NIH Public Access
Author Manuscript
Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

Published in final edited form as:
Birth Defects Res A Clin Mol Teratol. 2012 September ; 94(9): 701–713. doi:10.1002/bdra.23060.

Nitrosatable drug exposure during the first trimester of pregnancy and selected congenital malformations

Jean D. Brender[1], **Martha M Werler**[2], **Mayura U Shinde**[1], **Ann M Vuong**[1], **Katherine E. Kelley**[2], **John C. Huber Jr.**[1], **Joseph R. Sharkey**[1], **John S. Griesenbeck**[3], **Paul A. Romitti**[4], **Sadia Malik**[5], **Lucina Suarez**[6], **Peter H. Langlois**[7], **Mark A. Canfield**[7], and **National Birth Defects Prevention Study**

[1]Texas A&M Health Science Center, School of Rural Public Health, College Station, TX

[2]Slone Epidemiology Center at Boston University, Boston, MA

[3]III Marine Expeditionary Force, Okinawa, Japan

[4]Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA

[5]Department of Pediatrics, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR

[6]Environmental Epidemiology and Disease Registries, Texas Department of State Health Services, Austin, TX

[7]Birth Defects Epidemiology and Surveillance Branch, Texas Department of State Health Services, Austin, TX

Abstract

BACKGROUND—Nitrosatable drugs can react with nitrite in the stomach to form *N*-nitroso compounds, and results from animal studies suggest that *N*-nitroso compounds are teratogens. With data from the National Birth Defects Prevention Study, the relation between prenatal exposure to nitrosatable drugs and limb deficiencies, oral cleft, and heart malformations in offspring was examined.

METHODS—Maternal reports of drugs taken during the first trimester of pregnancy were classified with respect to nitrosatability for mothers of 741 babies with limb deficiencies, 2,774 with oral cleft malformations, 8,091 with congenital heart malformations, and 6,807 without major congenital malformations. Nitrite intake was estimated from maternal responses to a food frequency questionnaire.

RESULTS—Isolated transverse limb deficiencies and atrioventricular septal defects were associated with secondary amine drug exposures (adjusted odds ratios [aOR] 1.51, 95% confidence limit [CI] 1.11, 2.06 and aOR 1.97, 95% CI 1.19, 3.26, respectively). Tertiary amines were associated with hypoplastic left heart syndrome (aOR 1.50, 95% CI 1.10, 2.04) and single ventricle (aOR 1.61, 95% CI 1.06, 2.45). These two malformations were also significantly associated with amide drugs. For several malformations, the strongest associations with nitrosatable drug use occurred among mothers with the highest estimated dietary nitrite intake, especially for secondary amines and atrioventricular septal defects (highest tertile of nitrite, aOR 3.30, 95% CI 1.44, 7.58).

Corresponding author: Jean D. Brender, PhD, Texas A&M Health Science Center, School of Rural Public Health, 219 SRPH Administration Building, College Station, TX 77843-1266, Telephone: 979-862-1573/Fax: 979-458-1877, jdbrender@srph.tamhsc.edu.

$watermark-text
$watermark-text
$watermark-text

CONCLUSION—Prenatal exposure to nitrosatable drugs may be associated with several congenital malformations, especially with higher nitrite intake. The possible interaction between nitrosatable drugs and dietary nitrite on risk of congenital malformations warrants further attention.

Keywords

congenital heart defects; congenital limb deficiency; nitrosatable drug; oral clefts; nitrites

INTRODUCTION

Various *N*-nitroso compounds have been observed to be teratogenic in animal models and may cause abnormal development through DNA alkylation of target organs (Bochert et al., 1985). In mice, defects associated with exposure to *N*-nitroso compounds have included exencephaly, cleft palate, (Platzek et al., 1983), limb malformations (Bochert et al., 1985), hydrocephalus, spina bifida, gastroschisis, and skeletal anomalies (Diwan, 1974). In rats, maternal exposure to such compounds resulted in increased incidence of limb malformations, neural tube defects, microcephalus, and hydrocephalus (Koyama et al., 1970). Frog embryos exposed to nitrosamines were noted to develop severe heart defects (Fort et al., 1991). In hamsters, nitrosamines crossed the placental barrier (Alaoui-Jamali et al., 1989), even at low doses (Jorquera et al., 1992).

Exposure to *N*-nitroso compounds occurs from exogenous sources, such as cured meats and smoked fish (Lijinsky, 1999), and through endogenous formation. Endogenous formation of *N*-nitroso compounds contributes approximately 45 to 75% of exposures to these compounds in humans (Tricker, 1997), and their formation depends on precursors such as nitrate, nitrite, and secondary/tertiary amines and amides. Results from numerous experimental studies have indicated that *N*-nitroso compounds can be formed *in vivo* by the reaction of nitrosatable amines or amides with nitrosating agents, such as nitrite, in an acidic environment like that found in the stomach (Preussman, 1984). From standard assays (Gillatt et al., 1984; Brambilla et al., 1985) and from simulated human gastric conditions (Ziebarth and Teichmann, 1980; Gillatt et al.,1985; Ohta et al., 1986; Sakai et al., 1984; Ziebarth et al., 1989), a variety of prescription and over-the-counter drugs have been identified as having secondary or tertiary amine or amide groups in their molecular structure that can react with nitrite to form nitrosamines and nitrosamides. In these tests, drugs with secondary amine or amide groups had greater yields of *N*-nitroso compounds than drugs containing tertiary amine groups. Furthermore, nitrosation of drugs with tertiary amines or amides resulted in the production of known carcinogens. Estimates of nitrosatable drug exposure during pregnancy have varied, but a recent study of nitrosatable drug exposure among control-mothers in the National Birth Defects Prevention Study (NBDPS) indicated that exposures to these drugs were fairly common during the first trimester (Brender et al., 2011a), with approximately 24% of NBDPS control-mothers taking one or more nitrosatable drugs during this period.

Although several studies examined associations between nitrosatable drug exposure and neural tube defects (Olshan and Faustman, 1989; Croen et al., 2001; Brender et al., 2004; Brender et al., 2011b) and craniosynostosis (Olshan and Faustman, 1989; Gardner et al., 1998; Kallen and Robert-Gnansia, 2005), only one study to date has been published that specifically examined these exposures and musculoskeletal or cardiovascular defects (Olshan and Faustman, 1989). Using data from the National Collaborative Perinatal Project, Olshan and Faustman (1989) noted that women who took any nitrosatable drugs during the first four months of pregnancy were slightly more likely to have offspring with

$watermark-text $watermark-text $watermark-text

$watermark-text

$watermark-text

$watermark-text

musculoskeletal and cardiovascular malformations, but data for oral clefts or limb deficiencies were not presented.

The formation of nitrosamines in the presence of a nitrosatable compound will occur to a much greater extent if the nitrite concentration is high (Choi, 1985), and nitrosatable compounds in combination with higher nitrite have been found to be more strongly associated with exencephaly and skeletal malformations in mice (Teramoto et al., 1980) and with neural tube defects in humans (Brender et al., 2004; Brender et al., 2011b). In this study, we examined 1) the relation between prenatal exposure during the first trimester of pregnancy to drugs classified as nitrosatable secondary amines, tertiary amines, or amides and limb deficiencies, oral cleft, and heart malformations; and 2) whether estimated dietary intake of nitrate and nitrite (based on dietary consumption during the year before pregnancy) modified the associations between nitrosatable drug use during pregnancy and these selected groups of malformations.

MATERIALS AND METHODS

Study Population

To investigate the relation between nitrosatable drug use during the first trimester and the selected congenital malformations, we used data from the NBDPS, a population-based case-control study of congenital malformations in the United States. Since the study's inception in 1997, a total of ten sites (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah) have participated in the NBDPS. Case births are identified from each site's population-based, birth defect surveillance system (Yoon et al., 2001) among live births (all sites), stillbirths (all sites except New York from 1997 to 1999 and New Jersey), and elective terminations (all sites except Massachusetts, New Jersey, and New York from 1997 to 1999) (Cogswell et al., 2009). Case-births were ineligible to be included in the NBDPS if they were adopted, in foster care, or had a deceased mother.

Case classification is standardized for the NBDPS as described by Rasmussen et al. (2003), and clinical information on potentially eligible cases are evaluated by a clinical geneticist at each study site and independently reviewed by one or more other clinical geneticists. Congenital malformations identified among case-births are further classified as multiple (more than one major malformation) or isolated (a single major malformation with or without minor malformations, a major malformation with other major malformations in the same organ system or body part, or a major malformation accompanied by other pathogenetically-related malformations) (Rasmussen et al., 2003). Case-births with a documented chromosomal abnormality or single gene disorder are excluded from the NBDPS. In addition to case-infants being classified as having isolated or multiple malformations, heart malformations of case-infants are classified with respect to their complexity including simple malformations, associations, or complex malformations as described by Botto et al. (2007). For the purposes of this study, we included case-births with oral cleft malformations (any/isolated), transverse and longitudinal limb deficiencies (any/isolated), or heart malformations (examined by etiologic subgroups) and control-infants with estimated delivery dates (EDDs) from October 1, 1997 through December 31, 2005. The majority of eligible cases were live births (98.8%) with only 94 (0.8%) stillborn and 47 (0.4%) pregnancy terminations.

In the NBDPS, control-infants (live births without any major congenital malformations and whose mothers resided in the study area at delivery) were either randomly selected from live birth certificates (Arkansas [for EDDs 2001 to present], Georgia [for EDDs 2001 to present], Iowa, Massachusetts, New Jersey, North Carolina, and Utah) or hospital records (Arkansas

$watermark-text

$watermark-text

$watermark-text

[for EDDs before 2001] California, Georgia [for EDDs before 2001], New York, and Texas) (Cogswell et al., 2009). Control-infants were not eligible if they had a major congenital malformation, were not residents of one of the geographic areas covered by the sites, were adopted or in foster care, had a deceased mother, or were stillborn (National Birth Defects Prevention Study Protocol, Centers for Disease Prevention, 2005). The institutional review boards at each site and the Centers for Disease Control and Prevention approved the NBDPS study protocol, and the institutional review boards of Texas A&M University and the Texas Department of State Health Services also approved this project on nitrosatable drugs and birth defects.

Data Collection

After informed consent was obtained, NBDPS participants were interviewed either in English or Spanish by female interviewers using a computer-assisted telephone interview (Yoon et al., 2001). The interview took about one hour to complete and included detailed questions regarding maternal health during the index pregnancy (including prescription and over-the-counter medications taken), diet (vitamin supplementation from three months before to the end of pregnancy, food consumption in the year before pregnancy and beverage consumption from three months before to the end of pregnancy), work characteristics, family demographics, and water use (Yoon et al., 2001). Interviews were targeted for completion within 6 months after the EDD with a maximum time from EDD to interview of no more than 24 months but no earlier than 6 weeks after the EDD.

Classification of Nitrosatable Drugs

As part of the interview, NBDPS participants were questioned about prescription and non-prescription drugs taken (including dates taken) for specific illnesses and diseases and about specific products from three months prior to the estimated date of conception to the date of birth of the index pregnancy. Reported drugs were linked to their active ingredients with the use of the Slone Epidemiology Center Drug Dictionary system (Kelley et al., 2003).

Methods used to classify drugs with respect to nitrosatability, functional groups, and indications have been discussed in detail in previous publications (Brender et al., 2011a,b). Briefly, all reported orally administered prescription and non-prescription medications and their active ingredients were identified, cross-referenced against previously compiled lists of nitrosatable medicinal compounds (Brambilla and Martelli, 2007; McKean-Cowdin et al., 2003)and categorized based on the presence of amine (secondary, tertiary) and amide functional groups in their chemical structures. The structures of all remaining active ingredients were evaluated for the presence of amine and amide functional groups and checked for any additional published evidence of nitrosatability using Medline and Internet sources. Finally, each component was categorized by its primary indication or therapeutic use and pharmacologic class. For the purposes of this study, we focused on drugs reported as taken during the first trimester of pregnancy, and unexposed women were defined as those who did not report taking drugs classified as nitrosatable during this period. Complete data on nitrosatable drug use and covariates were available for 94.0 to 95.1% of the various case group and control participants.

Estimation of Dietary Intake of Nitrates and Nitrites

In the NBDPS, several portions of the questionnaire elicit information about dietary intake in the year before pregnancy including a 58-item food frequency questionnaire that was adapted from the short Willett food frequency questionnaire (Willett et al., 1985; Willett et al., 1987) and additional detailed questions about consumption of breakfast cereals from three months before to the end of pregnancy. From these sources of information, we estimated dietary intake of nitrate and nitrite in mg/day using methods described in detail

$watermark-text

$watermark-text

$watermark-text

elsewhere (Griesenbeck et al., 2009; Griesenbeck et al., 2010). Briefly, 1) weighted means for nitrates and nitrites in mg/100 g were calculated for each food item based on the relevant literature; 2) the respective means were multiplied by the serving size in grams assigned to each food; 3) nitrates and nitrites in each serving size were multiplied by the number of servings per month; and 4) nitrates and nitrites across all food items were summed and then divided by 30 to obtain daily intake of dietary nitrate and nitrite in mg for each participant. In addition to dietary intake of nitrates and nitrites, an estimate of total dietary nitrites (exogenous sources and endogenously formed nitrites from conversion of nitrates to nitrites) was calculated by the method suggested by Choi (1985), in which total daily nitrite = dietary nitrite intake + 5% of dietary nitrate intake. Dietary nitrite and total nitrite intakes were categorized into tertiles based on the control-mothers' distributions. Consistent with other dietary studies with the NBDPS population (Yang et al., 2008; Carmichael et al., 2010), we excluded women with daily caloric intakes of less than 500 or more than 5000 kilocalories in analyses of nitrosatable drug exposure stratified by dietary nitrite and total nitrite. Complete data for any nitrosatable drug use stratified by total nitrite intake were available for 92.6 to 94.4% of participating case group and control mothers.

Data Analysis

In the final database developed for analysis, each record provided information on a unique mother-child pair. Logistic regression was used to estimate odds ratios (OR) for limb deficiencies, oral cleft malformations and heart malformations in relation to reported use of drugs during the first trimester that were classified as secondary amines, tertiary amines, or amides; each of these functional groups were analyzed separately. Women who did not report taking any drugs during the first trimester that were classified as nitrosatable served as the reference group in all analyses. For each major group of defects (limb deficiencies, oral cleft malformations, and heart malformations), covariates were selected in the logistic models based on their association with the specific malformation group and with maternal factors associated with nitrosatable drug use (study site, maternal race/ethnicity, education, age) as noted in a recent publication of factors related to nitrosatable drug use in NBDPS control mothers (Brender et al., 2011a). In the interest of simplicity of displaying results, covariates were consistently included in the regression analyses for the various malformations under each major congenital malformation group. Covariates in the analyses of limb deficiencies included maternal age (< 18, 18–19, 20–24, 25–29, 30–34, 35 years or older), maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, other), maternal education (<12 years, 12 years, 13–15 years, >15 years), study site, and multivitamin supplementation during the first trimester (yes/no). Covariates in the oral cleft malformation analyses included the same demographic characteristics as for limb deficiencies, but also included any reported maternal smoking one month prior to conception through the first trimester (yes/no) and folic acid supplementation during the first trimester (yes/no). In logistic models for congenital heart malformations, maternal race/ethnicity, education, study site, smoking status, and multivitamin use during the first trimester were included in all the models on the relation between maternal nitrosatable drug use and these malformations. Nitrosatable drug exposure was further stratified by tertiles of dietary nitrite and total nitrite, and the odds ratios in these analyses were also adjusted for total energy intake in addition to the aforementioned covariates. Because the patterns of results were mostly similar with stratification by dietary nitrite and total nitrite, we present the associations between nitrosatable drug exposure and birth defects stratified by total nitrite only.

Additive and multiplicative interaction was assessed for the associations of birth defects with nitrosatable drugs that appeared to vary by estimated daily intake of dietary nitrite/total nitrite. Additive interaction was examined with methods discussed by Andersson et al.

$watermark-text

$watermark-text

$watermark-text

(2005) in which the relative excess risk due to interaction (RERI) and the attributable proportion (AP) due to interaction were calculated along with their respective 95% confidence intervals. Significant additive interaction was considered present if either or both measures differed from zero and their 95% confidence intervals excluded zero.

To assess multiplicative interaction, the product terms of the nitrosatable drug functional groups with dietary nitrite and total nitrite were included in the logistic models. We examined the primary indications/pharmacologic classes of drugs contained in functional groups that were most strongly associated with birth defects among women with higher nitrite intake, The purpose of these analyses were to determine whether such associations were attributed to one or two drugs rather than a broader effect which would support the *N*-nitroso hypothesis.

RESULTS

NBDPS participants with EDDs during 1997–2005 included 741 mothers with babies with limb deficiencies, 2,774 with babies with oral clefts, 8,091 with babies with congenital heart malformations, and 6,807 control mothers with babies with no major congenital malformations. Maternal participation rates for limb deficiency cases, oral cleft cases, congenital heart malformation cases, and control births were respectively 69%, 74%, 69%, and 66%. On average, the time between EDD and interview was somewhat shorter for control mothers (9 months) than for case-mothers (11 months). Median time from EDD to interview was 10 months for mothers with babies with either limb deficiencies or heart malformations, 9 months for mothers with babies with oral clefts, and 8 months for control-mothers. Compared with control mothers, case mothers were more likely to be Hispanic if they had babies with limb deficiencies, less likely to be non-Hispanic black if they had babies with oral clefts, somewhat less educated (across all defect categories examined), and more likely to have smoked in early pregnancy, especially if they had babies with oral clefts (Table 1). Similar proportions (ranging from 82.9% to 86.2%) of case and control mothers reported any use of multivitamin or folic acid-containing supplements during the first trimester.

Exposure during the first trimester to any nitrosatable drugs varied slightly by cases and controls with 23.6% of the control mothers, 26.4% of the mothers with babies with limb deficiencies, and 25.3% of mothers with babies with either oral defect or heart malformations reporting use of these drugs. Nitrosatable amides were only slightly associated with the various phenotypes of oral clefts examined, with the strongest association noted between amides and cleft palate (adjusted OR [aOR] 1.27, 95% CI 1.00, 1.62) (Table 2). Mothers with babies with longitudinal limb deficiency or transverse limb deficiency were respectively 1.5 (95% CI 1.04, 2.04) and 1.4 (95% CI 1.07, 1.90) times more likely than control mothers to report taking nitrosatable secondary amines, and these associations persisted with restriction to isolated defects.

Prenatal use of nitrosatable amides and tertiary amines were associated with several types of congenital heart malformations in the study population (Table 3). Heart malformations associated with tertiary amines included hypoplastic left heart syndrome (aOR 1.50, 95% CI 1.10, 2.04) (data not shown) and single ventricle, which includes double inlet left or double inlet right ventricles (aOR 1.61, 95% CI 1.06, 2.45). Mothers with babies with these same malformations were also more likely to take nitrosatable amide drugs than control mothers (aORs respectively, 1.49 [95% CI 1.02, 2.17] and 1.84 [95% CI 1.15, 2.95]) as were mothers with babies with left ventricular outflow tract obstruction (group that included hypoplastic left heart syndrome) (aOR 1.31, 95% CI 1.02, 1.69) and septal defects (aOR 1.24, 95% CI 1.04, 1.49). First trimester exposure to drugs classified as secondary amines was associated

$watermark-text

$watermark-text

$watermark-text

with atrioventricular septal defects (aOR 1.97, 95% CI 1.19, 3.26). A significant negative association was noted between tertiary amines and anomalous pulmonary venous return (aOR 0.42, 95% CI 0.21, 0.83).

With stratification by dietary nitrite and total nitrite, amides were most strongly associated with isolated cleft palate among births to mothers with the highest estimated intakes of nitrite (data not shown) and total nitrite (aORs respectively of 1.71 [95% CI 1.09, 2.68] and 1.57 [95% CI 1.01, 2.45]) (Table 4), and significant additive interaction was noted between exposures to amides and nitrite (AP 0.43, 95% CI 0.12, 0.75) and total nitrite (AP 0.38, 95% CI 0.02, 0.73). Isolated cleft palate was associated with amide drugs across several indication/pharmacologic groups including antiemetics, anti-infectives, and stimulants (data not shown). Nitrite intake had minimal effects on the relation between amide drug exposure and other types of oral clefts.

In contrast, exposures to secondary and tertiary amines were most strongly associated with isolated longitudinal limb deficiencies in the lowest tertile of total nitrite intake, and the odds ratios in the highest tertile of intake were less than 1.00 (Table 5). No consistent patterns were observed between exposure to these drugs and isolated transverse limb deficiencies by tertile of total nitrite intake.

Stronger associations between tertiary amine or amide drug exposures and several congenital heart malformations were also noted among study participants with higher intake of total nitrite (Table 6), most notably for those with babies with a single ventricle (significant additive interaction present between amide drug exposures and nitrite) and conotruncal heart defects (significant additive and multiplicative interaction both present for tertiary amine and amide drug exposures). Mothers of babies with conotruncal heart defects were more likely than control women to take several types of tertiary amine drugs including antiemetic antihistamines, antiepileptics, and anti-infectives, but the only nitrosatable amides associated with these defects to any appreciable degree were sulfonamide anti-infectives. Mothers of babies with single ventricle were more likely than control mothers to take antiemetic, anti-infective, and stimulant drugs classified as amides.

For atrioventricular septal defects, odds ratios in relation to secondary amine drug exposure for the first, second, and third tertiles of total nitrite intake were respectively 1.16 (95% CI 0.43, 3.13), 1.88 (95% CI 0.72, 4.92), and 3.30 (95% CI 1.44, 7.58). Asthma medications and decongestants classified as secondary amines were associated with these defects. Left ventricular outflow tract obstruction defects were the only group of heart defects examined in which the odds ratios associated with tertiary amine and amide exposures were highest among women with the lowest estimated intake of total nitrite.

DISCUSSION

In this large, population-based case-control study, first trimester exposure to nitrosatable drugs was associated with several types of congenital malformations including limb deficiencies and atrioventricular septal defects with secondary amines; cleft lip with cleft palate, hypoplastic left heart syndrome, and single ventricle with tertiary amines; and cleft palate alone, hypoplastic left heart syndrome, septal heart defects, and single ventricle with amides. Furthermore, among mothers with the highest estimated intake of total nitrite, case mothers of babies with conotruncal heart defects were more likely than control mothers to take drugs classified as either nitrosatable amides or tertiary amines. Higher nitrite intake also strengthened the associations between nitrosatable drugs and other congenital malformations, specifically atrioventricular defects with secondary amines and cleft palate and single ventricle with amides.

$watermark-text

$watermark-text

$watermark-text

We know of only one other published study examining the risk of cardiovascular and musculoskeletal malformations in relation to prenatal exposure to nitrosatable drugs. In the National Collaborative Perinatal Project, women who took nitrosatable drugs during the first four months of pregnancy were 1.28 times more likely (95% CI 0.62, 2.64) to have infants with cardiovascular malformations and 1.33 times more likely (95% CI 1.05, 1.70) to have infants with musculoskeletal malformations (Olshan and Faustman, 1989); risk estimates for oral cleft malformations were not reported. Because of the small numbers of births with these outcomes, specific malformations within each of these broad classifications were not examined, nor was nitrosatable drug exposure categorized by functional group. In the present study, we noted an odds ratio of 1.07 (95% CI 0.99, 1.16) for any type of congenital heart malformation and an odds ratio of 1.34 (95% CI 1.09, 1.65) for any type of isolated limb deficiency in relation to any nitrosatable drug exposure during the first trimester. We found stronger associations between nitrosatable drug use and these birth defects when we examined functional groups of nitrosatable drugs (secondary and tertiary amines, amides) in relation to specific malformations, and some of these associations also appeared to be modified by dietary nitrite intake.

Within functional groups of nitrosatable drugs that showed stronger associations with birth defects among women with higher estimated dietary nitrite intake, a broad range of indications and pharmacologic classes were represented, supporting our hypothesis that the endogenous formation of *N*-nitroso compounds might increase risk for congenital malformations. However, risk estimates in relation to the specific drug indications and/or pharmacologic classes were often compatible with the null because of the small numbers of exposed women.

With respect to nitrosatable amides, several types of anti-infectives within this functional group were associated with cleft palate alone and several congenital heart malformations in the study population. In an earlier examination of the use of anti-infectives within the NBDPS population (EDDs of 1997–2003) in which the exposure period was defined as one month before conception through the first trimester, mothers of babies with any type of oral cleft were twice as likely as control mothers (95% CI 0.6, 6.7) to take tetracycline antibiotics (Crider et al., 2009). In a retrospective cohort study conducted in Tennessee (Cooper et al., 2009), women who were prescribed doxycycline during the first four months of pregnancy were nearly three times more likely (relative risk [RR] 2.96, 95% CI 0.75, 11.67) to give birth to babies with oral cleft defects than women not prescribed antibiotics during this period, but this study had insufficient numbers of exposed mothers to examine risk specifically for cleft palate.

Clindamycin was the only macrolide anti-infective drug in this study that was classified as a nitrosatable amide (also classified as a tertiary amine) and was significantly associated with isolated cleft palate. Although several experimental studies with various animal models found no evidence of increased prevalence of cleft palate with maternal exposure to clindamycin (Gray et al., 1972; Bollert et al., 1974), no published epidemiologic studies were identified that specifically examined risk of cleft palate in relation to prenatal exposure to this drug. Results from animal and human studies have indicated the teratogenic potential as being either “none” or “unlikely” for various beta lactam antibiotics (several of which were classified in this study as nitrosatable amides) (Nahum et al., 2006). However, prenatal exposure to this group of antibiotics was associated with single ventricle heart defects in the NBDPS study population. In an earlier study with the NBDPS population, Crider et al. (2009) noted an elevated odds ratio for atrial septal defects (aOR 1.9, 95% CI 1.1, 3.2) with exposure to cephalosporins one month before conception through the end of the first trimester.

$watermark-text

$watermark-text

$watermark-text

Single ventricle defects were also associated with NBDPS mothers' use of the cough suppressant dextromethorphan which was classified as a nitrosatable amide and tertiary amine. There has been considerable disagreement on whether this drug is a potential teratogen (Ferencz et al., 1997; Andaloro et al., 1998; Holmes, 1999; Xu et al., 2011). Several studies found no association between prenatal use of this drug and major congenital malformations (Einarson et al., 2001; Martinez-Frias and Rodriguez-Pinilla, 2001), but these studies lacked sufficient sample sizes to examine associations with specific congenital heart malformations.

Drugs classified as secondary amines were associated with atrioventricular septal defects and specifically asthma (albuterol, epinephrine, and terbutaline) and decongestant (ephedrine and pseudoephedrine) medications within this nitrosatable functional group. Ephedrine, one of the secondary amine decongestants, has been linked to cardiac anomalies in animal studies (Nishikawa et al., 1985; Werler, 2006), but empirical evidence from epidemiologic studies is lacking regarding the association of such drugs with cardiovascular malformations. In a retrospective cohort study, Kallen and Olausson (2007) found that women who reported the use of any type of anti-asthmatic drugs at the first maternal health care visit (usually week 10–12) were 1.3 times more likely (95% CI 1.00, 1.61) than women who did not report taking these drugs to give birth to babies with severe cardiac malformations. In a case-control study conducted in the UK, Tata et al. (2008) noted that asthmatic mothers of babies with circulatory system malformations were more likely than asthmatic control mothers to have taken one or more asthma medications during pregnancy (aOR 1.27, 95% CI 1.02, 1.58). Medications reported, however, included a wide-range of pharmacologic classes. In a case-control study of congenital heart defects in New York state (Lin et al., 2009), case-mothers were more likely than control mothers to use bronchodilators (OR 2.20, 95% CI 1.05, 4.61), a category in which over half (58%) of the drugs reported taken by the cases included one of the asthma medications classified as secondary amines in the present study.

One of the strengths of the present study was the large numbers of birth defect cases available for analysis, including samples sizes that were sufficient to allow analyses with isolated birth defects. For example, with secondary amine, tertiary amine, and amide drug exposures, we had 80% power to detect minimum detectable ORs of 1.4, 1.4, and 1.5 respectively for isolated cleft palate; ORs of 1.6, 1.6, and 1.7 respectively for isolated transverse limb deficiency; and ORs of 1.3, 1.3, and 1.4 respectively for isolated conotruncal heart defects. On the other hand, we could detect odds ratios only between 2.0 and 3.0 with 80% power for more rare defects such as isolated atrioventricular septal defect and Ebstein's anomaly.

This study had other limitations. Approximately one-third of the eligible control mothers and 24% to 31% of the eligible case mothers did not participate in the study. Participating control mothers, however, tended to be representative of their base populations with respect to age and smoking and differed only slightly by race/ethnicity and education (Cogswell et al., 2009). Within the NBDPS, each participating study site attempts to capture 100% of eligible birth defect cases from their respective sources of ascertainment (e.g., live births, fetal deaths, and terminations [for most participating study sites]), although the representativeness of participating case mothers has not yet been reported.

We estimated dietary intake of nitrate and nitrite from a food frequency questionnaire that was subject to the participants' recall and also might not have captured all dietary sources of these food contaminants (Griesenbeck et al., 2010). For the purposes of examining nitrite intake as a potential effect modifier of the relation between nitrosatable drug use and birth defects, such an approach was probably adequate in classifying dietary nitrite into high,

$watermark-text

$watermark-text

$watermark-text

intermediate, and low levels of intake. In the NBDPS, participants are questioned about frequency of foods eaten during the year before conception which might have resulted in some misclassification of foods consumed during the first trimester of pregnancy. On the other hand, this misclassification was most likely nondifferential with regards to outcome status because the same period of dietary assessment was used for all NBDPS participants. Furthermore, results from another study indicated that average consumption of vegetables and meat did not significantly differ by time as measured by consecutive seven-day dietary records before pregnancy, and in weeks 6, 10, 26, and 38 of pregnancy (Cuco et al., 2006). Vegetables and meats are major sources respectively of nitrates and nitrites.

Although we used several extensive reviews of nitrosatable drugs (Brambilla and Martelli, 2007; McKean-Cowdin et al., 2003) as well as searched the more recent literature for additional reports of the nitrosatability of various drugs, exposures to some of these drugs may have been missed. Components of some of the drugs reported used might have not been tested for nitrosatability, and results of such tests might have not been published.

Another potential limitation of this study was the possibility of recall bias in which mothers of malformed offspring might have been more likely to recall drug exposures during the first trimester than mothers of non-malformed offspring. Some studies have found little evidence for differential recall for several classes of drugs that have nitrosatable products such as antibiotics (Werler et al., 1989; Feldman et al., 1989; Delgado-Rodriguez et al., 1995); antinauseants (Delgado-Rodriguez et al., 1995) and analgesics (Feldman et al., 1989). Several methodologic features of the NBDPS and this study may have reduced recall bias. First, women were asked about medications by indication for use and were also prompted with lists of medications. This two level approach to assess drug use has been shown to be more accurate than either type of question alone (Mitchell et al., 1986). Second, it is not common knowledge that preformed *N*-nitroso compounds and products of endogenous nitrosation are possible teratogens. Therefore, it is unlikely that differential reporting accuracy would occur with respect to drug nitrosatability. Furthermore, women were asked about use of medications during pregnancy, but the reported drugs were classified with respect to nitrosatability subsequent to the interviews. If recall bias accounted for the findings in this study, it would be expected that the odds ratios for the congenital malformations studied in relation to nitrosatable drug exposure would be elevated across all functional groups of drugs by tertiles of nitrite and total nitrite intake, a pattern that was not observed in this study. Finally, we examined nitrosatable drug exposure among study participants during the month prior to conception and excluded from such analyses women who took these drugs during the first trimester. We observed that most of the birth defects associated with nitrosatable drug exposure during the first trimester were not associated with exposure to such drugs taken during the month before conception (odds ratios close to 1.0 for most associations and all 95% confidence intervals compatible with the null), except for amide drugs and septal defects (AOR 1.79, 95% CI 1.18, 2.71)

This study involved multiple analyses and many comparisons, especially with respect to drug exposures stratified by dietary nitrite intake. Although analyses of nitrosatable drug-nitrite interactions were performed in accordance with *a priori* hypotheses, some of the significant findings might have been due to chance from multiple comparisons. In study analyses, 95% confidence intervals were determined for 60 associations between nitrosatable drug exposure and birth defects (45 for isolated and 15 for non-isolated birth defects). Three associations would be expected by chance alone. Ten statistically significant associations (AORs greater than 1.0 and 95% confidence intervals excluded the null) were observed including cleft lip with cleft palate defect with tertiary amines; longitudinal and transverse limb deficiencies (isolated and non-isolated) with secondary amines; atrioventricular septal defects with secondary amines; hypoplastic left heart syndrome with

$watermark-text

$watermark-text

$watermark-text

tertiary amines; and left ventricular outflow tract (including hypoplastic left heart syndrome), septal, and single ventricle heart defects with amides. To assess interaction between nitrosatable drug functional groups and total nitrite with birth defects, 24 statistical tests were conducted. Six statistically significant interactions were noted, while only one might have been expected by chance.

To our knowledge, this is the first study to examine the relation between prenatal exposure to functional groups of nitrosatable drugs (amides, secondary and tertiary amines) and specific phenotypes of limb deficiencies, oral cleft defects, and congenital heart malformations. Furthermore, we stratified nitrosatable drug exposure by dietary nitrite to examine whether higher intake of nitrite strengthened associations with nitrosatable drugs, a finding that would support the hypothesis that endogenous formation of nitrosamines/nitrosamides may function as human teratogens. Several associations of nitrosatable drugs with cleft palate and with various heart defects were more pronounced with higher estimated intake of dietary nitrite, although this pattern was not present for other defects examined and even reversed for longitudinal limb deficiencies and hypoplastic left heart syndrome. Generally, epidemiologic studies of drugs and birth defects have focused on such exposures without consideration of concomitant exposures to environmental contaminants and the potential endogenous formation of teratogens. With nitrites and nitrosatable compounds, empirical data from at least one animal model demonstrated that nitrite and a nitrosatable compound only functioned as teratogens if given together (Teramoto et al., 1980). Several findings in this study lend support that a similar phenomenon might operate in humans, and many comparisons are presented to inform future researchers on this topic. The associations detected between birth defects and prenatal exposure to nitrosatable drugs in conjunction with higher dietary intake of nitrite warrant further attention.

Acknowledgments

Sponsors of research: This research was supported by Award Numbers 5R01ES015634 and 3R01ES015634-03S1 from the National Institute of Environmental Health Sciences; Centers for Disease Control and Prevention, Birth Defects Branch Cooperative Agreement U50/CCU613232; and Texas Department of State Health Services Contract 2012-039849.The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Environmental Health Sciences or the National Institutes of Health.

Coding of drug information in the National Birth Defects Prevention Study used the Slone Drug Dictionary under license from the Slone Epidemiology Center of Boston University, Boston, Massachusetts. We thank the participating families, staff, and scientists from all sites in the National Birth Defects Prevention Study. We also thank Dr. Roberta McKean-Cowdin, Norris Comprehensive Cancer Center/Department of Preventive Medicine, University of Southern California, for sharing the list of nitrosatable drugs developed by the late Dr. William Lijinsky for the childhood cancer studies. We express our appreciation to Michelle Steck, Texas A&M Health Science Center, School of Rural Public Health, for her assistance in developing the nitrate and nitrite estimates for the food frequency questionnaire and to Amy Hanson, University of Texas Health Science Center at Houston, School of Public Health, for her assistance in the development of the nitrosatable drug files.

This work was supported by the National Institute of Environmental Health Sciences at the National Institutes of Health (5R01ES015634 and 3R01ES015634-03S1), the Centers for Disease Control and Prevention/Texas Center for Birth Defects Research and Prevention (Cooperative Agreement U50/CCU613232), and Texas Department of State Health Services Contract 2012-039849.The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Environmental Health Sciences or the National Institutes of Health.

References

Alaoui-Jamali MA, Rossignol G, Schuller HM, Castonguay A. Transplacental genotoxicity of a tobacco-specific N-nitrosoamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, in Syrian golden hamster. Mutat Res. 1989; 223:65–72. [PubMed: 2654630]

$watermark-text

$watermark-text

$watermark-text

Andaloro VJ, Monaghan DT, Rosenquist TH. Dextromethorphan and other N-methyl-D-aspartate receptor antagonists are teratogenic in the avian embryo mode. Pediatr Res. 1998; 43:1–7. [PubMed: 9432105]

Andersson T, Alfredsson L, Kallberg H, et al. Calculating measures of biological interaction. Eur J Epidemiol. 2005; 20:575–9. [PubMed: 16119429]

Bochert G, Platzek T, Blankenburg G, et al. Embryotoxicity induced by alkylating agents: left-sided preponderance of paw malformations induced by acetoxymethyl-methynitrosamine in mice. Arch Toxicol. 1985; 56:139–150. [PubMed: 3977594]

Bollert JA, Gray JE, Highstrete JD, et al. Teratogenicity and neonatal toxicity of clindamycin 2-phosphate in laboratory animals. Toxicol Appl Pharmacol. 1974; 27:322–329. [PubMed: 4854622]

Botto LD, Lin AE, Riehle-Colarusso T, et al. Seeking causes: classifying and evaluating congenital heart defects in etiologic studies. Birth Defects Res A Clin Mol Teratol. 2007; 79:714–727. [PubMed: 17729292]

Brambilla G, Cajelli E, Finollo R, et al. Formation of DNA-damaging nitroso compounds by interaction of drugs with nitrite. A preliminary screening for detecting potentially hazardous drugs. J Toxicol Environ Health. 1985; 15:1–24. [PubMed: 3981659]

Brambilla G, Martelli A. Genotoxic and carcinogenic risk to humans of drug-nitrite interaction products. Mutat Res. 2007; 635:17–52. [PubMed: 17157055]

Brender JD, Olive JM, Felkner M, et al. Dietary nitrites and nitrates, nitrosatable drugs, and neural tube defects. Epidemiology. 2004; 15:330–336. [PubMed: 15097014]

Brender JD, Kelley KE, Werler MM, et al. Prevalence and patterns of nitrosatable drug use among U.S. women during early pregnancy. Birth Defects Res A Clin Mol Teratol. 2011a; 91:258–264. [PubMed: 21472845]

Brender JD, Werler MM, Kelley KE, et al. Nitrosatable drug exposure during early pregnancy and neural tube defects in offspring. Am J Epidemiol. 2011b; 174:1286–95. [PubMed: 22047825]

Carmichael SL, Rasmussen SA, Lammer EJ, et al. Craniosynostosis and nutrient intake during pregnancy. National Birth Defects Prevention Study. Birth Defects Res A Clin Mol Teratol. 2010; 88:1032–9. [PubMed: 20842649]

Choi BC. N-Nitroso compounds and human cancer. Am J Epidemiol. 1985; 121:737–43. [PubMed: 4014165]

Cogswell ME, Bitsko RH, Anderka M, et al. Control selection and participation in an ongoing, population-based, case-control study of birth defects. Am J Epidemiol. 2009; 170:975–985. [PubMed: 19736223]

Cooper WO, Hernandez-Diaz S, Arbogast PG, et al. Antibiotics potentially used in response to bioterrorism and the risk of major congenital malformations. Paediatr Perinat Epidemiol. 2009; 23:18–28. [PubMed: 19228311]

Crider KS, Cleves MA, Reefhuis J, et al. Antibacterial medication use during pregnancy and risk of birth defects. Arch Pediatr Adolesc Med. 2009; 163:978–985. [PubMed: 19884587]

Croen LA, Todoroff K, Shaw GM. Maternal exposure to nitrate from drinking water and diet and risk for neural tube defects. Am J Epidemiol. 2001; 153:325–331. [PubMed: 11207149]

Cuco G, Fernandez-Ballart J, Sala J, et al. Dietary patterns and associated lifestyles in preconception, pregnancy and postpartum. Eur J Clin Nutr. 2006; 60:364–371. [PubMed: 16340954]

Delgado-Rodriguez M, Gomez-Olmedo M, Bueno-Cavanillas A, et al. Recall bias in a case-control study of low birth weight. J Clin Epidemiol. 1995; 48:1133–1140. [PubMed: 7636515]

Diwan BA. Strain-dependent teratogenic effects of 1-ethyl-1-nitrosourea in inbred strains of mice. Cancer Res. 1974; 34:151–157. [PubMed: 4203459]

Einarson A, Lyszkiewicz D, Koren G. The safety of dextromethorphan in pregnancy. Results of a controlled study. Chest. 2001; 119:466–469. [PubMed: 11171724]

Feldman Y, Koren G, Mattice K, et al. Determinants of recall and recall bias in studying drug and chemical exposure in pregnancy. Teratology. 1989; 40:37–45. [PubMed: 2763209]

Ferencz C, Correa-Villase XA, Loffredo CA, et al. Genetic and environmental risk factors of major cardiovascular malformations: The Baltimore-Washington Infant Study 1981–1989. Perspect Pediatr Cardio. 1997; 5:50–62.

$watermark-text

$watermark-text

$watermark-text

Fort DJ, Rayburn JR, DeYoung DJ, et al. Assessing the efficacy of an aroclor 1254-induced exogenous metabolic activation system for FETAX. Drug Chem Toxicol. 1991; 14:143–160. [PubMed: 1889373]

Gardner JS, Guyard-Boileau B, Alderman BW, et al. Maternal exposure to prescription and non-prescription pharmaceuticals or drugs of abuse and craniosynostosis. Int J Epidemiol. 1998; 27:64–67. [PubMed: 9563695]

Gillatt PN, Hart RJ, Walters CL, et al. Susceptibilities of drugs to nitrosation under standardized chemical conditions. Food Chem Toxicol. 1984; 22:269–274. [PubMed: 6539274]

Gillatt PN, Palmer RC, Smith PL, et al. Susceptibilities of drugs to nitrosation under simulated gastric conditions. Food Chem Toxicol. 1985; 23:849–855. [PubMed: 4043885]

Gray JE, Weaver RN, Bollert JA, et al. The oral toxicity of clindamycin in laboratory animals. Toxicol Appl Pharmacol. 1972; 21:516–531. [PubMed: 5047049]

Griesenbeck JS, Steck MD, Huber JC Jr, et al. Development of estimates of dietary nitrates, nitrites, and nitrosamines for use with the short Willett food frequency questionnaire. Nutr J. 2009; 8(1): 16. [PubMed: 19348679]

Griesenbeck JS, Brender JD, Sharkey JR, et al. Maternal characteristics associated with the dietary intake of nitrates, nitrites, and nitrosamines in women of child-bearing age: a cross-sectional study. Environ Health. 2010; 9(1):10. [PubMed: 20170520]

Holmes LB. Introduction. Teratology. 1999; 60:56. [PubMed: 10440773]

Jorquera R, Castonguay A, Schuller HM. Placental transfer of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone instilled intratracheally in Syrian golden hamsters. Cancer Res. 1992; 52:3273–80. [PubMed: 1596885]

Kallen B, Olausson PO. Use of anti-asthmatic drugs during pregnancy. 3. Congenital malformations in the infants. Eur J Clin Pharmacol. 2007; 63:383–388. [PubMed: 17279357]

Kallen B, Robert-Gnansia E. Maternal drug use, fertility problems, and infant craniostenosis. Cleft Palate Craniofac J. 2005; 42:589–593. [PubMed: 16241169]

Kelley KE, Kelley TP, Kaufman DW, et al. The Slone Drug Dictionary: a research driven pharmacoepidemiology tool. Pharmacoepidemiol Drug Saf. 2003; 12:S168–S198.

Koyama T, Handa J, Handa H, et al. Methynitrosourea-induced malformations of the brain in SD-JCL rat. Arch Neurol. 1970; 22:342–347. [PubMed: 5461423]

Lijinsky W. N-nitroso compounds in the diet. Mutat Res. 1999; 443:129–138. [PubMed: 10415436]

Lin S, Herdt-Losavio M, Gensburg L, et al. Maternal asthma, asthma medication use, and the risk of congenital heart defects. Birth Defects Res A Clin Mol Teratol. 2009; 85:161–168. [PubMed: 19067406]

Martinez-Frias ML, Rodriguez-Pinilla E. Epidemiologic analysis of prenatal exposure to cough medicines containing dextromethorphan: no evidence of human teratogenicity. Teratology. 2001; 63:38–41. [PubMed: 11169553]

McKean-Cowdin R, Pogoda JM, Lijinsky W, et al. Maternal prenatal exposure to nitrosatable drugs and childhood brain tumours. Int J Epidemiol. 2003; 32:211–217. [PubMed: 12714539]

Mitchell AA, Cottler LB, Shapiro S. Effect of questionnaire design on recall of drug exposure in pregnancy. Am J Epidemiol. 1986; 123:670–6. [PubMed: 3953545]

Nahum GG, Uhl K, Kennedy DL. Antibiotic use in pregnancy and lactation: what is and is not known about teratogenic and toxic risks. Obstet Gynecol. 2006; 107:1120–1138. [PubMed: 16648419]

Nishikawa T, Bruyere HJ Jr, Takagi Y, et al. Cardiovascular teratogenicity of ephedrine in chick embryos. Toxicol Lett. 1985; 29:59–63. [PubMed: 4082206]

Ohta T, Asabe Y, Takitani S. Identification of mutagenic nitrosation products of antipyrine and evaluation of their formation under model stomach conditions. Chem Pharm Bull (Tokyo). 1986; 34:3866–3872. [PubMed: 3815608]

Olshan AF, Faustman EM. Nitrosatable drug exposure during pregnancy and adverse pregnancy outcome. Int J Epidemiol. 1989; 18:891–899. [PubMed: 2621027]

Platzek T, Bochert G, Rahm U. Embryotoxicity induced by alkylating agents. Teratogenicity of acetoxymethyl-methylnitrosamine: dose-response relationship, application route dependency and phase specificity. Arch Toxicol. 1983; 52:45–69. [PubMed: 6838376]

$watermark-text

$watermark-text

$watermark-text

Preussmann R. Occurrence and exposure to N-nitroso compounds and precursors. IARC Sci Publ. 1984; 57:3–15. [PubMed: 6398296]

Sakai A, Inoue T, Tanimura A. Formation of volatile nitrosamines by drug-nitrite interactions under physiological conditions. Gann. 1984; 75:245–252. [PubMed: 6724228]

Rasmussen SA, Olney RS, Holmes LB, et al. Guidelines for case classification for the National Birth Defects Prevention Study. Birth Defects Res A Clin Mol Teratol. 2003; 67:193–201. [PubMed: 12797461]

Tata LJ, Lewis SA, McKeever TM, et al. Effect of maternal asthma, exacerbations, and asthma medication use on congenital malformations in offspring: a UK population-based study. Thorax. 2008; 63:981–987. [PubMed: 18678701]

Teramoto S, Saito R, Shirasu Y. Teratogenic effects of combined administration of ethylenethiourea and nitrite in mice. Teratology. 1980; 21:71–78. [PubMed: 7385057]

Tricker AR. N-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. Eur J Cancer Prev. 1997; 6:226–268. [PubMed: 9306073]

Werler MM, Pober BR, Nelson K, Holmes LB. Reporting accuracy among mothers of malformed and nonmalformed infants. Am J Epidemiol. 1989; 129:415–421. [PubMed: 2643303]

Werler WM. Teratogen update: pseudoephedrine. Birth Defects Res A Clin Mol Teratol. 2006; 76:445–452. [PubMed: 16933214]

Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol. 1985; 122:51–65. [PubMed: 4014201]

Willett WC, Reynolds RD, Cottrell-Hoehner S, et al. Validation of a semi-quantitative food frequency questionnaire: comparison with a 1-year diet record. J Am Diet Assoc. 1987; 87:43–7. [PubMed: 3794132]

Xu Z, Williams FE, Liu MC. Developmental toxicity of dextromethorphan in zebrafish embryos/larvae. J Appl Toxicol. 2011; 31:157–163. [PubMed: 20737414]

Yang W, Shaw GM, Carmichael SL, et al. Nutrient intakes in women and congenital diaphragmatic hernia in their offspring. Birth Defects Research A Clin Mol Teratol. 2008; 82:131–8.

Yoon PW, Rasmussen SA, Lynberg MC, et al. The National Birth Defects Prevention Study. Public Health Rep. 2001; 116 (Suppl1):32–40. [PubMed: 11889273]

Ziebarth D, Teichmann B. Nitrosation of orally administered drugs under simulated stomach conditions. IARC Sci Publ. 1980; 31:231–244. [PubMed: 7228255]

Ziebarth D, Schramm T, Toppel A. Drugs from the class of tricyclic antidepressives and antiepileptics, nitrosatable under simulated human gastric conditions. Arch Geschwulstforsch. 1989; 59:257–263. [PubMed: 2802933]

$watermark-text $watermark-text $watermark-text

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

Table 1

Selected Maternal Characteristics of Limb, Oral Cleft and Heart Defects Cases and Controls in the National Birth Defects Prevention Study, 1997–2005

Characteristics of participants	Controls n= 6807		Limb deficiency defects n= 741		Oral cleft defects n=2774		Congenital heart defects n=8091	
	No.	%	No.	%	No.	%	No.	%
Race-ethnicity								
Non-Hispanic white	4026	59.1	416	56.1	1724	62.2	4698	58.1
Non-Hispanic black	771	11.3	74	10.0	183	6.6	930	11.5
Hispanic	1502	22.1	204	27.5	659	23.8	1868	23.1
Asian/Pacific Islander	200	2.9	14	1.9	82	3.0	216	2.7
Other	279	4.1	33	4.5	118	4.3	361	4.5
Missing	29	0.4	0	0	8	0.3	18	0.2
Education (years)								
<12	1130	16.6	126	17.0	525	18.9	1399	17.3
12	1652	24.3	200	27.0	778	28.1	2091	25.8
13–15	1806	26.5	208	28.1	731	26.4	2196	27.1
>15	2110	31.0	195	26.3	711	25.6	2270	28.1
Missing	109	1.6	12	1.6	29	1.1	135	1.7
Age at delivery (years)								
<18	255	3.8	31	4.2	90	3.2	271	3.4
18–19	478	7.0	59	8.0	200	7.2	489	6.0
20–24	1552	22.8	188	25.4	704	25.4	1842	22.8
25–29	1807	26.6	190	25.6	738	26.6	2130	26.3
30–34	1759	25.8	185	25.0	626	22.6	2086	25.8
>34	956	14.0	88	11.9	416	15.0	1272	15.7
Missing	0	0.0	0	0.0	0	0.0	1	0.0
Study site								
Arkansas	848	12.5	70	9.5	310	11.2	1208	14.9
California	858	12.6	120	16.2	450	16.2	864	10.7
Georgia	735	10.8	78	10.5	333	12.0	994	12.3
Iowa	759	11.2	80	10.8	306	11.0	769	9.5

$watermark-text $watermark-text $watermark-text

Characteristics of participants	Controls n= 6807		Limb deficiency defects n= 741		Oral cleft defects n=2774		Congenital heart defects n=8091	
	No.	%	No.	%	No.	%	No.	%
Massachusetts	859	12.6	92	12.4	389	14.0	1102	13.6
North Carolina	412	6.1	16	2.2	101	3.6	295	3.6
New Jersey	574	8.4	84	11.3	192	6.9	548	6.8
New York	601	8.8	54	7.3	249	9.0	580	7.2
Texas	792	11.6	97	13.1	348	12.6	1242	15.4
Utah	369	5.4	50	6.7	96	3.5	489	6.0
Smoking[a]								
No	5444	80.0	582	78.5	2074	74.8	6344	78.4
Yes	1274	18.7	150	20.2	675	24.3	1629	20.1
Missing	89	1.3	9	1.2	25	0.9	118	1.5
Folic acid-containing supplement use[b]								
No	860	12.6	97	13.1	416	15.0	1101	13.6
Yes	5782	84.9	621	83.8	2299	82.9	6766	83.6
Missing	165	2.4	23	3.1	59	2.1	224	2.8
Multivitamin use[b]								
No	785	11.5	92	12.4	371	13.4	999	12.3
Yes	5865	86.2	629	84.9	2350	84.7	6882	85.1
Missing	157	2.3	20	2.7	53	1.9	210	2.6

[a] Any smoking one month prior to conception through the first trimester of pregnancy.

[b] Refers to use during the first trimester of pregnancy.

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

$watermark-text $watermark-text $watermark-text

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

Table 2

Exposure to Nitrosatable Drugs During the First Trimester and Selected Non-cardiac Congenital Malformations, National Birth Defects Prevention Study, 1997–2005

Defect group	Type of drug exposure	Cases		Controls		Unadjusted OR[b]	95% CI	Adjusted OR[c]	95% CI
		No.	%[a]	No.	%[a]				
Cleft lip alone	No nitrosatable drugs	449	74.0	4826	76.3	1.00	Referent	1.00	Referent
	Secondary amines	92	17.0	791	14.1	1.25	0.99, 1.58	1.18	0.93, 1.50
	Tertiary amines	78	14.8	781	13.9	1.07	0.83, 1.38	1.07	0.82, 1.38
	Amides	55	10.9	496	9.3	1.19	0.89, 1.60	1.16	0.86, 1.57
Cleft lip alone, isolated	No nitrosatable drugs	417	73.0	4826	76.3	1.00	Referent	1.00	Referent
	Secondary amines	89	17.6	791	14.1	1.30	1.02, 1.66	1.22	0.96, 1.57
	Tertiary amines	76	15.4	781	13.9	1.13	0.87, 1.45	1.12	0.86, 1.46
	Amides	53	11.3	496	9.3	1.24	0.92, 1.67	1.20	0.88, 1.63
Cleft lip with cleft palate	No nitrosatable drugs	852	75.2	4826	76.3	1.00	Referent	1.00	Referent
	Secondary amines	137	13.9	791	14.1	0.98	0.81, 1.19	1.01	0.83, 1.23
	Tertiary amines	162	16.0	781	13.9	1.17	0.98, 1.41	1.25	1.03, 1.51
	Amides	102	10.7	496	9.3	1.16	0.93, 1.46	1.21	0.96, 1.52
Cleft lip with cleft palate, isolated	No nitrosatable drugs	726	74.8	4826	76.3	1.00	Referent	1.00	Referent
	Secondary amines	118	14.0	791	14.1	0.99	0.80, 1.22	1.00	0.81, 1.24
	Tertiary amines	139	16.1	781	13.9	1.18	0.97, 1.44	1.23	1.00, 1.51
	Amides	87	10.7	496	9.3	1.17	0.92, 1.48	1.19	0.93, 1.52
Cleft palate alone	No nitrosatable drugs	670	74.5	4826	76.3	1.00	Referent	1.00	Referent
	Secondary amines	121	15.3	791	14.1	1.10	0.90, 1.36	1.06	0.86, 1.31
	Tertiary amines	115	14.7	781	13.9	1.06	0.86, 1.31	1.08	0.86, 1.34
	Amides	90	11.8	496	9.3	1.31	1.03, 1.66	1.27	0.99, 1.61
Cleft palate alone, isolated	No nitrosatable drugs	545	75.8	4826	76.3	1.00	Referent	1.00	Referent
	Secondary amines	88	13.9	791	14.1	0.99	0.78, 1.25	0.93	0.73, 1.19
	Tertiary amines	83	13.2	781	13.9	0.94	0.74, 1.20	0.93	0.73, 1.20
	Amides	68	11.1	496	9.3	1.21	0.93, 1.59	1.15	0.88, 1.52
Longitudinal limb deficiency	No nitrosatable drugs	196	72.9	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	46	19.0	814	14.2	1.42	1.02, 1.97	1.46	1.04, 2.04
	Tertiary amines	31	13.7	800	14.0	0.97	0.66, 1.43	0.98	0.66, 1.46

$watermark-text $watermark-text $watermark-text

Defect group	Type of drug exposure	Cases		Controls		Unadjusted OR[b]	95% CI	Adjusted OR[c]	95% CI
		No.	%[a]	No.	%[a]				
	Amides	20	9.3	505	9.3	0.99	0.62, 1.59	1.02	0.64, 1.64
Longitudinal limb deficiency, isolated	No nitrosatable drugs	106	69.3	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	27	20.3	814	14.2	1.54	1.00, 2.36	1.50	0.97, 2.33
	Tertiary amines	20	15.9	800	14.0	1.16	0.72, 1.88	1.11	0.67, 1.82
	Amides	14	11.7	505	9.3	1.29	0.73, 2.26	1.22	0.69, 2.17
Transverse limb deficiency	No nitrosatable drugs	292	73.0	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	63	17.8	814	14.2	1.30	0.98, 1.73	1.42	1.07, 1.90
	Tertiary amines	48	14.1	800	14.0	1.01	0.74, 1.38	1.17	0.85, 1.61
	Amides	36	11.0	505	9.3	1.20	0.84, 1.72	1.32	0.91, 1.89
Transverse limb deficiency, isolated	No nitrosatable drugs	241	71.9	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	55	18.6	814	14.2	1.38	1.02, 1.87	1.51	1.11, 2.06
	Tertiary amines	40	14.2	800	14.0	1.02	0.72, 1.44	1.20	0.84, 1.71
	Amides	32	11.7	505	9.3	1.29	0.89, 1.89	1.42	0.97, 2.09

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Percentages for no nitrosatable drugs are based on total participants with complete information while percentages for a given nitrosatable group exclude other nitrosatable groups in the denominator.

[b]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[c]For oral clefts, ORs adjusted for Center, maternal age, race/ethnicity, education, folic acid supplementation, and smoking. For limb malformations, ORs adjusted for Center, maternal age, race/ethnicity, education, and multivitamin supplementation.

$watermark-text $watermark-text $watermark-text

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

Table 3

Exposure to Nitrosatable Drugs During the First Trimester and Isolated Congenital Heart Malformations, National Birth Defects Prevention Study, 1997–2005

Defect group[a]	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[d]	95% CI
		No.	%[b]	No.	%[b]				
Conotruncal defects	No nitrosatable drugs	775	76.7	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	113	12.7	814	14.2	0.88	0.71, 1.09	0.88	0.71, 1.09
	Tertiary amines	123	13.7	800	14.0	0.98	0.80, 1.20	0.99	0.80, 1.22
	Amides	81	9.5	505	9.3	1.02	0.80, 1.30	1.01	0.79, 1.30
Right ventricular outflow tract obstruction	No nitrosatable drugs	571	74.4	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	106	15.7	814	14.2	1.12	0.90, 1.40	1.08	0.86, 1.35
	Tertiary amines	113	16.5	800	14.0	1.22	0.98, 1.51	1.14	0.92, 1.43
	Amides	64	10.1	505	9.3	1.09	0.83, 1.44	1.05	0.80, 1.39
Left ventricular outflow tract obstruction	No nitrosatable drugs	583	73.1	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	111	16.0	814	14.2	1.15	0.93, 1.43	1.05	0.84, 1.31
	Tertiary amines	127	17.9	800	14.0	1.34	1.09, 1.65	1.23	0.99, 1.52
	Amides	82	12.3	505	9.3	1.37	1.07, 1.76	1.31	1.02, 1.69
Septal defects	No nitrosatable drugs	1544	74.8	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	255	14.2	814	14.2	1.00	0.86, 1.16	1.00	0.86, 1.17
	Tertiary amines	286	15.6	800	14.0	1.14	0.98, 1.32	1.08	0.92, 1.25
	Amides	200	11.5	505	9.3	1.26	1.06, 1.50	1.24	1.04, 1.49
Single ventricle/complex (includes double inlet left or double inlet right ventricles)	No nitrosatable drugs	118	70.7	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	22	15.7	814	14.2	1.13	0.71, 1.79	1.13	0.71, 1.81
	Tertiary amines	30	20.3	800	14.0	1.56	1.04, 2.35	1.61	1.06, 2.45
	Amides	22	15.7	505	9.3	1.82	1.14, 2.89	1.84	1.15, 2.95
Atrioventricular septal defect	No nitrosatable drugs	61	63.5	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	22	26.5	814	14.2	2.18	1.33, 3.57	1.97	1.19, 3.26
	Tertiary amines	18	22.8	800	14.0	1.81	1.07, 3.09	1.62	0.94, 2.79
	Amides	9	12.9	505	9.3	1.44	0.71, 2.91	1.27	0.62, 2.58
Anomalous pulmonary venous return	No nitrosatable drugs	137	82.0	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	21	13.3	814	14.2	0.93	0.58, 1.48	0.94	0.59, 1.52
	Tertiary amines	9	6.2	800	14.0	0.40	0.20, 0.80	0.42	0.21, 0.83

$watermark-text $watermark-text $watermark-text

Defect group[a]	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[d]	95% CI
		No.	%[b]	No.	%[b]				
	Amides	9	6.2	505	9.3	0.64	0.32, 1.26	0.67	0.34, 1.33
Ebstein's anomaly	No nitrosatable drugs	45	69.2	4920	76.2	1.00	Referent	1.00	Referent
	Secondary amines	11	19.6	814	14.2	1.48	0.76, 2.87	1.56	0.79, 3.09
	Tertiary amines	12	21.1	800	14.0	1.64	0.86, 3.11	1.84	0.95, 3.59
	Amides	4	8.2	505	9.3	0.87	0.31, 2.42	0.92	0.33, 2.61

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Unless otherwise indicated, defects are simple heart defects without major extracardiac malformations.

[b] Percentages for no nitrosatable drugs are based on total participants with complete information while percentages for a given nitrosatable group exclude other nitrosatable groups in the denominator.

[c] Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[d] Adjusted for maternal race/ethnicity, education, smoking status, study site, and multivitamin use during the first trimester.

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

$watermark-text $watermark-text $watermark-text

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

Table 4

Exposure to Nitrosatable Drugs During the First Trimester of Pregnancy and Isolated Oral Clefts by Estimated Intake of Total Nitrites, National Birth Defects Prevention Study, 1997–2005

Type of malformation	Total nitrite intake mg/day[a]	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[d]	95% CI
			No.	%[b]	No.	%[b]				
Cleft lip alone	< 3.02	No nitrosatable drug exposure	138	72.6	1543	75.7	Referent	Referent	Referent	Referent
		Secondary amines	34	19.8	263	14.6	1.45	0.97, 2.15	1.38	0.91, 2.07
		Tertiary amines	23	14.3	254	14.1	1.01	0.64, 1.61	1.02[e]	0.63, 1.64
		Amides	16	10.4	147	8.7	1.22	0.71, 2.10	1.19	0.68, 2.08
	3.02–4.56	No nitrosatable drug exposure	137	74.9	1523	74.0	Referent	Referent	Referent	Referent
		Secondary amines	26	16.0	282	15.6	1.02	0.66, 1.59	1.01	0.65, 1.59
		Tertiary amines	23	14.4	280	15.5	0.91	0.58, 1.45	0.93[e]	0.58, 1.49
		Amides	18	11.6	181	10.6	1.11	0.66, 1.85	1.13	0.67, 1.91
	>4.56	No nitrosatable drug exposure	134	70.9	1622	78.8	Referent	Referent	Referent	Referent
		Secondary amines	28	17.3	229	12.4	1.48	0.96, 2.28	1.25	0.79, 1.96
		Tertiary amines	30	18.3	231	12.5	1.57	1.03, 2.39	1.46[e]	0.93, 2.27
		Amides	19	12.4	157	8.8	1.46	0.88, 2.43	1.36	0.80, 2.31
Cleft lip with cleft palate	< 3.02	No nitrosatable drug exposure	235	72.5	1543	75.7	Referent	Referent	Referent	Referent
		Secondary amines	42	15.2	263	14.6	1.05	0.74, 1.49	1.02	0.71, 1.46
		Tertiary amines	48	17.0	254	14.1	1.24	0.89, 1.74	1.21	0.85, 1.72
		Amides	30	11.3	147	8.7	1.34	0.88, 2.03	1.34	0.87, 2.07
	3.02–4.56	No nitrosatable drug exposure	224	71.3	1523	74.0	Referent	Referent	Referent	Referent
		Secondary amines	39	14.8	282	15.6	0.94	0.65, 1.35	0.96	0.66, 1.40
		Tertiary amines	52	18.8	280	15.5	1.26	0.91, 1.75	1.34	0.95, 1.90
		Amides	35	13.5	181	10.6	1.31	0.89, 1.94	1.31	0.87, 1.96
	>4.56	No nitrosatable drug exposure	243	79.4	1622	78.8	Referent	Referent	Referent	Referent
		Secondary amines	36	12.9	229	12.4	1.05	0.72, 1.53	1.12	0.75, 1.66
		Tertiary amines	36	12.9	231	12.5	1.04	0.71, 1.52	1.18	0.79, 1.75
		Amides	21	8.0	157	8.8	0.89	0.56, 1.44	1.00	0.61, 1.64
Cleft palate alone	< 3.02	No nitrosatable drug exposure	189	78.8	1543	75.7	Referent	Referent	Referent	Referent
		Secondary amines	31	14.1	263	14.6	0.96	0.64, 1.44	0.93	0.61, 1.40

$watermark-text $watermark-text $watermark-text

Type of malformation	Total nitrite intake mg/day[a]	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[d]	95% CI
			No.	%[b]	No.	%[b]				
		Tertiary amines	23	10.9	254	14.1	0.74	0.47, 1.16	0.76	0.48, 1.21
		Amides	16	7.8	147	8.7	0.89	0.52, 1.52	0.90[e]	0.52, 1.57
	3.02–4.56	No nitrosatable drug exposure	161	70.6	1523	74.0	Referent	Referent	Referent	Referent
		Secondary amines	34	17.4	282	15.6	1.14	0.77, 1.69	1.07	0.72, 1.60
		Tertiary amines	36	18.3	280	15.5	1.22	0.83, 1.78	1.23	0.82, 1.82
		Amides	23	12.5	181	10.6	1.20	0.76, 1.91	1.19[e]	0.74, 1.91
	>4.56	No nitrosatable drug exposure	176	76.5	1622	78.8	Referent	Referent	Referent	Referent
		Secondary amines	22	11.1	229	12.4	0.89	0.56, 1.41	0.85	0.52, 1.36
		Tertiary amines	23	11.6	231	12.5	0.92	0.58, 1.45	0.88	0.55, 1.42
		Amides	29	14.2	157	8.8	1.70	1.11, 2.61	1.57[e]	1.01, 2.45

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Total nitrites = daily dietary nitrite intake + 5% of daily nitrate intake.

[b] Percentages for no nitrosatable drugs are based on total participants with complete information while percentages for a given nitrosatable group exclude other nitrosatable groups in the denominator.

[c] Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[d] Adjusted for maternal age, race/ethnicity, education, smoking, study center, calorie intake, and folic acid supplementation during the first trimester.

[e] Significant additive interaction (95% confidence levels for RERI and/or AP exclude 0).

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

$watermark-text $watermark-text $watermark-text

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

Table 5

Exposure to Nitrosatable Drugs During the First Trimester of Pregnancy and Isolated Limb Deficiencies by Estimated Intake of Total Nitrites, National Birth Defects Prevention Study, 1997–2005

Type of malformation	Total nitrite intake mg/day[a]	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[d]	95% CI
			No.	%[b]	No.	%[b]				
Longitudinal limb deficiency	< 3.02	No nitrosatable drug exposure	29	55.8	1584	75.8	Referent	Referent	Referent	Referent
		Secondary amines	16	35.6	268	14.5	3.26	1.75, 6.09	3.51[e]	1.83, 6.75
		Tertiary amines	9	23.7	259	14.1	1.90	0.89, 4.06	2.00	0.91, 4.43
	3.02–4.56	No nitrosatable drug exposure	32	72.7	1555	73.9	Referent	Referent	Referent	Referent
		Secondary amines	6	15.8	291	15.8	1.00	0.42, 2.42	1.03[e]	0.42, 2.52
		Tertiary amines	7	18.0	289	15.7	1.18	0.51, 2.69	1.29	0.55, 3.05
	> 4.56	No nitrosatable drug exposure	40	78.4	1642	78.5	Referent	Referent	Referent	Referent
		Secondary amines	4	9.1	238	12.7	0.69	0.24, 1.95	0.62[e]	0.21, 1.79
		Tertiary amines	4	9.1	236	12.6	0.70	0.25, 1.96	0.60	0.21, 1.74
Transverse limb deficiency	< 3.02	No nitrosatable drug exposure	80	67.8	1584	75.8	Referent	Referent	Referent	Referent
		Secondary amines	23	22.3	268	14.5	1.70	1.05, 2.75	1.86	1.12, 3.07
		Tertiary amines	19	19.2	259	14.1	1.45	0.87, 2.44	1.69	0.99, 2.89
	3.02–4.56	No nitrosatable drug exposure	81	73.6	1555	73.9	Referent	Referent	Referent	Referent
		Secondary amines	17	17.4	291	15.8	1.12	0.66, 1.92	1.25	0.72, 2.17
		Tertiary amines	11	12.0	289	15.7	0.73	0.38, 1.39	0.83	0.43, 1.60
	> 4.56	No nitrosatable drug exposure	74	74.7	1642	78.5	Referent	Referent	Referent	Referent
		Secondary amines	14	15.9	238	12.7	1.31	0.73, 2.35	1.51	0.82, 2.78
		Tertiary amines	10	11.9	236	12.6	0.94	0.48, 1.85	1.20	0.59, 2.43

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Total nitrites = daily dietary nitrite intake + 5% of daily nitrate intake.

[b] Percentages for no nitrosatable drugs are based on total participants with complete information while percentages for a given nitrosatable group exclude other nitrosatable groups in the denominator.

[c] Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[d] Adjusted for maternal age, race/ethnicity, education, study site, calorie intake, and multivitamin supplementation during the first trimester.

[e] Significant multiplicative interaction ($p < 0.05$).

$watermark-text $watermark-text $watermark-text

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.

Table 6

Exposure to Nitrosatable Drugs During the First Trimester of Pregnancy and Isolated Congenital Heart Defects by Estimated Intake of Total Nitrites, National Birth Defects Prevention Study, 1997–2005

Type of defect[a]	Total nitrite intake mg/day[b]	Type of drug exposure	Cases		Controls		Unadjusted OR[d]	95% CI	Adjusted OR[e]	95% CI
			No.	%[c]	No.	%[c]				
Conotruncal defects	< 3.02	No nitrosatable drug exposure	282	81.5	1584	75.8	Referent	Referent	Referent	Referent
		Tertiary amines	34	10.8	259	14.1	0.74	0.50, 1.08	0.71[f,g]	0.48, 1.04
		Amides	18	6.0	149	8.6	0.68	0.41, 1.12	0.64[f,g]	0.39, 1.07
	3.02–4.56	No nitrosatable drug exposure	231	75.0	1555	73.9	Referent	Referent	Referent	Referent
		Tertiary amines	38	14.1	289	15.7	0.89	0.61, 1.28	0.96 [f,g]	0.66, 1.40
		Amides	24	9.4	185	10.6	0.87	0.56, 1.37	0.94 [f,g]	0.60, 1.48
	> 4.56	No nitrosatable drug exposure	235	73.4	1642	78.5	Referent	Referent	Referent	Referent
		Tertiary amines	47	16.7	236	12.6	1.39	0.99, 1.96	1.48[f,g]	1.04, 2.12
		Amides	37	13.6	159	8.8	1.63	1.11, 2.38	1.67[f,g]	1.12, 2.48
Right ventricular outflow tract obstruction	< 3.02	No nitrosatable drug exposure	187	72.5	1584	75.8	Referent	Referent	Referent	Referent
		Tertiary amines	36	16.1	259	14.1	1.18	0.81, 1.72	1.15	0.78, 1.71
		Amides	21	10.1	149	8.6	1.19	0.74, 1.93	1.20	0.74, 1.96
	3.02–4.56	No nitrosatable drug exposure	205	74.0	1555	73.9	Referent	Referent	Referent	Referent
		Tertiary amines	42	17.0	289	15.7	1.10	0.77, 1.57	1.05	0.73, 1.51
		Amides	21	9.3	185	10.6	0.86	0.54, 1.38	0.87	0.54, 1.41
	> 4.56	No nitrosatable drug exposure	163	76.5	1642	78.5	Referent	Referent	Referent	Referent
		Tertiary amines	32	16.4	236	12.6	1.37	0.91, 2.04	1.25	0.82, 1.91
		Amides	19	10.4	159	8.8	1.20	0.73, 1.99	1.13	0.68, 1.89
Left ventricular outflow tract obstruction	< 3.02	No nitrosatable drug exposure	214	73.0	1584	75.8	Referent	Referent	Referent	Referent
		Tertiary amines	56	20.7	259	14.1	1.60	1.16, 2.21	1.52	1.09, 2.13
		Amides	30	12.3	149	8.6	1.49	0.98, 2.26	1.44	0.94, 2.20
	3.02–4.56	No nitrosatable drug exposure	175	68.6	1555	73.9	Referent	Referent	Referent	Referent
		Tertiary amines	45	20.5	289	15.7	1.38	0.97, 1.97	1.24	0.87, 1.79
		Amides	24	12.1	185	10.6	1.15	0.73, 1.81	1.13	0.71, 1.80
	> 4.56	No nitrosatable drug exposure	178	79.8	1642	78.5	Referent	Referent	Referent	Referent

$watermark-text $watermark-text $watermark-text

Type of defect[a]	Total nitrite intake mg/day[b]	Type of drug exposure	Cases		Controls		Unadjusted OR[d]	95% CI	Adjusted OR[e]	95% CI
			No.	%[c]	No.	%[c]				
		Tertiary amines	24	11.9	236	12.6	0.94	0.60, 1.47	0.90	0.56, 1.44
		Amides	21	10.6	159	8.8	1.22	0.75, 1.97	1.18	0.72, 1.95
Septal defects	< 3.02	No nitrosatable drug exposure	494	74.6	1584	75.8	Referent	Referent	Referent	Referent
		Tertiary amines	98	16.6	259	14.1	1.21	0.94, 1.56	1.15	0.88, 1.49
		Amides	70	12.4	149	8.6	1.51	1.11, 2.04	1.45	1.06, 1.99
	3.02–4.56	No nitrosatable drug exposure	487	74.4	1555	73.9	Referent	Referent	Referent	Referent
		Tertiary amines	95	16.3	289	15.7	1.05	0.81, 1.35	0.99	0.76, 1.29
		Amides	63	11.5	185	10.6	1.09	0.80, 1.47	1.11	0.81, 1.52
	> 4.56	No nitrosatable drug exposure	521	74.8	1642	78.5	Referent	Referent	Referent	Referent
		Tertiary amines	92	15.0	236	12.6	1.23	0.95, 1.59	1.18	0.90, 1.56
		Amides	63	10.8	159	8.8	1.25	0.92, 1.70	1.23	0.89, 1.69
Single ventricle/complex (includes double inlet left or double inlet right ventricles)	< 3.02	No nitrosatable drug exposure	45	73.8	1584	75.8	Referent	Referent	Referent	Referent
		Tertiary amines	9	16.7	259	14.1	1.22	0.59, 2.53	1.28	0.61, 2.71
		Amides	5	10.0	149	8.6	1.18	0.46, 3.02	1.33[f]	0.51, 3.47
	3.02–4.56	No nitrosatable drug exposure	34	69.4	1555	73.9	Referent	Referent	Referent	Referent
		Tertiary amines	14	29.2	289	15.7	2.22	1.17, 4.18	2.55	1.30, 4.99
		Amides	4	10.5	185	10.6	0.99	0.35, 2.82	0.99[f]	0.34, 2.89
	> 4.56	No nitrosatable drug exposure	36	69.2	1642	78.5	Referent	Referent	Referent	Referent
		Tertiary amines	7	16.3	236	12.6	1.35	0.60, 3.07	1.30	0.55, 3.06
		Amides	11	23.4	159	8.8	3.16	1.58, 6.32	3.30[f]	1.59, 6.84
Atrioventricular septal defect[h]	< 3.02	No nitrosatable drug exposure	25	69.4	1584	75.8	Referent	Referent	Referent	Referent
		Secondary amines	5	16.7	268	14.5	1.18	0.45, 3.11	1.16	0.43, 3.13
	3.02–4.56	No nitrosatable drug exposure	18	72.0	1555	73.9	Referent	Referent	Referent	Referent
		Secondary amines	6	25.0	291	15.8	1.78	0.70, 4.53	1.88	0.72, 4.92
	> 4.56	No nitrosatable drug exposure	17	53.1	1642	78.5	Referent	Referent	Referent	Referent
		Secondary amines	10	37.0	238	12.7	4.06	1.84, 8.97	3.30	1.44, 7.58

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Unless otherwise indicated, malformations are simple heart defects without major extracardiac defects.

[b]Total nitrite = daily dietary nitrite intake + 5% of daily nitrate intake.

$watermark-text $watermark-text $watermark-text

[c] Percentages for no nitrosatable drugs are based on total participants with complete information on all covariables while percentages for a given nitrosatable group exclude other nitrosatable groups in the denominator.

[d] Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[e] Adjusted for maternal race/ethnicity, education, study site, smoking status, multivitamin supplementation during the first trimester, and caloric intake.

[f] Significant additive interaction (95% confidence levels for RERI and/or AP exclude 0).

[g] Significant multiplicative interaction ($p < 0.05$).

[h] Tertiary amines and amides not included because of numerous cells with less than 5 exposed case-mothers.

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2013 September 01.