

Supplemental Material

Prenatal Nitrate Intake from Drinking Water and Selected Birth Defects in Offspring of Participants in the National Birth Defects Prevention Study

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Supplemental Material, Table S1. Maternal daily nitrate intake from drinking water and selected birth defects in offspring among participants who only drank tap water from municipal water supplies

Type of Birth defect	Daily nitrate intake from water ^a (mg/day)	Cases No. (%)	Controls No. (%)	Unadjusted OR ^b (95%CI)	Adjusted OR ^b (95%CI)	<i>p</i> -value for linear trend
Any neural tube defect ^c	< 0.91	25 (16.7)	125 (18.9)	1.00	1.00	0.230
	0.91 – 4.9	38 (25.3)	199 (30.2)	0.95 (0.55, 1.66)	0.91 (0.52, 1.60)	
	≥ 5.0	87 (58.0)	336 (50.9)	1.29 (0.79, 2.11)	1.25 (0.76, 2.06)	
Spina bifida ^c	< 0.91	12 (12.1)	125 (18.9)	1.00	1.00	0.037
	0.91 – 4.9	27 (27.3)	199 (30.2)	1.41 (0.69, 2.89)	1.40 (0.68, 2.89)	
	≥ 5.0	60 (60.6)	336 (50.9)	1.86 (0.97, 3.57)	1.93 (0.99, 3.76)	
Anencephaly ^c	< 0.91	9 (24.3)	125 (18.9)	1.00	1.00	0.510
	0.91 – 4.9	9 (24.3)	199 (30.2)	0.63 (0.24, 1.63)	0.59 (0.23, 1.56)	
	≥ 5.0	19 (51.4)	336 (50.9)	0.79 (0.35, 1.78)	0.69 (0.30, 1.61)	
Any limb deficiency ^{d,e}	< 1.0	4 (6.6)	114 (17.2)	1.00	1.00	0.030
	1.0 – 5.41	19 (31.2)	215 (32.4)	2.52 (0.84, 7.58)	2.43 (0.80, 7.43)	
	≥ 5.42	38 (62.3)	335 (50.5)	3.23 (1.13, 9.26)	3.19 (1.09, 9.35)	
Any oral cleft defect ^{e,f}	< 1.0	47 (16.9)	114 (17.3)	1.00	1.00	0.191
	1.0 – 5.41	74 (26.5)	214 (32.4)	0.84 (0.55, 1.29)	0.80 (0.52, 1.25)	
	≥ 5.42	158 (56.6)	333 (50.4)	1.15 (0.78, 1.70)	1.15 (0.77, 1.72)	
Cleft lip without cleft palate ^{e,f}	< 1.0	8 (11.6)	114 (17.3)	1.00	1.00	0.022
	1.0 – 5.41	15 (21.7)	214 (32.4)	1.00 (0.41, 2.43)	0.96 (0.39, 2.37)	
	≥ 5.42	46 (66.7)	333 (50.4)	1.97 (0.90, 4.30)	1.96 (0.88, 4.36)	
Cleft palate ^{e,f}	< 1.0	12 (15.0)	114 (17.3)	1.00	1.00	0.092
	1.0 – 5.41	20 (25.0)	214 (32.4)	0.89 (0.42, 1.88)	0.93 (0.43, 2.02)	
	≥ 5.42	48 (60.0)	333 (50.4)	1.37 (0.70, 2.67)	1.55 (0.78, 3.10)	
Conotruncal ^{c,g}	< 1.0	15 (15.8)	114 (17.2)	1.00	1.00	0.252
	1.0 – 5.41	25 (26.3)	215 (32.4)	0.88 (0.45, 1.74)	0.92 (0.46, 1.83)	
	≥ 5.42	55 (57.9)	335 (50.5)	1.25 (0.68, 2.29)	1.29 (0.69, 2.41)	

Type of Birth defect	Daily nitrate intake from water ^a (mg/day)	Cases No. (%)	Controls No. (%)	Unadjusted OR ^b (95%CI)	Adjusted OR ^b (95%CI)	<i>p</i> -value for linear trend
Right ventricular outflow tract obstruction ^{c,g}	< 1.0	9 (11.1)	114 (17.2)	1.00	1.00	0.076
	1.0 – 5.41	24 (29.6)	215 (32.4)	1.41 (0.64, 3.14)	1.46 (0.65, 3.28)	
	≥ 5.42	48 (59.3)	335 (50.5)	1.81 (0.86, 3.82)	1.90 (0.89, 4.01)	
Left ventricular outflow tract obstruction ^{c,g}	< 1.0	17 (17.4)	114 (17.2)	1.00	1.00	0.944
	1.0 – 5.42	33 (33.7)	215 (32.4)	1.03 (0.55, 1.93)	1.12 (0.60, 2.12)	
	> 5.42	48 (49.0)	335 (50.5)	0.96 (0.53, 1.74)	1.06 (0.58, 1.92)	
Septal defects ^{c,g}	< 1.0	55 (17.8)	114 (17.2)	1.00	1.00	0.311
	1.0 – 5.42	117 (37.9)	215 (32.4)	1.13 (0.76, 1.67)	1.09 (0.72, 1.63)	
	> 5.42	137 (44.3)	335 (50.5)	0.85 (0.58, 1.24)	0.87 (0.59, 1.30)	

^a For neural tube defects, water nitrate intake one month preconception to one month post-conception was estimated. For limb, oral cleft and congenital heart defects, water nitrate intake during one month preconception through the first trimester was estimated.

^b Crude and adjusted odds ratio include only cases and controls with complete information for covariates.

^c Adjusted for maternal race/ethnicity, education, study center and folic acid supplementation.

^d Adjusted for maternal race/ethnicity, education, age, multivitamin supplementation, and study center.

^e Isolated defect

^f Adjusted for maternal race/ethnicity, education, age, folic acid supplementation during the first trimester, maternal smoking, and study center.

^g Adjusted for maternal race/ethnicity, education, multivitamin use during the first trimester, maternal smoking, and study center.

Supplemental Material, Table S2. Nitrate in municipal drinking water source of tap water drinkers^a and selected birth defects in offspring, Iowa and Texas participants of the National Birth Defects Prevention Study

Type of birth defect	Nitrate ^b (mg/L)	Cases No. (%)	Controls No. (%)	Unadjusted OR ^c (95% CI)	Adjusted OR ^c (95% CI)	<i>p</i> -value for linear trend
Any neural tube defect ^d	< 5.0	67 (43.2)	353 (51.6)	1.00	1.00	0.794
	5.0–15.0	72 (46.5)	205 (30.0)	1.85 (1.27, 2.69)	1.82 (1.24, 2.67)	
	> 15.0	16 (10.3)	126 (18.4)	0.67 (0.37, 1.20)	0.66 (0.36, 1.21)	
Spina bifida ^d	< 5.0	40 (38.8)	353 (51.6)	1.00	1.00	0.501
	5.0–15.0	52 (50.5)	205 (30.0)	2.24 (1.43, 3.50)	2.42 (1.52, 3.84)	
	> 15.0	11 (10.7)	126 (18.4)	0.77 (0.38, 1.55)	0.69 (0.33, 1.42)	
Anencephaly ^d	< 5.0	20 (52.6)	353 (51.6)	1.00	1.00	0.736
	5.0–15.0	13 (34.2)	205 (30.0)	1.12 (0.55, 2.30)	0.92 (0.44, 1.93)	
	> 15.0	5 (13.2)	126 (18.4)	0.70 (0.26, 1.91)	0.85 (0.29, 2.49)	
Any limb deficiency ^{e,f}	< 5.0	28 (44.4)	337 (48.8)	1.00	1.00	0.329
	5.0–15.0	23 (36.5)	229 (33.2)	1.21 (0.68, 2.15)	1.08 (0.59, 1.99)	
	> 15.0	12 (19.1)	124 (18.0)	1.16 (0.57, 2.36)	1.53 (0.71, 3.33)	
Any oral cleft defect ^{f,g}	< 5.0	127 (44.9)	335 (48.8)	1.00	1.00	0.269
	5.0–15.0	109 (38.5)	229 (33.3)	1.26 (0.92, 1.71)	1.22 (0.89, 1.68)	
	> 15.0	47 (16.6)	123 (17.9)	1.01 (0.68, 1.49)	1.18 (0.78, 1.79)	
Cleft lip without cleft palate ^{f,g}	< 5.0	29 (40.3)	335 (48.8)	1.00	1.00	0.026
	5.0–15.0	22 (30.6)	229 (33.3)	1.11 (0.62, 1.98)	1.07 (0.59, 1.96)	
	> 15.0	21 (29.2)	123 (17.9)	1.97 (1.08, 3.59)	2.31 (1.20, 4.47)	
Cleft palate ^{f,g}	< 5.0	32 (40.0)	335 (48.8)	1.00	1.00	0.344
	5.0–15.0	36 (45.0)	229 (33.3)	1.65 (0.99, 2.73)	1.75 (1.03, 2.98)	
	> 15.0	12 (15.0)	123 (17.9)	1.02 (0.51, 2.05)	1.10 (0.52, 2.29)	

Type of birth defect	Nitrate ^b (mg/L)	Cases No. (%)	Controls No. (%)	Unadjusted OR ^c (95% CI)	Adjusted OR ^c (95% CI)	<i>p</i> -value for linear trend
Conotruncal heart defects ^{f,h}	< 5.0	50 (49.0)	337 (48.8)	1.00	1.00	0.536
	5.0–15.0	39 (38.2)	229 (33.2)	1.15 (0.73, 1.80)	1.15 (0.72, 1.83)	
	> 15.0	13 (12.8)	124 (18.0)	0.71 (0.37, 1.35)	0.70 (0.35, 1.37)	
Right ventricular outflow tract obstruction ^{f,h}	< 5.0	33 (39.3)	337 (48.8)	1.00	1.00	0.210
	5.0–15.0	32 (38.1)	229 (33.2)	1.43 (0.85, 2.39)	1.44 (0.83, 2.51)	
	> 15.0	19 (22.6)	124 (18.0)	1.56 (0.86, 2.85)	1.41 (0.74, 2.70)	
Left ventricular outflow tract obstruction ^{f,h}	< 5.0	44 (44.4)	337 (48.8)	1.00	1.00	0.278
	5.0–15.0	30 (30.3)	229 (33.2)	1.00 (0.61, 1.64)	1.12 (0.67, 1.86)	
	> 15.0	25 (25.3)	124 (18.0)	1.54 (0.91, 2.63)	1.37 (0.78, 2.39)	
Septal defects ^{f,h}	< 5.0	201 (62.8)	337 (48.8)	1.00	1.00	0.103
	5.0–15.0	79 (24.7)	229 (33.2)	0.58 (0.42, 0.79)	0.58 (0.39, 0.88)	
	> 15.0	40 (12.5)	124 (18.0)	0.54 (0.36, 0.80)	0.81 (0.47, 1.40)	

OR (odds ratio); CI (confidence interval). ^a Women who reported drinking any municipal tap water during one month preconception to one month post-conception for neural tube defects, and one month preconception through the first trimester for limb, oral cleft and congenital heart defects.

^b For neural tube defects, nitrate in drinking water one month preconception to one month post-conception was estimated. For limb, oral cleft and congenital heart defects, nitrate in drinking water during one month preconception through the first trimester was estimated. ^c Crude and adjusted odds ratio include only cases and controls with complete information for covariates. ^d Adjusted for maternal race/ethnicity, education, study center and folic acid supplementation during period one month before through one month post-conception. ^e Adjusted for maternal race/ethnicity, education, age, multivitamin use during the first trimester, and study center. ^f Isolated defect. ^g Adjusted for maternal race/ethnicity, education, age, folic acid supplementation during the first trimester, maternal smoking, and study center. ^h Adjusted for maternal race/ethnicity, education, multivitamin use during the first trimester, maternal smoking, and study center.

Supplemental Material, Table S3. Associations between nitrosatable drug use (versus no use) and birth defects according to estimated nitrate intake from drinking water during early pregnancy

Congenital malformation	Nitrate ^a (mg/day)	Nitrosatable drug exposure during the first trimester				Unadjusted OR ^b (95% CI)	Adjusted OR ^b (95% CI)	Attributable proportion due to interaction	<i>p</i> -value for multiplicative interaction term
		Cases		Controls					
		Yes	No	Yes	No				
Any neural tube defect ^c	< 0.91	13	54	37	330	2.15 (1.07, 4.30)	2.54 (1.20, 5.37)	-0.29 (-1.27, 0.69)	0.283
	0.91 – 4.99	11	54	59	301	1.04 (0.51, 2.11)	1.26 (0.60, 2.65)		
	≥ 5.0	20	75	61	313	1.37 (0.78, 2.41)	1.20 (0.66, 2.19)		
Any limb deficiency ^{d,e}	< 1.0	3	19	66	297	0.71 (0.20, 2.47)	0.72 (0.19, 2.70)	0.32 (-0.40, 1.03)	0.440
	1.0 – 5.41	8	20	79	280	1.42 (0.60, 3.34)	1.12 (0.45, 2.79)		
	≥ 5.42	12	29	86	276	1.33 (0.65, 2.71)	1.20 (0.57, 2.53)		
Cleft lip with or without cleft palate ^{e,f}	< 1.0	16	75	66	297	0.96 (0.53, 1.75)	0.97 (0.52, 1.84)	-0.003 (-0.69, 0.68)	0.988
	1.0 – 5.41	20	66	78	280	1.09 (0.62, 1.90)	0.99 (0.54, 1.80)		
	≥ 5.42	28	88	85	276	1.03 (0.63, 1.69)	1.01 (0.60, 1.69)		
Cleft palate ^{e,f}	< 1.0	10	18	66	297	2.50 (1.10, 5.66)	2.89 (1.15, 7.25)	-0.24 (-1.17, 0.69)	0.391
	1.0 – 5.41	5	26	78	280	0.69 (0.26, 1.86)	0.60 (0.20, 1.78)		
	≥ 5.42	16	38	85	276	1.37 (0.73, 2.57)	1.31 (0.67, 2.54)		
Conotruncal heart defect ^{e,g}	< 1.0	12	45	66	297	1.20 (0.60, 2.39)	1.39 (0.66, 2.91)	-0.88 (-2.56, 0.80)	0.182
	1.0 – 5.41	7	32	79	280	0.78 (0.33, 1.82)	0.86 (0.35, 2.13)		
	≥ 5.42	10	52	86	276	0.62 (0.30, 1.27)	0.59 (0.28, 1.23)		
Right ventricular outflow tract obstruction ^{e,g}	< 1.0	8	27	66	297	1.33 (0.58, 3.07)	1.36 (0.56, 3.31)	-1.24 (-3.32, 0.84)	0.095
	1.0 – 5.41	10	21	79	280	1.69 (0.76, 3.73)	2.25 (0.94, 5.40)		
	≥ 5.42	8	45	86	276	0.57 (0.26, 1.26)	0.56 (0.25, 1.28)		
Left ventricular outflow tract obstruction ^{e,g}	< 1.0	9	35	66	297	1.16 (0.53, 2.52)	1.06 (0.47, 2.39)	-0.15 (-1.29, 0.99)	0.892
	1.0 – 5.41	11	45	79	280	0.87 (0.43, 1.75)	0.81 (0.39, 1.69)		
	≥ 5.42	12	39	86	276	0.99 (0.49, 1.97)	0.83 (0.41, 1.70)		

Congenital malformation	Nitrate ^a (mg/day)	Nitrosatable drug exposure during the first trimester				Unadjusted OR ^b (95% CI)	Adjusted OR ^b (95% CI)	Attributable proportion due to interaction	<i>p</i> -value for multiplicative interaction term
		Cases		Controls					
		Yes	No	Yes	No				
Septal heart defect ^{c,g}	< 1.0	47	154	66	297	1.37 (0.90, 2.09)	1.53 (0.97, 2.42)		
	1.0 – 5.41	43	157	79	280	0.97 (0.64, 1.48)	1.03 (0.66, 1.61)		
	≥ 5.42	41	110	86	276	1.20 (0.78, 1.84)	1.19 (0.76, 1.88)	-0.20 (-0.93, 0.53)	0.655

OR (odds ratio); CI (confidence interval).

^aFor neural tube defects, water nitrate intake one month preconception to one month post-conception was estimated. For limb, oral cleft and congenital heart defects, water nitrate intake one month preconception through the first trimester was estimated.

^bCrude and adjusted odds ratio (OR) include only cases and controls with complete information for covariates.

^cAdjusted for maternal race/ethnicity, education, study center and folic acid supplementation.

^dAdjusted for maternal race/ethnicity, education, age, multivitamin supplementation, and study center.

^eIsolated defect.

^fAdjusted for maternal race/ethnicity, education, age, folic acid supplementation, smoking, and study center.

^gAdjusted for maternal race/ethnicity, education, multivitamin supplementation, smoking, and study center.

Supplemental Material, Table S4. Associations between nitrosatable drug use (versus no use) and birth defects according to estimated total nitrite intake from diet and drinking water during early pregnancy

Type of birth defect	Total nitrite intake ^a (mg/day)	Nitrosatable drug exposure during the first trimester				Unadjusted OR ^b (95% CI)	Adjusted OR ^b (95% CI)	Attributable proportion due to interaction ^c	<i>p</i> -value for multiplicative interaction term
		Cases		Controls					
		Yes	No	Yes	No				
Any neural tube defect ^d	≤ 4.74	27	112	111	597	1.30 (0.81, 2.07)	1.41 (0.87, 2.29)	0.31 (-0.21, 0.83)	0.396
	> 4.74	16	67	41	313	1.82 (0.97, 3.44)	1.76 (0.90, 3.43)		
Cleft lip without cleft palate ^e	≤ 4.78	14	52	159	536	0.91 (0.49, 1.68)	0.80 (0.42, 1.52)	0.60 (0.15, 1.05)	0.074
	> 4.78	13	23	65	284	2.47 (1.19, 5.13)	2.01 (0.90, 4.48)		
Cleft lip with cleft palate ^e	≤ 4.78	29	134	159	536	0.73 (0.47, 1.13)	0.76 (0.48, 1.21)	0.34 (-0.41, 1.09)	0.533
	> 4.78	12	52	65	284	1.01 (0.51, 2.00)	1.13 (0.54, 2.37)		
Cleft palate ^e	≤ 4.78	20	69	159	536	0.98 (0.58, 1.66)	0.95 (0.55, 1.64)	0.63 (0.32, 0.95) ^h	0.019
	> 4.78	19	30	65	284	2.77 (1.47, 5.22)	2.51 (1.24, 5.06)		
Any limb deficiency ^f	≤ 4.78	14	52	161	537	0.90 (0.49, 1.66)	1.00 (0.53, 1.89)	0.47 (0.05, 0.89)	0.154
	> 4.78	14	35	65	283	1.74 (0.89, 3.42)	1.64 (0.80, 3.35)		
Conotruncal heart defect ^g	≤ 4.78	33	114	161	537	0.97 (0.63, 1.48)	0.98 (0.64, 1.52)	-0.13 (-1.02, 0.76)	0.769
	> 4.78	13	64	65	283	0.88 (0.46, 1.70)	0.80 (0.40, 1.61)		
Right ventricular outflow tract obstruction ^g	≤ 4.78	31	88	161	537	1.17 (0.75, 1.83)	1.15 (0.72, 1.82)	-0.26 (-1.26, 0.74)	0.565
	> 4.78	11	54	65	283	0.89 (0.44, 1.79)	0.83 (0.40, 1.74)		
Left ventricular outflow tract obstruction ^g	≤ 4.78	28	109	161	537	0.86 (0.55, 1.35)	0.82 (0.52, 1.30)	0.19 (-0.47, 0.85)	0.578
	> 4.78	14	56	65	283	1.09 (0.57, 2.07)	0.96 (0.49, 1.92)		
Septal heart defect ^g	≤ 4.78	125	373	161	537	1.12 (0.85, 1.46)	1.19 (0.90, 1.58)		

Type of birth defect	Total nitrite intake ^a (mg/day)	Nitrosatable drug exposure during the first trimester				Unadjusted OR ^b (95% CI)	Adjusted OR ^b (95% CI)	Attributable proportion due to interaction ^c	<i>p</i> -value for multiplicative interaction term
		Cases		Controls					
		Yes	No	Yes	No				
	> 4.78	63	244	65	283	1.12 (0.76, 1.65)	1.09 (0.72, 1.64)		
Atrioventricular septal defect ^g	≤ 4.78	8	13	161	537	2.05 (0.84, 5.04)	1.93 (0.76, 4.87)	0.03 (-0.42, 0.47)	0.955
	> 4.78	7	6	65	283	5.08 (1.65, 15.6)	5.10 (1.40, 18.6)		
Single ventricle ^g	≤ 4.78	5	24	161	537	0.69 (0.26, 1.85)	0.74 (0.27, 2.02)	0.50 (-0.17, 1.16)	0.230
	> 4.78	7	11	65	283	2.77 (1.03, 7.42)	3.25 (1.13, 9.31)		
								0.75 (0.32, 1.18)	0.063

OR (odds ratio); CI (confidence interval).

^aTotal nitrite intake = dietary nitrite intake + 5% (dietary nitrate intake + nitrate from drinking water). Contribution from nitrate in drinking water estimated for one month prior through one month postconception for NTDs and one month prior to conception through the first trimester for the other congenital malformations. ^bCrude and adjusted odds ratio include only cases and controls with complete information for covariates and calorie intake between 500-5000 kcal. ^cInteraction assessed on dichotomized categories of total nitrite intake: highest tertile versus the two lower tertiles of intake combined. ^dAdjusted for maternal race/ethnicity, education, study center, folic acid supplementation during period one month before through one month post conception, and caloric intake. ^eAdjusted for maternal race/ethnicity, education, age, folic acid supplementation during the first trimester, maternal smoking, study center, and caloric intake. ^fAdjusted for maternal race/ethnicity, education, age, multivitamin use during the first trimester, study center, and caloric intake. ^gAdjusted for maternal race/ethnicity, education, multivitamin use during the first trimester, maternal smoking, study center, and caloric intake. ^hSignificant relative excess risk due to interaction (RERI) 1.60 (0.11, 3.09) was observed for cleft palate.