DISSERTATION

# EXPOSURE TO WATER DISINFECTION BY-PRODUCTS AND HYPOSPADIAS RISK: AN EVALUATION OF EXPOSURE ASSESSMENT TECHNIQUES

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY THOMAS JOSEPH LUBEN ENTITLED "EXPOSURE TO WATER DISINFECTION BY-PRODUCTS AND HYPOSPADIAS RISK: AN EVALUATION OF EXPOSURE ASSESSMENT TECHNIQUES" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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## ABSTRACT OF DISSERTATION

**EXPOSURE TO DISINFECTION BY-PRODUCTS AND HYPOSPADIAS RISK:**
AN EVALUATION OF EXPOSURE ASSESSMENT TECHNIQUES

Epidemiologic studies have illustrated an association between disinfection by-products (DBPs) and increased risks of adverse reproductive outcomes, including birth defects. One of the largest constraints in these studies has been exposure misclassification. This dissertation presents the validation of an approach to link study participants to exposure data in such epidemiologic studies, evaluates and compares five techniques that have been proposed for reducing exposure misclassification in epidemiologic studies of DBPs, and presents the results of two population-based case-control studies of a genitourinary tract birth defect and DBP exposure during a critical period of gestation.

This study was designed to reduce exposure misclassification while testing the following hypothesis: women who give birth to infants with hypospadias are more likely to have been exposed to higher DBP concentrations than women that gave birth to infants without hypospadias. The specific aims of this study were as follows:

1. Develop a GIS for linking cases and controls to the water utility from which they receive their tap water during the vulnerable period of gestation for each birth or residence at time of birth;
2. Validate the GIS linking procedure by providing the residential addresses to the utilities to which they were linked to see if billing records and/or distribution boundaries include the subjects' addresses;
3. Compare different methods of assigning DBP exposure to study participants using routinely collected monitoring data and interview data from the NBDPS aimed at reducing exposure misclassification;

4. Determine whether exposure to DBPs increases the risk for development of hypospadias among ARHMS and NBDPS participants after accounting for potential interactions and confounding factors using linear regression models and routinely collected monitoring data;
5. Determine whether exposure to DBPs increases the risk for development of hypospadias among NBDPS participants after accounting for exposure via the ingestion, inhalation and dermal routes of exposure using interview data and routinely collected monitoring data.

Many epidemiologic studies concerning DBPs and adverse reproductive outcomes use community water system (CWS) monitoring data to estimate exposure. Use of such data requires linkage of residence location to a specific CWS and associated monitoring data during a given exposure period. The inability to perform this linkage successfully can lead to exposure misclassification and/or reduced sample size. We geocoded 3,886 residences obtained from the Arkansas Health Department and used boundary data from the 2000 U.S. Census Incorporated Place/Census Designated Places to link each of the residences included in the study to one or more CWS. In order to validate the linking procedure, we provided residential addresses to the CWS to which they were linked to see if billing records and/or distribution boundaries include the addresses. To explore potential bias, we compared demographic characteristics of participants linked to a U.S. Census Place name to participants not linked, and participants that were validated as linked to a specific CWS to those not validated. We successfully geocoded 97.2% of the addresses. The remaining 2.8% of addresses could not be geocoded because they were listed as post office boxes or the addresses were incomplete. We linked 71.4% of the geocoded addresses to a U.S. Census Placename. Of those addresses linked to a Placename, we linked 97.4% to at least one CWS. We validated 81.4% of these addresses as being correctly linked to the CWS. We found no bias for age, education, or

ethnicity between linked and unlinked participants. However, unlinked participants were more likely to be white and to live in a rural census block group. We found that the participants whose linkage we could not validate were more likely to be white and to live in a rural census block group. These results demonstrate that this method of assessing exposure to DBPs can be utilized in a large, population-based epidemiologic study.

Systematic reviews of the relationship between DBPs and adverse reproductive outcomes consistently identify exposure misclassification as an important limitation to accurate risk estimation. In the last few years researchers have developed exposure assessment metrics utilizing monitoring data while taking into consideration spatial variability, temporal variability, and personal water use into consideration. These metrics have been used with an expectation of decreasing exposure misclassification when compared to the use of quarterly CWS monitoring data alone. We compared five metrics used in assigning exposure to DBPs for a study of hypospadias in Arkansas. These included a weighting metric based on spatial variability of DBP concentrations throughout a water system, a threshold metric that excluded participants linked to a water utility classified as having high spatial variability, a spline regression technique that interpolated between sampling dates to account for temporal variability, the inclusion of water consumption data and an exposure index that included data on water consumption and shower and bath duration. We compared each of these metrics to the use of routinely collected water utility monitoring data to assess exposure to DBPs using Pearson correlation coefficients, cross-classification of exposure quintiles, and risk estimation. Pearson correlation coefficients ranged from 0.36 when water consumption data was used

to 0.97 when the spline regression technique was used. When we used a cross-classification technique to see if the different exposure metrics would cause participants to move from one exposure quintile to another, we found that the incorporation of water consumption data caused 75% of participants to change quintile while the use of the spline regression technique only caused 26% of participants to change quintile. Odds ratios (ORs) were computed for the study population using monitoring data and the 5 different metrics to assign exposure. There were no statistical differences between ORs calculated using monitoring data and ORs calculated using the 5 metrics, though this may be due to small sample size. We found that using spline regression had the least effect on exposure assessment, while the largest change in exposure assessment was observed when water consumption data was incorporated.

There is considerable evidence from both toxicological studies in animals and human epidemiology studies, to suggest that exposure to DBPs may be associated with genitourinary birth defects. Hypospadias is a birth defect in which the urethral opening occurs on the ventral side of the penis, as a result of abnormal urethral closure and is one of the most common congenital anomalies in the United States. We investigated the relationship of exposure to DBPs between gestational weeks 6 and 16 and risk of hypospadias. We obtained 651 cases from the Arkansas Reproductive Health Monitoring System and 1,277 controls from birth certificates provided by the Arkansas Department of Health born between January 1, 1998 and December 31, 2002. We collected quarterly DBP concentrations from 263 CWS throughout Arkansas and used spline regression to estimate daily DBP concentrations for each CWS. Exposure estimates were obtained by

averaging the daily DBP estimates between gestational weeks 6 and 16. We used logistic regression to obtain crude and adjusted ORs and 95% confidence intervals (CIs) for the relationship between DBPs and hypospadias. We examined exposure to total trihalomethanes (TTHMs), the sum of the 5 most prevalent haloacetic acids (HAAs) and 6 individual species of HAAs. None of the unadjusted or adjusted analyses produced statistically significant ORs. We conducted a subset analysis with 36 cases and 225 controls enrolled in the National Birth Defect Prevention Study that allowed us to refine our exposure assessment of TTHMs by including data on average volume of water ingested per day and average frequency and duration of bathing and showering per week. We found a statistically significant association for the highest tertile of exposure (OR 3.45 CI 1.05-11.35). A test for trend revealed a statistically significant dose-response relationship ($p<0.05$). These results suggest that exposure to TTHMs during gestation may be related to hypospadias risk and emphasize the necessity of including water use data when assessing exposure to DBPs.

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# 1. Introduction

## 1.1 Background and Aim

Disinfection by-products (DBPs) are formed when chlorine reacts with organic matter in water. DBPs were discovered in drinking water in 1974. There are several classes of DBPs, including trihalomethanes (THMs), haloacetic acids (HAAs), and haloacetonitriles (HANs). The chemical classes form during water disinfection and the proportional distributions of individual chemicals within a class vary by distribution systems. A variety of factors influence DBP formation, including precursor type, precursor concentration, chlorine dosage, temperature, pH, bromide concentrations and reaction time. Humic and fulvic acids are the predominant precursors found in natural waters. Due to differing levels of organic matter, DBP concentrations are generally lower in ground water compared to surface water.

In the 1990s, the U.S. Environmental Protection Agency (USEPA) focused upon the potential adverse health effects of DBPs in both toxicological and epidemiological arenas. Since then, epidemiologic studies have illustrated an association between DBPs and increased risks of adverse reproductive outcomes, including neural tube, cardiac, respiratory system, urinary tract and oral cleft defects.

One of the largest constraints in these studies has been exposure misclassification. Researchers have examined many different methods of exposure assessment to try to avoid misclassification. These include basing exposure on the source of the water entering a community water system (CWS), the type of treatment used in disinfection, the

treatment and color of the water, routine CWS monitoring data, and predicted DBP concentrations using linear regression modeling.

While the most reliable method of exposure assessment is through personal exposure monitoring, this is rarely feasible in epidemiologic studies due to financial and other constraints. In most tap water epidemiologic studies the alternative is to consider CWS monitoring data in conjunction with data collected on water consumption and use. Federal regulations require CWS to measure DBP levels at distribution system sampling sites on at least a quarterly basis.

The overall goal of this dissertation was to examine methods for linking CWS monitoring data to a specific time period during a woman's pregnancy while minimizing exposure misclassification in order to estimate risk of hypospadias. The specific objectives of this dissertation were to 1) conduct a feasibility study to see if a geographic information system (GIS) was effective in linking study participants to the CWS from which they receive their tap water, and validate this linkage by surveying individual CWS; 2) compare methods of exposure assessment that have been used in recent epidemiologic studies of DBPs, and; 3) conduct two population-based case-control studies of hypospadias, evaluating DBP exposure during a critical period of gestation, to determine if a relationship exists and whether magnitude of risk depends on exposure dose.

## 1.2 Dissertation Overview

This dissertation is organized into three related projects regarding epidemiologic evaluation of DBPs and prenatal exposure. Therefore, each chapter is organized

individually and includes an introduction, materials and methods, results, discussion and reference section.

The review of literature, Chapter 2, presents the information related to epidemiologic investigation of DBPs and birth defects that is presently available. Included within this chapter are comprehensive evaluations of previous epidemiologic studies of DBPs and birth defects and relevant exposure assessment literature.

Chapter 3 presents the feasibility of using a GIS to link participants in an epidemiologic study of birth defects with the CWS from which they receive their tap water. This was accomplished by linking U.S. Census Place names with CWS names, and was validated by surveying 263 CWS in Arkansas and asking them to confirm service to participants that had been linked to their system.

Chapter 4 compares five distinct methods for assessing exposure to DBPs that have been presented in recent epidemiologic studies to a common technique of linking quarterly monitoring data from a CWS to a trimester of a participant's pregnancy in order to assign exposure to DBPs. Two of these exposure assessment techniques attempt to account for spatial variability of DBP concentrations within a CWS distribution system, while another technique attempts to account for temporal variability of DBP concentrations within a CWS distribution system. The fourth and fifth exposure assessment techniques estimate dose to DBPs via the ingestion route of exposure, and the ingestion, inhalation and dermal routes of exposure, respectively. The objective of this study was to compare

how the application of these exposure assessment techniques might change the risk estimate due to the exposure assigned to a participant in an epidemiologic study of DBPs to the exposure assigned when quarterly monitoring data from a CWS was used.

In the fifth chapter the methods and results of two population-based case-control studies examining the relationship between exposure to DBPs and hypospadias are presented. The first case-control study utilizes birth defect registry data for cases and birth certificate data for controls, and while demographic characteristics from the birth defect registry and birth certificates were evaluated and adjusted for when appropriate, no data on individual water use or behaviors was available. The second, smaller case-control study uses data for cases and controls collected as part of the National Birth Defects Prevention Study (NBDPS), which includes data on individual water use and behavior. We used both of these studies to investigate the hypothesis that children born with hypospadias were exposed to higher concentrations of DBPs during a critical period of gestation.

Chapter 6 provides a summary of findings and a discussion of recommendations for future research efforts.

## Chapter 2

## 2. Literature Review

### 2.1 Introduction and Background

Congenital anomalies are the leading cause of infant mortality in the United States (Hoyert *et al.*, 2001). Hypospadias is one of the most frequent congenital malformations with an incidence around one per 125 to one per 300 live male births, which has increased in some countries during the last decades (Paulozzi *et al.*, 1997). During this same period, the discovery of a large number of by-products, resulting from tap water chlorination techniques, have raised concern about the potential health effects that might result from exposures to these compounds (Rook, 1974; Bellar *et al.*, 1974). In particular, there are concerns over the potential ability of these disinfection by-products (DBPs) to cause adverse birth outcomes. This concern is supported by three epidemiologic studies that found an association between precursors to DBPs and urinary tract defects (Aschengrau *et al.*, 1993; Magnus *et al.*, 1999; Hwang *et al.*, 2002). Because of the ubiquitous nature these by-products in chlorinated drinking water, and the high proportion of people that consume chlorinated water from community water sources, it is important that the possible teratogenic effects of DBPs be investigated.

### 2.2 Characterization, Etiology, and Environmental Epidemiology of Genitourinary Birth Defects

Birth defects are the leading cause of infant mortality in the United States (Hoyert *et al.*, 2001), affecting 1 of every 33 live births (Araneta *et al.*, 2003). While many important risk factors have been identified, the underlying etiology of two-thirds of all birth defects

remains unclear (Rasmussen *et al.*, 2003; Araneta *et al.*, 2003). For the past decade there has been an increasing interest in the role of environmental exposures, including DBPs, in the etiology of these defects (Aschengrau *et al.*, 1993; Bove *et al.*, 1995; Dodds *et al.*, 1999; Klotz & Pyrch, 1999; Magnus *et al.*, 1999; Dodds & King, 2001; Cedergren *et al.*, 2002; Hwang *et al.*, 2002; Shaw *et al.*, 2003). These studies have examined the relationship between exposure to DBPs and respiratory tract, oral cleft, cardiac, central nervous system, and urinary tract defects.

Birth defect surveillance is useful for monitoring the distribution of and changes in birth defect incidence suggesting environmental influences (Watkins *et al.*, 1996). Most birth defect monitoring systems record data on four congenital anomalies affecting the genitourinary system. These are hypospadias, renal agenesis, bladder exstrophy and obstructive genitourinary defect.

Hypospadias is one of the most common congenital anomalies in the United States, occurring in approximately 1 in 250 newborns (Paulozzi *et al.*, 1997). Hypospadias is a birth defect in which the urethral opening occurs on the ventral side of the penis, as a result of abnormal urethral closure at approximately week eight to week fourteen of gestation. Chordee, curvature of the penis, particularly when proximal, often accompanies hypospadias. The more proximal the location and the greater the associated chordee, the more functional impairment results (Dolk *et al.*, 2004). In some glanular cases, the deformity is only cosmetic. Although understanding of the development of the urogenital system has improved in recent years, and a variety of mechanisms involving

endocrine disruptors and genetic impairment have been proposed for hypospadias, their actual contribution to hypospadias etiology remains elusive (Paulozzi, 1999; Moller & Weidner, 1999).

### 2.3 Case Classification of Isolated and Syndrome-Related Birth Defects

The etiologic heterogeneity of birth defects has long been recognized, and such heterogeneity may complicate epidemiologic studies designed to identify causes of birth defects (Rasmussen *et al.*, 2003). Isolated birth defects have been shown to differ in their epidemiology and etiology from defects associated with additional major defects (Rasmussen *et al.*, 2003). Inclusion of infants with different causes in the study of a birth defect may dilute the magnitude of an observed association toward the null (Khoury *et al.*, 1992).

Two types of preferred case classification exist: (1) infants with the same defect are classified for analysis into separate groups, based on whether the defect is isolated, one of multiple congenital anomalies, or associated with a syndrome; and (2) infants with anatomically different, but presumed pathogenetically similar, defects are combined. The first type of case classification is usually utilized to create presumably more homogenous categories, while the second type of case classification is often used to increase the power of the study (Rasmussen *et al.*, 2003). Rasmussen *et al.*, (2003) recommend that case classification is best carried our by a clinical geneticist/dysmorphologist when possible.

## 2.4 Comparison of Birth Certificate and Birth Defect Registry Data for Use in an Epidemiologic Study

Birth certificates are an attractive source of information about birth defects because they are universal (one certificate per child), standardized across the United States, and inexpensive and convenient for case ascertainment (Watkins *et al.*, 1996). A 1989 revision of the US Standard Birth Certificate has much to do with this. Pre-1989 standard birth certificates used an open-ended question format that was poorly completed and difficult to analyze, whereas the 1989 revision replaced the open-ended format with a checkbox format to simplify and improve the reporting of birth defects and other birth information. It has been reported that this revision has improved the reporting of birth outcome information on vital records (Frost *et al.*, 1984; Teperi *et al.*, 1991), though Watkins *et al.* (1996) found little improvement in the sensitivity (9% pre-1989 vs. 14% post 1989 revision) of birth certificates for detecting congenital anomalies in their study in the metropolitan Atlanta area. Based on these findings, birth certificates should be used cautiously for case ascertainment in case-control studies, and may be unsuitable for epidemiologic studies (Watkins *et al.*, 1996).

There are limitations in both the quality and completeness of data from birth certificates. A better alternative for case ascertainment in a case-control study of birth defects is data from a birth defect registry. Surveillance of defects by a registry provides data for epidemiological research to identify causes or risk factors such as drugs, nutritional factors, environmental exposures, maternal illnesses and genetic factors (Watkins *et al.*, 1996). Klotz & Pyrch (1999), Dodds *et al.* (1999), and Magnus *et al.* (1999) chose to use

birth defect registries in the ascertainment of cases for their case-control studies of birth defects and exposure to DBPs, while controls for their studies were selected using birth certificate records. This leads to the possibility of introducing bias if the information on the birth certificates differs in accuracy from information in the birth defect registry.

Overall, the accuracy of birth certificates depends on the variable of interest. Birth weight, date of birth, plurality, birth order, mother's race, Apgar score, county of birth, mother's county of residence, mother's marital status, mother's date of birth, and method of delivery appear to be very accurately reported on birth certificates when compared to the "gold standard" medical records (Buescher *et al.*, 1993; Piper *et al.*, 1993). Reporting for month prenatal care began, number of prenatal care visits, and weight gain during pregnancy is considered fair to good when compared to medical records (Buescher *et al.*, 1993; Piper *et al.*, 1993). Finally, birth certificates could also be considered fair to good in their reporting for tobacco use, obstetrical procedures, and events of labor and delivery, and poor for medical history and alcohol use (Buescher *et al.*, 1993; Piper *et al.*, 1993). It should be noted, however, that available evidence indicates that alcohol use is underreported in both medical records and birth certificates (Buescher *et al.*, 1993).

### 2.5 Timing of Teratogenic Insult and Birth Defect Development

The stage of development of an embryo when an environmental agent is present determines its susceptibility to a teratogen. The most critical period in development is when cell division, cell differentiation, and morphogenesis are at their peak (Moore &

Persaud, 1998). Environmental disturbances during the first two weeks after fertilization may interfere with cleavage of the zygote and implantation of the blastocyst and/or cause early death and spontaneous abortion of the embryo; however, they are not known to cause congenital anomalies (Moore & Persaud, 1998). Instead, teratogens acting during the first two weeks will kill the embryo, or their disruptive effects are compensated for by powerful regulatory properties of the early embryo (Carlson, 1994).

A detailed description of the critical periods of human development can be found elsewhere (Moore & Persaud, 1998). Briefly, formation of the early embryonic structures occurs in the first two weeks of development. The organogenic period, beginning with week three and continuing through week eight, contains the development of the embryo and is the time when teratogenic agents may induce major congenital anomalies. Other physiologic effects, including minor morphological anomalies, are likely to result from disruption of development during the fetal period, from week nine through week 38. The most sensitive period for disruptions that can result in genitourinary defects is from week seven through week nine (See Figure 2.2.3.1).

## 2.6 Secular Trends of Genitourinary Defects

In recent decades, an increase of the birth frequency of several genitourinary defects has been reported for several countries worldwide (Czeizel, 1985; Matlai & Beral, 1985; Dolk, 1998; Paulozzi, 1999; Toppari *et al.*, 2001; Pierik *et al.*, 2002; Boisen *et al.*, 2004). In a study of 29 birth defect registries from 21 countries, Paulozzi (1999) found marked

increases in the prevalence of hypospadias in two American systems, Denmark, Norway, Finland, Sweden, and Japan. In his analyses, Paulozzi was unable to compare prevalence rates from individual countries directly due to differences in registry methods, genetic variation, and other factors, but was able to compare trends in hypospadias prevalence for each country between 1974 and 1996. While few systems showed consistent upward or downward trends, the author was able to identify wide inter-country variation in the rates of hypospadias and cryptorchidism (Paulozzi, 1999). The increasing prevalence of congenital cryptorchidism is supported further by British cohort data that demonstrated a 35% increase in the birth prevalence in 1984-1988 when compared with cohort data from the late 1950's, and a strikingly high prevalence of cryptorchidism in Denmark today compared to 40 years ago. (Boisen *et al.*, 2004).

More recently, Nelson *et al.* (2005) found a significant increase in the incidence of hypospadias and other congenital penile anomalies, with striking variation by race, region and socio-economic status (SES).

It is noteworthy, however, that the frequency of hypospadias in Spain and France has remained quite stable between 1978 and 1995 (Paulozzi, 1999; Martinez-Frias *et al.*, 2004). One possible explanation for the increasing prevalence of genitourinary defects in some countries and not others if DBPs are involved in the etiology of this defect is the consumption of bottled water. According to Belot (2000), Western Europe leads all regions of the world in consumption of bottled water. Approximately 46% of the water consumed in this region is bottled water. The region with the next highest consumption

of bottled water is North America, with just 20%. Bottled water consumption is 8% in Eastern Europe, and only 2% in Asia. In regions where bottled water consumption is high, these populations have lower exposure to tap water contaminants. These consumption rates correspond moderately with country-wide trends in hypospadias prevalence. Specifically, the United States, Denmark, Finland, Norway, Sweden, and Japan drink less bottled water (and presumably more tap water) and have documented increasing prevalence of hypospadias. Alternatively, France and Spain consume more bottled water (and presumably less tap water) and have seen no change in the prevalence of these birth defects.

### 2.7 Embryogenesis of Male Reproductive Tract and Morphogenesis of Hypospadias

The embryogenesis of the male reproductive tract and the morphogenesis of hypospadias have been described elsewhere in detail (Baskin, 2004). In summary, formation of the external male genitalia is a complex developmental process involving genetic programming, cell differentiation, hormonal signaling, enzyme activity and tissue remodeling. Before week seven or eight of gestation the external genitalia remain in the indifferent stage, with male and female genitalia essentially indistinguishable.

Under the influence of testosterone in response to a surge of luteinizing hormone from the pituitary, masculinization of the external genitalia takes place around week eight of gestation. At the molecular level testosterone must be converted to 5 α-dihydrotestosterone (DHT) by the type 2 5 α-reductase enzyme for complete differentiation of the penis with a male-type urethra and glans. Testosterone binds to the

androgen receptor (AR) and induces changes in conformation. This conformation change is also required for AR dimerization, DNA binding, and transcriptional activation, all necessary for testosterone to be expressed.

With the expression of testosterone, one of the first signs of masculinization is an increase in the distance between the anus and genital structures. This is followed by elongation of the phallus, formation of the penile urethra from the urethral groove and development of the prepuce. The urethral groove on the ventral surface of the phallus is between the paired urethral folds. By the twelfth week the urethral folds have completely fused in the midline of the ventrum of the penile shaft. During the sixteenth week of gestation the glandular urethral opening appears. The entire male urethra, including the glandular urethra, is formed by dorsal growth of the urethral plate into the genital tubercle and ventral growth and fusion of the urethral folds.

Several factors, including genetic defects, hormone receptor impairment, enzyme impairment, environmental contaminants and endocrine disruptors can interfere with proper fusion of the urethral folds and result in hypospadias. Genetic defects that interfere with the conversion of testosterone to DHT by 5 α-reductase may disrupt the proper fusion of the urethral folds and result in hypospadias. Defects in the AR or mutations in the AR coding sequence, though rare, could also lead to hypospadias. Also, defects in three major enzymes in the biosynthetic pathway of testosterone (3 β-hydroxysteroid dehydrogenase, 17 α-hydroxylase and 17,20-lyase) have been associated with hypospadias. Finally, environmental contaminants can mimic or antagonize

hormones or otherwise interfere with the development and function of the reproductive system. It is important to note that the contribution of all of these factors do not explain the etiology of 100% of hypospadias cases. The etiology of hypospadias remains unclear in many cases.

## 2.8 Demographic Variables Associated with Genitourinary Birth Defects

Researchers have investigated the relationship between genitourinary defect risk and several demographic variables. No relationship was found for education level, social class distribution, or proportion of the population working in industry vs. farming and forestry in a study conducted in Finland (Aho *et al.*, 2003). In the same study, Aho *et al.* (2003) actually found lower rates of hypospadias in remote areas when compared to cities of Finland. There have been contradicting findings when maternal age has been investigated in relation to hypospadias. Vrijheid *et al.* (2003) and Aho *et al.* (2003) have reported decreasing trends of hypospadias cases with maternal age. On the other hand, several studies have found that older mothers were more likely to give birth to a son with hypospadias (Fisch *et al.*, 2001; Flores-Luèvano *et al.*, 2003). A possible explanation for these discrepant results is that women at the extremes of their reproductive ages (i.e. less than 20 or over 35 years) are more likely at risk to give birth to an infant with this defect.

Previous studies of hypospadias and smoking have suggested either no association or a reduced risk. In a recent study using data from the National Birth Defects Prevention

Study (NBDPS) no association between maternal smoking and hypospadias was found for the low, medium or high smoking exposure categories (Carmichael *et al.*, 2005a).

Several other factors have been associated with increases in risk of genitourinary defects. Parikh *et al.* reported two significant associations, black race and diabetes, with renal agenesis in their analyses of Colorado birth certificates in 2002. Working during pregnancy was associated with increased risk of giving birth to a son with hypospadias (OR 4.68; 95% CI 1.15-18.9) (Flores-Luèvano, *et al.*, 2003). Also, both subfertility and maternal obesity have been associated with adverse reproductive outcomes, including some birth defects (Honein *et al.*, 2003). When examined together, Honein *et al.* (2003) found a strong association between obstructive congenital renal anomalies and having a joint exposure to high body mass index (BMI) and subfertility (OR 5.8, 95% CI 2.0-16.3). No association was observed for women who had only one of these two exposures when compared to the referent group. Women who are both subfertile and overweight/obese likely have a different hormonal profile compared to average weight/under weight women who are not subfertile. Hormone profiles were found to be associated with increased hypospadias risk in a study that related the intake of progestins during pregnancy to hypospadias risk (Carmichael *et al.*, 2005b). In the study using data from the NBDPS, Carmichael *et al.* (2005b) found that pregnancy-related progestin intake between weeks four and fourteen of gestation were associated with an almost four-fold increase in hypospadias risk.

Many types of birth defects appear to differ in prevalence by sex, with most occurring more frequently among males. Defects of the reproductive and urinary systems, for example, are far more prevalent among males than among females (Lary & Paulozzi, 2001; Parikh *et al.*, 2002). In their 2001 analyses, Lary and Paulozzi found that, in general, total sex organ defects are 8.55 times as likely to occur in males as in females and total urinary tract defects are 62% higher in males than in females. Ten of the thirteen significant urinary tract defects were more prevalent among males, with relative risks ranging from 1.71 to 46.68.

In humans, the male gonads begin to differentiate during week seven of development, and the testes begin to secrete testosterone during week eight of development. The female gonads, on the other hand, do not begin to differentiate until week twelve of development. Testosterone levels are much higher in the male fetus than in the female fetus after week eight of development (Reyes *et al.*, 1974; Winter *et al.*, 1977). Abnormal levels of testosterone and other hormones produced by the male reproductive tract after testicular differentiation may account for the large excess of defects of the male reproductive system. Increased levels of these hormones in the male fetus after testicular differentiation may influence the development of organs and tissues in other systems as well, leading to sex differences in the prevalence of some defects (Lary & Paulozzi, 2001).

Although biological differences in the development of the male and female reproductive systems may account for much of the substantial sex differences in the prevalence of

reproductive system defects, differences in the diagnosis of male and female reproductive system defects may account for some of the differences. Most of the female reproductive tract is internal, thus many structural abnormalities may not be detected in infancy or early childhood, leading to a lower reported prevalence of female reproductive system defects relative to male reproductive system defects (Lary & Paulozzi, 2001). Additionally, defects of the urinary tract are probably etiologically linked to development of the male reproductive system and thus tend to be more prevalent among males (Lary & Paulozzi, 2001).

Prenatal mortality is greater among malformed female conceptuses and also may contribute to the higher prevalence of malformed males among liveborn infants (McKeown and Lowe, 1951; Naeye *et al.*, 1971; Machin, 1975).

### 2.9 Environmental Exposures Associated with Genitourinary Birth Defects

In the past, environmental factors were generally ruled out as causes for genitourinary defects. For example, familial clustering of hypospadias among first-degree relatives has been perceived as under the influence of a strong genetic and heritable factor. However, in light of the growing number of recent reports, environmental influences on this and other genitourinary defects are now being considered as well.

### 2.9.1 Animal Studies

Toxicological studies examining environmental exposures that may result in genitourinary defects have examined pesticides that are believed to affect the endocrine system, synthetic estrogens present in the environment, and prescription drugs. The results of these studies have consistently identified adverse reproductive outcomes in association with exposure to these compounds.

Vinclozolin is a dicarboximide fungicide that acts as an antiandrogen, impairing the development of the reproductive systems in male rat pups, including undescended testes (Gray *et al.*, 1994; Kelce *et al.*, 1997; Wolf *et al.*, 2000). Prenatal exposure to vinclozolin was indicated to possibly prevent the closure of penile folds and induce feminization of the external genitalia, such as a decreased ano-genital distance, hypospadias, cleft phallus, nipple retention, undescended testes, small or absent accessory glands, and the development of a vaginal pouch in male rat offspring (Shono *et al.*, 2004; Gray *et al.*, 2001). The abnormal sexual differentiation induced by vinclozolin in these studies is likely to occur in human males if the fetus is exposed to vinclozolin or its active metabolites during the critical period of sexual differentiation (Shono *et al.*, 2004).

Studies in which dichlorodiphenyldichloroethylene (DDE), a metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT), was administered to rats during development affected androgen-dependent aspects of male development such that it reduced anogenital distance, caused nipple retention and induced hypospadias (You *et al.*, 1998). It is believed that these outcomes occur due to the ability of DDE to inhibit the binding of

androgens to the androgen receptor and to exert antiandrogenic effects in perinatal rats at a dose of 100 mg/kg/day administered to pregnant darns (You et al., 1998).

Approximately 50% (range 40% – 57%) of male rat fetuses exposed to different concentrations of synthetic estrogens (17-α-ethinyl estradiol (17-EE) or diethylstilbestrol (DES)) exhibited hypospadias, compared to 0% of controls (Kim *et al.*, 2004). Synthetic estrogen exposure had no effect on the female fetus. The authors hypothesized that exposure to estrogenic compounds known for their ability to disrupt reproduction, so-called estrogenic endocrine disrupters, were responsible for these results.

Finasteride is a therapeutic treatment for benign prostatic hyperplasia and male androgenic alopecia. Pre- or postnatal exposure to finasteride has been shown to alter male reproductive development and function, including hypospadias in animals (Clark *et al.*, 1990). Finasteride is a specific inhibitor of type 2 5 α-reductase, the enzyme that converts testosterone (T) to the more potent androgen receptor agonist dihydrotestosterone (DHT). In utero exposure to androgen receptor antagonists and T biosynthesis inhibitors have induced permanent effects on androgen-sensitive end points such as anogenital distance, nipple retention, and malformations of the male rat reproductive tract. Bowman *et al.* (2003) detected that 30% of rats exposed to 1.0 mg/kg/day of finasteride in utero displayed hypospadias, but that no hypospadias were observed in the 0, 0.01, or 0.1 mg/kg/day dose groups. A clear dose-response was observed, with the incidences of hypospadias at 30, 48, and 88% in the 1, 10 and 100 mg/kg/day dose groups, respectively (Bowman *et al.*, 2003). Prenatal finasteride

exposure significantly impaired testicular descent, also with a dose response pattern. Approximately 3, 23, and 73% of the adult males displayed ectopic testes in the 1.0, 10, and 100 mg/kg/day dose groups, respectively (Bowman *et al.*, 2003).

### 2.9.2 Human Studies

A number of human epidemiologic studies have explored the relationship between genitourinary tract defects and environmental exposures. In fact, the recorded temporal trend and geographic differences are compatible with an environmental etiology (Boisen *et al.*, 2004). Positive relationships have been found between these defects and the use of recreational drugs (including alcohol and cigarettes), oral contraceptives, DES, electric blankets, and prescription drugs. Furthermore, occupation, geographic area of residence, women that have experienced a flu or illness during their pregnancy, and subfertile women with a high BMI have also been demonstrated to have a higher risk of having a baby with a genitourinary birth defect. On the other hand, the use of folic acid and other multivitamins have been shown to reduce the risk of having a baby with a genitourinary birth defect. Finally, no association was found between exposure to pesticides and the risk of having a baby with a genitourinary birth defect. These studies are explored further below.

The lack of maternal multivitamin use (Li *et al.*, 1995c), maternal subfertility (Li, 1999), coexposure to subfertility and a high body mass index (Honein *et al.*, 2003), maternal use of cocaine (Chavez *et al.*, 1989; Brouhard, 1994; Battin *et al.*, 1995; Kashiwagi *et al.*,

2003), angiotensin-converting enzyme inhibitors (Centers for Disease Control and Prevention, 1997; Mastrobattista, 1997; Ratnapalan & Koren, 2002), oral contraceptives (Li *et al.*, 1995b), alcohol (Moore *et al.*, 1997), electric blankets (Li *et al.*, 1995a), and cigarettes (Li *et al.*, 1996) during pregnancy have all been associated with the occurrence of renal anomalies.

Hernandez-Diaz *et al.* (2000) found that women exposed to folic acid antagonists, and specifically to antiepileptic drugs, during their first trimester of pregnancy were more likely to give birth to an infant with a urinary tract defect (OR 2.1; 95% CI 1.2-3.7; OR 2.5; 95%CI 1.2-5.0, respectively). The sons of women exposed to DES have a broad range of reproductive tract problems, including hypospadias (McLachlan, 1979). The DES effects are probably not the result of genetic damage, but instead the result of disrupted gene expression elicited by xenobiotic compounds *in utero*. However, two recent studies have had conflicting results when examining in utero exposure to DES and risk of hypospadias. One of the studies does not support an increased risk of hypospadias among the sons exposed to DES in utero, as has previously been reported (Palmer *et al.*, 2005). The other found a statistically significant increase in hypospadias among boys that had been exposed to DES in utero (Pons *et al.*, 2005),

Abe *et al.* (2003) found that the infants of mothers who reported experiencing a flu-like illness and/or an episodic illness during the first trimester also showed an increased risk of renal anomalies, with an adjusted odds ratio (AOR) of 1.71 (95% CI 1.15-2.52). After stratifying by gender, the authors observed that the associations between renal anomalies

among male infants were distinctly different from those observed in female infants. Male infants appeared more likely to have renal anomalies when their mothers were exposed to a fever (AOR 2.26, 95% CI 1.22-4.19) or medication (AOR 1.75, 95% CI 1.01-3.04), or to both medication and fever (AOR 2.68, 95% CI 1.35-5.31). In contrast, female infants appeared to be more at risk of renal anomalies when their mothers were exposed to a flu-like illness and/or an episodic illness (AOR 2.14, 95% CI 1.08-4.25), but neither to a fever (AOR 2.74, 95% CI 1.20-6.24) nor to medication (AOR 2.64, 95% CI 1.03-6.73) (Abe *et al.*, 2003).

Vrijheid *et al.*, 2003, found little evidence for a relation between maternal occupational exposure to potential endocrine disrupting chemicals and risk of hypospadias in offspring, though there was an indication for an increased risk of hypospadias in the offspring of hairdressers and occupations exposed to phthalates. Araneta *et al.* (2003) found elevated risks for hypospadias among infants conceived post Gulf War to Gulf War and non-deployed female veterans (OR 6.4, 95% CI 1.5-26.8), but found no association for infants born to their male counterparts (OR 1.0, 95% CI 0.6-1.9). A 1994 report by the U.S. General Accounting Office identified that 21 reproductive toxicants and teratogenic agents were present in the Gulf War environment (United States General Accounting Office, 1994). These chemical agents include petroleum solutions, insecticides, arthropod –borne pathogens, sarin, mustard gas, prophylactic drugs (pyridostigmine bromide), and other medications and vaccines.

In two Sicilian towns in which residents are exposed to industrial and agricultural pollutants, the incidence of hypospadias was significantly higher than expected (Bianca *et al.*, 2003). The incidence of hypospadias in these two towns was 3.8 (95% CI 2.16-6.14) and 2.3 (95% CI 1.48-3.43) times higher, respectively, than expected for each of the populations. Pollutants from both of the areas included compounds with proven estrogenic activity, including pesticides and their metabolites, polychlorinated biphenyls and dioxins, and some phthalates.

There is consistent evidence that multivitamin supplements (including folic acid) have been associated with a decrease in risk of giving birth to a child with a genitourinary defect (Botto *et al.*, 2004). Specifically, the use of multivitamin supplements was associated with reductions in the risks of urinary tract defects (OR 0.6, 95% CI 0.4-0.9) (Werler *et al.*, 1999). Furthermore, supplementation that began even after pregnancy was clinically recognizable was associated with reduced risks of urinary tract defects.

Maternal use of valproic acid, an anticonvulsant, increases the risk for hypospadias (Bradai & Robert, 1998; Arpino *et al.*, 2000). Interestingly, valproic acid did not show any evidence of a relationship with genitourinary birth defects in male rats (Kallen, 2004). This leads to the hypothesis that valproic acid may not cause hypospadias by an antiandrogenic effect. Instead, the mechanism may be a direct effect on the penis, on the embryonic testicle, or on the gonadotrophic simulation of the early embryonic testicle to androgen production. In man, the latter occurs from placental gonadotrophins, in rats

from hypophyseal gonadotrophins which could be one explanation for the species difference.

Flores-Luèvano *et al.* (2003) investigated the relationship between exposure to DDT/DDE and risk of hypospadias, but found no significant association (OR 1.13; 95% CI 0.24-5.29).

### 2.10 Previous Epidemiologic Studies of Water Contaminants and Birth Defects

The effects of organic chemical contaminants in drinking water on human birth outcomes are largely unknown, although these contaminants and their more toxic metabolites are known to cross the placenta. Inter- and intra-country spatial variability in the prevalence of congenital malformations has sparked numerous investigations of water quality and water contaminants as playing a part in the etiology of these defects. As early as 1957, Penrose speculated that "the geographical variations observed in the incidence [of birth defects] might suggest a ... causal agent, such as the presence or absence of trace elements in the water supply." To date, water hardness, trace element concentrations, nitrates, and industrial solvents, though not always consistently, have been associated with birth defects in epidemiologic studies.

Lowe *et al.* (1971) found evidence of an inverse relation between the frequency of malformations of the central nervous system (CNS) and the hardness of local water supplies (hard water areas tend to have lower malformation rates than soft water areas).

Further, they showed a negative correlation between the mean annual perinatal mortality rate from anencephalus and estimates of the mean total hardness of the water supply in 58 county boroughs in England and Wales. In a similar study in 1981, Bound *et al.* concluded that soft water was not likely to be a primary factor in the etiology of anencephalus, but ceded the possibility the water hardness might mitigate the effect of other etiological factors. They suggested that their findings would be compatible with an etiologic effect from a trace element.

In 1981, Elwood and Coldman reported no significant association of anencephalus risk with any of 14 trace elements that were studied, including copper. Alternatively, in an ecologic analysis, Morton *et al.* (1976) found a significant negative association between two trace elements in water, copper and barium, and CNS malformations. The results of a case-control study from rural South Australia suggested that consumption of local groundwater during pregnancy was associated with a significantly increased risk of birth defects in offspring (Dorsch *et al.*, 1984). These waters are typically very hard with high calcium, magnesium and nitrate content.

In studies conducted in California and Australia, an association between maternal periconceptual exposure to nitrate from drinking water and diet and an elevated risk for neural tube defects was found (Dorsch *et al.*, 1984; Croen *et al.*, 2001). Ericson *et al.* (1988) were unable to verify nitrate as a risk factor for neural tube defects in their study in Sweden. Similarly, in 2002 Cedergren *et al.* found no excess risk for cardiac defects with an increase in nitrate concentrations in their study, also conducted in Sweden.

Goldberg *et al.* (1990) described an association between the proportion of offspring with congenital heart disease and contact with water contaminated with industrial chemicals in Tucson, AZ. Trichloroethylene (TCE) was the contaminant most elevated in the drinking water. Ingested TCE is almost completely absorbed by the gut, and is metabolized in the liver to its major metabolite, trichloroacetic acid (TCAA). Excretion of metabolites by the kidney is usually complete within 3 days (Nomiyama, 1971). Metabolites have been shown to cross the placenta readily into the fetal circulation and amniotic fluid (Bernstine & Mayer, 1954; Bernstine *et al.*, 1954; Beppu, 1968; Helliwell & Hutton, 1950). Parents who lived or worked in the defined area (2 zip codes in the Tucson Valley) during the period of active contamination were assumed to be exposed to contaminated water through its consumption. Goldberg *et al.* (1990) found that the proportion of cases of congenital heart disease among live births was statistically significantly greater for mothers who had first trimester residential exposure to the contaminated water area (6.8/1,000) than for mothers whose first trimester exposure was limited to the uncontaminated portion of the Tucson Valley (2.64/1,000). The difference in proportion was statistically significant ($p<0.001$; 95% CI 1.14 to 4.14). The authors concluded that if TCE caused cardiac malformations, direct action on the fetus is one possible explanation.

Also in 1990, Wrensch *et al.* investigated whether an increase in birth defects might be related to contamination of drinking water by toxic wastes in a community near San Jose, CA. TCE, the contaminant in the highest concentration, was measured in the water

supply at a concentration 8.5 times higher than the state action level. A hydrogeologic exposure assessment was performed to estimate the concentrations of TCE in the water supply and water flow within the water distribution system via computerized models. Comparisons of exposed and unexposed areas and time periods did not support the hypothesis that exposure to contaminated drinking water was related to the risk of birth defects.

In a related study, Claxton *et al.* (1988) compared rates of cardiac defects in the same community near San Jose, CA with rates in the rest of the county and found an excess of major cardiac defects in the service area of the contaminated water system. Ten babies with major cardiac defects were born to residents of this district, representing an excess of six cases over the number expected in this period based on rates in the remainder of the county (RR 2.6; p=0.01). The distribution of cases suggested that an excess of cardiac cases existed in several of the census tracts surrounding the study areas. A comparison of the distributions of the cardiac defect cases in time and space with the distribution patterns of the water supply and contaminants led the authors to conclude that the TCE contamination was an unlikely explanation for this excess.

## 2.11 Characterization of Disinfection By-Products (DBPs)

Chlorine, used to disinfect water, reacts with natural organic matter in surface waters, resulting in the formation of a complex mixture of chemicals, including trihalomethanes (THMs), haloacetic acids (HAAs), haloketones (HKs), and haloacetonitriles (HANs). THMs consist of four individual species, chloroform, bromodichloromethane (BDCM),

dibromochloromethane (DBCM), and bromoform. HAAs include nine substances but the most common are monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), TCAA, monobromoacetic acid (MBAA), and dibromoacetic acid (DBAA). Stage I of the US Disinfectant/Disinfection by-product (D/DBP) rule (USEPA, 1998) requires utilities to comply with a maximum contaminant limit (MCL) for total THMs of 80 μg/L based on the annual average of four samples collected quarterly at the extremities of the distribution system. In the Stage I of the USEPA D/DBP rule the maximum acceptable levels for HAAs are 60 μg/L based on a quarter running annual average of the five most common species (identified above).

DBPs were discovered in drinking water in 1974 (Rook, 1974; Bellar *et al.*, 1974). The chemical classes form during water disinfection and the proportional distributions of individual chemicals within a class vary by distribution systems. A variety of factors influence DBP formation, including precursor type, precursor concentration, chlorine dosage, temperature, pH, bromide concentrations and reaction time (Golfinopoulos & Arhonditsis, 2002). Humic and fulvic acids are the predominant precursors found in natural waters (Morrow & Minear, 1987). Due to differing levels of organic matter, DBP concentrations are generally lower in ground water compared to surface water (Arora *et al.*, 1997).

### 2.12 Toxicological Studies of Exposure to DBPs

There is considerable evidence from both toxicological studies in animals and human epidemiology studies, to suggest that exposure to DBPs may be associated with

genitourinary birth defects. Animal studies have consistently demonstrated an association between exposure to HAAs and adverse effects in the male reproductive system (Linder *et al.*, 1994a, Linder *et al.*, 1994b, Linder *et al.*, 1995, Linder *et al.*, 1997a; Linder *et al.*, 1997b, Veeramachaneni *et al.*, 2000, Christian *et al.*, 2002). Testicular toxicity following acute exposure to DBAA, including effects such as epididymal sperm motility and morphology in rats at a dose of 270 mg/kg/day (Linder *et al.*, 1994a), acute spermatotoxicity in rats at a dose of 1250 mg/kg/day (Linder *et al.*, 1994b), impaired reproductive competence at doses as low as 10 mg/kg/day and sperm quality in rats at doses ranging from 50 to 250 mg/kg/day (Linder *et al.*, 1995), histopathologic changes in testis and epididymis of rats at doses ranging from 10 to 250 mg/kg/day (Linder *et al.*, 1997b), impaired sexual function and fertility in male rabbits (Veeramachaneni *et al.*, 2000), and altered sperm production and epididymal tubule changes in rats at doses of 52.4 and 132.0 mg/kg/day (Christian *et al.*, 2002) has been demonstrated. It should be noted that the doses administered in these animal studies are magnitudes of order higher than the estimated typical human consumption of DBAA, which has been reported to be 0.0001 mg/kg/day (Christian *et al.*, 2002). Based on the high multiples of human exposure required to produce effects in male rats, Christian and colleagues suggest that DBAA should not be identified as a human reproductive or developmental risk.

Furthermore, DCAA has been implicated in delayed spermiation and distorted sperm motility and morphology in rats at doses ranging from 54 to 3000 mg/kg/day (Linder *et*

*al.*, 1997a) and bromochloroacetic acid (BCAA) has been associated with transient sub-fertility in rats (Luft *et al.*, 2000).

When mice were exposed to sub-chronic, low-dose (0, 5, and 50 mg/kg/day) levels of DBAA from gestation day 15 through nursing, Weber *et al.* (2006) found no significant differences between control and dosed animals in daily sperm production, testicular sperm counts, epididymal sperm reserves, or morphology of seminiferous epithelium.

## 2.13 Previous Epidemiological Studies of DBPs and Birth Defects

### 2.13.1 Epidemiologic Studies of Reproductive Outcomes with Qualitative Assessment of DBPs

Human epidemiologic studies have offered evidence of a positive relationship between DBP concentrations and the risk of genitourinary birth defects. Aschengrau *et al.*, (1993) conducted a case-control study among women who delivered infants during August 1977 through March 1980 at Brigham and Women's hospital in Massachusetts. This investigation analyzed drinking water quality and adverse reproductive outcomes, including 1,039 congenital anomaly cases. The results of the study indicated that pregnant women using chlorinated surface water had an increased risk of giving birth to children with urinary tract defects, compared with women that used chloraminated water (OR = 4.1; 95% CI 1.2-14.1).

In 1999, Magnus *et al.*, found an odds ratio of 1.99 (95% CI: 1.10-3.57) in a case-control study that evaluated exposure to DBPs and risk of urinary tract defects using water color

and chlorination status in the exposure assessment. This study linked the Norwegian waterworks registry, containing 1994 data on chlorination practice and color (an indicator for natural organic matter), with the Medical Birth Registry for 1993-1995, and included 141,077 births, of which 1.8% (2,608) had birth defects. The exposed group in the study was made up of those that received water classified as having high color and that was disinfected using chlorination, whereas the referent group were those that received water classified as having low color and receiving no chlorination treatment.

Hwang *et al.*, (2002) conducted a nationwide cross-sectional study of 285,631 Norwegian births occurring in 1993-1998 to assess the effect of DBPs on specific birth defects, including urinary tract defects. Exposure was based on municipal-level water quality information on chlorination practices and the humic content of raw water (use of chlorination and color) and the mother's place of residence during pregnancy. The authors found a slightly increased, though not significant, risk of urinary tract defects, with adjusted odds ratio of 1.61 (95% CI: 0.94-2.76) for the medium-exposure (chlorination used as method of disinfection and water color 10-19.9 mg Pt/L) and 1.35 (95% CI: (0.65-2.80) for the high exposure (chlorination used as method of disinfection and water color $\geq$20 mg Pt/L) categories. The odds ratio was 1.46 (95% CI 1.00-2.13) when the medium and high exposure groups were combined.

### 2.13.2 Epidemiologic Studies of Reproductive Outcomes with Quantitative Assessment of DBPs

Bove *et al.* (1995) undertook a cross-sectional study of 75 New Jersey towns to determine whether elevated levels of THMs were associated with increased prevalence of

congenital anomalies. The 75 towns were served by 49 water utilities. Tap water sample data for each water company serving the 75 towns were used to estimate quarterly levels of total THMs. For all birth defects grouped together, the odds ratio was 1.75 (50% CI 1.56-1.96) at total THM levels >80 – 100 ppb, but, at >100 ppb, the odds ratio fell to 1.04 (50% CI 0.81-1.35). When these levels were combined, the odds ratio was 1.57 (50% CI 1.42-1.75). At >80 ppb, odds ratio for CNS defects and its subgroup neural tube defects (NTDs) were 2.59 (50% CI 2.05-3.28) and 2.96 (50% CI 2.00-4.39), respectively. For oral cleft defects, the odds ratio was 3.17 (50% CI 2.05-4.89) at total THM levels >100 ppb. At >80 ppb, the odds ratio for major cardiac defects was 1.83 (50% CI 1.38-2.43). The trends for cleft defects and major cardiac defects were not monotonic, and the continuous TTHM variable did not support a trend.

Dodds *et al.* (1999) examined the relation between total THM level and adverse birth outcomes in the Canadian province of Nova Scotia between 1988 and 1995. They conducted a retrospective cohort study using a population-based perinatal database and the results of water quality monitoring data. Individual exposures to THMs during the first two months of pregnancy were estimated by linking mother's residence at delivery to the geographic area served by each water facility for cleft defects and cardiac defects. Average total THM levels from 1 month before and 1 month after conception were used in the analysis of NTDs. Intermediate levels of total THMs around the time of conception were moderately associated with reduced risks of NTDs (RR 0.41, 95% CI 0.17-1.00). The authors found little evidence of any important association between total THM exposure during early pregnancy and cleft defects (RR 1.01, 95% CI 0.55-1.86) or

cardiac defects (RR 0.78, 95% CI 0.58-1.05). Misclassification of estimated total THM levels, the possibility that other classes of DBPs are responsible for the effect, spatial variability of THMs, residential mobility, and lack of individual behavior data were listed as limitations of the study.

A 1999 investigation was designed to evaluate risk factors for NTDs by estimating exposure to drinking water at the critical time for neural tube closure (first month after conception) for each subject (Klotz & Pyrch, 1999). Tap water sampling and interviews were undertaken 4 months after the due date in order to correspond to the critical period for neural tube development 1 year earlier. The authors found evidence that exposure to THMs or some other feature or component of surface water, but not HAAs or HANs may be a risk factor for NTDs. When comparing the highest total THM tertile with the lowest tertile, the authors found an unadjusted prevalence odds ratio (POR) of 1.6 (95% CI 0.9-2.7). When the analysis was restricted to subjects for whom the residence during early gestation was known, the overall strength of this association increased slightly (POR 1.7, 95% CI 1.0-3.1). When stringent criteria for case definition (isolated vs. non-isolated) and residency data were applied simultaneously, the POR for total THMs was 2.1 (95% CI 1.1-4.0).

Dodds & King (2001) examined the effect of exposure of two trihalomethanes, chloroform and BDCM, on the risk of congenital anomalies. They used average chloroform and BDCM concentrations from 1 month before to 1 month after conception in analyses of NTDs, concentrations during the first 2 months after conception in the

analyses of cardiac defects and cleft defects, and the average concentrations 3 months before conception were used in the analyses of chromosomal abnormalities. The authors found differences in the risk associated with exposure to chloroform and BDCM for each of the congenital anomalies under study. For NTDs, the risk seems to be increased with high exposure to BDCM but not chloroform. A protective effect found for exposure to BDCM on cardiovascular defects was surprising to the authors and difficult to explain. No significant associations were found for cleft defects or chromosomal abnormalities.

In 2003, Shaw *et al.* used maternal addresses that could be matched to a municipal water company to estimate exposure to THMs during the period 3 months before to 3 months after conception. They found the risk of NTDs to be inversely associated with total THM exposure in one study set, however this pattern was not observed in a second, smaller data set. The authors noted that the observed effect estimates might have been biased owing to non-differential classification of exposures, and cited their inability to investigate the potential effects of other chemical compounds, namely HAAs, as a major limitation.

### 2.14 Assessing Exposure to DBPs in Environmental Epidemiology Studies

One of the largest constraints in epidemiologic studies of birth defects has been exposure misclassification. Researchers have examined many different methods of exposure assessment to try to avoid misclassification. These include basing exposure on the source of the water entering a community water system (CWS) (Aschengrau *et al.*, 1993), the type of treatment used in disinfection (Kanitz *et al.*, 1996; Kallen and Robert, 2000), the

treatment and color of the water (Magnus *et al.*, 1999; Hwang *et al.*, 2002), routine CWS monitoring data (Kramer *et al.*, 1992; Bove *et al.*, 1995; Savitz *et al.*, 1995; Waller *et al.*, 1998; Gallagher *et al.*, 1998; Klotz and Pyrch, 1999; Dodds and King, 2001), and predicted DBP concentrations using linear regression modeling (Dodds *et al.*, 1999; King *et al.*, 2000; Shimazu *et al.*, 2005).

While the most reliable method of exposure assessment is through personal exposure monitoring, this is rarely feasible in epidemiologic studies due to financial and other constraints (Arbuckle *et al.*, 2002). In most tap water epidemiologic studies the alternative is to consider CWS monitoring data in conjunction with data collected on water consumption and use. Federal regulations require all CWS that add a primary or residual disinfectant other than ultraviolet (UV) light or deliver water that has been disinfected by a primary or residual disinfectant other than UV to measure DBP levels at distribution system sampling sites on a quarterly basis (EPA, 2006). These regulatioins require monitoring at sites with the highest concentrations of TTHM and HAA5 in each system's distribution system on a regular schedule that is determined by source water type and system size (EPA, 2006). Still, using monitoring data to accurately assign DBP levels to individual residences in a water distribution system requires knowledge of the hydraulic relationship between the sampling point and the residence location (Gallagher *et al.*, 1998; Nuckols *et al.*, 2001). Often times, such information is not available and CWS-wide averages or proximity relationships between the sampling point and the residence are used to assign a DBP concentration to the individual's water supply. Such an approach can lead to significant misclassification of exposure depending on the degree

of spatial and temporal variation in DBP concentrations across the CWS distribution system (Symons *et al.*, 1982; Singer *et al.*, 1995; Stone, 1996; Gallagher *et al.*, 1998; Waller *et al.*, 2001; Nuckols *et al.*, 2001; Lynberg *et al.*, 2001).

In the case of DBPs in tap water, the exposures are not categorical, but occur on a continuum from negligible to substantial for any particular measure of DBP exposure. Generally speaking, epidemiologic studies of DBPs have used categories rather than continuous variables, in part because the quality of exposure assessment has not warranted the precision provided by a continuous exposure variable (Froese *et al.*, 2002). Inaccurate and imprecise exposure estimates in epidemiologic studies may lead to loss of power and precision, and attenuation in health risk estimates (Armstrong, 1998; Swan & Waller, 1998). The extent to which this happens depends on the relation between the exposure index that is used and the "true exposure".

### 2.14.1 Distribution and Correlation of Specific By-Products in Community Water Systems (CWS)

Among DBPs, THMs generally occur in the highest concentration (Krasner *et al.*, 1989; Williams *et al.*, 1997), with HAAs occurring in the second highest concentration. Total THMs, however, do not necessarily provide a good proxy measure of exposure to specific by-products, particularly brominated species (Serodes *et al.*, 2003; King *et al.*, 2004). Recent expert panel reviews have suggested that brominated species should be a focus of epidemiologic studies (USEPA, 1998). Due to the sparse data on concentrations of individual species of THMs and other classes of DBPs, researchers have looked to find

relationships between these and total THMs. The results of these investigations are presented here.

Total THMs were found to correlate highly with chloroform (r=0.96-0.98) and moderately with BDCM (r=0.26 - 0.63) and total HAAs (r=0.52 - 0.74) (King *et al.*, 2004; Golfinopoulos & Arhonditsis, 2002; Keegan *et al.*, 2001; Whitaker *et al.*, 2003). Villanueva *et al.* (2003) found a statistically significant correlation between total THM and HAA levels ($r=0.86$, $p<0.0005$). Total HAAs were highly correlated with TCAA and DCAA ($r>0.85$) (King *et al.*, 2004). Whitaker *et al.* (2003) found a moderate positive correlation between chloroform concentrations in tap water and the simulated uptake of chloroform, as estimated by serum chloroform concentrations for the whole population. They also found a lack of correlation between the brominated THMs and the total THM level, which suggests that total THM concentrations may not provide a good marker for these THMs and other bromine containing DBPs.

It should be noted that these correlations may differ significantly by distribution system and geographic region, and thus the Pearson correlation coefficients presented above should not be considered representative of relationships in all distribution systems and/or geographic regions.

The relevant period for the possible association of chemical exposures with adverse developmental outcomes is believed to be only three months (the first trimester of pregnancy for congenital malformations). The clear seasonal variation in the data means

that it is essential to the accuracy of exposure assessments to gain estimates of average CWS DBP levels over this period. Where seasonal fluctuations are large, estimation of average DBP levels over longer periods (e.g. annual average DBP levels) will be inaccurate. With few samples taken in each CWS throughout the year, reliable estimation of a mean water utility DBP level for such a short period is difficult

### 2.14.2 Spatial Variability of DBPs in Community Water Systems (CWS)

The degree of intra-system variation in water supply DBP levels is a very important factor in the design of an epidemiologic study (Lynberg *et al.*, 2001). Intra-system spatial variation in DBP concentrations results from formation and degradation processes occurring over time (Stevens *et al.*, 1989) and space (Chen & Weisel, 1998). Spatial variability in THM formation has been reported, with distribution system levels up to two (Rodriguez & Serodes, 2001), and three (Sohn *et al.*, 2001) times higher than finished water leaving the treatment plant, and up to four times higher when water temperatures exceeded 15° C (Rodriguez & Serodes, 2001). Conversely, for non-volatile compounds, such as haloacetic acids, maximum concentrations have been found at sampling locations closest to the point of disinfection (Chen & Weisel, 1998). Reductions in HAAs and other nonvolatile DBPs within the distribution system can result from abiotic and biotic degradation (Krasner *et al.*, 1989; Singer, 1994).

When intra-system variation is small, CWS means are likely to be more accurate, even with few measurements (Keegan *et al.*, 2001). When samples from a utility are limited, and intra-system variability is high, estimates are more likely to be inaccurate. This can

lead to exposure misclassification when CWS means are linked to an individual within the distribution system (Keegan *et al.*, 2001).

THM values for individual household samples in the same distribution system can vary widely. For example, THM values ranged from 13 to 84 μg/L and period distribution system averages ranged from 30 to 56 μg/L in a single distribution system (King *et al.*, 2004). On average, household samples differed from the distribution system mean by 8.0 μg/L, and the variance in household samples increased with higher mean THM levels (King *et al.*, 2004).

These intra-system differences highlight the limitation of using town average concentrations as a surrogate for maternal exposure to DBPs. Specifically, Whitaker *et al.* (2003) and King *et al.* (2004) used individual-level water usage data to estimate DBP exposure during pregnancy and reported exposure misclassification rates in excess of 40% for CWS average THM classification.

Hinckley *et al.* (2005) developed a method for a priori selection of study sites with low spatial variability using state and national public water utility datasets as a means to reduce exposure misclassification in epidemiologic studies of DBPs. This type of site selection, when applicable, may aid in decreasing exposure misclassification due to deficient data quality and improve our understanding of the reproductive risks associated with DBPs.

#### 2.14.2.1 Using GIS to Link Participant Residence with Environmental Exposure in Environmental Epidemiology

From an epidemiologic perspective, analyses at the individual level are preferred due to inherent strengths of case-control and cohort designs in making inferences regarding causality. The geocoding of individuals to a specific location instead of to a generalized region (town, municipality) also decreases the chance of non-differential misclassification with respect to exposure (Dearwent *et al.*, 2001). The validity of epidemiologic studies using a geographic information system (GIS) and geocoding methods depends on the proportion of addresses that can be geocoded as well as the positional accuracy of the geocoding success (Bonner *et al.*, 2003). Dearwent *et al.*, (2001) examined two geocoding models, the street centerline model, and the residential parcel model. The street centerline data model consists of digitized road networks and related address ranges. Vectors approximate the center of each road segment and data describing address ranges for each side of the street are associated with these vectors. Residential addresses are linked to these vectors as they are geocoded. For the residential parcels model, georeferencing to the center of residential parcels is considered to be a reference by which street centerline data were compared. Using data collected in two separate case-control analyses of cancer incidence and asthma prevalence in Jefferson County, Alabama the authors demonstrated that using the street centerline model, 89.3% of the study subjects' residences could be successfully geocoded, and that this model preformed better than the residential parcel model, which resulted in the geocoding of only 69.7% of the same study subjects' residences. The street centerline model, however, relies on several assumptions that may affect accuracy. These assumptions include: (1)

"parcel homogeneity" – wherein the partitioning of address ranges occurs through the uniform allocation of individual addresses along each road segment; and (2) the accurate and consistent assessment of residential location using street networks as a reference.

Positional inaccuracy of geocoded addresses may be an important source of exposure misclassification in environmental epidemiology studies (Bonner *et al.*, 2003). Positional accuracy has been shown to be somewhat better for the urban addresses than for the nonurban addresses when using the street centerline model, though the difference was small (Bonner *et al.*, 2003). Overall, the positional accuracy of addresses geocoded with the street centerline model was good. Bonner *et al.*, (2003) determined that the majority of the addresses were located within 100 meters of the real addresses as determined by on site global positioning system (GPS) latitude and longitude measurements. They also found that positional accuracy was not different for cases and controls, suggesting that errors resulting from the geocoding of addresses may not result in differential misclassification of exposure.

### 2.14.3 Temporal Variability of DBPs in Community Water Systems (CWS)

Overall, models incorporating year, season, or month showed that none of these explained more than 3% of the total variation in total THMs. Still, Golfinopoulos & Arhonditsis (2002) detected a seasonal effect in their investigation of DBPs. Most of the variability was attributed to temperature. THM concentrations also illustrated significant seasonal trends characterized by summer highs and winter lows, while values during

spring and autumn were lying at intermediate levels (Whitaker *et al.*, 2003; Toroz & Uyak, 2005). Water temperatures and raw water quality would be expected to vary according to season, and may explain this trend. Higher water temperatures favor THM production; thus, it is expected that THM levels will tend to be higher during summer months (Whitaker *et al.*, 2003). Seasonal variation also occurs in the levels and characteristics of the natural organic matter present in the water.

Whitaker *et al.* (2003) found that there was wide variation in chloroform levels from one month to the next whereas levels of the brominated THMs (BDCM, DBCM, and bromoform) were considerably more stable in their investigation of three CWS in the United Kingdom. They suggest that brominated THM levels may be more stable because these THMs typically form fairly rapidly, while chloroform formation increases over time. The authors conclude that chloroform levels may be more likely to be affected by residence time and chlorine residual in the distribution system and show wider spatial variation.

In order to examine temporal variability, King *et al.* (2004) took two samples, one year apart from each other, from 24 locations representing 12 distribution systems and total THM levels ranging from 14 to 141 μg/L. The correlation between original and 1-year post samples was 0.90 for total THMs with an average absolute difference of 9.8 μg/L.

### 2.14.4 Routes of Exposure to DBPs in Tap Water

Meaningful exposure to DBPs occurs through water ingestion, inhalation, and dermal absorption (Weisel & Jo, 1996; Backer *et al.*, 2000; Lynberg *et al.*, 2001; Xu & Weisel, 2005; Nuckols et al., 2005). This is important because the route of exposure affects the rates of metabolism and therefore the compounds potential toxicity (Weisel & Jo, 1996).

It has been demonstrated that dermal absorption is an important route of exposure to some DBPs in water when showering, bathing and swimming (Aggazotti *et al.*, 1990; Jo *et al.*, 1990; Lindstrom *et al.*, 1997; Gordon *et al.*, 1998; Xu *et al.*, 2002; Xu & Weisel, 2005), where THM exposure through a 10 minute shower may equate to the exposure received from consumption of 2 liters of water (Weisel & Jo, 1996). Kerger *et al.* (2000) report that THM release and corresponding average airborne concentrations are about three times higher on average for a typical shower compared to a bath. Although water temperature appeared to have relatively little influence on shower exposure concentrations, water flow rate and shower duration were determined to be key to the mass loading of THMs from water into the shower enclosure.

Little information exists for the multiple exposure routes for HAAs, though it is commonly accepted that exposure to nonvolatile DBPs such as HAAs, is primarily through ingestion (Nieuwenhuijsen *et al.*, 2000; Kaur *et al.*, 2004). Xu & Weisel (2003) did report that HAAs are expected to be completely ionized in tap water at a pH close to 7 and therefore are nonvolatile at ambient and typical shower water temperatures. Thus, they reported that inhalation exposure to HAAs during showering would occur

predominantly through respiratory uptake of shower-generated aerosols. The authors found that the inhalation dose of HAAs was much lower than corresponding daily average ingestion dose of the compound (less than 0.5%), which indicates that the potential inhalation exposure to particulate HAAs during showering is not expected to contribute significantly to total exposure.

Of THMs, HAAs and HKs, HAAs were the least permeable when permeability coefficients for dermal exposure were estimated, and were two orders of magnitude smaller than those of THMs (Xu *et al.*, 2002). The authors note, however, that the permeability of HAAs might have been overestimated in their study because HAAs penetrated the skin slowly; therefore a relatively long experiment duration was employed (24-48 hours). They suggest that the exposure duration to water should be minimized in subsequent studies in order to truly characterize the permeability coefficient for HAAs. In general, the ionized form of a chemical penetrates the skin poorly. This may explain the much lower permeability of HAAs compared to THMs (Xu *et al.*, 2002).

Based on this knowledge, risk estimates from exposure to DBPs, which can be calculated for population distribution from existing water usage patterns and concentrations, need to consider the dermal absorption and inhalation routes of exposure for THMs and HKs, but not for HAAs (Xu *et al.*, 2002; Xu & Weisel, 2005).

### 2.14.5 Contribution of Individual Water-Use Behavior Data for Assessing Exposure to DBPs in Tap Water

A large proportion of the variability of water intake and other habits during pregnancy depends on differences among subjects (Barbone *et al.*, 2002). Exposure assessment should start with a valid history of personal water use that is concurrent with the pertinent exposure period for the outcome under investigation (Swan & Waller, 1998; Barbone *et al.*, 2002). The differences in individual water use activities can significantly influence the level of THMs entering the blood stream (Lynberg *et al.*, 2001).

Backer *et al.* (2001) examined exposure to THMs via the dermal, inhalation and ingestion routes by measuring THM concentrations in whole blood samples provided by thirty-one study participants before and after drinking, showering and bathing activities. They found that the highest levels of THMs were found in the blood samples from people who took ten minute showers, whereas the lowest levels were found in the blood samples from people whoo drank one liter of water in ten minutes. This indicated that dermal and inhalation exposures associated with showering and bathing in addition to water consumption are important in accounting for an individual's exposure assessment, and that including only exposure to THMs from ingestion could under estimate and individual's exposure assessment.

Barbone *et al.* (2002) undertook a validation study of individual exposure assessment to DBPs in the last three months of pregnancy and found that intra-personal variability was very low for daily intake of tap water at home and at work and for shower and bath

frequency and duration. Information on total water mainly attributable to water other than tap water at work was more variable. Overall, the authors found that questionnaires developed to assess exposure to DBPs in tap water during pregnancy provide valid and reliable estimates of water intake.

King *et al.* (2004) developed an exposure measure, which assumed an equivalency of absorbed dose such that 1 liter of ingested water was equivalent to a 5 minute shower (Weisel & Jo, 1996) and a 15 minute bath (Kerger *et al.*, 2000). A 50% reduction in THM intake through cold water-based drinks was applied when a carbon filter was indicated. A reduction of 70% was applied to the THM ingestion estimate for boiled hot water drinks (Kuo *et al.*, 1997; Wu *et al.*, 2001). The influence of water-use behaviors on exposure assessment was evaluated through comparison of household THM level with the total exposure metric. Less than half the subjects (44.7%) were classified in the same quintile for both exposure measures.

In their study, Kaur *et al.* (2004) found considerable variation in exposure to tap water among pregnant women. Further, they determined that exposure of pregnant women to chlorinated water likely differed by geographical locations, due to differing water characteristics, as well as cultural differences. Finally, the results of this study allowed the authors to determine that a questionnaire was a valid method for estimating exposure to water in their comparison the "gold standard" seven-day diary.

Nuckols *et al.* (2005) measured blood and exhaled air concentrations of THMs in participants before and after they conducted 14 common household water use activities, including ingestion of hot and cold tap water, showering, clothes washing, hand washing, bathing, dish washing, and indirect shower exposure. They found that concentrations of TTHMs in blood and exhaled breath doubled after a ten minute showeror a twenty minute bath. Increases in the concentration of TTHMs were evident for other common household water use behaviors, but not with the same magnitude. These results emphasized the necessity for the inclusion of data on showering and bathing in the exposure assessment portion of epidemiologic studies of THMs.

### 2.14.6 Exposure Misclassification in Reproductive Epidemiology Studies Due to Residential Mobility

Women may change residences between exposure to potential teratogens and pregnancy outcome, and this can lead to migration bias whereby true excess risk is underestimated (Dolk *et al.*, 1998). Unlike most chronic effects of exposure to harmful chemicals, teratogenic effects may be detected as early as a few months after exposure to the teratogen. Thus, the potential for migration bias is limited. Still, estimates of the proportion of mothers who change residence during pregnancy may range between 20% and 32% (Khoury *et al.,* 1998; Nuckols *et al.*, 2004; Zender *et al.*, 2001) and of these, about 50% move less than 1 kilometer (Dolk, 1998). The results of the Nuckols *et al.* (2004) study indicated that 97% of the expectant study participants that moved during their pregnancy, moved to a new residence served by the same CWS as their previous residence.

### 2.14.7 Biomarkers in Epidemiologic Studies of DBPs

Optimally, DBP biomarkers should be sensitive and specific to the exposure of interest, readily accessible, inexpensive and technically feasible to measure; have an elimination half-life appropriate to the exposure window of interest; be indicative of exposure duration, intensity, and pattern; and be consistently and quantitatively related to exposure (Arbuckle *et al.*, 2002). Some of the difficulties in developing reliable biomarkers of exposure for DBPs are: the large number of compounds; variability in the bioavailability of these compounds; differences in the rate of absorption through lungs; skin and the gut; and differences in their persistence in the body (Sim, 2002).

#### 2.14.7.1 Biomarkers of Exposure

A major prospect for improved exposure assessment for epidemiologic studies of adverse reproductive outcomes is to validate a biomarker of exposure to DBPs (Froese *et al.*, 2002). Biomarkers of DBPs and/or their metabolites in blood, exhaled breath, and urine have been examined.

Lynberg *et al.* (2001) and Nuckols *et al.* (2005) demonstrated that it was possible to quantify blood levels of THMs at very low concentrations. Furthermore, they were able to document elevated background levels of individual THMs in human tissue, detect differences in blood levels of THMs between populations served by different water utilities, and determine that THM levels in blood correlate with levels found in an individual's water supply. Specifically, for each THM species, the concentration found in the blood samples was about 1/1,000 the level found in the resident's tap water.

In a 1996 study, Weisel & Jo found that, following ingestion of chlorinated municipal water, none of the breath samples had measurable levels of chloroform, but levels in exhaled breath were elevated after both inhalation and dermal exposures during showering and dermal exposure during bathing.

Urinary TCAA has been proposed as a biomarker of chronic ingestion exposure to HAAs from chlorinated drinking water (Froese *et al.*, 2002; Kim *et al.*, 1999; Weisel *et al.*, 1999). Weisel *et al.* (1999) evaluated how well drinking water concentrations measured within homes correlated with biomarker concentrations, and water concentrations combined with questionnaire data about activities related to water use. They enrolled 49 female subjects who had previously participated in a case-control study on NTDs in the study and examined two sets of biomarkers – exhaled breath measurements of THMs at the time of visit and showering, and urinary DCAA and TCAA excretion rates and creatinine-normalized concentrations. They found that the majority of background exhaled breath concentrations for THMs were below detection limits (1 $\mu g/m^3$). Due to small numbers, no statistically significant difference in breath concentrations could be determined between high and low water concentration groups. Neither the DCAA first-morning urine excretion rates nor the DCAA creatinine-normalized concentrations were significantly different between the two groups, though the TCAA urinary excretion rates and creatinine-normalized concentrations were. The TCAA urinary excretion rate was statistically related to the ingestion exposure with an adjusted $r^2$ of 0.532 ($p<0.0001$). The authors concluded that urinary TCAA rates demonstrate a dose-response relationship

with exposure and appear to be a valid biomarker of TCAA ingestion exposure from chlorinated water during routine household use over a 48-hour time period, whereas urinary DCAA does not.

TCAA concentrations were easily detected in the first morning urine samples analyzed for the 10 participants in a pilot exposure/intervention study conducted by Froese *et al.* (2002). The results, however, did indicate substantial intra- and inter-individual variability in both TCAA ingestion and excretion rates. A major contribution to the observed variability is the variability in source and volume of water consumed for each individual and across all individuals in the study. Ultimately, confidence in a relationship between ingestion and excretion must be established for urinary TCAA to provide any value for DBP exposure assessment. The failure of TCAA to decline to expected low levels raises concerns about the specificity of urinary TCAA levels as a marker of drinking water ingestion. Despite several qualifiers, TCAA remains the most promising prospect for a biomarker of ingestion exposure to DBPs in drinking water.

Calafat *et al.* (2003) found detectable concentrations on TCAA in 76% of archived urinary samples from a national reference population (n=402). This was not surprising considering the widespread use of chlorination of tap water. Because of TCAA's low dermal and inhalation absorption (Kim & Weisel, 1998), this suggests that a large portion of the study population was exposed to TCAA from ingestion of chlorinated drinking water. Statistically significant correlations were found between urinary creatinine-adjusted TCAA and blood levels of 1,1,1-trichloroethane (TRI) and TCE (r=0.32 and

r=0.43, respectively), suggesting that TCAA levels may be associated with these chemicals, at least to a certain extent.

### 2.14.7.2Biomarkers of Susceptibility

From over 2000 likely single gene birth defect syndromes in humans, the gene has been isolated in only five percent and mapped in a further five percent (Winter, 1996). Multifactorial disorders, in contrast, are characterized by high levels of genetic complexity and probable gene-environment interactions (Ellsworth *et al.*, 1997). Susceptibility genes for multifactorial disorders tend to be common in the general population (allele frequency $\geq$1%), and are usually associated with low risk for complex disease. However, these susceptibility genes may interact with specific environmental factors to produce a markedly increased risk of disease (Manson & Carr, 2003). A multifactorial pattern is the most consistent explanation for familial clustering of defects such as severe hypospadias.

5 α-reductase is the enzyme responsible for converting testosterone (T) to dihydrotestosterone (DHT). Two distinct isoenzymes have been described, 5 α-reductase types 1 and 2. The corresponding genes have been identified and named SRD5A1 and SRD5A2, respectively. Both isoenzymes are expressed in genital skin, prostate and seminal vesicle. Mutations of the SRD5A2 gene have been shown to lead to masculinization defects of varying degree between completely female, patients with micropenis or hypospadias, and completely male with overt signs of

undermasculinization (Tria *et al.*, 2004). The elevation of the T/DHT ratio is associated with this gene mutation.

The role of T and DHT in formation of the male genital tract is vividly illustrated in men with congenital SRD5A2 enzyme deficiency, who are known as male pseudohermaphrodites (Griffen & Wilson, 1989; Imperato-McGinley *et al.*, 1991; Zhu *et al.*, 1998). They have a normal XY karyotype but ambiguous external genitalia.

The coincidence of increased T/DHT ratios and hypospadias without mutations in the SRD5A2 gene or the androgen gene suggest potential, unidentified mutations in the SRD5A1 gene (Tria *et al.*, 2004). Tria *et al.* (2004) failed to detect a particular mutation in this gene in their genomic analysis of the SRD5A1 gene in patients with hypospadias and an elevated T/DHT ratio (n=10).

Codner *et al.* (2004) hypothesize that some cases of apparent idiopathic hypospadias may be the consequence of 3β-hydroxysteroid dehydrogenase gene type II (HSD3B2) gene mutations, which may affect androgen production during fetal life. HSD3B2 is expressed in the adrenal gland, ovary, and testis. They performed a complete hormonal and molecular study of the HSD3B2 gene in a large group of patients with apparent idiopathic hypospadias. Overall, the authors did not observe definite evidence of a steroidogenic profile suggestive of HSD3B2 or androgen insensitivity in 90 cases with genital abnormalities studied. They did find two patients with hypospadias and a heterozygous missense mutation in the HSD3B2 gene, resulting in partial loss of enzymatic activity.

The authors conclude that it is possible that during the first trimester of pregnancy, this mutation might interfere with the migration of the urethral meatus to the tip of the glans penis. Direct confirmation of this hypothesis, however, cannot be concluded from this study.

The 17β-hydroxysteroid dehydrogenase gene type III (HSD17B3) isozyme is found exclusively in the microsomes of the testes, where it converts the weak androgen, androstenedione, into T. Familial male pseudohermaphrodism due to autosomal recessive deficiency in the HSD17B3 isozyme has been reported (Castro-Magana *et al.*, 1993). Deficiency in HSD17B3 activity is rarely diagnosed at birth, and is usually discovered at puberty in genetic males reared as females. A total of 20 allelic variants have been described in the HSD17B3 gene in subjects with HSD17B3 isozyme deficiency, most of which cause complete loss of enzyme activity (Manson & Carr, 2003).

The androgen receptor (AR) gene plays a critical role in male sexual differentiation by mediating the biological effects of gonadal androgens. There are more than 150 mutations described in the AR gene that give rise to androgen insensitivity syndrome (MacLean *et al.*, 1995). Most reports of AR gene mutations in individuals with hypospadias have included patients with additional genitourinary malformations. Mutations of the AR coding sequence are infrequent in patients with isolated hypospadias (Allera *et al.*, 1995; Klocker *et al.*, 1995; Sutherland *et al.*, 1996).

These findings indicate that the gene mutations in the SRD5A2, HSD17B3, and the AR genes may not be common in patients drawn from the general population with nonsyndromic hypospadias. Until larger studies are undertaken, the role of genetic factors in genes controlling androgen action and metabolism will not be clearly understood (Manson & Carr, 2003).

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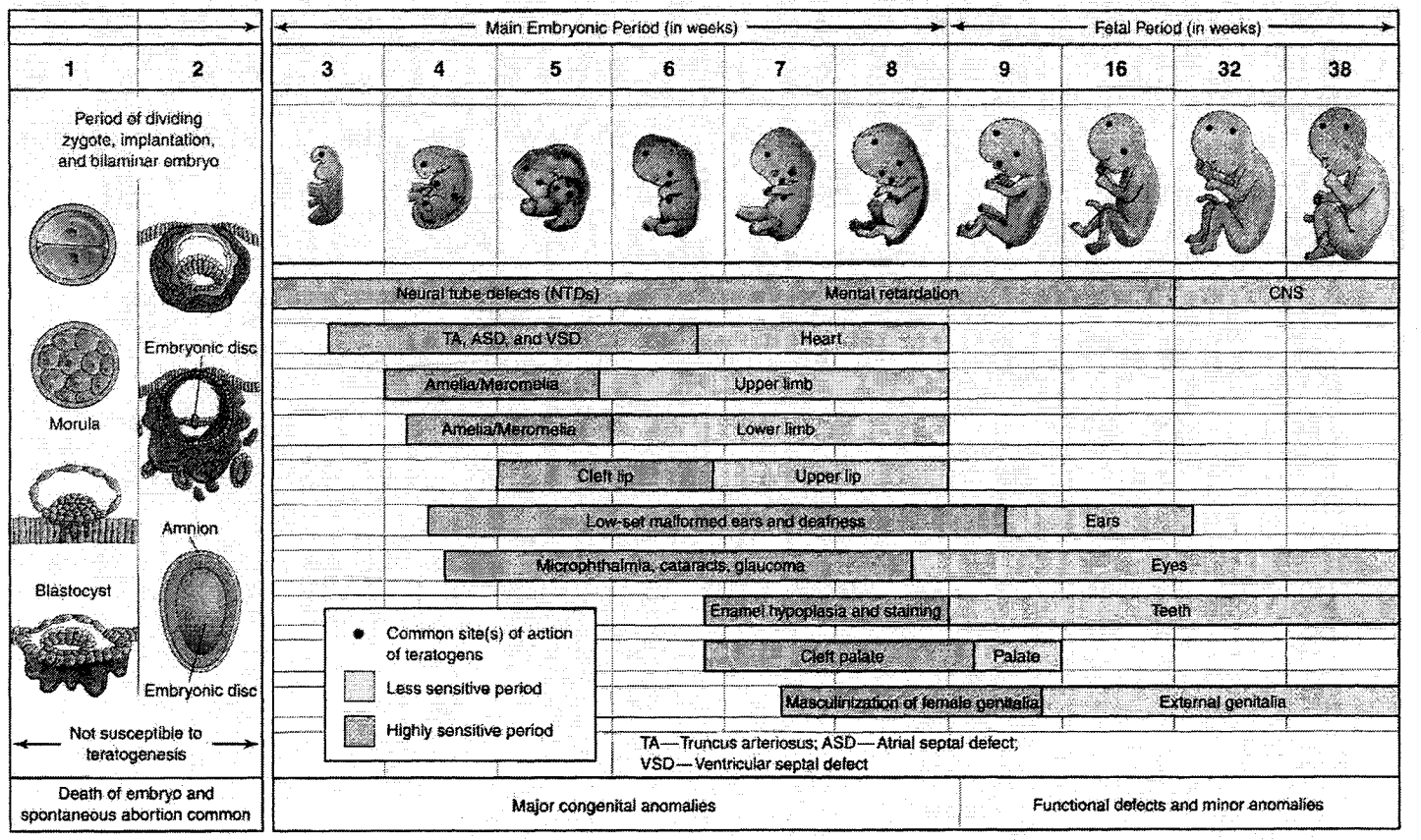
Figure 2.2.3.1: Schematic illustration of critical periods in human development. From: Moore KL, Persaud TVN. *Before we are born. Essentials of embryology and birth defects.* 5th edition. W.B. Saunders Company. Philadelphia, PA. 1998.




■ Figure 9–12. Schematic illustration of critical periods in human prenatal development. During the first 2 weeks of development, the embryo is usually not susceptible to teratogens; a teratogen either damages all or most of the cells, resulting in death of the embryo, or damages only a few cells, allowing the conceptus to recover and the embryo to develop without birth defects. Mauve denotes highly sensitive periods when major defects may be produced (e.g., amelia, absence of limbs). Green indicates stages that are less sensitive to teratogens when minor defects may be induced (e.g., hypoplastic thumbs).

## Chapter 3

# 3. Validation Study of Geocoding Procedure Used to Link Participants' Residences to a Community Water System in a Study of Genitourinary Birth Defects and DBPs in Arkansas

## 3.1 Introduction

Chlorine, used to disinfect water, reacts with natural organic matter in surface waters, resulting in the formation of a complex mixture of chemicals, including trihalomethanes (THMs), haloacetic acids (HAAs), haloketones (HKs), and haloacetonitriles (HANs). Collectively, these compounds are referred to as disinfection by-products (DBPs). Human epidemiologic studies have offered evidence of a positive relationship between DBP concentrations and the risk of certain cancers (McGeehin *et al.*, 1993; King and Marrett, 1996; Koivusalo *et al.*, 1997; Doyle *et al.*, 1997; Cantor *et al.*, 1998; Yang *et al.*, 1998; King *et al.*, 2000a) and adverse reproductive outcomes (Kramer *et al.*, 1992; Aschengrau *et al.*, 1993; Bove *et al.*, 1995; Savitz *et al.*, 1995; Kanitz *et al.*, 1996; Reif *et al.*, 1996; Waller *et al.*, 1998; Gallagher *et al.*, 1998; Swan *et al.*, 1998; Klotz and Pyrch, 1999; Magnus *et al.*, 1999; Dodds *et al.*, 1999; King *et al.*, 2000b; Kallen and Robert, 2000; Dodds and King, 2001). One of the largest constraints in these studies has been exposure misclassification.

While the most reliable method of exposure assessment is through personal exposure monitoring, this is rarely feasible in epidemiologic studies due to financial and other constraints (Arbuckle *et al.*, 2002). In most tap water epidemiologic studies the

alternative is to consider community water systems (CWS) monitoring data in conjunction with data collected on water consumption and use. Federal regulations require CWS to measure DBP levels at distribution system sampling sites on at least a quarterly basis (USEPA, 2003).

Many epidemiologic studies concerning DBPs and adverse reproductive outcomes use CWS monitoring data to estimate exposure (Aschengrau *et al.*, 1993; Bove *et al.*, 1995; Savitz *et al.*, 1995; Kanitz *et al.*, 1996; Waller *et al.*, 1998; Dodds *et al.*, 1999; Klotz & Pyrch, 1999; King *et al.*, 2000b; Dodds & King, 2001). Use of such data requires linkage of residence location to a specific CWS and associated monitoring data during a given exposure period. The inability to perform this linkage successfully can lead to exposure misclassification and/or reduced sample size.

The validity of epidemiologic studies using a geographic information system (GIS) and geocoding methods depends on the proportion of addresses that can be geocoded as well as the positional accuracy of the geocoding success (Bonner *et al.*, 2003; Nuckols *et al.*, 2004; Ward *et al.*, 2005). Positional inaccuracy of geocoded addresses may be an important source of exposure misclassification in environmental epidemiology studies (Bonner *et al.*, 2003; Ward *et al.*, 2005). Bonner *et al.* (2003) found that positional accuracy has been shown to be somewhat better for the urban addresses than for the non-urban addresses, though the difference was small, while Ward *et al.* (2005) reported that the positional error for rural addresses was substantially larger in their comparison of two geocoding methods.

Given the importance of the proportion of addresses geocoded, the positional accuracy of these geocoded addresses, and the role that the use of geocoded addresses may play in reducing non-differential exposure misclassification in epidemiological studies, the primary objective of the current investigation was to validate a geocoding method developed to link study participants to the CWS from which they receive their tap water. In particular, we were interested in the proportion of addresses that we were able to geocode, evaluating the use of U.S. Census Place boundaries as surrogates for CWS boundaries in order to link CWS with geocoded addresses, comparing the proportion of rural and non-rural addresses that we were able to link to a CWS, and investigating the problem of clustered utilities in correctly linking a geocoded address with a CWS.

## 3.2 Methods

### 3.2.1 Geocoding Procedure for Participants' Residences

We submitted street address, city, state, and zip code data from 3,886 residences that we obtained from the Arkansas Health Department for use in an epidemiologic study to an independent research corporation for geocoding purposes. Matchmaker® SDK Professional software (Geographic Data Technologies, Inc. (GDT), Lebanon, NH) was employed in the geocoding process. The geocoding process attaches census as well as coordinate information to address files, allowing us to derive information from address files.

The geocoding process included running an input file containing the 3,886 addresses through an initial batch match process to automatically link all records with clean addresses to latitudinal and longitudinal coordinates. For those addresses that could not be linked in this manner, we used an interactive matching process to manually change address information, such as eliminating an apartment number or changing a street prefix or suffix. For addresses that we were unable to geocode through the batch and interactive match processes, we used a manual match procedure to increase the link percentage. We linked records to a central street segment when the address number was unavailable or could not be linked, and the street was entirely contained in a single U.S. Census Block Group. We linked records to the nearest intersection in cases where the street number was not defined. We used the ZIP code centroid of a residence to link records that could not be linked otherwise. We assessed the degree to which each residence was urban or rural based on the demographic data for the U.S. Census Block Group to which the geocoded location of each residence was linked.

### 3.2.2 Census Place and Community Water System Linking Procedure

We downloaded the 2000 U.S. Census Incorporated Place/Census Designated Places GIS file for Arkansas to a GIS from the Internet (**http://www.census.gov/geo/www/cob/pl2000.html**). We compared the CWS names for Arkansas to the list of Census Place names and identified those with common names. We joined the boundary data from the 2000 U.S. Census Incorporated Place/Census Designated Places and the geocoded participant locations in the GIS based on spatial location. Finally, we created a file that contained all possible CWS links for each of the residences included in the study.

### 3.2.3 Determining Community Water System Cluster Status

We defined CWS within our study area as clustered with one or more CWS if the Census place boundary surrounding each CWS was located within a prescribed distance of the Census place boundary of another CWS. For example, we selected the city of Little Rock as a test site for determining CWS cluster status. We plotted the geographic centroid of Little Rock in a GIS and then clipped the layer using a 30 km radius around the centroid of Little Rock. Using that area as a test area, we applied different buffer distances (1 km, 2 km, 4 km). We found 4 km to be the minimum distance that coded all Census place polygons in the test region as "clustered". We used this buffer distance to identify other clustered CWS in our study area. Thus, if the Census place boundary linked to any CWS was within 4 km of a Census place boundary linked to another CWS, we considered the two CWS to be clustered. We examined the results of the cluster analysis based on utility size.

### 3.2.4 Survey Validation of Linked Residences and Community Water Systems

In order to validate the linking procedure described previously, we provided 100% of the residential addresses to the CWS to which they were linked to see if billing records and/or distribution boundaries included the addresses. If an address was matched to one or more clustered CWS, we sent the address to each CWS for confirmation of service. If the CWS confirmed that it served the address, we assumed that the address had been correctly linked to the utility. If the utility could not confirm that it served an address, we

assumed that the address had been incorrectly linked to a utility. We quantified the overall proportions of addresses correctly and incorrectly linked to water utilities.

To explore potential bias, we compared demographic characteristics of participants linked to a U.S. Census Place name to participants not linked, and participants that were validated as linked to a specific CWS to those not validated. These demographic characteristics were maternal and paternal age, race, ethnicity and education, maternal smoking and rural/urban residence. We analyzed demographic characteristics available from birth certificate records and U.S. Census records as categorical variables. We compared the distribution of demographic characteristics between groups to determine if differences were statistically significant using chi-square tests with one degree of freedom.

## 3.3 Results

### 3.3.1 Geocoding Procedure for Participants' Residences

Of the 3,886 addresses provided to the independent research corporation 177 were located outside of our study area and no attempt was made to geocode them. We were able to successfully geocode 3,607 (97.2%) of the remaining addresses. We linked 2,909 addresses (80.6%) to an exact point within a street segment, 198 addresses (5.5%) to an intersection closest to the given address, and 500 addresses (13.9%) to the geographic centroid of the given or imputed zip code associated with the address. We could not geocode 52 addresses (1.4%) because they were listed as post office boxes. We could not

geocode an additional 16 addresses (0.4%) because they were incomplete or recorded as "don't know address". We could not geocode the remaining 34 (0.9%) addresses for some "other reason".

### 3.3.2 Census Place and Community Water System Linking Procedure

Of the 3,607 addresses that were successfully geocoded, we linked 2,574 (71.4%) to a U.S. Census Place name within our study area. We could not link the remaining 1,033 (28.6%) to a U.S. Census Place name.

Of the 2,574 addresses that we successfully linked to a U.S. Census Place name, we linked 97% to at least one CWS; 1,681 (65%) were linked to one CWS, 613 (24%) to two CWS, and 214 (8%) to three CWS. We could not link 61 (2%) of the Census Place names to any of the CWS.

### 3.3.3 Determining Community Water System Cluster Status

We linked participants' residences from the epidemiologic study to 263 different CWS in Arkansas. We found 196 (74.5%) of the CWS to be clustered within four kilometers with at least one other CWS.

The results of the cluster analysis when stratified by utility size revealed all of the large utilities (serving greater than 100,000 persons; n=5) to be clustered. We found 73.5% of the small utilities (retail population $\leq$ 10,000) and 24% of medium utilities (retail population >10,000 - $\leq$100,000) to be clustered.

### 3.3.4 Survey Validation of Linked Residences and Community Water Systems

We sent participants' addresses to 263 CWS representatives to validate that those addresses were, in fact, served by that CWS. We sent 1,686 addresses to a single utility, 613 addresses to 2 utilities and 214 addresses to 3 utilities. Collectively, we sent 3,554 non-exclusive addresses to different CWS representatives for validation of water service. We received responses for 3,186 (89.6%) of these addresses from a total of 191 (72.6%) of the CWS contacted.

Of the 1,686 addresses sent to a single CWS for validation, we received responses for 1,436 (85.2%). We confirmed that 1,182 (82.3%) of these addresses were served by the CWS. We could not confirm 214 (14.9%) of the addresses, and we could not validate 40 (2.8%) of the addresses due to insufficient (i.e. P.O. boxes) or incomplete addresses.

Of the 613 addresses that were sent to 2 CWS for validation, we received responses from both CWS for 515 (84.0%) addresses, a response from only one of the CWS for 90 (17.7%), and responses from neither of the CWS for 8 (1.3%) of the addresses. Of the 515 addresses for which we received responses from both of the CWS contacted, 416 (80.8%) of the addresses had one positive (address was served by the CWS) and one negative (address was not served by the CWS) response. We received two negative responses for 37 (7.2%) of the addresses and two positive responses for 27 (5.4%) of the addresses. The remaining 35 (6.8%) of the addresses could not be validated by at least one of the CWS due to insufficient or incomplete addresses.

We sent 214 addresses to three CWS for validation. Of these, responses from all three CWS accounted for 201 (93.9%) of the addresses, and responses from two of the three CWS were received for 13 (6.1%) of the addresses. Of the 201 addresses for which we received responses from all three of the CWS contacted, 153 (76.1%) of the addresses had one positive and two negative responses. 22 (10.9%) of the addresses received three negative responses, and 14 (7.0%) of the addresses received two positive and one negative responses. The remaining 12 (6.0%) of the addresses could not be validated by at least one of the utilities due to insufficient or incomplete addresses.

Collectively, the necessary responses for validation were received from the CWS for 2,152 (85.7%) addresses. Of these, we were able to validate 1,751 (81.4%) addresses as being correctly linked to a CWS.

We found no bias for age, education, or ethnicity between linked and unlinked participants. However, unlinked participants were more likely to be white ($p<0.0001$) and more likely to live in a rural census block group ($p<0.0001$). We present these results in Table 3.3.5.2. The results of the bias analysis for participants who were linked to a CWS and validated compared to those whose address that were linked to a CWS but could not be validated are presented in Table 3.3.5.3. We found that the participants whose linkage we could not validate were more likely to be white ($p<0.0001$) and were more likely to live in a rural census block group ($p<0.0088$).

## 3.4 Discussion

We successfully geocoded 97% of the study participant addresses, which is slightly higher than the proportion geocoded in a recent Iowa study (Ward *et al.*, 2005). We were able to link 97.4% of all addresses linked to a Place name with at least one CWS, an indication of the effectiveness of our method. In our validation of using a GIS to link study participants to a CWS for use in the exposure assessment portion of an epidemiologic study, we found that the method correctly linked over 81% of the participants. These results demonstrate that this method of determining exposure to DBPs can be effectively employed, even in rural states such as Arkansas.

We identified the rural nature of Arkansas during the design phase of this study, and chose to utilize the GIS method of linking study participants to a CWS in Arkansas so the results could be compared to similar investigations being undertaken in Texas and New York (Nuckols, unpublished data). According to the 2000 U.S. Census classification, 58.6% of our participants resided in urban areas of Arkansas, and the remaining 41.4% lived in rural areas of Arkansas.

There were several significant differences in the demographic characteristics of those participants that were linked with a U.S. Census Place name when compared to those that could not be linked. Most notable is the difference in rural versus urban residence. On average, those that could not be linked to a Place name resided in a U.S. Census Block Group (CBG) that was characterized as over 92% rural in the 2000 U.S. Census. In contrast, those that were linked to a Place name resided in a CBG that was only 32% rural on average (See Table 3.3.5.2). We found a similar difference when we compared

participants linked to a CWS and validated compared to those linked to a CWS and not validated, though the magnitude of the difference was much smaller. It is likely that many residences out side of a U.S. Census Place name receive their tap water from a private well, and therefore we would not expect to match them to a CWS.

The difference in rural and urban residences could explain why white participants were less likely to be linked than Black participants or participants of other races. In our study population there is a greater proportion of white residents in rural Arkansas compared to urban Arkansas (96% and 83%, respectively; data not shown). The difference in the ability to link rural addresses might account for the difference in the ability to link white participants. Paternal education was also statistically different ($p<0.05$) among those participants linked and those not linked, though the actual difference in average education was less than one year. This may also be related to rural living, assuming that those fathers in rural Arkansas are slightly less educated than fathers in urban Arkansas.

Data on water source (i.e. private well or CWS) was collected for 25% of the participants' residences in this study. Of those questioned, 62% reported not receiving tap water from a private well, 3% reported receiving tap water from a private well, and 35% did not respond to the question, or responded "unknown water source". We do not know what proportion of those that did not respond, or responded "unknown water source" may have received their tap water from a private well. This may be due to the design of the question, which only asked about private well use. Due to the low percentage of participants reporting private well use and the high percentage that did not

respond or did not know their water source, we attempted to link participants' residences with a CWS regardless of their response to the question about the source of their tap water. Had we known the water source for each of the residences, we would have only tried to link those reported to receive tap water from a CWS, and possibly would have improved our linkage success. The fact that the water source of participants geocoded to a location outside of a Census Placename was unknown in many cases is a limitation to this study and may have caused the effectiveness of our linking and validating procedures to appear to be less than it actually was.

The validation success rate did not differ greatly for those addresses linked to one, two, or three utilities (range = 76% - 82%). It was interesting, however, to find that the lowest success rate was found for those addresses linked to three utilities (76%), even though we had the greatest response from those CWS (94%). The highest success rate was found for utilities linked to just one utility (82%). This indicates that while CWS that were clustered were more likely to respond to our survey, the validation was most successful among CWS that were not clustered.

GIS are increasingly being used in epidemiologic studies of environmental exposures and health. We found that our method of linking geocoded addresses to a U.S. Census Place name, and in turn, linking the Place name to a CWS was effective over 81% of the time. The largest limitation was the inability to correctly link rural addresses that had been geocoded, to a U.S. Census Place name. Additionally, linking geocoded addresses to clustered utilities and validating these linkages proved to be an effect way to link

participants to the correct utility. Overall, the use of GIS for linking participants in epidemiologic studies to a Census Place name and then a CWS was feasible in a large, population-based epidemiologic study.

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Table 3.3.5.1: Results of Survey Validation of Linked Residences and Community Water Systems

| | Number of Addresses Sent to CWS for Confirmation of Service | Number of Addresses with Responses from All CWS (%) | Number of Addresses Confirmed as Correctly Linked to CWS | Number of Addresses That Could Not Be Confirmed as Correctly Linked to CWS | Number of Addresses That Were Incomplete or Insufficient for Confirmation by CWS |
|---|---|---|---|---|---|
| Total | 2,513 | 2,152 (85.6) | 1,751 | 314 | 87 |
| 1 CWS | 1,686 | 1,436 (85.2) | 1,182 | 214 | 40 |
| 2 CWS | 613 | 515 (84.0) | 416 | 64 | 35 |
| 3 CWS | 214 | 201 (93.9) | 153 | 36 | 12 |

Table 3.3.5.2 Comparison of Demographic Characteristics between Participants Linked to a U.S. Census Place Name and those Not Linked to a U.S. Census Place Name

| | Not Linked | Linked | P-value |
|---|---|---|---|
| % Cases (N) | 33.9 (373) | 33.7 (845) | 0.9668 |
| % Controls (N) | 66.1 (726) | 66.3 (1663) | 0.9537 |
| AVG Maternal Age (StDev) | 25.1 (5.65) | 25.2 (5.7) | 0.5762 |
| AVG Paternal Age (StDev) | 28.3 (7.0) | 28.3 (6.3) | 0.9624 |
| Maternal Race | | | |
| % White (N) | 90.8 (998) | 80.4 (2016) | **<0.0001** |
| % Black (N) | 8.7 (96) | 18.8 (472) | 0.0615 |
| % Other (N) | 0.5 (5) | 0.8 (20) | 0.9589 |
| Paternal Race | | | |
| % White (N) | 94.9 (1043) | 85.4 (2141) | **<0.0001** |
| % Black (N) | 4.9 (53) | 13.1 (328) | 0.1837 |
| % Other (N) | 0.3 (3) | 1.6 (39) | 0.8327 |
| % Maternal Hispanic (N) | 3.8 (42) | 4.4 (110) | 0.9176 |
| % Maternal Non-Hispanic (N) | 96.2 (1057) | 95.6 (2398) | 0.6063 |
| % Paternal Hispanic (N) | 3.9 (43) | 4.3 (108) | 0.9507 |
| % Paternal Non-Hispanic (N) | 96.1 (1056) | 95.7 (2400) | 0.7593 |
| AVG Maternal Education (StDev) | 12.4 (2.3) | 12.7 (2.3) | 0.0549 |
| % < 12 grade (N) | 19.2 (211) | 19.7 (494) | 0.9203 |
| % 12 grade (N) | 48.3 (531) | 41.7 (1046) | 0.1209 |
| % some college (N) | 27.8 (306) | 32.2 (808) | 0.3737 |
| % post graduate (N) | 4.7 (51) | 6.4 (160) | 0.7700 |
| AVG Paternal Education (StDev) | 12.2 (2.3) | 12.9 (2.4) | **<0.0001** |
| % < 12 grade (N) | 18.9 (208) | 15.6 (391) | 0.5701 |
| % 12 grade (N) | 53.8 (591) | 44.3 (1111) | **0.0343** |
| % some college (N) | 24.3 (267) | 31.5 (790) | 0.1973 |
| % post graduate (N) | 3.0 (33) | 8.6 (216) | 0.3964 |
| % No Smoke (N) | 78.6 (864) | 82.2 (2062) | 0.1755 |
| % Smoke (N) | 21.4 (235) | 17.8 (446) | 0.4895 |
| AVG Cigarettes (StDev) | 2.7 (6.7) | 2.1 (5.7) | 0.0876 |
| AVG Rural* (StDev) | 92.6 (19.3) | 32.2 (40.7) | **<0.0001** |

*Average percent of residences considered rural in the Census Block Group that contains the index address, from 2000 U.S. Census.

Table 3.3.5.3 Comparison of Demographic Characteristics between Participants Linked to a CWS and Validated and those Linked to a CWS and Not Validated

| | Not Validated | Validated | P-value |
|---|---|---|---|
| % Cases (N) | 19.6 (79) | 31.7 (555) | **0.0155** |
| % Controls (N) | 80.4 (322) | 68.3 (1196) | **<0.001** |
| AVG Maternal Age (StDev) | 25.3 (5.3) | 25.4 (5.9) | 0.9888 |
| AVG Paternal Age (StDev) | 28.8 (6.5) | 28.6 (6.5) | 0.9789 |
| Maternal Race | | | |
| % White (N) | 82.7 (332) | 73.3 (1284) | **<0.001** |
| % Black (N) | 14.3 (57) | 26.5 (464) | **0.0295** |
| % Other (N) | 0.3 (1) | 0.2 (3) | 0.9868 |
| Paternal Race | | | |
| % White (N) | 71.1 (285) | 63.9 (1119) | **0.020** |
| % Black (N) | 6.6 (26) | 13.9 (243) | 0.1829 |
| % Other (N) | 0.0 (0) | 0.3 (5) | 0.9024 |
| % Maternal Hispanic (N) | 4.3 (17) | 5.4 (95) | 0.8451 |
| % Maternal Non-Hispanic (N) | 94.0 (377) | 93.8 (1642) | 0.8844 |
| % Paternal Hispanic (N) | 5.0 (20) | 4.4 (77) | 0.9125 |
| % Paternal Non-Hispanic (N) | 73.4 (294) | 73.4 (1285) | 1.000 |
| AVG Maternal Education (StDev) | 12.5 (2.5) | 12.7 (2.5) | 0.9682 |
| % < 12 grade (N) | 19.9 (80) | 15.6 (273) | 0.3951 |
| % 12 grade (N) | 41.2 (165) | 38.7 (678) | 0.5623 |
| % some college (N) | 28.9 (116) | 33.7 (590) | 0.3072 |
| % post graduate (N) | 6.3 (25) | 7.3 (128) | 0.8549 |
| AVG Paternal Education (StDev) | 12.4 (2.7) | 13.0 (2.5) | 0.9052 |
| % < 12 grade (N) | 11.0 (44) | 9.1 (159) | 0.7206 |
| % 12 grade (N) | 37.9 (152) | 31.8 (557) | 0.1717 |
| % some college (N) | 19.3 (77) | 26.2 (197) | 0.1700 |
| % post graduate (N) | 5.6 (22) | 7.9 (138) | 0.6777 |
| % No Smoke (N) | 84.4 (338) | 83.0 (1453) | 0.5304 |
| % Smoke (N) | 15.3 (61) | 16.6 (291) | 0.8018 |
| AVG Cigarettes (StDev) | 10.2 (6.5) | 12.1 (8.3) | 0.6827 |
| AVG Rural* (StDev) | 32.9 (39.8) | 14.8 (28.7) | **0.0088** |

*Average percent of residences considered rural in the Census Block Group that contains the index address, from 2000 U.S. Census.

**Chapter 4**

# 4. Assigning Utility-Based Disinfection By-Product Concentrations to a Critically Important Period of Gestation for a Study of Hypospadias in Arkansas

## 4.1 Introduction

Systematic reviews of the relationship between disinfection by-products (DBPs) and adverse reproductive outcomes consistently identify non-differential misclassification of individual exposure as an important limitation to accurate risk estimation (Reif *et al.*, 1996; Nieuwenhuijsen *et al.*, 2000; Bove *et al.*, 2002; Arbuckle *et al.*, 2002). While the most reliable method of exposure assessment is through personal exposure monitoring, this is rarely feasible in epidemiologic studies due to financial, time, and power constraints (Arbuckle *et al.*, 2002). In most tap water epidemiologic studies the alternative is to consider community water system (CWS) monitoring data in conjunction with data collected on water consumption and use.

Many epidemiologic studies of DBPs have focused on exposure to one class of DBPs, trihalomethanes (THM). Spatial variability in THM concentrations has been reported, with distribution system levels up to two (Rodriguez and Serodes 2001), and three (Sohn *et al.* 2001) times higher than finished water leaving the treatment plant. This level of spatial variability can lead to inaccurate estimates of THM concentrations in the residence of a study participant when utility means are used. When intra-system variation is small, utility means are likely to more accurately represent concentrations at individual residences, even with few measurements (Keegan *et al.* 2001). High spatial

variability can lead to exposure misclassification when CWS means are linked to an individual within the distribution system (Keegan *et al.* 2001; Nuckols *et al.* 2004). These intra-system differences highlight the limitation of using utility-wide average concentrations as a surrogate of maternal exposure to DBPs.

Temporal variability of DBPs is significant in epidemiologic studies because when seasonal fluctuations are large, the estimation of average THM levels over long periods (annual or quarterly THM levels) will be inaccurate (Whitaker *et al.* 2003). With few samples taken in each CWS throughout the year, the reliable estimation of a mean CWS THM level for a short period within one season is difficult. Such estimates are needed in reproductive studies when critical exposure periods for an endpoint are often measured in weeks. For this reason, formal statistical modeling of specific THM levels, such as that described by Dodds *et al.* (1999) has been proposed to improve the reliability and accuracy of estimates of exposure to THM.

The differences in individual water use activities can significantly influence the level of THMs entering the blood stream (Lynberg *et al.* 2001). Household water use (e.g., showering and bathing) can result in significant indoor air concentrations of DBPs (Gordon *et al.* 1998; Wilkes *et al.* 1996) and subsequent DBP exposure through inhalation will vary for different individuals within a household (Wilkes *et al.* 1996). For this reason, exposure assessment should start with a valid history of personal water use that is concurrent with the pertinent exposure period for the outcome under investigation (Barbone *et al.* 2002; Swan and Waller 1998).

In the last few years researchers have developed exposure assessment metrics utilizing monitoring data while taking into consideration spatial variability (Waller *et al.*, 2001; Nuckols *et al.*, 2004; Hinckley *et al.*, 2005), temporal variability (Klotz & Pyrch, 1999; Dodds *et al.,* 1999; Dodds & King, 2001; Golfinopoulos & Arhonditsis, 2002; Whitaker *et al.,* 2003; King *et al.*, 2004), personal tap water consumption (Waller *et al.*, 2001), and an exposure index that takes personal consumption, as well as showering and bathing time into consideration (King *et al.*, 2004). These metrics have been used with an expectation of decreasing non-differential exposure misclassification when compared to the use of quarterly CWS monitoring data alone.

In this study we evaluate and compare exposure assessment classifications and risk estimates for hypospadias due to exposure to total trihalomethanes when using different exposure assessment metrics for a study population in Arkansas.

## 4.2 Methods

### 4.2.1 Participant and Exposure Data

We collected case and control data under the auspices of the Arkansas Reproductive Health Monitoring Study (ARHMS) in the state of Arkansas in conjunction with data from the National Birth Defect Prevention Study (NBDPS). The participants used in this analysis are a subset from a larger population-based case-control study presented in Chapter 5. Cases included males born with secondary and tertiary hypospadias in Arkansas during the study period. We used a GIS procedure described in Chapter 3 to

link cases and controls to specific CWS and measured TTHM concentrations. Participants that could not be linked to CWS monitoring data during their exposure window were excluded from the study. Data from cases (n=40) and controls (n=243) born between January 1, 1998 and December 31, 2002, were linked to 55 different CWS throughout Arkansas. We collected TTHM concentrations reported as part of quarterly CWS monitoring data from these CWS for the same period. We used these measured TTHM concentrations to estimate exposure to TTHM between gestational weeks six and sixteen for each participant.

### 4.2.2 Exposure Assessment Metrics

#### 4.2.2.1 Exposure window concentration from CWS Monitoring Data

We averaged all of the available TTHM concentrations from monitoring data of the CWS to which a case or control was linked during weeks six to sixteen of gestation. We refer to these concentrations as exposure window concentrations. These exposure window concentrations do not take into consideration spatial or temporal variability or personal consumption/water use patterns. We refer to this manner of assessing exposure as the referent metric.

#### 4.2.2.2 Assessing Spatial Variability within a Community Water System

For the purpose of comparison we assessed spatial variability in exposure window concentrations using two different exposure assessment techniques: a weighting metric and a threshold metric. Waller *et al.* (2001) describe a weighting metric that can be used to account for spatial variability within a CWS distribution system. This weighting

metric is designed to minimize the contribution of exposure window concentrations whose DBP measurements varied widely across their CWS (i.e., sampling events with high spatial variability). The metric uses an algorithm to assign a weighting factor between 0.0 and 1.0 to exposure window concentrations such that each observation contributes the value of a weighting variable to the frequency count. The weighting variable value is designed so that the larger the spatial variation, the smaller the weight it contributes to a risk estimate. The equation we used follows:

Weight = 1 – (StDev/ mean TTHM across all samples from the Study Database) (Eq. 1)

Where,
Weight = weighting factor of exposure window concentration, between 0 and 1
StDev = standard deviation of exposure window concentration
Study Database[1] = all samples measured for THM for all CWS in the study area

The threshold metric (Nuckols *et al.*, 2004) is designed to identify exposure window concentrations with low spatial variation within the individual samples collected in each exposure window. Low spatial variation can be defined by satisfying any one of three very specific "threshold" criteria based on algorithms designed to calculate: (1) whether analytical results from all samples in the exposure window are less than a user-specified concentration; and (2) whether the difference between the minimum and maximum concentration is within a user- specified range; and (3) whether the coefficient of variation (CV) (using the log10 exposure window concentration and standard deviation) is less than a user-specified threshold. The latter criterion allows exposure window concentrations with high average TTHM concentrations and relatively large ranges in individual sample TTHM concentrations to be retained in the risk analysis.

[1] In the Waller study, the database was for all samples from 441 locations in 79 utilities over the period 1989-1992

In our study, if any one of the following criteria was satisified, the requirement for low spatial variability was considered to be met: (1) Include only exposure window concentrations comprised of samples with TTHM concentrations <20 μg/L. This decision was based on the published literature in which the 0-20 μg/L TTHM concentration is often used as the referent group and considered to contribute little or no health risks in reproductive studies (Bove *et al.*, 1995; Gallagher *et al.*., 1998; Klotz and Pyrch, 1999). (2) Include only exposure window concentrations in which the range across individual samples in TTHM concentration was $\leq$10 μg/L. We applied this criterion to achieve tightness of fit around the median. Furthermore, Waller *et al.*, (2001) conducted a subset analysis to control for spatial variability in which the range of all samples in an exposure window was less than 20 μg/L and the results from this subset showed a modest increase in the risk estimate for a high-level exposure group. We chose to use half this level as a conservative approach. (3) The coefficient of variation (CV) must be <0.05. This cut-point was determined by examining the relationship between CV and standard deviation for all exposure window concentrations in our database for which measurements of TTHM were greater than 60 μg/L. We observed that exposure window concentrations with a CV less than 0.05 tended to have standard deviations of less than 15 μg/L, while exposure window concentrations with a CV greater than 0.05 tended to have a standard deviation greater than 15 μg/L. We repeated this analysis for all exposure window concentrations for which measurements of TTHM were greater than 80 μg/L, and the observation held.

#### 4.2.2.3 Assessing Temporal Variability Within a Community Water System

In order to take into consideration temporal variability between quarterly averages, we employed a spline regression technique including month and individual distribution system characteristics to estimate daily DBP values (Dodds *et al.*, 1999). This metric, which interpolates between CWS monitoring data concentrations, uses a polynomial function to join temporally adjacent data points with a smoothed line (Greenland, 1998) and allowed us to approximate the daily TTHM concentration in a CWS. Specifically, all of the samples measured at each CWS were entered into a data matrix and a smoothing parameter was assigned. We used SAS (version 9.1; Cary, NC) to manipulate this matrix and create segments through each of the points representing the samples measured during our study period for each CWS. Then the segments were joined together and the smoothing parameter was used to smooth the line. This allowed us to estimate a TTHM concentration for the CWS for any day during our study period for which there was a known, measured concentration before and after. These daily concentrations were averaged for the exposure window of interest (weeks six to sixteen of gestation) for each participant. This method was applied to TTHM for each of the utilities linked with a case and/or control.

#### 4.2.2.4 Integrating Participant-Level Data into a Personal Consumption Index

We used detailed personal consumption data from the water module of the NBDPS questionnaire to estimate exposure to TTHM in tap water via ingestion. These data include the number of glasses of filtered and unfiltered tap water, bottled water, and hot and cold drinks consumed at work and at home reported by each participant. The number

of glasses of unfiltered tap water consumed per day was multiplied by the estimated exposure window concentration during the clinically important exposure period for the CWS linked to the participant's residence during this time period. We refer to this product as the personal consumption index (PCI). The PCI is a measure of dose resulting from ingestion only. The units for the PCI are micrograms of DBP per day.

#### 4.2.2.5 Integrating Participant-Level Data into a Multiple Exposure Index

We employed a Multiple Exposure Index (MEI) using an exposure measure developed previously by King *et al.* (2004) in order to account for exposure to TTHM via the ingestion, inhalation and dermal routes of exposure. The MEI is measured in units of micrograms per day. The index is based on an assumption that one liter of ingested water is equivalent to a five minute shower or to a fifteen minute bath. This assumption is derived from published studies of exposure to THMs via showering and bathing (Kerger *et al.* 2000; Weisel and Jo 1996). The index also suggests a 50% reduction in TTHM intake through cold water-based drinks when use of a carbon filter was indicated, and a reduction of 70% for boiled hot water drinks (Kuo *et al.* 1997; Wu *et al.* 2001). We used these estimates to create an exposure variable based in equivalent eight-ounce glasses of water ingested. We used the product of the number of equivalent eight-ounce glasses per day and the exposure window concentration as the MEI exposure metric for each study participant. For example, a five-minute shower was considered to administer the same dose as ingesting one liter of water. One liter of water was assumed to be equal to four eight-ounce glasses of water. Thus for each five minutes of showering per day, four equivalent eight-ounce glasses were added to the MEI. Similarly, four equivalent eight-

ounce glasses were added to the MEI for each fifteen minutes spent bathing, one equivalent eight-ounce glass was added to the MEI for each glass of unfiltered tap water ingested, 0.5 equivalent eight-ounce glasses were added to the MEI for each glass of filtered tap water ingested, and 0.3 equivalent eight-ounce glasses were added to the MEI for each boiled hot water drink ingested.

### 4.2.3 Comparison of Exposure Assessment Metrics

#### 4.2.3.1 Correlation between Exposure Assessment Metrics

We tested for correlations between each of the different exposure metrics in our study and the referent metric and for variations in the magnitude of the metrics when compared to the referent metric. These alternate metrics included the weighting metric, threshold metric, spline regression, PCI and MEI. We calculated Pearson correlation (r) values for each comparison.

The higher the correlation between an alternate exposure assessment metric and the referent metric, the less of an effect the exposure assessment metric had on changing the exposure assessment for participants.

#### 4.2.3.2 Cross-Classification in Exposure Quintiles of Exposure Assessment Metrics

In order to evaluate the differences in exposure assessment when using the alternate exposure assessment metrics, we evaluated the contribution of the different exposure assessment techniques to the change in the exposure variable by comparing exposure quintiles for each of these metrics to the exposure quintiles established using the referent metric. Pearson correlation (r) coefficients were calculated for these variables on a

continuous exposure scale and a weighted kappa statistic was used to compare the agreement between exposure quintiles. The weighted kappa statistic quantifies the degree of discrepancy between exposure assignments based on the two metrics being compared, and can range from 0 to 1.0. A value of 0 indicates no agreement better than chance and a value of 1.0 indicates perfect agreement between the two exposure methods. For example, participants that are classified into the same exposure quintile using the two different exposure metrics will receive a weight of 1.0. However, if a participant is grouped into two different exposure quintiles when two different exposure metrics are compared and the quintiles are close to one another, the weight will be closer to 1.0, whereas if the quintiles are further away from one another the weight will be closer to 0. The higher the correlation between quintiles of an alternate exposure assessment metric and the quintiles of the referent metric, the less of an effect the exposure assessment metric had on changing the exposure assessment for participants. Additionally, we were able to tell if the exposure assessment metric over- or under-estimated exposure compared to the referent metric.

#### 4.2.3.3 Risk Estimates for Exposure Assessment Metrics

Using unconditional logistic regression, we calculated risk estimates (odds ratios (ORs)) for hypospadias as a function of each of the exposure assessment metrics as the independent variable. We also determined if a significant difference in the risk estimate was observed across the different metrics. We computed ORs using continuous and categorical exposure variables for all of the exposure assessment metrics.

#### 4.2.3.4 Sensitivity Analysis of Multiple Exposure Index

We adjusted the correlations between dose from ingesting one liter of water and doses from a fifteen-minute bath or a five-minute shower reported by King *et al.* (2004) to evaluate what effect, if any, this might have on the risk estimates. Whereas King and colleagues assumed an equivalency of absorbed dose such that one liter of ingested water was equivalent to a five minute shower and a fifteen minute bath, we calculated risk estimates assuming that 0.5 liters, two liters and five liters of ingested water was equivalent to a five minute shower and a fifteen minute bath and compared the risk estimates to see how a range of these correlations might affect our results.

## 4.3 Results

### 4.3.1 Percent Contribution to the MEI

In creating the MEI, we found that the mean daily exposure to tap water through consumption, showering, and bathing was an absorbed dose equivalent to the ingestion of 13 eight-ounce glasses of unfiltered tap water on average, ranging from 0 to over 77 (Table 4.3.1.1). In our study water consumption (ingestion) accounted for 18% of exposure to tap water according to the MEI, while showering and bathing (dermal and inhalation exposure) accounted for an average of 63% and 15%, respectively (Table 4.3.1.1).

### 4.3.2 Correlation between Exposure Assessment Metrics

Correlations between the different exposure assessment metrics are presented in Table 4.3.2.1. Pearson correlation coefficients (r) ranged from 0.36 to 0.97. The referent metric was correlated highly with daily estimates from the threshold metric (r=1.0) and the spline regression (r=0.97), somewhat correlated with the weighting metric and the MEI (r=0.79 and r=0.63, respectively) and correlated poorly with the PCI (r=0.36).

### 4.3.3 Cross-Classification in Exposure Quintiles of Exposure Assessment Metrics

In Tables 4.3.3.1a-e we present the results of the analyses in which we compared the exposure quintiles for subjects based on exposure window concentrations alone with the weighting metric, threshold metric, spline regression estimates, the PCI and the MEI. We present agreement of classification into the same quintile with both exposure assessment techniques on the diagonal of the table. These values ranged from 100% (threshold metric) to 25% (PCI). Less than half of the subjects were classified in the same quintile for both exposure measures when we examined the weighting metric (38%), the PCI (25%), and the MEI (43%). The spline regression yielded a 76% agreement. When we examined the direction of shift in exposure quintiles for the weighting metric we found that 33% moved up one quintile (i.e. weighting factor exposure measure was higher than monitoring data alone), while 11% moved down one quintile (i.e. weighting factor exposure measure was lower than monitoring data alone). When examining the PCI quintiles we found that 13% moved up one quintile and 18% moved up two quintiles, while 21% moved down one quintile and 9% moved down two quintiles. We observed the shift in direction to be balanced for the remaining exposure assessment metrics. The weighted kappa values representing agreement beyond chance ranged from 0.14 (PCI) to 1.00 (threshold metric). The weighted kappa value for the PCI when compared to

exposure window concentration was below 0.20, indicating that the degree of agreement beyond chance is slight (McGinn *et al.*, 2004). With weighted kappa values of 0.49 and 0.50 the degree of agreement beyond chance for the weighting metric and the MEI, respectively, with the referent metric can be characterized as moderate (McGinn *et al.*, 2004). The weighted kappa value was 0.82 for the spline regression and the referent metric, indicating an almost perfect degree of agreement beyond chance (McGinn *et al.*, 2004). The weighted kappa value was 1.00 for the threshold metric and the referent metric, indicating perfect agreement of the two exposure assessment techniques.

### 4.3.4 Risk Estimates for Exposure Assessment Metrics

Risk estimates (ORs) were calculated using each of the different exposure assessment metrics with exposure on a continuous scale. The results of these analyses are presented in Table 4.3.4.1. An OR could not be calculated when using the threshold metric due to small numbers. All other ORs were close to 1.00 and were not significantly different from one another.

In Table 4.3.4.2 we present the results of the risk estimate analyses for the different exposure assessment metrics with exposure on a categorical scale, divided by quartiles. ORs could not be calculated when using the threshold metric due to small numbers. The highest ORs were calculated when the third quartile (Q3) was compared to the referent quartile (Q1) for the referent, weighting and MEI metrics. The highest OR for the PCI metric was calculated when the second quartile (Q2) was compared to the referent quartile (Q1). The highest OR for the spline regression metric was calculated when the

fourth quartile (Q4) was compared to the referent quartile (Q1). Only the results of the spline regression analyses suggested a dose-response relationship between exposure to TTHM and hypospadias, though none of these risk estimates were statistically significant at the 95% confidence level.

We had sufficient data to include 202 study participants when using the referent exposure metric. When we used the weighting metric, we only had sufficient data to include 110 study participants, a reduction in our sample size of 45%. When we used the threshold metric, we only had sufficient data to include 16 study participants, a reduction in our sample size of 92%. We were able to increase our sample size by 40% to 282 study participants by using the spline regression metric. The sample size was reduced by 2% (N=198) and 3% (N=195) when using the PCI and MEI metrics, respectively.

### 4.3.5 Sensitivity Analysis of the Multiple Exposure Index

In Table 4.3.5.1 we present the results of a sensitivity analysis that examined how modifying the MEI might affect risk estimation. When we calculated risk estimates assuming that internal dose of a five minute shower or a fifteen minute bath were equivalent to ingestion of 0.5 liters, two liters, and five liters of water. We did not find any statistically significant differences in the risk estimation.

## 4.4 Discussion

Epidemiologic studies of DBPs and reproductive outcomes have been hampered by misclassification of exposure (Hinckley et al. 2005). We examined five metrics that have been proposed to decrease this exposure misclassification and compared them to the

commonly utilized method of linking quarterly sampling data collected at multiple CWS sampling sites to meet regulatory requirements to either the first or third trimester of gestation. We used three different methods for comparing the alternate exposure techniques to the referent technique. These included Pearson correlation coefficients, cross-classification of exposure quintiles and estimates of risk in the form of ORs. Exposure estimates made using the PCI metric were consistently the most different from those made using the referent metric in the three comparisons. Conversely, exposure estimates made using the spline regression metric were consistently the least different from those made using the referent metric in the three comparisons. Finally, the use of the spline regression technique allowed us to estimate exposure for a greater number of study participants, thus increasing our sample size and, presumably, our statistical power. Our sample size was reduced when the weighting, threshold, PCI and MEI metrics were utilized, however the magnitude of these reductions varied immensely.

It is not surprising that exposure estimates made using the PCI metric differed the most from those made using the referent metric in the comparisons. The PCI metric incorporates individual-level data on water consumption at home and at work, and allows for the estimation of TTHM dose in the exposure assessment. Recent studies have suggested that individual water use data in conjunction with quarterly sampling data collected at multiple sampling sites to meet regulatory requirements may aid in diminishing a portion of the exposure misclassification introduced when compared to the use of quarterly sampling data alone (Backer et al. 2000; Barbone et al. 2002; Corley et al. 2000; Gordon 1998; Kaur et al. 2004; Kerger et al. 2000; King et al. 2004; Lynberg et

al. 2001; Nuckols et al. 2005; Waller et al. 2001; Weisel 1992, 1999; Zender et al. 2001). It is notable that the PCI metric differed more from the referent metric than did the MEI metric. Both of these metrics allow for the estimation of TTHM dose in the exposure assessment. But because the MEI metric included exposures to TTHM via the ingestion, inhalation, and dermal routes and the fact that our analyses demonstrated that a larger percentage of the estimated dose is received from showering and bathing than consumption (78% vs. 18%), we would have expected exposure assessments made using the MEI metric to differ more from those made using the referent metric than those using the PCI metric.

In this study we found that, on average, 78% of potential exposure to TTHMs calculated using the MEI was due to exposure other than ingestion, and that 63% of potential exposure to DBPs came from showering alone. These results demonstrate the necessity of gathering water use and behavior data in order to reduce exposure misclassification in future epidemiologic studies concerning DBPs and adverse reproductive outcomes. Furthermore, the range in exposure values calculated by the MEI (0 – 77 equivalent glasses, mean=13, standard deviation=11.5; see Table 1) reveals the degree of variability that showering and bathing can introduce in addition to exposure by ingestion alone.

The spline regression metric varied the least from the referent metric in the three comparisons. One possible explanation for this result is that temporal variability of TTHM concentrations was small during our ten week exposure windows. Perhaps if the exposure windows had been longer, temporal variability may have increased and we

would have seen more of a difference between exposure estimates made using the spline regression metric and the referent metric. Another possible explanation for this result is that quarterly measurements are adequate for estimating exposure to TTHMs and a metric accounting for temporal variability of TTHMs in CWS systems is unnecessary. Water temperatures and raw water quality would be expected to vary according to season, and introduce temporal variability. Higher water temperatures favor THM production; thus, it is expected that THM levels will tend to be higher during summer months, lower in winter months, and intermediate during spring and autumn (Toroz & Uyak, 2005; Whitaker *et al.*, 2003; Golfinopoulos & Arhonditsis, 2002). Still, models incorporating year, season, or month showed that none of these explained more than 3% of the total variation in TTHMs (Keegan *et al.,* 2001). If this is indeed the case, it might explain why the spline regression and referent metrics performed similarly in estimating exposure. Finally, it is possible that temporal variability of DBP concentrations was present in our study area, but that the spline regression metric is not an appropriate metric for detecting and controlling for it. Validation of the spline regression metric in needed in future epidemiologic studies. The magnitude of temporal variability present in DBP concentrations is likely to change drastically based on the geographic location, water source, and hydrogeologic characteristics of the CWS in the study. These factors need to be considered when determining if temporal variability of DBP concentrations need to be accounted for in epidemiologic studies.

Exposure estimates made using the weighting and threshold metrics, accounting for spatial variability of TTHMs, differed from those made using the referent metric and

from each other. Greater detail in CWS monitoring data is required in order for the weighting and threshold metrics to be applied. CWS collect quarterly sampling data at multiple sampling sites to meet regulatory requirements. If the CWS only reports the average TTHM concentration from these multiple sampling sites the weighting and threshold metrics cannot be calculated because each requires a measure of the standard deviation of this average. Also, it should be noted that the threshold metric does not change the magnitude of the exposure estimates made using the referent metric, but limits the inclusion of study participants to those linked to a CWS where spatial variability has been deemed to be low using the criteria of the threshold metric. We only had the necessary data to assess exposure using the weighting and threshold metrics for 110 study participants. Of these, only 16 participants were linked to exposure data that met the threshold metric's criteria for low spatial variability (1 case and 15 controls). The exposure data for these 16 participants came from eight different CWS. There is a potential for the introduction of bias to the study if the study participants linked to these eight CWS differ in some manner from the study participants linked to the 263 CWS throughout Arkansas. Because exposure estimates were available for only one case and due to the potential for the introduction of bias to the study, risk estimates were not calculated using the threshold metric. While the threshold metric has proved effective in other studies (Nuckols *et al.*, 2004), our sample size was not large enough to support its use.

While the approaches employed to account for spatial variability of TTHMs within a distribution system and approaches that included individual-level data on water

consumption and use produced results different from those yielded by using exposure window concentrations alone, we can not say which of these is closest to estimating the true level of exposure. Additionally, the each of these techniques for minimizing exposure misclassification requires validation. Our results support work by others that have suggested the importance of water use data and the incorporation of exposure from the dermal and inhalation routes as well as due to ingestion in epidemiologic studies of DBPs and adverse reproductive outcomes (Backer et al. 2000; Barbone et al. 2002; Corley et al. 2000; Gordon et al. 1998; Kaur et al. 2004; Kerger et al. 2000; King et al. 2004; Lynberg et al. 2001; Nuckols et al. 2005; Waller et al. 2001; Weisel et al. 1992, 1999; Zender et al. 2001).

The biologically effective dose associated with different routes of exposure is likely to vary for specific volatile DBPs and health effects of interest (King et al. 2004). Additionally, the route of exposure affects the rates of metabolism and therefore the compound's potential toxicity (Weisel and Jo 1996). In their 2000 study, Backer *et al.* found that most of the ingested dose of TTHM was excreted or metabolized within ten minutes rather than absorbed into the blood. By contrast, they found that the inhalation and dermal routes of exposure resulted in a more direct distribution to the blood and higher concentrations of TTHM in the blood. Water-use behaviors or activities are also likely to have an effect on the biologically effective dose. Kerger *et al.* (2000) demonstrated that average airborne concentrations of THM are about three times higher for a typical shower compared to a typical bath. Also, many water-use behaviors, including hand dish-washing, exhibit a high degree of inter-participant variation in blood

THM concentrations (Nuckols *et al.*, 2005). Still, exposure indices such as the MEI should be exercised and interpreted with extreme caution. The MEI was developed using the best available correlations to estimate exposure via the ingestion, inhalation and dermal routes, though this exposure index has yet to be validated. Additionally, the MEI did not include other water use behaviors for which data was collected by the NBDPS water module. These data include the frequency of bathing children, bathing pets, washing dishes by hand or with a dishwasher, washing clothes by hand, sauna/hot tub use, and swimming. We are unable to determine what effect, if any, these variables may have exhibited had they been included in the MEI. It is likely that they would have increased the estimated doses to TTHM as well as inter-participant variation (Nuckols et al., 2005).

A limitation of this study was the sample size. We were able to link 27 cases and 175 controls to TTHM concentrations during their exposure window using the referent metric. While this small sample size limited power in the study, it is important to remember that the objective of this study was to compare methods of exposure assessment, and not to assess risk. We did use risk estimates in our comparison of exposure assessment techniques, and due to the small sample size there were small numbers of cases in several of the analyses when a categorical exposure scale was used The limited power led to risk estimates that were accompanied by wide confidence intervals. These data were used to compare methods of exposure assessment and the only inferences that should be drawn from the study characterize how the methods relate to one another. Because the sample

size was small and statistical power was low, comparisons made using these risk estimates should be interpreted cautiously.

We conducted a sensitivity analysis to examine the effect of altering the assumed equivalency of internal dose for one liter of ingested water and a five minute shower or a fifteen minute bath. When we calculated risk estimates assuming that 0.5 liters, two liters and five liters of ingested water were equivalent to a five minute shower or a fifteen minute bath we did not find any statistically significant differences in the risk estimates. This might indicate that because of our small sample size we did not have sufficient statistical power to detect a difference in risk estimates calculated using the different dose equivalencies. This sensitivity analysis should be repeated with a larger sample population with sufficient statistical power to see how changes in these dose equivalencies could affect the outcome of epidemiologic studies.

A variety of approaches to improve exposure assessment in epidemiologic studies of DBPs and adverse reproductive outcomes have been proposed in recent years (Dodds et al. 1999; Dodds & King, 2001; Golfinopoulos & Arhonditsis, 2002; Hinckley et al. 2005; Klotz and Pyrch 1999; King et al. 2004; Nuckols et al. 2004, 2005; Waller et al. 2001; Whitaker et al. 2003). While personal exposure monitoring via the use of biomarkers in blood, urine, or exhaled breath would be optimal, these methods have yet to be validated, would require a prospective cohort study, and often prove too costly for large epidemiologic studies. Recent studies have suggested that data on water consumption and/or use in conjunction with quarterly sampling data collected at multiple sampling

sites to meet regulatory requirements may aid in diminishing a portion of the exposure misclassification introduced when compared to this quarterly sampling data alone (Backer et al. 2000; Barbone et al. 2002; Corley et al. 2000; Gordon 1998; Kaur et al. 2004; Kerger et al. 2000; King et al. 2004; Lynberg et al. 2001; Nuckols et al. 2005; Waller et al. 2001; Weisel 1992, 1999; Zender et al. 2001). The comparison of five exposure assessment metrics in this study supports this finding.

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Table 4.3.1.1: Percent contribution of water use behaviors to Multiple Exposure Index

| | MEI (equivalent glasses) | Water Consumption (%) | Minutes Showering (%) | Minutes Bathing (%) |
|---|---|---|---|---|
| Mean | 13 | 18 | 63 | 15 |
| Std Dev | 12 | 2 | 26 | 21 |
| | | | | |
| Minimum | 0 | 0 | 0 | 0 |
| Quartile 1 | 6 | 7 | 49 | 1 |
| Median | 12 | 19 | 67 | 6 |
| Quartile 3 | 18 | 33 | 84 | 20 |
| Maximum | 77 | 72 | 100 | 100 |

Table 4.3.2.1: Pearson correlation coefficients (r) for comparison of referent metric with five alternate exposure assessment metrics

| | N | Pearson Correlation (r) |
|---|---|---|
| Weighting Metric | 110 | 0.79 |
| Threshold Metric | 16 | 1.00 |
| Spline Regression | 110 | 0.97 |
| Personal Consumption Index | 80 | 0.36 |
| Multiple Exposure Index | 80 | 0.63 |

Table 4.3.3.1a: Comparison of exposure classification using classification in exposure quintiles of TTHM level from quarterly monitoring data with and without the weighting metric (N=110).

| | TTHM Level with Weighting Metric (% participants by quintile) | | | | |
|---|---|---|---|---|---|
| TTHM Level from Monitoring Data (quintile) | 1 | 2 | 3 | 4 | 5 |
| 1 | **13** | 7 | 0 | 0 | 0 |
| 2 | 2 | **5** | 10 | 2 | 0 |
| 3 | 5 | 3 | **5** | 6 | 2 |
| 4 | 0 | 4 | 1 | **6** | 9 |
| 5 | 0 | 1 | 5 | 5 | **9** |

Weighted Kappa= 0.49 (95% CI 0.39-0.59). Percent Agreement= 38.2%.
Results in **BOLD** represent percent agreement between the two exposure assessment techniques for the quintile

Table 4.3.3.1b: Comparison of exposure classification using classification in exposure quintiles of TTHM level from quarterly monitoring data with and without the threshold metric. (N=16)

| | TTHM Level with Threshold Metric (% participants by quintile) | | | | |
|---|---|---|---|---|---|
| TTHM Level from Monitoring Data (quintile) | 1 | 2 | 3 | 4 | 5 |
| 1 | **3** | 0 | 0 | 0 | 0 |
| 2 | 0 | **3** | 0 | 0 | 0 |
| 3 | 0 | 0 | **4** | 0 | 0 |
| 4 | 0 | 0 | 0 | **3** | 5 |
| 5 | 0 | 0 | 0 | 0 | **3** |

Weighted Kappa= 1.00; Percent Agreement= 100.0%.
Results in **BOLD** represent percent agreement between the two exposure assessment techniques for the quintile

Table 4.3.3.1c: Comparison of exposure classification using classification in exposure quintiles of TTHM level from quarterly monitoring data with and without the daily estimates from spline regression. (N=110)

| | TTHM Level with Daily Estimates (% participants by quintile) | | | | |
|---|---|---|---|---|---|
| TTHM Level from Monitoring Data (quintile) | 1 | 2 | 3 | 4 | 5 |
| 1 | **15** | 5 | 0 | 0 | 0 |
| 2 | 4 | **14** | 2 | 0 | 0 |
| 3 | 0 | 2 | **15** | 4 | 0 |
| 4 | 1 | 0 | 3 | **13** | 4 |
| 5 | 0 | 0 | 0 | 4 | **16** |

Weighted Kappa= 0.82 (95% CI 0.76-0.89). Percent Agreement= 73.6
Results in **BOLD** represent percent agreement between the two exposure assessment techniques for the quintile

Table 4.3.3.1d: Comparison of exposure classification using classification in exposure quintiles of TTHM level from quarterly monitoring data with and without the glasses of unfiltered tap water per day. (N=80)

| | TTHM Level * glasses per day (% participants by quintile) | | | | |
|---|---|---|---|---|---|
| TTHM Level from Monitoring Data (quintile) | 1 | 2 | 3 | 4 | 5 |
| 1 | **5** | 4 | 8 | 4 | 1 |
| 2 | 4 | **4** | 4 | 5 | 3 |
| 3 | 4 | 9 | **3** | 3 | 5 |
| 4 | 6 | 4 | 5 | **5** | 3 |
| 5 | 1 | 0 | 1 | 4 | **9** |

Weighted Kappa= 0.14 (95% CI -0.01-0.31). Percent Agreement= 25.0%.
Results in **BOLD** represent percent agreement between the two exposure assessment techniques for the quintile

Table 4.3.3.1e: Comparison of exposure classification using classification in exposure quintiles of TTHM level from quarterly monitoring data with and without the Multiple Exposure Index. (N=80)

| | TTHM Level * MEI (% participants by quintile) | | | | |
|---|---|---|---|---|---|
| TTHM Level from Monitoring Data (quintile) | 1 | 2 | 3 | 4 | 5 |
| 1 | **13** | 5 | 4 | 0 | 0 |
| 2 | 5 | **4** | 8 | 1 | 1 |
| 3 | 3 | 5 | **5** | 6 | 4 |
| 4 | 0 | 5 | 4 | **10** | 4 |
| 5 | 0 | 1 | 0 | 3 | **11** |

Weighted Kappa= 0.50 (95% CI 0.37-0.63). Percent Agreement= 42.5%.
Results in **BOLD** represent percent agreement between the two exposure assessment techniques for the quintile

Table 4.3.4.1: Risk estimates for TTHM concnetrations from monitoring data with referent and five alternate exposure assessment techniques on a continuous scale

| Exposure Assessment Technique | Unit Increase in TTHM | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|---|
| Referent | 20.0 µg/L | 202 (27) | 1.00 | 0.70-1.42 |
| Weighting Metric | 20.0 µg/L | 110 (16) | 0.92 | 0.51-1.66 |
| Threshold Metric | 20.0 µg/L | 16 (1) | NA | NA |
| Spline Regression | 20.0 µg/L | 267 (38) | 1.01 | 0.74-1.39 |
| PCI | 20.0 µg/Day | 173 (25) | 1.09 | 0.85-1.39 |
| MEI | 20.0 µg/Day | 170 (25) | 1.06 | 1.00-1.12 |

Table 4.3.4.2: Risk estimates for TTHM concentrations from monitoring data with referent and five alternate exposure assessment techniques on a categorical scale by quartiles (Q)

| Exposure Metric | Estimated TTHM Level | N (Cases) | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|---|
| Referent | Q1 (0-28 μg/L) | 50 (7) | 1.0 | Referent |
| | Q2 (28-42 μg/L) | 51 (7) | 0.98 | 0.32-3.02 |
| | Q3 (42-56 μg/L) | 49 (8) | 1.20 | 0.40-3.60 |
| | Q4 (>56 μg/L) | 52 (5) | 0.65 | 0.19-2.21 |
| Weighting Metric | Q1 (0-28 μg/L) | 18 (3) | 1.0 | Referent |
| | Q2 (28-42 μg/L) | 28 (4) | 0.87 | 0.17-4.47 |
| | Q3 (42-56 μg/L) | 31 (6) | 1.60 | 0.34-7.44 |
| | Q4 (>56 μg/L) | 33 (3) | 0.73 | 0.14-3.89 |
| Threshold Metric | Q1 (0-28 μg/L) | 5 (0) | 1.0 | Referent |
| | Q2 (28-42 μg/L) | 5 (0) | NA | NA |
| | Q3 (42-56 μg/L) | 3 (0) | NA | NA |
| | Q4 (>56 μg/L) | 3 (1) | NA | NA |
| Spline Regression | Q1 (0-28 μg/L) | 77 (12) | 1.0 | Referent |
| | Q2 (28-42 μg/L) | 71 (8) | 0.69 | 0.26-1.80 |
| | Q3 (42-54 μg/L) | 68 (9) | 0.83 | 0.33-2.10 |
| | Q4 (>54 μg/L) | 66 (11) | 1.08 | 0.44-2.65 |
| PCI | Q1 (No Exposure[†]) | 103 (10) | 1.0 | Referent |
| | Q2 (>0-22 μg/Day) | 29 (6) | 2.43 | 0.80-7.36 |
| | Q3 (22-50 μg/Day) | 31 (5) | 1.79 | 0.56-5.70 |
| | Q4 (>50 μg/Day) | 35 (4) | 1.20 | 0.35-4.10 |
| MEI | Q1 (No Exposure[±]) | 62 (5) | 1.0 | Referent |
| | Q2 (>0 – 107 μg/Day) | 38 (6) | 2.14 | 0.60-7.56 |
| | Q3 (107-173 μg/Day) | 48 (8) | 2.28 | 0.70-7.48 |
| | Q4 (>173 μg/Day) | 47 (6) | 1.67 | 0.48-5.84 |

[†] Participants reported consuming only bottled water or average TTHM concentration was below detection limit during exposure window

[±] Average TTHM concentration was below detection limit during exposure window

Table 4.3.5.1: Sensitivity analysis of multiple exposure index: estimating risk on a categorical scale by tertiles (T) assuming that internal dose equivalent for a 5-minute shower and a 15-minute bath vary between 0.5 liters and 5 liters ingested tap water (N=274)

| | 1 Liter | | 0.5 Liters | | 2 Liters | | 5 Liters | |
|---|---|---|---|---|---|---|---|---|
| TTHM Dose* (μg/Day) | N Cases | OR (95% CI) (compared to TTHM<20 μg/L Category) | N Cases | OR (95% CI) (compared to TTHM<20 μg/L Category) | N Cases | OR (95% CI) (compared to TTHM<20 μg/L Category) | N Cases | OR (95% CI) (compared to TTHM<20 μg/L Category) |
| No Exposure | 7 | 1.0 (1.0) | 7 | 1.0 (1.0) | 7 | 1.0 (1.0) | 7 | 1.0 (1.0) |
| T1 | 9 | 1.97 (0.69-5.61) | 11 | 2.40 (0.87-6.61) | 10 | 2.19 (0.78-6.11) | 8 | 1.71 (0.59-5.02) |
| T2 | 10 | 2.06 (0.74-5.76) | 8 | 1.65 (0.57-4.82) | 11 | 2.31 (0.84-6.35) | 13 | 2.68$^{\pm}$ (1.01-7.16) |
| T3 | 12 | 2.52 (0.93-6.83) | 11 | 2.31 (0.84-6.35) | 9 | 1.86 (0.65-5.29) | 9 | 1.93 (0.68-5.50) |

*Product of 8-ounce equivalent glasses from ingestion, showering and bathing and TTHM concentration during exposure window

$^{\pm}$Statistically significant at the 95% confidence level

## Chapter 5

## 5. Maternal Exposure to Disinfection By-Products during Gestation and Hypospadias among Infants

### 5.1 Introduction

Chlorine used to disinfect water reacts with natural organic matter in surface waters, resulting in the formation of a complex mixture of chemicals including trihalomethanes (THM) and haloacetic acids (HAA). Collectively, these compounds are referred to as disinfection by-products (DBPs). THMs consist of four individual species, chloroform, bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform. These four species are collectively referred to as total trihalomethanes (TTHM). HAAs include nine substances but the five most common are dibromoacetic acid (DBAA), dichloroacetic acid (DCAA), monobromoacetic acid (MBAA), monochloroacetic acid (MCAA), and trichloroacetic acid (TCAA). These five species of HAAs are often referred to as HAA5. Human epidemiologic studies have offered evidence of a positive relationship between DBP concentrations and the risk of certain cancers (McGeehin *et al.*, 1993; King and Marrett, 1996; Koivusalo *et al.*, 1997; Doyle *et al.*, 1997; Cantor *et al.*, 1998; Yang *et al.*, 1998; King *et al.*, 2000a) and adverse reproductive outcomes, including neural tube, cardiac, respiratory system, urinary tract and oral cleft defects (Shaw *et al.*, 1991; Aschengrau *et al.*, 1992; Bove *et al.*, 1995; Magnus *et al.*, 1999; Dodds *et al.*, 1999; Klotz & Pyrch, 1999; Dodds & King, 2001; Cedergren *et al.*, 2002; Hwang *et al.*, 2002; Shaw *et al.*, 2003).

Birth defects are the leading cause of infant mortality in the United States (Hoyert *et al.*, 2001), affecting 1 of every 33 live births (Araneta *et al.*, 2003). While many important risk factors have been identified, the underlying etiology of two-thirds of all birth defects remains unclear (Rasmussen *et al.*, 2003; Araneta *et al.*, 2003). For the past decade there has been an increasing interest in the role of DBPs in the etiology of these defects (Aschengrau *et al.*, 1993; Bove *et al.*, 1995; Dodds *et al.*, 1999; Klotz & Pyrch, 1999; Magnus *et al.*, 1999; Dodds & King, 2001; Cedergren *et al.*, 2002; Hwang *et al.*, 2002; Shaw *et al.*, 2003). These studies have examined the relationship between exposure to DBPs and respiratory tract, oral cleft, cardiac, central nervous system, and urinary tract defects.

There is considerable evidence from both toxicological studies in animals and human epidemiology studies, to suggest that exposure to DBPs may be associated with genitourinary birth defects. Animal studies have consistently demonstrated an association between exposure to HAAs and adverse effects in the male reproductive system (Linder *et al.*, 1994a, Linder *et al.*, 1994b, Linder *et al.*, 1995, Linder *et al.*, 1997a; Linder *et al.*, 1997b, Veeramachaneni *et al.*, 2000, Christian *et al.*, 2002). Acute exposures to DBAA, DCAA and BCAA have resulted in testicular toxicity (Linder *et al.*, 1994a), acute spermatotoxicity (Linder *et al.*, 1994b), impaired reproductive competence and sperm quality (Linder *et al.*, 1995), histopathologic changes in testis and epididymis (Linder *et al.*, 1997b), impaired sexual function and fertility (Veeramachaneni *et al.*, 2000), altered sperm production and epididymal tubule changes (Christian *et al.*, 2002), delayed spermiation and distorted sperm motility and morphology (Linder *et al.*, 1997a)

and transient sub-fertility (Luft *et al.*, 2000) in animal studies. In contrast, sub-chronic, low-dose exposure to DBAA, has not been found to affect daily sperm production, testicular sperm counts, epididymal sperm reserves, or morphology of seminiferous epithelium (Weber, *et al.*, 2006).

Human epidemiologic studies have offered evidence of a positive relationship between DBP concentrations and the risk of genitourinary birth defects. Aschengrau *et al.*, (1993) found that pregnant women using chlorinated surface water had an increased risk of giving birth to children with urinary tract defects, compared with women that used chloraminated water (OR = 4.1; 95% CI 1.2-14.1). In 1999, Magnus *et al.*, found an odds ratio of 1.99 (95% CI: 1.10-3.57) in a case-control study that evaluated exposure to DBPs and risk of urinary tract defects using water color and chlorination status in the exposure assessment. Hwang *et al.*, (2002) found a slightly increased, though not statistically significant, risk of urinary tract defects, with an adjusted odds ratio of 1.61 (95% CI: 0.94-2.76) for the medium-exposure category (chlorination used as method of disinfection and water color 10-19.9 mg Pt/L) and 1.35 (95% CI: (0.65-2.80) for the high exposure category (chlorination used as method of disinfection and water color ≥20 mg Pt/L) categories. The odds ratio was 1.46 (95% CI 1.00-2.13) when the medium and high exposure categories were combined.

Hypospadias is one of the most common congenital anomalies in the United States, occurring in approximately 1 in 250 newborns (Paulozzi *et al.*, 1997). Hypospadias is a birth defect in which the urethral opening occurs on the ventral side of the penis, as a

result of abnormal urethral closure at approximately week eight to week fourteen of gestation, and can be classified as primary, secondary or tertiary based on the location of the urethral opening (See Figure 5.1.1). Although understanding of the development of the genitourinary system has improved in recent years, and a variety of mechanisms involving endocrine disruptors and genetic impairment have been proposed for hypospadias, their actual contribution to hypospadias etiology remains elusive (Paulozzi, 1999; Moller & Weidner, 1999).

In this study we evaluated the risk associated with exposure to two groups of DBPs as well as individual species of DBPs during a specific period of gestation and giving birth to an infant with hypospadias. These analyses included participants from the Arkansas Reproductive Health Monitoring System. In a sub-set analysis, we examined the effect of incorporating data on water use behaviors to approximate dose and the estimation of risk in epidemiologic studies of DBPs and hypospadias.

## 5.2 Methods

### 5.2.1 ARHMS Subjects

We conducted a population-based case-control study in Arkansas. Cases were obtained using the Arkansas Reproductive Health Monitoring System (ARHMS). ARHMS is one of the oldest active birth defects surveillance systems in the United States. ARHMS monitors and collects data on birth defects throughout the state. As an active health surveillance system, ARHMS Health Information Specialists visit 83 hospitals in Arkansas and regional hospitals that provide obstetrical or pediatric care for case identification and abstraction. All AHRMS cases were abstracted from hospital records

and only cases diagnosed by physicians were included in the registry. Cases in our study included all infants born, fetal deaths or terminations between January 1, 1998 and December 31, 2002 diagnosed with primary, secondary and tertiary hypospadias (ICD 9 codes 752.600-752.627). We obtained information for 651 hypospadias cases born during our study period from ARHMS.

We obtained a database of birth certificate records for all births in Arkansas during our study period from the Arkansas Center for Health Statistics at the Arkansas Department of Health (ADH). Two controls without a congenital malformation diagnosis were selected randomly from the birth certificate records for each case. Controls were frequency matched with cases based on race and gender. We collected birth certificate information for 1,277 controls from ADH.

### 5.2.2 Subjects in NBDPS Analyses

Sub-set analyses were conducted using data for ARHMS subjects that also participated in the National Birth Defects Prevention Study (NBDPS). Initiated in 1997, the NBDPS is a case-control study of birth defects risk factors; it is being conducted in eight geographic areas across the United States, including Arkansas. The NBDPS provides a unique and unprecedented opportunity to study the relationship between birth defects and DBPs. Methods for assessing exposure to DBPs in the NBDPS included obtaining information on both personal water use habits (through a telephone interview) and tap water characteristics. To obtain information on personal water use, a water module was developed for inclusion as an integral part of the NBDPS computer assisted telephone interview (CATI). All NBDPS participants were asked about water consumption (at

home and at work) and water use (bathing, swimming, household habits). At least 100 NBDPS controls were randomly selected from birth certificates without major malformations and within Arkansas each year between 1998 and 2002. We analyzed data for 114 secondary and tertiary hypospadias cases and each of the 628 population controls enrolled in the NBDPS during our study period. All of the NBDPS cases are included as cases in the ARHMS analyses. While derived from the same source population and selected in similar manners, the controls for the NBDPS and ARHMS analyses are not the same.

### 5.2.3 Exposure Data And Exposure Assignment

DBP concentrations reported as part of quarterly community water system (CWS) monitoring data were collected from 263 CWS throughout Arkansas during our study period. The spline regression technique described in Chapter 4 was used to estimate daily DBP values for TTHM, HAA5, DBAA, DCAA, MBAA, MCAA, TCAA, and bromochloroacetic acid (BCAA) during the study period. This method interpolates between quarterly DBP averages, using a polynomial function to join temporally adjacent data points with a smoothed line (Greenland, 1998). Daily DBP concentrations were estimated from this smoothed line and these estimates were averaged over the defined exposure window of interest to approximate exposure to DBPs.

Next, the procedures outlined in Chapter 3 were used to geocode each participant's residences and link the residences to the CWS from which they were most likely to receive their tap water. The geocoding process included running an input file containing

the 3,886 addresses through an initial batch match process to automatically link all records with clean addresses to latitudinal and longitudinal coordinates. These coordinates were used to link residences with U.S. Census Place names. The CWS names for Arkansas were compared to the list of U.S. Census Place names. Shared Place names and CWS names were identified and residences were linked to one or more CWS. For cases and controls in the ARHMS analyses, the residence at date of birth was used in the linking procedure and for NBDPS cases and controls the residence at the date of conception was used in the linking procedure.

Exposure estimates were obtained by averaging the daily DBP estimates produced by the spline regression technique from the CWS to which the participant was linked between weeks 6 and 16 of gestation, an estimate of the biologically appropriate time period for hypospadias development.

To evaluate exposures to DBPs, both continuous and categorized levels for TTHM, HAA5, and six individual species of HAA were used. Individual species of THM could not be examined due to the low number of Arkansas CWS collecting data on these chemicals during our study period (See Table 5.2.3.1). Quartiles were used to develop the categories for DBPs with the lowest quartile serving as the referent category. The distribution of TTHM, HAA5 and the six species of HAA were characterized by their mean and standard deviation across the entire study period.

### 5.2.4 Using Exposure Data to Estimate Dose

Two quantitative measures to estimate dose to DBPs were examined. The Personal Consumption Index (PCI) described in Chapter 4 was used to evaluate the effect of exposure to DBPs via the ingestion route of exposure and DBP concentration during the exposure window for cases and controls in the NBDPS analyses. Additionally, the Multiple Exposure Index (MEI), also described in Chapter 4, was used to evaluate the effect of exposure to DBPs via the dermal, inhalation and ingestion routes of exposure (via consumption, showering and bathing) and TTHM concentration during the exposure window for cases and controls in the NBDPS analyses. These analyses were not conducted for participants in the ARHMS analyses due to the lack of water use behavior data for these participants. Analyses with the MEI were conducted for exposure to TTHMs only. The MEI, as described by King *et al.* (2004a) was developed for TTHMs and takes into account the volatile nature of these compounds and the ingestion, inhalation and dermal routes of exposure. Little information exists for the multiple exposure routes for HAAs, though it is commonly accepted that exposure to nonvolatile DBPs such as HAAs, is primarily through ingestion (Nieuwenhuijsen *et al.*, 2000; Kaur *et al.*, 2004). Xu & Weisel (2003) report that HAAs are completely ionized in tap water at a pH close to 7 and thus are nonvolatile at ambient and typical shower water temperatures. They reported that inhalation exposure to HAAs during showering would occur predominantly through respiratory uptake of shower-generated aerosols and are not expected to contribute significantly to total exposure. Also, HAAs were the least permeable class of DBPs when permeability coefficients for dermal exposure were estimated, and were two orders of magnitude smaller than those of TTHMs (Xu *et al.*,

2002). In general, the ionized form of a chemical penetrates the skin poorly. This may explain the much lower permeability of HAAs compared to TTHMs (Xu *et al.*, 2002). Based on these findings, we did not believe that the MEI developed for TTHMs could be applied to HAAs.

The PCI accounted for the average number of eight-ounce glasses of unfiltered tap water reportedly consumed at home and at work each day. The influence of differing amounts of ingested water on exposure assessment was evaluated through comparison of estimated CWS-wide tap water DBP concentrations with the PCI. We obtained ORs and 95 % CIs for the relationships between DBPs and the outcome of hypospadias with the PCI.

The MEI assumed an equivalency of absorbed dose such that one liter of ingested water was equivalent to a five minute shower (Weisel & Jo, 1996) and a fifteen minute bath (Kerger *et al.*, 2000). A 50% reduction in TTHM intake through cold water-based drinks was applied when a carbon filter was indicated. A reduction of 70% was applied to the TTHM ingestion estimate for boiled hot water drinks (Kuo *et al.*, 1997; Wu *et al.*, 2001). These equivalent measures were used to create an exposure variable that was based on ingestion of equivalent eight-ounce glasses. For example, a five-minute shower was considered to be equivalent to the dose received by ingesting one liter of water. We assumed one liter of water to be equal to four eight-ounce glasses of water. Thus for each five minutes of showering per day, four eight-ounce equivalent glasses were added to the MEI. Similarly, four eight-ounce equivalent glasses were added to the MEI for each

fifteen minutes spent bathing, one eight-ounce equivalent glass was added to the MEI for each glass of unfiltered tap water ingested, 0.5 eight-ounce equivalent glasses were added to the MEI for each glass of filtered tap water ingested, and 0.3 eight-ounce equivalent glasses were added to the MEI for each boiled hot water drink ingested. The influence of different water-use behaviors on exposure assessment was evaluated through comparison of household tap water TTHM level with the MEI. We obtained ORs and 95 % CIs for the relationships between TTHM and hypospadias with the MEI.

### 5.2.5 Evaluation of Potential Confounders

Information on potential confounders was abstracted for cases from the birth defect registry records and information on potential confounders for ADH controls from birth certificate records. A list of these variables can be found in Table 5.2.4.1.

Information on additional potential confounders for NBDPS cases and controls was abstracted from the NBDPS database including information on both personal water use habits via the CATI. A list of these variables can be found in Table 5.2.4.1.

### 5.2.6 Unadjusted and Adjusted Analyses of Risk

The mean daily DBP concentration during the exposure window was used to estimate risk of hypospadias for the participants in the ARHMS analyses and the NBDPS analyses. Unconditional logistic regression was used to obtain crude odds ratios (ORs) and 95 percent confidence intervals (CIs) for the relationship between DBPs and hypospadias. The only variables entered into the logistic models were the mean of the

estimated daily DBP concentrations during the participant's exposure window and case/control status.

Those potential confounders that were associated with hypospadias at the <0.25 level in the unadjusted model with average DBP concentration during the exposure window were retained for analyses in multivariate logistic regression models for participants in the ARHMS and NBDPS analyses. A backwards selection method was used to create multivariate models for participants such that potential confounders that were statistically significant at the $p<0.10$ level or that changed the odds ratio of another covariate by 10% or more were retained in the models (See Appendices 1 and 2 for detailed results of analyses). Multivariate logistic regression analyses were used to determine if DBP exposures between weeks six and sixteen of gestation increased the risk of hypospadias for participants. After adjustment for potential confounders, adjusted ORs and 95 percent CIs for the relationships between DBPs and the outcome of hypospadias were obtained. When appropriate, the Mantel extension chi square test was performed to test for a statistically significant trend between estimated exposure level and risk.

### 5.2.7 Potential for Effect Modification

All of the pair-wise interactions between the covariates retained in the multivariate model and the exposure variable for participants in the ARHMS and NBDPS analyses were evaluated for effect modification. Though none of the pair-wise interactions were statistically significant in the multivariate model at the $p<0.25$ level, we did evaluate several of the pair-wise interactions that approached significance in the multivariate

model in order to evaluate potential effect modification. For participants in the ARHMS analyses the additive and multiplicative interactions between the exposure variable and maternal age were evaluated. For participants in the NBDPS analyses the additive and multiplicative interactions between the exposure variable and maternal race and maternal body mass index (BMI) were evaluated.

### 5.2.8 Influence Analyses

#### 5.2.8.1 Potential for Selection Bias Due to Out-of-State Obstetrical Care Among the Residents of Seven Northeastern Arkansas Counties

Fewer cases of birth defects were reported than expected in seven eastern Arkansas counties included in our study area (Mosley *et al.,* 2002), which could introduce bias into our study. These counties were Craighead, Crittenden, Cross, Lee, Mississippi, Poinsett and St. Francis. Mosley *et al.* (2002) hypothesized that some residents of northeastern Arkansas sought medical care across the state line in western Tennessee where no collaborative agreement with ARHMS existed. Selection bias could have resulted if differences exist between cases and controls with regard to the proportion seeking out-of-state obstetrical care (Rothman and Greenland, 1998). We established an *a priori* criterion of 10% to determine if controlling for this factor was necessary (Rothman and Greenland, 1998). The multivariate model was run both with and without these seven counties and it was determined that the difference in reporting for these seven counties did not need to be controlled for. All results include cases and controls from all counties within Arkansas.

#### 5.2.8.2 Potential for Exposure Misclassification Due to Linkage with Incorrect Community Water System (CWS)

Cases and controls were assigned exposure based on their linkage to a CWS and the monitoring data collected by this CWS. This linkage procedure is described in Chapter 3. An incorrect linkage between a study subject and a CWS could result in exposure misclassification. In order to avoid this, we contacted each CWS to validate if it served the residential addresses that had been linked to it. These results are described in Chapter 3 also. We were able to validate over 81% of residential addresses as being correctly linked to a CWS when the necessary responses from the CWS were received. We established an *a priori* criterion of 10% to determine if including only those participants with a validated linkage to a CWS would produce results different from those if all participants were included (Rothman and Greenland, 1998). The multivariate model was run with participants whose residential-CWS linkage had been validated and with all participants. We determined that there was no difference between participants with a validated linkage and all participants. In order to preserve sample size and power the analyses include all cases and controls.

#### 5.2.8.3 Potential for Reporting Bias Due to Disparate Case Definition in ARHMS and NBDPS Analyses

Cases of hypospadias in the ARHMS analyses included primary, secondary, and tertiary hypospadias, whereas cases of hypospadias in the NBDPS analyses were limited to secondary and tertiary hypospadias. In order to rule out any bias that could have resulted due to the differences in case definition for the two populations we established an *a priori* criterion of 10% to determine if controlling for this factor was necessary (Rothman and

Greenland, 1998). We ran the multivariate model for all hypospadias cases in the ARHMS analyses, primary hypospadias cases only, secondary hypospadias cases only, and secondary and tertiary hypospadias cases together. Tertiary hypospadias alone could not be evaluated due to the small number of cases with this diagnosis. It was determined that it was not necessary to control for the difference in case severity. All cases of hypospadias were included in the analyses.

#### 5.2.8.4 Potential for Exposure Misclassification Due to Exposure Window Length

Although hypospadias is believed to occur as a result of an abnormal urethral closure at approximately week eight to week fourteen of gestation, weeks six to sixteen of gestation were used as the exposure window in this study. This was done to include any insult due to DBP exposure that could play a role in the etiology of hypospadias. In order to rule out any bias due to exposure misclassification that could have resulted due to the exaggerated exposure window the unadjusted model for all hypospadias cases was run with a six-week exposure window (week eight to week fourteen of gestation) and a two-week exposure window (week ten to week twelve of gestation). The risk estimates from these analyses were compared with the risk estimates using the ten-week exposure window (week six to week sixteen). The ORs were similar and 95% confidence limits were overlapping when different exposure windows were used, suggesting that changing the length of the exposure window did not affect risk estimation. All of the results presented reflect the use of a ten week exposure window (week six to week sixteen of gestation).

## 5.3 Results

### 5.3.1 Exposure Data: Distribution of DBP Concentrations

The five-year distribution of DBP concentrations by quartile for 263 CWS in Arkansas is presented in Table 5.3.1.1. Concentrations for chlorinated species of THMs and HAAs were higher than those for brominated species of DBPs during the study period. The relatively low contribution of brominated species of THM to the total TTHM concentration were similar to results reported by Lynberg *et al.* (2001) for Cobb County, Georgia, though the concentrations of TTHMs and THMs species in Cobb County were generally higher than those in Arkansas.

### 5.3.2 Geocoding Participant Residence and Linking to CWS

79.1% of ARHMS cases (n=515) were successfully geocoded and 62.1% (n=320) of the geocoded ARHMS cases were successfully linked to DBP data in their exposure windows. 79.3% of ARHMS controls (n=1,013) were successfully geocoded and 60.6% (n=614) of the geocoded ARHMS controls were successfully linked to DBP data in their exposure windows. While geocoding results for the NBDPS participants were higher than that for the ARHMS participants, the results of linking the participants to DBP data within their exposure windows were lower. 82.4% of NBDPS cases (n=94) were successfully geocoded and 40.4% (n=38) of the geocoded NBDPS cases were successfully linked to DBP data in their exposure windows. 96% of NBDPS controls (n=604) were successfully geocoded and 34.1% (n=206) of the geocoded NBDPS controls were successfully linked to DBP data in their exposure windows.

### 5.3.3 Participant Characteristics

The demographic characteristics of ARHMS and NBDPS participants are summarized in Table 5.3.3.1. The majority of subjects was white, nulliparous, greater than 20 years old, and had at least a high school education. Most participants gained at least 20 pounds during their pregnancy. The gestational age of the majority of infants was between 36 and 40 weeks, and the birth weight for most of the infants was greater than 2,500 grams. Eighty percent of women did not smoke during pregnancy. The characteristics of cases and controls were similar for ARHMS and NBDPS participants.

### 5.3.4 Potential Confounder Selection

The results of potential confounder selection for inclusion in the multivariate model for ARHMS participants are presented in Appendix 1. The results for significance testing of each potential confounder in the model alone and in the model with TTHM, HAA5 and DBAA are presented. Results for significance testing with the other HAA species were similar to those for DBAA and are not presented here. Paternal race, maternal ethnicity, paternal ethnicity, maternal education, 1-minute Apgar score, 5-minute Apgar score, gestation age, number of previous births now living, sum of previous live births now living and dead, number of cigarettes per day and complications of labor/delivery were found to be statistically significant at the $p<0.25$ level, and were therefore included in the model building procedure using the backwards selection technique. All of these variables were entered into the logistic regression model at the same time. The least statistically significant variable was removed one at a time. If the removal of the variable changed

the odds ratio of another variable by 10% or more, it was reentered into the model and the next least statistically significant variable was removed. This was done until the only remaining variables in the model were statistically significant at the $p<0.10$ level or changed the OR of another variable by 10% or more when removed. The multivariate model for ARHMS participants was adjusted for gestational age, number of cigarettes smoked per day during pregnancy, maternal education level, maternal ethnicity and paternal ethnicity.

The results of potential confounder selection for inclusion in the multivariate model for NBDPS participants are presented in Appendix 2. The results for significance testing of each potential confounder in the model alone and in the model with TTHM, HAA5 and DBAA are presented. Results for significance testing with the other HAA species were similar to those for DBAA and are not presented here. Maternal body mass index, number of showers per week, birth weight, maternal race, plurality, multivitamin use during pregnancy and folic acid supplementation during pregnancy were found to be significantly associated with hypospadias at the $p<0.25$ level when tested in the model with the exposure variable alone, and were therefore included in the model building procedure using the backwards selection technique. All of these variables were entered into the logistic regression model at the same time. The least statistically significant variable was removed one at a time. If the removal of the variable changed the odds ratio of another variable by 10% or more, it was reentered into the model and the next least statistically significant variable was removed. This was done until the only remaining variables in the model were statistically significant at the $p<0.10$ level or changed the OR

of another variable by 10% or more when removed. The multivariate model for NBDPS participants was adjusted for body mass index, birth weight, maternal race and plurality.

### 5.3.5 Unadjusted and Adjusted Analyses of Risk

Tables 5.3.5.1 and 5.3.5.2 present crude ORs and 95 percent CIs for hypospadias according to individual DBP with exposure assessment on continuous and categorical scales, respectively, for ARHMS participants and NBDPS participants. None of the unadjusted analyses produced statistically significant ORs ($p<0.05$) when the associations between DBPs and hypospadias were examined. These analyses were repeated using the weighting metric described in Chapter 4 in conjunction with the spline regression technique. The results were similar and can be found in Appendix 3a. Tables 5.3.5.3 and 5.3.5.4 present adjusted ORs and 95% CIs for hypospadias according to individual DBP with exposure assessment on continuous and categorical scales, respectively, for ARHMS participants and NBDPS participants. All ORs for ARHMS participants were adjusted for gestational age, number of cigarettes smoked per day during pregnancy, maternal education level, maternal ethnicity and paternal ethnicity. All ORs for NBDPS participants were adjusted for body mass index, birth weight, maternal race and plurality. None of the adjusted analyses produced statistically significant associations between DBP exposure and hypospadias at the 95 percent confidence level.

### 5.3.6 Evaluation of Effects of Dose

The results of PCI analyses for NBDPS participants for TTHM, HAA5, BCAA, DCAA, MCAA and TCAA, where dose is estimated as the product of DBP concentration during the exposure window and number of eight-ounce glasses of unfiltered tap water consumed per day are presented in Table 5.3.6.1 through Table 5.3.6.6. The results of these analyses are presented with the exposure assessment on a continuous scale. Results for DBAA and MBAA are not presented due to the low number of estimated concentrations above detection limits. None of the risk estimates were statistically significant ($P<0.05$).

The results of PCI analyses for NBDPS participants for TTHM, HAA5, BCAA, DCAA, MCAA and TCAA, where dose is estimated as the DBP concentration during the exposure window multiplied by the number of eight-ounce glasses of unfiltered tap water consumed per day are presented in Table 5.3.6.7 through Table 5.3.6.12. The results of these analyses are presented with the exposure assessment on a categorical scale, using tertiles of PCI as the cut points for the categories. Results for DBAA and MBAA are not presented due to the low number of estimated concentrations above detection limits. We found a statistically significant association between the lowest tertile of estimated TTHM dose and hypospadias (OR=2.44, 95% CI 1.02-5.88; Table 5.3.6.7), though there were no statistically significant associations with TTHM at higher tertiles. Similarly, we found a statistically significant association between the lowest tertile of estimated BCAA dose and hypospadias (OR=3.24, 95% CI 1.13-9.25; Table 5.3.6.9), although there were no statistically significant associations with BCAA at the higher tertiles.

The results of MEI analyses for NBDPS participants for TTHM, where dose is estimated as the product of DBP concentration during the exposure window and number of equivalent eight-ounce glasses of unfiltered tap water, incorporating ingestion, inhalation and dermal routes of exposure are presented in Tables 5.3.6.13 and 5.3.6.14. Table 5.3.6.13 presents the results with exposure assessment on a continuous scale. We found a statistically significant association between estimated TTHM dose and hypospadias for a 20 μg/day increase in estimated TTHM dose (OR 1.06 CI 1.01-1.11). This analysis was repeated using the weighting metric to account for spatial variability of TTHMs. The results were similar to those without the weighting metric and can be found in Appendix 3b.

The results of analyses of exposure to TTHM as a categorical variable based on estimated dose divided into tertiles are presented in Table 5.3.6.14. When the analysis was repeated with the exposure treated as a categorical variable, a statistically significant association was found for the highest tertile (OR 3.45; 95% CI 1.05-11.35). A Mantel extension chi square test was used to test for trend and a statistically significant dose-response relationship was found (chi square value =3.87, p = 0.049). This analysis was repeated using the weighting metric to account for spatial variability of TTHMs. The results were similar to those without the weighting metric and can be found in Appendix 3c.

### 5.3.7 Evaluation for Effect Modification

The interaction term between maternal education and HAA5 and individual species of HAA concentrations approached significance in the multivariate model for AHRMS participants and was evaluated for potential effect modification. There was no indication of a statistically significant interaction between maternal education and TTHM or BCAA, so these interactions were not evaluated. The results for the multiplicative interaction between maternal education and HAA5, DBAA, DCAA, MBAA, MCAA and TCAA are presented in Appendices 4a through 4f, respectively. For HAA5, DCAA, and MCAA the association appeared to increase as the DBP concentration increased, but decreased when DBP concentration was held constant and maternal education increased, though none of these associations was statistically significant and the 95 percent confidence limits were overlapping. Conversely, for DBAA, MBAA, and TCAA the association appeared to increase as the DBP concentration increased, and also increased when DBP concentration was held constant and maternal education increased, though none of these associations was statistically significant and the 95 percent confidence limits were overlapping. When evaluated for additive interactions we determined that there was a synergistic additive interaction between maternal education and HAA5, DBAA, DCAA, MCAA, and TCAA, but no interaction between maternal education and MBAA (data not shown).

The multiplicative interactions between maternal race and maternal BMI with DBP concentration approached significance in the multivariate model for NBDPS participants and were evaluated for potential effect modification. There were no indications of statistically significant interactions between maternal race and DBAA or MCAA, so these

interactions were not evaluated. The results for the interaction between maternal race and TTHM, HAA5, BCAA, DCAA, MCAA and TCAA are presented in Appendices 5a through 5f, respectively. For each of these interactions the association appeared to increase as the DBP concentration increased, and increased when DBP concentration was held constant and maternal race varied, though none of these associations was statistically significant. In each case the OR was highest for white mothers, lower for Black mothers, and lowest for mothers of another race. When examined for additive interactions we found a synergistic additive interaction between maternal race and TTHM, HAA5, BCAA, DCAA, MCAA and TCAA (data not shown).

The results for the multiplicative interaction between maternal BMI and TTHM, HAA5, BCAA, DBAA, DCAA, MBAA, MCAA, and TCAA are presented in Appendices 6a through 6h, respectively. For TTHM, BCAA, and DBAA the association appeared to increase as the DBP concentration increased, and increased when DBP concentration was held constant and maternal BMI increased, though none of these associations was statistically significant. Conversely, for HAA5, DCAA, MBAA, MCAA and TCAA the association appeared to increase as the DBP concentration increased, but decreased when DBP concentration was held constant and maternal BMI increased, though none of these associations was statistically significant. When examined for additive interactions we found an antagonistic additive interaction between maternal BMI and TTHM and MCAA, no additive interaction between maternal BMI and HAA5, DCAA and TCAA, and a synergistic additive interaction between maternal BMI and BCAA, DBAA, MBAA (data not shown).

### 5.3.8 Sensitivity Analyses

The results examining the potential for exposure misclassification due to an exaggerated exposure window in are presented in Appendices 7 and 8. Appendices 7a through 7d contain the results of the unadjusted and adjusted analyses with a six-week exposure window with the exposure assessment on both continuous and categorical scales. Appendices 8a through 8d contain the results of the unadjusted and adjusted analyses with a two-week exposure window with the exposure assessment on both continuous and categorical scales. As stated previously, when these results of these appendices were compared with Tables 5.3.5.1 and 5.3.5.2, no statistically significant differences in risk estimation was found. A ten-week exposure window was used in all of the analyses (gestational week six through gestational week sixteen).

## 5.4 Discussion

Past epidemiologic studies have offered evidence of a positive relationship between DBP concentrations and birth defects (Shaw *et al.,* 1991; Aschengrau *et al.,* 1993; Bove *et al.,* 1995; Magnus *et al.,* 1999; Dodds *et al.,* 1999; Klotz & Pyrch, 1999; Dodds & King, 2001; Cedergren *et al.*, 2002; Hwang *et al.*, 2002; Shaw *et al.*, 2003). We examined a specific birth defect, hypospadias, and found a suggestion of a weak association with BCAA and TTHM when personal water consumption was taken into account and a statistically significant association with TTHM when water-use behaviors (consumption, bathing and showering) were taken into account. We did not find any statistically significant ORs for the association between hypospadias and any species of DBP concentration alone.

Synergistic additive interactions between maternal education and HAA5, DBAA, DCAA, MCAA and TCAA were detected, such that the two risk factors do not appear to act independently, but that the absolute increase in risk due to DBP exposure depends on maternal education level. The combination of less maternal education and exposure to high DBP concentrations may be associated with a greater risk of hypospadias. One possible explanation for this would be that mothers with lower levels of education might be more likely to participate in risky behaviors during pregnancy (i.e. drinking or smoking), or they might have less access to prenatal care, or prenatal vitamins (including folic acid). Synergistic additive interactions between maternal race and TTHM, HAA5, BCAA, DCAA, MCAA and TCAA also were detected, such that the two risk factors do not appear to act independently, but that the absolute increase in risk due to DBP exposure depends on maternal race. The combination of being white and exposure to high DBP concentrations may be associated with a greater risk of hypospadias. A likely explanation for this would be that there is a genetic component to hypospadias risk.

It is difficult to explain why a statistically significant association was found for hypospadias and exposure to the lowest tertiles of BCAA and TTHM when personal water consumption was taken into account, when this association did not persist for the higher tertiles of exposure to these compounds. It is possible that the significant results at the lowest tertiles may be due to chance. The statistically significant association found for hypospadias and exposure to the highest tertile of TTHM when water-use behaviors were taken into account supports our a priori hypothesis that hypospadias is related to

DBP exposure. This association was strengthened by the positive test for trend indicating a dose-response relationship. Still, we cannot rule out that this significant association was found due to chance alone.

The biological mechanisms to support an association between exposure to DBPs and hypospadias are not well understood. Non-environmental factors associated with hypospadias include complex genetic syndromes, chromosomal abnormalities, 5α-reductase type 2 deficiency, androgen insensitivity, and impairment of the androgen receptor (Utsch *et al.*, 2003; Baskin *et al.*, 2001), and account for only a small percentage of hypospadias cases (Baskin, 2004). Among those without genetic mutations, fetal exposure to chemicals that affect the endocrine system may play a role in the etiology of hypospadias (Baskin *et al.*, 2001). Estrogenic and anti-androgenic substances are implicated in disturbing normal urethral development (Baskin, 2004; Toppari and Skakkebaek, 2000). These substances can disrupt the activation of hormone dependent pathways required for male genital development and induce abnormalities of the penis (Steinhardt, 2004).

The basis for assessing exposure in past epidemiologic studies of DBPs and birth defects has varied, including using municipal-level water quality on chlorination practice and the humic content of raw water (Hwang *et al.*, 2002; Magnus *et al.*, 1999), routine DBP monitoring data from individual CWS (Shaw *et al.*, 2003; Dodds and King, 2001; Dodds *et al.*, 1999), and home tap water samples (Klotz and Pyrch, 1999). We used quarterly DBP monitoring data from individual CWS in determining exposure for ARHMS and

NBDPS participants, but found no statistically significant associations with hypospadias. Additionally, we used interview data from the NBDPS in conjunction with the quarterly DBP monitoring data to estimate dose of DBPs via the ingestion route of exposure with the PCI and to estimate dose of TTHM via the ingestion, inhalation, and dermal routes of exposure with the MEI. The results of these analyses differed from those with DBP concentrations alone, and we found a statistically significant association between TTHM concentration and hypospadias when the MEI was used.

We are aware of one previous epidemiologic study of DBPs and birth defects which also estimated dose to DBPs. In 1999, Klotz and Pyrch examined exposure to DBPs and neural tube defects (NTDs). They surveyed a majority of their cases and controls, and with the responses to their survey used ingestion of hot and cold beverages to estimate the dose of DBPs. They also collected data on frequency and length of showering and bathing to account for the inhalation and dermal routes of exposure. They found no association with NTDs when adjusting for ingestion of hot and cold beverages, while adjusting for cold beverages alone did not change the risk estimates calculated using DBP concentration alone. They also found no association with NTDs when controlling for reported showering and bathing time.

Recently, the use of questionnaire data to estimate dose via the ingestion, inhalation and dermal routes of exposure has become more common in epidemiologic studies of DBPs and adverse reproductive outcomes. The procedures for estimating dose used in this study are based on methods developed by King *et al.* (2004a), and utilized in an

epidemiologic study by King *et al.* (2004b) of exposure to DBPs and risk of stillbirth. Infante-Rivard (2004) multiplied the product of the frequency of showers per week and duration of each shower, with the average levels of THMs in a study examining gene polymorphisms and fetal growth. In 2003, Shaw *et al.* estimated the daily average volume of cold water consumed at home and combined this with average THM levels in order to estimate exposure. All of these studies used system-wide DBP averages in the exposure assessment.

The use of the PCI and the MEI in this study allowed for the incorporation of data on the ingestion, inhalation and dermal routes of exposure for DBPs, and produced results different from those for DBP concentration alone. However, there are several disadvantages to these indices. The PCI and MEI are based on reported, average water use behaviors, which are subject to reporting bias, and may not reflect specific temporal changes in these behaviors linked to exposure. For example, consuming more glasses of water one day than another, or taking several more baths one week than another, especially during the critical window for hypospadias development, could be crucial in exposure assessment. Water-use behaviors were not recorded with this level of detail by the NBDPS questionnaire. Also, the MEI assumes that the ingestion of one liter of water produces an internal dose equivalent to that of a five-minute shower or a fifteen-minute bath (King *et al.*, 2004a). These equivalencies were developed using the best available knowledge but need to be validated, and the intra- and inter-participant variation of these equivalencies needs to be examined. Finally, the NBDPS questionnaire collects information on many other water-use behaviors, including hand washing, washing dishes,

clothes, children and pets, and swimming that may contribute to THM dose (Nuckols *et al.*, 2005), which have not been incorporated into the MEI.

Historically, windows of exposure have been important in epidemiologic investigations of thalidomide, retinoic acid (Vitamin A), maternal rubella, and radiation (O'Rahilly and Muller, 2001). Few congenital abnormalities are observed when exposures occur during the first two weeks of gestation because the teratogen either damages the majority of cells, resulting in cell and embryonic death, or affects only a few cells that can be repaired without resultant birth defects (Moore and Persaud, 1998). After the first two weeks, the tissue or organ that is most susceptible to malformation is the part undergoing critical development when the teratogen is active. The critical window for external genitalia development is estimated to be between gestational weeks six and sixteen with weeks eight through ten the most critical (Moore and Persuad, 1998).

Typically, epidemiologic studies of DBPs have used the first trimester as the exposure window for birth defects (Shaw *et al.*, 1991; Aschengrau *et al.*, 1993; Bove *et al.*, 1995; Magnus *et al.*, 1999; Dodds *et al.*, 1999; Klotz & Pyrch, 1999; Dodds & King, 2001; Cedergren *et al.*, 2002; Hwang *et al.*, 2002; Shaw *et al.*, 2003). Very few epidemiologic studies of DBPs have examined the effect of using exposure windows with varying lengths, despite the well-known temporal variability of DBPs and the critical windows for central nervous, cardiac, respiratory and genitourinary system development. We chose to use a ten-week exposure window, beginning with the sixth week and running through the sixteenth week of gestation. In doing so it is possible that we may have

introduced exposure misclassification by including exposures to DBPs that occurred before or after the insult(s) that lead to hypospadias. Additionally, using a larger exposure window could have led to over-estimation or under-estimation of the true DBP value during the critical time window in the etiology of hypospadias. In order to evaluate potential exposure misclassification, we chose to do the analyses with two additional exposure windows; a six week exposure window beginning at gestational week eight through gestational week fourteen, and a two week exposure window beginning at gestational week ten and continuing through gestational week twelve. When we used DBP concentrations within these exposure windows of varying length to estimate exposure, the results were similar for all of the exposure window lengths.

Selection bias is a limitation that is inherent in almost all case-control studies. In this study cases were obtained from a birth defects registry while controls were selected as a random sample from birth certificate records. While the controls were unlikely to have a tendency toward or away from DBP exposure, data collection was different for cases and controls. There are limitations in both the quality and completeness of data from birth certificates. This leads to the possibility of introducing bias if the information on the birth certificates or information from the birth defect registry is affected by confounder misclassification. This could bias the results of the study either toward or away from the null hypothesis.

Information bias is another potential limitation to the study. It is possible that there could be misclassification of study subjects with respect to hypospadias status. Primary hypospadias can often go unnoticed, or may not be reported by all physicians. If this

were to occur, some of the study subjects that were chosen to be controls might actually be cases. We would expect this type of misclassification to be non-differential with respect to exposure, which generally leads to a bias toward the null hypothesis. If this occurred in our study, it might help to explain why we did not find a strong association between exposure to DBPs and hypospadias risk.

Confounder misclassification is another potential bias that may affect case-control studies. As we have noted, there is a difference in the accuracy of information gathered from birth certificates and birth defect registries. If there was a difference in the accuracy of one of the variables which we included in the multivariate analyses this could have led to bias in our study. However, because there was no statistical difference between the unadjusted and adjusted analyses in our study, it is unlikely that confounder mislclassification played a large role in our results.

Another limitation of this study is low power for statistical analyses of the NBDPS participants due to the small number of hypospadias cases in the NBDPS that could be linked to DBP concentrations in their exposure window. Using our sample size for this case-control study and having approximately 25 percent of the population unexposed during the exposure window, we had 43 percent power to detect an odds ratio of 3.0. At 80 percent power, for an odds ratio of 3.0, we would have needed to link 70 cases and 420 controls to exposure data in their exposure windows. To detect an odds ratio of 1.5 with 80 percent power for the association between TTHM exposure and hypospadias we would have needed 359 cases and 2,154 controls.

Further research is required to characterize the association between hypospadias and exposure to DBPs. Specifically, a larger sample size is needed to increase the power of the statistical analyses. The NBDPS is a data-rich source for studies of DBPs and birth defects. Including several participating states instead of limiting the study area to one state would increase the sample size and power. Exposure indices such as the MEI should be interpreted with extreme caution. The biologically effective dose associated with different routes of exposure is likely to vary for specific volatile DBPs and health effects of interest (King *et al.*, 2004a). Additionally, the route of exposure affects the rates of metabolism and therefore the compound's potential toxicity (Weisel & Jo, 1996). It is possible that the results from physiologically-based pharmaco-kinetic (PBPK) models might aid in the refinement of indices such as the MEI and lead to a more accurate estimation of internal dose to DBPs from multiple routes of exposure. While the investigation of exposure windows with varying length did not produce statistically different risk estimates in this study, the evaluation of the effect the exposure window can have on epidemiologic studies of DBPs and adverse reproductive outcomes should be explored further. Finally, the reduction in power for analyses of DBAA and MBAA due to low reported concentrations and non-detectable concentrations of these HAAs was a limitation to this study. This study should be repeated in a geographic area where brominated species of HAA are at high enough levels to avoid this problem, especially because of the reported effects of brominated species of HAAs on the male reproductive system in animal studies.

Although the relationship between exposure to DBPs and risk of hypospadias is still unclear, this study suggests a positive association between TTHM dose and hypospadias. We found a positive association between hypospadias and an increase in TTHM dose as low as 20 μg/day. An increase in dose of this amount is readily achievable by a large proportion of the population, even when TTHM concentrations remain below the maximum contaminant limit (MCL) of 80 μg /L maintained by the U.S. EPA. Due to the wide potential for exposure to DBPs and the increasing incidence of hypospadias, this relationship requires further investigation.

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Figure 5.1.1: Schematic of different degrees of severity of hypospadias. Anterior hypospadias is also referred to as primary, middle as secondary, and posterior as tertiary (Manson and Carr, 2003).

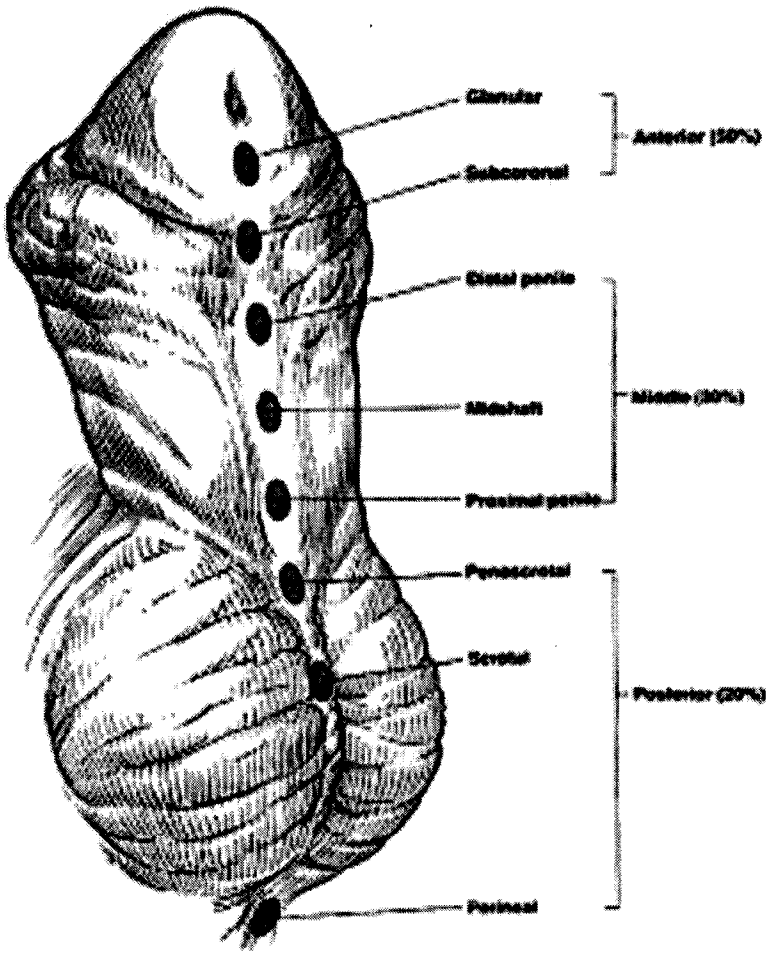

Table 5.2.3.1: Number of ARHMS and NBDPS participants with individual THM monitoring data available in their exposure window. 1998-2002.

| Trihalomethane Species | ARHMS Participants (Cases) | NBDPS Participants (Cases) |
|---|---|---|
| TTHM | 934 (320) | 267 (38) |
| BDCM | 57 (23) | 24 (2) |
| BF | 59 (24) | 24 (2) |
| CF | 59 (24) | 24 (2) |
| DBCM | 49 (22) | 22 (2) |

Table 5.2.4.1: Available variables evaluated for significance with average of daily DBP estimates during exposure window for ARHMS and NBDPS participants

| Potential Confounders | |
|---|---|
| ARHMS | NBDPS |
| Maternal age | Maternal age |
| Maternal race | Maternal race |
| Paternal race | Paternal race |
| Maternal education | Maternal education |
| Weight gain during pregnancy | Weight gain during pregnancy |
| Birth weight | Birth weight |
| Gestational age at delivery | Gestational age at delivery |
| Parity | Parity |
| Smoking | Smoking |
| Paternal age | Maternal height |
| Maternal ethnicity | Maternal weight |
| Paternal ethnicity | Infant's length at birth |
| Paternal education | Coffee consumption |
| Marital status | Tea consumption |
| One-minute apgar score | Glasses of water consumed per day |
| Five-minute apgar score | Dishwashing by hand |
| Plurality | Dishwashing with an automatic dishwasher |
| Type of delivery | Washing clothes by hand |
| Month of gestation prenatal care began | Bathing children |
| Number of prenatal visits | Bathing pets |
| Alcohol consumption during pregnancy | Loads of laundry per week |
| | Time spent in shower |
| | Number of baths per week |
| | Time spent bathing |
| | Time spent in swimming pool |
| | Times in a pool during first trimester |
| | History of seizures |
| | Respiratory illness |
| | Kidney, bladder, or urinary tract infection |
| | Surgery during pregnancy |
| | Previous stillbirths |
| | Previous abortions |
| | Previous miscarriages |
| | Use of oral birth control |
| | Use of fertility treatments |
| | Nausea during pregnancy |
| | Multivitamin supplementation |
| | Vitamin A supplementation |
| | Retinol supplementation |

Table 5.2.4.1 (Cont.): Available variables evaluated for significance with average of daily DBP estimates during exposure window for ARHMS and NBDPS participants

| | |
|---|---|
| | Beta carotene supplementation |
| | B6 supplementation |
| | B12 supplementation |
| | Folic acid supplementation |
| | Vitamin C supplementation |
| | Vitamin D supplementation |
| | Vitamin E supplementation |
| | Iron supplementation |
| | Calcium supplementation |
| | Zinc supplementation |
| | Selenium supplementation |
| | Prenatal vitamin supplementation |
| | Soda consumption |
| | Maternal marijuana use |
| | Paternal marijuana use |
| | Hot tub use |
| | Hot bath use |
| | Sauna use |
| | Pool use |
| | Drinking and cooking with tap water |
| | Infant's head circumference at birth |

Table 5.3.1.1 Five-year distribution of DBP concentrations by quartiles for 263 CWS in Arkansas (1998-2002)

| | TTHM | BF | CF | DBCM | BDCM | HAA5 | BCAA | DBAA | DCAA | MBAA | MCAA | TCAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 42.0 | 2.1 | 34.7 | 2.37 | 5.2 | 28.9 | 2.3 | 0.7 | 14.2 | 0.1 | 2.3 | 11.6 |
| St.Dev. | 32.5 | 10.8 | 56.2 | 8.12 | 5.1 | 26.9 | 2.4 | 2.1 | 14.2 | 1.1 | 2.6 | 13.4 |
| Min | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q1 | 21.2 | 0 | 2.1 | 0 | 1.6 | 16.5 | 1.2 | 0 | 3.7 | 0 | 0 | 2.2 |
| Q2 | 38.0 | 0 | 23.9 | 0 | 4.8 | 26.5 | 2.0 | 0 | 12.8 | 0 | 2.4 | 9.7 |
| Q3 | 56.9 | 0 | 48.2 | 1.6 | 7.3 | 38.9 | 2.8 | 0 | 19.4 | 0 | 3.8 | 15.4 |
| Max | 401 | 236 | 392 | 135 | 60.7 | 368 | 25.0 | 45.0 | 132 | 32.5 | 33.1 | 220 |

Table 5.3.3.1: Demographic characteristics of ARHMS and NBDPS cases and controls

| | ARHMS | | | NBDPS | | |
|---|---|---|---|---|---|---|
| | Total | Cases (%) | Controls (%) | Total | Cases (%) | Controls (%) |
| Participants | 934 | 320 (100) | 614 (100) | 244 | 38 (100) | 206 (100) |
| Maternal Age | | | | | | |
| <20 years | 146 | 47 (14.7) | 99 (16.1) | 38 | 7 (18.4) | 31 (15.0) |
| 20 – 29 years | 544 | 184 (57.5) | 360 (58.6) | 142 | 17 (44.7) | 125 (60.7) |
| >=30 years | 244 | 89 (27.8) | 155 (25.2) | 64 | 14 (36.8) | 50 (24.3) |
| Maternal Race | | | | | | |
| White | 732 | 262 (81.9) | 470 (76.5) | 175 | 29 (76.3) | 146 (70.9) |
| Black | 194 | 52 (16.3) | 142 (23.1) | 49 | 5 (13.2) | 44 (21.4) |
| Other | 8 | 6 (1.9) | 2 (0.3) | 12 | 1 (2.6) | 11 (5.3) |
| Missing | 0 | 0 (0.0) | 0 (0.0) | 8 | 3 (7.9) | 5 (2.4) |
| Maternal Education | | | | | | |
| <12 years | 168 | 41 (12.8) | 127 (20.7) | 31 | 1 (2.6) | 30 (14.6) |
| 12 years | 370 | 134 (41.9) | 236 (38.4) | 83 | 20 (52.6) | 63 (30.6) |
| >12 years | 361 | 116 (36.3) | 245 (39.9) | 130 | 17 (44.7) | 113 (54.9) |
| Missing | 35 | 29 (9.1) | 6 (1.0) | 0 | 0 (0.0) | 0 (0.0) |
| Weight Gain During Pregnancy | | | | | | |
| <15 pounds | 68 | 23 (7.2) | 45 (7.3) | 25 | 5 (13.2) | 20 (9.7) |
| 15 – 20 pounds | 100 | 31 (9.7) | 69 (11.2) | 31 | 7 (18.4) | 24 (11.7) |
| 21 – 35 pounds | 362 | 110 (34.4) | 252 (41.0) | 101 | 11 (28.9) | 90 (43.7) |
| >35 pounds | 285 | 96 (30.0) | 189 (30.8) | 86 | 15 (39.5) | 71 (34.5) |
| Missing | 119 | 60 (18.8) | 59 (9.6) | 1 | 0 (0.0) | 1 (0.5) |
| Birth Weight | | | | | | |
| <1500 grams | 68 | 23 (7.2) | 45 (7.3) | 25 | 5 (13.2) | 20 (9.7) |
| 1500 – 2500 grams | 100 | 31 (9.7) | 69 (11.2) | 31 | 7 (18.4) | 24 (11.7) |
| 2501 – 3500 grams | 500 | 171 (53.4) | 329 (53.6) | 126 | 14 (36.8) | 112 (54.4) |
| 3501 – 4500 grams | 317 | 106 (33.1) | 211 (34.3) | 72 | 14 (36.8) | 58 (28.2) |
| >4500 grams | 20 | 6 (1.9) | 14 (2.3) | 1 | 0 (0.0) | 1 (0.5) |
| Missing | 0 | 0 (0.0) | 0 (0.0) | 28 | 3 (7.9) | 25 (12.1) |
| Gestational Age | | | | | | |
| <25 weeks | 2 | 1 (0.3) | 1 (0.2) | 0 | 0 (0.0) | 0 (0.0) |
| 25 - 30 weeks | 11 | 6 (1.9) | 5 (0.8) | 1 | 0 (0.0) | 1 (0.5) |
| 31 – 35 weeks | 49 | 21 (6.6) | 28 (4.6) | 14 | 2 (5.3) | 12 (5.8) |
| 36 – 40 weeks | 793 | 267 (83.4) | 526 (85.7) | 198 | 31 (81.6) | 167 (81.1) |
| >40 weeks | 76 | 24 (7.5) | 52 (8.5) | 30 | 4 (10.5) | 26 (12.6) |
| Missing | 3 | 1 (0.3) | 2 (0.3) | 1 | 1 (2.6) | 0 (0.0) |
| Parity | | | | | | |
| 1st Pregnancy | 400 | 137 (42.8) | 263 (42.8) | 102 | 20 (52.6) | 82 (39.8) |
| 2nd Pregnancy | 278 | 88 (27.5) | 190 (30.9) | 94 | 12 (31.6) | 82 (39.8) |
| 3rd Pregnancy | 143 | 47 (14.7) | 96 (15.6) | 33 | 2 (5.3) | 31 (15.0) |
| 4th Pregnancy | 52 | 11 (3.4) | 41 (6.7) | 11 | 2 (5.3) | 9 (4.3) |
| 5th or greater pregnancy | 34 | 11 (3.4) | 23 (3.7) | 4 | 2 (5.3) | 2 (1.0) |
| Missing | 27 | 26 (8.1) | 1 (0.2) | 0 | 0 (0.0) | 0 (0.0) |
| Smoking during pregnancy | | | | | | |
| Yes | 147 | 52 (16.3) | 95 (15.5) | 61 | 9 (23.7) | 52 (25.2) |
| No | 755 | 241 (75.3) | 514 (83.7) | 183 | 29 (76.3) | 154 (74.8) |
| Missing | 32 | 27 (8.4) | 5 (0.8) | 0 | 0 (0.0) | 0 (0.0) |

Table 5.3.5.1: Crude odds ratios and 95% confidence intervals for DBPs with exposure assessment on continuous scale for ARHMS and NBDPS participants 1998-2002

| | | ARHMS | | | NBDPS | | |
|---|---|---|---|---|---|---|---|
| Variable | Unit Increase (μg/L) | N (Cases) | Odds Ratio | 95% Confidence Interval | N (Cases) | Odds Ratio | 95% Confidence Interval |
| TTHM | 20 | 934 (320) | 1.05 | 0.94–1.17 | 267 (38) | 1.01 | 0.74-1.39 |
| HAA5 | 10 | 801 (277) | 1.03 | 0.95–1.12 | 224 (32) | 0.92 | 0.72-1.19 |
| BCAA | 1 | 663 (233) | 0.96 | 0.86–1.07 | 221 (29) | 1.00 | 0.80-1.25 |
| DBAA | 1 | 709 (250) | 0.94 | 0.84–1.04 | 228 (30) | 0.99 | 0.82-1.20 |
| DCAA | 5 | 708 (249) | 1.03 | 0.96–1.11 | 231 (31) | 1.02 | 0.82-1.28 |
| MBAA | 1 | 719 (253) | 1.00 | 0.91–1.11 | 233 (31) | 0.89 | 0.65-1.22 |
| MCAA | 1 | 695 (245) | 0.99 | 0.91–1.07 | 225 (30) | 1.13 | 0.91-1.41 |
| TCAA | 5 | 715 (250) | 1.05 | 0.97-1.14 | 232 (30) | 0.97 | 0.76-1.23 |

Table 5.3.5.2: Crude odds ratios and 95% confidence intervals for DBPs with exposure assessment on categorical scale by quartiles for ARHMS and NBDPS participants 1998-2002

| | | ARHMS | | | NBDPS | | |
|---|---|---|---|---|---|---|---|
| Variable | Concentration (μg/L) | N (Cases) | Odds Ratio | 95% Confidence Interval | N (Cases) | Odds Ratio | 95% Confidence Interval |
| TTHM | <27 | 237 (82) | 1.0 | 1.0 | 55 (9) | 1.0 | 1.0 |
| | 27 - <41.5 | 233 (80) | 1.01 | 0.69-1.48 | 71 (8) | 0.99 | 0.37-2.70 |
| | 41.5 - <55.3 | 212 (66) | 0.84 | 0.57-1.24 | 68 (9) | 1.04 | 0.39-2.78 |
| | 55.3 – 270 | 252 (92) | 1.12 | 0.77-1.64 | 66 (11) | 1.13 | 0.40-3.15 |
| HAA5 | <17.3 | 207 (70) | 1.0 | 1.0 | 47 (11) | 1.0 | 1.0 |
| | 17.3 - <28.4 | 194 (66) | 1.01 | 0.67-1.53 | 56 (5) | 0.48 | 0.17-1.41 |
| | 28.4 - <36 | 151 (47) | 1.14 | 0.76-1.71 | 56 (5) | 0.55 | 0.20-1.47 |
| | 36 – 155 | 249 (94) | 1.05 | 0.69-1.59 | 55 (10) | 0.80 | 0.28-2.26 |
| BCAA | <1.4 | 171 (60) | 1.0 | 1.0 | 49 (8) | 1.0 | 1.0 |
| | 1.4 - <2.0 | 175 (66) | 1.04 | 0.66-1.63 | 56 (3) | 0.36 | 0.09-1.44 |
| | 2.0 – <2.6 | 151 (57) | 1.10 | 0.70-1.71 | 51 (6) | 0.64 | 0.21-1.97 |
| | 2.6 - <10 | 166 (50) | 0.77 | 0.49-1.23 | 59 (12) | 1.47 | 0.55-3.93 |
| DBAA | <0.5 | 551 (198) | 1.0 | 1.0 | 235 (34) | 1.0 | 1.0 |
| | 0.5 – 13 | 158 (52) | 0.88 | 0.60-1.28 | 48 (6) | 0.93 | 0.36-2.42 |
| DCAA | <7.9 | 159 (54) | 1.0 | 1.0 | 46 (8) | 1.0 | 1.0 |
| | 7.9 - <14.5 | 173 (62) | 0.84 | 0.54-1.30 | 63 (9) | 0.98 | 0.36-2.66 |
| | 14.5 - <18.8 | 150 (52) | 0.90 | 0.58-1.39 | 57 (4) | 0.86 | 0.31-2.38 |
| | 18.8 – 107 | 226 (81) | 0.94 | 0.61-1.44 | 57 (10) | 0.96 | 0.29-3.10 |
| MBAA | <0.5 | 493 (180) | 1.0 | 1.0 | 190 (30) | 1.0 | 1.0 |
| | 0.5 – 6.0 | 226 (73) | 0.83 | 0.59-1.16 | 93 (10) | 0.68 | 0.30-1.51 |
| MCAA | <3.3 | 334 (127) | 1.0 | 1.0 | 91 (11) | 1.0 | 1.0 |
| | 3.3 – 11.0 | 361 (118) | 0.83 | 0.57-1.21 | 111 (15) | 1.25 | 0.48-3.29 |
| TCAA | <3.8 | 155 (52) | 1.0 | 1.0 | 47 (9) | 1.0 | 1.0 |
| | 3.8 - <10.2 | 206 (69) | 1.05 | 0.68-1.63 | 63 (5) | 0.39 | 0.118-1.29 |
| | 10.2 - <14.1 | 153 (48) | 1.12 | 0.72-1.73 | 55 (6) | 0.48 | 0.17-1.35 |
| | 14.1 – 80 | 201 (81) | 1.29 | 0.83-1.99 | 60 (9) | 0.96 | 0.36-2.56 |

Table 5.3.5.3: Adjusted odds ratios and 95% confidence intervals for DBPs with exposure assessment on continuous scale for ARHMS and NBDPS participants 1998-2002

| Variable | Unit Increase (μg/L) | N (Cases) | Odds Ratio | 95% Confidence Interval | N (Cases) | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|---|---|---|---|
| TTHM | 20 | 728 (248) | 0.94 | 0.82-1.07 | 193 (31) | 0.95 | 0.64-1.41 |
| HAA5 | 10 | 633 (221) | 0.95 | 0.86-1.05 | 154 (24) | 0.98 | 0.72-1.33 |
| BCAA | 1 | 524 (184) | 0.90 | 0.78-1.04 | 152 (21) | 0.95 | 0.70-1.29 |
| DBAA | 1 | 560 (198) | 0.94 | 0.83-1.07 | 157 (23) | 0.80 | 0.48-1.33 |
| DCAA | 5 | 559 (197) | 0.98 | 0.88-1.07 | 157 (23) | 1.10 | 0.82-1.47 |
| MBAA | 1 | 570 (201) | 1.04 | 0.92-1.16 | 159 (23) | 0.94 | 0.65-1.35 |
| MCAA | 1 | 546 (193) | 0.97 | 0.88-1.06 | 157 (22) | 1.00 | 0.76-1.31 |
| TCAA | 5 | 569 (201) | 0.99 | 0.89-1.09 | 158 (22) | 0.99 | 0.74-1.33 |

*Adjusted for gestational age, number of cigarettes smoked per day during pregnancy, maternal education level, maternal ethnicity and father's ethnicity

** Adjusted for body mass index, birth weight, maternal race and plurality

Table 5.3.5.4: Adjusted odds ratios and 95% confidence intervals for DBPs with exposure assessment on categorical scale by quartiles for ARHMS and NBDPS participants 1998-2002

| Variable | Concentration (μg/L) | N (Cases) | Odds Ratio | 95% Confidence Interval | N (Cases) | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|---|---|---|---|
| TTHM | <27 | 170 (67) | 1.0 | 1.0 | 41 (8) | 1.0 | 1.0 |
| | 27 - <41.5 | 193 (68) | 0.90 | 0.58-1.39 | 51 (6) | 0.42 | 0.12-1.49 |
| | 41.5 - <55.3 | 169 (51) | 0.69 | 0.44-1.09 | 49 (7) | 0.61 | 0.20-1.87 |
| | 55.3 – 270 | 196 (62) | 0.74 | 0.41-1.17 | 46 (9) | 0.91 | 0.28-2.93 |
| HAA5 | <17.3 | 150 (58) | 1.0 | 1.0 | 37 (8) | 1.0 | 1.0 |
| | 17.3 - <28.4 | 158 (55) | 0.85 | 0.53-1.38 | 35 (4) | 0.75 | 0.21-2.73 |
| | 28.4 - <36 | 123 (41) | 0.86 | 0.53-1.38 | 41 (4) | 0.40 | 0.10-1.53 |
| | 36 – 155 | 202 (67) | 0.73 | 0.45-1.20 | 33 (7) | 1.55 | 0.43-5.54 |
| BCAA | <1.4 | 127 (48) | 1.0 | 1.0 | 32 (7) | 1.0 | 1.0 |
| | 1.4 - <2.0 | 133 (47) | 0.81 | 0.47-1.37 | 39 (1) | 0.06 | 0.01-0.69 |
| | 2.0 – <2.6 | 129 (49) | 0.99 | 0.60-1.66 | 39 (4) | 0.43 | 0.10-1.79 |
| | 2.6 - <10 | 135 (40) | 0.68 | 0.40-1.16 | 39 (9) | 1.07 | 0.30-3.84 |
| DBAA | <0.5 | 444 (161) | 1.0 | 1.0 | 167 (28) | 1.0 | 1.0 |
| | 0.5 – 13 | 119 (37) | 0.80 | 0.51-1.25 | 33 (4) | 0.90 | 0.27-2.99 |
| DCAA | <7.9 | 109 (41) | 1.0 | 1.0 | 31 (5) | 1.0 | 1.0 |
| | 7.9 - <14.5 | 148 (54) | 0.70 | 0.42-1.15 | 41 (6) | 0.93 | 0.24-3.56 |
| | 14.5 - <18.8 | 115 (40) | 0.75 | 0.44-1.26 | 42 (4) | 0.80 | 0.22-2.98 |
| | 18.8 – 107 | 187 (62) | 0.66 | 0.39-1.10 | 36 (8) | 2.28 | 0.55-9.40 |
| MBAA | <0.5 | 391 (143) | 1.0 | 1.0 | 136 (12) | 1.0 | 1.0 |
| | 0.5 – 6.0 | 179 (58) | 0.85 | 0.58-1.25 | 64 (7) | 0.50 | 0.18-1.40 |
| MCAA | <3.3 | 248 (99) | 1.0 | 1.0 | 65 (10) | 1.0 | 1.0 |
| | 3.3 – 11.0 | 298 (94) | 0.73 | 0.47-1.14 | 71 (9) | 0.91 | 0.28-2.97 |
| TCAA | <3.8 | 107 (39) | 1.0 | 1.0 | 34 (7) | 1.0 | 1.0 |
| | 3.8 - <10.2 | 167 (61) | 1.05 | 0.63-1.74 | 38 (4) | 0.32 | 0.07-1.45 |
| | 10.2 - <14.1 | 130 (40) | 0.84 | 0.50-1.41 | 35 (4) | 0.26 | 0.06-1.11 |
| | 14.1 – 80 | 165 (61) | 1.01 | 0.60-1.69 | 45 (7) | 1.02 | 0.30-3.41 |

*Adjusted for gestational age, number of cigarettes smoked per day during pregnancy, maternal education level, maternal ethnicity and father's ethnicity

** Adjusted for body mass index, birth weight, maternal race and plurality

Table 5.3.6.1: Odds ratio for TTHM and personal consumption index on continuous scale for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated Increase in TTHM Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 20 | 261 (36) | 1.09 | 0.90-1.31 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average TTHM concentration during exposure window

Table 5.3.6.2: Odds ratio for HAA5 and personal consumption index on continuous scale for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated Increase in HAA5 Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 20 | 219 (30) | 1.09 | 0.82-1.46 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average HAA5 concentration during exposure window

Table 5.3.6.3: Odds ratio for BCAA and personal consumption index on continuous scale for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated Increase in BCAA Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 2 | 216 (27) | 1.07 | 0.77-1.50 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average BCAA concentration during exposure window

Table 5.3.6.4: Odds ratio for DCAA and personal consumption index on continuous scale for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated Increase in DCAA Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 10 | 226 (29) | 1.18 | 0.88-1.58 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average DCAA concentration during exposure window

Table 5.3.6.5: Odds ratio for MCAA and personal consumption index on continuous scale for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated Increase in MCAA Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 3 | 220 (28) | 1.28 | 0.80-2.04 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average MCAA concentration during exposure window

Table 5.3.6.6: Odds ratio for TCAA and personal consumption index on continuous scale for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated Increase in TCAA Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 3 | 227 (28) | 1.04 | 0.94-1.14 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average TCAA concentration during exposure window

Table 5.3.6.7: Odds ratios for TTHM and personal consumption index on categorical scale by tertiles for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated TTHM Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| No Exposure[†] | 127 (14) | 1.0 | 1.0 |
| T1 (>0-22.05) | 43 (10) | 2.44[±] | 1.02-5.88 |
| T2 (22.06-49.62) | 44 (5) | 1.03 | 0.36-3.00 |
| T3 (49.63-223.25) | 45 (7) | 1.48 | 0.57-3.88 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average TTHM concentration during exposure window

[†] Participants reported consuming only bottled water or average TTHM concentration was below detection limit during exposure window

[±]Statistically significant at the 95% confidence level

Table 5.3.6.8: Odds ratios for HAA5 and personal consumption index on categorical scale by tertiles for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated HAA5 Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| No Exposure[†] | 97 (10) | 1.0 | 1.0 |
| T1 (>0-14.2) | 34 (7) | 2.26 | 0.78-6.50 |
| T2 (14.21-31.68) | 40 (4) | 0.97 | 0.29-3.28 |
| T3 (31.68-150) | 42 (8) | 2.05 | 0.75-5.62 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average HAA5 concentration during exposure window

[†] Participants reported consuming only bottled water or average HAA5 concentration was below detection limit during exposure window

Table 5.3.6.9: Odds ratios for BCAA and personal consumption index on categorical scale by tertiles for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated BCAA Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| No Exposure[†] | 100 (9) | 1.0 | 1.0 |
| T1 (>0-1.14) | 33 (8) | 3.24[±] | 1.13-9.25 |
| T2 (1.15-2.67) | 39 (2) | 0.55 | 0.11-2.65 |
| T3 (2.67-15.02) | 41 (8) | 2.45 | 0.87-6.88 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average BCAA concentration during exposure window

[†] Participants reported consuming only bottled water or average BCAA concentration was below detection limit during exposure window

[±]Statistically significant at the 95% confidence level

Table 5.3.6.10: Odds ratios for DCAA and personal consumption index on categorical scale by tertiles for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated DCAA Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| No Exposure† | 102 (10) | 1.0 | 1.0 |
| T1 (>0-5.96) | 36 (7) | 2.22 | 0.78-6.36 |
| T2 (5.97-16.00) | 42 (4) | 0.97 | 0.29-3.28 |
| T3 (16.01-64) | 41 (8) | 2.23 | 0.81-6.13 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average DCAA concentration during exposure window

† Participants reported consuming only bottled water or average DCAA concentration was below detection limit during exposure window

Table 5.3.6.11: Odds ratios for MCAA and personal consumption index on categorical scale by tertiles for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated MCAA Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| No Exposure† | 180 (22) | 1.0 | 1.0 |
| T1 (>0-0.84) | 26 (6) | 2.16 | 0.78-5.95 |
| T2 (0.85-2.46) | 39 (5) | 1.06 | 0.37-2.99 |
| T3 (2.47-17) | 38 (7) | 1.62 | 0.64-4.13 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average MCAA concentration during exposure window

† Participants reported consuming only bottled water or average MCAA concentration was below detection limit during exposure window

Table 5.3.6.12: Odds ratios for TCAA and personal consumption index on categorical scale by tertiles for NBDPS participants, 1998-2002 (Ingestion Only)

| Estimated TCAA Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| No Exposure† | 171 (23) | 1.0 | 1.0 |
| T1 (>0-5.40) | 34 (5) | 1.11 | 0.39-3.16 |
| T2 (5.41-12.80) | 39 (5) | 0.95 | 0.34-2.67 |
| T3 (12.81-65) | 39 (7) | 1.41 | 0.56-3.56 |

*Estimated Dose=Glasses of unfiltered water consumed per day * average TCAA concentration during exposure window

† Participants reported consuming only bottled water or average TCAA concentration was below detection limit during exposure window

Table 5.3.6.13: Odds ratio for TTHM and multiple exposure index on continuous scale for NBDPS participants, 1998-2002 (Ingestion, Showering, Bathing)

| Estimated Increase in TTHM Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 20 | 258 (36) | 1.06± | 1.01-1.11 |

*Estimated Dose=Number of eight-ounce equivalent glasses from ingestion, bathing and showering * average TTHM concentration during exposure window

±Statistically significant at the 95% confidence level

Table 5.3.6.14: Odds ratios for TTHM and multiple exposure index on categorical scale by tertiles for NBDPS participants, 1998-2002 (Ingestion, Showering, Bathing)

| Estimated TTHM Dose (μg/Day) | N (Cases) | OR (compared to TTHM<20 μg/L Category)* | 95% Confidence Interval |
|---|---|---|---|
| No Exposure† | 65 (4) | 1.0 | 1.0 |
| T1 (>0 – 106.76) | 60 (9) | 2.69 | 0.78-9.25 |
| T2 (106.77-173.14) | 64 (10) | 2.82 | 0.84-9.52 |
| T3 (173.15-807.92) | 65 (12) | 3.45± | 1.05-11.35 |

Mantel Extension Chi Square = 3.8730, p-value = 0.0491

*Estimated Dose=Number of eight-ounce equivalent glasses from ingestion, bathing and showering * average TTHM concentration during exposure window

†Average TTHM concentration was below detection limit during exposure window

±Statistically significant at the 95% confidence level

## Chapter 6

## Conclusions

The overall goal of this dissertation was to examine methods for linking community water system (CWS) monitoring data to a specific time period during a woman's pregnancy while minimizing exposure misclassification in order to estimate risk of hypospadias. The specific objectives of this dissertation were to 1) conduct a feasibility study to see if a geographic information system (GIS) was effective in linking study participants to the CWS from which they receive their tap water, and validate this linkage by surveying individual CWS; 2) compare methods of exposure assessment that have been used in recent epidemiologic studies of disinfection by-products (DBPs), and; 3) conduct two population-based case-control studies of hypospadias, evaluating DBP exposure during a critical period of gestation, to determine if a relationship exists and whether magnitude of risk depends on exposure dose.

In the GIS feasibility study we were successful in geocoding 97% of the study participant addresses, which is slightly higher than the proportions geocoded in recent studies. When we went on to validate this method of linking particpants' addresses to the CWS from which they receive their tap water we found that the method correctly linked over 81% of the participants' addresses to a CWS. Overall, these results demonstrate that this method of determining exposure to DBPs can be effectively employed, even in rural states such as Arkansas.

When we compared the methods of exposure assessment we found that all five techniques produced results different from those yielded by using exposure window averages alone. Additionally, the inclusion of data on spatial variability seemed to be important, though the difference between the two metrics accounting for spatial variability (weighting metric and threshold metric) needs to be explored further. Finally, the comparison of exposure metrics seems to suggest the importance of personal water use/behavior data and the incorporation of exposure from dermal and inhalation routes as well as ingestion in epidemiologic studies of DBPs and adverse reproductive outcomes.

In our population-based case-control studies we found no association between hypospadias and DBPs for Arkansas Reproductive Health Monitoring System (ARHMS) participants in either the unadjusted or adjusted analyses. There was a suggestion of a weak association between hypospadias and total rihalomethanes (TTHMs) and bromochloroacetic acid (BCAA) for National Birth Defects Prevention Study (NBDPS) participants when water consumption data were included, though these association were found for only the lowest tertiles of exposure and may be due to chance. We found a statistically significant association for hypospadias and exposure to the highest tertile of TTHM for NBDPS when water-use behaviors were taken into account, supporting our *a priori* hypothesis that hypospadias is related to DBP exposure. This association was strengthened by the positive test for trend indicating a dose-response relationship.

Our results support previous findings that have found a positive relationship between exposure to DBPs and hypospadias, although this relationship only became clear when

we used exposure assessment techniques to estimate internal daily dose of DBPs. We found no statistically significant associations when we used DBP concentration alone in the exposure assessment. These results emphasize the importance of the availability of water-use and behavior data, especially regarding activities that lend to significant exposure via the ingestion, inhalation, and dermal routes of exposure, in epidemiologic studies of DBPs. Future research directions should include further evaluation of each of these routes of exposure and how they may contribute to internal dose of THMs and HAAs. In particular, future studies should focus on individual species of THMs and HAAs that have had significant findings in toxicological studies of reproductive outcomes. The findings in this dissertation can serve as a foundation to these studies and will provide some practical and theoretical guidance to many future epidemiologic investigations of DBPs and birth defects.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| 17-EE | 17-α-ethinyl estradiol |
| ADH | Arkansas Department of Health |
| AOR | Adjusted Odds Ratio |
| AR | Androgen Receptor |
| ARHMS | Arkansas Reproductive Health Monitoring System |
| AVG | Average |
| BDCM | Bromodichloromethane |
| BF | Bromoform |
| BMI | Body Mass Index |
| CATI | Computer-Assisted Telephone Interview |
| CBG | Census Block Group |
| CF | Chloroform |
| CI | Confidence Interval |
| CNS | Central Nervous System |
| CV | Coefficient of Variation |
| CWS | Community Water System |
| D/DBP | Disinfectant/Disinfection By-Product |
| DBAA | Dibromoacetic Acid |
| DBCM | Dibromochloromethane |
| DBP | Disinfection By-Product |
| DCAA | Dichloroacetic Acid |
| DDE | Dichlorodiphenyldichloroethylene |
| DDT | Dichlorodiphenyltrichloroethane |
| DES | Diethylstilbestrol |
| DHT | 5 α-dihydrotestosterone |
| GIS | Geographic Information System |
| GPS | Global Positioning System |
| HAA | Haloacetic Acid |
| HAA5 | The sum of five most prevalent haloacetic acids (MCAA, MBAA, DCAA, DBAA, TCAA) |
| HAN | Haloacetonitrile |
| HK | Haloketone |
| HSD17B3 | 17β-hydroxysteroid dehydrogenase gene type III |
| HSD3B2 | 3β-hydroxysteroid dehydrogenase gene type II |
| KM | Kilometer |
| MBAA | Monobromoacetic Acid |
| MCAA | Monochloroacetic Acid |
| MCL | Maximum Contaminant Level |
| MEI | Multiple Exposure Index |
| NBDPS | National Birth Defects Prevention Study |
| NTD | Neural Tube Defect |
| OR | Odds Ratio |
| PCI | Personal Consumption Index |

| | |
|---|---|
| PO | Post Office |
| POR | Prevalence Odds Ratio |
| R | Pearson's Correlation Coefficient |
| RR | Relative Risk |
| SES | Socio-Economic Status |
| STDEV | Standard Deviation |
| T | Testosterone |
| TCAA | Trichloroacetic Acid |
| TCE | Trichloroethylene |
| THM | Trihalomethane |
| TTHM | Total Trihalomethanes |
| USEPA | Unites States Environmental Protection Agency |

Appendix 1: Analyses of Potential Confounders from ARHMS Dataset for Use in Multivariate Model

Highlighted variables were included in initial multivariate model

| | | Variable Alone in Model | | | Variable + TTHM in Model | | | Variable + HAA5 in Model | | | Variable + DBAA in Model | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Unit | Odds Ratio | 95% Confidence Interval | P-Value | Odds Ratio | 95% Confidence Interval | P-Value | Odds Ratio | 95% Confidence Interval | P-Value | Odds Ratio | 95% Confidence Interval | P-Value |
| Maternal Age (years) | 5 | 1.077 | 0.99 – 1.17 | **0.0793** | 1.028 | 0.92-1.15 | 0.6411 | 1.059 | 0.93-1.20 | 0.3681 | 1.098 | 0.96-1.25 | **0.1656** |
| Paternal Age (years) | 5 | 1.047 | 0.97 – 1.13 | 0.2618 | 1.017 | 0.91-1.14 | 0.7769 | 1.041 | 0.92-1.18 | 0.5246 | 1.006 | 0.88-1.15 | 0.9311 |
| Maternal Race | 1 | 0.915 | 0.72 – 1.16 | 0.4613 | 0.829 | 0.60-1.14 | **0.2491** | 0.802 | 0.56-1.15 | **0.2337** | 0.8 | 0.54-1.18 | 0.2625 |
| Paternal Race | 1 | 1.298 | 0.99 – 1.69 | **0.0516** | 1.214 | 0.88-1.68 | **0.2422** | 1.28 | 0.91-1.79 | **0.152** | 1.329 | 0.92-1.93 | **0.1353** |
| Maternal Hispanic | 1 | 0.452 | 0.26 – 0.80 | **0.0063** | 0.618 | 0.32-1.20 | **0.1562** | 0.459 | 0.23-0.93 | **0.0314** | 0.524 | 0.25-1.08 | **0.0795** |
| Paternal Hispanic | 1 | 0.42 | 0.22 – 0.79 | **0.0074** | 0.47 | 0.21-1.04 | **0.0624** | 0.367 | 0.16-0.84 | **0.0184** | 0.534 | 0.24-1.21 | **0.1318** |
| Maternal Education (years) | 1 | 1.049 | 1.01 – 1.10 | **0.0298** | 1.052 | 0.99-1.12 | **0.091** | 1.073 | 1.01-1.14 | **0.0271** | 1.086 | 1.02-1.16 | **0.0124** |
| Paternal Education (years) | 1 | 1.037 | 0.99 – 1.09 | **0.1268** | 1.02 | 0.96-1.09 | 0.5465 | 1.05 | 0.98-1.12 | **0.1541** | 1.029 | 0.96-1.10 | 0.4306 |
| Marital Status | 1 | 0.862 | 0.70 – 1.07 | **0.174** | 0.887 | 0.66-1.20 | 0.4299 | 0.827 | 0.60-1.15 | **0.2536** | 0.885 | 0.63-1.25 | 0.4822 |
| 1-Minute Apgar Score | 1 | 0.822 | 0.77 – 0.88 | **<.0001** | 0.797 | 0.72-0.88 | **<0.0001** | 0.783 | 0.70-0.87 | **<0.001** | 0.782 | 0.70-0.88 | **<0.0001** |
| 5-Minute Apgar Score | 1 | 0.822 | 0.74 – 0.92 | **0.0005** | 0.781 | 0.67-0.92 | **0.0023** | 0.8 | 0.68-0.94 | **0.0059** | 0.809 | 0.68-0.97 | **0.022** |
| Weight Gain (pounds) | 5 | 1.028 | 0.99 – 1.07 | **0.1472** | 1.019 | 0.97-1.07 | 0.4718 | 1.028 | 0.97-1.09 | 0.3259 | 1.039 | 0.98-1.10 | **0.1871** |
| Birth Weight (grams) | 100 | 0.985 | 0.97 – 0.99 | **0.0142** | 0.99 | 0.97-1.01 | 0.2892 | 0.993 | 0.97-1.01 | 0.4884 | 0.992 | 0.99-1.01 | 0.4353 |
| Gestational Age (weeks) | 2 | 0.883 | 0.82 – 0.96 | **0.0018** | 0.863 | 0.77-0.97 | **0.0147** | 0.857 | 0.76-0.97 | **0.0145** | 0.817 | 0.71-0.94 | **0.0045** |
| Method of Delivery | 1 | 1.08 | 0.99 – 1.18 | 0.0907 | 1.13 | 0.99-1.28 | **0.0603** | 1.082 | 0.94-1.24 | 0.2565 | 1.076 | 0.93-1.24 | **0.2267** |
| Prenatal Care (yes/no) | 1 | 0.996 | 0.93 – 1.07 | 0.904 | 1.045 | 0.95-1.15 | 0.3665 | 1.043 | 0.94-1.16 | 0.4294 | 1.045 | 0.94-1.17 | 0.4353 |
| Number of Prenatal Care Visits | 1 | 1.009 | 0.99 – 1.03 | 0.4707 | 1.016 | 0.98-1.05 | 0.3634 | 1.008 | 0.97-1.05 | 0.688 | 1.008 | 0.97-1.05 | 0.7058 |

Appendix 1 (Cont.): Analyses of Potential Confounders from ARHMS Dataset for Use in Multivariate Model

| | | Variable Alone in Model | | | Variable + TTHM in Model | | | Variable + HAA5 in Model | | | Variable + DBAA in Model | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Unit** | **Odds Ratio** | **95% CI** | **P-Value** | **Odds Ratio** | **95% CI** | **P-Value** | **Odds Ratio** | **95% CI** | **P-Value** | **Odds Ratio** | **95% CI** | **P-Value** |
| Number of Previous Births Now Living | 1 | 0.947 | 0.87 – 1.04 | **0.2471** | 0.937 | 0.83-1.06 | 0.2956 | 0.913 | 0.75-1.05 | **0.203** | 0.895 | 0.78-1.03 | **0.1199** |
| Sum of Previous Live Births Now Living and Dead | 1 | 0.935 | 0.85 – 1.03 | **0.1707** | 0.923 | 0.81-1.05 | **0.2334** | 0.906 | 0.78-1.05 | **0.1859** | 0.889 | 0.77-1.03 | **0.1245** |
| Number of Terminations | 1 | 1 | 0.88 – 1.14 | 0.9967 | 1.099 | 0.90-1.34 | 0.3566 | 1.079 | 0.87-1.34 | 0.4855 | 1.201 | 0.97-1.49 | **0.0927** |
| Number of Previous Live Births Now Dead | 1 | 0.699 | 0.37 – 1.31 | 0.2633 | 0.777 | 0.33-1.81 | 0.5577 | 0.428 | 0.13-1.39 | **0.1577** | 0.469 | 0.15-1.52 | **0.2071** |
| Tobacco Use During Pregnancy (yes/no) | 1 | 1.101 | 0.85 – 1.43 | 0.4676 | 0.856 | 0.59-1.24 | 0.4109 | 0.807 | 0.54-1.20 | 0.2854 | 0.711 | 0.74-1.07 | **0.1033** |
| Number of Cigarettes per Day | 5 | 1.021 | 0.94 – 1.11 | 0.6243 | 1.122 | 0.99-1.28 | **0.0798** | 1.138 | 0.99-1.31 | **0.0628** | 1.153 | 1.00-1.33 | **0.0458** |
| Alcohol Use During Pregnancy | 1 | 0.507 | 0.17 – 1.52 | **0.2245** | 0.964 | 0.18-5.30 | 0.9667 | 0.72 | 0.12-4.36 | 0.7205 | 0.384 | 0.05-2.90 | 0.3538 |
| Number of Alcoholic Drinks per Day | 1 | 1.441 | 0.48 – 4.32 | 0.5136 | <0.001 | <0.001->999.99 | 0.8026 | <0.001 | <0.001->999.99 | 0.9255 | <0.001 | <0.001->999.99 | 0.8283 |
| Medical Risk Factors (yes/no) | 1 | 0.787 | 0.63 – 0.99 | **0.0405** | 0.747 | 0.54-1.03 | **0.0738** | 0.823 | 0.58-1.16 | 0.2668 | 0.837 | 0.58-1.21 | 0.3471 |
| Obstetric Procedures performed (yes/no) | 1 | 0.74 | 0.45 – 1.21 | **0.2304** | 1.269 | 0.70-2.32 | 0.4372 | 1.106 | 0.57-2.16 | 0.7669 | 1.252 | 0.63-2.50 | 0.5231 |
| Complications of Labor/Delivery (yes/no) | 1 | 0.793 | 0.64 – 0.99 | **0.0357** | 0.709 | 0.52-0.96 | **0.0263** | 0.731 | 0.53-1.01 | **0.0578** | 0.729 | 0.52-1.03 | **0.0727** |
| Abnormal Conditions of Newborn (yes/no) | 1 | 0.835 | 0.58 – 1.21 | 0.3419 | 0.74 | 0.45-1.21 | **0.2266** | 0.794 | 0.48-1.32 | 0.3756 | 0.826 | 0.48-1.42 | 0.4906 |

Appendix 2: Analyses of Potential Confounders from NBDPS Dataset for Use in Multivariate Model

| | | Variable Alone in Model | | | Variable + TTHM in Model | | | Variable + HAA in Model | | | Variable + DBAA in Model | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Unit | Odds Ratio | 95% CI | P-Value | Odds Ratio | 95% CI | P-Value | Odds Ratio | 95% CI | P-Value | Odds Ratio | 95% CI | P-Value |
| Mother's Height (CM) | 20 | 0.645 | 0.245-1.700 | 0.3757 | 0.527 | 0.20-1.41 | **0.203** | 0.662 | 0.22-1.98 | 0.4601 | 0.566 | 0.19-1.69 | 0.3074 |
| Mother's Weight (KG) | 5 | 1.03 | 0.940-1.129 | 0.5298 | 1.038 | 0.94-1.15 | 0.4559 | 1.044 | 0.95-1.15 | 0.3885 | 1.052 | 0.95-1.16 | 0.3174 |
| Body Mass Index | 3 | 1.075 | 0.927-1.247 | 0.3385 | 1.096 | 0.94-1.28 | **0.243** | 1.101 | 0.94-1.29 | **0.237** | 1.113 | 0.95-1.31 | **0.19** |
| Baby's Length (CM) | 5 | 1.01 | 0.476-2.143 | 0.98 | 1.034 | 0.47-2.26 | 0.934 | 0.878 | 0.39-1.97 | 0.7532 | 0.758 | 0.34-1.70 | 0.5016 |
| Baby's Head Circumference (CM) | 5 | 1.352 | 0.45-4.09 | 0.5931 | 1.421 | 0.45-4.46 | 0.5468 | 0.951 | 0.30-3.04 | 0.933 | 0.73 | 0.21-2.50 | 0.616 |
| Birth Weight (Grams) | 100 | 0.944 | 0.889-1.003 | **0.063** | 0.945 | 0.89-1.01 | **0.072** | 0.946 | 0.88-1.01 | **0.114** | 0.931 | 0.87-1.00 | **0.043** |
| Gestational Age (Weeks) | 2 | 0.971 | 0.702-1.343 | 0.859 | 0.999 | 0.71-1.40 | 0.9973 | 0.942 | 0.66-1.35 | 0.7445 | 0.907 | 0.64-1.30 | 0.5909 |
| Mother's Age (Years) | 5 | 1.149 | 0.869-1.518 | 0.3292 | 1.141 | 0.85-1.53 | 0.3755 | 1.169 | 0.85-1.62 | 0.3465 | 1.109 | 0.79-1.56 | 0.5511 |
| Number of Previous Pregnancies | 1 | 0.929 | 0.731-1.181 | 0.5483 | 0.961 | 0.76-1.22 | 0.7416 | 0.89 | 0.67-1.19 | 0.4298 | 0.941 | 0.70-1.26 | 0.6802 |
| Parity | 1 | 0.975 | 0.701-1.356 | 0.8806 | 1 | 0.72-1.39 | 0.9986 | 0.887 | 0.58-1.35 | 0.5712 | 0.915 | 0.60-1.39 | 0.6776 |
| Weight Before Pregnancy (LBs) | 10 | 1.027 | 0.944-1.116 | 0.5372 | 1.034 | 0.95-1.13 | 0.4597 | 1.039 | 0.95-1.14 | 0.3937 | 1.047 | 0.96-1.15 | 0.3196 |
| Weight Change During Pregnancy (Pounds) | 5 | 0.99 | 0.923-1.063 | 0.7825 | 0.989 | 0.92-1.06 | 0.7551 | 0.974 | 0.90-1.06 | 0.523 | 0.977 | 0.90-1.06 | 0.5869 |
| When Pregnancy Recognized (Weeks) | 1 | 1.011 | 0.931-1.098 | 0.7945 | 1.012 | 0.93-1.10 | 0.7838 | 0.972 | 0.87-1.08 | 0.6037 | 0.966 | 0.86-1.08 | 0.5383 |

Appendix 2 (Cont.): Analyses of Potential Confounders from NBDPS Dataset for Use in Multivariate Model

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Coffee Consumption During Pregnancy (cups/week) | 10 | 0.927 | 0.813-1.056 | 0.2555 | 0.943 | 0.82-1.08 | 0.3876 | 0.941 | 0.81-1.09 | 0.4154 | 0.975 | 0.84-1.13 | 0.7341 |
| Tea Consumption During Pregnancy (cups/week) | 10 | 1.009 | 0.930-1.094 | 0.8307 | 1.014 | 0.94-1.10 | 0.737 | 0.964 | 0.87-1.07 | 0.4987 | 0.985 | 0.89-1.10 | 0.7841 |
| Total Residences During Pregnancy | 1 | 0.868 | 0.558-1.350 | 0.5305 | 0.908 | 0.59-1.40 | 0.6641 | 0.862 | 0.53-1.40 | 0.5459 | 0.894 | 0.56-1.44 | 0.6428 |
| Wash/Rinse Dishes by Hand (Times/Week) | 3 | 1.036 | 0.91-1.18 | 0.6037 | 1.03 | 0.90-1.18 | 0.6704 | 0.99 | 0.85-1.16 | 0.9043 | 0.989 | 0.84-1.17 | 0.8974 |
| Wash Dishes with Auto. Dishwasher (Times/Week) | 3 | 1.03 | 0.797-1.331 | 0.8228 | 0.978 | 0.74-1.30 | 0.8758 | 1.006 | 0.77-1.32 | 0.6981 | 1.035 | 0.78-1.37 | 0.8089 |
| Wash Clothes by Hand (Times/Week) | 1 | 1.006 | 0.714-1.417 | 0.9715 | 1.007 | 0.72-1.40 | 0.9655 | 1.01 | 0.72-1.42 | 0.9555 | 0.999 | 0.69-1.44 | 0.9949 |
| Bathe Children (Times/Week) | 1 | 1.004 | 0.937-1.075 | 0.9198 | 1.012 | 0.95-1.09 | 0.7262 | 0.985 | 0.91-1.07 | 0.713 | 0.988 | 0.91-1.07 | 0.7705 |
| Bathe Pet (Times/Week) | 1 | 0.675 | 0.210-2.169 | 0.5088 | 0.733 | 0.25-2.12 | 0.5676 | 0.417 | 0.08-2.30 | 0.3152 | 0.526 | 0.11-2.61 | 0.4315 |
| Loads of Laundry in Machine (Loads/Week) | 5 | 0.904 | 0.632-1.293 | 0.5811 | 0.924 | 0.64-1.33 | 0.6726 | 0.909 | 0.63-1.31 | 0.6053 | 0.883 | 0.60-1.30 | 0.5279 |
| Number of Showers per Week | 1 | 1.085 | 1.01-1.16 | 0.0221 | 1.076 | 1.00-1.16 | 0.0432 | 1.115 | 1.03-1.21 | **0.008** | 1.106 | 1.10-1.20 | **0.016** |
| AVG Minutes Spent in Shower (for each shower event) | 10 | 1.019 | 0.978-1.062 | 0.3622 | 1.018 | 0.98-1.06 | 0.4156 | 1.026 | 0.98-1.07 | **0.232** | 1.023 | 0.98-1.07 | 0.2988 |
| Baths per Week | 1 | 0.992 | 0.906-1.086 | 0.8561 | 0.998 | 0.91-1.09 | 0.9661 | 1.003 | 0.91-1.11 | 0.9498 | 0.991 | 0.90-1.10 | 0.8601 |
| AVG Minutes Spent in Bath (for each bath event) | 15 | 0.999 | 0.951-1.049 | 0.9578 | 1.002 | 0.96-1.05 | 0.9351 | 1.006 | 0.96-1.06 | 0.829 | 1.005 | 0.95-1.06 | 0.8612 |

Appendix 2 (Cont.): Analyses of Potential Confounders from NBDPS Dataset for Use in Multivariate Model

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AVG Minutes Spent in Pool (for each pool visit) | 30 | 0.966 | 0.858-1.088 | 0.5686 | 0.962 | 0.86-1.08 | 0.5127 | 0.956 | 0.84-1.08 | 0.4797 | 0.953 | 0.84-1.08 | 0.4575 |
| Number of Times Used Pool in First Trimester | 1 | 0.97 | 0.92-1.03 | 0.3003 | 0.971 | 0.92-1.03 | 0.3009 | 0.976 | 0.92-1.03 | 0.3778 | 0.98 | 0.93-1.03 | **0.465** |
| Mother's Education (years) | 1 | 1.02 | 0.735-1.415 | 0.9072 | 0.989 | 0.71-1.38 | 0.9499 | 1.173 | 0.80-1.71 | 0.4068 | 1.161 | 0.79-1.70 | 0.442 |
| Birth Weight Category | 1 | 0.297 | 0.125-0.706 | **0.006** | 0.332 | 0.14-0.80 | **0.015** | 0.274 | 0.10-0.73 | **0.009** | 0.256 | 0.10-0.67 | **0.006** |
| Mother's Age Category | 1 | 1.114 | 0.862-1.440 | 0.4085 | 1.112 | 0.85-1.46 | 0.441 | 1.122 | 0.83-1.51 | 0.4477 | 1.069 | 0.78-1.46 | 0.6759 |
| Mother's Race | 1 | 0.632 | 0.295-1.354 | **0.238** | 0.616 | 0.29-1.33 | **0.217** | 0.611 | 0.27-1.40 | **0.244** | 0.637 | 0.28-1.46 | 0.2854 |
| Father's Race | 1 | 1.014 | 0.535-1.921 | 0.9659 | 0.999 | 0.52-1.90 | 0.9969 | 1.065 | 0.52-2.17 | 0.8627 | 1.066 | 0.52-2.17 | 0.8611 |
| Smoke during pregnancy (yes/no) | 1 | 0.973 | 0.450-2.105 | 0.9451 | 0.914 | 0.41-2.05 | 0.8273 | 0.988 | 0.42-2.35 | 0.9773 | 0.981 | 0.39-2.44 | 0.968 |
| Plurality | 1 | 22.667 | 2.462-208.650 | **0.006** | 22.94 | 2.49-211-65 | **0.006** | 16.221 | 1.63-161.57 | **0.018** | 17.841 | 1.78-178.68 | **0.014** |
| Mother Seizures during Pregnancy (Yes/No) | 1 | 1.546 | 0.316-7.558 | 0.5905 | 1.777 | 0.35-8.94 | 0.4854 | 1.534 | 0.31-7.60 | 0.6 | 2.289 | 0.44-12.05 | 0.3285 |
| Mother Resp. Illness during Pregnancy (Yes/no) | 1 | 1.147 | 0.570-2.309 | 0.7014 | 1.128 | 0.55-2.33 | 0.7439 | 0.817 | 0.38-1.74 | 0.6018 | 0.909 | 0.42-2.00 | 0.8128 |
| Mother Kidney, Bladder, Urinary Tract Infection during Pregnancy (yes/no) | 1 | 0.671 | 0.294-1.530 | 0.3425 | 0.651 | 0.27-1.56 | 0.3353 | 0.528 | 0.19-1.45 | **0.214** | 0.591 | 0.22-1.63 | 0.3085 |
| Mother Any Injury during Pregnancy (yes/no) | 1 | 0.927 | 0.306-2.814 | 0.8941 | 0.85 | 0.24-3.01 | 0.8015 | 0.892 | 0.25-3.20 | 0.8607 | 0.984 | 0.27-3.55 | 0.9804 |
| Mother Any Surgery during Pregnancy (yes/no) | 1 | 0.286 | 0.037-2.192 | **0.228** | 0.297 | 0.04-2.29 | **0.244** | 0.465 | 0.06-3.71 | 0.4703 | 0.491 | 0.06-3.92 | 0.5027 |

Appendix 2 (Cont.): Analyses of Potential Confounders from NBDPS Dataset for Use in Multivariate Model

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mother Any Scan during Pregnancy (yes/no) | 1 | 0.619 | 0.208-1.841 | 0.3881 | 0.444 | 0.13-1.53 | **0.197** | 1.049 | 0.34-3.27 | 0.9344 | 0.675 | 0.19-2.38 | 0.5412 |
| Previous Stillbirths | 1 | <0.001 | <0.001->999.99 | 0.9886 | <0.001 | <0.001->999.99 | 0.9901 | <0.001 | <0.001->999.99 | 0.9848 | <0.001 | <0.001->999.99 | 0.9854 |
| Previous Abortions | 1 | 0.649 | 0.187-2.248 | 0.4948 | 0.7 | 0.20-2.44 | 0.5763 | 0.505 | 0.11-2.27 | 0.3727 | 0.601 | 0.13-2.71 | 0.5074 |
| Previous Miscarriage | 1 | 1.041 | 0.467-2.322 | 0.9216 | 1.138 | 0.51-2.57 | 0.7554 | 1.104 | 0.46-2.63 | 0.8239 | 1.351 | 0.56-3.25 | 0.5018 |
| Any Birth Control | 1 | 0.905 | 0.408-2.011 | 0.8072 | 0.863 | 0.37-2.00 | 0.7308 | 1.265 | 0.53-3.04 | 0.5991 | 1.271 | 0.53-3.06 | 0.592 |
| Any Fertilization Treatment | 1 | 2.139 | 0.654-6.995 | **0.209** | 2.126 | 0.65-6.99 | **0.214** | 2.01 | 0.51-7.86 | 0.3157 | 2.09 | 0.54-8.13 | 0.2877 |
| Nausea During Pregnancy | 1 | 1.19 | 0.552-2.564 | 0.6577 | 1.188 | 0.55-2.59 | 0.6647 | 0.899 | 0.39-2.07 | 0.802 | 0.853 | 0.37-1.98 | 0.7123 |
| Ultrasound during Pregnancy | 1 | 0.517 | 0.117-2.286 | 0.3844 | 0.566 | 0.13-2.52 | 0.4546 | <0.001 | <0.001->999.99 | 0.9648 | <0.001 | <0.001->999.99 | 0.9621 |
| Blood Test During Pregnancy | 1 | 0.611 | 0.275-1.357 | **0.226** | 0.537 | 0.24-1.23 | **0.141** | 0.657 | 0.27-1.62 | 0.3615 | 0.6 | 0.24-1.53 | 0.2842 |
| Amniocentesis During Pregnancy | 1 | 1.767 | 0.354-8.825 | 0.4879 | 1.732 | 0.34-8.73 | 0.5058 | 0.988 | 0.12-8.50 | 0.9915 | 1.129 | 0.12-10.25 | 0.9143 |
| CVS | 1 | <0.001 | <0.001->999.99 | 0.9886 | <0.001 | <0.001->999.99 | 0.9886 | <0.001 | <0.001->999.99 | 0.9913 | <0.001 | <0.001->999.99 | 0.9917 |
| Multivitamin Use During Pregnancy | 1 | 2.594 | 1.178-5.714 | **0.018** | 2.281 | 1.01-5.15 | **0.048** | 2.674 | 1.06-6.73 | **0.037** | 2.639 | 1.06-6.60 | **0.038** |
| Vitamin A Supplement During Pregnancy (yes/no) | 1 | 2.051 | 0.208-20.223 | 0.5383 | 2.08 | 0.21-20.88 | 0.5339 | <0.001 | <0.001->999.99 | 0.9877 | <0.001 | <0.001->999.99 | 0.9881 |
| Beta carotene Supplement During Pregnancy (yes/no) | 1 | <0.001 | <0.001->999.99 | 0.9901 | <0.001 | <0.001->999.99 | 0.9901 | <0.001 | <0.001->999.99 | 0.9877 | <0.001 | <0.001->999.99 | 0.9881 |
| B6 Supplement During Pregnancy (yes/no) | 1 | <0.001 | <0.001->999.99 | 0.9886 | <0.001 | <0.001->999.99 | 0.9885 | <0.001 | <0.001->999.99 | 0.9849 | <0.001 | <0.001->999.99 | 0.9854 |
| B12 Supplement During Pregnancy (yes/no) | 1 | <0.001 | <0.001->999.99 | 0.9872 | <0.001 | <0.001->999.99 | 0.9885 | <0.001 | <0.001->999.99 | 0.9877 | <0.001 | <0.001->999.99 | 0.9881 |

Appendix 2 (Cont.): Analyses of Potential Confounders from NBDPS Dataset for Use in Multivariate Model

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Folic Acid Supplement During Pregnancy (yes/no) | 1 | 1.697 | 0.684-4.209 | 0.2539 | 1.762 | 0.70-4.41 | **0.226** | 1.845 | 0.68-5.01 | 0.2297 | 2.008 | 0.74-5.46 | **0.172** |
| Vitamin C Supplement During Pregnancy (yes/no) | 1 | 1.677 | 0.588-4.780 | 0.3336 | 1.764 | 0.61-5.10 | 0.2946 | 1.836 | <0.001->999.99 | 0.3792 | 1.9 | 0.50-7.27 | 0.3484 |
| Vitamin D Supplement During Pregnancy (yes/no) | 1 | <0.001 | <0.001->999.99 | 0.9901 | <0.001 | <0.001->999.99 | 0.9901 | <0.001 | <0.001->999.99 | 0.9877 | <0.001 | <0.001->999.99 | 0.9881 |
| Vitamin E Supplement During Pregnancy (yes/no) | 1 | 0.541 | 0.068-4.307 | 0.5615 | 0.536 | 0.07-4.28 | 0.5566 | 0.906 | 0.11-7.71 | 0.9281 | 0.943 | 0.11-7.95 | 0.9567 |
| Iron Supplement During Pregnancy (yes/no) | 1 | 1.019 | 0.497-2.088 | 0.9594 | 1.129 | 0.54-2.35 | 0.7468 | 1.116 | 0.50-2.51 | 0.7915 | 0.966 | 0.42-2.24 | 0.9349 |
| Calcium Supplement During Pregnancy | 1 | 1.304 | 0.467-3.642 | 0.6129 | 0.846 | 0.24-2.99 | 0.7958 | 1.789 | 0.61-5.22 | 0.2872 | 1.454 | 0.46-4.62 | 0.5257 |
| Zinc Supplement During Pregnancy (yes/no) | 1 | <0.001 | <0.001->999.99 | 0.9901 | <0.001 | <0.001->999.99 | 0.9901 | <0.001 | <0.001->999.99 | 0.9913 | <0.001 | <0.001->999.99 | 0.9881 |
| Prenatal Vitamin Supplement During Pregnancy | 1 | 0.731 | 0.152-3.511 | 0.695 | 0.728 | 0.15-3.54 | 0.6936 | 0.59 | 0.12-3.00 | 0.5242 | 0.666 | 0.14-3.24 | 0.6142 |
| Drink Soda During Pregnancy (yes/no) | 1 | 0.677 | 0.216-2.126 | 0.5041 | 0.683 | 0.22-2.16 | 0.5162 | 0.758 | 0.21-2.81 | 0.6784 | 0.676 | 0.18-2.53 | 0.5611 |
| Father Marijuana Use During Pregnancy (yes/no) | 1 | 1.022 | 0.371-2.814 | 0.967 | 1.149 | 0.41-3.20 | 0.791 | 0.763 | 0.22-2.71 | 0.6755 | 0.78 | 0.22-2.77 | 0.7002 |
| Mother Marijuana Use During Pregnancy (yes/no) | 1 | <0.001 | <0.001->999.99 | 0.975 | <0.001 | <0.001->999.99 | 0.977 | <0.001 | <0.001->999.99 | 0.9683 | <0.001 | <0.001->999.99 | 0.9684 |

Appendix 2 (Cont.): Analyses of Potential Confounders from NBDPS Dataset for Use in Multivariate Model

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Use Hot Tub During Pregnancy (yes/no) | 1 | 1.237 | 0.528-2.897 | 0.6251 | 1.087 | 0.44-2.69 | 0.8576 | 1.337 | 0.50-3.60 | 0.5646 | 1.077 | 0.38-3.05 | 0.8895 |
| Use Hot Bath During Pregnancy (yes/no) | 1 | 0.825 | 0.41-1.66 | 0.5884 | 0.747 | 0.36-1.54 | 0.4284 | 1.077 | 0.38-3.05 | 0.8895 | 0.678 | 0.30-1.52 | **0.344** |
| Use Sauna During Pregnancy (yes/no) | 1 | 0.78 | 0.093-6.519 | 0.8189 | 0.768 | 0.09-6.43 | 0.808 | <0.001 | <0.001->999.99 | 0.9779 | <0.001 | <0.001->999.99 | 0.9785 |
| Times Mother Used Pool in First Trimester | 1 | 0.987 | 0.478-2.038 | 0.9715 | 1.036 | 0.49-2.18 | 0.9264 | 1.334 | 0.62-2.87 | 0.4619 | 1.268 | 0.58-2.78 | 0.553 |
| Drink or Cook with Tap Water at Home | 1 | 0.972 | 0.393-2.405 | 0.9517 | 0.909 | 0.35-2.39 | 0.8459 | 1.103 | 0.44-2.79 | 0.8355 | 1.016 | 0.38-2.71 | 0.9754 |

Appendix 3a: Crude odds ratios and 95% confidence intervals for DBPs with exposure assessment on continuous scale using spline regression and weighting metrics for ARHMS and NBDPS participants 1998-2002

| | | ARHMS | | | NBDPS | | |
|---|---|---|---|---|---|---|---|
| Variable | Unit Increase (μg/L) | N (Cases) | Odds Ratio | 95% Confidence Interval | N (Cases) | Odds Ratio | 95% Confidence Interval |
| TTHM | 20 | 321 (102) | 1.16 | 0.83-1.64 | 108 (16) | 0.84 | 0.39-1.82 |
| HAA5 | 10 | 296 (95) | 1.10 | 0.90-1.34 | 93 (12) | 0.76 | 0.41-1.42 |
| BCAA | 1 | 223 (76) | 1.02 | 0.83-1.26 | 84 (10) | 0.89 | 0.53-1.52 |
| DBAA | 1 | 256 (85) | 0.97 | 0.76-1.24 | 90 (11) | 0.83 | 0.45-1.55 |
| DCAA | 5 | 256 (85) | 1.15 | 0.93-1.43 | 92 (12) | 0.90 | 0.49-1.65 |
| MBAA | 1 | 262 (87) | 1.01 | 0.80-1.28 | 93 (12) | 1.25 | 0.56-2.82 |
| MCAA | 1 | 247 (83) | 1.02 | 0.85-1.21 | 87 (12) | 0.82 | 0.50-1.35 |
| TCAA | 5 | 258 (84) | 1.10 | 0.90-1.34 | 93 (12) | 0.91 | 0.54-1.53 |

Appendix 3b: Odds ratios for TTHM and multiple exposure index on continuous scale with weighting metric for NBDPS participants, 1998-2002 (Ingestion, Showering, Bathing)

| Estimated Increase in TTHM Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 20 | 105 (15) | 1.02 | 0.91-1.14 |

*Estimated Dose=Number of eight-ounce equivalent glasses from ingestion, bathing and showering * average TTHM concentration during exposure window

Appendix 3c: Odds ratios for TTHM and multiple exposure index on categorical scale by tertiles with weighting metric for NBDPS participants, 1998-2002 (Ingestion, Showering, Bathing)

| Estimated TTHM Dose* (μg/Day) | N (Cases) | OR (compared to TTHM<20 μg/L Category) | 95% Confidence Interval |
|---|---|---|---|
| No Exposure[†] | 27 (3) | 1.0 | 1.0 |
| T1 (>0 – 106.76) | 17 (3) | 1.39 | 0.24-7.93 |
| T2 (106.77-173.14) | 36 (6) | 1.70 | 0.39-7.39 |
| T3 (173.15-807.92) | 25 (3) | 1.20 | 0.23-6.34 |

*Estimated Dose=Number of eight-ounce equivalent glasses from ingestion, bathing and showering * average TTHM concentration during exposure window

[†] Average TTHM concentration was below detection limit during exposure window

Appendix 4a: Evaluation of effect modification of HAA5 and maternal education with hypospadias for ARHMS participants 1998-2002

| HAA5 Concentration (μg /L) | Maternal Education Level (years) | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 10 | <12 | 1.174 | 0.95-1.45 |
| 10 | 12 | 0.862 | 0.75-0.99 |
| 10 | >12 | 0.633 | 0.43-0.94 |
| 30 | <12 | 1.887 | 0.89-4.01 |
| 30 | 12 | 1.018 | 0.73-1.42 |
| 30 | >12 | 0.549 | 0.32-0.94 |
| 50 | <12 | 2.228 | 0.78-6.34 |
| 50 | 12 | 1.030 | 0.59-1.78 |
| 50 | >12 | 0.476 | 0.23-0.97 |

Appendix 4b: Evaluation of effect modification of DBAA and maternal education with hypospadias for ARHMS participants 1998-2002

| DBAA Concentration (μg /L) | Maternal Education Level (years) | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 1.0 | <12 | 0.861 | 0.68-1.09 |
| 1.0 | 12 | 1.025 | 0.84-1.26 |
| 1.0 | >12 | 1.221 | 0.72-2.07 |
| 3.0 | <12 | 0.584 | 0.24-1.40 |
| 3.0 | 12 | 0.829 | 0.55-1.24 |
| 3.0 | >12 | 1.175 | 0.55-2.50 |
| 5.0 | <12 | 0.472 | 0.14-1.57 |
| 5.0 | 12 | 0.731 | 0.37-1.43 |
| 5.0 | >12 | 1.130 | 0.41-3.14 |

Appendix 4c: Evaluation of effect modification of DCAA and maternal education with hypospadias for ARHMS participants 1998-2002

| DCAA Concentration (μg /L) | Maternal Education Level (years) | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 10 | <12 | 1.173 | 0.78-1.77 |
| 10 | 12 | 1.029 | 0.83-1.27 |
| 10 | >12 | 0.902 | 0.72-1.13 |
| 15 | <12 | 1.271 | 0.68-2.36 |
| 15 | 12 | 1.043 | 0.76-1.43 |
| 15 | >12 | 0.856 | 0.61-1.20 |
| 20 | <12 | 1.376 | 0.60-3.14 |
| 20 | 12 | 1.058 | 0.69-1.62 |
| 20 | >12 | 0.813 | 0.52-1.28 |

Appendix 4d: Evaluation of effect modification of MBAA and maternal education with hypospadias for ARHMS participants 1998-2002

| MBAA Concentration (μg /L) | Maternal Education Level (years) | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 1.5 | <12 | 0.899 | 0.63-1.28 |
| 1.5 | 12 | 1.014 | 0.84-1.22 |
| 1.5 | >12 | 1.142 | 0.91-1.44 |
| 2.5 | <12 | 0.838 | 0.46-1.51 |
| 2.5 | 12 | 1.130 | 0.85-1.51 |
| 2.5 | >12 | 1.248 | 0.85-1.84 |
| 5.0 | <12 | 0.702 | 0.22-2.29 |
| 5.0 | 12 | 1.046 | 0.56-1.99 |
| 5.0 | >12 | 1.558 | 0.72-3.37 |

Appendix 4e: Evaluation of effect modification of MCAA and maternal education with hypospadias for ARHMS participants 1998-2002

| MCAA Concentration (μg /L) | Maternal Education Level (years) | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 1.0 | <12 | 1.038 | 0.83-1.29 |
| 1.0 | 12 | 0.983 | 0.88-1.10 |
| 1.0 | >12 | 0.932 | 0.82-1.06 |
| 3.0 | <12 | 1.118 | 0.58-2.15 |
| 3.0 | 12 | 0.950 | 0.68-1.32 |
| 3.0 | >12 | 0.808 | 0.55-1.19 |
| 5.0 | <12 | 1.204 | 0.40-3.58 |
| 5.0 | 12 | 0.919 | 0.53-1.59 |
| 5.0 | >12 | 0.701 | 0.37-1.33 |

Appendix 4f: Evaluation of effect modification of TCAA and maternal education with hypospadias for ARHMS participants 1998-2002

| TCAA Concentration (μg /L) | Maternal Education Level (years) | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 10 | <12 | 1.498 | 0.98-2.30 |
| 10 | 12 | 1.108 | 0.89-1.39 |
| 10 | >12 | 0.820 | 0.62-1.07 |
| 15 | <12 | 1.834 | 0.97-3.48 |
| 15 | 12 | 1.166 | 0.83-1.63 |
| 15 | >12 | 0.742 | 0.49-1.11 |
| 20 | <12 | 2.245 | 0.96-5.27 |
| 20 | 12 | 1.228 | 0.79-1.92 |
| 20 | >12 | 0.672 | 0.39-1.16 |

Appendix 5a: Evaluation of effect modification of TTHM and maternal race with hypospadias for NBDPS participants 1998-2002

| TTHM Concentration (μg /L) | Maternal Race | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 20 | White | 1.093 | 0.69-1.74 |
| 20 | Black | 0.640 | 0.23-1.76 |
| 20 | Other | 0.375 | 0.05-2.99 |
| 40 | White | 1.196 | 0.47-3.02 |
| 40 | Black | 0.410 | 0.05-3.09 |
| 40 | Other | 0.140 | 0.00-8.91 |
| 60 | White | 1.307 | 0.33-5.24 |
| 60 | Black | 0.262 | 0.01-5.44 |
| 60 | Other | 0.053 | 0.00-26.60 |

Appendix 5b: Evaluation of effect modification of HAA5 and maternal race with hypospadias for NBDPS participants 1998-2002

| HAA5 Concentration (μg /L) | Maternal Race | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 10 | White | 1.091 | 0.77-1.55 |
| 10 | Black | 0.732 | 0.32-1.68 |
| 10 | Other | 0.492 | 0.09-2.69 |
| 30 | White | 1.300 | 0.46-3.69 |
| 30 | Black | 0.393 | 0.03-4.74 |
| 30 | Other | 0.119 | <0.01-19.52 |
| 50 | White | 1.548 | 0.27-8.81 |
| 50 | Black | 0.211 | <0.01-13.37 |
| 50 | Other | 0.029 | <0.01-141.5 |

Appendix 5c: Evaluation of effect modification of BCAA and maternal race with hypospadias for NBDPS participants 1998-2002

| BCAA Concentration (μg /L) | Maternal Race | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 1.5 | White | 1.002 | 0.63-1.60 |
| 1.5 | Black | 0.420 | 0.07-2.68 |
| 1.5 | Other | 0.176 | <0.01-7.40 |
| 2.5 | White | 1.004 | 0.46-2.19 |
| 2.5 | Black | 0.114 | <0.01-11.94 |
| 2.5 | Other | 0.055 | <0.01-28.10 |
| 5.0 | White | 1.007 | 0.21-4.78 |
| 5.0 | Black | 0.056 | <0.01-26.63 |
| 5.0 | Other | 0.003 | <0.01-789.5 |

Appendix 5d: Evaluation of effect modification of DCAA and maternal race with hypospadias for NBDPS participants 1998-2002

| DCAA Concentration (μg /L) | Maternal Race | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 10 | White | 1.542 | 0.77-3.08 |
| 10 | Black | 0.685 | 0.15-3.13 |
| 10 | Other | 0.304 | 0.01-6.90 |
| 15 | White | 1.914 | 0.68-5.39 |
| 15 | Black | 0.567 | 0.06-5.53 |
| 15 | Other | 0.168 | <0.01-18.12 |
| 20 | White | 2.376 | 0.60-9.46 |
| 20 | Black | 0.469 | 0.02-9.78 |
| 20 | Other | 0.093 | <0.01-47.60 |

Appendix 5e: Evaluation of effect modification of MCAA and maternal race with hypospadias for NBDPS participants 1998-2002

| MCAA Concentration (μg /L) | Maternal Race | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 1.0 | White | 1.094 | 0.82-1.45 |
| 1.0 | Black | 0.335 | 0.08-1.39 |
| 1.0 | Other | 0.103 | <0.01-1.79 |
| 3.0 | White | 1.310 | 0.56-3.07 |
| 3.0 | Black | 0.038 | <0.01-2.69 |
| 3.0 | Other | 0.001 | <0.01-5374 |
| 5.0 | White | 1.569 | 0.38-6.48 |
| 5.0 | Black | 0.004 | <0.01-5.19 |
| 5.0 | Other | <0.001 | <0.01-18.38 |

Appendix 5f: Evaluation of effect modification of TCAA and maternal race with hypospadias for NBDPS participants 1998-2002

| TCAA Concentration (μg /L) | Maternal Race | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 10 | White | 1.115 | 0.58-2.13 |
| 10 | Black | 0.693 | 0.13-3.83 |
| 10 | Other | 0.430 | 0.01-13.65 |
| 15 | White | 1.178 | 0.45-3.11 |
| 15 | Black | 0.576 | 0.04-7.49 |
| 15 | Other | 0.282 | <0.01-50.41 |
| 20 | White | 1.244 | 0.34-4.53 |
| 20 | Black | 0.480 | 0.01-14.66 |
| 20 | Other | 0.185 | <0.01-186.2 |

Appendix 6a: Evaluation of effect modification of TTHM and maternal BMI with hypospadias for NBDPS participants 1998-2002

| TTHM Concentration (μg /L) | BMI | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 20 | 16 - 20.55 | 0.862 | 0.43-1.75 |
| 20 | 20.56 - 23.23 | 0.923 | 0.58-1.46 |
| 20 | 23.24 - 27.67 | 0.989 | 0.66-1.49 |
| 20 | 27.68 - 54.5 | 1.060 | 0.58-1.95 |
| 40 | 16 - 20.55 | 0.743 | 0.18-3.05 |
| 40 | 20.56 - 23.23 | 0.852 | 0.34-2.13 |
| 40 | 23.24 - 27.67 | 0.978 | 0.43-2.22 |
| 40 | 27.68 - 54.5 | 1.123 | 0.33-3.79 |
| 60 | 16 - 20.55 | 0.640 | 0.08-5.32 |
| 60 | 20.56 - 23.23 | 0.787 | 0.20-3.11 |
| 60 | 23.24 - 27.67 | 0.968 | 0.28-3.30 |
| 60 | 27.68 - 54.5 | 1.190 | 0.19-7.38 |

Appendix 6b: Evaluation of effect modification of between HAA5 and maternal BMI with hypospadias for NBDPS participants 1998-2002

| HAA5 Concentration (μg /L) | BMI | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 10 | 16 - 20.55 | 1.155 | 0.61-2.19 |
| 10 | 20.56 - 23.23 | 1.068 | 0.72-1.59 |
| 10 | 23.24 - 27.67 | 0.988 | 0.72-1.37 |
| 10 | 27.68 - 54.5 | 0.914 | 0.55-1.51 |
| 30 | 16 - 20.55 | 1.539 | 0.23-10.49 |
| 30 | 20.56 - 23.23 | 1.219 | 0.37-4.01 |
| 30 | 23.24 - 27.67 | 0.965 | 0.37-2.54 |
| 30 | 27.68 - 54.5 | 0.765 | 0.17-3.42 |
| 50 | 16 - 20.55 | 2.052 | 0.08-50.23 |
| 50 | 20.56 - 23.23 | 1.391 | 0.19-10.11 |
| 50 | 23.24 - 27.67 | 0.943 | 0.19-4.74 |
| 50 | 27.68 - 54.5 | 0.639 | 0.05-7.77 |

Appendix 6c: Evaluation of effect modification of between BCAA and maternal BMI with hypospadias for NBDPS participants 1998-2002

| BCAA Concentration (μg /L) | BMI | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 1.5 | 16 - 20.55 | 0.663 | 0.22-1.96 |
| 1.5 | 20.56 - 23.23 | 0.772 | 0.38-1.55 |
| 1.5 | 23.24 - 27.67 | 0.899 | 0.56-1.45 |
| 1.5 | 27.68 - 54.5 | 1.047 | 0.56-1.95 |
| 2.5 | 16 - 20.55 | 0.504 | 0.08-3.07 |
| 2.5 | 20.56 - 23.23 | 0.737 | 0.29-1.85 |
| 2.5 | 23.24 - 27.67 | 0.837 | 0.38-1.85 |
| 2.5 | 27.68 - 54.5 | 0.719 | 0.27-1.88 |
| 5.0 | 16 - 20.55 | 0.254 | <0.01-9.45 |
| 5.0 | 20.56 - 23.23 | 0.421 | 0.04-4.35 |
| 5.0 | 23.24 - 27.67 | 0.700 | 0.14-3.42 |
| 5.0 | 27.68 - 54.5 | 1.164 | 0.14-9.20 |

Appendix 6d: Evaluation of effect modification of DBAA and maternal BMI with hypospadias for NBDPS participants 1998-2002

| DBAA Concentration (μg /L) | BMI | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 1.0 | 16 - 20.55 | 0.538 | 0.09-3.36 |
| 1.0 | 20.56 - 23.23 | 0.632 | 0.19-2.10 |
| 1.0 | 23.24 - 27.67 | 0.743 | 0.38-1.44 |
| 1.0 | 27.68 - 54.5 | 0.872 | 0.49-1.57 |
| 3.0 | 16 - 20.55 | 0.156 | <0.01-37.91 |
| 3.0 | 20.56 - 23.23 | 0.253 | <0.01-9.31 |
| 3.0 | 23.24 - 27.67 | 0.410 | 0.06-3.01 |
| 3.0 | 27.68 - 54.5 | 0.663 | 0.11-3.85 |
| 5.0 | 16 - 20.55 | 0.045 | <0.01-427.9 |
| 5.0 | 20.56 - 23.23 | 0.101 | <0.01-41.19 |
| 5.0 | 23.24 - 27.67 | 0.226 | <0.01-6.27 |
| 5.0 | 27.68 - 54.5 | 0.505 | 0.03-9.46 |

Appendix 6e: Evaluation of effect modification of DCAA and maternal BMI with hypospadias for NBDPS participants 1998-2002

| DCAA Concentration (μg /L) | BMI | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 10 | 16 - 20.55 | 1.366 | 0.42-4.47 |
| 10 | 20.56 - 23.23 | 1.313 | 0.62-2.79 |
| 10 | 23.24 - 27.67 | 1.261 | 0.69-2.32 |
| 10 | 27.68 - 54.5 | 1.211 | 0.49-2.99 |
| 15 | 16 - 20.55 | 1.597 | 0.27-9.45 |
| 15 | 20.56 - 23.23 | 1.504 | 0.49-4.66 |
| 15 | 23.24 - 27.67 | 1.416 | 0.57-3.53 |
| 15 | 27.68 - 54.5 | 1.333 | 0.34-5.16 |
| 20 | 16 - 20.55 | 1.867 | 0.17-19.98 |
| 20 | 20.56 - 23.23 | 1.723 | 0.38-7.77 |
| 20 | 23.24 - 27.67 | 1.590 | 0.47-5.37 |
| 20 | 27.68 - 54.5 | 1.467 | 0.24-8.92 |

Appendix 6f: Evaluation of effect modification of MBAA and maternal BMI with hypospadias for NBDPS participants 1998-2002

| MBAA Concentration (μg/L) | BMI | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 1.5 | 16 - 20.55 | 1.205 | 0.36-4.02 |
| 1.5 | 20.56 - 23.23 | 1.088 | 0.50-2.38 |
| 1.5 | 23.24 - 27.67 | 0.982 | 0.53-1.81 |
| 1.5 | 27.68 - 54.5 | 0.887 | 0.38-2.08 |
| 2.5 | 16 - 20.55 | 1.364 | 0.18-10.15 |
| 2.5 | 20.56 - 23.23 | 1.056 | 0.36-3.09 |
| 2.5 | 23.24 - 27.67 | 0.970 | 0.35-2.68 |
| 2.5 | 27.68 - 54.5 | 0.818 | 0.20-3.40 |
| 5.0 | 16 - 20.55 | 1.859 | 0.03-103.1 |
| 5.0 | 20.56 - 23.23 | 1.323 | 0.10-17.90 |
| 5.0 | 23.24 - 27.67 | 0.941 | 0.12-7.16 |
| 5.0 | 27.68 - 54.5 | 0.669 | 0.04-11.55 |

Appendix 6g: Evaluation of effect modification of MCAA and maternal BMI with hypospadias for NBDPS participants 1998-2002

| MCAA Concentration (μg/L) | BMI | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 1.0 | 16 - 20.55 | 1.872 | 1.05-3.35 |
| 1.0 | 20.56 - 23.23 | 1.302 | 0.89-1.89 |
| 1.0 | 23.24 - 27.67 | 0.905 | 0.67-1.22 |
| 1.0 | 27.68 - 54.5 | 0.629 | 0.41-0.97 |
| 3.0 | 16 - 20.55 | 6.561 | 1.15-37.59 |
| 3.0 | 20.56 - 23.23 | 2.205 | 0.72-6.80 |
| 3.0 | 23.24 - 27.67 | 0.741 | 0.30-1.84 |
| 3.0 | 27.68 - 54.5 | 0.249 | 0.07-0.92 |
| 5.0 | 16 - 20.55 | 22.996 | 1.25-421.8 |
| 5.0 | 20.56 - 23.23 | 3.737 | 0.57-24.38 |
| 5.0 | 23.24 - 27.67 | 0.607 | 0.13-2.76 |
| 5.0 | 27.68 - 54.5 | 0.099 | 0.01-0.88 |

Appendix 6h: Evaluation of effect modification of TCAA and maternal BMI with hypospadias for NBDPS participants 1998-2002

| TCAA Concentration (μg/L) | BMI | Odds Ratio | 95% Confidence Interval |
|---|---|---|---|
| 10 | 16 - 20.55 | 1.140 | 0.38-3.38 |
| 10 | 20.56 - 23.23 | 1.064 | 0.54-2.11 |
| 10 | 23.24 - 27.67 | 0.994 | 0.53-1.88 |
| 10 | 27.68 - 54.5 | 0.928 | 0.34-2.50 |
| 15 | 16 - 20.55 | 1.217 | 0.24-6.21 |
| 15 | 20.56 - 23.23 | 1.098 | 0.39-3.07 |
| 15 | 23.24 - 27.67 | 0.991 | 0.38-2.57 |
| 15 | 27.68 - 54.5 | 0.894 | 0.20-3.95 |
| 20 | 16 - 20.55 | 1.300 | 0.15-11.40 |
| 20 | 20.56 - 23.23 | 1.133 | 0.29-4.46 |
| 20 | 23.24 - 27.67 | 0.988 | 0.28-3.52 |
| 20 | 27.68 - 54.5 | 0.861 | 0.12-6.25 |

Appendix 7a: Crude odds ratios and 95% confidence intervals for DBPs with exposure assessment on continuous scale for ARHMS and NBDPS participants 1998-2002
EXPOSURE WINDOW: 8-14 WEEKS

| | | ARHMS | | NBDPS | |
|---|---|---|---|---|---|
| Variable | Unit Increase (μg/L) | Odds Ratio | 95% Confidence Interval | Odds Ratio | 95% Confidence Interval |
| TTHM | 20 | 1.05 | 0.94-1.17 | 1.03 | 0.75-1.40 |
| HAA5 | 10 | 1.03 | 0.95-1.12 | 0.92 | 0.71-1.18 |
| BCAA | 1 | 0.94 | 0.83-1.05 | 0.99 | 0.79-1.24 |
| DBAA | 1 | 0.93 | 0.83-1.03 | 0.99 | 0.82-1.19 |
| DCAA | 5 | 1.03 | 0.95-1.10 | 1.02 | 0.82-1.28 |
| MBAA | 1 | 1.02 | 0.93-1.12 | 0.92 | 0.70-1.21 |
| MCAA | 1 | 0.98 | 0.91-1.07 | 1.11 | 0.89-1.38 |
| TCAA | 5 | 1.04 | 0.96-1.13 | 0.97 | 0.77-1.23 |

Appendix 7b: Crude odds ratios and 95% confidence intervals for DBPs with exposure assessment on categorical scale by quartiles for ARHMS and NBDPS participants 1998-2002

EXPOSURE WINDOW: 8-14 WEEKS

| | ARHMS | | | NBDPS | | |
|---|---|---|---|---|---|---|
| Variable | Concentration (μg/L) | Odds Ratio | 95% Confidence Interval | Concentration (μg/L) | Odds Ratio | 95% Confidence Interval |
| TTHM | <26.5 | 1.0 | 1.0 | <27.86 | 1.0 | 1.0 |
| | 26.5-41.6 | 1.07 | 0.74-1.54 | 27.86-41.73 | 0.67 | 0.25-1.81 |
| | 41.6-55.8 | 0.88 | 0.61-1.28 | 41.73-54.07 | 1.03 | 0.41-2.55 |
| | 55.8-243 | 1.20 | 0.84-1.72 | 54.07-145 | 1.13 | 0.46-2.75 |
| HAA5 | <16.99 | 1.0 | 1.0 | <17.36 | 1.0 | 1.0 |
| | 16.99-28.2 | 1.01 | 0.71-1.46 | 17.36-28.27 | 0.63 | 0.24-1.67 |
| | 28.2-35.9 | 1.01 | 0.71-1.44 | 28.27-32.95 | 0.51 | 0.18-1.45 |
| | 35.9-160 | 1.12 | 0.28-1.60 | 32.95-110 | 1.14 | 0.49-2.64 |
| BCAA | <1.41 | 1.0 | 1.0 | <1.41 | 1.0 | 1.0 |
| | 1.41-2.03 | 1.29 | 0.90-1.86 | 1.41-2.07 | 0.30 | 0.09-1.06 |
| | 2.03-2.64 | 1.14 | 0.79-1.66 | 2.07-2.62 | 0.85 | 0.35-2.08 |
| | 2.64-10 | 0.82 | 0.56-1.20 | 2.62-11 | 1.14 | 0.49-2.64 |
| DBAA | 0 | 1.0 | 1.0 | 0 | 1.0 | 1.0 |
| | >0 | 0.84 | 0.57-1.23 | >0 | 0.72 | 0.27-1.95 |
| DCAA | <7.84 | 1.0 | 1.0 | <5.63 | 1.0 | 1.0 |
| | 7.84-14.61 | 1.01 | 0.70-1.45 | 5.63-13.75 | 0.91 | 0.34-2.26 |
| | 14.61-18.91 | 1.05 | 0.73-1.51 | 13.75-17.08 | 0.53 | 0.18-1.51 |
| | 18.91-108 | 1.02 | 0.71-1.48 | 17.08-58 | 1.14 | 0.49-2.68 |
| MBAA | 0 | 1.0 | 1.0 | 0 | 1.0 | 1.0 |
| | >0 | 0.85 | 0.60-1.20 | >0 | 0.60 | 0.25-1.42 |
| MCAA | 0 | 1.0 | 1.0 | <0.5 | 1.0 | 1.0 |
| | 0-1.93 | 1.34 | 0.94-1.91 | 0.5-1.79 | 1.51 | 0.39-5.79 |
| | 1.93-3.21 | 0.84 | 0.57-1.22 | 1.79-2.74 | 0.88 | 0.35-2.20 |
| | 3.21-12 | 0.99 | 0.68-1.44 | 2.74-8 | 1.01 | 0.42-2.41 |
| TCAA | <3.70 | 1.0 | 1.0 | <2.49 | 1.0 | 1.0 |
| | 3.70-10.86 | 1.05 | 0.73-1.52 | 2.49-10.70 | 0.43 | 0.15-1.21 |
| | 10.86-14.82 | 1.18 | 0.82-1.71 | 10.70-13.60 | 0.52 | 0.20-1.39 |
| | 14.82-70 | 1.17 | 0.82-1.69 | 13.60-45 | 0.82 | 0.35-1.93 |

Appendix 7c: Odds ratios for TTHM and multiple exposure index on continuous scale for NBDPS participants, 1998-2002 (Ingestion, Showering, Bathing)
EXPOSURE WINDOW: 8-14 WEEKS

| Estimated Increase in TTHM Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 20 | 257 (36) | 1.06± | 1.02-1.29 |

*Estimated Dose=Number of eight-ounce equivalent glasses from ingestion, bathing and showering * average TTHM concentration during exposure window
±Statistically significant at the 95% confidence level

Appendix 7d: Odds ratios for TTHM and multiple exposure index on categorical scale by tertiles for NBDPS participants, 1998-2002 (Ingestion, Showering, Bathing)
EXPOSURE WINDOW: 8-14 WEEKS

| Estimated TTHM Dose (μg/Day) | N (Cases) | OR (compared to TTHM<20 μg/L Category)* | 95% Confidence Interval |
|---|---|---|---|
| No Exposure† | 64 (4) | 1.0 | 1.0 |
| T1 (>0 – 106.76) | 62 (9) | 2.55 | 0.74-8.75 |
| T2 (106.77-173.14) | 62 (10) | 2.88 | 0.85-9.74 |
| T3 (173.15-807.92) | 65 (12) | 3.40± | 1.03-11.16 |

Mantel Extension Chi Square = 3.9305, p-value = 0.0474
*Estimated Dose=Number of eight-ounce equivalent glasses from ingestion, bathing and showering * average TTHM concentration during exposure window
† Average TTHM concentration was below detection limit during exposure window
±Statistically significant at the 95% confidence level

Appendix 8a: Crude odds ratios and 95% confidence intervals for DBPs with exposure assessment on continuous scale for ARHMS and NBDPS participants 1998-2002
EXPOSURE WINDOW: 10-12 WEEKS

| | | ARHMS | | NBDPS | |
|---|---|---|---|---|---|
| Variable | Unit Increase (μg/L) | Odds Ratio | 95% Confidence Interval | Odds Ratio | 95% Confidence Interval |
| TTHM | 20 | 1.05 | 0.94-1.17 | 1.03 | 0.76-1.40 |
| HAA5 | 10 | 1.03 | 0.95-1.12 | 0.92 | 0.71-1.18 |
| BCAA | 1 | 0.94 | 0.84-1.06 | 1.00 | 0.80-1.25 |
| DBAA | 1 | 0.94 | 0.84-1.05 | 0.99 | 0.82-1.20 |
| DCAA | 5 | 1.03 | 0.96-1.11 | 1.02 | 0.82-1.27 |
| MBAA | 1 | 1.03 | 0.95-1.12 | 0.93 | 0.73-1.19 |
| MCAA | 1 | 0.99 | 0.91-1.07 | 1.09 | 0.87-1.35 |
| TCAA | 5 | 1.05 | 0.97-1.14 | 0.97 | 0.76-1.23 |

Appendix 8b: Crude odds ratios and 95% confidence intervals for DBPs with exposure assessment on categorical scale by quartiles for ARHMS and NBDPS participants 1998-2002

EXPOSURE WINDOW: 10-12 WEEKS

| | ARHMS | | | NBDPS | | |
|---|---|---|---|---|---|---|
| Variable | Concentration (μg/L) | Odds Ratio | 95% Confidence Interval | Concentration (μg/L) | Odds Ratio | 95% Confidence Interval |
| TTHM | <26.55 | 1.0 | 1.0 | <27.50 | 1.0 | 1.0 |
| | 26.55-41.53 | 1.08 | 0.75-1.55 | 27.50-41.64 | 0.85 | 0.32-2.26 |
| | 41.53-56.01 | 0.88 | 0.61-1.28 | 41.64-53.93 | 1.26 | 0.51-3.11 |
| | 56.01-240 | 1.23 | 0.85-1.76 | 53.93-144 | 1.10 | 0.44-2.78 |
| HAA5 | <17.5 | 1.0 | 1.0 | <17.54 | 1.0 | 1.0 |
| | 17.5-28.13 | 1.03 | 0.71-1.47 | 17.54-28.37 | 0.65 | 0.24-1.72 |
| | 28.13-36.19 | 0.99 | 0.69-1.42 | 38.37-32.98 | 0.65 | 0.24-1.72 |
| | 36.19-160 | 1.10 | 0.77-1.57 | 32.98-111 | 1.01 | 0.42-2.40 |
| BCAA | <1.41 | 1.0 | 1.0 | <1.41 | 1.0 | 1.0 |
| | 1.41-2.03 | 1.33 | 0.92-1.91 | 1.41-2.06 | 0.19 | 0.04-0.84 |
| | 2.03-2.65 | 1.14 | 0.79-1.66 | 2.06-2.61 | 0.85 | 0.35-2.08 |
| | 2.65-10 | 0.84 | 0.57-1.24 | 2.61-11 | 1.11 | 0.48-2.56 |
| DBAA | 0 | 1.0 | 1.0 | 0 | 1.0 | 1.0 |
| | >0 | 0.82 | 0.53-1.27 | >0 | 0.81 | 0.30-2.21 |
| DCAA | <7.90 | 1.0 | 1.0 | <5.59 | 1.0 | 1.0 |
| | 7.90-14.59 | 0.99 | 0.69-1.44 | 5.59-13.77 | 0.79 | 0.31-2.03 |
| | 14.59-19.11 | 1.06 | 0.73-1.53 | 13.77-17.11 | 0.66 | 0.25-1.79 |
| | 19.11-110 | 0.99 | 0.69-1.44 | 17.11-58 | 1.18 | 0.50-2.77 |
| MBAA | 0 | 1.0 | 1.0 | 0 | 1.0 | 1.0 |
| | >0 | 0.99 | 0.65-1.50 | >0 | 0.28 | 0.07-1.21 |
| MCAA | <0.5 | 1.0 | 1.0 | <0.5 | 1.0 | 1.0 |
| | 0.5-1.94 | 1.31 | 0.92-1.87 | 0.5-1.79 | 0.92 | 0.37-2.26 |
| | 1.94-3.29 | 0.88 | 0.60-1.28 | 1.79-2.80 | 0.66 | 0.25-1.77 |
| | 3.29-12 | 0.99 | 0.68-1.44 | 2.80-8 | 0.92 | 0.37-2.26 |
| TCAA | <3.82 | 1.0 | 1.0 | <2.48 | 1.0 | 1.0 |
| | 3.82-10.84 | 0.95 | 0.65-1.39 | 2.48-10.83 | 0.45 | 0.16-1.26 |
| | 10.84-14.93 | 1.24 | 0.86-1.79 | 10.83-13.58 | 0.35 | 0.11-1.08 |
| | 14.93-70 | 1.13 | 0.78-1.63 | 13.58-46 | 1.08 | 0.48-2.46 |

Appendix 8c: Odds ratios for TTHM and multiple exposure index on continuous scale for NBDPS participants, 1998-2002 (Ingestion, Showering, Bathing)
EXPOSURE WINDOW: 10-12 WEEKS

| Estimated Increase in TTHM Dose * (μg/Day) | N (Cases) | OR | 95% Confidence Interval |
|---|---|---|---|
| 20 | 257 (36) | 1.06± | 1.01-1.30 |

*Estimated Dose=Number of eight-ounce equivalent glasses from ingestion, bathing and showering * average TTHM concentration during exposure window
±Statistically significant at the 95% confidence level

Appendix 8d: Odds ratios for TTHM and multiple exposure index on categorical scale by tertiles for NBDPS participants, 1998-2002 (Ingestion, Showering, Bathing)
EXPOSURE WINDOW: 10-12 WEEKS

| Estimated TTHM Dose (μg/Day) | N (Cases) | OR (compared to TTHM<20 μg/L Category)* | 95% Confidence Interval |
|---|---|---|---|
| No Exposure† | 64 (4) | 1.0 | 1.0 |
| T1 (>0 – 106.76) | 62 (9) | 2.55 | 0.74-8.75 |
| T2 (106.77-173.14) | 60 (10) | 3.00 | 0.89-10.15 |
| T3 (173.15-807.92) | 66 (12) | 3.33± | 1.01-10.95 |

Mantel Extension Chi Square = 3.8518, p-value = 0.0497
*Estimated Dose=Number of eight-ounce equivalent glasses from ingestion, bathing and showering * average TTHM concentration during exposure window
† Average TTHM concentration was below detection limit during exposure window
±Statistically significant at the 95% confidence level