCA		
<1	89	179
≥1	1	2
Vinyl Chloride		
<1	90	181
≥1	0	0
Benzene		
<1	89	181
≥1	1	0
1,2- DCA		
<1	89	181
≥1	1	0
1,1- DCE		
<1	90	180
≥1	0	1
cis-1,2-DCE		
<1	88	178
≥1	2	3
trans-1,2-DCE		
<1	90	181
≥1	0	0
Carbon Tetrachloride		
<1	90	180
≥1	0	1
Total A280 Contaminants		
<5	87	177
5–<10	0	3
≥10	3	1

Table 6.—Odds ratios: Concentrations of total THMs based on public monitoring data concurrent with critical period for NTDs (N = 360).

		Cases (N = 112)	Controls (N = 248)	Unadjusted Odds Ratio	95% CI
Water Source	Ground	39	105	(1.0)	---
	Surface	57	105	1.5	0.9-2.5
	Mixed	16	38	1.1	0.5-2.4
THMs in 20 ppb Increments	<20	47	124	(1.0)	---
	20–<40	12	45	0.7	0.3-1.5
	40–<60	17	27	1.7	0.8-3.5
	60–<80	23	30	2.0	1.0-4.0
	80+	13	22	1.6	0.7-3.6
THM Tertiles ppb	<5	39	92	(1.0)	---
	5–<40	20	77	0.6	0.3-1.2
	40+	53	79	1.6	0.9-2.7

Adjusting for risk factors from birth certificate data (maternal age, race, ethnicity, education, pregnancy history, prenatal care, gestational age) did not change ORs by ≥ 10%.

Table 7.—Odds ratios: Concentrations of total THMs estimated by public monitoring data concurrent with critical period for NTDs: Subjects for whom residency during this time is known (N = 273).

		Cases (N = 90)	Controls (N = 183)	Unadjusted Odds Ratio	95% CI
Water Source	Ground	31	78	(1.0)	---
	Surface	45	73	1.6	0.9-2.8
	Mixed	14	32	1.1	0.5-2.5
THMs in 20 ppb Increments	<20	37	93	(1.0)	---
	20–<40	10	32	0.8	0.3-1.9
	40–<60	15	22	1.9	0.7-3.9
	60–<80	16	19	1.7	0.9-4.9
	80+	13	17	1.8	0.7-4.4
THM Tertiles	<5 ppb	30	70	(1.0)	---
	5–<40	17	55	0.7	0.3-1.5
	40+	43	58	1.7	1.0-3.1

Adjusting for risk factors from birth certificate data (maternal age, race, ethnicity, education, pregnancy history, prenatal care, gestational age) did not change ORs by ≥ 10%.

Table 8.—Odds ratios: Isolated defect cases only concentrations of total THMs estimated by public monitoring data concurrent with critical period for NTDs (N = 345).

		Cases (N = 97)	Controls (N = 248)	Unadjusted Odds Ratio	95% CI
Water Source	Ground	33	105	(1.0)	---
	Surface	49	105	1.5	0.9-2.5
	Mixed	15	38	1.3	0.6-2.7
THMs in 20 ppb Increments	<20	40	124	(1.0)	---
	20–<40	11	45	0.8	0.3-1.7
	40–<60	17	27	1.9	0.9-4.2
	60–<80	16	30	1.7	0.8-3.5
	80+	13	22	1.8	0.8-4.2
THM Tertiles	<5 ppb	32	92	(1.0)	---
	5–<40	19	77	0.7	0.4-1.4
	40+	46	79	1.7	0.9-3.0

Adjusting for risk factors from birth certificate data (maternal age, race, ethnicity, education, pregnancy history, prenatal care, gestational age) did not change ORs by ≥ 10%.

Table 9.—Odds ratios: Concentrations of total THMs estimated by public monitoring data concurrent with critical period for NTDs. Subjects for whom residence during this time is known and excluding multiple defects from cases (N = 264).

		Cases (N = 81)	Controls (N = 183)	Unadjusted Odds Ratio	95% CI
Water Source	Ground	26	78	(1.0)	---
	Surface	42	73	1.7	0.9-3.2
	Mixed	13	32	1.2	0.5-2.8
THMs in 20 ppb Increments	<20	31	93	(1.0)	---
	20–<40	9	32	0.9	0.3-2.1
	40–<60	15	22	2.0	0.9-4.7
	60–<80	14	19	2.2	0.9-5.3
	80+	12	17	2.1	0.8-5.3
THM Tertiles ppb	<5	24	70	(1.0)	---
	5–<40	16	55	0.9	0.4-1.9
	40+	41	58	2.1	1.1-4.0

Adjusting for risk factors from birth certificate data (maternal age, race, ethnicity, education, pregnancy history, prenatal care, gestational age) did not change ORs by ≥ 10%.

Table 10.—Odds ratios: Concentrations of total THMs estimated by public monitoring data concurrent with critical period for NTDs. Spina bifida (isolated) cases only (N = 319).

		Cases (N = 71)	Controls (N = 248)	Unadjusted Odds Ratio	95% CI
Water Source	Ground	24	105	(1.0)	---
	Surface	35	105	1.5	0.8-2.8
	Mixed	12	38	1.4	0.6-3.2
THMs in 20 ppb Increments	<20	30	124	(1.0)	---
	20–<40	8	45	0.8	0.3-1.8
	40–<60	12	27	1.8	0.8-4.3
	60–<80	10	30	1.4	0.5-3.3
	80+	11	22	2.1	0.8-5.0
THM Tertiles ppb	<5	24	92	(1.0)	---
	5–<40	14	77	0.7	0.3-1.5
	40+	33	79	1.6	0.8-3.1

Table 11.—Odds ratios for individual trihalomethanes and sum of three brominated THMs. Concentrations based on public monitoring (N = 357).

		Cases (N = 111)	Controls (N = 246)	Unadjusted Odds Ratio	95% CI
Chloro-form ppb	<23	39	97	(1.0)	---
	2–<35	30	77	1.0	0.5-1.8
	35+	42	72	1.5	0.8-2.6
Bromo-dichloro-methane ppb	<1	32	64	(1.0)	---
	1–<8	28	89	0.6	0.3-1.2
	8+	47	71	1.3	0.7-2.4
Dibromo-chloro-methane ppb	<0.6	36	86	(1.0)	---
	0.6–<2	37	94	0.9	0.5-1.7
	2+	38	66	1.4	0.8-2.5
Bromo-form ppb	<0.5	29	59	(1.0)	---
	0.5–<1.0	64	136	1.0	0.5-1.7
	1.0+	18	51	0.7	0.3-1.5
Brominated THMs ppb	<3	35	85	(1.0)	---
	3–<10	26	80	0.8	0.4-1.5
	10+	50	81	1.5	0.9-2.6

Excludes three subjects for whom data were not available or applicable.

Table 12.—Odds ratios: Controlling for key birth certificate characteristics*: Maternal years of education, Hispanic, African American, and onset of prenatal care (N = 296). Concentrations of total THMs based on public monitoring data concurrent with critical period for NTDs.

		Cases (N = 76)	Controls (N = 220)	Unadjusted Odds Ratio	95% CI
Water Source	Ground	27	95	(1.0)	---
	Surface	38	92	1.5	0.8-2.8
	Mixed	11	33	1.5	0.6-4.1
THMs in 20 ppb Increments	<20	34	112	(1.0)	---
	20–<40	8	37	0.6	0.2-1.7
	40–<60	10	23	1.7	0.6-4.3
	60–<80	15	28	1.5	0.6-3.6
	80+	9	20	1.6	0.5-4.7
THM Tertiles ppb	<5	29	83	(1.0)	---
	5–<40	13	66	0.6	0.2-1.3
	40+	34	71	1.4	0.7-2.7

*Binary variables.

Table 13.—Odds ratios: Controlling for key birth certificate factors*: Maternal years of education, Hispanic, African American, onset of prenatal care (N = 223). Concentrations of total THMs estimated by public monitoring data concurrent with critical period for NTDs. Subjects for whom residence during this time is known and excluding multiple defects from cases.

		Cases (N = 57)	Controls (N = 166)	Adjusted Odds Ratios	95% CI
Water Source	Ground	18	70	(1.0)	---
	Surface	31	67	2.0	0.9-4.4
	Mixed	8	29	1.3	0.4-3.9
THMs in 20 ppb Increments	<20	22	84	(1.0)	---
	20–<40	6	28	0.8	0.2-2.5
	40–<60	10	19	2.5	0.9-7.3
	60–<80	10	19	2.3	0.7-7.3
	80+	9	16	2.5	0.8-8.2
THM Tertiles ppb	<5	18	63	(1.0)	---
	5–<40	10	49	0.7	0.2-2.0
	40+	29	54	2.3	1.0-5.2

*Binary factors.

Table 14.—Odds ratios: Concentrations of total THMs for tap water sampled one year after critical period for NTDs (N = 271).

		Cases (N = 90)	Controls (N = 181)	Unadj. Odds Ratio	95% CI	Adj. Odds Ratio*	95% CI
Water Source	Ground	32	78	(1.0)	---	(1.0)	---
	Surface	45	72	1.5	0.8-2.8	1.6	0.9-2.9
	Mixed	13	31	1.0	0.4-2.4	0.9	0.4-2.2
Total Chlorine in Tap Water ppm †	<0.5	50	112	(1.0)	---	(1.0)	---
	>0.5	34	52	1.5	0.8-2.6	1.5	0.8-2.7
THMs in 20 ppb Increments	<20	42	95	(1.0)	---	(1.0)	---
	20–<40	20	40	1.1	0.6-2.3	1.1	0.5-2.4
	40–<60	17	23	1.7	0.8-3.7	1.8	0.8-3.8
	60–<80	8	12	1.5	0.5-4.4	1.8	0.7-4.7
	80+	3	11	0.6	0.1-2.5	0.7	0.2-2.7
THM Tertiles ppb	<5	30	74	(1.0)	---	(1.0)	---
	5–<40	32	61	1.3	0.7-2.5	1.3	0.7-2.6
	40+	28	46	1.5	0.8-3.0	1.7	0.9-3.8

* Adjusted for late onset of prenatal care
† not available for all samples

Table 15.—Odds ratios: Concentrations of total THMs for tap water sampled one year after critical period for NTDs, isolated defect cases only (N = 262).

		Cases (N = 81)	Controls (N = 181)	Unadj. Odds Ratio	95% CI	Adj. Odds Ratio*	95% CI
Water Source	Ground	27	78	(1.0)	---	(1.0)	---
	Surface	42	72	1.7	0.9-3.2	1.8	1.0-3.4
	Mixed	12	31	1.1	0.5-2.6	1.0	0.4-2.5
Total Chlorine ppm †	<0.5	43	112	(1.0)	---	(1.0)	---
	>0.5	32	52	1.6	0.9-2.9	1.7	0.9-3.1
THMs in 20 ppb Increments	<20	37	95	(1.0)	---	(1.0)	---
	20–<40	18	40	1.2	0.6-2.3	1.1	0.5-2.5
	40–<60	16	23	1.8	0.9-3.8	1.9	0.9-4.3
	60–<80	7	12	1.5	0.5-4.1	1.8	0.7-5.1
	80+	3	11	0.7	0.2-2.7	0.8	0.2-3.2
THM Tertiles ppb	<5	25	74	(1.0)	---	(1.0)	---
	5–<40	30	61	1.5	0.7-2.9	1.35	0.7-3.1
	40+	26	46	1.7	0.8-3.4	1.9	1.0-4.0

* Adjusted for late onset of prenatal care
† not available for all samples

Table 16.—Odds ratios: Concentrations of total THMs for tap water: Includes only individuals with residential tap water sampled at index home (N = 198).

		Cases (N = 70)	Controls (N = 128)	Unadj. Odds Ratio	95% CI	Adj.* Odds Ratio	95% CI
Water Source	Ground	28	64	(1.0)	---	(1.0)	---
	Surface	32	43	1.7	0.9-3.4	1.7	0.8-3.6
	Mixed	10	21	1.1	0.4-2.9	1.2	0.4-3.3
Total Chlorine in Tap Water ppm	<0.5	42	86	(1.0)	---	(1.0)	---
	>0.5	23	27	1.7	0.9-4.0	1.8	0.8-3.8
THMs in 20 ppb Increments	<20	29	70	(1.0)	---	(1.0)	---
	20–<40	12	25	1.3	0.6-2.9	1.1	0.4-2.7
	40–<60	10	12	2.0	0.7-5.4	2.0	0.7-5.7
	60–<80	5	5	2.2	0.5-9.4	2.4	0.5-11.1
	80+	3	8	0.8	0.2-3.7	0.9	0.2-4.1
THM Tertiles ppb	<5	26	61	(1.0)	---	(1.0)	---
	5–<40	25	42	1.4	0.7-2.9	1.4	0.6-3.3
	40+	19	25	1.8	0.8-4.1	2.0	0.9-4.9

*Adjusted for late onset of prenatal care.

Table 17.—Haloacetic acids (HAA): Odds ratios: Concentrations in tap water and estimated quantity ingested daily (N = 176 sampled, 154 interviewed*).

		Cases (N = 62 sampled, 56 intvd)	Controls (N = 114 sampled, 98 intvd)	Unadjusted Odds Ratio	95% CI
HAA in Water (tertiles, ppb)	<3	21	39	(1.0)	---
	3–<35	18	38	0.9	0.4-2.0
	35+	23	37	1.2	0.5-2.6
HAAs Ingested (tertiles ug/d)	<2	15	34	(1.0)	---
	2–<18	24	33	1.7	0.7-4.0
	18+	17	31	1.2	0.5-3.2

* Sampling commenced in the 11th month of fieldwork. Subjects living in counties or municipalities served exclusively by groundwater were not tested for these compounds and were placed in the lowest tertile. For subjects whose mothers were not interviewed, ingested quantities could not be estimated.

Table 18.—Haloacetonitriles (HAN): Odds ratios: Concentrations estimated by tap water sampling one year after critical period (N = 241*).

		Cases (N = 81)	Controls (N = 118)	Unadjusted Odds Ratio	95% CI
HAN Tertiles ppb	<0.5	24	56	(1.0)	---
	0.5–<3.0	26	46	1.3	0.6-2.8
	3.0+	31	58	1.3	0.6-2.5

* Sampling began in third month of fieldwork. Subjects residing in counties or municipalities served only by groundwater were assumed to be in the lowest tertile.

Adjusting for risk factors from birth certificate data (maternal age, race, ethnicity, education, pregnancy history, prenatal care, gestational age) and interviews (ingested quantity estimates) did not change ORs by ≥ 10%.

Table 19.—Odds ratios: Nitrate concentrations of sampled tap water (N = 265).

		Cases (N = 89)	Controls (N = 176)	Unadjusted Odds Ratio	95% CI
Nitrates ppm	<1	56	106	(1.0)	---
	1–<2	21	39	1.0	0.5-2.0
	2–<4	8	23	0.7	0.3-1.7
	4+	4	8	1.0	0.2-3.7
Nitrate Tertiles	<0.3	23	48	(1.0)	---
	0.3–<1.0	34	60	1.2	0.6-2.4
	1.0+	32	68	1.0	0.5-2.0

Table 20.—"A-280" VOC concentrations (ppb) in tap water samples by case/control status (N = 273).

Contaminant	Cases (N = 90)	Controls (N = 181)
PCE		
<1	88	178
≥ 1	2	3
TCE		
<1	89	179
≥ 1	1	2
1,1,1-TCA		
<1	89	179
≥ 1	1	2
Vinyl Chloride		
<1	90	181
≥ 1	0	0
Benzene		
<1	89	181
≥ 1	1	0
1,2- DCA		
<1	89	181
≥ 1	1	0
1,1- DCE		
<1	90	180
≥ 1	0	1
cis-1,2-DCE		
<1	88	178
≥ 1	2	3
trans-1,2-DCE		
<1	90	181
≥ 1	0	0
Carbon Tetrachloride		
<1	90	180
≥ 1	0	1
Total A280 Contaminants		
<5	87	177
5–<10	0	3
≥ 10	3	1

Table 21.—Risk factors from interview tested as potential confounders (N = 247).*

		Cases N = 82	Controls N = 165	Odds Ratio	95% CI
Fever Before Conception	No	64	143	(1.0)	---
	Yes	7	6	2.6	0.7-9.9
Influenza and Fever Before Conception	No	69	149	(1.0)	---
	Yes	3	2	3.3	0.4-39.4
Asthma or Allergy Before Conception	No	57	134	(1.0)	---
	Yes	15	17	2.1	0.9-4.7
Antihistamines Before Conception	No	63	140	(1.0)	---
	Yes	8	9	2.0	0.6-6.1
Multivitamins, or Folate Before Conception	Yes	26	38	(1.0)	---
	No	55	124	0.7	0.4-1.2
Mother Worked Outside Home During Year Before Birth	No	35	35	(1.0)	---
	Yes	47	129	0.4	0.2-0.7
Mother Exposed to Solvents thru Home or Work	No	68	146	(1.0)	---
	Yes	3	3	2.1	0.3-16.4
Father Exposed to Solvents thru Work	No	67	140	(1.0)	---
	Yes	4	9	0.9	0.2-3.5
Mother Exposed to Paint thru Home or Work	No	65	132	(1.0)	---
	Yes	6	17	0.7	0.2-2.0
Father Exposed to Paint thru Work	No	69	145	(1.0)	---
	Yes	2	4	1.1	0.1-7.5
Residential Chemical Exposures via Hobby	No	60	137	(1.0)	---
	Yes	13	13	2.3	0.9-57
Residential Pesticide Use	No	45	108	(1.0)	---
	Yes	25	39	1.5	0.8-3.0

Table 21.—Continued.

		Cases N = 82	Controls N = 165	Odds Ratio	95% CI
Time at Swimming Pool (total hours) Before Conception	none	60	130	(1.0)	---
	1–<24	14	25	1.2	0.5-2.6
	>24	8	10	1.7	0.6-5.2
Tobacco Use	No	64	126	(1.0)	---
	Yes	17	38	0.9	0.4-1.8
Mothers' Age at Birth of Subject	20-29	37	76	(1.0)	---
	<20	13	11	1.7	0.6-4.7
	30+	34	76	0.9	0.5-1.7
More than Three Previous Live Births	No	69	156	(1.0)	---
	Yes	10	9	2.5	0.9-7.3
Any Previous Stillbirth or Miscarriage	No	72	160	(1.0)	---
	Yes	5	4	2.8	0.6-14.4
Prenatal Care Began after First Trimester	No	63	134	(1.0)	---
	Yes	19	31	1.3	0.6-2.6
Low Income or Public Assistance	No	35	100	(1.0)	---
	Yes	47	65	2.1	1.1-3.0
12 or Fewer Years of School	No	40	102	(1.0)	---
	Yes	42	63	1.7	1.0-3.0
African American	No	70	136	(1.0)	---
	Yes	12	29	0.8	0.4-1.8
Hispanic	No	59	133	(1.0)	---
	Yes	20	29	1.6	0.8-3.1

* Unless otherwise specified, all factors refer to maternal characteristics or exposure during six-month period from three months before conception through first trimester. Factors presented include standard sociodemographic, factors previously reported to be associated with NTDs (see text), and factors for which ORs >1.5 or <0.67 were observed. Interview responses for which less than three subjects were exposed were omitted. Because of missing data on many of the items, not all totals equal N = 247.

Table 22.—Confounding of THM odds ratios by interview variables: Highest tertile (>40 ppb) vs lowest tertile (≤5 ppb) total THM exposure estimated from public monitoring data.

Stratified by Maternal Risk Factors:	Cases >40 ppb	Controls >40 ppb	Odds Ratio of Risk Factor	Odds Ratio of THM	95% CI	p of Common Odds
None (not adjusted)	37	53	---	1.5	0.80-2.99	---
Residential Pesticide Use, 3 Months Before Conception Through First Trimester	33	47	1.5	1.7	0.86-3.58	1.0
Asthma or Allergy Before Conception	33	47	2.1	1.7	0.86-3.49	1.0
Worked Outside Home During Year Before Birth	37	53	0.4	1.3	0.68-2.67	1.0
All 3 Above	33	47	---	1.6	0.74-3.29	0.98

Table 23.—Effect modification of THM odds ratios by vitamin intake response from interview. (Highest vs lowest tertile of total THM exposure estimated from public monitoring data).

Stratum of Maternal Risk Factor:	Cases >40 ppb THMs	Controls >40 ppb THMs	Odds Ratio of >40 ppb THMs	95% CI	p of Common Odds
Folate or Multivitamins Taken Before Conception	9	18	0.47	0.13-1.66	---
Folates/Multivitamins Not Taken	27	33	2.59	1.15-6.00	---
Strata Combined	36	51	1.53	0.79-2.99	0.018

Table 24.—Percentage of mothers who responded that they took multivitamins in the 3 months before becoming pregnant with index.

Dates of Birth	Percentage of Cases	Percentage of Controls
January 1993 - June 1993	22	23
July 1993 - December 1993	28	20
January 1994 - June 1994	33	26
July 1994 - December 1994	39	23

Table 25.—Percentage of subjects interviewed according to key demographic and reproductive variables.

	Cases		Controls	
	Total N	Interviewed %	Total N	Interviewed %
Total	112	73	248	67
Age of Mother:				
<20	16	69	29	48
20 - 29	54	68	121	63
>29	42	81	98	78
Education of Other (years):				
≤11				
12	27	67	49	47
13+	38	68	80	59
	33	82	110	83
Month Prenatal Care Began:				
1st - 2nd				
3rd - 4th	45	78	143	73
≥5th (or none)	27	82	59	63
	31	58	46	54

APPENDICES

Appendix A

Exposure Assessment Methodology for Public Water Systems

For all subjects receiving public water, the New Jersey Department of Environmental Protection (NJDEP) monitoring databases are utilized to estimate both past and present exposures to trihalomethanes (THMs), volatile organic compounds (VOCs) and nitrate.

As required by Federal and State regulations, most public water systems submit THM monitoring results to the NJDEP on a quarterly basis; therefore, seasonal data are available for those water systems. Most public water systems submitted VOC sampling results to the NJDEP on a semiannual basis prior to 1993. However, some water systems were required to monitor annually. As a result of regulatory changes, all public water systems were required to take four consecutive quarterly samples for VOCs between 1993 and 1995 unless they were granted a waiver or qualified for annual sampling. Nitrate sampling results were submitted to the NJDEP on a triennial basis prior to 1993. As of 1993, monitoring frequency was increased to annual sampling for groundwater systems and quarterly sampling for surface water systems.

When a public water system is not required to monitor, contaminant levels are estimated based on the source of water utilized (groundwater, surface water, or mixed supply), when possible. When a public water system purchases water from another system and is not required to monitor that supply itself, sampling data are obtained from the supplier system.

For most subjects, distribution samples are used. When distribution samples are not available, plant tap (treated water) samples are used. After 1993, point of entry samples are used, particularly for VOCs and nitrates.

Public water monitoring locations are selected that are most representative of the source(s) of water reaching the index residence for both past and present exposure time frames. Representative monitoring locations are identified with the assistance of the respective water purveyor. When more than one source of water (or point of entry) supplies the index residence, an average of the sampling data for these sources is used. If one of these sources provides most of the water to the index residence, then the sampling data from that "lead" source are used. When the same monitoring location is not sampled during both exposure time frames, different locations are selected that are both representative of the source(s) of water reaching the index residence. When a public water system provides purchased water (from another system) to the index residence and does not monitor that water supply itself, a representative monitoring location is selected from those utilized by the supplier system. When an alternate tap water sampling location is used as a surrogate for the index residence, a monitoring location is selected that is representative of the source of water reaching both the surrogate location and index residence.

Monitoring periods are selected that are representative of the time period and season of interest (in order to account for temperature and seasonal influences on THM levels) for both past and present exposure time frames. When the time period of interest falls equally close to two monitoring dates, sampling data that are most representative of the time period/season of interest are used. If sampling data from the representative monitoring period are missing or incomplete, another monitoring period is used that is most representative of the time period/season of interest. In some instances, sampling data from the same monitoring period are used for both exposure time frames. When the monitoring period closest to the time period of interest is not representative of the source of water (or point of entry) reaching the index residence, sampling data from another monitoring period are used that are representative of water quality at the index residence.

GENERAL INFORMATION

Time period of interest:

For each interviewed subject, the time period (and season) of interest is identified based on study questionnaire information and from vital records (birth or fetal death certificates). The time period of interest corresponds with both the *past* (the three months prior to conception and the first trimester of pregnancy) and *present* (one year after the critical time period/4th week of gestation) exposure time frames.

For non-interviewed subjects, the time period of interest is determined based on information from vital records.

Index residence:

For each interviewed subject, the index residence is identified based on study questionnaire information and from vital records. The index residence is the address where the subject lived during the time period of interest. When the subject lived at two or more addresses during the time period of interest, the index residence is identified as the address where the subject lived around the 4th week of gestation.

For non-interviewed subjects, the index residence is assumed to be the subject's address at birth/delivery, as indicated on vital records, when address history could not be ascertained directly from the subject. When a street address is not available (i.e., post office box) from vital records, the index residence is based on municipality information.

Source of water:

For each interviewed subject, the source of water (public water system versus a private well) for the index residence during the time frame of interest is identified based on information from the New Jersey Department of Environmental Protection (NJDEP) and the respective water purveyor.

For non-interviewed subjects, the source of water is determined based on comparing the index residence to water purveyor billing records and private well records.

When an alternate tap water sampling location is used as a surrogate for the index residence, it is first confirmed with the respective water purveyor that the surrogate location is representative of the source of the water reaching the index residence.

TOTAL TRIHALOMETHANES

Source of data:

For all subjects receiving public water, the NJDEP trihalomethane monitoring database is utilized to estimate both past and present exposures to trihalomethanes (THMs).

Monitoring requirements:

As required by Federal and State regulations, most public water systems submit monitoring results to the NJDEP on a quarterly basis; therefore, seasonal data are available for those water systems.

When quarterly monitoring results are not required, i.e., due to a water systems small size (< 3300 services) or water source (groundwater only), THM levels are estimated based on available information, when possible. For example, those water systems utilizing only groundwater sources are assumed to contain minimal or no detectable THM levels (< 0.5 ppb). Those water systems with surface or mixed (groundwater and surface water) sources are assumed to contain detectable levels of THMs (> 0.5 ppb).

When a public water system purchases water from another system and is not required to monitor that supply itself, sampling data are obtained from the supplier system.

Sample type:

For most subjects, distribution samples (D) are used. In some instances, these D samples are maximum residence time samples, those collected from the furthest points in the distribution system where THM levels are expected to be the highest. When D samples are not available, plant tap (treated water) samples (P) are used.

Monitoring location:

THM monitoring locations are selected that are closest in the water distribution system to the index residence for both past and present exposure time frames. Representative monitoring locations are identified with the assistance of the respective water purveyor.

For most subjects, sampling data from the same monitoring location are used for both exposure time frames. When the same monitoring location is not sampled during both exposure time frames, different locations are selected that are both representative of the source of water reaching the index residence.

When an alternate tap water sampling location is used as a surrogate for the index residence, a monitoring location is selected that is representative of the source(s) of water reaching both the surrogate location and index residence.

When a public water system provides purchased water (from another system) to the index residence and does not monitor that water supply itself, a representative monitoring location is selected from those utilized by the supplier system, that typically being at the point of entry to the purchaser distribution system.

Monitoring period:

For most subjects, THM monitoring periods are selected that are representative of the season of interest (the season encompassing both past and present exposure time frames) in order to account for temperature and seasonal influences on THM levels.

When the time period of interest falls equally close to two monitoring dates, sampling data that are most representative of the season of interest are used.

When sampling data from the representative monitoring period are missing or incomplete, such as when results are not submitted, another monitoring period is used from a prior or subsequent year that is representative of the season of interest.

OTHER VOLATILE ORGANIC COMPOUNDS

Source of data:

For all subjects receiving public water, the NJDEP A-280 monitoring database is utilized to estimate both past and present exposures to volatile organic compounds (VOCs).

Monitoring requirements:

As required by Federal and State regulations, most public water systems submitted sampling results to the NJDEP on a semiannual basis prior to 1993. For these water systems, there are two sampling periods: January through June and July through December. Some water systems were required to monitor annually.

Between 1993 and 1995, all public water systems were required to take four consecutive quarterly samples for volatile organic compounds unless a waiver was granted by the State (based on the likelihood that the system would be impacted by VOC contamination) or the water system qualified for reduced (annual) sampling (based on previous sampling data).

When a public water system purchases water from another system and is not required to monitor that supply itself, sampling data are obtained from the supplier system.

Sample type:

Prior to 1993, distribution samples (D) are used for most subjects. When D samples are not available, plant tap (treated water) samples (P) are used.

Beginning in 1993, samples collected at the point of entry (POE) to the distribution system, such as wells or treatment plants, are used for all subjects.

Monitoring location:

A-280 monitoring locations are selected that are most representative of the source(s) of water reaching the index residence for both past and present exposure time frames. For example, D and P sampling locations are selected that receive the same source(s) of water as the index residence. POE sampling locations are chosen that provide water to the index residence. Representative sampling locations are identified with the assistance of the respective water purveyor.

For most subjects, D and P sampling data from the same monitoring location are used for both exposure time frames. When the same monitoring location is not sampled during both exposure time frames, different locations are selected that are both representative of the source(s) of water reaching the index residence. POE sampling data are obtained from the same monitoring location/source(s) for both exposure time frames for most subjects.

When an alternate tap water sampling location is used as a surrogate for the index residence, a monitoring location is selected that is representative of the source(s) of water reaching both the surrogate location and index residence.

When a public water system provides purchased water (from another system) to the index residence and does not monitor that water supply itself, a representative monitoring location is selected from those utilized by the supplier system, that typically being at the point of entry to the purchaser distribution system.

When more than one source of water (i.e., a mixture of more than one well and/or surface water source) or point of entry supplies the index residence, an average of the sampling data for all sources is used. If one of these sources provides most of the water to the index residence, then the sampling data from that "lead" source are used.

Monitoring period:

For most subjects, VOC monitoring periods are selected that are closest in time to the time period of interest for both past and present exposure time frames.

When the time period of interest falls equally close to two monitoring dates, sampling data from the monitoring date before the critical time period are used.

When sampling data from the monitoring period closest to the time period of interest are missing or incomplete, such as when results are not submitted, another monitoring period is used that is as close in time as possible to the period of interest. In some instances, sampling data from the same monitoring period are used for both past and present exposure time frames.

When sampling data from the monitoring period closest to the time period of interest are not representative of the source of water (or point of entry) reaching the index residence, such as when a change in water source occurred within the system, another monitoring period is used that is representative of water quality at the index residence.

NITRATES

Source of data:	For all subjects receiving public water, the NJDEP nitrate monitoring database is utilized to estimate both past and present exposures to nitrate.
Monitoring requirements:	As required by Federal and State regulations, most public water systems submitted sampling results to the NJDEP on a triennial basis prior to 1993. As of 1993, monitoring frequency was increased to annual sampling for groundwater systems and quarterly sampling for surface water systems. When a public water system purchases water from another system and is not required to monitor that supply itself, sampling data are obtained from the supplier system.
Sample type:	Prior to 1993, distribution samples (D) are used for most subjects. When D samples are not available, plant tap (treated water) samples (P) are used. Beginning in 1993, samples collected at the point of entry (POE) to the distribution system, such as wells or treatment plants, are used for all subjects.
Monitoring locations:	Nitrate monitoring locations are selected that are most representative of the source(s) of water reaching the index residence for both past and present exposure time frames. For example, D and P sampling locations are selected that received the same source(s) of water as the index residence. POE sampling locations are chosen that provide water to the index residence. Representative sampling locations are identified with the assistance of the respective water purveyor. For most subjects, D and P sampling data from the same monitoring location are used for both exposure time frames. When the same monitoring location is not sampled during both exposure time frames, different locations are selected that are both representative of the source(s) of water reaching the index residence. POE sampling data are used from the same monitoring location/source(s) for both exposure time frames for most subjects. When an alternate tap water sampling location is used as a surrogate for the index residence, a monitoring location is selected that is representative of the source(s)of water reaching both the surrogate location and index residence When a public water system provides purchased water (from another system) to the index residence and does not monitor that water supply itself, a representative monitoring location is selected from those utilized by the supplier system, that typically being at the point of entry to the purchaser distribution system. When more than one source of water (i.e., a mixture of more than one well and/or surface water source) or point of entry supplies the index residence, an average of the sampling data for all sources is used. If one of these sources provides most of the water to the index residence, then the sampling data from that "lead" source are used.

Monitoring period:

For most subjects, nitrate monitoring periods are selected that are closest in time to the time period of interest for both past and present exposure time frames.

When the time period of interest falls equally close to two monitoring dates, sampling data from the monitoring date before the critical time period are used.

When sampling data from the monitoring period closest to the time period of interest are missing or incomplete, such as when results were not submitted, another monitoring period is used that is as close in time as possible to the period of interest. In some instances, sampling data from the same monitoring period are used for both past and present exposure time frames.

When sampling data from the monitoring period closest to the time period of interest are not representative of the source of water (or point of entry) reaching the index residence, such as when a change in water source occurred within the system, another monitoring period is used that is representative of water quality at the index residence.

Disinfection By-Products Included in USEPA 551 and 552 Laboratory Assays

METHOD 551	METHOD 552
Trihalomethanes Chloroform Bromodichloromethane Dibromochloromethane Bromoform Haloacetonitriles Dibromoacetonitrile Dichloroacetonitrile Bromochloroacetonitrile Trichloroacetonitrile Propanones 1,1-Dichloropropanone 1,1,1-Trichloropropanone Chloropicrin	Haloacetic acids Monochloroacetic acid Monobromoacetic acid Dichloroacetic acid Dibromoacetic acid Bromochloroacetic acid Trichloroacetic acid Dalapon

Correlation Between THM Metrics

For illustrative purpose, correlations were derived between total THM estimates from the three different sources of THM data:

"public past" = monitoring data concurrent with early pregnancy
"public current" = monitoring data one year later
"tap current" = tapw water sampling one year later.

The correlations between the estimates indicate that when surrogate sampling locations are excluded, there are approximately equivalent correlations between the metrics with common locations and with common time. These equivalent correlations support the notion that both "public past" and "tap current" are each reasonable estimates for water quality in household tap water exposure during early pregnancy (the metric which is being estimated).

Surface/mixed Water: Including Surrogate Tap Sampling Locations

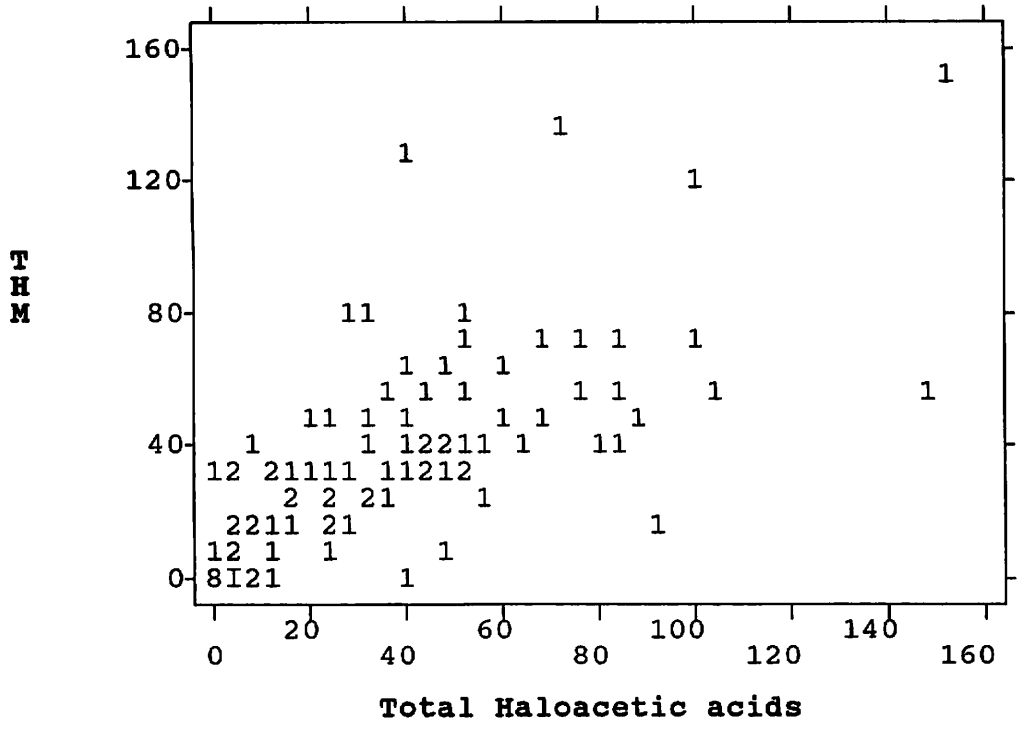
Public Past and Public Current (different years)	.84
Tap Current and Public Current (different locations)	.80
Tap Current and Public Past (different location and year)	*.75*

Surface/mixed Water, Only Index Residences

Public Past and Public Current (different years)	.84
Tap Current and Public Current (different location)	.83
Tap Current and Public Past (different location and year)	*.75*

(All correlations increase when groundwater samples are included).

Plot of Total Trihalomethanes with Total Haloacetic Acids among Controls (mixed and surface water sources only, N = 111)



Surface or mixed sources
HAA: 6 compounds (EPA method 552)
THM: 4 compounds (EPA method 524.2)

Appendix B

Distribution of Interview Type

INTERVIEW TYPE	CASES	CONTROLS	TOTAL
HOME VISIT	74 (90%)	147 (89%)	221 (89%)
TELEPHONE	8 (10%)	18 (11%)	26 (11%)
TOTAL	82 (100%)	165 (100%)	247 (100%)

Distribution of Interview Length

INTERVIEW LENGTH	CASES	CONTROLS	TOTAL
FULL	70 (85)	145 (89%)	215 (87%)
ABBREVIATED	12 (15%)	20 (12%)	32 (13%)
TOTAL	82 (100%)	165 (100%)	247 (100.0%)

Water Sampling Locations

LOCATION	CASES	CONTROLS	TOTAL
INDEX	72 (79%)	131 (72%)	203 (74%)
FACILITY	19 (21%)	51 (28%)	70 (26%)
TOTAL	91 (100%)	182 (100%)	273 (100%)

For four subjects (2 cases and 2 controls) residences for the critical time period were known but we were still unable to collect tap water samples, even at surrogate locations. The reasons were: one spoke only a dialect of Chinese for which no translator could be found before the family moved out of contact; two had private wells; one was reported to the birth defects too late to be fielded by the study.

Date Differences: Target Versus Actual Tap Water Sampling Date by Participant Status*

	CASES	*CONTROLS*
< 31 days	62 (69%)	129 (71%)
31-60 days	14 (16%)	35 (19%)
61-90 days	6 (7%)	7 (4%)
>90 days	8 (9%)	10 (6%)

Season Differences: Target Versus Actual Tap Water Sampling Season by Participant Status**

	CASES	*CONTROLS*
Same season	68 (76%)	137 (76%)
Different season	22 (24%)	44 (24%)

* If two years had elapsed, difference in days = (days - 365).
**Winter = Jan-Mar; Spring = Apr-Jun; Summer = Jul-Sep; Fall = Oct-Dec

Trip Blanks Completed for Field Sampling QA/QC

MONTH	NUMBER OF TRIP BLANKS
7/93	13
8/93	6
9/93	7
2/94	2
6/94	2
8/94	4
9/94	1
2/95	4
3/95	2

Total number trip blanks = 41

Appendix C

BIOLOGICAL MONITORING

Selection of Participants

The biological monitoring portion of the study draws from a subset of original study participants in the highest and lowest categories of total trihalomethane concentrations for residential tap water samples drawn in the course of the study fieldwork for both cases and controls. Criteria for eligibility to participate includes the following:

1) participation, including an interview and tap water sample, in the original study.
2) public water source.
3) tap water sampling obtained from current residence which was the same as, or located in close proximity to, the index address.
4) participant was either English or Spanish-speaking.
5) no known contra-indications to follow-up.
6) tap water results of total trihalomethanes levels less than 10 ug/L, between 25 and 39 ug/L for samples collected during non-summer months, greater than 40 ug/L, or total trihalomethanes levels less than 40 ug/L and the total of dichloroacetic acid (DCAA) plus trichloroacetic acid (TCAA) greater than 49.99ug/L.

Contacting Potential Participants

NJDHSS study staff were responsible for selecting and contacting potential participants eligible for biological monitoring. The recontact was timed to optimize the likelihood of conducting the home visit in the same month or season as the target date. Correspondence included a letter, a form for the addressee to indicate her decision regarding participation, and a postage paid envelope to return the form to the NJDHSS.

In the event that no response was received within two weeks of mailing, an attempt was made to contact the potential participant by telephone. If contact could not be made by telephone, a certified letter was sent.

Upon receipt of a positive response to participate in the biological monitoring, the NJDHSS staff relayed pertinent information to the UMDNJ staff so that they could schedule an appointment for a home visit. This process continued until a total of 50 individuals had agreed to participate.

When the UMDNJ staff received a positive response from NJDHSS, they contacted the individual by telephone to schedule an appointment for a home visit. At this time, the participant was asked to collect their first urine sample on the morning of the scheduled visit. She was also asked to collect a breath sample at the end of her shower or bath either on the evening before or the morning of the visit. The participant was given brief instructions for the sampling and informed that the sampling equipment and specific instructions would be delivered to their home address prior to the day of the scheduled appointment.

Notification of Sampling Results

A letter with the results of individual environmental and biological samples was sent to each participant by the UMDNJ staff. If the total trihalomethanes were detected above the current New Jersey Maximum Contaminant level, or if the total of DCAA and TCAA exceeded the proposed standard, a copy of the participant results letter was sent to NJDHSS.

The NJDHSS staff then notified the Bureau of Safe Drinking Water in the NJDEP of these levels with the collection date, municipality, county, and water supplier. No personal information was included.

ELIGIBLE INDIVIDUALS

TARGET MONTH	HIGH *CASE* (contacted)	HIGH *CONTROL*	MODERATE *CASE* (contacted)	MODERATE *CONTROL*	LOW *CASE* (contacted)	LOW *CONTROL*	TOTAL
October	2 (2)	1 (1)	2 (2)	1 (1)	2 (2)	3 (3)	11 (11)
November	2 (2)	2 (2)		2 (2)	2 (2)	4 (3)	12 (11)
December	2 (2)	4*(4)	1 (1)		1 (1)	6 (2)	14 (10)
January		2 (2)	1 (1)	4 (4)	1	4 (1)	12 (8)
February	1 (1)		1 (1)		6 (5)	4 (2)	12 (9)
March		1 (1)	1 (1)	1 (1)		3 (1)	6 (4)
April	1 (1)	1 (1)		1 (1)		1	4 (3)
May	2 (2)	1 (1)	1 (1)		5 (2)	5 (1)	14 (7)
June	3 (3)				3 (3)	3 (3)	9 (9)
July	1 (1)	5 (5)			2 (2)	2 (2)	10 (10)
August	1 (1)	4 (4)			1 (1)	4 (4)	10 (10)
September	2 (2)	4 (4)		1 (1)		3 (2)	10 (9)
TOTAL ELIGIBLE	**17**	**25**	**7**	**10**	**23**	**42**	**124**
TOTAL CONTACTED	**17**	**25**	**7**	**10**	**18**	**24**	**101**

THM results: High = 40+ ug/L Moderate = 25-39.99 ug/L Low = <10 ug/L
*Includes (1) THAA result >50 ug/L; TTHM <40 ug/L

Biomonitoring of Chlorination By Products Exposure Associated with Water Use

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EXECUTIVE SUMMARY

The research described in this report was undertaken in support of a case control study of neural tube birth defects and disinfection by-products (DBPs) in drinking water, to evaluate whether biomarkers of exposure to DBPs could be identified and whether the uncertainty in exposure classification could be reduced by using questionnaire information on the activities that involve water use compared to solely using water concentrations as a surrogate of exposure. Additional goals were to measure the body burden of the two most abundant categories of DBPs, trihalomethanes and haloacetic acids, in a subset of the highest and lowest exposed cases and controls to determine whether differences in the body burden of the cases and controls exist, albeit two years after the pregnancy, and if differences existed in populations receiving different levels of DBPs in their water at their residence.

Measurements of air, water and exhale breath of chloroform, bromodichloromethane, chlorodibromomethane and bromoform and of water and urine dichloroacetic acid and trichloroacetic acid were made for forty-nine participants. Elevated breath concentrations of the trihalomethanes were observed, particularly after showering, for subjects in the high exposure group. Elevated urinary trichloroacetic acid, but not urinary dichloroacetic acid, was identified. No clear differences were documented between the biological markers of the cases and controls, but the number of subjects in the study was small. The dose response association between trichloroacetic acid water concentration and urinary excretion of trichloroacetic acid was strengthened when exposure estimates, based on the household water used, were used in place of the water concentration. This stronger association justifies the use exposure estimates based on questionnaire data in epidemiological studies instead of just the water concentrations delivered to a home to reduce misclassification of subjects.

INTRODUCTION

Chlorination of drinking water results in the production of a series of compounds referred to as disinfection by-products (DBPs). A number of adverse health effects have been identified with some of the DBP compounds in animals (Lynch and Bull 1992, ILSI 1995) and a weak association between chlorination of water and several adverse health outcomes in humans, including: bladder and renal cancer and adverse reproductive outcomes have been suggested by epidemiological studies (Cantor et al 1987, Kramer et al 1992, McGeehin et al 1993, Bove et al 1995). The epidemiological studies have relied upon chloroform or trihalomethane measurements or estimates in the use of chlorinated water vs water disinfected by other means to assess exposure to chlorinated DBPs in the selected cohorts. The reliance of the water concentration of a single compound or group of compounds as a surrogate of exposure to the DBPs can lead to misclassification of the exposure if the exposure differs for different DBPs, thus, weakening an epidemiologist's ability to detect an effect. In addition, misclassification also results from using the water concentrations delivered to a residence rather than an exposure estimate, since water use is multi-fold, and varies across individuals. DBPs exposure is multi-route, with the significance of each route dependent upon the physico-chemical properties of the individual DBP. Further water use within a population can vary (e.g. ingest different amounts, shower or bath for different time periods). Thus a single exposure surrogate or categorization would not completely describe the exposure to all DBPs or to a population.

The largest exposure characterization study to date of volatile organic compounds, which included the trihalomethanes (THMs), one of the two most prevalent groups of DBPs in

chlorinated drinking water, was the Total Exposure Assessment Methodology (TEAM) study (Wallace et al 1984, Hartwell et al. 1987, Wallace et al. 1987). The results from the TEAM study demonstrated that environmental exposures to THMs occur primarily within one's own home and that drinking water concentrations of THMs were highly correlated with exhaled breath, a measure of the body burden of these compounds. The results of the TEAM study also suggested that a predominant source of exposure to chloroform and bromo- dichloromethane was transmission of these compounds from the residential drinking water via ingestion and from various uses of hot water, while the subject was in close proximity to the source (i.e. in a shower stall), which resulted in both volatilization and subsequent inhalation exposure and dermal contact with water (Wallace et al.1987). Subsequent studies have confirmed that exposure to chloroform while showering results in a dose equivalent to that of ingestion and elevates the body burden, as measured by exhaled breath, for up to several hours after showering from both inhalation and dermal exposures (Andleman 1985, Jo et al. 1990a,b, Weisel et al. 1992). Ingestion of water containing THMs while resulting in an internal dose does not elevate the breath concentration of chloroform due to the rapid metabolism of that compound in the liver after ingestion (Weisel and Jo 1996). Weisel and Jo (1996) further hypothesized that the potential health risk from exposure to substances in water would depend upon the route of exposure and whether the parent compound, a short lived metabolite or a long lived metabolite was the biologically active agent since those factors affect the amount of that agent that reaches the target tissue.

Ingestion of chemicals within water can be associated with elevated urinary excretion

levels. Drinking water contaminated with trichloroethene and/or tetrachloroethene resulted in higher concentration of their urinary metabolites (Zigli et al. 1985, Vartiainen et al. 1993). Vartiainen et al. (1993) were able to identify statistically significant differences in the excretion rates of dichloroacetic and trichloroacetic acid, which they attributed to exposure to drinking water containing up to 212 μg/l of trichloroethene and 180 μg/l of tetrachloroethene. Thus urinary excretion of HAAs can reflect increased body burden resulting from exposure to compounds within drinking water.

The research described in this report, undertaken in support of a case control study of neural tube birth defects and DBPs in drinking water, was to evaluate whether biomarkers of exposure to DBPs could be identified and whether the uncertainty in exposure classification could be reduced by using questionnaire information to quantify water use compared to solely using water concentrations as a surrogate of exposure. Additional goals were to measure the body burden of the two most abundant categories of DBPs, trihalomethanes and haloacetic acids, in a subset of study participants whose tap water contained the highest and lowest concentrations of THMs. Both cases and controls were included to determine whether differences existed in the body burden of the two groups, albeit, for many of the participants, two years had elapsed since their earlier pregnancy, and if differences existed in populations receiving different levels of DBPs in their water at their residence. To accomplish this a comparison of the water concentration data alone and water concentrations in combination with questionnaire data was done for two different classes of water disinfection by-products, trihalomethanes (THMs) and haloacetic acids (HAAs), based on biomarker measurements: exhaled breath concentrations of

THMs and urinary HAAs levels. The two groups of chlorinated DBPs studied, the four trihalomethanes (chloroform, bromodichloromethane, chlorodibromomethane and bromoform) and two haloacetic acids (dichloroacetic acid and trichloroacetic acid) were chosen since they are the most abundant DBPs in chlorinated drinking water and have been found to cause adverse toxicological effects in animal studies (REF). All compounds were measured in the water, the THMs were measured in the air and breath and the HAAs were measured in urine.

EXPERIMENTAL DESIGN

Subject selection

Subjects who participated in the New Jersey neural tube defect study were stratified according to the concentration of chlorinated DBPs in water samples collected during visits conducted as part of the case control study. Individuals whose THM tap water concentrations were at the extreme ends of these concentration levels were contacted by the NJDOH by mail, until a total of 50 individuals (with approximately equal numbers of cases and controls) agreed to participate, with approximately equal numbers in the high and low concentration groups. The potential subjects were then contacted by telephone by EOHSI staff to arrange for a home visit. Attempts were made to visit these subjects in the season of their conception two years prior. Air, water, urine and breath samples were collected from each subject, for which they were compensated $50. A questionnaire was administered to determine their water usage and potential confounding exposures during the 48 hours prior to the visit and for comparison to the previously administered questionnaire. The questions asked were a subset of those queried previously, except that they applied to a much shorter time period.

Environmental Samples

Indoor air concentrations of the THMs were measured within the home at the time of the visit. The air samples was collected for fifteen minutes on an adsorbent trap containing carboxen 569 attached to a calibrated constant-flow portable pump set at a flow rate of 1.0 l/min. These adsorbents retain organic compounds with a wide range of volatility, including the four THMs. The air samples were analyzed for trihalomethanes using thermal desorption (Perkin Elmer ATD400) coupled to gas chromatography/mass spectrometry (GC/MS) (Hewlett Packard 5890GC/5970MSD). The VOCs were quantified using ions characteristic of the compound of interest with the ratios of the ions in each identified peak being compared to expected values to confirm the peak identification based on the retention time.

Water samples were collected into clean 40 ml vials with teflon-faced septa screw top closures from the cold water faucet located in the kitchen, after allowing the water to run for 1 minute. The water samples were stored immediately in a cooler with blue ice packs. Care was taken to avoid bubble formation. For homes having water filtration system in the kitchen, a water sample was taken in from the bathroom tap, if it was not filtered. No quenching of the chlorine was done, but rather, the samples were analyzed or extracted for THMs and HAAs the same day the water was collected. The THMs were analyzed by purge and trap followed by GC/MS. The haloacetic acids were analyzed using a modification of EPA methods as described below.

THM Analysis by Purge and Trap

Five or ten milliliters of water were placed in a glass purge and trap device to which an adsorbent trap was attached using a teflon union. Helium was flowed at 40 ml/min for 20 minutes to transfer the THMs to the trap. The adsorbent trap was analyzed by thermal desorption coupled to GC/MS in an analogous fashion to the air samples.

HAA Analysis

Water samples were collected in glass bottles and stored in a cooler until the samples were returned to the laboratory where they were kept in a refrigerator (4°C) until extracted. The samples were warmed to ambient temperature and five ml transferred into a 40 ml glass vial containing 2-bromopropionic acid solution in MTBE as an internal standard. An half ml aliquot of concentrated sulfuric acid was added dropwise and the vial was well shaken. After the mixture was cooled to room temperature, 7 ml of MTBE were added to the vial and the resulting mixture was mechanically shaken for 7 minutes. When the two layers were clearly separated, 5 ml of the upper (MTBE) layer were transferred into a 15 ml conical centrifuge tube and the solvent was evaporated to 2 ml using a mild stream of nitrogen gas (UHP/Zero grade, Air Products, Inc.). Then, 2 ml of a 10% H_2SO_4/MeOH solution and 2 ml of MTBE were placed in the tube and the contents well mixed. The resulting solution was heated in a water bath at approximately 60°C for 1 hour. After the tube cooled to room temperature, 5 ml of a saturated aqueous Na_2SO_4 solution were added to the tube and the resulting mixture was shaken in a vortex mixer for 10 seconds. One ml of the upper (MTBE) layer was transferred into an auto sampler vial and 2 μl of the water extract were injected into a GC (30 m DB1701 column, J&W) equipped with an Electron Capture Detector (ECD ^{63}Ni).

Urine Samples

Whole void urine samples were collected using clean, new polypropylene containers. The urine samples were obtained at the time of the visit and from first morning samples which were collected by the subject on the day of a visit. A collection bottle for the first morning urine sample, along with instructions for collection and storage, were sent to the subject prior to the scheduled visit. Participants were reminded to collect the sample through a telephone call on the evening before the visit. The time of sample collection and of the last time that the subject urinated were recorded on the bottle label. The samples were placed in the portable cooler for transport back to the laboratory and extracted the same day. The samples were analyzed for haloacetic acids in the same manner as the water samples except that ethyl ether was used for the initial extraction rather than MTBE, because it provides a cleaner interface with the urine; and 2μl of the extract were analyzed. The urine samples were analyzed for creatinine following the procedure outlined by Baselt (1988), to normalize for void volumes and calculation of excretion rates. Total void samples were collected and the times were recorded by the participants. HAA concentrations and creatinine corrected HAA concentrations were calculated for the urine collected at the time of the visit and the first morning urine. In addition excretion rate was calculated for the first morning urine by multiplying the HAA concentration by the volume of urine collected to obtain the total amount of HAAs in the urine, then dividing that amount by the time between the first morning urine void and the previous void, as indicated by the subject.

Breath Samples

Breath samples were collected at two times during the study. Alveolar breath samples were collected during the visit to determine the background breath levels of the subjects. The background alveolar breath samples were collected using a portable system which composites exhaled breath over several minutes (Weisel et al. 1992). To collect a sample an individual breathes through a new mouthpiece that was connected to a disinfected non-rebreathing valve. Room air, purified through NIOSH certified charcoal filters for removing volatile organic compounds (VOCs), was inspired and the expired breath was directed by the non-rebreathing valve into a 5 meter, 1.2 cm internal diameter polyethylene tube. The breath was continuously withdrawn from the polyethylene tube in a last in (alveolar air) first out fashion onto an adsorbent trap which was connected to a constant flow sampling pump. The THMs contained in the breath were collected by the trap, and the trap was analyzed by thermal desorption coupled to GC/MS in a manner analogous to the air samples.

Post shower breath samples were collected by having the subject blow into a tedlar® air sampling bag at the completion of a shower either the evening before or morning of the visit. The sampling bag and instructions were sent to the subject prior to the visit. The interpretation of exactly when after the showered to collect the sample, however, varied among the subjects from immediately after the shower to 20 minutes later. The time delay between the exposure (shower) and when the breath sample was collected is an important determinant of the breath concentration. The subjects were therefore grouped into one of three categories, dependent upon when they collected the breath sample: a) immediately; b) more than five but less than twenty

minutes after showering; and c) greater than 20 minutes after showering. The timing of the shower breath sample was determined either during our visit or by contacting the participant afterwards. Some misclassification could have resulted in the assignment of the category. To quantify the breath levels, 1 to 2 L of the breath in the sampling bag was transferred to an adsorbent trap using a personal sampling pump, as soon as the bag was returned to the laboratory. Storage test demonstrated that the THMs are stable in the sampling bag for up to 48 hours. The breath was then analyzed for trihalomethanes by thermal desorption GC/MS.

Quality Control/Quality Assurance of the Environmental & Biological Samples

Quality assurance in the sample collection and analysis is an integral part of the work done at EOHSI. The status of each instrument used was checked daily before use. The response of the instruments was verified to be within preestablished criteria. The calibration curves were prepared using external standards and checked daily using a solution containing a known quantity of the target compounds. The calculated concentrations of the known solution was to be within $\pm 20\%$ of the expected value for the calibration curve to be considered valid for any particular day. If the $\pm 20\%$ criterium was not met, a new calibration of the instrument was done. Quality control charts were maintained of the instruments' responses. Blank adsorbent traps were transported and analyzed along with the sample traps on all field collection days. Duplicate samples were collected and analyzed from 10% of the homes. Calibration of sampling pumps was done before and after each use.

Questionnaire

A questionnaire was administered to the subjects to ascertain their water usage and other potential exposures to compounds that could contribute to the body burden of the biomarkers being measured. The questionnaire (Appendix A) used was developed jointly with NJ DHSS and contained many of the questions relevant to water use that were asked previously during the interview conducted as part of the case-control study. The questions used during this study were based on a 48 hour recall, while a longer time frame had been asked about during the case control study. The differences in time periods were because the biomarkers being examined are short-term, (minutes to hours), while the information quiered for the case control study pertained to the overall exposure to the subject over a several month time period.

Communication of the Results to the Participants

A letter containing the results of the environmental and biological samples, along with any existing standards (current or proposed) for any compounds were sent to each participant. When the water concentrations exceeded the standard, which is based on the running average of samples collected quarterly, NJDHSS also notified NJDEP, which then contacted the water utility involved. These results, along with the caveats that average water concentrations rather than values from single samples, should be considered when interpreting any health concerns were related to the participant. Telephone numbers of both the Principal Investigator and NJDHSS personnel were provided to the participant if any questions arose concerning the values. Examples of the letters sent are given in Appendix B.

RESULTS AND DISCUSSION

Based on the total THMs concentration measured during this study the forty nine participants were divided into an high exposure group (n=20) and a low exposure group (n=29), with 27 of the participants being controls from the case-control study and 22 being cases. The fiftieth subject was used to check whether changes in the sampling protocol during the project may have affected any of the results. No alterations in the results were observed. A TTHMs concentration of 25 μg/l was used as the cut point to distinguish the high and low exposure groups. The time of year for contacting the individuals was within one month of the target date of conception, usually two years previously, which coincided with the time of the visit during the case-control study. Sixteen of the visits or 33% occurred within one month of the target date (i.e. assumed date of conception two years earlier. Samples of water, air, and background breath samples were obtained from all participants. However, instrumental failure prevented one air and three background breath samples from being analyzed. Time of visit urine samples were collected from 44 of the 49 subjects (90%) while first morning urine samples were obtained from 47 of the 49 participants (96%). Five of the first morning urine samples were questionable as to their status, with three having very low volumes (< 100 ml) suggesting that incomplete voids were collected and two not being true first morning urine (one collected at 8:55 am but the previous void was at 8:20 am and a second collected at 2:30 pm). Post shower breath samples were obtained from 36 of the 49 participants (73%). The low collection rate was due to problems with the sampler originally chosen for use in the project (a new method was not available for the first 12 subjects.) For the participants who were provided a revised sampler, 36 of the 37 (97%) collected samples.

The concentrations of the THMs measured in the air and water samples are given in Table 1, THMs concentrations in the background breath and post shower breath are given in Table 2; the water dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) concentrations are given in Table 3, DCAA and TCAA concentrations for the first morning urine (FMU) and time of visit urine (VU) are given in Table 4; and the DCAA and TCAA creatinine normalized values and excretion rates for the FMU and VU are given in Table 4. Answers to the questionnaire are given in Appendix A. As expected, the distributions of the environmental and biological samples were predominantly bimodal, since the participants were selected to form two groups, one exposed to tap water with minimal THMs concentrations, and a second exposed to tap water with high THMs concentrations. Within each exposure group (high and low water concentration) the distributions were usually log normal rather than normal. Comparisons of mean concentrations were therefore done either using non-parametric tests or on the log transformed data.

COMPARISON OF CASES AND CONTROLS

The individual THM and total trihalomethanes (TTHM) concentrations of the twenty nine of the subjects with mean TTHM water concentrations below 25μg/L were compared to the twenty subjects whose TTHM were above that level. Since the cohort selection criteria was to have two distinct groups with differing TTHM water concentrations, it was expected that the mean water concentrations of the individual DBPs of the two groups would be different as well. The concentration of each of the individual THMs and dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) were statistically different between the two groups, with the exception of bromoform, which was uniformly low throughout the data set ($<5\mu$g/L) (Table 1).

Table 1
Trihalomethane Concentrations in Environmental Samples

No	I.D.	Type	Sampling Date	Air Concentration µg/m**3				Water Concentration µg/L				
				$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$	TTHMs
1	255	2	2/7/95	<0.12	0.086	<0.29	<0.066	<0.14	2.04	1.97	0.54	4.55
2	191	2	2/9/95	<0.12	<0.040	<0.27	<0.062	<0.14	0.10	<0.33	<0.076	<.5
3	222	2	2/20/95	<0.088	0.23	<0.20	<0.046	<0.14	<0.048	<0.33	<0.076	<.5
4	389	1	3/14/95	0.36	0.11	0.13	0.096	0.04	0.07	0.49	0.61	1.21
5	381	2	3/30/95	0.84	0.34	0.67	0.40	0.26	0.30	0.80	0.26	1.62
6	325	1	4/3/95	0.16	<0.016	<0.11	<0.025	<0.072	<0.024	0.14	0.17	0.31
7	245	2	4/10/95	<0.047	<0.016	<0.11	<0.025	0.55	<0.024	<0.17	<0.038	0.55
8	188	2	4/12/95	0.18	0.027	<0.15	<0.033	24.96	5.70	1.65	<0.038	32.3
9	275	2	4/18/95	0.14	<0.014	<0.099	<0.022	0.22	0.31	1.22	0.30	2.05
10	272	2	5/3/95	0.45	<0.016	<0.11	<0.025	35.7	2.70	1.05	0.25	39.7
11	386	2	5/8/95	0.35	<0.016	<0.11	<0.026	12.63	2.43	0.80	<0.038	15.8
12	376	2	5/10/95	1.48	0.32	0.30	0.062	26.06	13.00	6.39	0.25	45.7
13	307	1	5/24/95	5.20	0.77	<0.11	<0.024	125.00	15.45	2.70	0.055	143
14	112	2	6/9/95	0.16	<0.015	<0.10	<0.023	39.54	4.12	0.21	<0.038	43.9
15	291	2	6/28/95	0.42	<0.012	<0.085	<0.019	0.37	0.1	<0.17	<0.038	0.47
16	259	1	7/6/95	NA	NA	NA	NA	34.65	7.59	2.25	<0.038	44.5
17	115	2	7/13/95	1.02	0.47	<0.30	<0.069	0.21	0.06	<0.17	<0.038	0.27
18	251	1	7/26/95	0.81	<0.024	<0.16	<0.037	67.57	14.38	2.34	<0.038	84.3
19	334	2	7/27/95	0.91	<0.048	<0.33	<0.075	0.85	0.27	0.17	0.08	1.37
20	290	1	8/8/95	<DL	<DL	<DL	<DL	0.77	0.5	0.44	<DL	1.71
21	267	1	8/10/95	0.12	<DL	<DL	<DL	86.2	19.07	9.7	1.47	116
22	288	1	8/15/95	0.059	<DL	<DL	0.026	0.21	0.28	0.88	1.51	2.88
23	313	2	8/16/95	0.4	0.58	<DL	<DL	0.18	<DL	<DL	0.03	0.21
24	343	2	8/22/95	<DL	<DL	<DL	<DL	23.85	7.19	2.75	0.12	33.9
25	124	1	8/28/95	0.56	0.43	<DL	0.058	0.37	0.27	1.32	2.29	4.25
26	295	1	9/11/95	9.96	2.98	2.25	3.25	201.2	47.72	9.62	0.83	259
27	172	2	9/12/95	0.28	<.047	<0.24	0.83	4.61	1.94	1.16	<0.13	7.71
28	147	2	9/18/95	0.77	2.84	3.64	3.36	22.92	1.03	1.29	1.49	26.7
29	116	1	10/2/95	1.83	3.84	4.82	4.67	1.88	1.33	2.18	1.84	7.23
30	144	2	10/16/95	2.59	0.26	0.51	<0.13	28.16	6.06	2.52	<0.13	36.7
31	348	2	10/18/95	0.45	<0.069	0.63	<0.13	<0.18	0.12	0.32	0.57	1.01
32	328	2	10/19/95	1.02	0.11	<0.36	<0.13	25.89	5.33	2.49	<0.13	33.7
33	487	1	10/23/95	0.12	<0.068	<0.35	<0.13	2.34	0.99	0.68	0.73	4.74
34	349	2	10/26/95	8.17	1.1	<0.35	<0.13	127	24.8	3.28	0.47	156
35	339	2	10/26/95	6.27	1.34	<0.35	<0.13	39.7	8.75	2.59	0.42	51.5
36	238	1	10/30/95	18.7	2.79	0.75	<0.13	86	9.91	1.08	<0.13	97
37	315	2	11/1/95	11.3	0.41	<0.35	<0.13	136	6.91	0.71	<0.13	143
38	148	1	11/2/95	<0.18	0.32	<0.36	<0.13	2.03	0.21	<0.36	<0.13	2.24
39	479	1	11/7/95	5.71	1.31	<0.36	<0.13	42.9	7.3	1.63	0.6	52.4
40	462	1	11/8/95	25.3	1.34	0.75	0.62	15.7	3.19	1.71	0.61	21.2
41	481	1	11/30/95	0.43	<0.046	<0.23	<0.085	28.1	5.87	1.25	<0.13	35.2
42	229	2	12/13/95	1.85	0.84	0.97	<0.10	3.53	3.73	2.77	0.14	10.2
43	418	2	12/21/95	0.41	0.6	<0.24	<0.087	5.75	3.67	1.62	<0.13	11
44	299	1	1/3/96	<0.12	0.77	<0.24	<0.086	<0.18	0.89	2.89	4.21	7.99
45	410	1	1/23/96	2.17	0.27	<0.23	0.11	<0.18	0.18	<0.36	<0.13	0.18
46	219	2	1/24/96	1.25	0.16	<0.23	0.1	21.8	11.7	1.41	0.21	35.1
47	413	1	1/29/96	0.5	<0.046	<0.23	0.11	0.8	0.22	<0.36	0.16	1.18
48	438	1	2/5/96	<0.12	<0.047	<0.24	<0.087	<0.18	<0.071	<0.36	<0.13	<.5
49	482	1	2/6/96	0.2	1.81	1.19	0.23	0.26	2.42	1.21	0.32	4.21
IDNumber assigned this study	Case/Control Study ID Number	Type 1 Case 2 Control		NA - not available								

Table 2
Trihalomethane Breath Concentrations

No	I.D.	Type	Sampling Date	Breath Concentrations (µg/m³)				Shower Concentrations (µg/m³)			
				$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
1	255	2	2/7/95	<0.10	<0.033	<0.23	<0.05	NA	NA	NA	NA
2	191	2	2/9/95	<0.09	<0.028	<0.20	<0.04	NA	NA	NA	NA
3	222	2	2/20/95	<0.19	<0.063	<0.44	<0.10	NA	NA	NA	NA
4	369	1	3/14/95	<0.12	<0.041	<0.29	<0.066	NA	NA	NA	NA
5	381	2	3/30/95	<0.12	<0.041	<0.28	<0.064	NA	NA	NA	NA
6	325	1	4/3/95	<0.12	<0.040	<0.28	<0.063	NA	NA	NA	NA
7	245	2	4/10/95	<0.12	<0.041	<0.28	<0.064	NA	NA	NA	NA
8	188	2	4/12/95	<0.12	<0.040	<0.28	<0.063	NA	NA	NA	NA
9	275	2	4/18/95	<0.12	<0.040	<0.28	<0.063	NA	NA	NA	NA
10	272	2	5/3/95	<0.12	<0.040	<0.28	<0.063	NA	NA	NA	NA
11	386	2	5/8/95	<0.12	<0.040	<0.28	<0.063	NA	NA	NA	NA
12	376	2	5/10/95	0.53	<0.033	<0.23	<0.05	NA	NA	NA	NA
13	307	1	5/24/95	<0.12	<0.040	<0.28	<0.063	40.16	0.62	<1.47	<0.33
14	112	2	6/9/95	<0.12	<0.041	<0.28	<0.064	36.07	3.30	<1.11	<0.25
15	291	2	6/28/95	NA	NA	NA	NA	3.9	<0.11	<0.79	<0.18
16	259	1	7/6/95	NA	NA	NA	NA	6.28	0.68	<0.71	<0.16
17	115	2	7/13/95	2.67	<0.23	<1.59	<0.36	1.45	<0.12	<0.87	<0.20
18	251	1	7/26/95	1.4	<0.24	<1.68	<0.38	98.58	13.97	1.13	<0.38
19	334	2	7/27/95	<0.24	<0.081	<0.56	<0.13	1.57	0.44	<0.82	<0.19
20	290	1	8/8/95	<DL	<DL	<DL	<DL	0.59	<0.35	2.23	<0.61
21	267	1	8/10/95	2.08	<DL	<DL	<DL	33.55	5.85	<1.70	<0.63
22	288	1	8/15/95	<DL	<DL	<DL	<DL	1.88	0.15	<1.71	<0.61
23	313	2	8/16/95	0.52	0.2	<DL	1.12	1.93	1.34	<1.71	<0.61
24	343	2	8/22/95	<DL	<DL	<DL	<DL	1.8	<0.34	<1.71	<0.6
25	124	1	8/28/95	1.46	<DL	<DL	<DL	1.04	<0.34	<1.71	0.20
26	295	1	9/11/95	11.5	13.5	19.93	23.86	6.49	1.57	<3.6	<1.31
27	172	2	9/12/95	<0.59	<0.24	<1.2	<0.44	1.5	<0.36	<1.80	<0.66
28	147	2	9/18/95	<1.78	9.7	<3.6	<1.31	<0.89	<0.36	<1.8	4.45
29	116	1	10/2/95	<1.76	11.77	<3.56	<1.30	3.17	6.58	7.23	4.95
30	144	2	10/16/95	<0.58	<0.23	<1.17	<0.42	NA	NA	NA	NA
31	348	2	10/18/95	<0.57	<0.23	<1.16	<0.42	<0.86	<0.34	<1.73	<0.63
32	328	2	10/19/95	<0.58	<0.23	<1.17	<0.42	73.60	17.81	11.32	<1.53
33	487	1	10/23/95	<0.86	0.78	<1.75	<0.64	<0.85	1.05	<1.71	<0.62
34	349	2	10/26/95	<0.87	<0.35	<1.76	<0.64	19.5	1.48	<1.77	<0.65
35	339	2	10/26/95	NA	NA	NA	NA	14.2	14.1	8.68	7.2
36	238	1	10/30/95	<1.75	<0.70	<3.53	<1.28	358	41.9	5.94	1.98
37	315	2	11/1/95	1.57	<0.35	<1.76	<0.64	506	26.4	3.25	<0.64
38	148	1	11/2/95	<0.88	<0.35	<1.77	<0.65	<0.88	<0.35	<1.77	<0.65
39	479	1	11/7/95	<0.87	<0.35	<1.76	<0.64	44.3	8.65	3.62	2.8
40	482	1	11/8/95	1.9	<0.71	<3.6	<1.31	58.9	4.54	3.29	2.85
41	481	1	11/30/95	<0.86	<0.34	<1.74	<0.63	7.96	2.82	<3.50	<1.27
42	229	2	12/13/95	<0.88	<0.35	<1.77	<0.65	2.6	7.9	<3.60	<1.31
43	418	2	12/21/95	<0.89	<0.36	<1.81	<0.66	17.8	10.9	6.09	<0.66
44	299	1	1/3/96	<0.88	<0.35	<1.77	<0.65	<0.89	<0.35	<1.79	<0.65
45	410	1	1/23/96	<0.88	<0.35	<1.77	<0.65	<0.88	<0.35	<1.78	<0.65
46	219	2	1/24/96	<0.86	<0.34	<1.74	<0.63	66.6	11	<1.74	0.77
47	413	1	1/29/96	<0.88	<0.35	<1.77	<0.65	<0.81	<0.32	<1.64	<0.60
48	438	1	2/5/96	<0.86	<0.34	<1.74	<0.63	<0.89	<0.35	<1.80	<0.66
49	482	1	2/6/96	<0.86	<0.34	<0.73	<0.63	<0.86	<0.34	<0.73	<0.63
IDNumber assigned this study	Case/Control Study ID Number	Type 1 Case 2 Control		NA - not available							

Table 3
Haloacetic Acid Water Concentrations (µg/l)

No	I.D.	Type	Sampling	Dichloroacetic Acid	Trichloroacetic Acid	Sum of Di & Trichloroacetic Acid
1	255	2	2/7/95	0.69	0.37	1.06
2	191	2	2/9/95	0.33	0.82	1.15
3	222	2	2/20/95	1.30	0.39	1.69
4	369	1	3/14/95	0.33	0.35	0.68
5	381	2	3/30/95	0.33	0.25	0.58
6	325	1	4/3/95	0.33	0.25	0.58
7	245	2	4/10/95	0.33	0.25	0.58
8	188	2	4/12/95	27.40	14.41	41.81
9	275	2	4/18/95	0.33	0.25	0.58
10	272	2	5/3/95	59.09	37.79	96.88
11	386	2	5/8/95	31.11	18.97	50.08
12	376	2	5/10/95	34.22	18.71	52.93
13	307	1	5/24/95	64.66	67.52	132.18
14	112	2	6/9/95	36.63	55.67	92.3
15	291	2	6/28/95	0.43	0.52	0.95
16	259	1	7/6/95	41.57	24.28	65.85
17	115	2	7/13/95	0.33	0.69	1.02
18	251	1	7/26/95	71.6	80.33	151.93
19	334	2	7/27/95	3.05	11.06	14.11
20	290	1	8/8/95	3.52	4.12	7.64
21	267	1	8/10/95	26.15	10.57	36.72
22	288	1	8/15/95	2.08	2.25	4.33
23	313	2	8/16/95	6.04	2.54	8.58
24	343	2	8/22/95	9.51	11.02	20.53
25	124	1	8/28/95	48.31	3.43	51.74
26	295	1	9/11/95	43.5	56.5	100
27	172	2	9/12/95	5.9	6.6	12.5
28	147	2	9/18/95	0.48	6.08	6.56
29	116	1	10/2/95	1.99	2.59	4.58
30	144	2	10/16/95	35.58	63.91	99.49
31	348	2	10/18/95	4.61	5.08	9.69
32	328	2	10/19/95	23.06	40.97	64.03
33	487	1	10/23/95	1.65	5.21	6.86
34	349	2	10/26/95	9.95	11.9	21.85
35	339	2	10/26/95	1.84	5.09	6.93
36	238	1	10/30/95	50.3	50.8	101.1
37	315	2	11/1/95	114	123	237
38	148	1	11/2/95	3.99	3.99	7.98
39	479	1	11/7/95	39.5	38.6	78.1
40	462	1	11/8/95	32.8	19.9	52.7
41	481	1	11/30/95	34.2	43.5	77.7
42	229	2	12/13/95	12.5	5.67	18.17
43	418	2	12/21/95	3.6	2.45	6.05
44	299	1	1/3/96	0.46	0.54	1
45	410	1	1/23/96	7.8	9.81	17.61
46	219	2	1/24/96	33	33.9	66.9
47	413	1	1/29/96	0.33	0.42	0.75
48	438	1	2/5/96	0.92	1.09	2.01
49	482	1	2/6/96	6.37	1.35	7.72
IDNumber assigned this study	Case/Control Study ID Number	Type 1 Case 2 Control		NA - not available		

Table 4 Urinary Haloacetic Acid								
No	I.D.	Sampling Date	First Morning Urine				Time of Visit Urine	
			Excretion Rate (ng/min)		Creatinine Normalized (ng/mg)		Creatinine Normalized (ng/mg)	
			DCAA	TCAA	DCAA	TCAA	DCAA	TCAA
1	255	2/7/95	0.20	1.40	0.19	1.33	0.35	1.54
2	191	2/9/95	0.27	2.11	0.22	1.77	0.32	2.41
3	222	2/20/95	<0.24	4.82	<0.25	5.11	0.18	6.22
4	369	3/14/95	0.12	3.26	0.12	3.27	0.23	0.95
5	381	3/30/95	0.27	1.24	0.29	1.33	0.43	1.28
6	325	4/3/95	2.65	14.07	2.93	15.57	0.46	11.69
7	245	4/10/95	0.053	0.27	0.13	0.66	0.095	1.15
8	188	4/12/95	0.55	24.96	0.21	9.59	NA	NA
9	275	4/18/95	<0.081	1.68	<0.10	2.10	0.14	2.06
10	272	5/3/95	1.48	8.26	1.17	6.59	0.63	5.40
11	386	5/8/95	0.81	17.75	0.88	19.25	1.13	15.71
12	376	5/10/95	2.11	6.23	2.84	8.42	1.08	5.22
13	307	5/24/95	1.09	12.23	0.84	9.40	0.29	6.29
14	112	6/9/95	1.24	18.14	1.23	17.97	0.95	11.62
15	291	6/28/95	0.47	6.67	0.67	9.49	3.05	68
16	259	7/6/95	0.86	9.27	1.32	10.51	0.59	1.76
17	115	7/13/95	0.74	2.99	1.76	7.09	0.77	4.24
18	251	7/26/95	0.9	1.6	3.27	5.81	1.54	5.43
19	334	7/27/95	0.49	2.04	1.17	4.84	1.5	6.33
20	290	8/8/95	1.54	3.17	2.08	4.3	NA	NA
21	267	8/10/95	2.69	3.88	4.21	6.06	1.95	4.01
22	288	8/15/95	1.27	3.95	3.06	9.5	2.9	8.94
23	313	8/16/95	2.9	4.66	3.91	6.27	4.98	11.5
24	343	8/22/95	0.76	8.44	0.97	10.71	2.59	23.65
25	124	8/28/95	1.52	18.21	1.62	19.42	4.22	18.38
26	295	9/11/95	2.56	20.65	5.71	46.17	1.88	8.16
27	172	9/12/95	7.18	28.32	7.04	27.75	NA	NA
28	147	9/18/95	0.86	6.35	1.18	8.73	2.96	7.15
29	116	10/2/95	2.75	1.40	6.23	3.16	2.86	3.24
30	144	10/16/95	NA	NA	NA	NA	3.25	7.30
31	348	10/18/95	2.55	7.28	1.99	5.68	1.64	7.75
32	328	10/19/95	1.87	11.02	2.79	16.46	1.26	8.19
33	487	10/23/95	1.08	2.13	1.06	2.08	1.17	4.13
34	349	10/26/95	1.71	10.2	1.98	11.8	1.16	6.94
35	339	10/26/95	2.88	9.54	2.32	7.71	2.96	9.74
36	238	10/30/95	0.43	1.61	0.81	3.03	1.79	7.32
37	315	11/1/95	0.86	1.7	1.95	3.86	2.04	4.67
38	148	11/2/95	1.01	16.4	1.77	28.8	1.59	35
39	479	11/7/95	6.6	30.6	2.85	13.2	1.09	6.99
40	462	11/8/95	1.08	6.31	0.76	4.44	1.08	6.68
41	481	11/30/95	NA	NA	NA	NA	7.38	15.9
42	229	12/13/95	0.44	0.46	1.84	1.92	2.23	3.58
43	418	12/21/95	1.05	4.86	3.64	16.8	1.9	7.21
44	299	1/3/96	0.42	2.2	0.72	3.81	0.53	13
45	410	1/23/96	0.47	1.46	0.485	1.51	0.3	1.3
46	219	1/24/96	0.75	5.22	0.74	5.17	NA	NA
47	413	1/29/96	0.49	3.95	0.39	3.14	0.308	2.22
48	438	2/5/96	0.73	5.51	0.7	5.28	0.96	5.78
49	482	2/6/96	0.65	2.76	0.62	2.61	NA	NA

Comparisons of the CBPs water concentrations for the cases and controls for the entire data set and within each group are given in Table 5. While the cases had somewhat higher chloroform and TTHM concentrations when all data were combined these were only statistically higher in the high exposure group. However, since these were not a random sample of the population it cannot be extrapolated to the entire case/control study. The only statistically significant differences in the means concentrations between the cases and controls for the low exposure group was for bromoform. This was the result of bromoform being detectable more frequently in the group of cases than in the control group. However, only five bromoform values exceeded 1 μg/L in entire data set.

The mean THM air concentration in the homes for the high and low exposure groups and for the cases and controls are provided in Table 6. As was observed during the TEAM study, homes with higher tap water THM concentration had higher mean air concentration for chloroform. This was confirmed with the high exposure and low exposure groups and the cases and controls for the high exposure group having statistically significant mean air concentrations for chloroform. While the mean air concentrations for the high exposure group for the other THMs were elevated compared to the low exposure groups, the differences were not statistically different, with many of the values being below the detection limit in both groups. This is consistent with volatilization from the water being the main source of THMs to the air, with chloroform the most prevalent THM and the most volatile. The higher air concentrations for cases in the high exposure group is also consistent with the higher mean water concentrations measured for that group.

Table 5
Comparison of Water Concentrations (μg/L)
Average $\pm$ Standard Deviation
Number of Samples Above the Detection Limit/Total Number

	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$	TTHMs	DCAA	TCAA
All Data							
Low	1.9±2.7 20/29*	0.9±1.2 24/29*	.89±.79 19/29*	.51±.91 17/29	6.3±7.0 24/29*	6.3±11 29/29*	3.8±5.2 29/29*
High	61±50 20/20*	11±10 20/20*	2.9±2.7 20/20*	.33±.45 11/20	90±71 20/20*	28±26 20/20*	40±30 20/20*
All Data							
Cases	32±52 17/22	3.±11 20/22	2.0±2.6 18/22	.7±1.0 14/22	50±78 20/22	22±24 22/22	19±25 22/22
Controls	22±35 23/27	4.2±5.5 24/27	1.4±1.4 21/27	.21±.32 14/27	33±45 24/27	17±25 27/27	18±27 27/27
Low Exposure Group							
Cases	.70±.83 8/13	.58±.68 11/13	.88±.83 9/13	0.9±1.3 9/13	5.3±4.9 11/13	6.0±4.8 13/13*	2.7±2.7 13/13
Controls	2.0±3.5 11/15	1.0±1.4 12/15	.84±.77 9/15	.15±.19 7/15	5.8±6.8 12/15	4.7±8.1 15/15*	3.7±5.3 15/15
High Exposure Group							
Cases	76±58 9/9*	15±13 9/9*	3.6±3.5 9/9	.41±.51 5/9	110±89 9/9*	45±15 9/9	44±23 9/9
Controls	46±40 12/12*	8.1±6.32 12/12*	2.2±1.6 12/12	.28±.41 7/12	67±48 12/12*	32±31 12/12	35±34 12/12

* statistically different at $p < .05$ (Mann Whitney Rank Sum Test)

Table 6 Comparison of Air Concentrations ($\mu g/m^3$) Average ± Standard Deviation Number of Samples Above the Detection Limit/Total Number				
	$CHCl_3$	$CHBrCl_2$	CHBr2Cl	CHBr3
All Data				
Low Exposure Group	0.5±0.6* 20/28	0.4±0.8 14/28	0.5±0.9 6/28	0.3±0.9 9/28
High Exposure Group	5.0±6.9* 19/20	0.8±1.0 14/20	0.6±0.9 6/20	0.4±1.0 5/20
Water Concentration - Low Group				
Cases	0.5±0.7 9/13	0.6±1.1 7/13	0.7±1.3 3/13	0.5±1.3 7/13
Controls	0.5±0.5 11/15	0.2±0.3 7/15	0.3±0.2 3/15	0.2±0.2 2/15
Water Concentration - High Group				
Cases	8.3±9.8* 8/8	1.2±1.2 5/8	0.6±0.7 3/8	0.6±1.1 2/8
Controls	2.8±3.7* 11/12	0.6±0.8 9/12	0.6±1.0 3/12	0.4±0.9 3/12
*two groups were statistically different at $p<.05$ (Mann Whitney Rank Sum Test)				

BIOMARKERS

Exhaled breath

The background breath concentrations for each of the four THMs were also compared, however the majority of the measurements were below the detection limit. Only five samples were above detection for chloroform for the high exposure group for the cases and controls, with mean values of 3.4±4.1 and 3.4±4.5, respectively. Fewer samples were detectable in the low exposure group and for the other THMs.

In addition to the background exhaled breath concentration, which only showed differences between the high and low groups for chloroform and TTHMs, post shower breath samples were collected. As previously discussed, directions were given to the participants to collect the breath sample at the end of their shower. However, the subjects interpreted this differently with some collecting the sample at the end of the shower while others collected their breath samples when they left the bathroom. These variations in collection protocol resulted in considerably different time lags between the end of the shower and when the breath sample was collected. The breath concentrations of THMs decline exponentially once a shower ceases. Thus the time lag between the end of the shower and when samples are collected greatly affects the breath concentration. To account for the time lag a questionnaire was sent to the participants to determine approximately how long after a shower the sample was collected and each subject was assigned to one of three groups which had the following approximate time lags: Group A: 0 to 5 minutes (immediately after showering), Group B: 5 to 20 minutes (after drying off and before leaving the bathroom) and Group C: greater than 20 minutes (after leaving the bathroom). The breath concentrations for each of the THMs by time lag Group, along with the TTHM water concentration and case/control status are given in Table 7. The concentration of each THM in the exhaled breath was compared to the THMs concentrations in the water for each Group separately, using different symbols for the cases and controls, in Figures 1 to 12. A general observation for all groups and compounds is that the participants showering with the water having the lowest THM concentrations had the lowest breath THM concentrations. A trend of

Table 7
THM Exhaled Breath Concentration After Shower

Group	Type	$CHCl_3$ $\mu g/m^3$	$CHBrCl_2$ $\mu g/m^3$	$CHBr_2Cl$ $\mu g/m^3$	$CHBr_3$ $\mu g/m^3$	TTHMS in Water $\mu g/L$
A	Case	0.88	0.35	1.8	<0.6	0.18
A	Case	0.59	<0.3	2.2	<0.6	1.7
A	Case	<1	<0.3	<1	<0.6	2.2
A	Case	2.8	6.6	7.2	5.0	7.2
A	Case	59	4.5	3.3	2.9	21
A	Case	44	8.7	3.6	2.8	52
A	Case	99	14	<1	<0.1	84
A	Control	1.9	1.3	<1	<0.6	0.21
A	Control	18	11	6.1	<0.6	11
A	Control	<1	<0.3	<1	4.5	26
A	Control	74	18	11	1.0	33
A	Control	67	11	<1	0.77	35
A	Control	36	3.3	<1	0.25	44
B	Case	<1	<0.3	<2	<0.6	1.2
B	Case	1.9	0.15	<2	<0.6	2.9
B	Case	<1	<0.3	<1	<0.6	4.2
B	Case	<1	<0.3	<2	<0.2	4.3
B	Case	1.0	1.1	<2	<0.6	4.7
B	Case	8	2.8	4	1	35
B	Case	6.3	0.68	<1	<0.2	44
B	Case	360	42	5.9	2.0	97
B	Case	34	5.9	<2	<0.6	120
B	Control	1.5	<0.1	<1	<0.2	0.27
B	Control	3.9	<0.1	<1	<0.2	0.47
B	Control	<1	<0.3	<2	<0.6	1.0
B	Control	1.6	0.44	<1	<0.2	1.4
B	Control	14	14	8.7	7.2	51
B	Control	506	26	3.3	<0.6	140
B	Control	20	1.5	<2	<0.6	160
C	Case	<1	<0.3	<2	<0.6	1.0
C	Case	<1	<0.3	<2	<0.6	8.0
C	Case	40	0.62	<1	<0.3	140
C	Control	1.5	<0.3	<2	<0.6	7.7
C	Control	2.6	7.9	<4	1	10
C	Control	1.8	<0.3	<1	<0.6	34
*	Case	6.5	1.6	<4	1	260

Figure 1

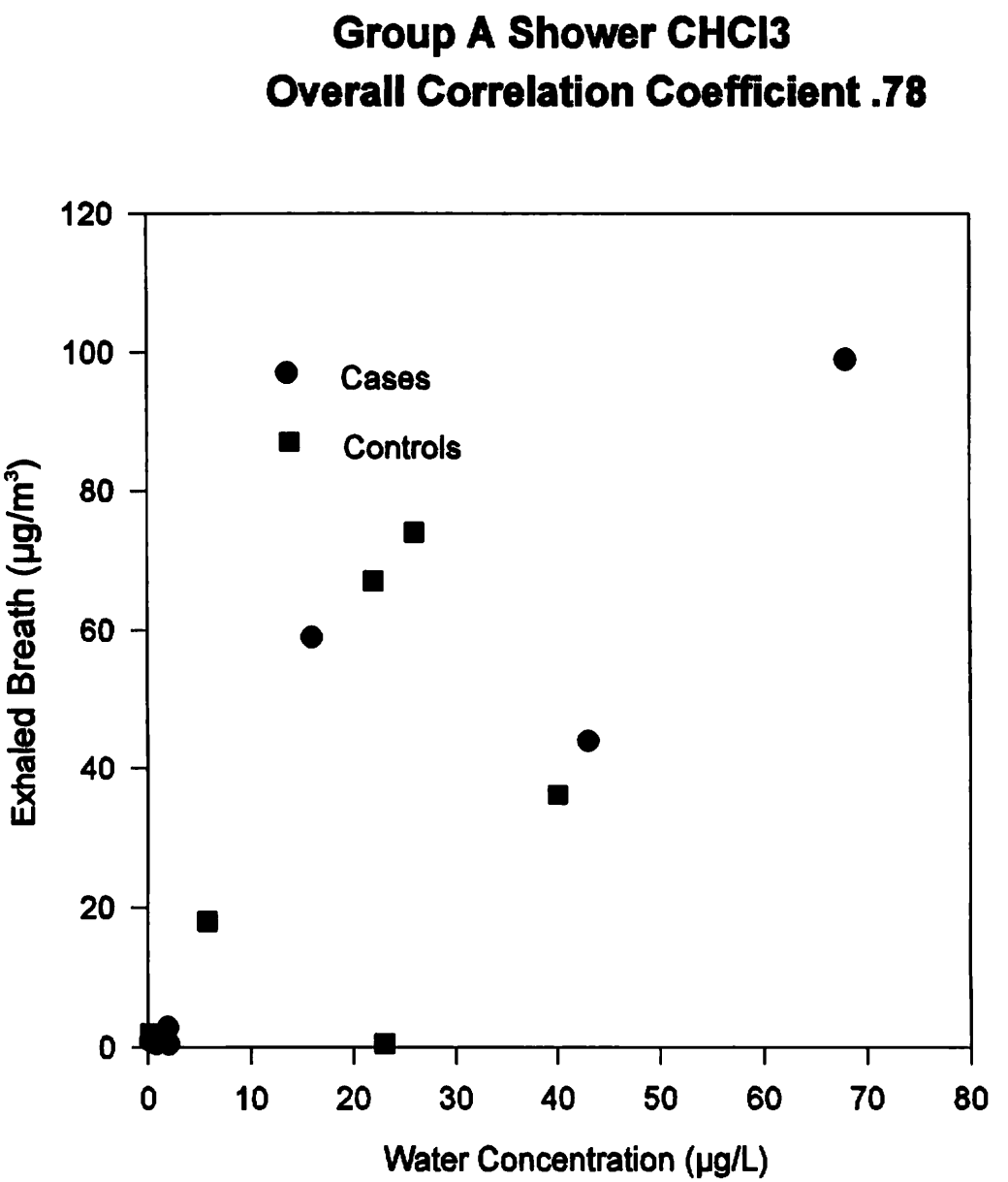

Group A Shower CHCl3
Overall Correlation Coefficient .78
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
Cases
Controls
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80

Figure 2

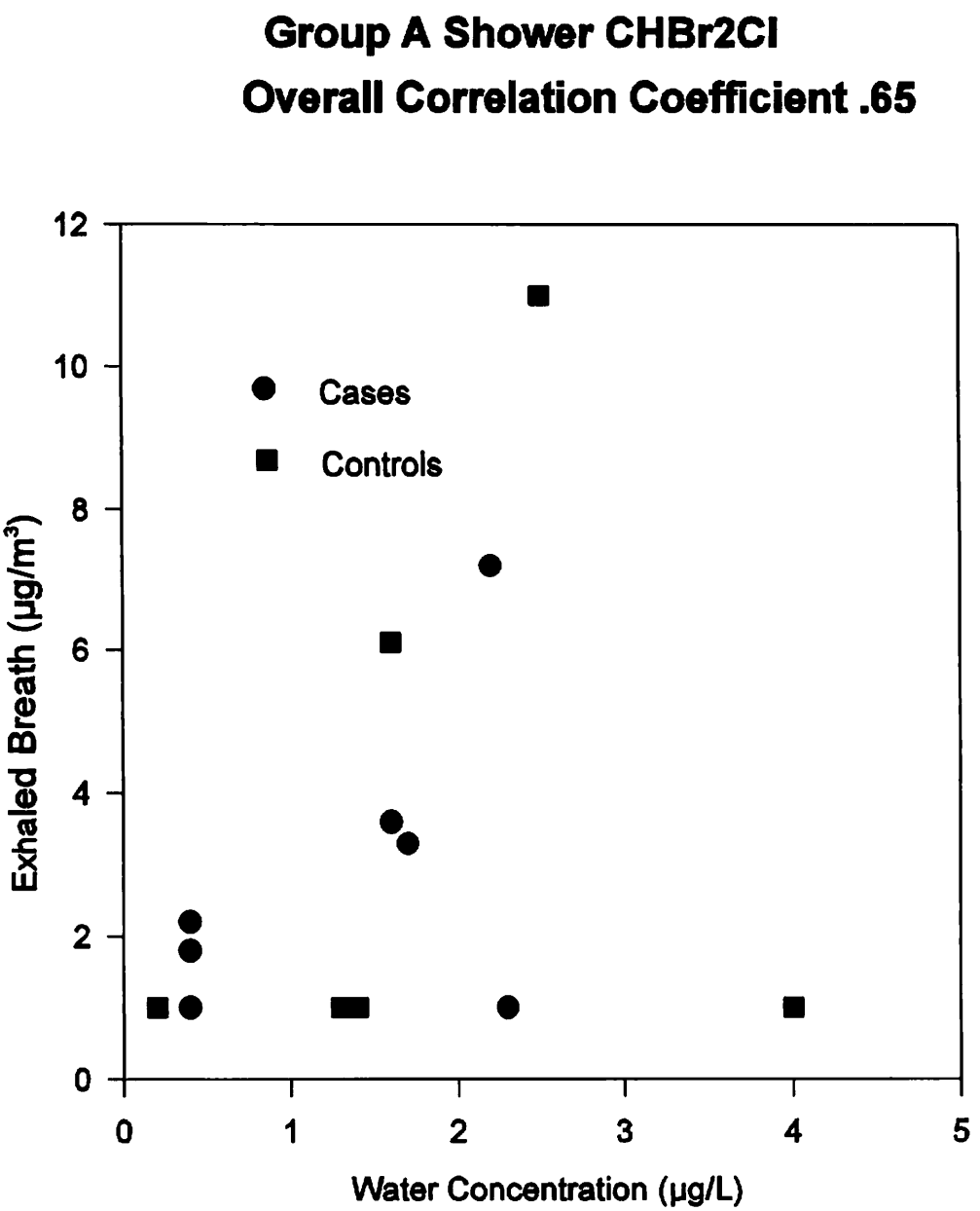

Group A Shower CHBr2Cl
Overall Correlation Coefficient .65
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
0
2
4
6
8
10
12
0
1
2
3
4
5

Figure 3

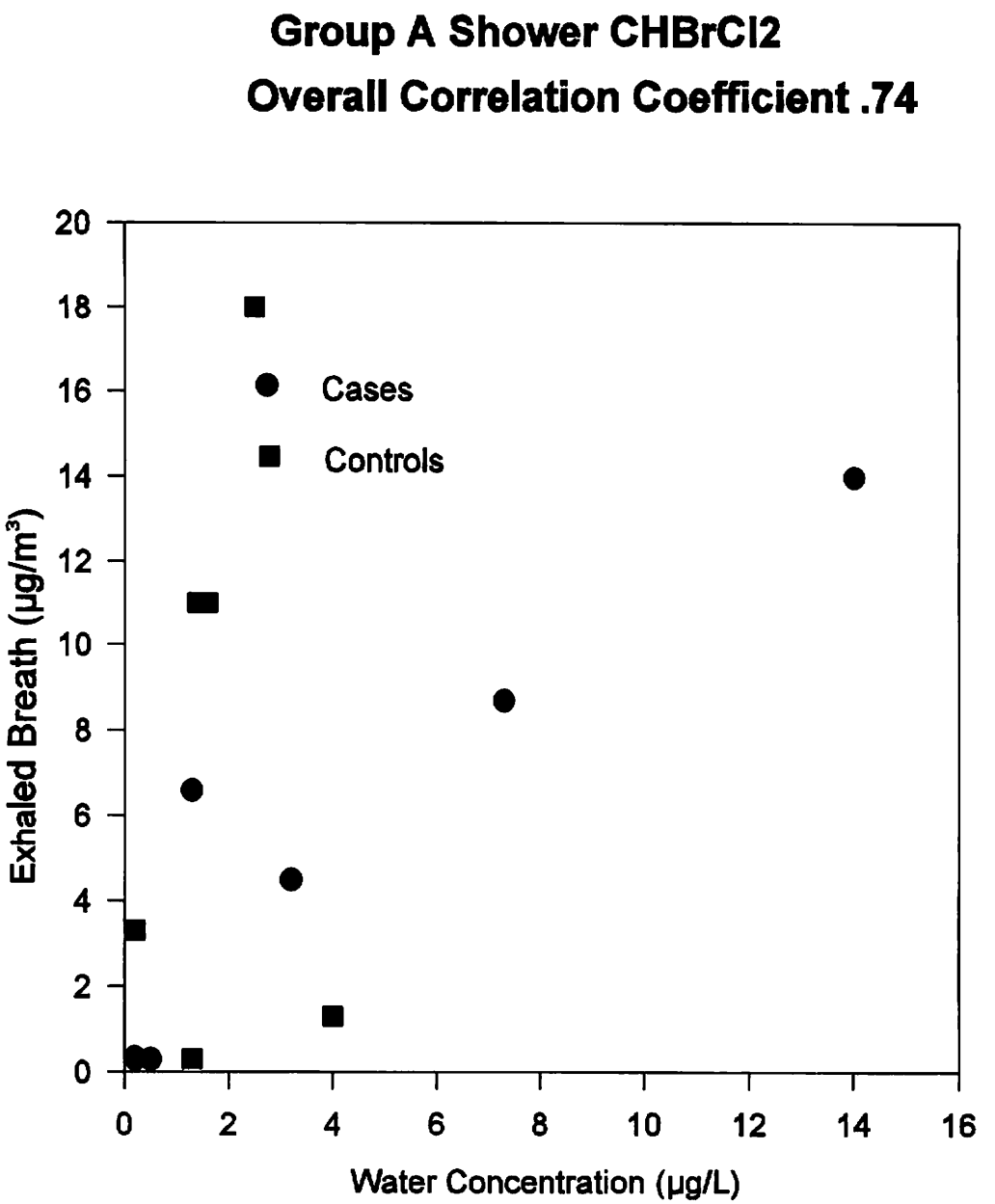

Group A Shower CHBrCl2
Overall Correlation Coefficient .74
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
0
2
4
6
8
10
12
14
16
18
20

Figure 4

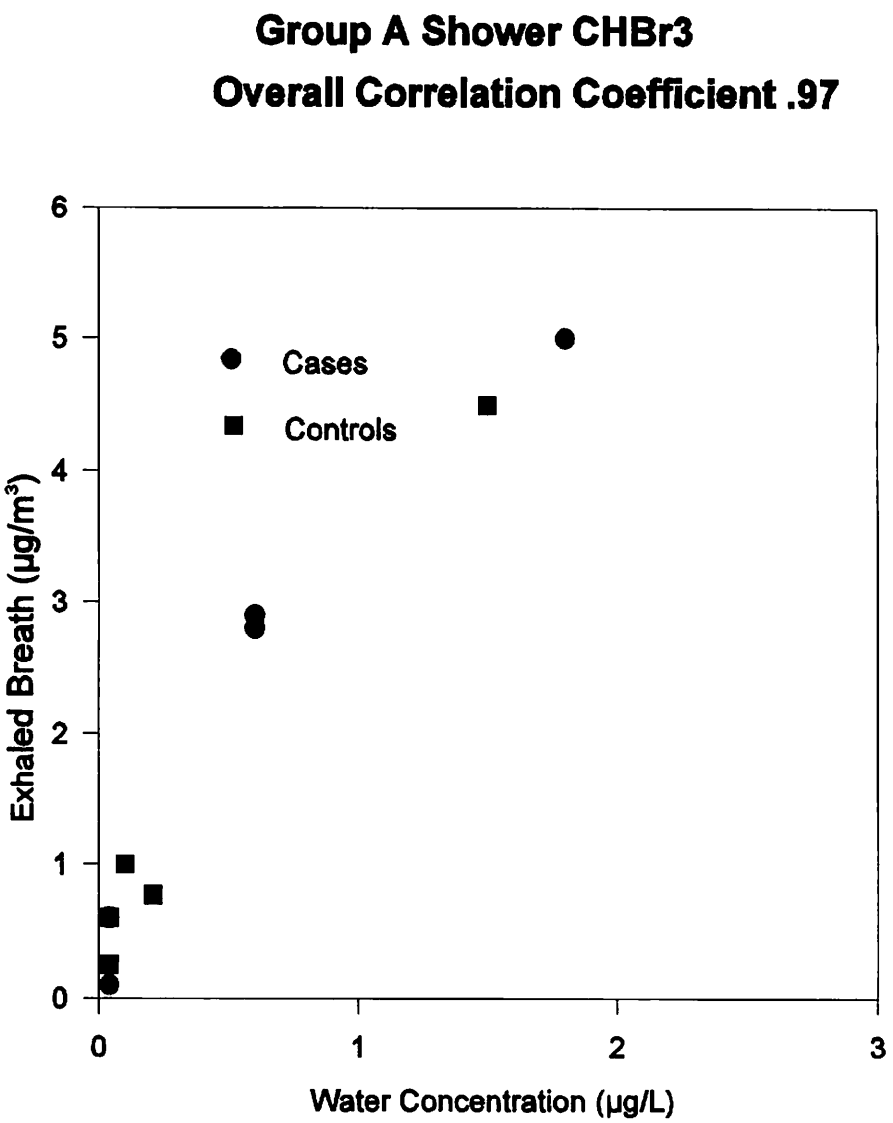

Group A Shower CHBr3
Overall Correlation Coefficient .97
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
0
1
2
3
4
5
6

Figure 5

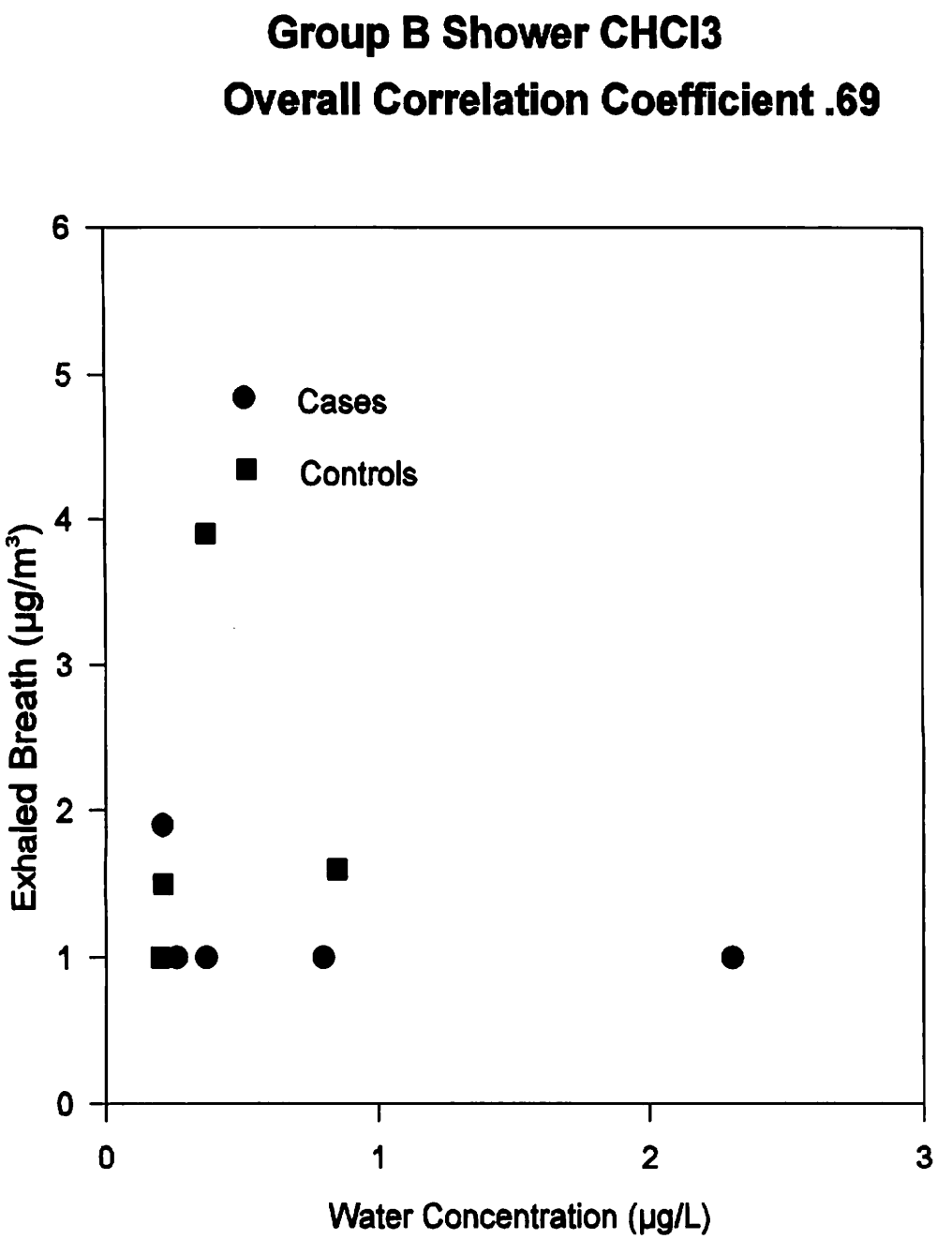

Group B Shower CHCl3
Overall Correlation Coefficient .69
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
0
1
2
3
4
5
6

Figure 6

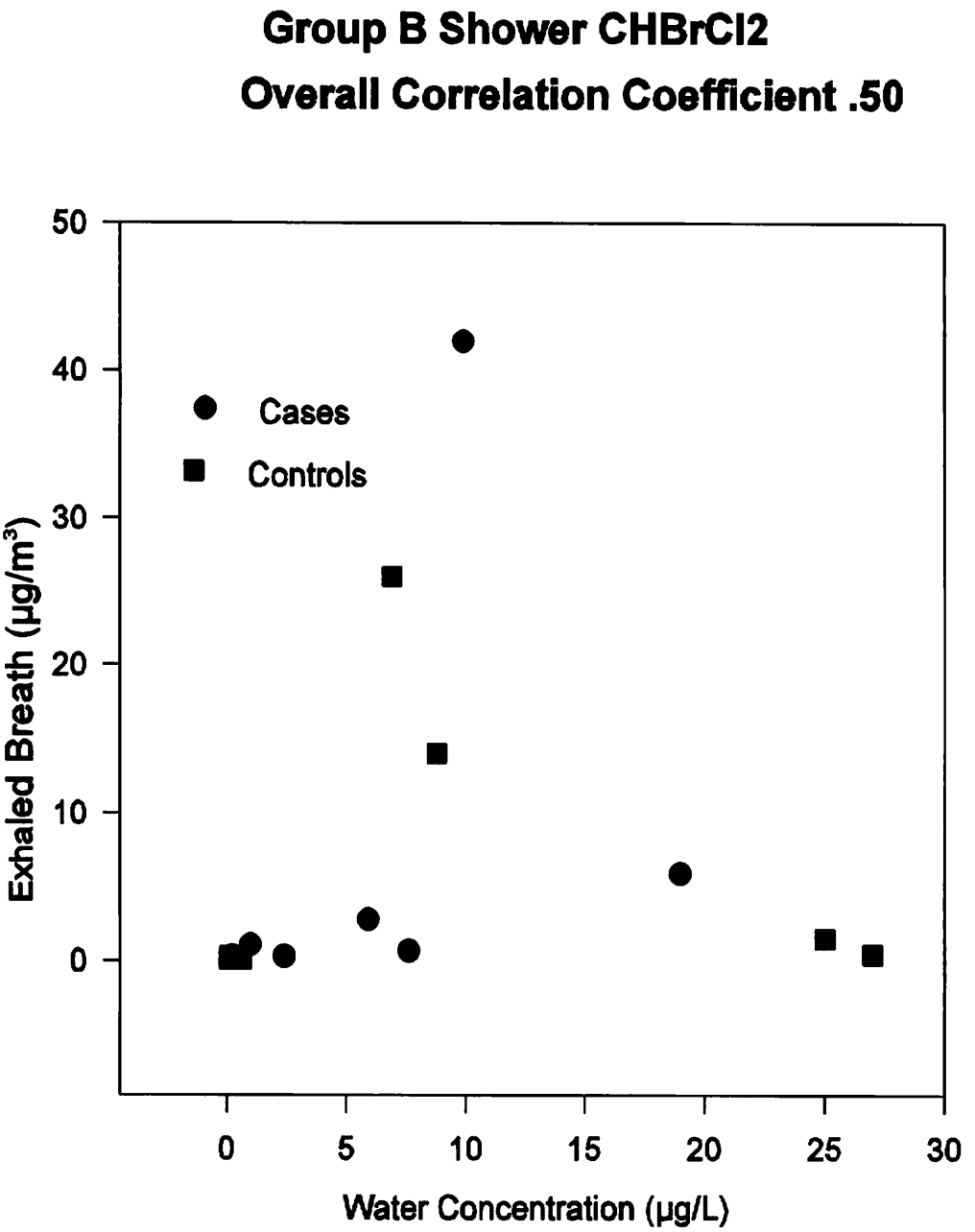

Group B Shower CHBrCl2
Overall Correlation Coefficient .50
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
50
40
30
20
10
0
0
5
10
15
20
25
30

Figure 7

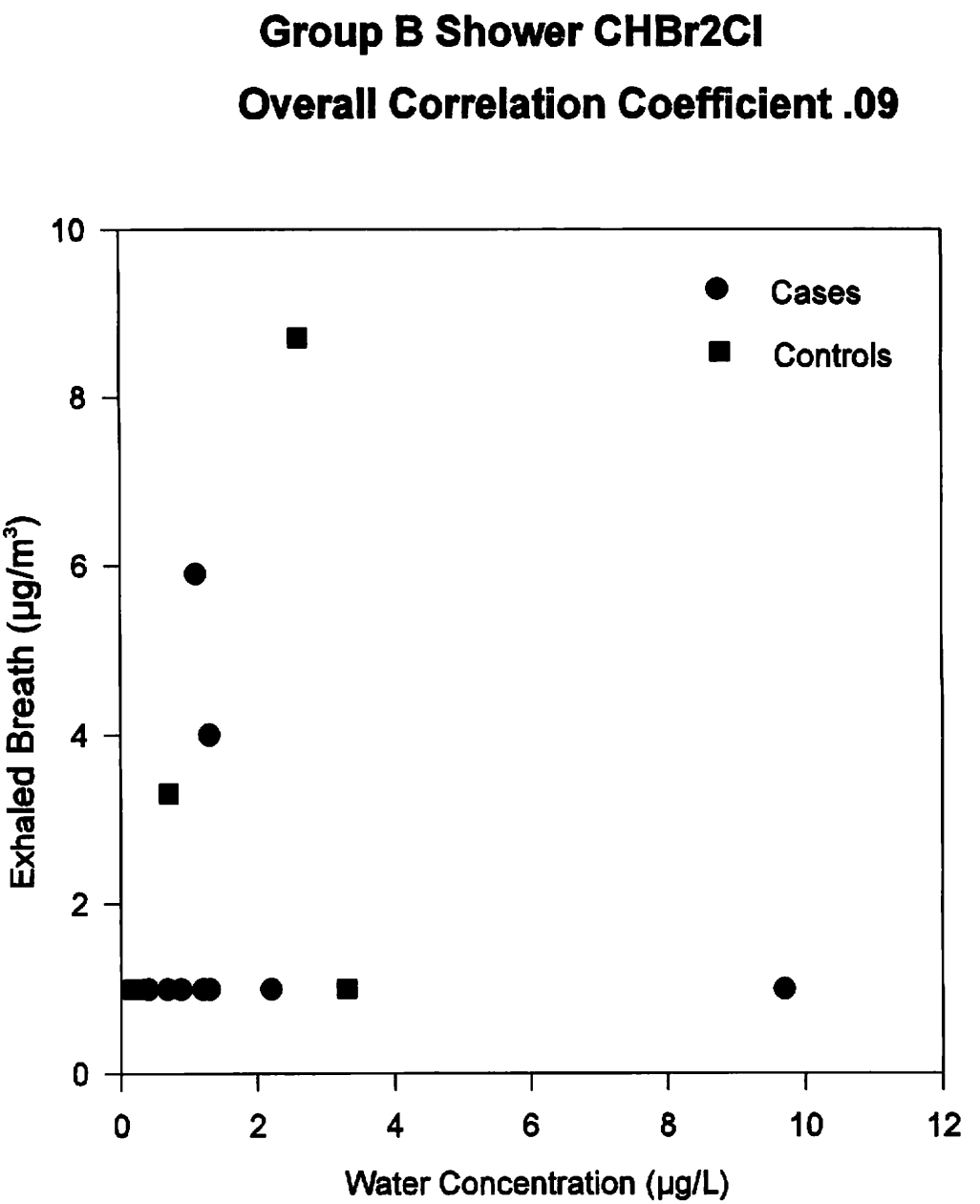

Group B Shower CHBr2Cl
Overall Correlation Coefficient .09
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
0
2
4
6
8
10
12

Figure 8

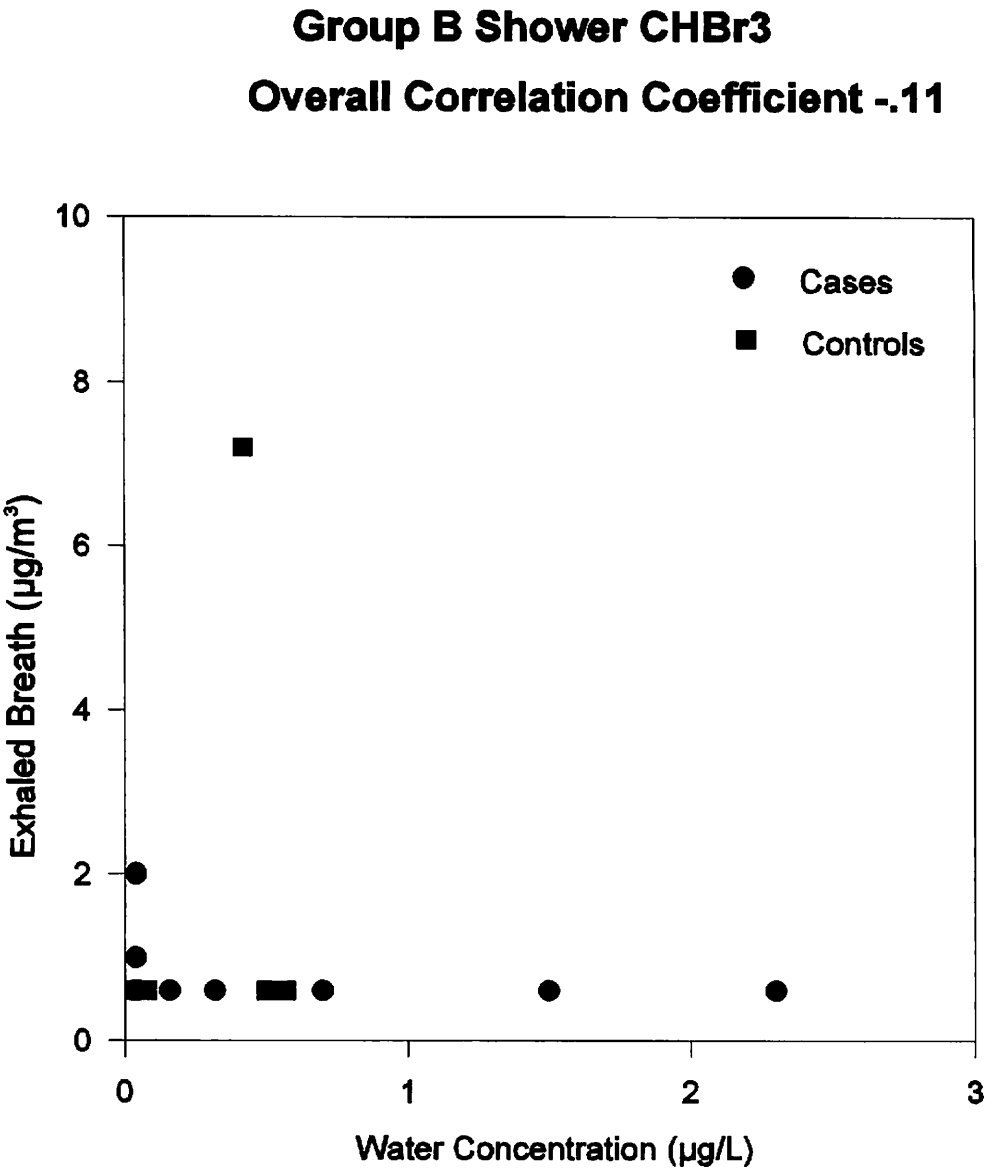

Group B Shower CHBr3
Overall Correlation Coefficient -.11
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
0
2
4
6
8
10
0
1
2
3

Figure 9

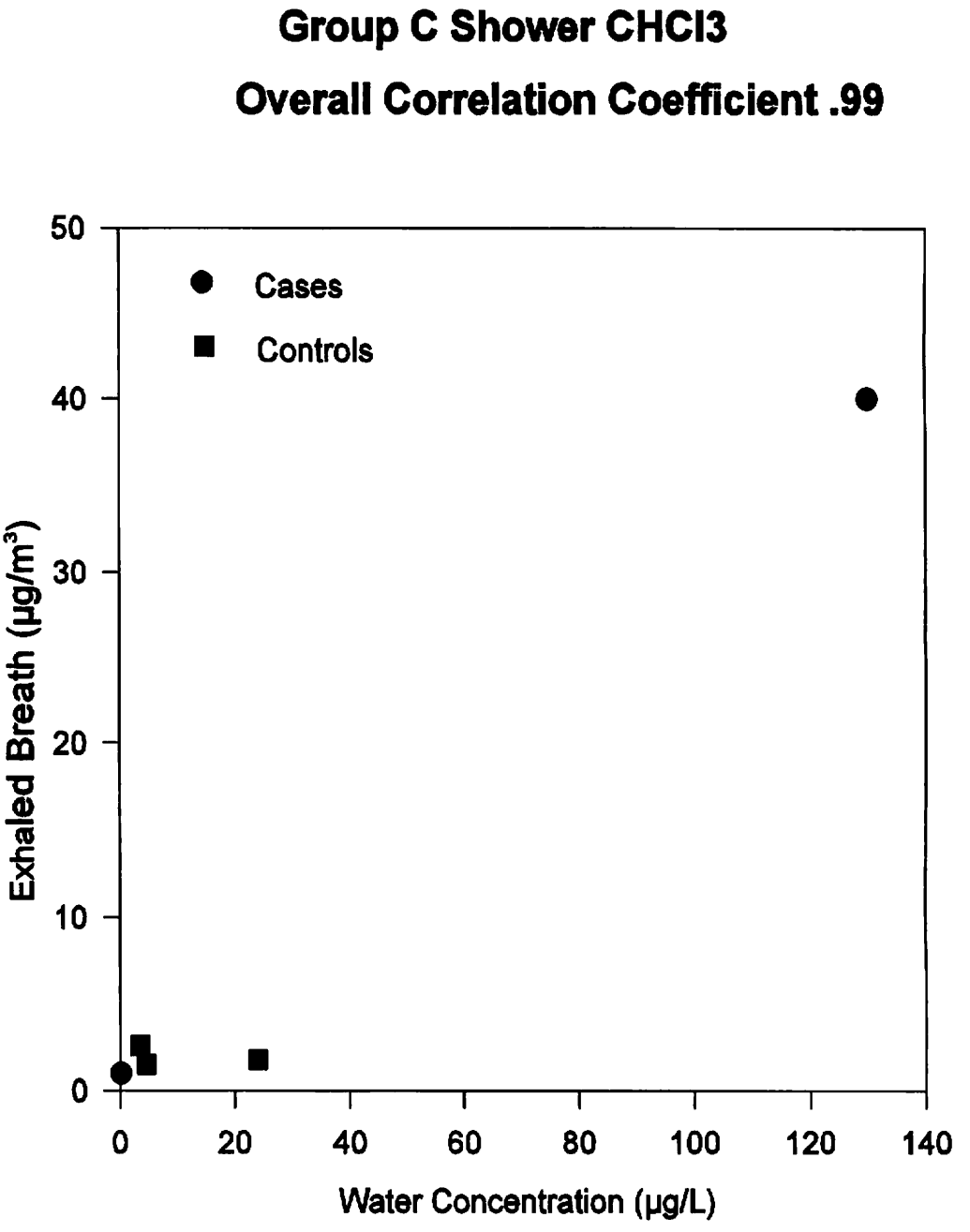

Group C Shower CHCl3
Overall Correlation Coefficient .99
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
0
10
20
30
40
50
0
20
40
60
80
100
120
140

Figure 10

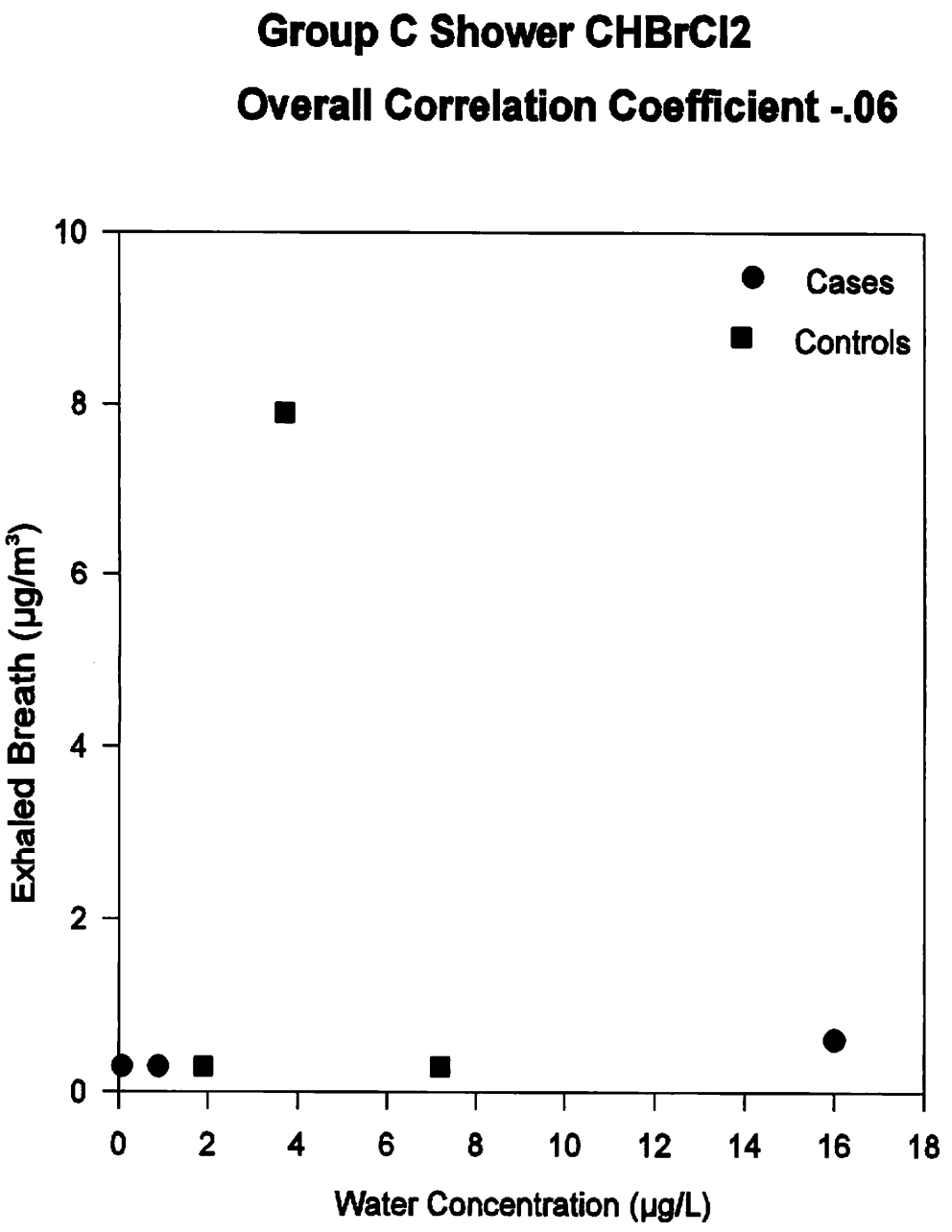

Group C Shower CHBrCl2
Overall Correlation Coefficient -.06
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
0
2
4
6
8
10
12
14
16
18

Figure 11

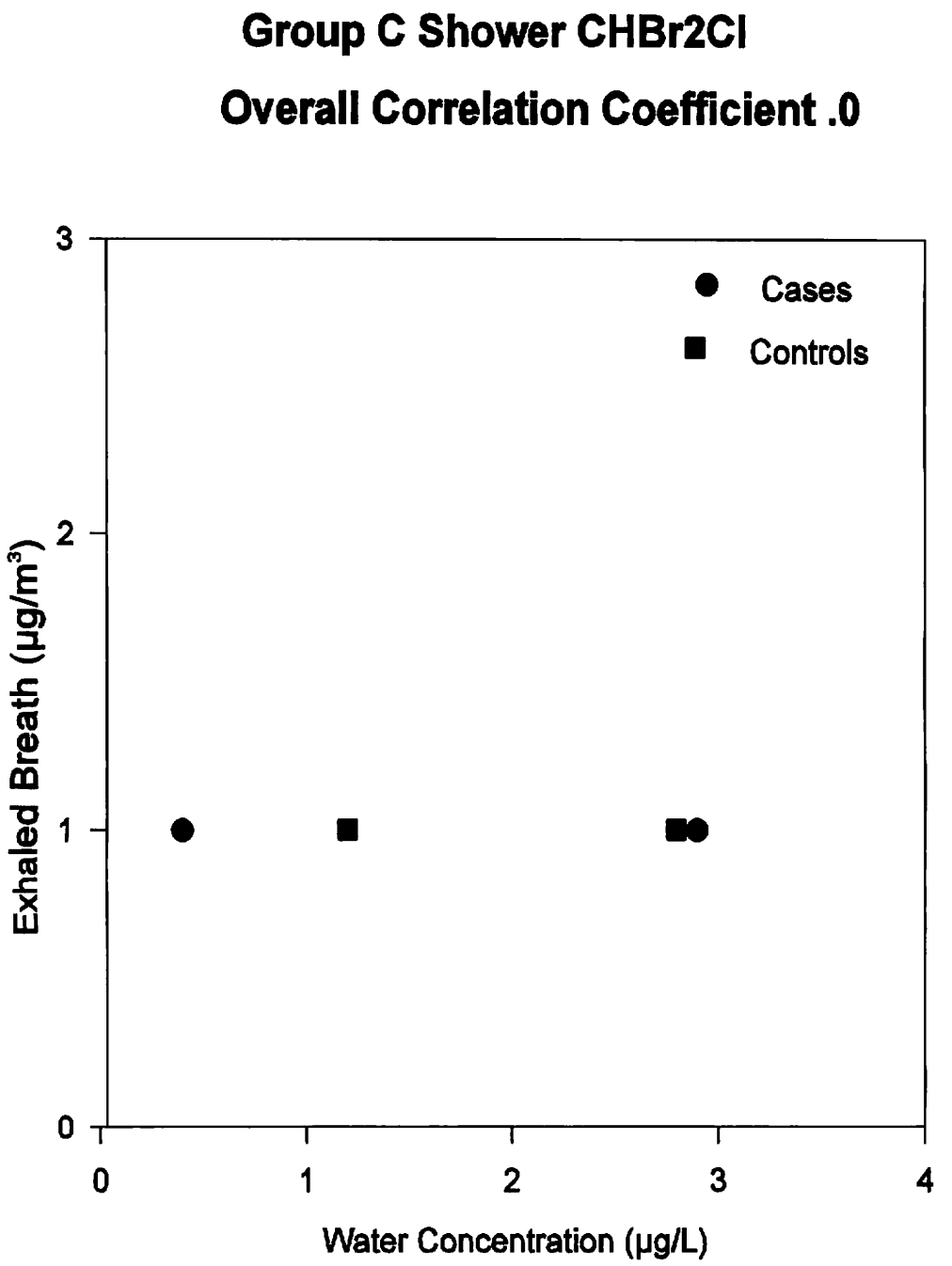
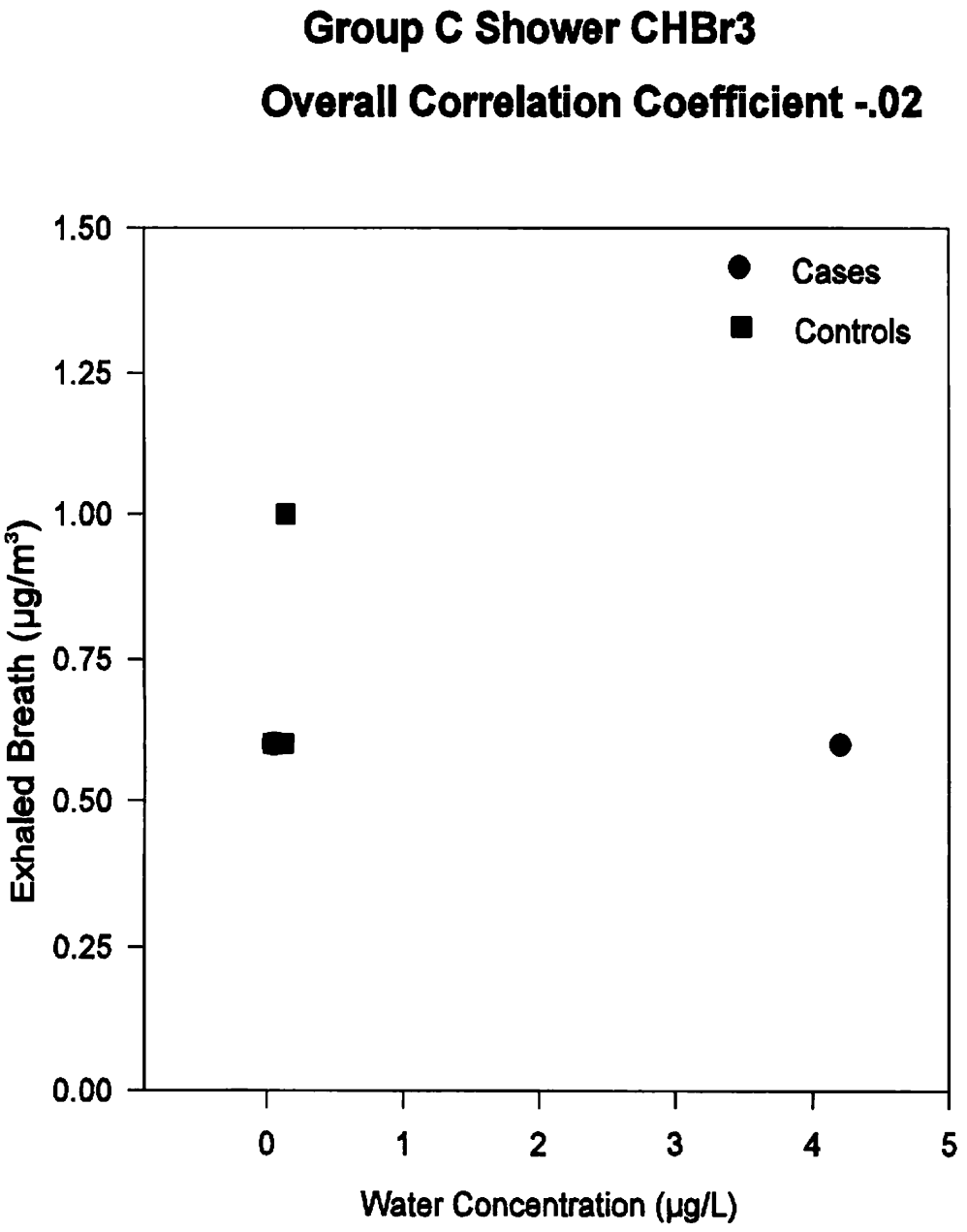

Group C Shower CHBr2Cl
Overall Correlation Coefficient .0
Cases
Controls
Exhaled Breath (µg/m³)
Water Concentration (µg/L)
0
1
2
3
4

Figure 12


Group C Shower CHBr3
Overall Correlation Coefficient -.02
Cases
Controls
Exhaled Breath (µg/m³)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
5
Water Concentration (µg/L)

increasing breath concentration with increasing water concentrations is also observed, though individual data points deviate from a linear relationship. The cause for the deviation is most probably the difference in the lag time between the end of the exposure and when the breath sample was collected, though differences in metabolic rates and dispersion of the THMS in the body could also contribute to scatter in the data. Chloroform and bromodichloromethane were the two most abundant THMs and show the strongest relationship, with statistically significant correlation coefficients (R) for Group A and B. The relationships for the other two compounds were evident for the Group A participants, but not Group B. Only five participants were in Group C and no consistent trend is observed. These observations are consistent with what would be expected since by twenty minutes the breath concentrations are starting to approach background values. The mean and median exhaled breath concentrations for each THM for the lower and higher half of the water concentrations for each group are given in Table 8. The mean breath concentrations for the higher water concentrations were always higher, usually statistically so, than those for the lower water concentrations, with the median values being greater as the lag time increased. This confirms that exhaled breath is a biomarker of exposure within a shower for all of the THMS, but its utility is somewhat limited by the need to quantify the time after a shower that the sample is collected.

A regression analysis of each THM was run, which included the exhaled breath of each THM as the dependent variable and the Group, water concentration, subjects' average shower duration, water temperature (hot, warm, cold) and case/control status as the independent variables. Only the water concentration was included as a predictive variable of chloroform

breath concentration, while group was predictive for dibromochloromethane. None of the variables were predictive for bromodichloromethane at a $p < .05$, though water concentration had a p of .06 in a regression analysis. Pearson Product Moment Correlation Coefficients and Spearman Rank Order Correlation Coefficients were calculated among the water concentration, exposure concentration (water concentration x shower duration) and breath concentration. Similar values were obtained by both methods. The values for the Spearman analysis are provided in Table 9. As expected the three primary THMS were highly correlated with one another, while $CHBr_3$ was not due to the large number of values below the detection limit which were assigned a constant value. All of the water concentrations were highly correlated with the exposure values ($R^2 > 0.9$), since the exposure values were calculated as the product of the water concentration multiplied by the time spent in the shower. The breath concentrations of each THM, except $CHBr_2Cl$, were correlated with both the exposure and water concentration of that THM, with little improvement between the exposure relationship compared to the water concentration. This could be because the actual time of the shower being considered was unknown so the average shower time the subjects reported on the questionnaire was used.

Table 8
Mean (Median) THM Breath Concentrations ($\mu g/m^3$) Following a Shower By Group Divided into the Highest and Lowest Water Concentrations in That Group

Group	Water Conc.	Number	$CHCl_3$	CHBrCl	$CHBr_2Cl$	$CHBr_3$
A	Low	6	4.2(1.5)	1.5(0.8)	1.3(1.0)	0.5(0.6
	High	7	47(58)	9.5(8.7)	3.7(3.6)	2.5(1.0)
B	Low	8	1.6(1.2)	0.4(0.3)	1.8(1.5)	0.6(0.4)
	High	8	120(17)	12(4.3)	3.3(2.6)	1.4(0.6)
C	Low	3	1.5(0.8)	0.3(0.3)	1.7(2.0)	0.5(0.6)
	High	3	14(2.6)	2.9(0.6)	2.3(2.0)	0.7(0.6)

Table 9
Correlation Coefficients of THMS in Water, Exposure and Exhaled Breath
Spearman Rank Order Correlation Coefficient, p Value

	Water Concentration (μg/L)				Exposure {(μg/l)-min}				Breath Concentration ($\mu g/m^3$)			
	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Water $CHCl_3$	-	.91,.001	.59,.001	-.08,.65	.95,.001	.87,.001	.57,.001	-.07,.68	.72,.001	.62,.001	.29,.08	.17,.33
$CHBrCl_2$		-	.78,.001	.07,.67	.87,.001	.94,.001	.71,.001	.09,.61	.69,.001	.62,.001	.25,.14	.18,.30
$CHBr_2Cl$			-	.40,.01	.59,.001	.77,.001	.91,.001	.41,.01	.39,.02	.37,.03	.17,.33	.17,.31
$CHBr_3$				-	-.02,.87	.09,.31	.39,.02	.98,.001	-.15,.37	-.01,.95	-.09,.58	.43,.009
Exposure $CHCl_3$					-	.92,.001	.66,.001	.02,.91	.74,.001	.59,.001	.25,.13	.16,.34
$CHBrCl_2$						-	.84,.001	.14,.41	.72,.001	.61,.001	.28,.13	.12,.48
$CHBr_2Cl$							-	.44,.006	.49,.002	.45,.006	.26,.12	.19,.25
$CHBr_3$								-	-.12,.49	-.26,.88	-.09,.62	.43,.007
Breath $CHCl_3$									-	.86,.001	.54,.001	.25,.13
$CHBrCl_2$										-	.66,.001	.33,.05
$CHBr_2Cl$											-	.25,.13
$CHBr_3$												-

Urinary Biomarkers

The urinary excretion rate and concentrations and water concentrations of DCAA and TCAA of the low and high exposure groups were compared (Table 10). While large differences in the mean concentrations were present for the water concentrations of these two species only the urinary TCAA concentration and excretion rates of first morning urine were statistically different.

Table 10
Excretion of Haloacetic Acids

	DCAA Water µg/l	[1]FMU DCAA ng/l	[1]FMU DCAA [3]Normalized ng/mg	[1]FMU DCAA Excretion Rate ng/min	[2]VU DCAA ng/l	[2]VU DCAA Normalized ng/mg
Low Exposure						
Mean	1.8±1.9*	2.1_2.4	1.5±1.5	1.1±1.0	1.9±1.9	1.4±1.3
Median	0.8	1.4	0.9	0.7	1.4	0.8
N >mdl	24	22	22	22	22	22
High Exposure						
Mean	36±24	2.9±2.3	2.0±1.7	1.7±1.8	2.4±1.4	1.8±1.6
Median	34	2.1	1.3	1.1	1.9	1.4
N>mdl	25	23	23	23	23	23
	TCAA Water µg/l	FMU TCAA ng/l	FMU TCAA Normalized ng/mg	FMU TCAA Excretion Rate ng/min	VU TCAA ng/l	VU TCAA Normalized ng/mg
Low Exposure						
Mean	2.0±1.9*	8.5±7.0*	6.5±6.3*	4.7±3.9*	13±17	9.8±15
Median	1.0	6.9	4.7	3.6	7.2	6.0
N >mdl	24	24	24	22	22	22
High Exposure						
Mean	34±29	17±14	11±1	11±9	12±8	8.2±5.5
Median	24	11	9	8	11	8
N>mdl	25	23	23	23	22	22

[1]FMU - first morning urine, [2]VU - urine collected during visit, [3] Normalized - normalized to creatinine
* - statistically different between high and low groups at $p < .01$

However, water concentration is not the best estimate of the exposure, since different amounts of water are ingested by different individuals, some people had filters on their tap water in the kitchen and the heating of water can change the concentrations of the HAA. The effectiveness of a filter is dependent upon the maintenance of the filter and the appropriateness of the filter material for removing HAAs. An average removal efficiency for HAAs of 80% was calculated for the study population by comparing the HAA concentration in water collected from the bathroom, which was not filtered, to that collected from the kitchen, in a subset of homes that indicated that the water was filtered. To determine the effect of heating water on the HAA concentration, water with known quantities of HAA were heated to boiling for five minutes to simulate the preparation of coffee, tea and soup. No change in the water concentration of DCAA was detected, after correcting for the change in water volume that resulted from the heating. However, TCAA decreased by an average 39%. Based on the above the exposure was calculated as follows:

$$EXPOSURE_{DCAA} = WaterConc[\times.2(if filtered)]\times(WaterIngested + HeatedWaterIngested)$$

$$EXPOSURE_{TCAA} = WaterConc[\times.2(if filtered)]\times(WaterIngested + HeatedWaterIngested\times.61))$$

The association observed between the urinary TCAA and exposure was strengthened compared to just using the water concentration, with no association observed for urinary DCAA compared to either water concentration or exposure. An additional confounder to the exposure estimate being made was that some of the participants worked outside of their home and consumed water, coffee and tea outside their home. This was an additional source of HAA that could not be accounted for using the current model, since the DCAA and TCAA water concentrations of the

water consumed outside the home were unknown (figure 13). The association between urinary TCAA/DCAA and exposure was therefore examined only for those participants who did not work outside their home (figures 14). A higher correlation for TCAA was observed for this subgroup than the entire data set, supporting the contention that the exposure calculated was confounded by the water ingested by individuals at work. A final confounder that was identified in the data set for the first morning urine (FMU) was that five of the subjects reported urine values of approximately 100 ml, which is abnormally low for a FMU, or did not actually provide the FMU based on the time of collection, as discussed in the methods section. Inclusion of these samples would bias the excretion rate calculation and the correlation calculated for urinary TCAA was stronger when they were excluded. In addition the creatinine normalized TCAA concentration was correlated to the ingestion exposure, with a correlation coefficient (R^2) of .14 ($p=.01$) for the entire data set of valid urine samples (figure 15), with an improvement to a correlation coefficient of 0.61 ($p<.01$) when only those subjects who ingested water at home were included (figure 16).

The dose response relationship between the urinary TCAA and exposure confirms the utility of the urinary TCAA as a biomarker of exposure. A lower limit linear relationship appears to exist between the exposure and the TCAA urinary excretion rate (figure 14). The slope of this line, 0.3 ng/min excreted per μg injected. The lower limit line represents the relationship between the TCAA ingestion from residential water sources and the excretion, with values above the line indicative of other sources of urinary TCAA. For the 48 hours over which the exposure data the total amount excreted per μg ingested would be 0.3ng/min x 60 min/hour x 48 hours or 0.9μg excreted per μg ingested, indicated that the ~90% of the TCAA ingested

in water is excreted, confirming the applicability of urinary TCAA as a biomarker of TCAA water ingestion.

Figure 13

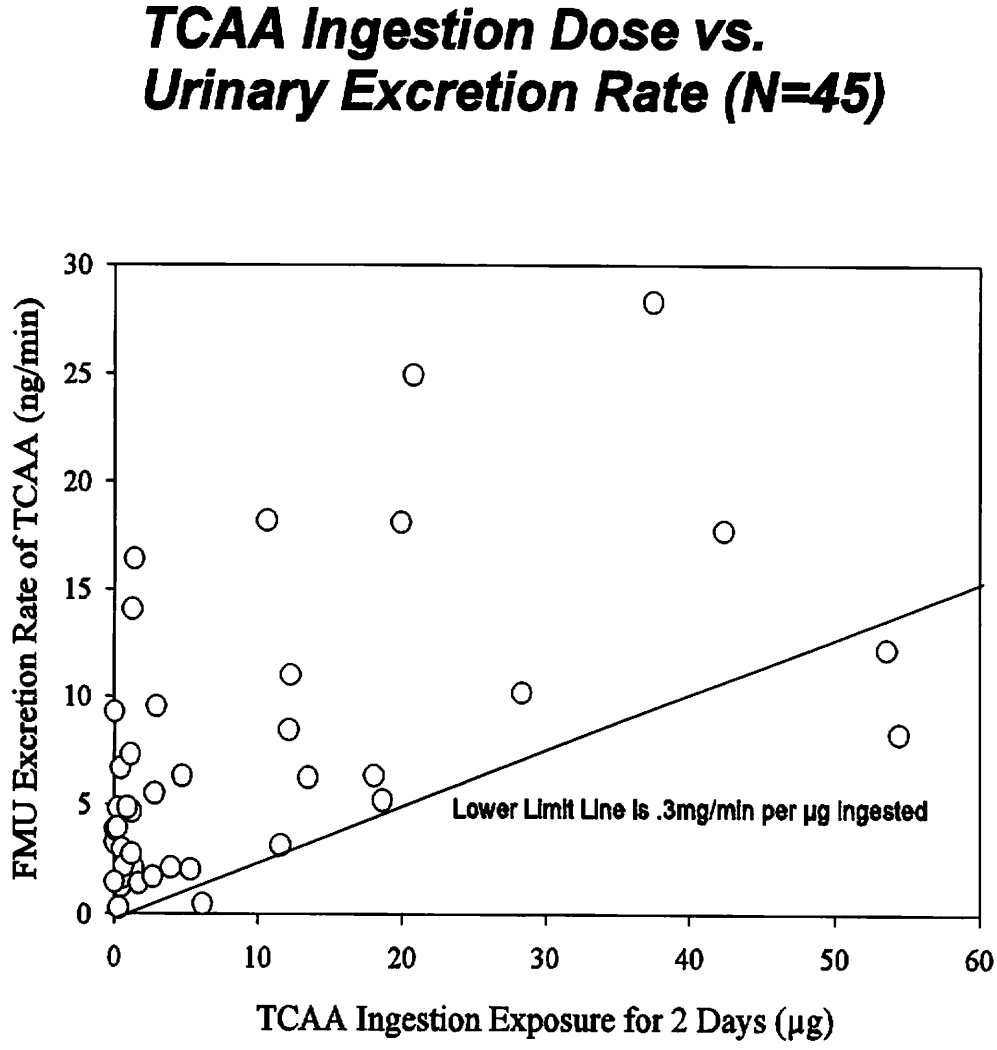
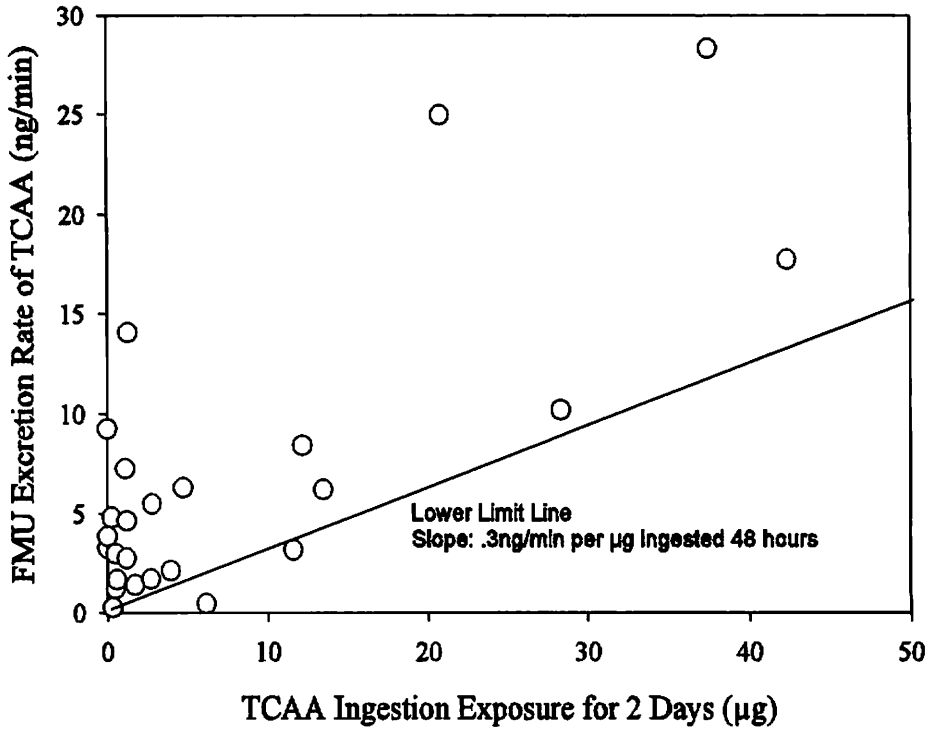

TCAA Ingestion Dose vs.
Urinary Excretion Rate (N=45)
FMU Excretion Rate of TCAA (ng/min)
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Lower Limit Line is .3mg/min per µg ingested
TCAA Ingestion Exposure for 2 Days (µg)

Figure 14

TCAA INGESTION DOSE vs. URINARY EXCRETION RATE EXCLUSIVE OF JOB WORKERS (N=27)



To evaluate other sources of urinary TCAA, the questionnaire included information on ingestion of water and other liquids prepared with water outside the home, dermal exposure to HAA and/or exposure to chlorinated compounds, such as trichloroethane, which are metabolized to TCAA. A step-wise regression analysis was done using urinary TCAA as the dependant variable, and the water concentration and answers to the questionnaire related to exposure to water and solvents as the independent variables. Only the water concentration was kept with a positive relationship to urinary TCAA excretion. Therefore, no other potential exposures that contributed to the urinary TCAA were identified in our cohort, confirming urinary TCAA as a good biomarker of exposure to TCAA in the water supply. The identification of urinary TCAA as biomarker of HAA exposure from water but not DCAA is consistent with measurements of the metabolism of HAA in animals. DCAA is metabolized rapidly in the liver, with near complete metabolism during the first pass as it leaves the gastrointestinal tract, while TCAA is metabolized much more slowly and would enter the blood stream without being completely metabolized following ingestion, thus is expected to be partially eliminated in the urine (Larson and Bull 1992).

Summary

Exhaled breath of THMs and urinary TCAA were demonstrated to be biomarkers of exposure to these compound originating in the residential tap water. While the background breath were slightly higher for the subjects in the high exposure group, a much stronger relationship between water concentration and exhaled breath for all THMs was determined after a shower, indicative that the majority of the THM dose is relatively short lived in the body.

Further, showering and bathing activities, in addition to ingestion, need to be considered for exposure determination of volatile or dermally absorbed compounds present in residential water. An exposure model for dichloroacetic acid and trichloroacetic acid, based on water ingestion including the amount of degradation of TCAA when water is heated for preparation of coffee, tea and soup, was a better predictor of the urinary excretion of TCAA than water concentration alone. No relationship for DCAA was identified. This confirms that exposure estimates, and not solely water concentrations, should be used when possible in epidemiological studies of adverse health effects of disinfection by-products in water.

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