**SUPPLEMENTAL** **TABLE 1**. Chi-square goodness-of-fit tests for representativeness of child and maternal characteristics

and maternal exposures for participants included in the analytical sample, National Birth Defects Prevention Study,

2000-2005

| **Characteristics** | **All control children** | | **Control children in the analytical sample** | | **Case children, all NTDs combined** | | **Case children in the analytical sample, all NTDs combined** | | **Control children** | **Case children, all NTDs combined** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Na*** | ***%*** | ***Na*** | ***%*** | ***Na*** | ***%*** | ***Na*** | ***%*** | ***p*** | ***p*** |
| **Totals** | **3974** |  | **649** |  | **667** |  | **111** |  |  |  |
| **Child characteristics** | | | | | | | | | | |
| Pregnancy outcome | | | | | | | | | | |
| Livebirth | 3973 | 100.0 | 649 | 100.0 | 453 | 68.0 | 69 | 62.2 | NC | <.01 |
| Fetal death (≥20 weeks gestation) | 0 | 0.0 | 0 | 0.0 | 56 | 8.4 | 3 | 2.7 |
| Induced abortion | 0 | 0.0 | 0 | 0.0 | 157 | 23.6 | 39 | 35.1 |
| *Missing* | 1 |  | 0 |  | 1 |  | 0 |  |
| Sex | | | | | | | | | | |
| Male | 2030 | 51.1 | 339 | 52.2 | 304 | 48.3 | 52 | 52.5 | .57 | .40 |
| Female | 1940 | 48.9 | 310 | 47.8 | 325 | 51.7 | 47 | 47.5 |
| *Missing* | 4 |  | 0 |  | 38 |  | 12 |  |
| Gestational age (weeks) | | | | | | | | | | |
| Preterm (<37) | 391 | 9.8 | 70 | 10.8 | 322 | 48.3 | 57 | 51.4 | .42 | .52 |
| Term (≥37) | 3582 | 90.2 | 579 | 89.2 | 345 | 51.7 | 54 | 48.6 |
| *Missing* | 1 |  | 0 |  | 0 |  | 0 |  |
| Family history of NTDs | | | | | | | | | | |
| First-degree relative | 6 | 0.2 | 1 | 0.2 | 6 | 0.9 | 1 | 0.9 | 1.00b | 1.00b |
| None | 3968 | 99.8 | 648 | 99.8 | 661 | 99.1 | 110 | 99.1 |
| *Missing* | 0 |  | 0 |  | 0 |  | 0 |  |
| Plurality | | | | | | | | | | |
| Single birth | 3854 | 97.0 | 622 | 95.8 | 637 | 95.5 | 104 | 93.7 | .09 | .35b |
| Multiple birth | 120 | 3.0 | 27 | 4.2 | 30 | 4.5 | 7 | 6.3 |
| *Missing* | 0 |  | 0 |  | 0 |  | 0 |  |
| **Maternal characteristics** | | | | | | | | | | |
| Age at delivery (years) | | | | | | | | | | |
| <20 | 366 | 9.2 | 37 | 5.7 | 70 | 10.5 | 3 | 2.7 | .01 | .01 |
| 20-34 | 3043 | 76.6 | 508 | 78.3 | 500 | 75.0 | 85 | 76.6 |
| ≥35 | 565 | 14.2 | 104 | 16.0 | 97 | 14.5 | 23 | 20.7 |
| *Missing* | 0 |  | 0 |  | 0 |  | 0 |  |
| Educational attainment at delivery (years) | | | | | | | | | | |
| <12 | 613 | 15.5 | 43 | 6.6 | 122 | 18.3 | 10 | 9.0 | <.01 | <.01 |
| 12 | 943 | 23.8 | 131 | 20.2 | 180 | 27.1 | 18 | 16.2 |
| >12 | 2405 | 60.7 | 475 | 73.2 | 363 | 54.6 | 83 | 74.8 |
| *Missing* | 13 |  | 0 |  | 2 |  | 0 |  |
| Race/Ethnicity | | | | | | | | | | |
| Non-Hispanic white | 2523 | 63.5 | 451 | 69.5 | 378 | 56.7 | 77 | 69.4 | <.01 | <.01 |
| Non-Hispanic black | 487 | 12.3 | 95 | 14.6 | 74 | 11.1 | 15 | 13.5 |
| Hispanic | 723 | 18.2 | 70 | 10.8 | 171 | 25.6 | 12 | 10.8 |
| Other | 241 | 6.1 | 33 | 5.1 | 44 | 6.6 | 7 | 6.3 |
| *Missing* | 0 |  | 0 |  | 0 |  | 0 |  |
| Gravidity | | | | | | | | | | |
| First pregnancy | 1162 | 29.2 | 188 | 29.0 | 179 | 26.8 | 38 | 34.2 | .01 | .15 |
| Second pregnancy | 1186 | 29.9 | 226 | 34.8 | 191 | 28.6 | 25 | 22.5 |
| Third or later pregnancy | 1625 | 40.9 | 235 | 36.2 | 297 | 44.5 | 48 | 43.2 |
| *Missing* | 1 |  | 0 |  | 0 |  | 0 |  |
| Pre-pregnancy BMI (kg/m2) | | | | | | | | | | |
| Underweight (<18.5) | 205 | 5.4 | 17 | 2.7 | 24 | 3.8 | 6 | 5.7 | .02 | .27b |
| Normal (18.5-<25.0) | 2094 | 54.7 | 370 | 58.2 | 316 | 50.2 | 60 | 56.6 |
| Overweight (25.0-<30.0) | 886 | 23.2 | 149 | 23.4 | 148 | 23.5 | 23 | 21.7 |
| Obese (≥30.0) | 642 | 16.8 | 100 | 15.7 | 142 | 22.5 | 17 | 16.0 |
| *Missing* | 147 |  | 13 |  | 37 |  | 5 |  |
| Study site | | | | | | | | | | |
| Arkansas | 641 | 16.1 | 124 | 19.1 | 105 | 15.7 | 20 | 18.0 | <.01 | <.01 |
| Georgia | 529 | 13.3 | 109 | 16.8 | 107 | 16.0 | 15 | 13.5 |
| Iowa | 534 | 13.4 | 143 | 22.0 | 104 | 15.6 | 25 | 22.5 |
| Massachusetts | 565 | 14.2 | 77 | 11.9 | 43 | 6.4 | 4 | 3.6 |
| New York | 401 | 10.1 | 13 | 2.0 | 49 | 7.3 | 3 | 2.7 |
| North Carolinac | 383 | 9.6 | 95 | 14.6 | 65 | 9.7 | 22 | 19.8 |
| Texas | 560 | 14.1 | 49 | 7.6 | 117 | 17.5 | 8 | 7.2 |
| Utahc | 361 | 9.1 | 39 | 6.0 | 77 | 11.5 | 14 | 12.6 |
| *Missing* | 0 |  | 0 |  | 0 |  | 0 |  |
| **Maternal exposures** | | | | | | | | | | |
| Periconceptional cigarette smokingd | | | | | | | | | | |
| No active or passive smoking | 2731 | 69.0 | 477 | 73.6 | 422 | 63.7 | 73 | 65.8 | <.01 | .64 |
| Active smoking only | 299 | 7.6 | 44 | 6.8 | 46 | 6.9 | 5 | 4.5 |
| Passive smoking only | 481 | 12.2 | 83 | 12.8 | 129 | 19.5 | 24 | 21.6 |
| Active and passive smoking | 447 | 11.3 | 44 | 6.8 | 66 | 10.0 | 9 | 8.1 |
| *Missing* | 16 |  | 1 |  | 4 |  | 0 |  |
| Periconceptional alcohol consumptiond | | | | | | | | | | |
| No drinking | 2639 | 67.0 | 401 | 62.3 | 463 | 70.2 | 70 | 64.8 | .03 | .38 |
| Binge drinking (≥4 drinks/occasion) | 417 | 10.6 | 82 | 12.7 | 77 | 11.7 | 13 | 12.0 |
| Drinking but no binge drinking | 884 | 22.4 | 161 | 25.0 | 120 | 18.2 | 25 | 23.1 |
| *Missing* | 34 |  | 5 |  | 7 |  | 3 |  |
| Prepregnancy dietary folate intake (μg/day) | | | | | | | | | | |
| <600 | 2852 | 71.8 | 489 | 75.3 | 499 | 74.8 | 86 | 77.5 | .04 | .52 |
| ≥600 | 1121 | 28.2 | 160 | 24.7 | 168 | 25.2 | 25 | 22.5 |
| *Missing* | 1 |  | 0 |  | 0 |  | 0 |  |
| Periconceptional folic acid containing supplement use | | | | | | | | | | |
| Yes | 2153 | 54.7 | 408 | 63.2 | 364 | 55.7 | 76 | 71.0 | <.01 | <.01 |
| No | 1783 | 45.3 | 238 | 36.8 | 289 | 44.3 | 31 | 29.0 |
| *Missing* | 38 |  | 3 |  | 14 |  | 4 |  |
| Periconceptional feverd | | | | | | | | | | |
| Yes | 179 | 4.9 | 28 | 4.8 | 46 | 7.8 | 9 | 9.8 | .85 | .49 |
| No | 3455 | 95.1 | 560 | 95.2 | 541 | 92.2 | 83 | 90.2 |
| *Missing* | 340 |  | 61 |  | 80 |  | 19 |  |
| Average shower duratione | | | | | | | | | | |
| <15 minutes | 2004 | 52.3 | 366 | 57.5 | 291 | 45.5 | 53 | 51.0 | .01 | .26 |
| ≥15 minutes | 1827 | 47.7 | 270 | 42.5 | 349 | 54.5 | 51 | 49.0 |
| *Missing* | 143 |  | 13 |  | 27 |  | 7 |  |

*Note:* Because of rounding, percentages might not total 100.

Abbreviations: BMI, body mass index; NC, not calculated; NTD, neural tube defect.

a Missing values not included in chi-square tests.

b Exact *p*-value.

c Includes estimated dates of delivery during 2003-2005.

d Periconceptional period defined as 1 month before conception and 1 month after conception.

e Assessed around the time the participant became pregnant.

**SUPPLEMENTAL TABLE 2**. Correlations of residential trihalomethane (THM) and haloacetic acid (HAA) concentrations in public tap water among participants

included in the analytical sample, National Birth Defects Prevention Study, 2000-2005

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Exposure (μg/L, B1-P1)a** | **TTHM** | **Bromo-form** | **Chloro-form** | **Bromo-dichloro-methane** | **Dibromo-chloro-methane** | **HAA5** | **Chloro-acetic acid** | **Dichloro-acetic acid** | **Trichloro-acetic acid** | **Bromo-acetic acid** | **Dibromo-acetic acid** |
| **TTHM** | 1.00 | 0.21 | 0.89 | 0.77 | 0.44 | 0.63 | 0.54 | 0.65 | 0.51 | 0.31 | 0.36 |
| Bromoform | 0.21 | 1.00 | -0.11 | 0.10 | 0.60 | 0.07 | 0.18 | 0.00 | -0.06 | 0.19 | 0.65 |
| Chloroform | 0.89 | -0.11 | 1.00 | 0.54 | 0.03 | 0.69 | 0.45 | 0.70 | 0.63 | 0.27 | 0.04 |
| Bromodichloromethane | 0.77 | 0.10 | 0.54 | 1.00 | 0.67 | 0.49 | 0.53 | 0.47 | 0.25 | 0.25 | 0.51 |
| Dibromochloromethane | 0.44 | 0.60 | 0.03 | 0.67 | 1.00 | 0.15 | 0.33 | 0.11 | -0.10 | 0.23 | 0.71 |
| **HAA5** | 0.63 | 0.07 | 0.69 | 0.49 | 0.15 | 1.00 | 0.68 | 0.95 | 0.87 | 0.31 | 0.28 |
| Chloroacetic acid | 0.54 | 0.18 | 0.45 | 0.53 | 0.33 | 0.68 | 1.00 | 0.55 | 0.45 | 0.32 | 0.44 |
| Dichloroacetic acid | 0.65 | 0.00 | 0.70 | 0.47 | 0.11 | 0.95 | 0.55 | 1.00 | 0.78 | 0.28 | 0.18 |
| Trichloroacetic acid | 0.51 | -0.06 | 0.63 | 0.25 | -0.10 | 0.87 | 0.45 | 0.78 | 1.00 | 0.17 | -0.02 |
| Bromoacetic acid | 0.31 | 0.19 | 0.27 | 0.25 | 0.23 | 0.31 | 0.32 | 0.28 | 0.17 | 1.00 | 0.20 |
| Dibromoacetic acid | 0.36 | 0.65 | 0.04 | 0.51 | 0.71 | 0.28 | 0.44 | 0.18 | -0.02 | 0.20 | 1.00 |

Abbreviations: B1, one month before conception; HAA5, group of five most common haloacetic acids; P1, one month after conception; TTHM, total trihalomethanes.

a Massachusetts and Utah did not report individual THM and HAA concentrations and were not included in the analysis of individual disinfection by-products.

**SUPPLEMENTAL TABLE 3**. Correlations of average daily maternal ingestion of trihalomethanes (THMs) and haloacetic acids (HAAs) among participants included

in the analytical sample, National Birth Defects Prevention Study, 2000-2005

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Exposure (μg/L, B1-P1)a** | **TTHM** | **Bromo-form** | **Chloro-form** | **Bromo-dichloro-methane** | **Dibromo-chloro-methane** | **HAA5** | **Chloro-acetic acid** | **Dichloro-acetic acid** | **Trichloro-acetic acid** | **Bromo-acetic acid** | **Dibromo-acetic acid** |
| **TTHM** | 1.00 | 0.46 | 0.84 | 0.90 | 0.64 | 0.78 | 0.58 | 0.72 | 0.68 | 0.54 | 0.58 |
| Bromoform | 0.46 | 1.00 | 0.03 | 0.42 | 0.76 | 0.20 | 0.24 | 0.13 | 0.05 | 0.41 | 0.83 |
| Chloroform | 0.84 | 0.03 | 1.00 | 0.64 | 0.15 | 0.86 | 0.61 | 0.83 | 0.87 | 0.43 | 0.14 |
| Bromodichloromethane | 0.90 | 0.42 | 0.64 | 1.00 | 0.77 | 0.59 | 0.44 | 0.53 | 0.45 | 0.54 | 0.68 |
| Dibromochloromethane | 0.64 | 0.76 | 0.15 | 0.77 | 1.00 | 0.25 | 0.25 | 0.18 | 0.06 | 0.46 | 0.87 |
| **HAA5** | 0.78 | 0.20 | 0.86 | 0.59 | 0.25 | 1.00 | 0.71 | 0.96 | 0.93 | 0.45 | 0.30 |
| Chloroacetic acid | 0.58 | 0.24 | 0.61 | 0.44 | 0.25 | 0.71 | 1.00 | 0.63 | 0.60 | 0.45 | 0.28 |
| Dichloroacetic acid | 0.72 | 0.13 | 0.83 | 0.53 | 0.18 | 0.96 | 0.63 | 1.00 | 0.86 | 0.37 | 0.23 |
| Trichloroacetic acid | 0.68 | 0.05 | 0.87 | 0.45 | 0.06 | 0.93 | 0.60 | 0.86 | 1.00 | 0.36 | 0.09 |
| Bromoacetic acid | 0.54 | 0.41 | 0.43 | 0.54 | 0.46 | 0.45 | 0.45 | 0.37 | 0.36 | 1.00 | 0.42 |
| Dibromoacetic acid | 0.58 | 0.83 | 0.14 | 0.68 | 0.87 | 0.30 | 0.28 | 0.23 | 0.09 | 0.42 | 1.00 |

Abbreviations: B1, one month before conception; HAA5, group of five most common haloacetic acids; P1, one month after conception; TTHM, total trihalomethanes.

a Massachusetts and Utah did not report individual THM and HAA concentrations and were not included in the analysis of individual disinfection by-products.

**SUPPLEMENTAL FIGURE 1**. Directed Acyclic Graph for adjustment variable selection

between drinking water disinfection by-products and neural tube defects (NTDs)

A diagram of a network

Description automatically generated

BMI=Body Mass Index; DBP=Disinfection by-product; U=Unknown

Minimal sufficient adjustment set for estimating the total effect of drinking water disinfection

by-products and neural tube defects: Maternal educational attainment at delivery, maternal

race/ethnicity, and study site.

The Directed Acyclic Graph was generated using the R package “DAGitty” (Textor et al., 2016).

DAGgitty color legend:

A screenshot of a computer screen

Description automatically generated

**DAGitty MODEL CODE:**

dag {

bb="-6.767,-12.319,7.753,7.543"

"Anticonvulsant use" [latent,pos="2.800,6.434"]

"DBP (drinking water)" [exposure,pos="-0.030,-3.180"]

"DBP (not drinking water)" [latent,pos="-4.870,-9.716"]

"Dietary Folate" [pos="6.676,4.919"]

"Family History NTD" [pos="6.716,-9.704"]

"Folic acid use" [pos="6.866,-2.422"]

"Infant sex" [pos="6.678,-6.750"]

"Pregnancy Outcome" [pos="-0.269,-8.171"]

"Race/Ethnicity" [adjusted,pos="-3.718,2.110"]

"Study Site" [adjusted,pos="-2.319,-9.771"]

"U2 (Genetics)" [latent,pos="4.454,-9.749"]

Age [pos="-5.542,-0.460"]

Alcohol [pos="-1.055,4.578"]

BMI [pos="1.068,6.371"]

Diabetes [latent,pos="4.592,6.213"]

Education [adjusted,pos="-1.189,-0.201"]

Fever [pos="-5.311,4.791"]

Gestation [pos="1.580,-7.742"]

Gravidity [pos="-5.492,-3.684"]

NTD [outcome,pos="3.767,-3.121"]

Plurality [pos="1.955,-9.749"]

Shower [pos="-4.093,-3.752"]

Smoking [pos="-3.116,4.706"]

U1 [latent,pos="7.303,1.185"]

Unknown [latent,pos="-0.207,-9.726"]

"Anticonvulsant use" -> "Folic acid use"

"Anticonvulsant use" -> NTD

"DBP (drinking water)" -> Gestation

"DBP (drinking water)" -> NTD

"DBP (not drinking water)" -> NTD

"Dietary Folate" -> NTD

"Family History NTD" -> NTD

"Folic acid use" -> NTD

"Infant sex" -> NTD

"Pregnancy Outcome" -> Gestation

"Pregnancy Outcome" <-> NTD

"Race/Ethnicity" -> "DBP (drinking water)"

"Race/Ethnicity" -> "Dietary Folate"

"Race/Ethnicity" -> "Folic acid use"

"Race/Ethnicity" -> Alcohol

"Race/Ethnicity" -> Diabetes

"Race/Ethnicity" -> Gestation

"Race/Ethnicity" -> NTD

"Race/Ethnicity" -> Shower

"Race/Ethnicity" -> Smoking

"Race/Ethnicity" <-> Education

"Study Site" -> "DBP (drinking water)"

"Study Site" -> "Race/Ethnicity"

"Study Site" -> Education

"Study Site" -> NTD

"Study Site" -> Unknown

"U2 (Genetics)" -> "Family History NTD"

"U2 (Genetics)" -> Alcohol

"U2 (Genetics)" -> BMI

"U2 (Genetics)" -> Diabetes

"U2 (Genetics)" -> Gestation

"U2 (Genetics)" -> NTD

"U2 (Genetics)" -> Plurality

"U2 (Genetics)" -> Smoking

Age -> "Dietary Folate"

Age -> "Folic acid use"

Age -> "U2 (Genetics)"

Age -> Education

Age -> Gestation

Age -> Gravidity

Age -> NTD

Age -> Shower

Age -> Smoking

Alcohol -> "Dietary Folate"

Alcohol -> BMI

Alcohol -> NTD

Alcohol <-> Education

Alcohol <-> Smoking

BMI -> NTD

Diabetes -> "Dietary Folate"

Diabetes -> "Folic acid use"

Diabetes -> NTD

Education -> "DBP (drinking water)"

Education -> "Dietary Folate"

Education -> "Folic acid use"

Education -> BMI

Education -> Diabetes

Education -> NTD

Fever -> NTD

NTD -> Gestation

Plurality -> Gestation

Plurality -> NTD

Shower -> "DBP (not drinking water)"

Smoking -> Education

U1 -> "Dietary Folate"

U1 -> "Folic acid use"

Unknown -> NTD

}