PERICONCEPTIONAL EXPOSURE TO TRIHALOMETHANES IN DRINKING WATER AND THE RISK OF NEURAL TUBE DEFECTS

THESIS

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DEDICATION

This thesis is dedicated in memory of my mother Carolyn for giving me the courage and strength to complete my goals. I will always remember your unfailing love and support. My only wish is that you could have lived long enough to see me graduate.

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TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

PERICONCEPTIONAL EXPOSURE TO TRIHALOMETHANES IN DRINKING WATER AND THE RISK OF NEURAL TUBE DEFECTS

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Trihalomethanes (THMs) are disinfection by-products found in drinking water that result when chlorine reacts with organic matter. An association between THMs and neural tube defects (NTDs) has been suggested in other studies. Using a case-control

design, this study investigated the relation between NTDs and THMs in offspring of Mexican-American women living in the 14 Texas counties bordering Mexico. Participants in the study, 184 case-women with an NTD-affected pregnancy and 225 control-women, were interviewed about their environmental and occupational exposures during the periconceptional period. Public drinking water monitoring data was matched to the mother's residence at the time of conception. Odds ratios were suggestive of an association among women with an NTD-affected pregnancy and periconceptional exposure to drinking water with THM levels greater than or equal to 50 μg/liter (OR=2.36); however, 95% confidence intervals were consistent with unity. Odds ratios for THM levels ≥ 50 μg/liter were also elevated for women who ingested tap water as opposed to bottled water (OR= 3.6, 95% CI 1.1-12.3). Logistic regression models examining THMs and other potential risk factors were not significant (p> 0.05). The study was limited by sparse drinking water THM monitoring data with very few case- and control-women in the high exposure categories. Further studies are warranted to investigate individual compounds of THMs as opposed to total THMs in an attempt to understand if individual compounds render exposed women more susceptible to NTD-affected pregnancies in this study population. The relation between THM exposure and source of drinking water (tap versus bottled water) also deserves further exploration.

CHAPTER 1

INTRODUCTION

Neural tube defects (NTDs) are serious birth defects of the spine and brain that result when the neural tube fails to close during the early weeks of gestation. Abnormal closure of the neural tube can occur anywhere along the neuroaxis from the brain down the length of the spinal cord and often results in neural tissue being exposed. The consequences are devastating birth defects such as anencephaly, spina bifida and encephalocele. It is believed a combination of genetics and environment play a role in the causation of NTDs. Previous epidemiological studies have associated NTD prevalence with a variety of factors; however, the exact causes of NTDs remain unclear.

One risk factor that has emerged consistently is an increased incidence of NTDs among Hispanics (Canfield, Annegers, Brender, Cooper & Greenberg, 1996; Hendricks, Simpson & Larsen, 1999; Shaw, Velie & Wasserman, 1997). For example, during the years 1993-95, in the fourteen Texas-Mexico border counties, prevalence of NTDs among Hispanic women in Texas (14.9 per 10,000) was significantly higher than the prevalence for Anglo women (10.6 per 10,000) (Hendricks, et al., 1999). The risk for Hispanics remained high, even after controlling for other NTD risk factors such as obesity and diabetes (Canfield, et al 1996; Shaw et al., 1997).

Because of the high rates of NTDs in the Texas-Mexico border counties, an area in which the majority of residents are Mexican-American, researchers with the Texas

Neural Tube Defect Project (TNTDP) have focused on identifying risk factors within this ethnic group. A major finding is that folic acid supplementation, known to dramatically reduce NTD risk, is essentially nonexistent in this population (Suarez, Hendricks, Cooper, Sweeney, Hardy & Larsen, 2000). Other risk factors that have been identified include: maternal occupational exposures where case-women were more likely to have exposures to solvents than control-women (OR = ∞) (Brender, Suarez, Hendricks, Baetz and Larsen, 2002), low levels of serum B_{12} (adjusted OR = 2.6) (Suarez, Hendricks, Felkner & Gunter, 2003), one or more stressful life events during the year before conception (OR = 2.9) (Suarez, Cardarelli & Hendricks, 2003), one or more episodes of periconceptional diarrhea (OR = 3.7) (Felkner, Hendricks, Suarez & Waller, 2003) and a strong NTD risk association found in women with periconceptional nitrosatable drug exposure coupled with a higher intake of dietary nitrite (OR = 7.5) (Brender, Olive, Felkner, Suarez, Marckwardt & Hendricks, 2004).

One environmental exposure that has not been explored on the Texas-Mexico border is trihalomethanes in drinking water. Exposures to chlorinated surface waters with high levels of organic matter have been associated with increased risk for NTD occurrence in other populations (Hwang, B.F., Magnus, P. and Jaakkola, J.J., 2002).

Disinfection by-products (DBPs) form when disinfectants used in water treatment react with natural organic and inorganic matter present in the water source (EPA, 2002). Different disinfection techniques produce different types and amounts of DBPs. For instance, chlorine, the most widely used disinfectant, reacts with naturally occurring organic and inorganic matter to produce trihalomethanes (THMs): chloroform, bromodichloromethane, dibromochloromethane and bromoform. Since DBPs were

discovered in 1974, numerous toxicological studies have been conducted to evaluate the potential risk of health problems associated with these products (EPA, 2002).

There are many types of DBPs, some regulated by the EPA and some not. The links of DBPs to both cancer and reproductive outcomes have resulted in more stringent regulations of all DBPs including total THMs. Prior to 2001, maximum contaminant level (MCL) for THM was 100 micrograms per liter (µg/l). In 2001, the EPA required that large surface water public water systems use treatment methods to reduce the formation of DBPs and to meet the standard MCL of 80 µg/l. This was done as a measure to protect human health (EPA, 2002).

Exposure to THMs has been associated with intrauterine growth retardation, reduced cranial circumference, and stillbirth as well as NTDs. From the preliminary data collected by Chisholm, Cook, Bower, Heyworth, and Weinstein (2004) for a study in Australia, women residents of reproductive age in areas with high exposures to THMs were found to be 26% more likely to have a child with birth defects. A study done in Norway demonstrated increasing risks of NTDs with increasing levels of chlorine in various water systems throughout the country (Magnus, Jaakkola, Skrondal, Alexander, Becher, Krogh and Dybing, 1999). Aschengrau, Zierler, and Cohen (1993) looked at the quality of drinking water by examining the levels of heavy metals and found an increased risk for birth defects (OR = 1.5). Bove, Fulcomer, Klotz, Esmart, Dufficy and Savrin (1995) assessed public drinking water contamination and birth outcomes and found a strong association between THMs and adverse birth outcomes. Bove, Shim, and Zeitz, in 2002, summarized several articles concerning exposure to a variety of drinking water contaminants and adverse pregnancy outcomes. They noted an overall moderate

association with THMs and NTDs; ORs ranged from 1.2 to 3.0. Dodds and King (2001); Hwang and Jaakkola, (2003); Kallen and Robert (2000); Klotz and Pyrch (1999) and Shaw, Ranatunga, Quach, Neri, Correa, and Neutra (2003) examined adverse perinatal outcomes in relation to THMs, all with mixed results.

Small sample sizes, limited geographic areas and poor control of potential confounders contributed to mixed results, as did misclassification of exposure. Since THMs are highly volatile, dermal absorption and inhalation as opposed to ingestion are also important routes of exposure. A recent study showed that showering and bathing provided a more significant route of uptake than did ingestion of tap water (Whitaker, Nieuwenhuijsen, and Best, 2003).

Objective

The objective of this study was to evaluate the association between NTDs and periconceptional exposure to THMs in public drinking water in Mexican-American women residing and delivering or terminating pregnancies between June 1995 and May 2000 in one of the 14 Texas counties bordering Mexico.

CHAPTER 2

METHODS

The Texas Department of Health (TDH) conducted a case control study from June 1995 through May 2000 as a part of the Texas Neural Tube Defect Project (TNTDP). In-person interviews provided data on medical history, biological and environmental factors for case women (NTD-affected pregnancies) and control-women (normal births). Participants in the study included women residing in a 14 county area along the Texas-Mexico border. Counties represented included Brewster, Cameron, El Paso, Hidalgo, Hudspeth, Jeff Davis, Kinney, Maverick, Presidio, Starr, Terrell, Val Verde, Webb, and Zapata.

Dr. Marilyn Felkner, epidemiologist with the TNTDP, granted permission to graduate student, Karen Moody, and her thesis chairperson, Dr. Jean Brender, to access data for this research project. Personal identifiers were not needed nor were they included in the data shared for this study. The Texas Department of State Health Services (DSHS) Institutional Review Board approved the original study and our use of this portion of the data. (see attached letters in Appendix)

Research Design

This study was a population-based case control study examining the relationship of maternal periconceptional exposure to THMs in public water systems and the risk of NTDs.

Study Population

Cases were defined as Mexican-American women giving birth to a child with an NTD, specifically, anencephaly (International Classification of Diseases, 9th Revision, Clinical Modification [ICD-CM] code 740), spina bifida (741) and encephalocele (742.0). Live births, stillbirths, spontaneous and induced abortions were included in the data collection. Case women must have resided in the fourteen-county Texas border area at the time of delivery. Active surveillance of hospitals, birthing centers, physician offices, abortion facilities and delivering midwives throughout the fourteen-county region identified case women. Control women were defined as Mexican-American women residing in the fourteen-county region, who had normal live births during the same time period described above. Control women were frequency matched to case women by index birth or pregnancy termination and hospital of delivery. A total of 184 case women and 225 control women participated in the TNTDP. Signed inform consent for each participant was obtained prior to the interview process.

Data Collection

Data extracted from the TNTDP questionnaire included medical history, demographic, environmental and biological factors as well as the date of conception. Prior to administering the questionnaires, dates of conception were estimated by the staff obstetrician/gynecologist using all gestational age estimates from the medical record. As a part of the data collection, public drinking water system monitoring data were matched to mother's residence at the time of conception. Ms. Karen Moody participated in this data collection as part of an internship at the TDH.

Estimates of Exposure to THMs in Drinking Water

Luben, Nuckols, Lynberg, Mendola and Wolf (2004) reported that obtaining proper exposure categories requires linkage of residence location to a specific water utility and associated monitoring data during a given exposure period. Issues important in assessing exposure to THMs are mobility during pregnancy and ingestion of tap versus bottled water (Nieuwenhuijsen, Toledano, Eaton, Fawell and Elliot, 2000).

The monitoring data for THMs in public water systems were obtained from the Texas Commission of Environmental Quality (TCEQ). These data were merged with the existent NTD study data, based on the mother's residence address at the time of conception. Two values were obtained from the TCEQ data based on each mother's conception date; a minimum level (the level of THM was at least this high) and a maximum level (the level of THM was no higher than this). Interpolation of these levels resulted in a 'most likely' level of THM in the drinking water at conception. The interpolation value served as the level of exposure for each mother at conception.

As stated earlier, ingestion, dermal absorption and inhalation are all potential routes of exposure to THMs, the most critical of those being dermal absorption and inhalation. Data was collected from the questionnaire on the mother's routine practice of drinking tap water or bottled water. Those women who drank tap water regularly were considered to have a higher exposure level to THMs, as it was assumed that they also showered, bathed and cooked with tap water. Therefore, women who showered, bathed and cooked with tap water but ingested bottled water regularly were considered to have a potentially lower level of exposure to THMs. As a comparison, an additional analysis was done to examine the association of NTDs and THM level >50μg/liter in relation to

the source of drinking water (bottled versus tap).

Confounding Factors

Factors, which may change, modify or confound the relationship between THMs and risk for NTD-birth were collected from the TNTDP database and adjusted for in this study. Previously published studies of exposures associated with NTDs (Brender, et al., 2004; Brender, et al., 2002; Dodds and King, 2001; Dodds, King, Woolcott and Pole, 1999; Frey and Hauser, 2003; Shaw, et al., 2003; Suarez, et al., 2000), identified maternal age and education, smoking, alcohol use, income, folate intake, solvent exposure, bottled water use and BMI as potential confounders.

Analysis

To establish appropriate THM exposure categories, TCEQ water monitoring data was utilized along with documented exposure relationships. SPSS version 11.5 (SPSS Inc., 2002) was used to perform descriptive statistics, which describe cases and controls, and obtain frequencies of sociodemographic characteristics. For the analysis, SPSS was utilized to calculate crude odds ratios (OR) and 95 percent confidence intervals. SPSS binary logistic regression was employed to examine the relationship between THM levels in drinking water and NTD risk with adjustment for age, education, smoking, income, folate intake, solvent exposure, bottled water use, BMI and alcohol. Odds ratios were also quantified using Computer Programs for Epidemiologists version 4.0 (PEPI) (Abramson and Gahlinger 2001).

Benefits of the Study

Population estimates in Texas for 2001 indicated a large Hispanic population

in this 14-county region as compared to the entire state of Texas (74.2 percent versus 44 percent). Nine of the fourteen counties had populations greater than 74.2 percent Hispanic. Likewise, the average birth rate in this region for 2001 is 18.5 per 1000 births, higher than that for the State of Texas at 17.1 per 1000. Nine of the fourteen counties exceeded the fourteen-county birth rate average for 2001. These characteristics for each county are summarized in Table 1 (Texas Department of State Health Services [DSHS], 2001). Based on population projections by the Texas State Data Center, the Hispanic population in this fourteen county region is predicted to rise 49% by the year 2040 (Texas Population Estimates and Projections Program at the Texas State Data Center and Office of the State Demographer, 2004). It has been established that Hispanics are at a particularly high risk for NTD-affected pregnancies.

Socioeconomic status has been shown to have an association with NTD-affected pregnancy (Frey and Hauser, 2003). On the average, approximately 28 percent of the population in this fourteen-county region lived below poverty versus 14.6 percent that lived below poverty in the entire state for 2001. The average per capita personal income in this border region was $15,778 compared to the state per capita personal income at $28,472 (DSHS, 2001), indicating poor socioeconomic status for this region.

In areas with high poverty, many existing sources of water may be inadequate, being stressed by withdrawals to meet human and environmental needs. Higher levels of chlorination may be needed to assure safer drinking water. This coupled with high levels of organic matter from an inadequate water source consequently may produce higher levels of THMs in drinking water.

Studies centered in other geographic areas have indicated an association between

NTDs and THMs in drinking water; however, this risk factor has not been studied in this geographic region of Texas. This research is especially important in a population already at-risk of NTDs due to ethnicity and socioeconomic status.

Table 1: Selected characteristics of counties within the study area - 2001

County	Total Population	% Hispanic	Birth Rate[a]	% Below Poverty	Per Capita Personal Income
Brewster	8905	44%	12.8	19.7%	$23,314
Cameron	344,621	84.7%	24.3	30.2%	$15,334
El Paso	688,263	79%	20.6	25.3%	$19,186
Hidalgo	591,083	88.8%	25.5	31.7%	$13,788
Hudspeth	3,411	73.3%	12.0	32.7%	$14,827
Jeff Davis	2,242	35.5%	8.0	16.6%	$17,300
Kinney	3,409	51%	11.1	23.4%	$6,530
Maverick	48,438	95%	22.8	32.4%	$12,258
Presidio	7,366	84.5%	20.2	30.8%	$14,302
Starr	54,591	97.6%	26.7	42.2%	$9,769
Terrell	1,020	48.9%	6.0	22.2%	$28,195
Val Verde	45,494	75.6%	18.8	24.3%	$17,466
Webb	201,256	94.4%	29.5	27.7%	$15,508
Zapata	12,593	85.3%	21.1	32.3%	$13,120

[a]Birth rate per 1000

CHAPTER 3

RESULTS

Descriptive Analysis

Among the 184 cases and 225 controls, water data was collected for 39 (38.2%) cases and 63 (61.8%) controls. Table 2 compares the characteristics of participants and non-participants by maternal age and education, household income, body mass index (BMI), preconception folic acid intake, smoking, alcohol use, bottled water use and periconceptional solvent exposure. Compared with non-participants, a higher proportion of participants were better educated; had a greater annual income; had a body mass index of greater than or equal to 30 kg/m^2 and were less likely to have periconceptional solvent exposure. Participants and non-participants were similar by age, folic acid intake, smoking, alcohol, and bottled water use. (No differences greater than 5.5%).

Of the study participants, 39 were case women and 63 were control women. Table 3 shows demographic characteristics for these women. Case women were slightly older than control women. Case-women ranged in age from 14 to 38 years with a mean of 26.3 years. Control women ranged in age from 13 to 38 years with a mean age of 23.5 years. Greater than 55% of cases and controls were 20-29 years of age. A higher proportion of case women were slightly poorer, had periconceptional solvent exposure, smoked, used bottled water, and had a BMI of greater than or equal to

30 kg/m^2 compared with control women. Case and control women were similar with respect to education, folic acid intake and alcohol use.

Table 2. Comparison of characteristics of participants to non-participants

	Water Sample Study Participants		Non-Participants	
	N	(%)	N	(%)
Maternal age (years)				
<20	21	(20.6)	80	(26.1)
20 – 24	37	(36.3)	102	(33.3)
25 – 29	23	(22.5)	76	(24.8)
30+	21	(20.6)	49	(16.0)
Education (years)				
< 7	7	(6.9)	55	(17.9)
7 – 11	39	(38.2)	102	(33.2)
12+	56	(54.9)	150	(48.9)
Household Income[a]				
<$10,000/year	21	(20.8)	140	(46.5)
$10,000 - 14,999/year	30	(29.7)	52	(17.3)
$15,000 - 24,999/year	23	(22.8)	58	(19.3)
$25,000 +	27	(26.7)	51	(16.9)
Body Mass Index[b]				
< 30 kg/m^2	71	(69.6)	239	(79.1)
≥ 30 kg/m^2	31	(30.4)	63	(20.9)
Preconception Folic Acid				
Yes	8	(7.8)	13	(4.2)
No	94	(92.2)	294	(95.8)
Preconception Smoking				
Yes	18	(17.6)	52	(16.9)
No	84	(82.4)	255	(83.1)
Preconception Alcohol Use				
Yes	31	(30.4)	87	(28.3)
No	71	(69.6)	220	(71.7)
Bottled Water Use				
Yes	36	(35.3)	109	(35.5)
No	66	(64.7)	198	(64.5)
Periconceptional Solvent Exposure[c]				
Yes	7	(6.9)	49	(16.0)
No	94	(93.1)	257	(84.0)

[a] Six non-participants and one participant missing on household income

[b] Five non-participants missing on body mass index

[c] One non-participant and one participant missing on periconceptional solvent exposure

Table 3. Comparison of cases and controls

	Case Women		Control Women	
	N	(%)	N	(%)
Maternal age (years)				
<20	5	(12.8)	16	(25.4)
20 – 24	14	(35.9)	23	(36.5)
25 – 29	8	(20.5)	15	(23.8)
30+	12	(30.8)	9	(14.3)
Education (years)				
< 7	3	(7.7)	4	(6.3)
7 – 11	14	(35.9)	25	(39.7)
12+	22	(56.4)	34	(54.0)
Household Income				
<$10,000/year	11	(28.9)	10	(15.9)
$10,000 - 14,999/year	12	(31.6)	18	(28.6)
$15,000 - $24,999/year	5	(13.2)	18	(28.6)
$25,000 +	10	(26.3)	17	(27.0)
Body Mass Index				
< 30 kg/m^2	21	(53.8)	50	(79.4)
≥ 30 kg/m^2	18	(46.2)	13	(20.6)
Preconception Folic Acid				
Yes	4	(10.3)	4	(6.3)
No	35	(89.7)	59	(93.7)
Preconception Smoking				
Yes	10	(25.6)	8	(12.7)
No	29	(74.4)	55	(87.3)
Preconception Alcohol Use				
Yes	14	(35.9)	17	(27.0)
No	25	(64.1)	46	(73.0)
Bottled Water Use				
Yes	17	(43.6)	19	(30.2)
No	22	(56.4)	44	(69.8)
Periconceptional Solvent Exposure				
Yes	5	(12.8)	2	(3.2)
No	34	(87.2)	60	(96.8)

The distribution of THM levels in drinking water for cases and controls is shown in Table 4. Case-women had a higher median (31.90 μg/liter) and mean (38.24 μg/liter) compared to the overall median and mean of 27.81 μg/liter and 33.97 μg/liter

respectively. Furthermore, the mean and median value for case-women was greater than the control-women median (27.22 μg/liter) and mean (31.33 μg/liter).

Table 4. Distribution of drinking water trihalomethane (THM) levels (μg/liter)

	N	Median	Mean	(Std. Dev.)	Range
Overall	102	27.81	33.97	22.83	3.34 – 101.67
Case-women	39	31.90	38.24	25.62	4.32 – 101.67
Control-women	63	27.22	31.33	20.69	3.34 – 94.82

Table 5 compares the average level of THMs between case and control-women. Based on overall mean and median values, 56% of case-women had THM levels above the median compared to 46% of control-women. Approximately 43% of case-women and 35% of control women had THM values above the mean. Based on control women distribution, 56% of case-women were above the control median value of 27.22 μg/liter compared to 49% of control women. About 54% of case-women were above the control mean value of 31.33 μg/liter compared to 40% of control women.

Table 5. Trihalomethane median and mean distribution based on control-women values[a]

	Below Median		Above Median		Below Mean		Above Mean	
	N	(%)	N	(%)	N	(%)	N	(%)
Case-women	17	(44)	22	(56)	18	(46)	21	(54)
Control-women	31	(51)	32	(49)	38	(60)	25	(40)

[a]Control-women trihalomethane level median = 31.33 μg/liter and mean = 27.22 μg/liter

Bivariate Analysis

THM levels were also compared between case- and control-women at 50 μg/l, a level based on cut points reported in previously published studies. Numbers of exposed cases and controls were insufficient to permit finer stratification by water THM levels.

An increased risk for NTD-affected pregnancy was observed among women with periconceptional exposure to drinking water with THM levels greater than 50 μg/liter, with an odds ratio (OR) = 2.4 and 95% confidence intervals (CI) 0.8 – 7.1 (Table 6).

Table 6. Association between drinking water trihalomethane levels[a] and NTDs

THM (μg/liter)	Case-women N	Control-women N	Odds Ratio	95% CI[b]
< 50	28	54	1.0	Referent
≥ 50	11	9	2.4	0.8 – 7.1

[a] Based on literature (50 μg/liter cut point)
[b] CI (Fisher's Exact Method)

Table 7 shows the odds ratios of the relation between THM and NTDs stratified by various characteristics. This table exhibits increasing risks for NTD-affected pregnancies associated with drinking water THM levels ≥ 50 μg/l for women <20 years of age (OR = 6.5) and for those women aged 25 – 29 years (OR = 8.4). Additionally, women with a household income of $10,000 to $14,999, as well as incomes of $15,000 to $24, 999, were four and eleven times (respectively) more likely to have drinking water THM levels ≥ 50 μg/l as well as an NTD-affected pregnancy. Increasing risks for NTD-affected pregnancies with higher THM levels in drinking water were also seen among women with 7 – 11 years of education, a body mass index <30 kg/m^2, no preconception folic acid intake and among those who did not drink bottled water. The associated p-values of the chi-square test for heterogeneity indicated no statistically significant effect modification among any of the factors. ($p > 0.05$). Due to no participants reported in high exposure categories, maternal age 20 – 24 years and maternal education group < 7 years risk estimates were not quantifiable. Additionally all 95% confidence intervals around odds ratios were consistent with unity with the exception of those stratified by

maternal education 7-11 years (OR = 13.2; 95% CI [2.6 – 67.2) and a body mass index of <30 kg/m^2 (OR = 3.7; 95% CI 1.1 - 12.7).

Table 7. Stratified odds ratios[a] for association of THM level and NTDs by risk factor

Stratification Characteristic	Case-women (N)	Control-women (N)	THM Level (µg/l) <50		THM Level (µg/l) ≥50
			Referent	**OR**	**(95% CI)**
Overall	39	63	1.0	2.4	(0.8-7.1)
Maternal Age (years)					
< 20	5	14		6.5	(0.7-57.8)
20-24	14	23		0	
25-29	8	15		8.4	(0.7-100.6)
30+	12	9		0.4	(0.01-2.4)
Household Income					
<$10,000	11	10		0.9	(0.1-5.8)
$10,000-$14,999	12	18		4.0	(0.6-26.7)
$15,000-$24,999	5	18		11.3	(0.8-168.0)
$25,000 +	10	17		1.2	(0.2-8.5)
Maternal Education (years)					
<7	3	4		0	
7 – 11	14	25		13.2	(2.6-67.2)
12+	22	34		0.8	(0.1-4.5)
Body Mass Index (kg/m^2)					
<30	21	50		3.7	(1.1-12.7)
30+	18	13		1.0	(0.2-5.2)
Preconception Folic Acid					
Yes	4	35		0.8	(0.4-1.3)
No	4	59		2.2	(0.80-6.2)
Preconception Smoking					
Yes	10	8		1.3	(0.2-10.5)
No	29	55		2.6	(0.8-8.1)
Preconception Alcohol					
Yes	14	17		1.3	(0.2-7.6)
No	25	46		3.1	(0.9-10.4)
Bottled Water					
Yes	17	19		1.1	(0.2-6.6)
No	22	44		3.6	(1.1-12.3)
Periconceptional Solvent Exposure					
Yes	5	34		0.6	(0.3-1.2)
No	2	60		0.9	(0.7-1.1)

[a] SPSS crosstabs risk estimates

Logistic regression was used to obtain odds ratios and 95% CI for neural tube defects associated with THM exposure from drinking water. Other variables previously

associated with NTDs were considered as covariates including maternal age, household income, bottled water use, smoking, body mass index and periconceptional solvent exposure. In the final logistic regression analyses, only BMI changed the risk estimate by more than 10%; therefore none of the other covariates were included in the final models.

Table 8 shows results from a sequence of four logistic regression models. The first model examined THM level and the association with NTDs (OR = 2.4, 95% CI 0.9-6.4). The Wald statistic for this model was 2.87 and was not significant ($p > 0.05$). The overall predicted percent correct was 64%. (28.2% of cases and 85.7% of controls predicted correctly).

In model two, the relation between THMs and NTDs was examined with adjustment for other potential risk factors (OR for THM exposure > 50 μg/liter was 2.3, 95% CI 0.7-7.6). The Wald statistic was 1.72 and was not significant (p>0.05). Other potential risk factors in this model include BMI level, maternal age (<20, 20-24, 25-29, 30+ years), household income (<$10,000, $10,000-$14,999, $15,000-$24,999, $25,000+), preconception smoking, use of bottled drinking water and periconceptional solvent exposure. All variables in this model were not statistically significant ($p >0.05$), ORs ranged from 0.7 for household income <$10,000 to 4.6 for maternal age <20 years, and all 95% CI were consistent with unity. With adjustment for these variables, the OR for THM changed less than 10 percent. The overall predicted percentage increased to 66% (79% of control-women and 44.7% of case-women predicted correctly).

Model three examined NTD risk in relation to THM level and BMI. The OR for THM was 2.3 (95% CI 0.8-6.6). BMI had an OR of 3.3 (95% CI 1.35-8.00). The Wald statistic for THM was 2.63 and was not significant ($p > 0.05$). The overall predicted percentage

for this model was 67% (46.2% of cases and 79.4% of controls predicted correctly).

Table 8. Logistic regression analysis of THM[a] and BMI[b] for case- and control-women

Model #	Predictor Variable	Beta	S. E.	Odds Ratio	95% CI	Wald Statistic	*p*
1	THM level [a]	-0.857	0.506	2.4	0.9-6.4	2.87	0.09
2	THM level [a]	0.810	0.618	2.3	0.7-7.6	1.72	0.19
	BMI level [b]	.597	.527	1.8	0.7-5.1	1.285	0.26
	Maternal Age (years)					4.130	0.25
	<20	1.533	.812	4.6	0.9-22.8	3.561	0.06
	20 – 24	0.470	.659	1.6	0.4-5.8	0.509	0.48
	25 – 29	0.949	.713	2.6	0.6-10.4	1.772	0.18
	30+						
	Household Income					1.357	0.72
	<$10,000	0.336	0.700	0.7	0.2-2.8	0.231	0.63
	$10,000-$14,999	0.117	0.629	1.1	0.3-3.9	0.034	0.85
	$15,000-$24,999	0.520	0.735	1.7	0.4-7.1	0.500	0.48
	$25,000 +						
	Preconception Smoking	-0.846	0.618	2.3	0.7-7.8	1.877	0.17
	Bottled Water Use	0.669	0.531	2.0	0.7-5.5	1.587	0.21
	Periconceptional Solvent Exposure	1.362	0.987	3.9	0.6-27.0	1.903	0.17
3	THM level [a]	.852	.526	2.34	0.84-6.57	2.63	0.10
	BMI level [b]	1.19	.454	3.29	1.35-8.00	6.88	0.01
4	THM level [a]	-1.397	1.850	0.25	0.01-9.30	0.570	0.45
	BMI level [b]	-1.215	1.959	0.30	0.01-13.80	0.384	0.53
	THM*BMI interaction	1.348	1.076	3.85	0.47-31.74	1.569	0.21

[a] Based on published studies cut points, <50 µg/liter and ≥50 µg/liter

[b] Based on BMI cut point <30 kg/m^2 and ≥30 kg/m^2

Model four incorporates THM level, BMI level and a THM*BMI interaction factor. THM level indicated an OR = 0.3; 95% CI (0.01-9.3). The Wald statistic was 0.570 and was not significant (p>0.05). BMI level values were similar to THM level values (OR = 0.3; 95% CI [0.01-13.8). The THM*BMI interaction factor had an OR of

3.85 but 95% CI is consistent with unity and it was not significant ($p > 0.05$). Based on the findings, model three appears to be the most parsimonious model.

In the final analysis, the relation between THM and NTDS and whether or not the mother drank bottled or tap water during the periconceptional period. Table 9 shows the results of this analysis. Women who drank tap water during the periconceptional period from a public water system with THMs greater than or equal to 50 μg/liter were more likely to have an NTD-affected pregnancy (OR = 3.6, 95% CI 1.1-12.3) compared with tap water drinkers drinking water with THM levels less than 50 μg/liter. For those women who drank bottled water, the odds ratio and 95% CI for NTDs associated with THM levels greater than or equal to 50 μg/liter were consistent with unity (OR = 1.1 95% CI 0.2-6.6).

Table 9. THM by source of drinking water (bottled versus tap) and risk estimates for NTDs

Source of Drinking Water	**Trihalomethane Level in Public Water System**	**Case-women**	**Control-women**	**Odds Ratio (95% C. I.)**
Tap Water	≥ 50 μg/liter	8	14	3.6 (1.1-12.3)
	< 50 μg/liter	6	38	
Bottled Water	≥ 50 μg/liter	3	14	1.1 (0.2-6.6)
	< 50 μg/liter	3	16	

Table 10 incorporates THM, BMI and source of drinking water (bottled water versus tap) into three logistic regression analyses. In the first model, THM, BMI and bottled drinking water were examined. THM has an OR = 2.4, 95% CI 0.9-6.8, the Wald statistic was 2.84 which was not significant ($p = 0.09$). In the second model, women who

drank tap water ('no' bottled drinking water) were selected, therefore only including those women who ingested tap water in the analysis. THM OR = 3.4, 95% CI (1.0-11.6). The Wald statistic for THM was 3.65 and approached significance ($p = 0.06$). The third model selected for bottled water drinkers and excludes those who ingested tap water. THM OR = 1.5, 95% CI (0.2-10.1). The Wald statistic for this model was 0.17, which was not significant. ($p = 0.68$).

Table 10. Stratified logistic regression analysis THM, BMI and bottled drinking water

Model #	**Variables**	**OR**	**(95% CI)**	**Wald**	***p***
1	THM	2.4	(0.9-6.8)	2.84	0.09
	BMI	3.0	(1.2-7.4)	5.60	0.02
	Drinking Water	0.7	(0.3-1.6)	0.88	0.35
2[a]	THM	3.4	(1.0-11.6)	3.65	0.06
	BMI	1.8	(0.5-6.2)	0.87	0.35
3[b]	THM	1.5	(0.2-10.1)	0.17	0.68
	BMI	5.3	(1.3-22.7)	5.15	0.02

[a] 'No' bottled drinking water selected

[b] Bottled drinking water selected

CHAPTER 4

DISCUSSION

Although findings in this study were consistent with other studies, the results of this study were inconclusive in determining whether maternal exposure to high levels of THMs (≥50 μg/liter) is a risk factor for NTD in offspring. While 95% confidence limits (.79 – 7.12) around the point estimate were consistent with unity and logistic regression models were not significant ($p>0.05$), women in this study whose public water supply measured ≥ 50 μg/liter were 2.4 times more likely to have offspring with a NTD, consistent with findings in previous studies. A higher percentage of case women than control women had drinking water THM levels above the overall mean and median as well as control-based mean and median indicating higher levels of THM exposure through drinking water were more likely among case- women rather than control-women.

In a separate analysis, NTD risk was examined in relation to THM level in drinking water by comparing women who drank bottled water to those who drank tap water. Elevated risks (OR = 3.6) were seen with women who drank tap water with THM levels ≥50 μg/liter as compared with those who drank tap water with THM levels <50μg/liter. Further analysis showed comparable risks when bottled water drinkers and tap-water drinkers were analyzed separately in logistic regression models. When tap-water drinkers were selected out, risk estimates equaled 3.4, whereas bottled water drinkers risks were only slightly elevated (OR = 1.5). Logistic models approached

significance. Further studies exploring drinking water source is warranted.

The study had several limitations including sparse drinking water THM monitoring data. Much of the water data (307) were missing or reported as zero. Some of the women reported a water source that was not public and THM levels could not ascertained. Of the 402 cases and controls interviewed, only 102 had THM drinking water data. Moreover, of the participants with drinking water THM data, very few were in high exposure categories resulting in imprecise risk estimates.

Compared with non-participants, participants (women with THM data) were better educated, had a greater annual income, less likely to have periconceptional solvent exposure and had a body mass index of greater than or equal to 30 kg/m^2. Although these variables were included as covariates in the risk estimates, these differences between participants and non-participants could have biased the odds ratios for NTDs associated with THM exposure in drinking water.

Several other potential limitations should be discussed. Case-women differed in several demographic characteristics. In particular, a higher proportion of case- women were slightly poorer, had periconceptional solvent exposure, had BMI of greater than or equal to 30 kg/m^2, used bottled water and smoked compared with control-women. These differences could have also biased the odds ratios. A higher percentage of control-women had water-monitoring data as compared to case-women (62% vs. 38.2% respectively). Limited numbers of case-women especially in the higher exposure categories resulted in odds ratios that were not quantifiable for some variables. Data with zero values were not considered in this study; inclusion of these values would have increased the size of the lower level exposure group, which could have altered the results.

The strengths of the current study include selection of exposure data consistent with the time and specific address of the periconceptional period. In addition, data on pregnancies was obtained for all live births, stillbirths, as well as spontaneous and induced abortions, improving the depth of the data. Using geocoding to match participant addresses to public water supply improved the accuracy of drinking water data obtained for the study. Furthermore, data was acquired for women who drank bottled water versus tap water, which to my knowledge is the only study to consider the relation between THMs in drinking water, drinking water source and the risk for NTDs in offspring.

Previous studies examining THM exposure and the association with NTD had mixed results. Shaw, et al (2003) conducted two large case-control studies in California where they examined THMs and selected congenital malformations including NTDs. They found no clear association between THM exposure and risks of NTDs, due to imprecise exposure measures coupled with a lack of information about other possible sources of THM exposure. Using different exposure categories for the two studies, both studies revealed very different patterns. In the first study, NTD was inversely associated with THM exposure (OR range 0.39-0.92) and in the second study there was no clear pattern of association between THM (OR >1.00 for lower exposure categories and <1.00 in the higher exposure categories).

Klotz and Pyrch (1999), in a study done in New Jersey, used public water monitoring records concurrent with the first month of gestation to assess exposure. They found the prevalence odds ratio for the highest tertile of THM compared with the lowest to be 1.6 and the 95% CI included 1.0 (0.9-2.70). Sensitivity analyses restricted to

isolated NTD cases and mothers with known residence at conception yielded stronger associations between THM and NTDs (OR 2.1, 95% CI 1.1-4.0). This study had similar findings to the present study in that exposure was assessed in a similar way and the odds ratios showed a positive association between THM exposure and NTD-affected pregnancies.

In New Jersey, a large cross-sectional study (Bove, et al 1995) was conducted using environmental and birth outcome databases. A total of 80,938 live births and 594 fetal deaths were studied. Similar to the present study, only one CNS defect was exposed to very high THM level. Although the ORs ranged from 2.59 to 2.69 with THM levels >80 μg/liter, instead of computing 95% CI, the authors calculated 50% confidence intervals possibly exaggerating the significance of their findings.

Total THM, which is under examination in this study, consists of 4 compounds, primarily, chloroform. A retrospective cohort study in Nova Scotia (Dodds & King, 2001) looked at two specific THM compounds (bromodichloromethane (BDCM) and chloroform) in relation to NTD risk. Using data from a perinatal database and routine water monitoring tests, women who were exposed to BDCM at ≥ 20 μg/liter were 2.5 times more likely to have an NTD-affected pregnancy than women without this exposure (95% CI 1.2-5.1). No association between chloroform and NTDs were noted in the study. Their findings could explain the lack of association in some studies that use total THMs as the exposure instead of individual THM compounds.

While this study showed some evidence for increased risk of NTD with periconceptional exposure to THM in drinking water, the results are imprecise as evidenced by lack of statistical significance and confidence intervals consistent with

unity. Sample size and insufficient numbers in high exposure categories limited the findings in this study. The findings in this study related to drinking water source merit additional research, particularly to address the significance of exposure routes (dermal absorption vs. ingestion). In addition, further research is warranted to determine if women who drink tap water with high levels THM may have a greater risk of NTDs in offspring. Other larger studies have found associations with NTD and exposure to THM, particularly BDCM. Future studies may be warranted in this population that focus on BDCM as the primary exposure to understand if this compound as opposed to total THM might render exposed women more susceptible to NTD-affected pregnancies.

APPENDIX

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Nicolas Curry, M.D., M.P.H.
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Dr. Jean Brender, Associate Professor & Ms. Karen Moody, Graduate Student
Department of Health Services Research
Texas State University
601 University Drive
San Marcos, Texas 78666-4616

Dear Ms. Moody and Dr. Brender:

Ms. Karen Moody, graduate student in the Health Services Research Program at Texas State University, has requested permission to use data from the Texas Department of Health (TDH) Texas Neural Tube Defects Project (TNTDP) for her master's thesis. She has indicated that Dr. Brender, a collaborator in the TNTDP from 1992-1998, will serve as chair for her thesis committee. I approve of her proposal to study "Periconceptional Exposure to Trihalomethanes and the Risk of Neural Tube Defects."

As part of the TNTDP research team, I grant both of you permission to use the data from the case-control component for the purpose of Ms. Moody's thesis project. You are permitted to access data that has been specifically abstracted and has had all personal identifiers removed for the purpose of this study.

Our Institutional Review Board for the Protection of Human Subjects has previously approved the TNTDP. If you need any additional information, please feel free to contact me at 512-458-7676.

Sincerely,

Marilyn Felkner

Marilyn Felkner, DrPH
Epidemiologist, Infectious Disease & Surveillance Division

TEXAS DEPARTMENT OF STATE HEALTH SERVICES

EDUARDO J. SANCHEZ, M.D., M.P.H.
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January 18, 2005

Karen M. Moody
705 Furlong Drive
Austin, Texas 78756-4129

Re: Review Exemption for Periconceptional Exposure to Trihalomethanes & the Risk of Neural Tube Defects, IRB# 05-001

Dear Ms. Moody:

Upon review of your submission for the above-referenced data request, the Department of State Health Services Institutional Review Board (DSHS IRB) determined that the request could be exempted from IRB review base on the Code of Federal Regulations 45 CFR 46.101(b)(4).

Further review of this data request by the DSHS IRB is not required unless the protocol changes *in the use of human subjects*. In that case, the data request must be resubmitted to this IRB for review. Please let this IRB know when the project is completed.

If you have any questions, please contact the DSHS IRB Administrator, Steven Lowenstein at (512) 458-7111, extension 2202, or toll-free at 1-888-777-5037, or e-mail at steven.lowenstein@dshs.state.tx.us. You may also visit our website at www.tdh.state.tx.us/irb.

Sincerely,

Linda Bultman, Ph.D.
Chair, DSHS Institutional Review Board
FWA00000694 / IRB00000993

sl.

cc: file (05-001)

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VITA

Karen McCulloch Moody was born in Texarkana, Texas, on February 11, 1958, the daughter of Amos and Carolyn McCulloch. She attended Texas High School in Texarkana, Texas. After high school she attended college at the University of Texas at Austin and the University of Oklahoma in Norman. She graduated with a B.S. in Physical Education May 1981 from the University of Oklahoma in Norman. Following several years of employment as a coach and teacher, she was accepted to the University of Texas Health Science Center Physical Therapy Program in San Antonio, Texas. She graduated with a B.S. in Physical Therapy in May 1986. During the following years, she was employed in several settings as a physical therapist before entering the Graduate College of Texas State University-San Marcos in January 2003. While attending Texas State, she was employed as an intern at Texas Department of State Health Services - Center for Health Statistics and as a graduate assistant under Dr. Jean Brender.

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This thesis was typed by Karen McCulloch Moody.