# PRENATAL EXPOSURE TO NITRATES, NITRITES, AND NITROSATABLE DRUGS AND PRETERM BIRTHS

A Dissertation

by

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## ABSTRACT

Nitrosatable drugs react with nitrite in the stomach to form *N*-nitroso compounds, observed in animal models to result in adverse pregnancy outcomes such as birth defects and reduced fetal weight. Previous studies examining prenatal exposure to medications classified as nitrosatable have observed an increased risk of preterm delivery. Vitamin C is a known nitrosation inhibitor.

Using data from mothers (controls) of babies without major birth defects from the National Birth Defects Prevention Study, we examined the relation between preterm births and: 1) prenatal nitrosatable drug usage; 2) dietary intake of nitrates/nitrites; 3) joint exposures to nitrosatable drugs and nitrate/nitrite intake; and 4) nitrosatable drugs and vitamin C intake among 496 case-mothers of preterm infants and 5398 control-mothers who delivered full term babies from 1997-2005.

An increased risk of preterm births was observed with secondary amine exposure during the second (adjusted hazard ratio (aHR) 1.37, [95% confidence interval (CI) 1.05, 1.79]) and third (aHR 1.34, [95% CI 1.02, 1.76]) trimester. A protective effect was detected with high levels of plant nitrites (aHR 0.72, [95% CI 0.53, 0.97]). Exposure to secondary amines and high levels of nitrite were associated with preterm births, having an increased risk with first (aHR 1.84, [95% CI 1.14, 2.98]), second (aHR 1.89, [95% CI 1.17, 3.07]), and third (aHR

2.00, [95% CI 1.22, 3.29]) trimester exposure. Lower risk of moderately preterm births was observed with second trimester amide exposure in conjunction with higher levels of dietary vitamin C (aHR 1.14, [95% CI 0.66, 1.98]) compared to <85 mg/day (aHR 2.08, [95% CI 1.25, 3.47]).

Prenatal exposure to nitrosatable drugs during the second and third trimester, particularly secondary amines, might increase risk of preterm delivery. In addition, nitrosatable drugs, especially secondary and tertiary amines, and higher levels of dietary nitrite (including animal, plant, and total) may increase risk of preterm births. However, dietary vitamin C intake ≥85 mg/day may attenuate the association between nitrosatable drug use during the second trimester and preterm and moderately preterm births. In this study population, daily vitamin C supplementation did not appear to confer the same benefits.

## DEDICATION

*“All parents believe their children can do the impossible. They thought it the minute we were born, and no matter how hard we’ve tried to prove them wrong, they all think it about us now. And the really annoying thing is, they’re probably right.”* - Cathy Guisewitte

To my parents, for everything I am.

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## NOMENCLATURE

| | |
|---|---|
| AED | Antiepileptic drug |
| aOR | Adjusted odds ratio |
| aHR | Adjusted hazard ratio |
| AP | Attributable proportion |
| aRR | Adjusted relative risk |
| BDS | Slone Epidemiology Center Birth Defect Study |
| BMI | Body mass index |
| CI | Confidence interval |
| CO | Carbon monoxide |
| EDD | Estimated date of delivery |
| FFQ | Food frequency questionnaire |
| GDM | Gestational diabetes |
| H2 | Histamine 2 |
| HBRA | Hypnotic benzodiazepine receptor agonist |
| hsCRP | High sensitivity C-Reactive protein |
| HR | Hazard ratio |
| NBDPS | National Birth Defects Prevention Study |
| $NO_2$ | Nitrogen dioxide |
| NRT | Nicotine replacement therapy |
| OAH | Other antihistamine |

| | |
|---|---|
| OR | Odds ratio |
| P1P3 | First trimester |
| P1P6 | First two trimesters |
| P1P9 | Entire pregnancy |
| P4P6 | Second trimester |
| P7P9 | Third trimester |
| $PM_{2.5}$ | Particulate matter of aerodynamic ≤2.5 μM |
| $PM_{10}$ | Particulate matter of aerodynamic ≤10 μM |
| PPROM | Preterm premature rupture of the membranes |
| RCT | Randomized controlled trial |
| RERI | Relative excess risk due to interaction |
| ROS | Reactive oxygen species |
| RR | Relative risk |
| SGA | Small for gestational age |
| $SO_2$ | Sulfur dioxide |
| sRR | Summary relative risk |
| SSRI | Selective serotonin reuptake inhibitor |
| USDA | United States Department of Agriculture |
| US | United States |

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# CHAPTER I

## INTRODUCTION

Prematurity is one of the most important predictors of an infant's health and survival. Infants born less than 37 completed weeks of gestation are considered preterm. According to the National Vital Statistics Report for 2006, preterm infants are 14.7 times more likely to die during the first year of life compared to full term infants.[1] They are also at an increased risk of an array of infant morbidities, ranging from neurodevelopmental and respiratory impairments to gastrointestinal complications.[2] Other complications include behavioral, cognitive, hearing, motor, visual, and socio-emotional function.[3] Although assisted ventilation, antenatal corticosteroid usage, and intensive care practices have improved the survival rates of premature infants, the prevalence of preterm births has increased by 31% in the United States (US) between 1981 and 2003.[4] This rise has been attributed to several factors, including changes in obstetric practice[5] and the use of assisted reproduction techniques.[6] Numerous environmental toxicants have been examined for their role in preterm births, of which the weight of evidence has only been sufficient for two: lead and tobacco smoke.[3] No study has examined nitrates, nitrites, and nitrosatable drugs and their association with preterm births although exposure through diet and drug usage are common.

Nitrites and nitrates are ubiquitous in food. Dietary sources are the main source of exposure to nitrates and nitrites. The estimates of total nitrite and nitrate intake in the U.S. are 0.77 mg and 76 mg per day, respectively.[7] Dietary consumption of these exposures contribute to nitric oxide levels as five percent of dietary nitrate is converted to nitrite and further converted to nitric oxide, a free radical found in higher levels in the blood and urine of women with preterm labor.[8] Nitric oxide may damage the collagen in the chorioamnion, resulting in preterm premature rupture of membranes.[9]

Approximately one quarter of the control women from the National Birth Defects Prevention Study (NBDPS) population reported taking at least one medication considered nitrosatable during the first trimester.[10] These nitrosatable amines or amides react with nitrosating agents like nitrites in the stomach to form N-nitroso compounds.[11] *N*-nitroso compounds have been observed in animal models to result in adverse pregnancy outcomes, such as reduced fetal weight[12] and birth defects;[13, 14] their effects on gestational age are not known as studies of adverse pregnancy outcomes did not focus on this aspect. Therefore, possible effects in humans need to be examined.

The specific objective of this project was to investigate the individual and joint associations of nitrosatable drug usage and dietary intake of nitrite/nitrate on the risk of preterm deliveries, and whether vitamin C, a known nitrosation inhibitor, plays a role in these relationships.

Specific aims included:

1. To determine whether nitrosatable drug usage during pregnancy increases the risk of preterm births (Chapter II)
2. To estimate the risk of preterm births associated with dietary nitrite/nitrate intake (Chapter III)
3. To examine whether the joint effects of maternal exposures to dietary nitrite/nitrate intake and nitrosatable drugs increases the risk of preterm deliveries (Chapter III)
4. To examine whether dietary or supplemental vitamin C plays a role in the relationship between nitrosatable drug usage and preterm births (Chapter IV)

**Literature Review**

Several factors have been associated with an increased risk of preterm births. Maternal predictors include intrauterine infections,[15-21] previous history of preterm births,[22-26] multiple pregnancy,[27, 28] single marital status,[29-36] and low socioeconomic status.[32, 33, 37-42] In addition, a U-shaped distribution has been observed between maternal age and preterm births,[43-46] with the highest rates occurring in women <20 and >35 years of age.[47] Whether this increase in preterm births among women <20 is due to a biological immaturity or a higher prevalence of other risk factors remains uncertain.

Racial ethnic differences have also been reported. The preterm birth rate among black women is twice that of white women in the U.S. even after controlling for maternal factors,[23, 48] with preterm birth rates ranging between 16-18% compared to 5-9%.[2] Reasons for this difference continue to be poorly understood as this disparity cannot be explained by the variation in socioeconomic status. McGrady *et al*.[49] noted that although the risk of preterm births declined with increasing levels of maternal education, the rate of preterm births remained higher among black women compared to whites. In addition, lower neighborhood socioeconomic status was not associated with preterm births in a study of U.S. black women.[50] It is suggested that the difference may be due to behavioral choices among groups; however, the proportion of black women who reported to have continued smoking or consuming alcohol during pregnancy is lower or similar to that of white women.[51-53] Additionally, among women who initiated prenatal care in the first trimester, black women continued to have the highest rate of preterm births.[3] These behavioral factors cannot fully account for the observed disparities.

Pre-pregnancy and pregnancy nutritional status may contribute to the risk of preterm births. Women with a low pre-pregnancy body mass index (BMI) are at higher risk of having a premature infant.[39, 54-57] However, the association between maternal overweight or obesity and preterm births remains uncertain. A few studies have reported an increased risk for preterm births in overweight or obese women[58-62] while others report no excess risk.[63-65]

Substance abuse during pregnancy and its association with preterm births is not clear. Several studies have observed no association between cocaine use and preterm births,[66-69] while others have reported a modest increase in risk.[70-78] However, results must be interpreted with caution since many of the confounding maternal behavioral factors were not controlled for, suggesting that the measure of effect may be overestimated. In addition, different criteria for exposure assessment have produced discordant results; a summary relative risk (sRR) for cocaine use and preterm births based on self-report data yielded a positive association (sRR 1.8, [95% Confidence Interval (CI) 1.2, 2.7], based on 7 studies) while urine screening did not (sRR 1.6, [95% CI 0.9, 2.6], based on 4 studies).[79]

It is well recognized that heavy prenatal alcohol exposure is harmful to the fetus,[80] but its relation to preterm births is not conclusive. Several studies lend support for excess risk of preterm births among heavy[58, 81-83] and moderate alcohol users,[84] whereas others did not.[85-89] Although there is a large body of literature on alcohol use and its relation to preterm births, many of the studies have methodological weaknesses preventing a definitive conclusion from being reached. For instance, several did not control for known confounding factors, and those which did had other limitations that prevent the generalizability of the results. Differences in study design, timing of exposure, and methods of exposure assessment may have contributed to the inconsistent findings.

Smoking is considered one of the most significant, modifiable causes of adverse pregnancy outcomes. Nicotine and carbon monoxide are two major compounds of significance, as these two vasoconstrictors are associated with placental damage and decreased uteroplacental blood flow, pathways leading to fetal growth restriction and preterm births.[2, 90] Serum carbon monoxide levels are three times higher in smokers, and fetal concentrations are twice that of maternal levels.[91] It is widely documented that smoking during pregnancy leads to fetal growth impairment,[92] causing an average birth weight reduction of 200 grams and doubling the risk of having a low birth weight baby.[93]

However, the association between smoking and preterm births is relatively modest,[94-100] with a significant dose response relationship observed.[95, 101, 102] Several studies have suggested a stronger association[103-106] while others report none.[107, 108] Environmental tobacco smoke has also been associated with a moderate increase in risk for preterm births among studies utilizing self-reports[109, 110] and biomarkers[111, 112] as exposure assessment measures, though a number of studies negate this conclusion.[113-118]

Of the metals and metalloids, lead exposure is the most well-known reproductive toxicant, crossing the placenta readily. The correlation between maternal and umbilical cord blood lead levels ranges from 0.55 to 0.92.[119] Though few studies report no increased risk of preterm births and umbilical cord blood lead levels,[120-122] the weight of evidence indicates a significant association.[123-125] Further, blood lead levels lower than the recommended level

of 10 μg/dl have also been reported to be associated with preterm births,[126-128] suggesting that levels below 6 μg/dl are still a concern.

Airborne particles released as industrial by-products from lead smelters are another vehicle of exposure; these aerosols can be absorbed directly in the lung, eventually resulting in deposition in the bone and teeth. Areas contaminated with high levels of lead have had an increase in frequencies of preterm births.[129, 130] An 11% increase in risk of preterm births was observed for every μg/dl increase in blood lead levels.[130] However, Factor-Litvak *et al*.[131] found no significant relationship after town of residence was included in the model, suggesting that there was another difference between the two towns besides blood lead levels that would account for the initial increase observed in preterm births among residents of the lead smelter community. This finding was corroborated in a study comparing births in five towns in Shoshone County to the rest of Idaho during three exposure periods: 1) pre-fire; 2) high exposure during a lead smelter facility fire; and 3) post-fire.[132]

Ambient air pollutants, including sulfur dioxide ($SO_2$), fine particulate matter (PM) of aerodynamic diameter ≤2.5 μM ($PM_{2.5}$) and coarse PM of aerodynamic diameter ≤10 μM ($PM_{10}$), nitrogen dioxide ($NO_2$), and carbon monoxide (CO), have been suspected as possible risk factors for preterm births. While few studies have reported null findings,[133, 134] most examining the relation between $SO_2$ and preterm births have found a positive association, with a 27% increase in preterm births [95% CI 1.16, 1.39] per 50 $\mu g/m^3$ increase in $SO_2$

concentration noted in a study of women in the Czech Republic[135] and a 21% [95% CI 1.01, 1.45] increase for each natural log μg/$m^3$ increase in $SO_2$ concentration in Beijing, China.[136] A slight increase in risk was also reported among women in the highest quartile of $SO_2$ exposure during the last month of pregnancy (adjusted odds ratio (aOR) 1.07, [95% CI 1.01, 1.14])[137] and among women who were exposed to 45.86-103.96 μg/$m^3$ of $SO_2$ during the first trimester (aOR 1.21, [95% CI 1.04, 1.42]).[138]

Similarly, $PM_{2.5}$ and $PM_{10}$ have also been implicated as potential risk factors. PM is emitted from a number of sources, such as residential heating, power plants, cars, and wood burning. Its entry into the body can lead to oxidative inflammation in the lungs and other organs, including the placenta, which increases the susceptibility of preterm labor.[139] To examine the relation between $PM_{2.5}$ exposure during pregnancy and preterm births, Kloog *et al*.[140] used predicted 10×10 km of $PM_{2.5}$ and residence-specific cumulative traffic density to assign exposure estimates. A 6% increase [95% CI 1.01, 1.13] in risk for preterm births was observed with every 10 μg/$m^3$ increase in $PM_{2.5}$. This positive association was also corroborated by Wu *et al*.[141] and Huynh *et al*.,[142] with the latter focusing on the first and last two months of gestation. However, several studies have reported no such relation between $PM_{2.5}$ exposure and preterm births.[133, 143, 144]

Further, $PM_{10}$ exposure during the first trimester was associated with higher risk of preterm births.[138, 145] Although Leem *et al*.[138] found no excess risk

with third trimester exposure to $PM_{10}$ levels between 47.07 and 65.63 μg/$m^3$, Suh *et al*.[146] noted an aOR of 1.09 [95% CI 1.03, 1.15] with third trimester levels of 16.53 μg/$m^3$. A 19% increase in preterm births per 50 μg increase in ambient $PM_{10}$ levels 6 weeks before birth was also observed among women who delivered in Southern California between 1989 and 1993,[147] while Sagiv *et al*.[134] reported null findings.

The relationship between preterm births and $NO_2$ and CO is less certain. An excess risk of preterm births was reported among women with prenatal exposure to $NO_2$ levels >46.2 μg/$m_3$.[148] Despite a similar finding with first trimester exposure levels between 56.22 and 80.58 μg/$m^3$ (aOR 1.24, [95% CI 1.09, 1.41]),[138] others have presented conflicting results; however, these studies focused on lower levels of $NO_2$ exposure.[133, 143, 145, 149] With regard to CO exposure during the first trimester of pregnancy, Leem *et al*.[138] and Wilhelm & Ritz[150] both noted an increase in risk with levels >1.11 mg/$m^3$. Conversely, other studies have reported null findings between levels of CO exposure and preterm births.[133, 137, 142] Inconsistent conclusions between studies investigating the relation between air pollutants and preterm births may be due to the heterogeneity of exposure assessment and comparison strategies.

The causes and mechanisms of preterm delivery are multifactorial and are hypothesized to be initiated by numerous mechanisms, including infection, inflammation, uterine overdistension, and stress.[2] These risk factors are believed to interact with one another, resulting in a transition from uterine

quiescence to preterm delivery. Obstetric precursors leading to preterm delivery include: inducing labor, spontaneous preterm labor with intact membranes, and preterm premature rupture of the membranes (PPROM).[2] PPROM is defined as "rupture of the chorioamniotic membranes before the onset of labor prior to 37 weeks of gestation"[151] and accounts for one third of all preterm deliveries.[152, 153] Although its causes are still not well understood, there have been some associations with sexually transmitted infections, vaginal bleeding, and smoking.

Higher levels of nitric oxide have been observed in the blood and urine of women with preterm labor or PPROM.[8] While this compound is necessary for implantation, embryo development, and vascular tone in the placenta,[154] high levels can lead to cell cycle arrest, apoptosis, and senescence.[155] As a reactive oxygen species (ROS), nitric oxide has been hypothesized to damage the collagen in the chorioamnion which would result in PPROM.[9] ROS are unstable molecules that inflict tissue damage in its attempt to reach a stable state by abstracting an electron from nearby molecules to pair with the single electron in its outer orbit. Several studies have observed collagen in several tissues to be the primary target for ROS damage.[9] As a biologically active membrane, the chorioamnion's collagenolytic enzymes are vulnerable to ROS, such as nitric oxide, stimulation.[156]

Nitric oxide is naturally produced by the body from cells of L-arginine; however, a portion is contributed by dietary consumption of nitrates and nitrites as five percent of dietary nitrate is converted to nitrite and further converted to

nitric oxide.[157] The major source of exposure to nitrates and nitrites is through food consumption, with vegetables contributing the most to nitrate levels and cured meats the most to nitrite.[7] Nitrate levels in vegetables vary depending on the plant's metabolic pattern, nitrate content in the soil, fertilizer usage, and seasonality. Nitrate intake also contributes to total nitrite levels by a conversion of nitrate (5%) to nitrite by bacteria in the mouth.[158]

*N*-nitroso compounds are formed endogenously when nitrosating agents, such as nitrites and nitrates, react with nitrosatable amines or amides in an acidic environment like the stomach.[11] Certain medications, which are classified as tertiary amines, secondary amines, or amides, are sources of nitrosatable compounds. Nitrosatable drug usage during the first trimester was observed in 24 percent of NBDPS control mothers.[10] Adverse pregnancy outcomes, such as birth defects and reduced fetal weight,[12-14] have been observed in mice exposed to *N*-nitroso compounds; its effects on gestational age are not known.

In addition, preterm birth rates have been observed to vary by ethnic origin, with non-Hispanic black women having almost twice the rate of non-Hispanic white women in the U.S., regardless of socioeconomic status.[2, 159] For the past decade, this racial gap has decreased slightly due to the 22.3% rise in preterm birth rates among non-Hispanic white women compared to the 3.6% increase observed in non-Hispanic blacks.[159] The disproportionate rates of preterm births among non-Hispanic blacks and the unexpected rise among non-Hispanic whites may be partially attributable to the higher prevalence of

nitrosatable drug usage, and subsequent higher levels of *N*-nitroso compounds, during early pregnancy among the two groups. The highest prevalence of use was observed among non-Hispanic whites, followed by women who described themselves as other and non-Hispanic black.[10] Ingestion of nitrosatable drugs may be a contributing factor in preterm deliveries and could possibly explain the preterm birth rates observed by ethnic origin.

Although no known study has examined the relation between nitrosatable drugs and preterm births, a number have investigated various drugs which have been classified as nitrosatable as outlined by Brambilla and Martelli.[160] Many of these prescription and nonprescription drugs were reported to have been taken by NBDPS control women during the first trimester of pregnancy.[10] A discussion of these medications, categorized by their amine or amide functional groups, is provided.

*Secondary Amines*

Albuterol

Albuterol is a bronchodilator, $\beta_2$-adrenergic receptor agonist, used to relax smooth muscle through the stimulation of cyclic adenosine monophosphate and the production of functional antagonism to bronchoconstriction.[161] Often used in conjunction with an inhaled steroid, albuterol relieves acute symptoms commonly associated with asthma, such as bronchospasm. Asthma, one of the most common chronic diseases among women in reproductive age, occurs in

3.7-8.4% of all pregnancies.[162] Asthma medications, such as albuterol, have warranted safety considerations with regard to adverse pregnancy outcomes.

Utilizing data from a large health maintenance organization in San Diego, Schatz *et al*.[163] identified 259 asthmatic women who used inhaled β-agonist bronchodilators and 101 asthmatic women who were not using this medication to compare perinatal outcomes. No significant difference was observed between asthmatic women who used inhaled bronchodilators and those who did not with regard to preterm births. A similar finding was observed in a prospective study of 824 asthmatic patients who were followed from 1978 to 1990.[164] In a larger cohort consisting of 2,123 asthmatic participants recruited from 16 centers of the National Institute of Child Health and Human Development Maternal Fetal Medicine Units Network, Schatz *et al*.[165] noted no significant relationship between use of inhaled β-agonists and preterm births. These null findings were corroborated by Bracken *et al*.[166] in a large prospective study, where no increase in risk for preterm births was observed among 529 women exposed to short-acting β-agonists even after adjusting for numerous factors, including number of asthma symptoms (aOR 1.01, [95% CI 1.00, 1.02]).

Studies addressing the effects of short-acting β-agonists, such as albuterol, do not associate an increased risk of preterm births with maternal exposure. This suggests that short-acting β-agonists are generally safe to use during pregnancy to treat maternal asthma.

Antidiabetic, Biguanides

Metformin is an oral hypoglycemic agent that improves insulin sensitivity by reducing fasting plasma glucose and insulin concentrations.[167] Its use during pregnancy among women with gestational diabetes (GDM) has been preferred to insulin for several reasons.[168, 169] Although insulin is effective, the medication requires multiple injections daily, there is an increased risk for hypoglycemia, and possible weight gain.[167, 168] With the prevalence of GDM increasing,[170, 171] it is important to concentrate on the safety of prenatal exposure to metformin, one of the most commonly used oral hypoglycemics during pregnancy.[172]

Studies comparing pregnancy outcomes in women with GDM treated with metformin or insulin have yielded conflicting results. Two studies observed a significantly lower frequency of preterm births among women treated with metformin compared to insulin.[168, 173] However, in one of the largest randomized clinical trials, preterm births was found to be more common in the metformin treated group ($P = 0.04$).[169] Though no differences in gestational age were noted between women treated with metformin or insulin in an open-label randomized controlled study conducted in Finland.[174] A similar observation was reported by Tertti *et al*.[175]

Previous studies have examined the association between preterm births and prenatal exposure to metformin among women with GDM; very few relate metformin treatment during pregnancy in women with type 2 diabetes. Utilizing the National Women's Hospital, 214 pregnancies in women with type 2 diabetes

were identified to compare pregnancy outcomes between metformin treated and non-treated women.[176] There was no difference between the two groups in terms of preterm births ($P$ = 0.7). A majority of the studies have reported that prenatal use of metformin appears to be safe with regard to preterm births.

Beta Blockers

Beta blockers, also referred to as beta-adrenergic blocking agents, prevent norepinephrine and epinephrine from binding to receptors on nerves, thus reducing heart rate and blood pressure. Classified as secondary amines, tertiary amines, and amides, these drugs are used for treating a number of conditions such as hypertension, heart failure, migraines, and tremors. There is contradictory evidence regarding treatment with beta blockers during pregnancy and preterm births.

In a nationwide, population-based cohort study using the Danish Fertility Database, over 900,000 births between 1995 and 2008 were obtained to explore the effects of prenatal exposure to beta blockers.[177] Exposure to beta blockers was defined as the redemption of at least two prescriptions between 6 months preconception and the 20$^{th}$ week of gestation (one had to have been redeemed after conception). An increased risk of preterm births was detected among women who were exposed to beta blockers during pregnancy (aOR 2.26, [95% CI 2.03, 2.52]). This finding was previously noted in a retrospective cohort of 436 pregnancies among 318 women attending the Antenatal Hypertension Clinic at City Hospital.[178] A higher proportion of preterm deliveries was found in women

who were in multiple antihypertensive drug regimens ($P < 0.001$). Mean gestational age at delivery in the drug treatment groups (atenolol, labetalol, methydopa, multiple drugs) were also significantly earlier compared to infants of untreated hypertensive women in a study conducted in England.[179]

Conversely, Easterling *et al*.[180] reported that the prematurity rate among women treated with atenolol before 18 weeks gestation was similar to the normal population. An earlier study of labetalol use, a nonselective beta blocker with alpha blocking effects, was examined to determine whether treatment would improve pregnancy outcomes. Prenatal use of labetalol was found to be associated with a higher frequency of fetal growth retardation, but not preterm births.[181] In addition, a meta-analysis of randomized trials of prenatal beta blocker use, published between 1966 and 1997, yielded a pooled odds ratio of 1.35 [95% CI 0.51, 3.60] with regard to preterm births.[182]

Fluoxetine and Paroxetine

Use of antidepressant medication among pregnant women has raised some concern as exposure has been associated with various adverse pregnancy outcomes. The prevalence of depression among pregnant women ranges from 10-20%,[183] and it is estimated that 4-10% of pregnant women in the U.S. and Canada are on medication therapy for depression.[184] Selective serotonin reuptake inhibitors (SSRIs), which include fluoxetine and paroxetine, are considered to be the first line of treatment among most patients with depression during pregnancy.[185, 186] Within the NBDPS, 2.8% of the control

mothers reported using SSRIs from three months preconception to the end of pregnancy.[187] Numerous studies have investigated prenatal use of SSRIs and adverse pregnancy outcomes.

Several studies have found a positive association between prenatal SSRI use and preterm births.[186, 188, 189] In a prospective cohort utilizing data from the Swedish Medical Birth Registry from 1995-2001, Kallen observed an increased risk for preterm births among mothers who had taken any type of antidepressant during pregnancy (aOR 1.96, [95% CI 1.60, 2.41]).[188] An even stronger relationship was observed with prenatal use of SSRIs (aOR 2.06, [95% CI 1.58, 2.69]). Further analyses excluding women who had taken other additional drugs, such as anticonvulsants and antihypertensives, did not change the overall finding (aOR 2.01, [95% CI 1.17, 3.46]). Expanding the data utilized in the previous study beyond 2001 to 2007, Reis & Kallen[189] identified 14,821 women who had been prescribed antidepressants during pregnancy or who had reported use of antidepressants early in pregnancy. Women who had delivered a preterm infant were 1.46 times more likely to have been prescribed prenatal SSRIs [95% CI 1.31, 1.63].

Similar findings were observed in a retrospective cohort study focusing on prescription SSRIs dispensed within one year prior to delivery. Using records from the Canadian province of Saskatchewan, 972 pregnant women were identified who had been given at least one SSRI within the year before delivery and 3,878 who had not received any SSRI within the same time frame.[186] Risk of

preterm births was higher among infants born to mothers receiving SSRI therapy (aOR 1.57, [95% CI 1.28, 1.92]).

Colvin *et al*.[190] further investigated the relation between SSRIs dispensed during pregnancy and pregnancy outcomes by linking data from population-based health datasets from Western Australia and a national pharmaceutical claims dataset. By using record linkage, Colvin *et al*. were able to obtain a larger study size with a rich source of information that not only allowed the examination of different types of SSRIs, but also the trimester these medications were dispensed. Approximately 4% of 96,968 pregnant women between 2002 and 2005 had an SSRI dispensed during pregnancy. In addition to confirming previous findings of a positive association between prenatal exposure to SSRIs and preterm births (aOR 1.43, [95% CI 1.24, 1.65]), Colvin *et al*. continued to observe an association for a number of SSRI drugs, including sertraline (aOR 1.62, [95% CI 1.30, 2.03]), citalopram (aOR 1.38, [95% CI 1.08, 1.77]), and paroxetine (aOR 1.41, [95% CI 1.02, 1.96]). These associations remained statistically significant even after refining the exposure window to the first trimester, with the greatest increase in risk observed with sertraline (aOR 1.74, [95% CI 1.33, 2.27]) and paroxetine (aOR 1.61, [95% CI 1.14, 2.28]). Second/third trimester exposure to SSRIs, as well as specific drugs, was not associated with preterm births.

Conversely, Chambers *et al*.[191] noted significantly higher rates of prematurity among mothers exposed to fluoxetine during the third trimester

(14.3%) compared to those who had early (first and second trimester) exposure (4.1%) and those who did not have any (5.9%) ($P$ = 0.03). Infants whose mothers reported third trimester fluoxetine use had higher rates of prematurity (adjusted relative risk (aRR) 4.8, [95% CI 1.1, 20.8]) compared to infants whose mothers reported early use of fluoxetine. Additionally, in a retrospective cohort study of 228,876 singleton pregnancies among women enrolled in the Tennessee Medicaid program from 1995 to 2007, SSRI and non-SSRI medication use in the second trimester were both independently associated with shorter gestational age ($P$ < 0.0001).[184] Since findings for SSRIs and non-SSRIs were similar with regard to gestational age, overall antidepressant use and its relation to gestational age was examined by the number of filled prescriptions among a subset of 68,007 nulliparous women. Antidepressant prescription filling during the second trimester was significantly associated with shorter gestational age, with the filling of 1, 2, or ≥3 prescriptions associated with a shorter gestational age of 2.6 [95% CI 1.3, 3.9], 5.8 [95% CI 3.9, 7.8], and 6.6 [95% CI 4.6, 8.6] days, respectively. However, filling antidepressant prescriptions during the third trimester was observed to be associated with longer gestational age, particularly among women who filled ≥3 prescriptions (aOR 6.4, [95% CI 5.5, 7.3]). This finding could have been observed since mothers at risk for preterm delivery due to antidepressant use may have already delivered, since refilling additional prescriptions in the last trimester would indicate that pregnancy continues well into the third trimester.

While many studies have reported an association between prenatal SSRI use and preterm births, several have noted no effect.[192-195] In addition, rates of preterm births among 138 pregnant women treated with SSRIs were lower than the national rate.[196] Although, this lower rate may have been due to the strict exclusion of women who used alcohol, nicotine, and recreational drugs, which would have resulted in a healthier population.

In a Finnish study based on population-based register data, no increased risk for preterm or very preterm births (<32 weeks of gestation) was observed among women with SSRI purchases in each trimester or during the second and third trimesters compared to those who had only first trimester exposure.[192] In addition, risk of delivering a preterm infant was not elevated among women who used SSRIs at the outset of pregnancy, but stopped prior to the end of the first trimester (aOR 1.12, [95% CI 0.47, 2.19]) or among those who continued to use SSRIs (aOR 1.27, [95% CI 0.59, 2.76]) compared to those without antidepressant use two months preconception through delivery.[194]

To control for confounding by depression, Oberlander *et al*.[193] compared infants of depressed mothers treated with SSRIs to infants of untreated depressed mothers and nonexposed controls. No difference was observed in the incidence of preterm births between treated and untreated depressed mothers after accounting for maternal illness severity using propensity score matching ($P = 0.61$).

Sit *et al*.[195] prospectively followed 21 mother-infant pairs enrolled in their parent study to explore the relationship of cord-maternal antidepressant levels and maternal depression with preterm births. Samples of umbilical cord and maternal blood were obtained at delivery and analyzed for total drug concentrations. In the parent study, Wisner *et al*.[197] concluded that infants who were exposed to either SSRIs or untreated maternal depression throughout the gestational period were more likely to be born preterm compared to those with partial or no exposure. However, Sit *et al*. did not observe a significant association between preterm births and cord-to-maternal parent drug concentration ratios (odds ratio (OR) 2.2, [95% CI 0.1, 54.6]) or cord-to-maternal metabolite concentration ratios (OR 0.2, [95% CI <0.001, 310.6]).

No definitive increase in risk of preterm births with prenatal use of SSRIs has emerged so far, though the preponderance of evidence does indicate a possible association.[184, 186, 188-191, 197] Though, a majority of the studies did not control for confounding by depression as the condition itself has been associated with preterm births.[198-201] Failing to account for mental illness severity may have attributed preterm births to SSRI exposure. Only two of the aforementioned studies have tried to tease out the role of untreated maternal depression and/or disease severity, reporting discordant results.[193, 197]

Migraine

Triptans are serotonin receptor agonists used to treat migraine symptoms by binding to various serotonin receptors thereby causing blood vessel constriction and neuronal inhibition.[202] Prenatal use of triptans to treat migraines are considered to be relatively safe compared to ergotamine and dihydroergotamine, which have been labeled with an "X" pregnancy warning in the U.S., designating them as contraindicated during pregnancy.[203]

Studies of triptan use during pregnancy with regard to preterm births are limited and present inconsistent results. Sumatriptan, the first member of its drug class, has been available longer than any of the other triptans.[204] As such, its relation with preterm births has been examined in two of the three studies. In the Swedish study, 658 infants whose mothers had used sumatriptan during pregnancy were identified and compared to infants exposed to other migraine drugs.[203] And although infants were slightly more likely to be preterm, the effect did not reach statistical significance (aOR 1.29, [95% CI 0.84, 1.97]). Olesen *et al*.[205] was able to lend support for this positive association utilizing data from the Danish registry. An elevated risk of preterm delivery was reported with prenatal sumatriptan exposure in comparison to those who received no treatment for their migraines (aOR 6.3, [95% CI 1.2, 32.0]) and healthy controls (aOR 3.3, [95% CI 1.3, 8.5]). However, these findings may actually be due to disease severity rather than actual drug exposure.

In the most recent study, Kallen *et al*.[206] broadened their focus to migraine drugs in general, including triptans and ergots. An increased risk for preterm births was observed among women exposed to migraine medications later in pregnancy (aOR 1.50, [95% CI 1.22, 1.84]) compared to mothers who were not exposed any time during pregnancy. However, this positive association may be driven by ergot exposure. Use of ergots during pregnancy has been restricted due to the possible risk of preterm births and hypertonic uterine contractions during delivery.[203]

Pseudoephedrine

Pseudoephedrine, a decongestant, is used to treat symptoms associated with upper respiratory infections, allergies, asthma, and rhinitis by narrowing blood vessels in the nasal passages. It is also used as a temporary relief of sinus congestion and pressure. Decongestants are one of the most commonly taken nonprescription drugs during pregnancy.[207] Among 7,563 case-mothers and control-mothers in the Slone Epidemiology Center Birth Defects Study (BDS) and 2,970 control-mothers in the NBDPS, pseudoephedrine was tied with ibuprofen as the second most commonly taken product during pregnancy, with at least 15% of women exposed.[208] In fact, pseudoephedrine use was higher during pregnancy than before conception. The prevalence of pseudoephedrine use increased from pre-pregnancy to the second trimester, but then dropped during the third trimester.[208] Despite the common use of decongestants during pregnancy, few studies have examined the relationship between its use and

preterm births. Of the two which have, both observed a reduced risk of preterm births with maternal decongestant use.[207, 209]

In the first study, Swedish register data was utilized to obtain information regarding maternal drug use and delivery outcomes.[209] No association was noted between first trimester exposure to decongestants and preterm births, but a reduced risk was observed with later pregnancy use (aOR 0.68, [95% CI 0.52, 0.88]). This inverse association was corroborated in a population-based retrospective study of 3,271 Massachusetts live-born births without major malformations by Hernandez *et al*.[207] A hazard ratio (HR) of 0.42 [95% CI 0.21, 0.84] was observed between second or third trimester decongestant use and preterm births. Kallen and Olausson[209] hypothesized that the protective association may be due to confounding by indication, similar to nausea and vomiting which have been associated with reduced risk of preterm births,[210] pregnancy rhinitis may also be an indicator of a healthy pregnancy. To explore this further, various combinations of asthma and decongestant exposures were examined. Non-asthmatic women who took decongestants had lower risk of preterm births compared to those unexposed.[207] Compared to non-asthmatic, non-decongestant exposed mothers, untreated asthmatic women had the highest risk (HR 1.8, [95% CI 1.2, 2.6]), whereas asthmatic women who took decongestants had no increase in risk (HR 1.0, [95% CI 0.50, 2.0]). Although confounding by indication was not supported, this protective association

between maternal decongestant use and preterm births requires further investigation.

Ranitidine

Ranitidine is among a group of drugs known as histamine 2 (H2) blockers. These medications are used to treat peptic ulcer and gastroesophageal reflux diseases by inhibiting gastric secretion. A handful of studies have documented the safety of acid-suppressing drug use during pregnancy and preterm births, with most reporting no association.

Two studies focused on H2 blocker exposure during the first trimester and its relation to preterm births.[211, 212] Both reported null findings, observing no difference between women exposed to H2 blockers during the first trimester and the controls. Matok *et al*.[213] further examined maternal H2 blocker exposure by each trimester of pregnancy by linking three databases containing information on medications dispensed and hospitalization records of women registered to Clait, a health maintenance organization in the Southern District of Israel. No increased risk was noted between any trimester of H2 blocker exposure and preterm births. Only one study reported contradictory findings. Utilizing data from 18 Teratology Information Services within the European Network of Teratology Information Services, Garbis *et al*.[214] found that the incidence of preterm births was higher among mothers who had taken H2 blockers than among those who had no exposure (relative risk (RR) 1.67, [95% CI 1.18, 2.35]). However, the

weight of evidence appears to suggest that prenatal use of H2 blockers is not associated with higher risk of preterm births.

Thiazide Diuretics

Thiazide diuretics are considered as secondary amines and amides. This medication is often used to treat hypertension and edema. Contradictory results were observed between the Danish and Scottish cohort data.[215] A positive association between prescription thiazide diuretic purchase during pregnancy and preterm births was observed in the Danish cohort (aOR 1.9, [95% CI 1.2, 3.0]). Although an increased risk of preterm births was observed in the Swedish cohort, the 95% CI was compatible with the null.

*Tertiary Amines*

Antiepileptics

Epilepsy is the most common neurological disorder in pregnant women,[216] with a prevalence ranging from 0.3 to 0.7%.[217] Although more than 90% of pregnancies in epileptic women occur without complications,[218] epileptic women are considered at high risk. Studies present conflicting results with prenatal antiepileptic drug (AED) use (e.g., carbamazepine and valproate) and preterm births.

A Danish study observed higher risk of preterm births among epileptic women treated with AEDs who also smoked (aOR 5.7, [95% CI 2.3, 14.2]) compared to nonepileptics, but no such association was observed among their

nonsmoking counterparts (aOR 0.5, [95% CI 0.1, 3.5]).[219] This positive association may have been driven by smoking rather than AEDs since smoking has been reported to be associated with infant prematurity.[94-100, 103-106] However, an increase risk of preterm births was reported with prenatal AED use even after adjusting for smoking and other factors by Veiby *et al*.[220] Further, women who had delivered a preterm infant were 80% more likely to have been exposed to carbamazepine during pregnancy [95% CI 1.4, 2.4]. In addition, an increased risk of delivery before 34 weeks of gestation was observed among epileptic women using AEDs (aOR 1.6, [95% CI 1.2, 2.1]) compared to nonepileptic women in a population-based cohort study utilizing data from the Medical Birth Registry of Norway.[217] Slightly elevated risk was observed with delivering an infant 34-36 weeks of gestation, but the 95% CI was compatible with the null.

On the other hand, Fonager *et al*.[221] and Lin *et al*.[222] did not find any evidence of an increased risk of preterm births with prenatal AED use in their study populations. Likewise were the conclusions of Katz *et al*.[223] and Viinikainen *et al*.,[218] despite using untreated epileptic women as their referent group to examine gestational age and preterm births, respectively. Although a positive association was reported among untreated epileptic women and preterm births (aOR 1.35, [95% CI 1.07, 1.71]),[222] previous studies report null findings between the condition and preterm births.[217, 219, 220]

Antiinfective, Macrolides

Macrolides are a group of antibiotics which include drugs such as azithromycin, erythromycin, and roxithromycin. These drugs are tertiary amines and amides. While the use of antiinfective drugs during pregnancy has decreased from 1998 to 2002 ($P \leq 0.05$ for trends), macrolides had an increasing trend of use.[224] Most notable was azithromycin, climbing from 0.04% in 1998 to 10.16% in 2002. Studies on prenatal macrolide present inconsistent conclusions with regard to preterm births.

In a randomized controlled trial (RCT) conducted in Jefferson County, Alabama, 624 healthy women who had a previous spontaneous delivery or who weighed <50 kg before pregnancy were enrolled to determine whether treatment of metronidazole and erythromycin reduces the incidence of preterm births among: 1) women at risk for preterm births; and 2) women with bacterial vaginosis.[225] Women were randomly assigned 2:1 to either antimicrobial therapy or an identical-appearing placebo containing lactose filler. A lower incidence of preterm births was observed among the treatment group compared to the placebo ($P = 0.01$). In addition, antimicrobial treatment reduced the rates of preterm births among women with bacterial vaginosis ($P = 0.006$). This protective association was not observed in a RCT examining the effect of antibiotic treatment on fetal fibronectin-positive women.[226] No difference was observed in preterm births between the antibiotic-treated and placebo-treated women (RR 0.99, [95% CI 0.71, 1.38]).

In a large, population-based register study in Norway, 180,120 women who were pregnant between 2004 and 2007 were linked to the Norwegian prescription database to determine prenatal exposure to several antibiotics (erythromycin, penicillin V, amoxicillin). No difference in preterm births was observed between the three antibiotic drug exposures and among women who had not taken any systemic antibiotics.[227] However, a slightly lower odds ratio was observed among women with prenatal exposure to erythromycin, but this result did not reach statistical significance (aOR 0.96, [95% CI 0.86, 1.07]). Kallen *et al*.[228] found similar conclusions, with no excess risk of preterm births among infants with fetal exposure to erythromycin.

Antihypertensives

Hydralazine and clonidine are antihypertensives that have tertiary amine functional groups. Hydralazine is an agent acting on arteriolar smooth muscles, decreasing peripheral resistance and lowering blood pressure. Clonidine is a centrally acting antiadrenergic agent, providing easier blood flow by decreasing the heart rate and relaxing blood vessels. There does not appear to be a study which examines the use of these two medications during pregnancy, either singly or in conjunction, and its relation to preterm births. However, utilizing the Swedish Medical Birth Registry, maternal use of antihypertensive drugs in early pregnancy and delivery outcomes were explored.[229] A higher risk of preterm births was noted among women who reported using antihypertensives during the first trimester (aOR 3.33, [95% CI 2.89, 3.84]). Though it is not evident if this

relationship does in fact exist between these two specific medications and preterm births since antihypertensive drug use also included beta blocking agents, calcium channel blockers, angiotensin-converting-enzyme inhibitors, and angiotensin II antagonists.

Calcium Channel Blockers

Calcium channel blockers are nitrosatable drugs that have been classified as tertiary amines and amides. These drugs prevent calcium from entering cells in the heart and blood vessel walls, thereby lowering blood pressure. Studies regarding its association with preterm births have presented discordant results. A significantly lower gestational age and an increase in prematurity were observed among women with first trimester exposure to calcium channel blockers compared to women who reported taking nonteratogenic medications.[230] This conclusion differs from that of Gulmezoglu & Hofmeyr's,[231] where a peto OR of 0.50 [95% CI 0.18, 1.40] was reported for the relation between flunarizine exposure and preterm births. The peto OR uses an inverse variance approach and is considered an alternative to the Mantel-Haenszel method.[232] The observed effect size was based on published and unpublished data for a clinical trial of 100 women randomly assigned to either the flunarizine or placebo group during their second trimester of pregnancy. However, nonsmokers were excluded from this clinical trial and preterm births was defined as <38 weeks of gestation.

Chlorpheniramine, Diphenhydramine, and Promethazine

Chlorpheniramine, diphenhydramine, and promethazine are first-generation antihistamines used to relieve symptoms of allergic reactions. Most first-generation antihistamines are generally considered safe to use during pregnancy. In an Israeli study conducted between 1995 and 2001, 1,406 pregnant women were prospectively followed to evaluate the rate of adverse pregnancy outcomes.[233] The study compared three exposure groups: 1) loratadine, a second-generation antihistamine; 2) other antihistamines (OAH), such as chlorpheniramine, promethazine, and hydroxyzine; and 3) the control group, those exposed to nonteratogenic agents. The rate of preterm births was not significantly different between the three groups ($P$ = 0.101).

While considered as antihistamines, diphenhydramine and promethazine possess antiemetic effects and are often used to treat nausea and vomiting during pregnancy. Utilizing data obtained from the Swedish Medical Birth Registry, drug use during pregnancy was prospectively ascertained between July 1, 1995 to 2002. Focusing on antiemetic drug use, including diphenhydramine, metoclopramide, and promethazine, Asker *et al*.[234] compared women who had been exposed during pregnancy to those who had given birth within the study period. A moderate decrease in risk of preterm births was observed with any antiemetic drug use during the first trimester (aOR 0.93, [95% CI 0.88, 0.98]). However, measures of effect varied by antiemetic drug use, with women who had taken diphenhydramine (aOR 1.18, [95% CI 1.06, 1.32]) and

promethiazine (aOR 1.13, [95% CI 1.02, 1.24]) both having higher risk of having preterm births. Though overall use of antiemetics during early pregnancy was associated with a more favorable outcome, it may not be due to a direct drug effect. The presence of nausea and vomiting during pregnancy indicates a well-functioning placenta, which may increase the likelihood of good pregnancy outcomes.[234]

Nicotine Replacement

Exposure to tobacco during pregnancy increases the risk of having a preterm infant.[94-100, 103-106] Approximately 12% of women continue to smoke during pregnancy.[235] Nicotine replacement therapy (NRT), including nicotine gum, patches, and inhalers, have been shown to be an effective treatment option to aid with smoking cessation by controlling nicotine dependency symptoms.[236, 237] However, the safety of prenatal NRT use with regard to preterm births is uncertain.

Wisborg *et al*.[238] found no difference in the rate of preterm births between NRT patch users and the placebo group in a small randomized controlled, intention-to-treat trial. Only 11% of the patients, however, completed the full 11-week course of therapy, which could explain the null findings. According to a study utilizing data from the 1988 National Health Interview Survey, cessation of smoking during the first trimester was found to reduce the incidence of preterm births by 26%.[239] Oncken *et al*.[240] corroborated this finding in a randomized, double-blinded, controlled trial comparing the use of NRT gum versus a placebo

in 194 women ≤26 weeks pregnant who smoked at least one cigarette a day. Women were either given a 2 mg NRT gum or an identical placebo for six weeks, followed by an additional six week taper period. A lower risk of preterm births was observed in the NRT group ($P$ = 0.027).

Conversely, a higher risk of preterm births was reported among women who were either prescribed or recommended NRT in a large sample of U.S. women (aOR 2.04, [95% CI 1.14, 3.63]).[236] Women who were exposed to NRT during the first 27 weeks of pregnancy were also observed to have a slightly higher proportion of preterm births compared to non-NRT users in a Danish study.[241] However, women prescribed NRT may be among the heaviest smokers, having the most difficult time with cessation. These individuals may not have used the NRT products consistently and could have continued smoking.

Opioids

Methadone is an opioid pain reliever used to treat severe ongoing pain and addiction to narcotic drugs (e.g., heroine). The treatment of choice for management of opiate dependence in pregnant women is methadone;[242] as such, its relationship with preterm births has been examined by a number of studies.

The rate of preterm births among women confirmed by a urine drug screen to have been prenatally exposed to methadone (29.1%) was reported to be three times the national average (11.1%).[243] This rate may have been inflated since women who were also exposed to illicit drugs were included. In an effort to

tease out this information, Arlettaz *et al*.[244] found that prenatal use of methadone alone resulted in an incidence of preterm births which was twice that of the general population. A fourfold higher incidence of prematurity was reported for babies born to mothers who used methadone in conjunction with additional drugs, such as heroin and cocaine.

An increased risk of preterm births was reported among women who received methadone maintenance treatment for opioid dependence compared to non-opioid dependent women.[245] In addition, a significant linear relationship was reported between maternal methadone dose (no dose, ≤58 mg/day, >58 mg/day) and preterm births ($P$ = 0.001). Even after controlling for factors generally associated with methadone dose, such as socioeconomic status and lower educational attainment, it remained a significant predictor of preterm delivery. Similar findings were reported in a retrospective cohort at a large maternity hospital.[242] Not only was methadone exposure associated with an increased risk of preterm births (aOR 2.47, [95% CI 1.97, 3.11]), but very preterm births as well (aOR 2.4, [95% CI 1.40, 4.34]). However, gestational age did not differ between women who continuously took methadone from conception to delivery and those that started taking methadone during the second/third trimester.[246] A longer duration of fetal methadone exposure does not appear to be associated with shorter gestational age.

*Amides*

Amoxicillin

Amoxicillin is a β-lactam antibiotic, commonly used to treat many different types of bacterial infections. It was among the top 10 medications taken during pregnancy among women in the BDS database between 1998 and 2004.[208] Although the use of amoxicillin has significantly decreased from 1998 to 2002, its use remains the highest in terms of antiinfective prescriptions filled by pregnant women based on the Quebec Pregnancy Registry.[224] Though a number of studies have focused on the safety of amoxicillin use during pregnancy and birth defects, few have explored other adverse pregnancy outcomes such as preterm births.

In a population-based study conducted in Denmark, 401 primiparous women were identified who had redeemed a prescription for amoxicillin during pregnancy.[247] Women who did not redeem any prescription three months preconception through the end of pregnancy served as the referent group. No association was noted between prenatal amoxicillin exposure and preterm births after adjusting for maternal age and smoking status (aOR 0.77, [95% CI 0.49, 1.21]). This finding was similar to two studies that examined the relation between pivampicillin and ampicillin (medications closely related to amoxicillin) and preterm births.[248, 249] No evidence suggests an excess risk of preterm delivery among mothers taking amoxicillin during pregnancy.

Benzodiazepine

Having both tertiary amine and amide functional groups, benzodiazepines are a type of anti-anxiety medication known as tranquilizers. Besides anxiety, benzodiazepines are commonly prescribed to treat seizures, insomnia, and muscle spasms. Use of prenatal benzodiazepines has been associated with preterm births in two studies.

Utilizing the Swedish Medical Birth Registrar to identify maternal characteristics associated with exposure to benzodiazepines or hypnotic benzodiazepine receptor agonists (HBRA) during pregnancy, Wikner *et al*.[250] found that women who were older, smoked, had lower education, and whose parity was either one or ≥4 were associated with higher use of either or both medications. In addition, an excess of preterm births was noted for women who used these medications in late pregnancy (aOR 1.48, [95% CI 1.26, 1.75]). An increased risk of preterm birth was also detected with both early and late exposure by Wikner *et al*.[251] The measure of effect was higher among neonates exposed later in pregnancy (aOR 2.57, [95% CI 1.92, 3.43]) compared to early exposure (aOR 1.48, [95% CI 1.26, 1.75]). However, 31% of the women who used benzodiazepines and/or HBRA were also taking antidepressants. Excluding women who also reported use of antidepressants yielded a lower odds ratio for preterm births which was compatible with the null (aOR 1.20, [95% CI 0.97, 1.50]).

Metoclopramide

Metoclopramide, an antiemetic, is widely used for nausea and vomiting among pregnant women. By blocking the dopamine receptor in the chemoreceptor trigger zone, metoclopramide is able to prevent nausea and vomiting normally triggered by most stimuli. Few studies have assessed the safety of prenatal metoclopramide use with regard to preterm births, with most finding no increase in risk.

In a retrospective cohort study in Israel, registered women of Clalit Health Services were identified if they had a singleton delivery at Soroka Medical Center between January 1998 and March 2007.[252] Information regarding medications dispensed during pregnancy and pregnancy outcomes were obtained by linking three databases. First trimester exposure to metoclopramide was not associated with preterm births with an aOR of 1.15 [95% CI 0.99, 1.34]. This finding is consistent with previous studies. Berkovitch *et al*.[253] prospectively enrolled 126 women who had reported taking metoclopramide during the first trimester of pregnancy. These women were then matched by age, smoking status, and alcohol use to a control group. No significant difference was observed in terms of gestational age at delivery ($P = 0.56$) and prematurity ($P = 0.70$). Not only did Sorensen *et al*.[254] observe similar null results with regard to first trimester exposure and preterm births in their study (aOR 1.46, [95% CI 0.8, 2.5]), but no increase risk in preterm births was observed with any metoclopromide exposure during pregnancy (aOR 1.02, [95% CI 0.62, 1.67]).

Only one study observed a positive association between early pregnancy exposure to metoclopromide and prematurity.[255] Expanding on their previous study,[253] a larger cohort consisting of 175 women were enrolled. Although no difference was noted with regard to gestational age ($P$ = 0.099), a significant relationship was now observed between early prenatal exposure to metoclopromide and preterm births (aRR 3.37, [95% CI 1.12, 10.12]).

Sulfamethoxazole

Sulfamethoxazole is an antibiotic once commonly prescribed to treat infections such as otitis media, conjunctivitis, and urinary tract infections. With the development of bacterial resistance, sulfamethoxazole is now used in combination with trimethoprim. This combination has a synergistic action and blocks various steps in the bacterial synthesis of dihydrofolic acid, which is necessary in DNA formation.[256] Although several studies have reported an associated between early pregnancy exposure to this medication and congenital malformations,[257-261] only one examined the risk of preterm births. Approximately 3.2% of pregnant women in the Canadian province Saskatchewan were exposed to trimethoprim/sulfamethoxazole, according to a population-based study conducted on a random sample of women giving birth between 1997 to 2000.[256] Utilizing the same population, Yang *et al*.[262] found an increase in risk for preterm births among women who were prenatally exposed to trimethoprim/sulfamethoxazole compared to those who had no exposure (aOR 1.51, [95% CI 1.10, 2.08]).

*Dietary Intake and Preterm Birth*

Dietary intake of nitrate/nitrite in combination with nitrosatable compounds may pose a risk for preterm births. The main source of dietary nitrite consists of cured meats, cereals, and baked goods; vegetables contribute the largest proportion to daily nitrate intake.[7] Previous studies regarding nitrate/nitrite exposure and preterm births have only focused on exposure levels from drinking water.[263-265] Super *et al*.[265] conducted a study in a rural region of southwest Africa and found no association between the incidence of preterm births and residing in an area with high levels of nitrates (>89 mg/L nitrate as nitrate). Conversely, in a population-based case-control study conducted in Prince Edward Island, Canada, a significant dose-response association was observed between nitrate levels in drinking water and prematurity.[263] An OR of 1.83 [95% CI 1.25, 2.68] was detected for prematurity with median nitrate levels as low as 13.7 mg/L nitrate as nitrate, a level which is below the current U.S. maximum contaminant level set for drinking water in public water supplies (45 mg/L nitrate as nitrate). Joyce *et al*.[264] also noted an increase in the prevalence of PPROM with moderate (0.553-1.55 mg/L nitrate as nitrate) (aOR 1.23, [95% CI 1.03, 1.52]) and high (>1.55 mg/L nitrate as nitrate) water nitrate levels (aOR 1.47, [95% CI 1.20, 1.79]).

Studies regarding dietary intake have focused mainly on different types of diets rather than exposures to nitrates and nitrites. A Mediterranean-type diet consists of vegetables, fruits, whole grains, nuts, legumes, fish, and use of olive

oil. Consumption of red meat, full-fat dairy products, and eggs are limited. While Haugen *et al*.[266] noted no association, a significantly lower incidence of preterm births was reported among mothers who adopted a Mediterranean-type diet from gestational week 17-20 to birth.[267] Mikkelsen *et al*.[268] reported a similar finding utilizing the Danish National Birth Cohort. In addition, Scholl *et al*.[269] examined the association of high-sensitivity C-reactive protein (hsCRP) and preterm births. Higher hsCRP concentrations are associated with a Western diet, which consists of high quantities of red meat and high cholesterol food items. A significant increase in risk for early preterm delivery (<34 weeks) was observed with the highest tertile of hsCRP (7.06-137.41 mg/L).

Vitamin C was examined to determine whether nitrosatable drug users with high vitamin C intake have a reduced risk of preterm births than those with lower intake. Vitamin C has been shown to inhibit endogenous formation of *N*-nitroso compounds. Ascorbic acid inhibits the formation of *N*-nitroso compounds by rapidly reducing nitrite to nitrous oxide, followed by the production of dehydroascorbic acid.[270] Animal models have further demonstrated vitamin C's ability to inhibit nitrosation as reduced risk for peripheral nervous system tumors in the offspring of pregnant mice and hamsters were observed when ascorbic acid was given in conjunction with ethylurea and nitrite.[271, 272] In a clinical trial of human volunteers, increased doses of ascorbic acid, starting from 1.76 mg to 1,000 mg, were administered along with combined exposures of nitrate and a nitrosatable precursor, proline.[273] A significant 44% reduction in *N*-nitroso

compound excretion was observed among individuals who were given vitamin C in conjunction with nitrate and proline compared to those without concomitant administration of vitamin C.[273]

In a recent study, Brender *et al*.[274] observed lower odds of anencephalic births among women who took daily vitamin C supplementation along with tertiary or secondary drug exposures. A reduction in risk was also noted for transverse limb deficiency in conjunction with secondary amine drug exposures, cleft lip without cleft palate with tertiary amine exposures, and several congenital heart defects in conjunction with tertiary amine and amide drug exposures with daily use of supplements containing vitamin C.[275] In addition, in a prospective cohort of pregnant women in North Carolina, total vitamin C intake preconceptionally and during the second trimester was examined for its relation with preterm births.[276] Although no association was noted between women with preconception or second trimester total vitamin C intakes of <10$^{th}$ percentile and overall preterm births, there was an increased risk of preterm births due to PPROM (RR 2.2, [95% CI 1.1, 4.5]) among those with total vitamin C intakes less than the 10$^{th}$ percentile preconceptionally. This finding aligns with other studies which have also reported a higher incidence of PPROM among women with low vitamin C levels.[277-279]

However, Steyn *et al*.[280] noted no difference in preterm births between women who received 250 mg of vitamin C and women who were given a matching placebo daily until 34 weeks gestation. Further, maternal

supplementation with vitamin C and E beginning at 9 to 16 weeks gestation did not reduce the risk of preterm births among nulliparous women in a RCT.[281] If a relationship is observed where nitrosatable drug users with high vitamin C intake have lower risks of preterm births than those with lower intake, then women who may need to take medications that are considered nitrosatable may be advised to increase their vitamin C intake.

**Methods**

A case-control study design was utilized to examine the relationship between prenatal exposures to nitrates, nitrites, and nitrosatable drugs and preterm births. Control mothers from the NBDPS, who had delivered infants without major congenital malformations, with estimated dates of delivery during 1997-2005 served as the source for both cases and controls. The NBDPS is an ongoing population-based case control study that has been conducted since 1997. As a collaborative study of the Centers for Disease and Control National Center on Birth Defects and Developmental Disabilities and ten birth defect surveillance registries including Arkansas, California, Georgia (metropolitan Atlanta), Iowa, New Jersey, New York, North Carolina, Massachusetts, Texas, and Utah, the NBDPS is the largest population study focused on birth defects in the nation.

NBDPS control-mothers served as the population source, with cases in the present study defined as preterm births (those who were born less than 37

weeks gestation) and controls as infants delivered at full term. Prescription and non-prescription drug use prior to conception and throughout pregnancy have been collected with a standardized questionnaire from the NBDPS and was the basis for the development of nitrosatable drug usage and classification. Nitrosatable drug usage during pregnancy was investigated to determine whether there is an increased risk for preterm births. Utilizing the food frequency questionnaire from the NBDPS, dietary intake of nitrates and nitrites was estimated to assess the risk of preterm births. Lastly, the role of vitamin C was examined in the relationship between preterm births and nitrosatable drugs.

*Source of Population*

Controls were identified using the NBDPS Data Analysis Tools release 7.04 that included births with an estimated date of delivery (EDD) from 1997 to 2005. Control-mothers within the NBDPS were randomly selected from birth certificates (Arkansas, for EDDs after 2000; Georgia, for EDDs after 2000; Iowa; Massachusetts; New Jersey; North Carolina; and Utah) or hospital records (Arkansas, for EDDs prior to 2001; California; Georgia, for EDDs prior to 2001; New York; and Texas).[282] A systematic random sampling scheme was utilized for control selection from hospitals, allowing for the selection to be proportional to the number of births in each hospital in the geographic area. Controls were unmatched to cases and had an EDD within the same year as cases. Control infants were excluded if a major birth defect was present, were not residents of

one of the covered geographical areas, were adopted or in foster care, had a deceased mother, or were stillborn.

Only control-mothers who had delivered infants during 1997-2005 without major congenital malformations were included in the present study. The study population was also restricted to singleton births as multiple births have been observed as a major risk factor for preterm deliveries.

*Case Definition*

Cases were defined as preterm births, those who were born less than 37 weeks gestation. Preterm births were be further categorized into very preterm (less than 33 weeks) and moderately preterm (33-36 weeks).

*Control Definition*

Control-births, for the purposes of this study, were NBDPS births with 37-41 weeks of gestation. Infants who are small for gestational age (SGA) were not included as control births since *N*-nitroso compounds have been observed to affect fetal weight.[12] Excluding SGA births increases the likelihood of detecting any true associations between nitrosatable drugs and preterm births.

*Exposure Assessment*

Mothers were contacted by mail with a packet which included an introductory letter, a pamphlet of frequently asked questions, a “Rights of

Research Subjects" fact sheet, a response list, a calendar covering the duration of their pregnancy, and compensation in the form of a $20 money order. Packets were sent to mothers six weeks after the infant's EDD. Mothers were contacted approximately ten days after the introductory packets were delivered to answer any potential questions, conduct the interview, and/or schedule a more convenient time to complete the interview. Interviews were targeted for completion within six months of the EDD, but no later than 24 months after.[283]

Requiring approximately an hour to complete, interviews were conducted in either Spanish or English by female interviewers using a computer-assisted telephone interview. Interviews are completed in one session or separate sessions if requested by the mothers. Verbal informed consent was obtained prior to the interview through a standard script. The interviews covered a variety of topics including chemical, infectious, nutritional, physical, and behavioral factors. Detailed questions regarding exposures three months preconception to the end of pregnancy were collected. Some were open-ended while most are structured with pre-coded response lists. Utilizing the calendar sent in the introductory packet, mothers were able to respond to questions regarding timing of exposures by date, month of pregnancy, or trimester.

*Dietary Assessment of Nitrate and Nitrite Intake*

Average food consumption throughout the year before conception was inquired during the NBDPS interview using the 58-item food frequency

questionnaire (FFQ) that was adapted from the short Willett FFQ. The Willett FFQ has been validated and reproduced in other studies and has been indicated to provide useful information about nutrient intake in women during pregnancy.[284] Information regarding cereal intake three months preconception through the third trimester was also obtained. Additional dietary questions (e.g., avocados, tortillas, etc.) were added to address the diverse diet of the study population.

Nutrient calculations were based on the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference 19. Daily intake of each food component was calculated based on the frequency of use and serving size. Foods and food groups in the FFQ were assigned codes which also correspond to USDA codes. Since estimates of nitrites and nitrates were not available in the USDA National Nutrient Database, values were estimated based on published literature for each food item/group by Griesenbeck *et al*.[285]

Briefly, estimation of dietary intake of nitrates and nitrites in milligrams per day were assigned based on the following procedures: 1) for each food item, weighted means (mg/100 g) were calculated based on relevant literature; 2) the number of serving sizes were multiplied by the weighted means; 3) values of nitrites and nitrates were multiplied by the number of monthly servings; and 4) levels of nitrates and nitrites were summed across all food items and divided by 30. Total dietary nitrite was calculated based on the formula [total nitrite = dietary nitrite intake + (0.05 × dietary nitrate intake)].[286] Nitrites, nitrates, and

total nitrites were further categorized into tertiles based on the control women's distribution that had a total daily caloric intake between 500-5000 kcal.

*Assessment of Nitrosatable Drugs*

Information regarding prescription and nonprescription drugs from three months preconception to the end of pregnancy was collected. Name of medication, dates of use, and frequency of use were obtained. The Slone Epidemiology Center Drug Dictionary system was used to link the reported medications to their active ingredient.[287] Drugs were then classified by their nitrosatibility, functional groups, and indications based on methods described by Brender *et al*.[10] The methodology for classification included: 1) active ingredients for all medications were identified; 2) drugs were cross-referenced with comprehensive nitrosatable medicinal compounds lists;[160, 288] 3) drugs were categorized based on the presence of functional groups (e.g., secondary amine, tertiary amine, or amide); and 4) drugs were further categorized by its primary indication (analgesic, cardiovascular) or therapeutic use (opioid, beta blocker). This study focused on exposures by trimester and month of gestation.

*Assessment of Vitamin C Intake*

Vitamin C's potential role in reducing preterm risk in the presence of nitrosatable drugs was examined since it is a known nitrosation inhibitor.[289, 290] The NBDPS questionnaire contains questions regarding supplemental vitamin

use (single, prenatal, and multivitamins) from three months preconception to the end of pregnancy. These questions were used to create variables for the last two trimesters, as was done previously for the first trimester of pregnancy, which included merging the Centers for Disease Control multivitamin and folic acid file to data from the NBDPS Data Analysis Tools release 7.04 on single vitamin and other preparations of vitamin C. Dietary intake of vitamin C was also available from the FFQ and the NBDPS Database Tools. Estimates were developed for supplemental and dietary vitamin C intake based on the FFQ and the nutrient database.

*Data Analyses*

Table 1 displays the minimum detectable odds ratios for preterm births in relation to selected exposures (Specific Aims #1-2) based on 500 affected controls within the NBDPS.

**Table 1.** Minimum Detectable Odds Ratio of Preterm Births in relation to Selected Exposures in NBDPS Controls

| Outcome[1] | Number of affected controls | Exposure[2] | **Odds ratio[3] Power** | |
|---|---|---|---|---|
| | | | **80%** | **90%** |
| Preterm births | 500 | Any Nitrosatable Drugs | 1.35 | 1.41 |
| | | Secondary Amines | 1.45 | 1.53 |
| | | Tertiary Amines | 1.45 | 1.54 |
| | | Amides | 1.57 | 1.67 |
| | | Dietary Nitrite or Total Nitrite | 1.32 | 1.37 |

[1] Number of controls (term births at 10th percentile or above birth weight for gestational age) – 5546
[2] Prevalence of exposure in comparison to women (full term, non-SGA deliveries): nitrosatable drugs (23.6%), secondary amines (12.4%), tertiary amines (12.2%), amides (7.6%), dietary nitrite or total nitrite tertiles (33.3%)
[3] Two tailed significance level of 0.05

Specific Aims #1-4

To determine which potential confounding variables to include in the models, forward selection was utilized. The following covariates were considered based on their potential associations with preterm births and the exposures of interest: maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, Native American, and other); maternal age at delivery in years (<18, 18-19, 20-24, 25-29, 30-34, and ≥35); maternal education in years (<12, 12, 13-15, ≥16); household income (<$10,000; $10,000-$20,000; $20,001-$30,000; $30,001-$40,000; $40,001-$50,000; and >$50,000); maternal active smoking (yes/no); pre-pregnancy body mass index (in $kg/m^2$, categorized as <18.5, 18.5-24.9, 25.0-29.9, and ≥30.0); caloric intake (kcal); parity (nulliparous, primiparous, and multiparious); maternal hypertension during pregnancy (yes/no); pre-pregnancy hypertension (yes/no); pre-pregnancy diabetes (yes/no); gestational diabetes (yes/no); infant gender (male/female); study site; vitamin use (yes/no); and folate supplementation (yes/no). Nonsignificant covariables as well as those that did not change the measure of effect by 10% or more were eliminated from the model.

Regardless of its results, maternal race/ethnicity, education, and study center were included in the final model since they have been observed as important predictors of nitrosatable drug use and dietary intake of nitrites and total nitrites.[10] Only cases and controls who had reported a daily caloric intake between 500-5000 kcal were included in analyses involving dietary intake.

These limits are recommended by Willett[291] and are consistent with previous dietary studies and those utilizing the NBDPS database.[274, 292-294] Complete case analyses was used (participants were included for crude and adjusted analyses only if complete information was available for all variables included in the final models). Complete data on nitrosatable drug use stratified by total nitrite intake was available for 1,132 (92.6%) case-mothers and 6,376 (93.7%) controls.

Specific Aims #1-2

Cox proportional hazards models were used to obtain hazard ratios (HR) and 95% CI for preterm births in relation to 1) dietary nitrate, nitrite, and total nitrite; 2) any nitrosatable drug use; 3) nitrosatable drug usage based on categories: secondary amines, tertiary amines, and amides; and 4) drug indication groups. For analyses involving nitrosatable drug usage, women who reported no nitrosatable drug usage anytime during pregnancy served as the referent group.

Specific Aims #3-4

To examine the joint effects of nitrosatable drug exposure and dietary intake of nitrates/nitrites, nitrosatable drug exposure was stratified by tertiles of dietary intake of nitrates/nitrites. Stratum specific HRs and 95% CIs were obtained. Nitrosatable drug exposure was stratified by dietary vitamin C (<85 mg/day or ≥85 mg/day, based on NIH recommendations for pregnant women) and supplemental vitamin C (none, <daily, and daily) for each trimester.

Multiplicative interaction was assessed by including the various product terms of nitrosatable drugs with dietary intake and/or vitamin C. Additive interaction was assessed using a program developed by Andersson *et al*.,[295] relying on the relative excess risk due to interaction (RERI) and the attributable proportion (AP) due to interaction along with the 95% CIs to determine whether additive interaction was present.

In the multivariable analyses, the following main effects and interactions were considered:

1. (nitrosatable drug groups) + (dietary nitrites) + (nitrosatable drugs groups) * (dietary nitrites)
2. (nitrosatable drug groups) + (dietary plant nitrites) + (nitrosatable drugs groups) * (dietary plant nitrites)
3. (nitrosatable drug groups) + (dietary animal nitrites) + (nitrosatable drugs groups) * (dietary animal nitrites)
4. (nitrosatable drug groups) + (total nitrites) + (nitrosatable drugs groups) * (total nitrites)
5. (nitrosatable drug groups) + (vitamin C supplement) + (nitrosatable drug groups) * (vitamin C supplement)
6. (nitrosatable drug groups) + (dietary vitamin C) + (nitrosatable drug groups) * (dietary vitamin C)

**Significance**

This study examined whether nitrosatable drug usage is associated with an increased risk of preterm deliveries, which has not been done in any epidemiologic study before. Only one identified study has examined nitrosatable drugs in relation to low birth weight in humans; however, gestational age was not considered.[296] Since previous studies have observed a positive association

between preterm births and drugs which are considered nitrosatable, it is reasonable to consider its possible implications on gestational age.

Previous studies regarding nitrate/nitrite exposure and preterm births have only focused on exposure levels from drinking water.[263, 264] Sources of dietary nitrate/nitrite have not been considered despite its major contribution to the total level of human exposure. Therefore, it is important to examine the impact of dietary nitrate and nitrite intake in relation to preterm births. This study is the first to examine dietary intake of nitrate/nitrite levels and its risk on preterm births using the food frequency data available within the NBDPS.

The proportion of infants born preterm in the U.S. has gradually been increasing.[297] It is hypothesized that survival limits for preterm infants has been reached using the current methods of neonatal intensive care.[298] At this point, more research should focus on understanding the etiology of preterm births so that we are able to pinpoint and estimate risk factors of preterm births to identify women who are more susceptible. Although the causes and mechanisms of preterm delivery are not fully understood, PPROM has been connected to one third of all cases.[151-153] As higher levels of nitric oxide have been observed in the blood and urine of women who had preterm births, it is necessary to examine dietary nitrate/nitrite intake as a portion is converted to nitric oxide.[157] Because every individual has some level of nitrate and nitrite exposure, it is pertinent to examine and estimate its association with preterm births. Its effects, even if

small, could potentially have a high attributable risk as exposure is extremely common.

If an association is observed between nitrosatable drugs and preterm births, then women considering over-the-counter nitrosatable drugs during pregnancy could be recommended non-nitrosatable drugs with similar therapeutic indications as possible alternatives. For women who are prescribed nitrosatable drugs, prenatal exposure may be unavoidable (e.g., antiepileptics). However, dietary and supplemental vitamin C may attenuate the association between nitrosatable drugs and preterm births. If a diminished association is observed with high vitamin C intake, then women prenatally exposed to nitrosatable drugs would be encouraged to take a daily vitamin C supplement. In addition, this research provides information that will allow healthcare providers to identify those who are at higher risk. This would allow possible mitigation strategies such as the usage of tocolytic agents to delay or arrest the progression of preterm labor. Preventing preterm births would not only reduce the associated economic costs and improve health outcomes, but it will save countless families from the emotional and financial hardships inflicted as a result of a preterm delivery.

# CHAPTER II

# NITROSATABLE DRUG EXPOSURE DURING PREGNANCY AND PRETERM BIRTHS

## Overview

Nitrosatable drugs react with nitrite in the stomach to form *N*-nitroso compounds, observed in animal models to result in adverse pregnancy outcomes such as birth defects and reduced fetal weight. Previous studies examining prenatal exposure to medications classified as nitrosatable have observed an increased risk of preterm delivery.

Using data from mothers (controls) of babies without major birth defects from the National Birth Defects Prevention Study, prenatal nitrosatable drug usage by trimester and month of gestation was examined in relation to preterm delivery among 496 case-mothers of preterm infants and 5398 control-mothers who delivered full term babies from 1997 to 2005.

Positive associations were observed with nitrosatable drug use following the first trimester of pregnancy, with the strongest relationship among exposures during the second trimester (adjusted hazard ratio (aHR) 1.37, [95% confidence interval (CI) 1.10, 1.70]). Of the nitrosatable functional groups, secondary amines were the most notable, with an increased risk observed among women who reported exposure during the second (aHR 1.37, [95% CI 1.05, 1.79]) and

third (aHR 1.34, [95% CI 1.02, 1.76]) trimester. When we examined nitrosatable drug usage by gestational month, the strongest associations were detected with usage during the sixth and seventh month of gestation, particularly with secondary and tertiary amines. Similar findings were observed with prenatal nitrosatable drug use in relation to moderately preterm births.

Prenatal exposure to nitrosatable drugs during the second and third trimester of pregnancy, particularly secondary amines, might increase risk of preterm delivery.

**Background**

Infants born less than 37 completed weeks of gestation are considered preterm. These infants are at higher risk of adverse health outcomes during their first year of life, behavioral dysfunctions in childhood, and long-term health effects spanning through adulthood.[159] Preterm infants also account for 75% of perinatal mortality,[2] making it the leading cause of perinatal morbidity and mortality in industrialized countries. Numerous environmental toxicants have been examined for their role in preterm births, of which the weight of evidence has only been sufficient for two: lead and tobacco smoke.[3]

*N*-nitroso compounds, including nitrosamines and nitrosamides, are formed when nitrosatable amines or amides react with nitrosating agents, such as nitrite, in the acidic environment of the stomach.[11] Though exogenous sources are responsible for some of the exposure to *N*-nitroso compounds,

endogenous formation is estimated to account for approximately 45-75% of total levels.[299] Certain nitrosatable drugs, classified as secondary amines, tertiary amines, and amides, contribute to the formation of *N*-nitroso compounds by reacting with nitrosating agents. Within the National Birth Defects Prevention Study (NBDPS), nitrosatable drug use during the first trimester of pregnancy was observed in 24% of the control mothers.[10] In the NBDPS study population, prenatal exposure to nitrosatable drugs during the first trimester was associated with several birth defects, including neural tube defects, limb deficiencies, cleft lip with cleft palate, cleft palate alone, single ventricle heart defects, atrioventricular septal defects, and hypoplastic left heart syndrome.[274, 294] Further, in animal models *N*-nitroso compounds have been observed to result in adverse pregnancy outcomes in mice, such as reduced fetal weight[12] and birth defects.[13, 14] Its effects on gestational age are not fully known as studies of adverse pregnancy outcomes did not focus on this aspect.

In addition, preterm birth rates have been observed to vary by ethnic origin, with non-Hispanic black women having almost twice the rate of non-Hispanic white women in the United States, regardless of socioeconomic status.[2, 159] For the past decade, this racial gap has decreased slightly due to the 22.3% rise in preterm birth rates among non-Hispanic white women compared to the 3.6% increase observed in non-Hispanic blacks.[159] The disproportionate rates of preterm births among non-Hispanic blacks and the unexpected rise among non-Hispanic whites may be partially attributable to the higher

prevalence of nitrosatable drug usage, and subsequent higher levels of *N*-nitroso compounds, during early pregnancy among the two groups. The highest prevalence of use was observed among non-Hispanic whites, followed by women who described themselves as other and non-Hispanic black.[10] Ingestion of nitrosatable drugs may be a contributing factor in preterm deliveries and could possibly explain the preterm birth rates observed by ethnic origin.

Although no known study has examined the relation between nitrosatable drugs and preterm births, a number have investigated various drugs which have been classified as nitrosatable.[160] Many of these prescription and nonprescription drugs were reported to have been taken by NBDPS control women during the first trimester of pregnancy.[10] Several medications within the sub-categories of nitrosatable drugs have been indicated as possible risk factors of preterm births. For secondary amines, several studies have observed a positive association with prenatal use of beta blockers,[177-179] anti-depressants,[184, 186, 188-191] thiazide diuretics,[215] and migraine[203, 205, 206] medications in relation to preterm births. Tertiary amine drugs, such as antiepileptics,[219, 220] antihypertensives,[229] calcium channel blockers,[230] nicotine replacement,[236, 241] and opioids[242-245] have also been implicated. Benzodiazepine[250, 251] and sulfamethoxazole[262] medications, classified amide drugs, have also been associated with preterm deliveries. Though evidence suggests an association for several nitrosatable drugs, some studies have yielded conflicting results.[180-182, 192-195, 217, 218, 221-223, 231, 238, 246]

To our knowledge, no study has examined the relation between prenatal use of nitrosatable drugs and risk of preterm births. Given the prevalence of nitrosatable drug use and the positive associations observed between preterm births and drugs that have been classified as nitrosatable in previous studies, we examined the relation between prenatal exposure to nitrosatable drugs by their molecular structure (secondary amines, tertiary amines, and amides), focusing on usage by trimester and month of gestation, and preterm births. Moderate and very preterm births were also examined.

## Methods

### *Study Population*

Data from control-mothers of babies without major birth defects within NBDPS, an on-going population-based, case-control study of major structural birth defects in the United States, were used to examine prenatal use of nitrosatable drugs by their molecular structure (secondary amines, tertiary amines, and amides) and their relation to preterm births. The NBDPS, which began in 1997, is comprised of 10 sites across the nation, including: Arkansas, California, Georgia, Iowa, Massachusetts, New York, and Texas (from 1998 to present); New Jersey (from 1998 to 2002); and North Carolina and Utah (from 2003 to present). Case-infants within NBDPS are identified from live births (all centers), stillbirths (all centers except New Jersey and New York from 1997 to 1999), and elective pregnancy terminations (all centers except Massachusetts,

New Jersey, and New York before 2000) from birth defect surveillance programs.[282]

NBDPS control-infants were live born without major birth defects, who were delivered in the same time frame and study area as the case births with major birth defects. They were randomly sampled from birth certificates (Arkansas and Georgia, for estimated delivery dates (EDDs) after 2000; Iowa; Massachusetts; New Jersey; North Carolina; and Utah) or hospital records (Arkansas, for EDDs before 2001; California; Georgia, for EDDs before 2001; New York; and Texas).[282] Control-infants were excluded if they were not liveborn, had a major birth defect, or were born outside the study area. Prospective study participants were further excluded if the infant was either adopted or in foster care or if the mother possessed at least one of the following characteristics: did not speak English or Spanish, participated in the NBDPS previously, was incarcerated, was a donor or a surrogate parent, was unable to answer questions, or was deceased.

For our study, we focused on NBDPS control-infants with EDDs between October 1, 1997 and December 31, 2005. We further restricted our analyses to singleton births as multiple births have been observed as a major risk factor for preterm deliveries. For the purposes of this study, case-infants were defined as preterm births, infants who were born less than 37 weeks gestation. Gestational age at delivery was taken from study participants' medical records or birth certificates. If these were not available, gestational age was calculated using the

EDD reported by the mother in the interview. If the previous methods did not provide gestational age, it was calculated using (in order of descending preference): ultrasound <14 weeks, last menstrual period, ultrasound >14 weeks, or standard neonatal exam. Infants with a gestational age between 33 and 36 weeks were further classified as moderately preterm. Infants with 37-41 weeks gestation served as control-infants. Infants who are small for gestational age (SGA) were excluded since N-nitroso compounds have been observed to affect fetal weight.[12] The institutional review boards in each state and the Centers for Disease Control and Prevention approved the NBDPS study protocol, and the institutional review board of Texas A&M University also approved this project on nitrosatable drugs and preterm births.

*Data Collection*

Following informed consent, interviews were conducted in either English or Spanish by trained female interviewers using a computer-assisted telephone interview.[283] Interviews were conducted 6 weeks to 24 months after the EDDs (or delivery of a full-term infant) and targeted for completion within 6 months of the EDD. The interview included detailed questions pertaining to maternal health during the index pregnancy (including medication usage), nutrition (food and beverage consumption), infections, and behavioral factors.

*Classification of Nitrosatable Drugs*

During the interview, the NBDPS collected information about prescription and non-prescription drug usage from three months prior to the estimated date of conception to the end of pregnancy, including medication name, frequency of use, and corresponding dates of usage. Reported medications were linked to their active ingredient utilizing the Slone Epidemiology Center Drug Dictionary system.[287] Classification methods used to categorize drugs with regard to their nitrosatability, functional groups, and indications have been described in detail by Brender *et al*.[10, 274] Briefly, the methodology for classification included: 1) identification of active ingredients for all medications; 2) cross-referencing the drugs with comprehensive nitrosatable medicinal compounds lists;[160, 288] 3) drug categorization based on the presence of functional groups (e.g., secondary amine, tertiary amine, or amide); and 4) further classification by its primary indication or therapeutic use and pharmacologic class. This study focuses on drugs reported to have been taken during pregnancy, concentrating on periods of exposure by trimester and month of gestation. Complete data on nitrosatable drug use and covariates were available for 477 (96.2%), 392 (95.8%), and 5194 (96.2%) mothers of preterm, moderately preterm, and full term infants, respectively.

*Covariates*

Covariate selection was based on factors associated with preterm births in previous studies and maternal factors associated with nitrosatable drug exposure.[10] Potential confounders assessed included maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, other), educational level (<12 years, 12 years, 13-15 years, >15 years), age (<18, 18-19, 20-24, 25-29, 30-34, ≥35 years), and smoking status (yes/no); body mass index (BMI) based on self-reported height and weight ($kg/m^2$), categorized according to NIH guidelines (underweight, normal, overweight, and obese); study site; infant gender (male/female); parity (nulliparous, primiparous, and multiparous); pre-pregnancy diabetes (yes/no); gestational diabetes (yes/no); and pre-pregnancy hypertension (yes/no). Nonsignificant covariates as well as those that did not change the hazard ratio by 10% or more were eliminated from the final model using forward selection.

*Statistical Analysis*

Descriptive analyses were performed to examine the distribution of potentially important covariates among case- and control-mothers. For the main analyses, time-to-event methods were employed since preterm delivery is a time-based outcome that depends on gestational age. Cox proportional hazards model was used to estimate hazard ratios (HR) and corresponding 95% confidence intervals (CI) for preterm and moderately preterm births in relation to

nitrosatable drug use. Exposure periods of interest were by trimester (first, second, and third) and month of gestation (first, second, third, etc.). Gestational age at birth, measured in weeks, was used as the underlying time variable in the survival analysis. Each woman remained in the risk set of giving birth to a preterm infant until delivery or gestational age of 37 weeks, whichever occurred first. In other words, women with term or post-term deliveries were censored at 37 weeks. Women who did not report taking any drugs classified as nitrosatable during pregnancy served as the reference group in all analyses. Maternal race/ethnicity, educational level, and age; study center; pre-pregnancy diabetes; and pre-pregnancy hypertension were included in the regression models as possible confounders. The analyses were restricted to singleton pregnancies with complete information on all covariates included in the final model. All statistical tests were two-sided, and findings were considered statistically significant at the 5% level if the CI did not include 1.00. A hazard ratio above 1.00 represents an increased probability of preterm birth, corresponding to a shorter period of gestation. We assessed the fit of the final model using Cox-Snell residuals analysis, link test, and a global test based on Schoenfeld residuals to assess violation of the proportional hazards assumption.[300] STATA version 12.0 was used for all analyses.

**Table 2.** Selected Maternal Characteristics of Preterm Cases and Controls in the National Birth Defects Prevention Study, 1997-2005

| Characteristics of Participants | Controls[a] (%) n=5398 | Preterm Cases[a] (%) n=496 |
|---|---|---|
| Race/ethnicity* | | |
| Non-Hispanic white | 3252 (60.2) | 277 (55.9) |
| Non-Hispanic black | 605 (11.2) | 74 (14.9) |
| Hispanic | 1180 (21.9) | 107 (21.6) |
| Asian/Pacific Islander | 155 (2.9) | 9 (1.8) |
| All others | 206 (3.8) | 29 (5.9) |
| Education (years) | | |
| <12 | 852 (15.8) | 91 (18.4) |
| 12 | 1288 (23.9) | 137 (27.6) |
| 13-15 | 1462 (27.1) | 124 (25.0) |
| >15 | 1723 (31.9) | 136 (27.4) |
| Missing | 73 (1.4) | 8 (1.6) |
| Age at delivery (years)* | | |
| <18 | 193 (3.6) | 24 (4.8) |
| 18-19 | 370 (6.9) | 40 (8.1) |
| 20-24 | 1223 (22.7) | 119 (24.0) |
| 25-29 | 1433 (26.6) | 142 (28.6) |
| 30-34 | 1459 (27.0) | 96 (19.4) |
| >34 | 720 (13.3) | 75 (15.1) |
| Study center* | | |
| Arkansas | 650 (12.0) | 88 (17.7) |
| California | 697 (12.9) | 57 (11.5) |
| Georgia | 597 (11.1) | 44 (8.9) |
| Iowa | 607 (11.2) | 56 (11.3) |
| Massachusetts | 672 (12.5) | 58 (11.7) |
| North Carolina | 321 (6.0) | 34 (6.9) |
| New Jersey | 449 (8.3) | 32 (6.5) |
| New York | 499 (9.2) | 33 (6.7) |
| Texas | 602 (11.2) | 68 (13.7) |
| Utah | 304 (5.6) | 26 (5.2) |
| Body mass index (kg/m$^2$) | | |
| <18.5 | 257 (4.8) | 29 (5.9) |
| 18.5–24.9 | 2904 (53.8) | 268 (54.0) |
| 25.0–29.9 | 1190 (22.1) | 99 (20.0) |
| >29.9 | 847 (15.7) | 86 (17.3) |
| Missing | 200 (3.7) | 14 (2.8) |
| Smoking | | |
| No | 4371 (81.0) | 382 (77.0) |
| Yes | 969 (18.0) | 107 (21.6) |
| Missing | 58 (1.1) | 7 (1.4) |
| Pre-pregnancy diabetes* | | |
| No | 5244 (97.2) | 475 (95.8) |
| Yes | 26 (0.5) | 10 (2.0) |
| Missing | 128 (2.4) | 11 (2.2) |
| Pre-pregnancy hypertension* | | |
| No | 4723 (87.5) | 393 (79.2) |
| Yes | 668 (12.4) | 102 (20.6) |
| Missing | 7 (0.1) | 1 (0.2) |
| Infant gender | | |
| Male | 2702 (50.1) | 243 (49.0) |
| Female | 2696 (49.9) | 253 (51.0) |

**Table 2** (continued)

| Characteristics of Participants | Controls[a] (%) n=5398 | Preterm Cases[a] (%) n=496 |
|---|---|---|
| Parity | | |
| Nulliparous | 2150 (39.8) | 207 (41.7) |
| Primiparous | 1816 (33.6) | 154 (31.1) |
| Multiparous | 1432 (26.5) | 135 (27.2) |

[a] NBDPS control women who gave birth to preterm infants (cases) and women who had full term infants without SGA (controls)

* Statistically significant differences in the distribution between cases and controls at $P < 0.05$.

## Results

A total of 496 eligible case-mothers who delivered a preterm infant (409 who delivered a moderately preterm infant) and 5398 control-mothers with an EDD from 1997 to 2005 participated in the NBDPS. The maternal participation rate among NBDPS controls mothers was 66%. Time to interview was consistent between mothers with preterm and full term deliveries, with a 7.7 month median length of time from the EDD to the interview. Control-mothers were significantly more likely than case-mothers to be non-Hispanic white and somewhat older at time of delivery, and less likely to have pre-pregnancy hypertension, pre-pregnancy diabetes, and live in Arkansas and Texas (Table 2).

Use of secondary amines anytime during pregnancy was associated with having a preterm delivery (adjusted hazard ratio (aHR) 1.31, [95% CI 1.05, 1.63]) (Table 3). Focusing on the first two trimesters of pregnancy, we observed that exposure to any nitrosatable drugs was associated with an increased risk of

**Table 3.** Exposure to Nitrosatable Drugs and Preterm Birth by Gestational Period, National Birth Defects Prevention Study, 1997-2005

| Gestational Period | Type of drug exposure | Cases No. | Cases % | Controls No. | Controls % | Unadjusted HR[a] | Unadjusted 95% CI | Adjusted HR[b] | Adjusted 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| P1P9 | No nitrosatable drugs | 272 | 57.0 | 3216 | 61.9 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 205 | 43.0 | 1978 | 38.1 | **1.21** | [**1.01, 1.45**] | 1.20 | [0.99, 1.44] |
| | Secondary amines | 127 | 31.8 | 1130 | 26.0 | **1.31** | [**1.06, 1.62**] | **1.31** | [**1.05, 1.63**] |
| | Tertiary amines | 104 | 27.7 | 1003 | 23.8 | 1.21 | [0.97, 1.52] | 1.18 | [0.93, 1.49] |
| | Amides | 82 | 23.2 | 759 | 19.1 | 1.25 | [0.98, 1.60] | 1.21 | [0.94, 1.56] |
| P1P6 | No nitrosatable drugs | 272 | 59.9 | 3216 | 65.7 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 182 | 40.1 | 1682 | 34.3 | **1.26** | [**1.05, 1.52**] | **1.25** | [**1.03, 1.51**] |
| | Secondary amines | 107 | 28.2 | 953 | 22.9 | **1.31** | [**1.05, 1.64**] | **1.32** | [**1.04, 1.66**] |
| | Tertiary amines | 96 | 26.1 | 856 | 21.0 | 1.31 | [**1.04, 1.65**] | 1.26 | [0.99, 1.61] |
| | Amides | 69 | 20.2 | 614 | 16.0 | 1.30 | [1.00, 1.70] | 1.27 | [0.97, 1.66] |
| P1P3 | No nitrosatable drugs | 272 | 68.3 | 3216 | 72.6 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 126 | 31.7 | 1215 | 27.4 | 1.22 | [0.98, 1.50] | 1.19 | [0.95, 1.48] |
| | Secondary amines | 73 | 21.2 | 639 | 16.6 | **1.34** | [**1.03, 1.73**] | 1.29 | [0.99, 1.69] |
| | Tertiary amines | 60 | 18.1 | 642 | 16.6 | 1.10 | [0.83, 1.46] | 1.04 | [0.77, 1.39] |
| | Amides | 46 | 14.5 | 389 | 10.8 | 1.37 | [1.00, 1.87] | 1.35 | [0.98, 1.86] |
| P4P6 | No nitrosatable drugs | 272 | 67.7 | 3216 | 74.3 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 130 | 32.3 | 1111 | 25.7 | **1.36** | [**1.10, 1.68**] | **1.37** | [**1.10, 1.70**] |
| | Secondary amines | 74 | 21.4 | 657 | 17.0 | **1.32** | [**1.02, 1.70**] | **1.37** | [**1.05, 1.79**] |
| | Tertiary amines | 63 | 18.8 | 513 | 13.8 | **1.42** | [**1.08, 1.87**] | **1.40** | [**1.06, 1.86**] |
| | Amides | 42 | 13.4 | 340 | 9.6 | **1.42** | [**1.03, 1.97**] | 1.39 | [1.00, 1.93] |

**Table 3** (continued)

| Gestational Period | Type of drug exposure | Cases No. | Cases % | Controls No. | Controls % | Unadjusted HR[a] | Unadjusted 95% CI | Adjusted HR[b] | Adjusted 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| P7P9 | No nitrosatable drugs | 272 | 71.2 | 3216 | 76.4 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 110 | 28.8 | 991 | 23.6 | **1.29** | [**1.03, 1.61**] | 1.26 | [1.00, 1.59] |
| | Secondary amines | 69 | 20.2 | 592 | 15.6 | **1.35** | [**1.04, 1.76**] | **1.34** | [**1.02, 1.76**] |
| | Tertiary amines | 45 | 14.2 | 429 | 11.8 | 1.22 | [0.89, 1.68] | 1.21 | [0.87, 1.67] |
| | Amides | 32 | 10.5 | 280 | 8.0 | 1.32 | [0.91, 1.90] | 1.28 | [0.88, 1.85] |

Abbreviations: HR, hazard ratio; CI, confidence interval; P1P9, entire pregnancy; P1P6, first two trimesters; P1P3, first trimester; P4P6, second trimester; P7P9, third trimester.

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

[b] Adjusted for study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.

having a preterm infant (aHR 1.25, [95% CI 1.03, 1.51]) compared to no nitrosatable drug use anytime during pregnancy, especially with secondary amine usage (aHR 1.32, [95% CI 1.04, 1.66]). Exposure during the first trimester of pregnancy to drugs classified as secondary amines was associated with preterm births (HR 1.34, [95% CI 1.03, 1.73]). However, after adjusting for a number of factors the 95% CI was compatible with the null (aHR 1.29, [95% CI 0.99, 1.69]). A higher proportion of case-mothers (32.3%) than control-mothers (25.7%) reported taking drugs classified as nitrosatable during the second trimester of pregnancy (aHR 1.37, [95% CI 1.10, 1.70]), particularly secondary (aHR 1.37, [95% CI 1.05, 1.79]) and tertiary amines (aHR 1.40, [95% CI 1.06, 1.86]). An increased risk of preterm births was detected with third trimester exposure to secondary amines (aHR 1.34, [95% CI 1.02, 1.76]).

Nitrosatable drug exposure was further examined by month of gestation (Table 4). Though no significant relationship was observed with regard to nitrosatable drug use during the first trimester and preterm births, we did note an increased risk among those who reported taking a nitrosatable drug during the first month of gestation (aHR 1.33, [95% CI 1.03, 1.72]), especially among amide users (aHR 1.70, [95% CI 1.14, 2.55]). Women with secondary amine usage during the fourth month of gestation had excess preterm births (aHR 1.40, [95% CI 1.03, 1.91]). A similar finding was observed with any nitrosatable use during

**Table 4.** Exposure to Nitrosatable Drugs and Preterm Birth by Month of Gestation, National Birth Defects Prevention Study, 1997-2005

| Gestational Month | Type of drug exposure | Cases No. | Cases % | Controls No. | Controls % | Unadjusted HR[a] | Unadjusted 95% CI | Adjusted HR[b] | Adjusted 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | No nitrosatable drugs | 272 | 76.8 | 3216 | 82.0 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 82 | 23.2 | 706 | 18.0 | **1.35** | [**1.05, 1.73**] | **1.33** | [**1.03, 1.72**] |
| | Secondary amines | 47 | 14.7 | 427 | 11.7 | 1.29 | [0.94, 1.75] | 1.25 | [0.91, 1.73] |
| | Tertiary amines | 33 | 10.8 | 343 | 9.6 | 1.13 | [0.79, 1.62] | 1.08 | [0.75, 1.57] |
| | Amides | 27 | 9.0 | 183 | 5.4 | **1.68** | [**1.13, 2.50**] | **1.70** | [**1.14, 2.55**] |
| 2 | No nitrosatable drugs | 272 | 78.4 | 3216 | 80.9 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 75 | 21.6 | 760 | 19.1 | 1.16 | [0.90, 1.50] | 1.09 | [0.84, 1.42] |
| | Secondary amines | 49 | 15.3 | 407 | 11.2 | **1.40** | [**1.04, 1.90**] | 1.34 | [0.97, 1.84] |
| | Tertiary amines | 34 | 11.1 | 383 | 10.6 | 1.05 | [0.73, 1.50] | 0.97 | [0.67, 1.41] |
| | Amides | 14 | 4.9 | 197 | 5.8 | 0.85 | [0.49, 1.45] | 0.80 | [0.47, 1.38] |
| 3 | No nitrosatable drugs | 272 | 76.6 | 3216 | 80.6 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 83 | 23.4 | 773 | 19.4 | 1.26 | [0.99, 1.61] | 1.23 | [0.95, 1.59] |
| | Secondary amines | 46 | 14.5 | 397 | 11.0 | 1.36 | [1.00, 1.86] | 1.36 | [0.98, 1.88] |
| | Tertiary amines | 41 | 13.1 | 396 | 11.0 | 1.22 | [0.88, 1.70] | 1.18 | [0.84, 1.66] |
| | Amides | 21 | 7.2 | 202 | 5.9 | 1.21 | [0.78, 1.89] | 1.19 | [0.76, 1.87] |
| 4 | No nitrosatable drugs | 272 | 76.6 | 3216 | 81.2 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 83 | 23.4 | 746 | 18.8 | **1.30** | [**1.02, 1.66**] | 1.28 | [0.99, 1.66] |
| | Secondary amines | 51 | 15.8 | 444 | 12.1 | 1.34 | [1.00, 1.81] | **1.40** | [**1.03, 1.91**] |
| | Tertiary amines | 37 | 12.0 | 361 | 10.1 | 1.20 | [0.85, 1.69] | 1.17 | [0.82, 1.67] |
| | Amides | 23 | 7.8 | 190 | 5.6 | 1.39 | [0.91, 2.13] | 1.35 | [0.88, 2.08] |
| 5 | No nitrosatable drugs | 272 | 76.6 | 3216 | 81.7 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 83 | 23.4 | 723 | 18.3 | **1.34** | [**1.05, 1.71**] | **1.33** | [**1.03, 1.71**] |
| | Secondary amines | 50 | 15.5 | 438 | 12.0 | 1.33 | [0.99, 1.80] | 1.34 | [0.99, 1.83] |

**Table 4** (continued)

| Gestational Month | Type of drug exposure | Cases No. | Cases % | Controls No. | Controls % | Unadjusted HR[a] | Unadjusted 95% CI | Adjusted HR[b] | Adjusted 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| | Tertiary amines | 40 | 12.8 | 320 | 9.1 | **1.44** | [**1.04, 2.01**] | 1.39 | [0.99, 1.96] |
| | Amides | 21 | 7.2 | 184 | 5.4 | 1.33 | [0.85, 2.07] | 1.32 | [0.84, 2.08] |
| 6 | No nitrosatable drugs | 272 | 74.5 | 3216 | 81.8 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 93 | 25.5 | 718 | 18.2 | **1.49** | [**1.18, 1.89**] | **1.49** | [**1.17, 1.90**] |
| | Secondary amines | 51 | 15.8 | 445 | 12.2 | 1.34 | [0.99, 1.81] | 1.37 | [1.00, 1.86] |
| | Tertiary amines | 44 | 13.9 | 322 | 9.1 | **1.57** | [**1.14, 2.16**] | **1.56** | [**1.12, 2.16**] |
| | Amides | 26 | 8.7 | 163 | 4.8 | **1.79** | [**1.20, 2.68**] | **1.74** | [**1.16, 2.61**] |
| 7 | No nitrosatable drugs | 272 | 73.7 | 3216 | 80.8 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 97 | 26.3 | 766 | 19.2 | **1.46** | [**1.16, 1.84**] | **1.43** | [**1.13, 1.82**] |
| | Secondary amines | 57 | 17.3 | 476 | 12.9 | **1.39** | [**1.04, 1.85**] | **1.40** | [**1.04, 1.87**] |
| | Tertiary amines | 40 | 12.8 | 327 | 9.2 | **1.41** | [**1.01, 1.97**] | 1.39 | [0.99, 1.96] |
| | Amides | 24 | 8.1 | 186 | 5.5 | 1.48 | [0.98, 2.25] | 1.42 | [0.93, 2.17] |
| 8 | No nitrosatable drugs | 272 | 78.2 | 3216 | 81.8 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 76 | 21.8 | 716 | 18.2 | 1.23 | [0.96, 1.59] | 1.20 | [0.92, 1.57] |
| | Secondary amines | 54 | 16.6 | 455 | 12.4 | **1.37** | [**1.02, 1.83**] | 1.35 | [1.00, 1.82] |
| | Tertiary amines | 32 | 10.5 | 324 | 9.2 | 1.16 | [0.80, 1.67] | 1.13 | [0.78, 1.65] |
| | Amides | 19 | 6.5 | 152 | 4.5 | 1.42 | [0.89, 2.27] | 1.40 | [0.87, 2.25] |
| 9 | No nitrosatable drugs | 272 | 94.1 | 3216 | 84.3 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 17 | 5.9 | 600 | 15.7 | **0.34** | [**0.21, 0.56**] | **0.34** | [**0.21, 0.56**] |
| | Secondary amines | 10 | 3.6 | 379 | 10.5 | **0.32** | [**0.17, 0.60**] | **0.32** | [**0.17, 0.61**] |
| | Tertiary amines | 6 | 2.2 | 268 | 7.7 | **0.27** | [**0.12, 0.61**] | **0.27** | [**0.12, 0.60**] |
| | Amides | 4 | 1.5 | 122 | 3.7 | 0.40 | [0.15, 1.07] | 0.40 | [0.15, 1.06] |

Abbreviations: HR, hazard ratio; CI, confidence interval.

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

[b] Adjusted for study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.

the fifth month of gestation compared to no prenatal nitrosatable drug use (aHR 1.33, [95% CI 1.03, 1.71]), though to a lesser extent. The strongest association was observed during the sixth month of gestation (aHR 1.49, [95% CI 1.17, 1.90]), most notably with tertiary amines (aHR 1.56, [95% CI 1.12, 2.16]) and amides (aHR 1.74, [95% CI 1.16, 2.61]). Risk of preterm delivery was also elevated with nitrosatable drug use during the seventh month (aHR 1.43, [95% CI 1.13, 1.82]), as with secondary amines (aHR 1.40, [95% CI 1.04, 1.87]). In contrast, nitrosatable drug exposure during the ninth gestational month yielded a protective association, suggesting a 66% lower hazard of preterm births (aHR 0.34, [95% CI 0.21, 0.56]). The strongest reduction in risk appeared with tertiary amine exposure (aHR 0.27, [95% CI 0.12, 0.60]) followed by secondary amines (aHR 0.32, [95% CI 0.17, 0.61]).

Overall, preterm births were associated with drugs classified as secondary amines across a broad range of indications, including asthma, cardiovascular, decongestants, and antidepressants during the second trimester (data not shown; associations reported are restricted to drugs with at least 5 exposed cases and 5 exposed controls). Use of asthma and cardiovascular medications during the second trimester were most strongly associated with delivering a preterm infant (aHR 2.15, [95% CI 1.47, 3.14] and aHR 3.04 [95% CI 1.40, 6.62], respectively).

Women with nitrosatable drug usage anytime during pregnancy had higher risk of delivering a moderately preterm infant compared to those with no

**Table 5.** Exposure to Nitrosatable Drugs and Moderately Preterm Birth by Gestational Period, National Birth Defects Prevention Study, 1997-2005

| Gestational Period | Type of drug exposure | Cases No. | Cases % | Controls No. | Controls % | Unadjusted HR[a] | Unadjusted 95% CI | Adjusted HR[b] | Adjusted 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| P1P9 | No nitrosatable drugs | 217 | 55.4 | 3216 | 61.9 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 175 | 44.6 | 1978 | 38.1 | **1.30** | [**1.06, 1.58**] | **1.29** | [**1.05, 1.58**] |
| | Secondary amines | 109 | 33.4 | 1130 | 26.0 | **1.41** | [**1.12, 1.78**] | **1.40** | [**1.10, 1.78**] |
| | Tertiary amines | 84 | 27.9 | 1003 | 23.8 | 1.23 | [0.96, 1.58] | 1.22 | [0.93, 1.58] |
| | Amides | 72 | 24.9 | 759 | 19.1 | **1.38** | [**1.06, 1.80**] | **1.35** | [**1.03, 1.77**] |
| P1P6 | No nitrosatable drugs | 217 | 58.7 | 3216 | 65.7 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 153 | 41.3 | 1682 | 34.3 | **1.33** | [**1.08, 1.64**] | **1.32** | [**1.07, 1.64**] |
| | Secondary amines | 90 | 29.3 | 953 | 22.9 | **1.39** | [**1.08, 1.77**] | **1.38** | [**1.07, 1.79**] |
| | Tertiary amines | 77 | 26.2 | 856 | 21.0 | **1.32** | [**1.02, 1.71**] | 1.30 | [0.99, 1.71] |
| | Amides | 59 | 21.4 | 614 | 16.0 | **1.40** | [**1.05, 1.86**] | **1.38** | [**1.03, 1.85**] |
| P1P3 | No nitrosatable drugs | 217 | 67.8 | 3216 | 72.6 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 103 | 32.2 | 1215 | 27.4 | 1.25 | [0.99, 1.58] | 1.23 | [0.96, 1.57] |
| | Secondary amines | 59 | 21.4 | 639 | 16.6 | **1.36** | [**1.02, 1.81**] | 1.30 | [0.96, 1.76] |
| | Tertiary amines | 46 | 17.5 | 642 | 16.6 | 1.06 | [0.77, 1.46] | 1.01 | [0.73, 1.41] |
| | Amides | 38 | 14.9 | 389 | 10.8 | **1.42** | [**1.01, 2.00**] | 1.42 | [1.00, 2.02] |
| P4P6 | No nitrosatable drugs | 217 | 66.6 | 3216 | 74.3 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 109 | 33.4 | 1111 | 25.7 | **1.43** | [**1.14, 1.80**] | **1.45** | [**1.14, 1.84**] |
| | Secondary amines | 62 | 22.2 | 657 | 17.0 | **1.39** | [**1.05, 1.84**] | **1.44** | [**1.07, 1.92**] |
| | Tertiary amines | 51 | 19.0 | 513 | 13.8 | **1.44** | [**1.06, 1.96**] | **1.45** | [**1.06, 1.99**] |
| | Amides | 35 | 13.9 | 340 | 9.6 | **1.49** | [**1.04, 2.13**] | **1.47** | [**1.03, 2.12**] |

**Table 5** (continued)

| Gestational Period | Type of drug exposure | Cases No. | Cases % | Controls No. | Controls % | Unadjusted HR[a] | Unadjusted 95% CI | Adjusted HR[b] | Adjusted 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| P7P9 | No nitrosatable drugs | 217 | 69.3 | 3216 | 76.4 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 96 | 30.7 | 991 | 23.6 | **1.41** | [**1.11, 1.80**] | **1.39** | [**1.08, 1.78**] |
| | Secondary amines | 61 | 21.9 | 592 | 15.6 | **1.50** | [**1.13, 1.99**] | **1.47** | [**1.10, 1.98**] |
| | Tertiary amines | 36 | 14.2 | 429 | 11.8 | 1.23 | [0.86, 1.75] | 1.22 | [0.85, 1.75] |
| | Amides | 29 | 11.8 | 280 | 8.0 | **1.50** | [**1.02, 2.21**] | 1.47 | [0.99, 2.18] |

Abbreviations: HR, hazard ratio; CI, confidence interval; P1P9, entire pregnancy; P1P6, first two trimesters; P1P3, first trimester; P4P6, second trimester; P7P9, third trimester.

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

[b] Adjusted for study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.

exposure (aHR 1.29, [95% CI 1.05, 1.58]), particularly with secondary amines (aHR 1.40, [95% CI 1.10, 1.78]) and amides (aHR 1.35, [95% CI 1.03, 1.77]) (Table 5). Similar associations were noted with nitrosatable drug exposure during the first two trimesters. Though no significant relationship was detected with nitrosatable drugs during the first trimester and moderately preterm deliveries, risk was elevated during the second trimester (aHR 1.45, [95% CI 1.14, 1.84]). In addition, significant associations were observed with every sub-category of nitrosatable drugs, with the strongest association occurring among women with amide exposure (aHR 1.47, [95% CI 1.03, 2.12]). A higher proportion of mothers of moderately preterm infants (30.7%) than control-mothers (23.6%) reported taking drugs identified as nitrosatable during the third trimester (aHR 1.39, [95% CI 1.08, 1.78]), particularly secondary amines (aHR 1.47, [95% CI 1.10, 1.98]).

Nitrosatable drug use was also examined by month of gestation in relation to moderately preterm births (Table 6). An elevated hazard ratio was observed with nitrosatable drug use during the first gestational month (aHR 1.36, [95% CI 1.02, 1.81]), especially with amides (aHR 1.70, [95% CI 1.07, 2.68]). Risk of delivering a moderately preterm infant was higher with secondary amine usage during the fourth month of pregnancy (aHR 1.44, [95% CI 1.02, 2.02]) and with overall nitrosatable drug use during the fifth month (aHR 1.36, [95% CI 1.03, 1.81]). Exposure during the sixth month of gestation was associated with moderately preterm births for overall nitrosatable drugs (aHR 1.61, [95% CI

**Table 6.** Exposure to Nitrosatable Drugs and Moderately Preterm Birth by Month of Gestation, National Birth Defects Prevention Study, 1997-2005

| Gestational Month | Type of drug exposure | Cases No. | Cases % | Controls No. | Controls % | Unadjusted HR[a] | Unadjusted 95% CI | Adjusted HR[b] | Adjusted 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | No nitrosatable drugs | 217 | 76.4 | 3216 | 82.0 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 67 | 23.6 | 706 | 18.0 | **1.38** | [**1.05, 1.82**] | **1.36** | [**1.02, 1.81**] |
| | Secondary amines | 40 | 15.6 | 427 | 11.7 | 1.38 | [0.98, 1.93] | 1.32 | [0.93, 1.87] |
| | Tertiary amines | 26 | 10.7 | 343 | 9.6 | 1.11 | [0.74, 1.67] | 1.08 | [0.71, 1.64] |
| | Amides | 21 | 8.8 | 183 | 5.4 | **1.65** | [**1.05, 2.58**] | **1.70** | [**1.07, 2.68**] |
| 2 | No nitrosatable drugs | 217 | 78.3 | 3216 | 80.9 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 60 | 21.7 | 760 | 19.1 | 1.17 | [0.88, 1.55] | 1.10 | [0.81, 1.48] |
| | Secondary amines | 39 | 15.2 | 407 | 11.2 | 1.41 | [1.00, 1.98] | 1.32 | [0.93, 1.89] |
| | Tertiary amines | 27 | 11.1 | 383 | 10.6 | 1.04 | [0.70, 1.55] | 0.98 | [0.65, 1.48] |
| | Amides | 10 | 4.4 | 197 | 5.8 | 0.76 | [0.40, 1.42] | 0.73 | [0.38, 1.38] |
| 3 | No nitrosatable drugs | 217 | 77.2 | 3216 | 80.6 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 64 | 22.8 | 773 | 19.4 | 1.22 | [0.93, 1.62] | 1.20 | [0.90, 1.61] |
| | Secondary amines | 35 | 13.9 | 397 | 11.0 | 1.31 | [0.92, 1.87] | 1.30 | [0.90, 1.88] |
| | Tertiary amines | 28 | 11.4 | 396 | 11.0 | 1.05 | [0.71, 1.55] | 1.03 | [0.69, 1.55] |
| | Amides | 17 | 7.3 | 202 | 5.9 | 1.23 | [0.75, 2.02] | 1.22 | [0.74, 2.02] |
| 4 | No nitrosatable drugs | 217 | 76.1 | 3216 | 81.2 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 68 | 23.9 | 746 | 18.8 | **1.34** | [**1.02, 1.75**] | 1.32 | [0.99, 1.75] |
| | Secondary amines | 42 | 16.2 | 444 | 12.1 | 1.39 | [1.00, 1.94] | **1.44** | [**1.02, 2.02**] |
| | Tertiary amines | 28 | 11.4 | 361 | 10.1 | 1.14 | [0.77, 1.69] | 1.12 | [0.75, 1.69] |
| | Amides | 19 | 8.1 | 190 | 5.6 | 1.44 | [0.90, 2.31] | 1.42 | [0.88, 2.28] |
| 5 | No nitrosatable drugs | 217 | 76.4 | 3216 | 81.7 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 67 | 23.6 | 723 | 18.3 | **1.36** | [**1.03, 1.78**] | **1.36** | [**1.03, 1.81**] |
| | Secondary amines | 40 | 15.6 | 438 | 12.0 | 1.34 | [0.96, 1.88] | 1.35 | [0.96, 1.91] |

**Table 6** (continued)

| Gestational Month | Type of drug exposure | Cases No. | Cases % | Controls No. | Controls % | Unadjusted HR[a] | Unadjusted 95% CI | Adjusted HR[b] | Adjusted 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| | Tertiary amines | 30 | 12.2 | 320 | 9.1 | 1.36 | [0.93, 1.99] | 1.34 | [0.90, 1.98] |
| | Amides | 17 | 7.3 | 184 | 5.4 | 1.35 | [0.83, 2.22] | 1.36 | [0.83, 2.25] |
| 6 | No nitrosatable drugs | 217 | 73.3 | 3216 | 81.8 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 79 | 26.7 | 718 | 18.2 | **1.60** | [**1.23, 2.06**] | **1.61** | [**1.24, 2.10**] |
| | Secondary amines | 42 | 16.2 | 445 | 12.2 | 1.39 | [1.00, 1.93] | **1.41** | [**1.01, 1.99**] |
| | Tertiary amines | 34 | 13.6 | 322 | 9.1 | **1.53** | [**1.06, 2.19**] | **1.55** | [**1.07, 2.24**] |
| | Amides | 22 | 9.2 | 163 | 4.8 | **1.91** | [**1.23, 2.96**] | **1.89** | [**1.21, 2.95**] |
| 7 | No nitrosatable drugs | 217 | 72.3 | 3216 | 80.8 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 83 | 27.7 | 766 | 19.2 | **1.57** | [**1.22, 2.02**] | **1.55** | [**1.19, 2.02**] |
| | Secondary amines | 49 | 18.4 | 476 | 12.9 | **1.50** | [**1.10, 2.05**] | **1.50** | [**1.09, 2.06**] |
| | Tertiary amines | 31 | 12.5 | 327 | 9.2 | 1.38 | [0.94, 2.01] | 1.37 | [0.93, 2.01] |
| | Amides | 21 | 8.8 | 186 | 5.5 | **1.63** | [**1.04, 2.55**] | **1.59** | [**1.01, 2.50**] |
| 8 | No nitrosatable drugs | 217 | 75.1 | 3216 | 81.8 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 72 | 24.9 | 716 | 18.2 | **1.47** | [**1.12, 1.92**] | **1.44** | [**1.09, 1.90**] |
| | Secondary amines | 52 | 19.3 | 455 | 12.4 | **1.66** | [**1.22, 2.24**] | **1.62** | [**1.19, 2.22**] |
| | Tertiary amines | 28 | 11.4 | 324 | 9.2 | 1.27 | [0.86, 1.88] | 1.26 | [0.84, 1.88] |
| | Amides | 18 | 7.7 | 152 | 4.5 | **1.69** | [**1.05, 2.74**] | **1.69** | [**1.03, 2.76**] |
| 9 | No nitrosatable drugs | 217 | 92.7 | 3216 | 84.3 | 1.00 | Referent | 1.00 | Referent |
| | Any nitrosatable drugs | 17 | 7.3 | 600 | 15.7 | **0.43** | [**0.26, 0.70**] | **0.43** | [**0.26, 0.71**] |
| | Secondary amines | 10 | 4.4 | 379 | 10.5 | **0.40** | [**0.21, 0.75**] | **0.40** | [**0.21, 0.76**] |
| | Tertiary amines | 6 | 2.7 | 268 | 7.7 | **0.34** | [**0.15, 0.76**] | **0.33** | [**0.15, 0.76**] |
| | Amides | 4 | 1.8 | 122 | 3.7 | 0.50 | [0.18, 1.33] | 0.49 | [0.18, 1.32] |

Abbreviations: HR, hazard ratio; CI, confidence interval.

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

[b] Adjusted for study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.

1.24, 2.10]) and across the board for all subtypes. Besides tertiary amines, similar associations were observed for the seventh and eighth month of gestation. However, a lower risk was detected with nitrosatable drug usage during the ninth gestational month (aHR 0.43, [95% CI 0.26, 0.71]), particularly with secondary (aHR 0.40, [95% CI 0.21, 0.76]) and tertiary amine use (aHR 0.33, [95% CI 0.15, 0.76]).

We also examined very preterm births, defined as infants with a gestational age less than 33 weeks, with respect to nitrosatable drug use by trimester and gestational month. A higher risk of delivering a very preterm infant was detected with tertiary amine use during the third month of gestation (HR 1.90, [95% CI 1.04, 3.47]), though the 95% CI was compatible with the null after adjustment (aHR 1.76, [95% CI 0.93, 3.32]) (data not shown). Despite some elevated point estimates with nitrosatable drug exposure, none were statistically significant. All graphical depictions based on the Cox-Snell residual analysis yielded results that support a reasonably well-fit model. Additionally, the link test and global test based on Schoenfeld residuals suggest no violation of the proportional hazards assumption.

**Comment**

In this study based on NBDPS control women with EDDs between 1997 and 2003, prenatal use of nitrosatable drugs was associated with preterm births. Positive associations were observed with exposure after the first trimester of

pregnancy, with the strongest relationship detected with second trimester exposure. Of the nitrosatable functional groups, secondary amines were the most notable, with an increased risk of having a preterm infant observed among women who reported secondary amine exposure during the second and third trimester of pregnancy. When we further examined exposure by month of gestation, the strongest associations were observed during the sixth and seventh month. However, a reduction in risk was found with nitrosatable drug use during the last month of gestation, particularly with secondary and tertiary amines. Since length of gestation was taken into account, we have no explanation for why we observed reduced associations between preterm births and nitrosatable drug use during the ninth gestational month.

For moderately preterm births, we observed similar results with nitrosatable drug exposure by trimester and month of gestation. However, there were stronger associations with moderately preterm births than that observed with all preterm births combined. In addition, ten more significant relationships were noted. Conversely, when we further examined risk of delivering a very preterm infant, no associations were found with prenatal nitrosatable drug use by trimester or month of gestation. Aside from: 1) amide exposure during the third trimester; 2) amide usage from the second gestational month onward; and 3) the last two gestational months, we had sufficient numbers to analyze the relation between nitrosatable drug use and very preterm births. Hazard ratios

were close to 1.00, and 95% CIs were compatible with the null for all remaining analyses involving nitrosatable drugs and very preterm births.

Overall, preterm births were associated with use of drugs classified as secondary amines across a broad range of indications, including asthma, cardiovascular, decongestants, and antidepressants during the second trimester in this study. Previous studies examining albuterol, an asthma medication, indicate no increased risk of preterm births with maternal exposure. In a cohort consisting of 2,123 asthmatic participants recruited from 16 centers of the National Institute of Child Health and Human Development Maternal Fetal Medicine Units Network, Schatz *et al*.[165] noted no significant relationship between use of inhaled β-agonists and preterm births. These null findings were corroborated by Bracken *et al*.[166] in a large prospective study among 529 women exposed to short-acting β-agonists even after adjusting for numerous factors, including number of asthma symptoms (aOR 1.01, [95% CI 1.00, 1.02]).

There is contradictory evidence regarding treatment with cardiovascular drugs, particularly beta blockers, during pregnancy and preterm births. In a population-based cohort study using the Danish Fertility Database, exposure to beta blockers was defined as the redemption of at least two prescriptions between 6 months preconception and the 20$^{th}$ week of gestation. An increased risk of preterm births was detected among women exposed to beta blockers during pregnancy (aOR 2.26, [95% CI 2.03, 2.52]).[177] This finding was previously noted in a retrospective cohort where a higher proportion of preterm deliveries

was found in women who were in multiple antihypertensive drug regimens ($P < 0.001$).[178] Conversely, an earlier study of labetalol use found that prenatal exposure was associated with a higher frequency of fetal growth retardation, but not preterm births.[181] In addition, a meta-analysis of randomized trials of prenatal beta blocker use, published between 1966 and 1997, yielded a pooled odds ratio of 1.35 [95% CI 0.51, 3.60] with regard to preterm births.[182]

No definitive increase in risk of preterm births with prenatal use of antidepressants has emerged so far, though the preponderance of evidence does indicate an association.[184, 186, 188-191, 197] In a prospective cohort utilizing data from the Swedish Medical Birth Registry from 1995-2001, Kallen[188] observed an increased risk among mothers who had taken any type of antidepressant during pregnancy (aOR 1.96, [95% CI 1.60, 2.41]). An even stronger relationship was observed with prenatal use of selective serotonin reuptake inhibitors (SSRIs) (aOR 2.06, [95% CI 1.58, 2.69]). Excluding women who had taken other drugs, such as anticonvulsants and antihypertensives, did not change the overall finding (aOR 2.01, [95% CI 1.17, 3.46]). Colvin *et al*.[190] confirmed previous findings between prenatal exposure to SSRIs and preterm births (aOR 1.43, [95% CI 1.24, 1.65]) and also observed an association for a number of SSRI drugs, including sertraline (aOR 1.62, [95% CI 1.30, 2.03]), citalopram (aOR 1.38, [95% CI 1.08, 1.77]), and paroxetine (aOR 1.41, [95% CI 1.02, 1.96]). These associations remained statistically significant even after refining the exposure window to the first trimester, with the greatest increase in

risk observed with sertraline (aOR 1.74, [95% CI 1.33, 2.27]) and paroxetine (aOR 1.61, [95% CI 1.14, 2.28]). Second/third trimester exposure to SSRIs, as well as specific drugs, was not associated with preterm births.

While many studies have reported an association between prenatal SSRI use and preterm births, several have noted no effect.[192-195] In a Finnish study based on population-based register data, no increased risk for preterm or very preterm births was observed among women with SSRI purchases in each trimester or during the second and third trimesters compared to those who had only first trimester exposure.[192] In addition, risk of delivering a preterm infant was not elevated among women who used SSRIs at the outset of pregnancy, but stopped prior to the end of the first trimester (aOR 1.12, [95% CI 0.47, 2.19]) or among those who continued to use SSRIs (aOR 1.27, [95% CI 0.59, 2.76]) compared to those without antidepressant use two months preconception through delivery.[194] To control for confounding by depression, Oberlander *et al*.[193] compared infants of depressed mothers treated with SSRIs to infants of untreated depressed mothers and nonexposed controls. No difference was observed in the incidence of preterm births between treated and untreated depressed mothers after accounting for maternal illness severity using propensity score matching ($P = 0.61$).

Contrary to the increased risk of preterm births observed with prenatal decongestant use in the present study, two previous studies have noted a protective effect.[207, 209] In the first study, Swedish register data was utilized to

obtain information regarding maternal drug use and delivery outcomes.[209] No association was noted between first trimester exposure to decongestants and preterm births, but a reduced risk was observed with later pregnancy use (aOR 0.68, [95% CI 0.52, 0.88]). This inverse association was corroborated in a population-based retrospective study (HR 0.42, [95% CI 0.21, 0.84]) between second or third trimester decongestant use and preterm births. This protective association may be due to confounding by indication, similar to nausea and vomiting which have also been associated with a reduced risk of preterm births,[210] as pregnancy rhinitis may also be an indicator of a healthy pregnancy. To explore this, various combinations of asthma and decongestant exposures were examined. Non-asthmatic women who took decongestants had lower risk of preterm births compared to those unexposed.[207] Compared to non-asthmatic, non-decongestant exposed mothers, untreated asthmatic women had the highest risk (HR 1.8, [95% CI 1.2, 2.6]) whereas asthmatic women who took decongestants had no increase in risk (HR 1.0, [95% CI 0.50, 2.0]).

Our study has several strengths, including the relatively large sample of preterm delivery cases and controls. In addition, our study population came from control women within the NBDPS, one of the largest collaborative population-based studies of birth defects in the United States. Utilizing control data from the NBDPS has three main advantages. First, control mothers selected in the NBDPS were those who delivered infants without any birth defects. Analyzing this population of control mothers eliminates the confounding effects of birth

defects as infants are more likely to be born preterm if congenital malformations are present.[301] In addition, an association was observed with nitrosatable drugs and dietary nitrite and total nitrite intake with selected birth defects in a two previous studies.[274, 294] Second, control data from this study has been found to be representative of their base populations with regard to maternal age, smoking status, and prevalence of diabetes mellitus, though slight differences were observed in terms of maternal race/ethnicity and education.[282] Time to interview is also consistent between mothers with preterm and full term deliveries as the point of reference is the EDD rather than the actual delivery date. If the actual date of delivery was used, then mothers of preterm births would have a shorter length of time to recall exposures prior to conception through the end of pregnancy than mothers who had delivered full term infants. Both mothers of preterm and full term infants had a 7.7 month median length of time from the EDD to the interview.

Another strength pertains to the various exposure periods which were investigated. Currently it is not known when the critical window of susceptibility for preterm births is. Though this question has been address by numerous studies, no clear answer has been identified. It is likely that the critical period of susceptibility would depend, partially, on the pathway which the exposure initiates its action.[3] As prenatal exposures vary in their chemical structure and biological activities, the critical period of exposure for preterm births may differ and be contingent upon the exposure itself. In the present study, we were able

to examine different periods of exposure for nitrosatable drugs by trimester and month of gestation.

The findings in our study are subject to several limitations. The first of which pertains to the potential maternal recall bias of drug exposures. In the original study, the primary outcome was birth defects, as such there was a concern that mothers of infants with birth defects may more likely recall drug exposures during pregnancy compared to mothers of infants without birth defects. However, our study population consisted only of control women from the NBDPS. In addition, little evidence has been found regarding differential recall of drugs classified as nitrosatable within the present study, particularly among antibiotics,[302, 303] antinauseants,[302] analgesics,[303] and benzodiazepines.[303] However, a 20% higher sensitivity was reported for antibiotics among case-mothers than in control-mothers.[304] To reduce recall bias, NBPDS utilized a two-level approach for drug assessment by asking participants about drug use by indication and subsequently prompting them with lists of medications. This approach has been shown to be more accurate for assessing drug use compared to asking either type of question individually.[305] Medications were classified based on their nitrosatability and further grouped based on their functional groups (secondary amines, tertiary amines, or amides) after the interviews. Given that participants were not directly questioned about nitrosatable drug usage, it is unlikely that recall bias is present. Though, it may be possible that some sub-types of nitrosatable drugs may have been recalled

differentially. In addition, exposure to some nitrosatable drugs might have been missed. While the present study utilized extensive reviews[160, 288] previous studies did not have available, some components may not been tested for its nitrosatability or results from such tests may not have been available and thus exposures may have been missed.

Secondly, information regarding several risk factors previously reported to influence the risk of delivering a preterm infant were not available for this study. Of particular concern is prior history of preterm delivery as recurrence is estimated to range from 15 to over 50%, depending on the number and gestational age of previous deliveries.[2, 306] Other risk factors include intrauterine infections,[15-21] marital status,[29-36] and psychological or social stress.[307, 308] Failure to account for a number of previously reported risk factors may have resulted in elevated point estimates. In addition, preterm births were treated as a single entity within the present study as we did not have information available for distinction by clinical presentation. Evidence has suggested that preterm deliveries consist of three clinical subtypes with partially heterogeneous etiologies, including spontaneous preterm delivery after preterm labor, medically indicated preterm delivery, and spontaneous preterm delivery after preterm premature rupture of fetal membranes (PPROM).[309, 310] However, examining preterm births as a group versus splitting them into subsets remains controversial.[311, 312] Splitting preterm births by clinical presentation has been supported by various researchers since preterm delivery can result from diverse

clinical pathways. For instance, rupturing of the membranes and spontaneous onset of labor is quite distinctive from fetal distress that should be handled with early delivery. However, it is argued that the conditions that prompt medical intervention for early delivery, such as preeclampsia and fetal growth restriction, have similar mechanisms as pathways resulting in spontaneous preterm births.[311, 313] Spontaneous preterm births are also motivated by the same predictors of medically indicated preterm births, including placenta abruption,[314] preeclampsia,[315] and restricted fetal growth.[316, 317] Therefore, grouping preterm births would offer an increase in statistical power since etiologies are shared.

Multiple analyses were performed to test the relation between prenatal nitrosatable drug use and preterm and moderately preterm births. In the study analyses, 95% CI were determined for 112 associations between nitrosatable drug use and the study outcome (56 for preterm births and 56 for moderately preterm births). Six statistically significant associations would be expected by chance alone. However, a total of 48 were observed (19 for preterm births and 29 for moderately preterm births).

In conclusion, findings from the present study suggest that prenatal exposure to nitrosatable drugs during the second and third trimester of pregnancy, especially secondary amines, might increase the risk of having a preterm delivery. To our knowledge, this is the first study to examine the relation between prenatal exposure to nitrosatable drugs, as well as various functional groups (amides, secondary and tertiary amines), and preterm births. As *N*-

nitroso compounds are formed when nitrosatable amines or amides react with nitrosating agents like nitrite, further research is needed to examine prenatal exposure to nitrosatable drugs in conjunction with dietary nitrite intake in relation to preterm births.

# CHAPTER III

# DIETARY NITRITES, NITROSATABLE DRUGS, AND PRETERM BIRTHS

## Overview

Nitrosatable drugs react with nitrite in the stomach to form *N*-nitroso compounds, observed to result in adverse pregnancy outcomes in animal models. An increased risk of preterm births has been detected with prenatal exposure to medications classified as nitrosatable.

Using data from mothers (controls) of babies without major birth defects from the National Birth Defects Prevention Study, dietary intake of nitrites was examined in relation to preterm births among 496 case-mothers of preterm infants and 5398 control-mothers with full term deliveries between 1997 and 2005. Exposure to nitrosatable drugs in conjunction with nitrite intake was also investigated. Dietary nitrite levels were estimated from a food frequency questionnaire.

A reduced risk was detected with high levels of plant nitrites (adjusted hazard ratio (aHR) 0.72, [95% confidence interval (CI) 0.53, 0.97]). Drugs classified as secondary amines in conjunction with high levels of nitrite were associated with preterm births, having an increased risk with first (aHR 1.84, [95% CI 1.14, 2.98]), second (aHR 1.89, [95% CI 1.17, 3.07]), and third (aHR

2.00, [95% CI 1.22, 3.29]) trimester exposure. HRs for tertiary amine use during the third trimester from the lowest tertile of nitrite intake to the highest were 0.67 [95% CI 0.35, 1.31] 1.25 [95% CI 0.71, 2.19], and 2.02 [95% CI 1.17, 3.49].

Prenatal exposure to nitrosatable drugs, particularly secondary and tertiary amines, in conjunction with higher levels of dietary nitrite (including animal, plant, and total) may increase risk of preterm births.

**Background**

One of the most important predictors of an infant's health and survival is gestational age. This measure provides a degree of prematurity as infants born before 37 weeks are considered preterm. These infants are at increased risk of gastrointestinal and respiratory complications and neurodevelopmental impairments.[2] According to the National Vital Statistics Report for 2006, preterm infants were 14.7 times more likely to die during the first year of life compared to full term infants.[1] Although survival rates for preterm infants have improved as a result of assisted ventilation, antenatal corticosteroid usage, and intensive care practices, preterm births have increased by 31% in the U.S. from 1981 to 2003,[4] though this rise may be due to changes in obstetric practice.[5]

The causes and mechanisms of preterm delivery are multifactorial and are hypothesized to be initiated by numerous mechanisms, including infection, inflammation, uterine overdistension, and stress.[2] These risk factors are believed to interact with one another, resulting in a transition from uterine

quiescence to preterm delivery. Obstetric precursors leading to preterm delivery include: inducing labor, spontaneous preterm labor with intact membranes, and preterm premature rupture of the membranes (PPROM).[2] PPROM is defined as "rupture of the chorioamniotic membranes before the onset of labor prior to 37 weeks of gestation"[151] and accounts for one third of all preterm deliveries.[152, 153] Higher levels of nitric oxide have been observed in higher levels in the blood and urine of women with preterm labor and PPROM.[8] While this compound is necessary for implantation, embryo development, and vascular tone in the placenta,[154] high levels can lead to cell cycle arrest, apoptosis, and senescence.[155] As a reactive oxygen species (ROS), nitric oxide has been hypothesized to damage the collagen in the chorioamnion which would result in PPROM.[9] ROS are unstable molecules that inflict tissue damage in its attempts to reach a stable state by abstracting an electron from nearby molecules to pair with the single electron in its outer orbit. Numerous studies have observed collagen in several tissues to be the primary target for ROS damage.[9] As a biologically active membrane, the chorioamnion's collagenolytic enzymes are vulnerable to ROS, such as nitric oxide, stimulation.[156]

Nitric oxide is naturally produced by the body; however, a portion is contributed by dietary consumption of nitrates and nitrites as five percent of dietary nitrate is converted to nitrite and further converted to nitric oxide.[157] The major source of exogenous exposure to nitrates and nitrites is through food consumption, with vegetables contributing the most to nitrate levels and cured

meats the most to nitrite.[7] Nitrate intake also contributes to total nitrite levels as approximately five percent of nitrate is endogenously converted to nitrite in the saliva and stomach.[158]

Dietary intake of nitrates and nitrites in conjunction with nitrosatable compounds may pose a risk for preterm births. Previous studies regarding nitrate and nitrite exposure in relation to preterm births have only focused on exposure levels from drinking water.[263-265] Super *et al*.[265] conducted a study in a rural region of southwest Africa and found no association between the incidence of preterm births and residing in an area with high levels of nitrates (>89 mg/L nitrate as nitrate). Conversely, in a population-based case-control study conducted in Prince Edward Island, Canada, a significant dose-response relationship was observed between nitrate levels in drinking water and prematurity.[263] An odds ratio of 1.83 [95% Confidence Interval (CI) 1.25, 2.68] was detected for prematurity with median nitrate levels as low as 13.7 mg/L nitrate as nitrate, a level which is below the current U.S. maximum contaminant level set for drinking water in public water supplies (45 mg/L nitrate as nitrate). Joyce *et al*.[264] also noted an increase in the prevalence of PPROM with moderate (0.553-1.55 mg/L nitrate as nitrate) (adjusted odds ratio (aOR) 1.23, [95% CI 1.03, 1.52]) and high (>1.55 mg/L nitrate as nitrate) water nitrate levels (aOR 1.47, [95% CI 1.20, 1.79]).

*N*-nitroso compounds are formed endogenously when nitrosatable amines or amides and nitrosating agents, such as nitrite, react in an acidic

environment like the stomach.[11] Certain medications, which are classified as tertiary amines, secondary amines, or amides, are sources of nitrosatable compounds. *N*-nitroso compounds have been observed to result in adverse pregnancy outcomes in mice, such as reduced fetal weight[12] and birth defects;[13, 14] its effects on gestational age are not known as previous studies did not examine this outcome.

Although no known study has examined the relation between nitrosatable drugs and preterm births, a number of studies have investigated various drugs which have been classified as nitrosatable as outlined by Brambilla and Martelli.[160] In a recent study of National Birth Defects Prevent Study (NBDPS) control women, drugs classified as nitrosatable were taken by 24% of the mothers during the first trimester of pregnancy.[10] Several medications within the sub-categories of nitrosatable drugs have been indicated as possible risk factors of preterm births. For secondary amines, several studies have observed a positive association with prenatal use of beta blockers,[177-179] anti-depressants,[184, 186, 188-191] thiazide diuretics,[215] and migraine medications[203, 205, 206] in relation to preterm births. Tertiary amine drugs, such as antiepileptics,[219, 220] antihypertensives,[229] calcium channel blockers,[230] nicotine replacement,[236, 241] and opioids[242-245] have also been implicated. In addition, prenatal use of benzodiazepine[250, 251] and sulfamethoxazole,[262] medications classified as amides, were associated with an increased risk of preterm births. Although evidence indicates a significant association for several of the nitrosatable drugs,

many studies have reported conflicting results.[180-182, 192-195, 217, 218, 221-223, 231, 238, 246] In the present study, we examined 1) the relation between maternal dietary intake of nitrites (animal, plant, and total) and preterm births; and 2) the relation between prenatal exposure to nitrosatable drugs (any nitrosatable drugs, and by their molecular structure, such as secondary amines, tertiary amines, and amides) in conjunction with dietary intake of nitrites and preterm births.

## Methods

### *Study Population*

The NBDPS, previously described by Yoon *et al*.,[283] is an ongoing population-based, case-control study of major structural birth defects in the United States. Since its inception in 1997, ten sites have participated, including: Arkansas, California, Georgia, Iowa, Massachusetts, New York, and Texas (from 1998 to present); New Jersey (from 1998 to 2002); and North Carolina and Utah (from 2003 to present). Case-infants within the NBDPS are identified from live births (all centers), stillbirths (all centers except New Jersey and New York from 1997 to 1999), and elective pregnancy terminations (all centers except Massachusetts, New Jersey, and New York before 2000) from birth defect surveillance programs. The present study utilizes NBDPS data from control-mothers of babies without major birth defects to examine the relation between prenatal exposure to nitrosatable drugs in conjunction with dietary intake of nitrites and preterm births.

NBDPS control-infants were live born without major birth defects and were delivered in the same time frame and study area as the case-infants with major birth defects. They were randomly sampled from birth certificates (Arkansas and Georgia, for estimated delivery dates (EDDs) after 2000; Iowa; Massachusetts; New Jersey; North Carolina; and Utah) or hospital records (Arkansas, for EDDs before 2001; California; Georgia, for EDDs before 2001; New York; and Texas).[282] Control-infants were excluded if they were not liveborn, had a major birth defect, or were born outside the study area. Prospective study participants were further excluded if the infant was either adopted or in foster care or if the mother possessed at least one of the following characteristics: did not speak English or Spanish, participated in the NBDPS previously, was incarcerated, was a donor or a surrogate parent, was unable to answer questions, or was deceased.

For our study, we focused on NBDPS control-infants with EDDs between October 1, 1997 and December 31, 2005. Analyses were further restricted to singleton births as multiple births have been observed to be major risk factor for preterm deliveries. Case-infants in the present study were defined as preterm births, infants who were born less than 37 weeks gestation. Gestational age at delivery was taken from study participants' medical records or birth certificates. Gestational age was calculated using the EDD reported during the interview if the previously mentioned documents could not provide this information. If gestational age still could not be obtained through these methods, it was

calculated using (in order of descending preference): ultrasound <14 weeks, last menstrual period, ultrasound >14 weeks, or standard neonatal exam. Infants with a gestational age between 33 and 36 weeks were further classified as moderately preterm. Control-infants were those with 37-41 weeks of gestation. Small for gestational age infants were excluded since *N*-nitroso compounds have been reported to affect fetal weight.[12] The institutional review boards in each state and the Centers for Disease Control and Prevention approved the NBDPS study protocol, and the institutional review board of Texas A&M University also approved this project on dietary nitrite, nitrosatable drugs, and preterm births.

*Data Collection*

Interviews were conducted in either English or Spanish by trained female interviewers using a computer-assisted telephone interview after informed consent was received.[283] Interviews were conducted 6 weeks to 24 months after the EDDs (or delivery of a full-term infant) and targeted for completion within 6 months of the EDD. The interview contained detailed questions pertaining to maternal health during the index pregnancy (including medication usage), nutrition (food and beverage consumption), infections, and behavioral factors.

*Classification of Nitrosatable Drugs*

Information about prescription and non-prescription drug usage, including medication name, frequency of use, and corresponding dates of usage were collected during the interview from three months prior to the estimated date of conception to the end of pregnancy. The Slone Epidemiology Center Drug Dictionary system was used to link reported medications to their active ingredient.[287] Classification methods employed to categorize drugs in terms of their nitrosatability, functional groups, and indications have been described in detail elsewhere.[10] Briefly, all reported orally administered prescription and non-prescription medications and their active ingredients were identified and cross-referenced against comprehensive nitrosatable medicinal compounds lists.[160, 288] These drugs were further categorized based on their chemical structure, whether an amine (secondary or tertiary) or amide functional group was present. Medline and internet sources were used to evaluate the presence of amine or amide functional groups of all remaining active ingredients. Lastly, each component was classified by primary indication or therapeutic use and pharmacologic class. This study focuses on drugs reported to have been taken during pregnancy, concentrating on periods of exposure by trimester.

*Estimation of Dietary Nitrates and Nitrites*

Women were questioned about their average food consumption throughout the year before conception using a 58-item food frequency

questionnaire (FFQ) that was adapted from the short Willett FFQ.[318, 319] The Willett FFQ has been validated and reproduced in other studies and has been indicated to provide useful information about nutrient intake in women during pregnancy.[284] Additional questions on consumption of breakfast cereals from three months preconception to the end of pregnancy were included. Questions on region-specific foods, such as avocados, tortillas, and refried beans, were also incorporated to address the diverse diet of the study population within the NBDPS. Nutrient calculations were based on the USDA National Nutrient Database for Standard Reference 19. Daily intake of each food component was calculated based on frequency of use and serving size. Since estimates of nitrites and nitrates were not available in the USDA National Nutrient Database, values were estimated based on published literature for each food item or group by Griesenbeck *et al*.[285] Briefly, estimation of dietary intake of nitrates and nitrites in milligrams per day were assigned based on the following procedures: 1) for each food item, weighted means (mg/100 g) were calculated based on relevant literature; 2) the number of serving sizes were multiplied by the weighted means; 3) values of nitrites and nitrates were multiplied by the number of monthly servings; and 4) levels of nitrates and nitrites were summed across all food items and divided by 30. Total dietary nitrite was calculated based on the following formula: total nitrite = dietary nitrite intake + (0.05 × dietary nitrate intake).[286] Nitrites, including animal nitrites, plant nitrites, and total nitrites, were further categorized into tertiles based on the control women's distribution who

reported a total caloric intake between 500-5000 kcal per day. These limits are consistent with previous dietary studies[291] and with what has been used with the NBDPS population.[293, 320] Complete data for any nitrosatable drug use stratified by nitrite intake were available for 471 (95%), 388 (94.9%), and 5132 (95.1%) mothers of preterm, moderately preterm, and full term infants, respectively.

*Covariates*

Covariate selection was based on factors associated with preterm births in previous studies and maternal factors associated with nitrosatable drug exposure.[10] Potential confounders assessed included maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, other), educational level (<12 years, 12 years, 13-15 years, >15 years), age (<18, 18-19, 20-24, 25-29, 30-34, ≥35 years), and smoking status (yes/no); body mass index (BMI) based on self-reported height and weight ($kg/m^2$), categorized according to NIH guidelines (underweight, normal, overweight, and obese); study site; infant gender (male/female); parity (nulliparous, primiparous, and multiparous); pre-pregnancy diabetes (yes/no); gestational diabetes (yes/no); and pre-pregnancy hypertension (yes/no). Nonsignificant covariates as well as those that did not change the hazard ratio by 10% or more were eliminated from the final model using forward selection.

*Statistical Analysis*

Descriptive analyses were performed to examine the distribution of several covariates among case- and control-mothers. For the main analyses, time-to-event methods were employed since preterm birth is a time-based outcome that depends on gestational age. Cox proportional hazards model was used to estimate hazard ratios (HR) and corresponding 95% confidence intervals (CI) for preterm and moderately preterm births in relation to dietary nitrites. The lowest tertile of each compound was used as the referent category in all analyses. Gestational age at birth, measured in weeks, was used as the underlying time variable. Each woman remained in the risk set of giving birth to a preterm infant until delivery or gestational age of 37 weeks, whichever occurred first. In other words, women with term or post-term deliveries were censored at 37 weeks. Maternal race/ethnicity, educational level, and age; study center; pre-pregnancy diabetes; pre-pregnancy hypertension; and caloric intake were included in the regression models as possible confounders. Analyses were restricted to singleton pregnancies with complete information on all covariates included in the final model. Statistical tests were two-sided, and findings were considered statistically significant at the 5% level if the CI did not include 1.00. A hazard ratio above 1.00 represents an increased probability of preterm birth, corresponding to a shorter period of gestation. We assessed the fit of the final model using Cox-Snell residuals analysis, link test, and a global test based on

Schoenfeld residuals to assess violation of the proportional hazards assumption.[300]

Nitrosatable drug use by trimester of pregnancy (any, secondary amines, tertiary amines, and amides) was further stratified by tertiles of dietary nitrite (plant nitrite, animal nitrite, and total nitrite), and HRs and 95% CIs were estimated for preterm births for each stratum. Women who reported no nitrosatable drug use during pregnancy served as the referent group for these analyses. Additive and multiplicative interaction was assessed for the associations of preterm births with nitrosatable drugs by dietary intake of nitrites. To determine whether significant additive interaction was present, we relied on measures of relative excess risk due to interaction (RERI) and attributable proportion due to interaction (AP).[295] If the 95% confidence intervals of either or both measures excluded 0, additive interaction was considered present, implying that the risk of preterm births attributable to the two risk factors in combination is greater than the sum of risks associated with each risk factor separately. Multiplicative interaction was assessed with the inclusion of product terms of nitrosatable drug groups with dietary nitrite intake in the Cox proportional hazards models and was considered significant if the *P* value was less than 0.05. STATA version 12.0 was used for all analyses.

**Table 7.** Selected Maternal Characteristics of Preterm Cases and Controls in the National Birth Defects Prevention Study, 1997-2005

| Characteristics of Participants | Controls[a] (%) n=5398 | Preterm Cases[a] (%) n=496 |
|---|---|---|
| Race/ethnicity* | | |
| Non-Hispanic white | 3252 (60.2) | 277 (55.9) |
| Non-Hispanic black | 605 (11.2) | 74 (14.9) |
| Hispanic | 1180 (21.9) | 107 (21.6) |
| Asian/Pacific Islander | 155 (2.9) | 9 (1.8) |
| All others | 206 (3.8) | 29 (5.9) |
| Education (years) | | |
| <12 | 852 (15.8) | 91 (18.4) |
| 12 | 1288 (23.9) | 137 (27.6) |
| 13-15 | 1462 (27.1) | 124 (25.0) |
| >15 | 1723 (31.9) | 136 (27.4) |
| Missing | 73 (1.4) | 8 (1.6) |
| Age at delivery (years)* | | |
| <18 | 193 (3.6) | 24 (4.8) |
| 18-19 | 370 (6.9) | 40 (8.1) |
| 20-24 | 1223 (22.7) | 119 (24.0) |
| 25-29 | 1433 (26.6) | 142 (28.6) |
| 30-34 | 1459 (27.0) | 96 (19.4) |
| >34 | 720 (13.3) | 75 (15.1) |
| Study center* | | |
| Arkansas | 650 (12.0) | 88 (17.7) |
| California | 697 (12.9) | 57 (11.5) |
| Georgia | 597 (11.1) | 44 (8.9) |
| Iowa | 607 (11.2) | 56 (11.3) |
| Massachusetts | 672 (12.5) | 58 (11.7) |
| North Carolina | 321 (6.0) | 34 (6.9) |
| New Jersey | 449 (8.3) | 32 (6.5) |
| New York | 499 (9.2) | 33 (6.7) |
| Texas | 602 (11.2) | 68 (13.7) |
| Utah | 304 (5.6) | 26 (5.2) |
| Body mass index ($kg/m^2$) | | |
| <18.5 | 257 (4.8) | 29 (5.9) |
| 18.5–24.9 | 2904 (53.8) | 268 (54.0) |
| 25.0–29.9 | 1190 (22.1) | 99 (20.0) |
| >29.9 | 847 (15.7) | 86 (17.3) |
| Missing | 200 (3.7) | 14 (2.8) |
| Smoking | | |
| No | 4371 (81.0) | 382 (77.0) |
| Yes | 969 (18.0) | 107 (21.6) |
| Missing | 58 (1.1) | 7 (1.4) |
| Pre-pregnancy diabetes* | | |
| No | 5244 (97.2) | 475 (95.8) |
| Yes | 26 (0.5) | 10 (2.0) |
| Missing | 128 (2.4) | 11 (2.2) |
| Pre-pregnancy hypertension* | | |
| No | 4723 (87.5) | 393 (79.2) |
| Yes | 668 (12.4) | 102 (20.6) |
| Missing | 7 (0.1) | 1 (0.2) |
| Infant gender | | |
| Male | 2702 (50.1) | 243 (49.0) |
| Female | 2696 (49.9) | 253 (51.0) |

**Table 7** (continued)

| Characteristics of Participants | Controls[a] (%) n=5398 | Preterm Cases[a] (%) n=496 |
|---|---|---|
| Parity | | |
| Nulliparous | 2150 (39.8) | 207 (41.7) |
| Primiparous | 1816 (33.6) | 154 (31.1) |
| Multiparous | 1432 (26.5) | 135 (27.2) |

[a] NBDPS control women who gave birth to preterm infants (cases) and women who had full term infants without SGA (controls)

* Statistically significant differences in the distribution between cases and controls at $P < 0.05$.

## Results

A total of 496 eligible case-mothers who delivered a preterm infant (409 who delivered a moderately preterm infant) and 5398 control-mothers with an EDD from 1997 to 2005 participated in the NBDPS. The participation rate among NBDPS controls mothers was 66%. Time to interview was consistent between mothers with preterm and full term deliveries, with both having a 7.7 month median length of time from the EDD to the interview. Control-mothers were significantly more likely than case-mothers to be non-Hispanic white and somewhat older at time of delivery, and less likely to have pre-pregnancy hypertension, pre-pregnancy diabetes, and live in Arkansas and Texas (Table 7).

The results of the unadjusted and adjusted Cox proportional hazards models for preterm births in relation to dietary intake of nitrates and nitrites are shown in Table 8. No increased risk of preterm deliveries was noted among women with estimated nitrate levels in the second or third tertile compared to levels <31.62 mg/day. Compared to the lowest tertile of nitrite intake, neither of

**Table 8.** Estimated Dietary Intake of Nitrates and Nitrites and Preterm Births, National Birth Defects Prevention Study, 1997-2005

| | Cases | | Controls | | Unadjusted HR[a] | | Adjusted HR[a,b] | | |
|---|---|---|---|---|---|---|---|---|---|
| Dietary Contaminant (mg/day) | No. | % | No. | % | HR | 95% CI | aHR | 95% CI | *P*-value trend |
| Nitrate | | | | | | | | | |
| <31.62 | 168 | 36.2 | 1709 | 33.8 | 1.00 | Referent | 1.00 | Referent | 0.222 |
| 31.62-52.30 | 155 | 33.4 | 1689 | 33.4 | 0.94 | [0.75, 1.17] | 0.94 | [0.75, 1.19] | |
| >52.30 | 141 | 30.4 | 1664 | 32.9 | 0.87 | [0.69, 1.09] | 0.85 | [0.65, 1.10] | |
| Nitrite | | | | | | | | | |
| <1.29 | 159 | 34.3 | 1717 | 33.9 | 1.00 | Referent | 1.00 | Referent | 0.379 |
| 1.29-1.92 | 153 | 33.0 | 1702 | 33.6 | 0.98 | [0.78, 1.22] | 0.93 | [0.74, 1.17] | |
| >1.92 | 152 | 32.8 | 1649 | 32.5 | 0.99 | [0.80, 1.24] | 0.88 | [0.66, 1.74] | |
| Animal Nitrite | | | | | | | | | |
| <0.74 | 157 | 33.6 | 1719 | 33.8 | 1.00 | Referent | 1.00 | Referent | 0.562 |
| 0.74-1.21 | 156 | 33.3 | 1712 | 33.7 | 1.00 | [0.80, 1.24] | 0.96 | [0.77, 1.21] | |
| >1.21 | 155 | 33.1 | 1657 | 32.6 | 1.02 | [0.82, 1.27] | 0.93 | [0.72, 1.20] | |
| Plant Nitrite | | | | | | | | | |
| <0.46 | 170 | 36.6 | 1706 | 33.6 | 1.00 | Referent | 1.00 | Referent | **0.032** |
| 0.46-0.71 | 157 | 33.8 | 1707 | 33.6 | 0.92 | [0.74, 1.15] | 0.90 | [0.71, 1.13] | |
| >0.71 | 138 | 29.7 | 1672 | 32.9 | 0.83 | [0.67, 1.04] | **0.72** | [**0.53, 0.97**] | |
| Total Nitrite[c] | | | | | | | | | |
| <3.04 | 167 | 36.0 | 1706 | 33.7 | 1.00 | Referent | 1.00 | Referent | 0.347 |
| 3.04-4.57 | 147 | 31.7 | 1696 | 33.5 | 0.89 | [0.71, 1.11] | 0.88 | [0.69, 1.11] | |
| >4.57 | 150 | 32.3 | 1660 | 32.8 | 0.93 | [0.74, 1.16] | 0.88 | [0.67, 1.16] | |

Abbreviations: HR, hazard ratio; CI, confidence interval.
[a] Crude and adjusted hazard ratios include only cases and controls with complete information for dietary contaminants and covariates, and who had a daily caloric intake between 500-5000 kcal.
[b] Adjusted for caloric intake, study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.
[c] Total nitrites = daily dietary nitrite intake + 5% of daily nitrate intake.

the upper two were associated with an increased risk of preterm births. Though nitrite intake from animal sources yielded similar null findings, a protective effect was noted for the highest tertile of nitrite intake from plant sources (aHR 0.72, [95% CI 0.53, 0.97]) compared to the lowest tertile. A significant linear trend (*P* = 0.032) was also observed in the association between plant nitrite and preterm births. Linear trend was assessed by treating the three levels of plant nitrite intake as a continuous variable in the hazards model and testing the significance

**Table 9.** Estimated Dietary Intake of Nitrates and Nitrites and Moderately Preterm Births, National Birth Defects Prevention Study, 1997-2005

| Dietary Contaminant (mg/day) | Cases | | Controls | | Unadjusted HR[a] | | Adjusted HR[a,b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | HR | 95%CI | aHR | 95%CI | *P*-value trend |
| Nitrate | | | | | | | | | |
| <31.62 | 139 | 36.4 | 1709 | 33.8 | 1.00 | Referent | 1.00 | Referent | 0.353 |
| 31.62-52.30 | 125 | 32.7 | 1689 | 33.4 | 0.91 | [0.72, 1.16] | 0.92 | [0.71, 1.19] | |
| >52.30 | 118 | 30.9 | 1664 | 32.9 | 0.88 | [0.69, 1.12] | 0.87 | [0.65, 1.17] | |
| Nitrite | | | | | | | | | |
| <1.29 | 138 | 36.1 | 1717 | 33.9 | 1.00 | Referent | 1.00 | Referent | 0.206 |
| 1.29-1.92 | 119 | 31.2 | 1702 | 33.6 | 0.87 | [0.68, 1.12] | 0.84 | [0.65, 1.08] | |
| >1.92 | 125 | 32.7 | 1649 | 32.5 | 0.94 | [0.74, 1.20] | 0.83 | [0.60, 1.14] | |
| Animal Nitrite | | | | | | | | | |
| <0.74 | 131 | 34.1 | 1719 | 33.8 | 1.00 | Referent | 1.00 | Referent | 0.580 |
| 0.74-1.21 | 126 | 32.8 | 1712 | 33.7 | 0.96 | [0.75, 1.23] | 0.93 | [0.73, 1.20] | |
| >1.21 | 127 | 33.1 | 1657 | 32.6 | 1.00 | [0.78, 1.28] | 0.93 | [0.70, 1.23] | |
| Plant Nitrite | | | | | | | | | |
| <0.46 | 141 | 36.8 | 1706 | 33.6 | 1.00 | Referent | 1.00 | Referent | **0.025** |
| 0.46-0.71 | 130 | 33.9 | 1707 | 33.6 | 0.92 | [0.73, 1.17] | 0.88 | [0.69, 1.13] | |
| >0.71 | 112 | 29.2 | 1672 | 32.9 | 0.81 | [0.64, 1.04] | **0.68** | [**0.49, 0.94**] | |
| Total Nitrite[c] | | | | | | | | | |
| <3.04 | 141 | 36.9 | 1706 | 33.7 | 1.00 | Referent | 1.00 | Referent | 0.322 |
| 3.04-4.57 | 117 | 30.6 | 1696 | 33.5 | 0.84 | [0.66, 1.07] | 0.83 | [0.64, 1.07] | |
| >4.57 | 124 | 32.5 | 1660 | 32.8 | 0.91 | [0.71, 1.15] | 0.87 | [0.64, 1.18] | |

Abbreviations: HR, hazard ratio; CI, confidence interval.
[a] Crude and adjusted hazard ratios include only cases and controls with complete information for dietary contaminants and covariates, and who had a daily caloric intake between 500-5000 kcal.
[b] Adjusted for caloric intake, study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.
[c] Total nitrites = daily dietary nitrite intake + 5% of daily nitrate intake.

of linearity with the *z*-test in STATA (equivalent to the Wald chi-square test). No association was detected with total nitrite levels (sum of dietary nitrite and 5% dietary nitrate intake) and preterm births. Dietary intake of nitrites was further examined in relation to moderately preterm births (Table 9). Analyses regarding moderately preterm births and dietary nitrate and nitrite resulted in similar findings, including a reduced risk among women with high levels of plant nitrite intake (aHR 0.68, [95% CI 0.49, 0.94]) and a significant linear trend (*P* = 0.025).

Nitrosatable drug use during the first, second, and third trimester of pregnancy was most strongly associated with preterm births among mothers with the highest estimated intake of nitrites (aHR 1.61, [95% CI 1.08, 2.41]; aHR 1.85, [95% CI 1.25, 2.73]; and aHR 1.89, [95% CI 1.26, 2.85], respectively) (Table 10). Of the nitrosatable drug groups, secondary amine usage in conjunction with high levels of nitrite intake was significantly associated with having a preterm delivery. An increased risk was observed with first (aHR 1.84, [95% CI 1.14, 2.98]), second (aHR 1.89, [95% CI 1.17, 3.07]), and third (aHR 2.00, [95% CI 1.22, 3.29]) trimester exposure. Additive (AP 0.70, [95% CI 0.29, 1.11]) and multiplicative interaction ($P$ = 0.011) was noted between secondary amine usage during the first trimester of pregnancy and nitrite intake. Hazard ratios for preterm births in relation to tertiary amine drug use during the last trimester of pregnancy for the first, second, and third tertiles of nitrite intake were 0.67 [95% CI 0.35, 1.31], 1.25 [95% CI 0.71, 2.19], and 2.02 [95% CI 1.17, 3.49], respectively; significant additive (AP 0.75, [95% CI 0.29, 1.21]) and multiplicative interaction ($P$ = 0.018) was observed between exposure to tertiary amines during the third trimester and nitrite intake. With regard to dietary nitrite intake from animal sources, a similar pattern of increasing risk was found with secondary and tertiary amine usage during every trimester of pregnancy. In particular, risk was higher among women with secondary amine use during the first trimester who also had high levels of animal nitrite intake (aHR 1.87, [1.20, 2.92]) compared to the lower two tertiles (aHR 0.68, [95% CI 0.37, 1.23] and

**Table 10.** Exposure to Nitrosatable Drugs by Trimester of Pregnancy and Preterm Births Stratified by Estimated Dietary Intake of Nitrites, National Birth Defects Prevention Study, 1997-2005

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| Nitrite | | | | | | | | | | |
| P1P3 | < 1.29 | No nitrosatable drug exposure | 97 | 74.1 | 1050 | 71.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 34 | 25.9 | 417 | 28.4 | 0.88 | [0.60, 1.30] | 0.88[d,e] | [0.59, 1.32] |
| | | Secondary amines | 16 | 14.2 | 220 | 17.3 | 0.79 | [0.47, 1.34] | 0.76[d,e] | [0.44, 1.32] |
| | | Tertiary amines | 17 | 14.9 | 222 | 17.5 | 0.83 | [0.50, 1.40] | 0.81 | [0.47, 1.38] |
| | | Amides | 16 | 14.2 | 123 | 10.5 | 1.36 | [0.80, 2.31] | 1.45 | [0.84, 2.49] |
| | 1.29-1.92 | No nitrosatable drug exposure | 87 | 65.4 | 1005 | 70.7 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 46 | 34.6 | 416 | 29.3 | 1.27 | [0.89, 1.81] | 1.28[d,e] | [0.88, 1.86] |
| | | Secondary amines | 28 | 24.4 | 215 | 17.6 | 1.48 | [0.97, 2.27] | 1.49[d,e] | [0.95, 2.33] |
| | | Tertiary amines | 22 | 20.2 | 203 | 16.8 | 1.24 | [0.77, 1.97] | 1.20 | [0.73, 1.96] |
| | | Amides | 16 | 15.5 | 135 | 11.8 | 1.34 | [0.79, 2.29] | 1.37 | [0.79, 2.36] |
| | > 1.92 | No nitrosatable drug exposure | 80 | 64.5 | 1070 | 74.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 44 | 35.5 | 360 | 25.2 | **1.61** | [**1.11, 2.32**] | **1.61**[d,e] | [**1.08, 2.41**] |
| | | Secondary amines | 27 | 25.2 | 194 | 15.4 | **1.83** | [**1.18, 2.82**] | **1.84**[d,e] | [**1.14, 2.98**] |
| | | Tertiary amines | 21 | 20.8 | 207 | 16.2 | 1.35 | [0.84, 2.19] | 1.27 | [0.75, 2.16] |
| | | Amides | 14 | 14.9 | 123 | 10.3 | 1.50 | [0.85, 2.65] | 1.61 | [0.88, 2.93] |
| P4P6 | < 1.29 | No nitrosatable drug exposure | 97 | 71.9 | 1050 | 73.9 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 38 | 28.1 | 370 | 26.1 | 1.10 | [0.75, 1.60] | 1.17 | [0.79, 1.72] |
| | | Secondary amines | 23 | 19.2 | 221 | 17.4 | 1.12 | [0.71, 1.76] | 1.19 | [0.74, 1.90] |
| | | Tertiary amines | 15 | 13.4 | 171 | 14.0 | 0.95 | [0.55, 1.63] | 0.95[d] | [0.54, 1.67] |
| | | Amides | 15 | 13.4 | 107 | 9.3 | 1.45 | [0.84, 2.49] | 1.60 | [0.92, 2.81] |
| | 1.29-1.92 | No nitrosatable drug exposure | 87 | 66.4 | 1005 | 72.3 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 44 | 33.6 | 386 | 27.7 | 1.31 | [0.91, 1.88] | 1.32 | [0.90, 1.92] |
| | | Secondary amines | 24 | 21.6 | 229 | 18.6 | 1.21 | [0.77, 1.90] | 1.25 | [0.78, 1.99] |
| | | Tertiary amines | 23 | 20.9 | 175 | 14.8 | 1.50 | [0.95, 2.37] | 1.41[d] | [0.87, 2.27] |
| | | Amides | 12 | 12.1 | 116 | 10.4 | 1.19 | [0.65, 2.17] | 1.25 | [0.67, 2.32] |

**Table 10** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | > 1.92 | No nitrosatable drug exposure | 80 | 64.0 | 1070 | 76.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 45 | 36.0 | 335 | 23.8 | **1.74** | [**1.21, 2.50**] | **1.85** | [**1.25, 2.73**] |
| | | Secondary amines | 25 | 23.8 | 195 | 15.4 | **1.67** | [**1.07, 2.62**] | **1.89** | [**1.17, 3.07**] |
| | | Tertiary amines | 23 | 22.3 | 160 | 13.0 | **1.83** | [**1.15, 2.91**] | **2.06**[d] | [**1.25, 3.40**] |
| | | Amides | 15 | 15.8 | 111 | 9.4 | **1.76** | [**1.01, 3.05**] | 1.58 | [0.89, 2.83] |
| P7P9 | < 1.29 | No nitrosatable drug exposure | 97 | 74.1 | 1050 | 75.1 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 34 | 25.9 | 349 | 24.9 | 1.04 | [0.70, 1.54] | 1.05 | [0.70, 1.56] |
| | | Secondary amines | 23 | 19.2 | 207 | 16.5 | 1.18 | [0.75, 1.86] | 1.22 | [0.76, 1.94] |
| | | Tertiary amines | 10 | 9.4 | 162 | 13.4 | 0.68 | [0.35, 1.29] | 0.67[d,e] | [0.35, 1.31] |
| | | Amides | 10 | 9.4 | 90 | 7.9 | 1.16 | [0.61, 2.23] | 1.19 | [0.62, 2.32] |
| | 1.29-1.92 | No nitrosatable drug exposure | 87 | 73.1 | 1005 | 74.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 32 | 26.9 | 343 | 25.4 | 1.08 | [0.72, 1.63] | 1.06 | [0.69, 1.61] |
| | | Secondary amines | 19 | 17.9 | 208 | 17.2 | 1.06 | [0.64, 1.74] | 1.07 | [0.64, 1.78] |
| | | Tertiary amines | 16 | 15.5 | 141 | 12.3 | 1.31 | [0.77, 2.23] | 1.25[d,e] | [0.71, 2.19] |
| | | Amides | 10 | 10.3 | 94 | 8.6 | 1.23 | [0.64, 2.36] | 1.16 | [0.59, 2.29] |
| | > 1.92 | No nitrosatable drug exposure | 80 | 66.1 | 1070 | 78.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 41 | 33.9 | 288 | 21.2 | **1.82** | [**1.25, 2.65**] | **1.89** | [**1.26, 2.85**] |
| | | Secondary amines | 24 | 23.1 | 169 | 13.6 | **1.83** | [**1.16, 2.88**] | **2.00** | [**1.22, 3.29**] |
| | | Tertiary amines | 19 | 19.2 | 123 | 10.3 | **1.96** | [**1.19, 3.23**] | **2.02**[d,e] | [**1.17, 3.49**] |
| | | Amides | 12 | 13.0 | 95 | 8.2 | 1.63 | [0.89, 2.99] | 1.68 | [0.89, 3.16] |
| Animal Nitrite | | | | | | | | | | |
| P1P3 | < 0.74 | No nitrosatable drug exposure | 96 | 74.4 | 1099 | 74.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 33 | 25.6 | 382 | 25.8 | 0.98 | [0.66, 1.46] | 0.96[d] | [0.64, 1.44] |
| | | Secondary amines | 13 | 11.9 | 204 | 15.7 | 0.74 | [0.41, 1.31] | 0.68[d,e] | [0.37, 1.23] |
| | | Tertiary amines | 13 | 11.9 | 196 | 15.1 | 0.76 | [0.43, 1.36] | 0.73 | [0.40, 1.33] |
| | | Amides | 19 | 16.5 | 109 | 9.0 | **1.88** | [**1.15, 3.07**] | **1.82** | [**1.09, 3.03**] |

**Table 10** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | 0.74-1.21 | No nitrosatable drug exposure | 90 | 68.7 | 1026 | 71.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 41 | 31.3 | 415 | 28.8 | 1.12 | [0.78, 1.62] | 1.05[d] | [0.71, 1.54] |
| | | Secondary amines | 25 | 21.7 | 219 | 17.6 | 1.29 | [0.83, 2.01] | 1.23[d,e] | [0.77, 1.95] |
| | | Tertiary amines | 23 | 20.4 | 211 | 17.1 | 1.23 | [0.78, 1.94] | 1.11 | [0.69, 1.80] |
| | | Amides | 12 | 11.8 | 134 | 11.6 | 1.02 | [0.56, 1.86] | 0.95 | [0.51, 1.76] |
| | > 1.21 | No nitrosatable drug exposure | 81 | 61.8 | 1015 | 71.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 50 | 38.2 | 398 | 28.2 | **1.55** | [**1.09, 2.20**] | **1.54**[d] | [**1.05, 2.25**] |
| | | Secondary amines | 33 | 29.0 | 208 | 17.0 | **1.94** | [**1.29, 2.90**] | **1.87**[d,e] | [**1.20, 2.92**] |
| | | Tertiary amines | 24 | 22.9 | 225 | 18.2 | 1.34 | [0.85, 2.11] | 1.22 | [0.74, 2.01] |
| | | Amides | 15 | 15.6 | 139 | 12.1 | 1.34 | [0.77, 2.33] | 1.35 | [0.76, 2.40] |
| P4P6 | < 0.74 | No nitrosatable drug exposure | 96 | 71.6 | 1099 | 75.5 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 38 | 28.4 | 356 | 24.5 | 1.20 | [0.82, 1.75] | 1.27 | [0.86, 1.87] |
| | | Secondary amines | 19 | 16.5 | 215 | 16.4 | 1.01 | [0.61, 1.64] | 1.06 | [0.64, 1.76] |
| | | Tertiary amines | 16 | 14.3 | 161 | 12.8 | 1.12 | [0.66, 1.91] | 1.14 | [0.66, 1.97] |
| | | Amides | 18 | 15.8 | 102 | 8.5 | **1.89** | [**1.14, 3.12**] | **2.01** | [**1.20, 3.38**] |
| | 0.74-1.21 | No nitrosatable drug exposure | 90 | 66.7 | 1026 | 73.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 45 | 33.3 | 364 | 26.2 | 1.39 | [0.97, 1.99] | 1.33 | [0.91, 1.93] |
| | | Secondary amines | 27 | 23.1 | 219 | 17.6 | 1.39 | [0.91, 2.14] | 1.37 | [0.88, 2.14] |
| | | Tertiary amines | 24 | 21.1 | 168 | 14.1 | **1.59** | [**1.01, 2.50**] | 1.43 | [0.90, 2.29] |
| | | Amides | 10 | 10.0 | 109 | 9.6 | 1.04 | [0.54, 2.00] | 0.94 | [0.48, 1.86] |
| | > 1.21 | No nitrosatable drug exposure | 81 | 64.8 | 1015 | 72.9 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 44 | 35.2 | 377 | 27.1 | 1.44 | [1.00, 2.07] | **1.54** | [**1.05, 2.27**] |
| | | Secondary amines | 26 | 24.3 | 214 | 17.4 | 1.50 | [0.96, 2.33] | **1.66** | [**1.04, 2.66**] |
| | | Tertiary amines | 21 | 20.6 | 181 | 15.1 | 1.42 | [0.88, 2.29] | 1.55 | [0.93, 2.60] |
| | | Amides | 14 | 14.7 | 125 | 11.0 | 1.39 | [0.79, 2.45] | 1.32 | [0.73, 2.40] |

**Table 10** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| P7P9 | < 0.74 | No nitrosatable drug exposure | 96 | 75.6 | 1099 | 77.0 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 31 | 24.4 | 329 | 23.0 | 1.06 | [0.71, 1.59] | 1.06 | [0.70, 1.61] |
| | | Secondary amines | 19 | 16.5 | 198 | 15.3 | 1.08 | [0.66, 1.77] | 1.11 | [0.67, 1.84] |
| | | Tertiary amines | 11 | 10.3 | 147 | 11.8 | 0.86 | [0.46, 1.61] | 0.85 | [0.45, 1.61] |
| | | Amides | 10 | 9.4 | 88 | 7.4 | 1.25 | [0.65, 2.40] | 1.26 | [0.65, 2.44] |
| | 0.74-1.21 | No nitrosatable drug exposure | 90 | 71.4 | 1026 | 75.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 36 | 28.6 | 339 | 24.8 | 1.21 | [0.82, 1.77] | 1.12 | [0.75, 1.69] |
| | | Secondary amines | 23 | 20.4 | 206 | 16.7 | 1.26 | [0.80, 2.00] | 1.25 | [0.77, 2.01] |
| | | Tertiary amines | 16 | 15.1 | 136 | 11.7 | 1.33 | [0.78, 2.26] | 1.21 | [0.69, 2.11] |
| | | Amides | 11 | 10.9 | 89 | 8.0 | 1.39 | [0.74, 2.60] | 1.17 | [0.61, 2.24] |
| | > 1.21 | No nitrosatable drug exposure | 81 | 66.4 | 1015 | 76.4 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 41 | 33.6 | 313 | 23.6 | **1.59** | [**1.09, 2.31**] | **1.70** | [**1.14, 2.55**] |
| | | Secondary amines | 25 | 23.6 | 180 | 15.1 | **1.68** | [**1.08, 2.64**] | **1.89** | [**1.17, 3.06**] |
| | | Tertiary amines | 18 | 18.2 | 143 | 12.4 | 1.53 | [0.92, 2.54] | 1.58 | [0.91, 2.74] |
| | | Amides | 11 | 12.0 | 102 | 9.1 | 1.32 | [0.70, 2.48] | 1.46 | [0.76, 2.81] |
| Plant Nitrite | | | | | | | | | | |
| P1P3 | < 0.46 | No nitrosatable drug exposure | 98 | 69.5 | 1020 | 70.4 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 43 | 30.5 | 428 | 29.6 | 1.04 | [0.73, 1.49] | 1.02 | [0.71, 1.48] |
| | | Secondary amines | 24 | 19.7 | 221 | 17.8 | 1.11 | [0.71, 1.74] | 1.09 | [0.69, 1.73] |
| | | Tertiary amines | 25 | 20.3 | 215 | 17.4 | 1.20 | [0.77, 1.86] | 1.14 | [0.72, 1.79] |
| | | Amides | 16 | 14.0 | 144 | 12.4 | 1.13 | [0.67, 1.93] | 1.12 | [0.65, 1.92] |
| | 0.46-0.71 | No nitrosatable drug exposure | 86 | 64.7 | 963 | 67.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 47 | 35.3 | 457 | 32.2 | 1.14 | [0.80, 1.63] | 1.17 | [0.81, 1.70] |
| | | Secondary amines | 27 | 23.9 | 249 | 20.5 | 1.21 | [0.78, 1.86] | 1.22 | [0.78, 1.91] |
| | | Tertiary amines | 22 | 20.4 | 260 | 21.3 | 0.95 | [0.60, 1.52] | 0.93 | [0.57, 1.52] |
| | | Amides | 17 | 16.5 | 134 | 12.2 | 1.38 | [0.82, 2.32] | 1.47 | [0.86, 2.52] |

**Table 10** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | > 0.71 | No nitrosatable drug exposure | 80 | 69.6 | 1156 | 78.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 35 | 30.4 | 311 | 21.2 | **1.61** | [**1.08, 2.40**] | **1.65** | [**1.08, 2.53**] |
| | | Secondary amines | 21 | 20.8 | 161 | 12.2 | **1.86** | [**1.15, 3.02**] | **1.91** | [**1.14, 3.23**] |
| | | Tertiary amines | 13 | 14.0 | 158 | 12.0 | 1.19 | [0.66, 2.14] | 1.19 | [0.63, 2.22] |
| | | Amides | 13 | 14.0 | 104 | 8.3 | 1.78 | [0.99, 3.21] | 1.78 | [0.97, 3.29] |
| P4P6 | < 0.46 | No nitrosatable drug exposure | 98 | 68.5 | 1020 | 72.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 45 | 31.5 | 386 | 27.4 | 1.20 | [0.84, 1.71] | 1.30 | [0.90, 1.88] |
| | | Secondary amines | 27 | 21.6 | 228 | 18.3 | 1.23 | [0.80, 1.88] | 1.33 | [0.86, 2.07] |
| | | Tertiary amines | 23 | 19.0 | 169 | 14.2 | 1.38 | [0.88, 2.17] | 1.41 | [0.88, 2.26] |
| | | Amides | 14 | 12.5 | 119 | 10.5 | 1.19 | [0.68, 2.07] | 1.29 | [0.73, 2.29] |
| | 0.46-0.71 | No nitrosatable drug exposure | 86 | 66.2 | 963 | 69.5 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 44 | 33.8 | 423 | 30.5 | 1.15 | [0.80, 1.66] | 1.11 | [0.76, 1.62] |
| | | Secondary amines | 23 | 21.1 | 257 | 21.1 | 1.00 | [0.63, 1.59] | 1.04 | [0.64, 1.67] |
| | | Tertiary amines | 23 | 21.1 | 211 | 18.0 | 1.20 | [0.76, 1.91] | 1.11 | [0.68, 1.80] |
| | | Amides | 14 | 14.0 | 118 | 10.9 | 1.30 | [0.74, 2.29] | 1.22 | [0.68, 2.18] |
| | > 0.71 | No nitrosatable drug exposure | 80 | 67.2 | 1156 | 80.3 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 39 | 32.8 | 284 | 19.7 | **1.92** | [**1.31, 2.82**] | **1.90** | [**1.27, 2.85**] |
| | | Secondary amines | 23 | 22.3 | 162 | 12.3 | **1.98** | [**1.24, 3.14**] | **2.10** | [**1.28, 3.44**] |
| | | Tertiary amines | 16 | 16.7 | 126 | 9.8 | **1.79** | [**1.04, 3.05**] | **1.95** | [**1.10, 3.44**] |
| | | Amides | 14 | 14.9 | 98 | 7.8 | **2.02** | [**1.14, 3.56**] | 1.76 | [0.98, 3.17] |
| P7P9 | < 0.46 | No nitrosatable drug exposure | 98 | 72.1 | 1020 | 74.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 38 | 27.9 | 348 | 25.4 | 1.12 | [0.77, 1.63] | 1.16 | [0.79, 1.70] |
| | | Secondary amines | 24 | 19.7 | 222 | 17.9 | 1.11 | [0.71, 1.74] | 1.16 | [0.74, 1.84] |
| | | Tertiary amines | 17 | 14.8 | 150 | 12.8 | 1.16 | [0.70, 1.95] | 1.15 | [0.68, 1.94] |
| | | Amides | 11 | 10.1 | 88 | 7.9 | 1.25 | [0.67, 2.33] | 1.32 | [0.70, 2.49] |

**Table 10** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | 0.46-0.71 | No nitrosatable drug exposure | 86 | 70.5 | 963 | 71.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 36 | 29.5 | 389 | 28.8 | 1.03 | [0.70, 1.52] | 1.00 | [0.67, 1.50] |
| | | Secondary amines | 23 | 21.1 | 222 | 18.7 | 1.15 | [0.72, 1.82] | 1.16 | [0.72, 1.87] |
| | | Tertiary amines | 15 | 14.9 | 180 | 15.8 | 0.93 | [0.54, 1.62] | 0.91 | [0.51, 1.62] |
| | | Amides | 12 | 12.2 | 111 | 10.3 | 1.19 | [0.65, 2.17] | 1.08 | [0.57, 2.04] |
| | > 0.71 | No nitrosatable drug exposure | 80 | 70.8 | 1156 | 82.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 33 | 29.2 | 244 | 17.4 | **1.89** | [**1.26, 2.83**] | **1.86** | [**1.20, 2.87**] |
| | | Secondary amines | 19 | 19.2 | 141 | 10.9 | **1.88** | [**1.14, 3.11**] | **1.96** | [**1.15, 3.34**] |
| | | Tertiary amines | 13 | 14.0 | 96 | 7.7 | **1.88** | [**1.05, 3.38**] | **1.96** | [**1.05, 3.68**] |
| | | Amides | 9 | 10.1 | 80 | 6.5 | 1.61 | [0.81, 3.21] | 1.54 | [0.76, 3.14] |
| Total Nitrite[c] P1P3 | < 3.04 | No nitrosatable drug exposure | 101 | 71.6 | 1033 | 71.3 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 40 | 28.4 | 416 | 28.7 | 0.98 | [0.68, 1.42] | 1.03 | [0.71, 1.52] |
| | | Secondary amines | 22 | 17.9 | 217 | 17.4 | 1.03 | [0.65, 1.63] | 1.06[d] | [0.66, 1.71] |
| | | Tertiary amines | 23 | 18.6 | 218 | 17.4 | 1.08 | [0.68, 1.69] | 1.08 | [0.68, 1.74] |
| | | Amides | 15 | 12.9 | 120 | 10.4 | 1.25 | [0.73, 2.15] | 1.36 | [0.78, 2.38] |
| | 3.04-4.57 | No nitrosatable drug exposure | 82 | 67.2 | 1003 | 70.0 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 40 | 32.8 | 429 | 30.0 | 1.13 | [0.78, 1.66] | 1.11 | [0.75, 1.66] |
| | | Secondary amines | 23 | 21.9 | 230 | 18.7 | 1.21 | [0.76, 1.93] | 1.19[d] | [0.73, 1.93] |
| | | Tertiary amines | 18 | 18.0 | 226 | 18.4 | 0.98 | [0.59, 1.63] | 0.85 | [0.49, 1.47] |
| | | Amides | 11 | 11.8 | 148 | 12.9 | 0.91 | [0.48, 1.70] | 0.93 | [0.49, 1.77] |
| | > 4.57 | No nitrosatable drug exposure | 81 | 64.8 | 1086 | 75.9 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 44 | 35.2 | 345 | 24.1 | **1.67** | [**1.16, 2.41**] | **1.64** | [**1.10, 2.42**] |
| | | Secondary amines | 26 | 24.3 | 181 | 14.3 | **1.89** | [**1.21, 2.94**] | **1.85**[d] | [**1.15, 2.99**] |
| | | Tertiary amines | 19 | 19.0 | 186 | 14.6 | 1.36 | [0.82, 2.24] | 1.37 | [0.80, 2.34] |
| | | Amides | 20 | 19.8 | 113 | 9.4 | **2.26** | [**1.39, 3.69**] | **2.14** | [**1.27, 3.59**] |

**Table 10** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| P4P6 | < 3.04 | No nitrosatable drug exposure | 101 | 70.1 | 1033 | 73.7 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 43 | 29.9 | 368 | 26.3 | 1.18 | [0.83, 1.69] | 1.33 | [0.92, 1.93] |
| | | Secondary amines | 24 | 19.2 | 217 | 17.4 | 1.13 | [0.72, 1.76] | 1.27 | [0.80, 2.02] |
| | | Tertiary amines | 19 | 15.8 | 162 | 13.6 | 1.19 | [0.73, 1.94] | 1.22 | [0.73, 2.04] |
| | | Amides | 15 | 12.9 | 106 | 9.3 | 1.39 | [0.81, 2.39] | 1.52 | [0.87, 2.67] |
| | 3.04-4.57 | No nitrosatable drug exposure | 82 | 67.2 | 1003 | 71.9 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 40 | 32.8 | 392 | 28.1 | 1.24 | [0.85, 1.80] | 1.25 | [0.84, 1.85] |
| | | Secondary amines | 24 | 22.6 | 228 | 18.5 | 1.27 | [0.81, 2.01] | 1.32 | [0.83, 2.11] |
| | | Tertiary amines | 23 | 21.9 | 186 | 15.6 | 1.47 | [0.93, 2.34] | 1.46 | [0.90, 2.37] |
| | | Amides | 7 | 7.9 | 131 | 11.6 | 0.67 | [0.31, 1.44] | 0.69 | [0.31, 1.50] |
| | > 4.57 | No nitrosatable drug exposure | 81 | 64.8 | 1086 | 76.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 44 | 35.2 | 331 | 23.4 | **1.72** | [**1.19, 2.49**] | **1.85** | [**1.25, 2.73**] |
| | | Secondary amines | 24 | 22.9 | 200 | 15.6 | 1.58 | [1.00, 2.49] | **1.78** | [**1.09, 2.89**] |
| | | Tertiary amines | 19 | 19.0 | 158 | 12.7 | 1.57 | [0.95, 2.59] | **1.77** | [**1.04, 3.00**] |
| | | Amides | 20 | 19.8 | 97 | 8.2 | **2.58** | [**1.58, 4.21**] | **2.36** | [**1.41, 3.96**] |
| P7P9 | < 3.04 | No nitrosatable drug exposure | 101 | 71.6 | 1033 | 75.1 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 40 | 28.4 | 342 | 24.9 | 1.18 | [0.82, 1.70] | 1.27 | [0.87, 1.85] |
| | | Secondary amines | 24 | 19.2 | 205 | 16.6 | 1.18 | [0.76, 1.84] | 1.32 | [0.83, 2.08] |
| | | Tertiary amines | 17 | 14.4 | 148 | 12.5 | 1.17 | [0.70, 1.95] | 1.23 | [0.72, 2.09] |
| | | Amides | 13 | 11.4 | 95 | 8.4 | 1.35 | [0.76, 2.40] | 1.35 | [0.75, 2.43] |
| | 3.04-4.57 | No nitrosatable drug exposure | 82 | 75.2 | 1003 | 74.0 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 27 | 24.8 | 353 | 26.0 | 0.94 | [0.61, 1.45] | 0.87 | [0.55, 1.36] |
| | | Secondary amines | 17 | 17.2 | 215 | 17.7 | 0.97 | [0.57, 1.63] | 0.95 | [0.55, 1.62] |
| | | Tertiary amines | 12 | 12.8 | 163 | 14.0 | 0.90 | [0.49, 1.65] | 0.87 | [0.46, 1.63] |
| | | Amides | 7 | 7.9 | 92 | 8.4 | 0.92 | [0.43, 2.00] | 0.77 | [0.34, 1.71] |

**Table 10** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | > 4.57 | No nitrosatable drug exposure | 81 | 66.9 | 1086 | 79.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 40 | 33.1 | 285 | 20.8 | **1.81** | [**1.24, 2.64**] | **1.85** | [**1.23, 2.78**] |
| | | Secondary amines | 25 | 23.6 | 164 | 13.1 | **1.96** | [**1.25, 3.07**] | **2.06** | [**1.27, 3.34**] |
| | | Tertiary amines | 16 | 16.5 | 115 | 9.6 | **1.79** | [**1.05, 3.06**] | **1.79** | [**1.01, 3.17**] |
| | | Amides | 12 | 12.9 | 92 | 7.8 | 1.70 | [0.93, 3.12] | 1.68 | [0.90, 3.15] |

Abbreviations: HR, hazard ratio; CI, confidence interval; P1P3, first trimester; P4P6, second trimester; P7P9, third trimester.

[a] Crude and adjusted hazard ratios include only cases and controls with complete information for dietary contaminants and covariates, and who had a daily caloric intake between 500-5000 kcal

[b] Adjusted for caloric intake, study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.

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

[d] Significant additive interaction (95% CIs for RERI and/or AP exclude 0).

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

aHR 1.23, [95% CI 0.77, 1.95]). Significant additive (AP 0.68, [95% CI 0.38, 0.97]) and multiplicative interaction ($P$ = 0.005) was noted between first trimester secondary amine exposure and dietary animal nitrite. In contrast, higher risk of preterm births was observed within the lowest tertile of animal nitrites among women who reported taking amides during the second trimester of pregnancy (aHR 2.01, [95% CI 1.20, 3.38]).

Only secondary amine usage during early pregnancy had a similar pattern of increasing risk for dietary nitrite levels from plant sources. An increased risk of delivering a preterm infant was observed with secondary amine usage during the first trimester among women with high levels of dietary nitrite from plant sources (aHR 1.91, [95% CI 1.14, 3.23]) compared to the lower two tertiles (aHR 1.09, [95% CI 0.69, 1.73] and aHR 1.22, [95% CI 0.78, 1.91]). Tertiary amine use during the last two trimesters of pregnancy was most strongly associated with preterm births among mothers with the highest estimated intake of plant nitrites (aHR 1.95, [95% CI 1.10, 3.44] and aHR 1.96, [95% CI 1.05, 3.68]). An overall pattern was observed where exposures to nitrosatable drugs and plant nitrites during the last two trimesters resulted in a decrease in the HRs for the second tertile followed by an increase in the highest tertile. When examining total nitrite intake, higher HRs were observed for preterm births in relation to secondary amine usage during the first and second trimester among women with high intake (aHR 1.85, [95% CI 1.15, 2.99] and aHR 1.78, [95% CI 1.09, 2.89]). Additive interaction was detected between secondary amine usage

during the first trimester of pregnancy and total nitrite intake (AP 0.45, [95% CI 0.01, 0.89]). Although an increasing pattern was not found with tertiary amine exposure during the third trimester of pregnancy and total nitrite intake, the highest tertile of total nitrite intake was associated with increased risk of preterm births (aHR 1.79, [1.01, 3.17]).

Results for nitrosatable drug exposure by trimester of pregnancy and moderately preterm births stratified by estimated dietary intake of nitrites are shown in Table 11. The strongest associations between moderately preterm births and exposure to secondary and tertiary amines were among women with nitrite levels in the upper two tertiles during the second trimester of pregnancy. Adjusted HRs for moderately preterm births in relation to secondary amines during the second trimester from the lowest to the highest tertile of nitrite were 1.28 [95% CI 0.78, 2.10], 1.35 [95% CI 0.80, 2.29], and 1.87 [95% CI 1.09, 3.20]. A similar pattern was observed with tertiary amines during the same time frame, with the highest risk observed among women with dietary nitrite in the highest tertile (aHR 2.31, [95% CI 1.34, 3.99]) compared to the lower two tertiles (aHR 0.96, [95% CI 0.53, 1.76] and aHR 1.27, [95% CI 0.72, 2.25]). Stronger associations were observed during the last trimester, with adjusted HRs of 0.70 [95% CI 0.35, 1.41], 1.08 [95% CI 0.55, 2.12], and 2.21 [95% CI 1.21, 4.01] for increasing levels of nitrite intake among women reporting tertiary amine use (AP 0.77, [95% CI 0.26, 1.29] for additive interaction; $P = 0.023$ for multiplicative interaction). Focusing on dietary nitrite from animal sources and moderately

**Table 11.** Exposure to Nitrosatable Drugs by Trimester of Pregnancy and Moderately Preterm Births Stratified by Estimated Dietary Intake of Nitrites, National Birth Defects Prevention Study, 1997-2005

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| Nitrite | | | | | | | | | | |
| P1P3 | < 1.29 | No nitrosatable drug exposure | 82 | 73.2 | 1050 | 71.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 30 | 26.8 | 417 | 28.4 | 0.92 | [0.61, 1.40] | 0.94 | [0.61, 1.44] |
| | | Secondary amines | 15 | 15.5 | 220 | 17.3 | 0.87 | [0.50, 1.52] | 0.85 | [0.48, 1.51] |
| | | Tertiary amines | 13 | 13.7 | 222 | 17.5 | 0.75 | [0.42, 1.35] | 0.75 | [0.41, 1.37] |
| | | Amides | 15 | 15.5 | 123 | 10.5 | 1.51 | [0.87, 2.62] | 1.65 | [0.94, 2.92] |
| | 1.29-1.92 | No nitrosatable drug exposure | 65 | 62.5 | 1005 | 70.7 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 39 | 37.5 | 416 | 29.3 | 1.44 | [0.97, 2.14] | 1.44 | [0.95, 2.19] |
| | | Secondary amines | 24 | 27.0 | 215 | 17.6 | **1.71** | [**1.07, 2.73**] | **1.67** | [**1.02, 2.74**] |
| | | Tertiary amines | 19 | 22.6 | 203 | 16.8 | 1.43 | [0.86, 2.39] | 1.38 | [0.80, 2.38] |
| | | Amides | 14 | 17.7 | 135 | 11.8 | 1.58 | [0.89, 2.81] | 1.60 | [0.89, 2.91] |
| | > 1.92 | No nitrosatable drug exposure | 65 | 67.0 | 1070 | 74.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 32 | 33.0 | 360 | 25.2 | 1.44 | [0.95, 2.21] | 1.49 | [0.94, 2.35] |
| | | Secondary amines | 18 | 21.7 | 194 | 15.4 | 1.51 | [0.90, 2.54] | 1.51 | [0.85, 2.66] |
| | | Tertiary amines | 14 | 17.7 | 207 | 16.2 | 1.12 | [0.63, 1.99] | 1.10 | [0.59, 2.05] |
| | | Amides | 9 | 12.2 | 123 | 10.3 | 1.20 | [0.60, 2.40] | 1.36 | [0.65, 2.82] |
| P4P6 | < 1.29 | No nitrosatable drug exposure | 82 | 70.7 | 1050 | 73.9 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 34 | 29.3 | 370 | 26.1 | 1.16 | [0.78, 1.73] | 1.22 | [0.81, 1.84] |
| | | Secondary amines | 21 | 20.4 | 221 | 17.4 | 1.21 | [0.75, 1.95] | 1.28 | [0.78, 2.10] |
| | | Tertiary amines | 13 | 13.7 | 171 | 14.0 | 0.97 | [0.54, 1.74] | 0.96[d] | [0.53, 1.76] |
| | | Amides | 14 | 14.6 | 107 | 9.3 | 1.59 | [0.90, 2.81] | 1.79 | [1.00, 3.21] |
| | 1.29-1.92 | No nitrosatable drug exposure | 65 | 65.0 | 1005 | 72.3 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 35 | 35.0 | 386 | 27.7 | 1.40 | [0.93, 2.11] | 1.40 | [0.91, 2.15] |
| | | Secondary amines | 19 | 22.6 | 229 | 18.6 | 1.29 | [0.77, 2.14] | 1.35 | [0.80, 2.29] |
| | | Tertiary amines | 16 | 19.8 | 175 | 14.8 | 1.40 | [0.81, 2.43] | 1.27[d] | [0.72, 2.25] |
| | | Amides | 10 | 13.3 | 116 | 10.4 | 1.33 | [0.68, 2.59] | 1.37 | [0.69, 2.72] |

**Table 11** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | > 1.92 | No nitrosatable drug exposure | 65 | 63.7 | 1070 | 76.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 37 | 36.3 | 335 | 23.8 | **1.77** | [**1.18, 2.64**] | **1.92** | [**1.25, 2.96**] |
| | | Secondary amines | 20 | 23.5 | 195 | 15.4 | 1.65 | [1.00, 2.73] | **1.87** | [**1.09, 3.20**] |
| | | Tertiary amines | 20 | 23.5 | 160 | 13.0 | **1.97** | [**1.19, 3.25**] | **2.31**[d] | [**1.34, 3.99**] |
| | | Amides | 11 | 14.5 | 111 | 9.4 | 1.60 | [0.84, 3.03] | 1.52 | [0.78, 2.96] |
| P7P9 | < 1.29 | No nitrosatable drug exposure | 82 | 71.9 | 1050 | 75.1 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 32 | 28.1 | 349 | 24.9 | 1.16 | [0.77, 1.74] | 1.16 | [0.77, 1.77] |
| | | Secondary amines | 22 | 21.2 | 207 | 16.5 | 1.34 | [0.83, 2.14] | 1.36 | [0.84, 2.20] |
| | | Tertiary amines | 9 | 9.9 | 162 | 13.4 | 0.72 | [0.36, 1.43] | 0.70[d,e] | [0.35, 1.41] |
| | | Amides | 10 | 10.9 | 90 | 7.9 | 1.37 | [0.71, 2.64] | 1.48 | [0.75, 2.89] |
| | 1.29-1.92 | No nitrosatable drug exposure | 65 | 72.2 | 1005 | 74.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 25 | 27.8 | 343 | 25.4 | 1.14 | [0.72, 1.80] | 1.09 | [0.68, 1.77] |
| | | Secondary amines | 16 | 19.8 | 208 | 17.2 | 1.20 | [0.69, 2.07] | 1.21 | [0.69, 2.13] |
| | | Tertiary amines | 11 | 14.5 | 141 | 12.3 | 1.21 | [0.64, 2.29] | 1.08[d,e] | [0.55, 2.12] |
| | | Amides | 8 | 11.0 | 94 | 8.6 | 1.32 | [0.63, 2.75] | 1.22 | [0.57, 2.61] |
| | > 1.92 | No nitrosatable drug exposure | 65 | 64.4 | 1070 | 78.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 36 | 35.6 | 288 | 21.2 | **1.97** | [**1.31, 2.97**] | **2.08** | [**1.33, 3.24**] |
| | | Secondary amines | 20 | 23.5 | 169 | 13.6 | **1.88** | [**1.14, 3.11**] | **2.05** | [**1.19, 3.56**] |
| | | Tertiary amines | 16 | 19.8 | 123 | 10.3 | **2.04** | [**1.18, 3.52**] | **2.21**[d,e] | [**1.21, 4.01**] |
| | | Amides | 11 | 14.5 | 95 | 8.2 | 1.85 | [0.98, 3.50] | 1.95 | [1.00, 3.82] |
| Animal Nitrite | | | | | | | | | | |
| P1P3 | < 0.74 | No nitrosatable drug exposure | 76 | 71.7 | 1099 | 74.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 30 | 28.3 | 382 | 25.8 | 1.13 | [0.74, 1.72] | 1.15 | [0.74, 1.78] |
| | | Secondary amines | 12 | 13.6 | 204 | 15.7 | 0.86 | [0.47, 1.57] | 0.82[d] | [0.44, 1.55] |
| | | Tertiary amines | 11 | 12.6 | 196 | 15.1 | 0.81 | [0.43, 1.53] | 0.85 | [0.44, 1.64] |
| | | Amides | 18 | 19.2 | 109 | 9.0 | **2.26** | [**1.35, 3.77**] | **2.31** | [**1.35, 3.94**] |

**Table 11** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | 0.74-1.21 | No nitrosatable drug exposure | 72 | 68.6 | 1026 | 71.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 33 | 31.4 | 415 | 28.8 | 1.13 | [0.75, 1.71] | 1.04 | [0.67, 1.60] |
| | | Secondary amines | 21 | 22.6 | 219 | 17.6 | 1.36 | [0.84, 2.21] | 1.26[d] | [0.76, 2.09] |
| | | Tertiary amines | 18 | 20.0 | 211 | 17.1 | 1.20 | [0.72, 2.02] | 1.06 | [0.62, 1.82] |
| | | Amides | 10 | 12.2 | 134 | 11.6 | 1.06 | [0.55, 2.05] | 0.96 | [0.49, 1.89] |
| | > 1.21 | No nitrosatable drug exposure | 65 | 63.1 | 1015 | 71.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 38 | 36.9 | 398 | 28.2 | 1.47 | [0.99, 2.20] | 1.47 | [0.96, 2.26] |
| | | Secondary amines | 24 | 27.0 | 208 | 17.0 | **1.77** | [**1.11, 2.83**] | 1.62[d] | [0.97, 2.70] |
| | | Tertiary amines | 17 | 20.7 | 225 | 18.2 | 1.18 | [0.69, 2.02] | 1.10 | [0.61, 1.97] |
| | | Amides | 10 | 13.3 | 139 | 12.1 | 1.12 | [0.57, 2.18] | 1.23 | [0.61, 2.46] |
| P4P6 | < 0.74 | No nitrosatable drug exposure | 76 | 69.1 | 1099 | 75.5 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 34 | 30.9 | 356 | 24.5 | 1.35 | [0.90, 2.03] | 1.47 | [0.96, 2.23] |
| | | Secondary amines | 18 | 19.2 | 215 | 16.4 | 1.20 | [0.72, 2.01] | 1.32 | [0.78, 2.25] |
| | | Tertiary amines | 14 | 15.6 | 161 | 12.8 | 1.24 | [0.70, 2.19] | 1.33 | [0.74, 2.39] |
| | | Amides | 17 | 18.3 | 102 | 8.5 | **2.26** | [**1.34, 3.82**] | **2.44** | [**1.42, 4.19**] |
| | 0.74-1.21 | No nitrosatable drug exposure | 72 | 66.7 | 1026 | 73.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 36 | 33.3 | 364 | 26.2 | 1.40 | [0.94, 2.08] | 1.31 | [0.86, 2.00] |
| | | Secondary amines | 21 | 22.6 | 219 | 17.6 | 1.36 | [0.84, 2.22] | 1.32 | [0.80, 2.18] |
| | | Tertiary amines | 17 | 19.1 | 168 | 14.1 | 1.42 | [0.83, 2.40] | 1.22 | [0.70, 2.12] |
| | | Amides | 8 | 10.0 | 109 | 9.6 | 1.04 | [0.50, 2.16] | 0.92 | [0.43, 1.98] |
| | > 1.21 | No nitrosatable drug exposure | 65 | 64.4 | 1015 | 72.9 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 36 | 35.6 | 377 | 27.1 | 1.47 | [0.98, 2.21] | **1.57** | [**1.03, 2.42**] |
| | | Secondary amines | 21 | 24.4 | 214 | 17.4 | 1.51 | [0.92, 2.47] | 1.63 | [0.96, 2.76] |
| | | Tertiary amines | 18 | 21.7 | 181 | 15.1 | 1.52 | [0.90, 2.56] | 1.71 | [0.98, 2.99] |
| | | Amides | 10 | 13.3 | 125 | 11.0 | 1.24 | [0.64, 2.42] | 1.27 | [0.64, 2.52] |

**Table 11** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| P7P9 | < 0.74 | No nitrosatable drug exposure | 76 | 73.1 | 1099 | 77.0 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 28 | 26.9 | 329 | 23.0 | 1.21 | [0.79, 1.87] | 1.26 | [0.81, 1.98] |
| | | Secondary amines | 18 | 19.2 | 198 | 15.3 | 1.29 | [0.77, 2.16] | 1.39 | [0.82, 2.37] |
| | | Tertiary amines | 9 | 10.6 | 147 | 11.8 | 0.89 | [0.44, 1.77] | 0.94 | [0.46, 1.91] |
| | | Amides | 10 | 11.6 | 88 | 7.4 | 1.58 | [0.82, 3.06] | 1.70 | [0.86, 3.33] |
| | 0.74-1.21 | No nitrosatable drug exposure | 72 | 70.6 | 1026 | 75.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 30 | 29.4 | 339 | 24.8 | 1.26 | [0.82, 1.93] | 1.15 | [0.74, 1.81] |
| | | Secondary amines | 20 | 21.7 | 206 | 16.7 | 1.38 | [0.84, 2.26] | 1.33 | [0.79, 2.23] |
| | | Tertiary amines | 12 | 14.3 | 136 | 11.7 | 1.25 | [0.68, 2.30] | 1.07 | [0.56, 2.03] |
| | | Amides | 9 | 11.1 | 89 | 8.0 | 1.43 | [0.71, 2.85] | 1.14 | [0.55, 2.35] |
| | > 1.21 | No nitrosatable drug exposure | 65 | 64.4 | 1015 | 76.4 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 36 | 35.6 | 313 | 23.6 | **1.74** | [**1.16, 2.61**] | **1.88** | [**1.21, 2.92**] |
| | | Secondary amines | 21 | 24.4 | 180 | 15.1 | **1.77** | [**1.08, 2.90**] | **1.97** | [**1.16, 3.35**] |
| | | Tertiary amines | 15 | 18.8 | 143 | 12.4 | 1.59 | [0.91, 2.78] | 1.70 | [0.93, 3.10] |
| | | Amides | 10 | 13.3 | 102 | 9.1 | 1.50 | [0.77, 2.92] | 1.73 | [0.86, 3.47] |
| Plant Nitrite P1P3 | < 0.46 | No nitrosatable drug exposure | 80 | 69.0 | 1020 | 70.4 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 36 | 31.0 | 428 | 29.6 | 1.07 | [0.72, 1.58] | 1.05 | [0.70, 1.57] |
| | | Secondary amines | 21 | 20.8 | 221 | 17.8 | 1.19 | [0.74, 1.93] | 1.16 | [0.71, 1.90] |
| | | Tertiary amines | 19 | 19.2 | 215 | 17.4 | 1.12 | [0.68, 1.84] | 1.06 | [0.63, 1.77] |
| | | Amides | 14 | 14.9 | 144 | 12.4 | 1.21 | [0.69, 2.14] | 1.20 | [0.67, 2.14] |
| | 0.46-0.71 | No nitrosatable drug exposure | 68 | 63.0 | 963 | 67.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 40 | 37.0 | 457 | 32.2 | 1.23 | [0.83, 1.82] | 1.30 | [0.86, 1.95] |
| | | Secondary amines | 21 | 23.6 | 249 | 20.5 | 1.19 | [0.73, 1.94] | 1.26 | [0.76, 2.09] |
| | | Tertiary amines | 17 | 20.0 | 260 | 21.3 | 0.93 | [0.55, 1.58] | 0.94 | [0.54, 1.65] |
| | | Amides | 15 | 18.1 | 134 | 12.2 | 1.54 | [0.88, 2.70] | 1.65 | [0.93, 2.93] |

**Table 11** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | > 0.71 | No nitrosatable drug exposure | 64 | 71.1 | 1156 | 78.8 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 26 | 28.9 | 311 | 21.2 | 1.50 | [0.95, 2.37] | 1.60 | [0.98, 2.61] |
| | | Secondary amines | 16 | 20.0 | 161 | 12.2 | **1.79** | [**1.04, 3.10**] | **1.86** | [**1.03, 3.37**] |
| | | Tertiary amines | 10 | 13.5 | 158 | 12.0 | 1.15 | [0.59, 2.24] | 1.21 | [0.59, 2.46] |
| | | Amides | 9 | 12.3 | 104 | 8.3 | 1.56 | [0.77, 3.13] | 1.64 | [0.79, 3.38] |
| P4P6 | < 0.46 | No nitrosatable drug exposure | 80 | 69.0 | 1020 | 72.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 36 | 31.0 | 386 | 27.4 | 1.18 | [0.79, 1.74] | 1.24 | [0.82, 1.86] |
| | | Secondary amines | 21 | 20.8 | 228 | 18.3 | 1.17 | [0.72, 1.89] | 1.23 | [0.75, 2.02] |
| | | Tertiary amines | 18 | 18.4 | 169 | 14.2 | 1.33 | [0.80, 2.21] | 1.33 | [0.78, 2.25] |
| | | Amides | 12 | 13.0 | 119 | 10.5 | 1.24 | [0.68, 2.28] | 1.35 | [0.73, 2.52] |
| | 0.46-0.71 | No nitrosatable drug exposure | 68 | 64.2 | 963 | 69.5 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 38 | 35.8 | 423 | 30.5 | 1.26 | [0.85, 1.87] | 1.23 | [0.81, 1.85] |
| | | Secondary amines | 19 | 21.8 | 257 | 21.1 | 1.05 | [0.63, 1.74] | 1.12 | [0.67, 1.90] |
| | | Tertiary amines | 20 | 22.7 | 211 | 18.0 | 1.32 | [0.80, 2.18] | 1.24 | [0.74, 2.09] |
| | | Amides | 12 | 15.0 | 118 | 10.9 | 1.41 | [0.77, 2.61] | 1.36 | [0.73, 2.56] |
| | > 0.71 | No nitrosatable drug exposure | 64 | 66.0 | 1156 | 80.3 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 33 | 34.0 | 284 | 19.7 | **2.05** | [**1.34, 3.11**] | **2.07** | [**1.33, 3.22**] |
| | | Secondary amines | 21 | 24.7 | 162 | 12.3 | **2.27** | [**1.39, 3.72**] | **2.45** | [**1.45, 4.15**] |
| | | Tertiary amines | 12 | 15.8 | 126 | 9.8 | 1.68 | [0.91, 3.11] | **1.97** | [**1.03, 3.78**] |
| | | Amides | 11 | 14.7 | 98 | 7.8 | **2.00** | [**1.06, 3.79**] | 1.78 | [0.92, 3.45] |
| P7P9 | < 0.46 | No nitrosatable drug exposure | 80 | 70.8 | 1020 | 74.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 33 | 29.2 | 348 | 25.4 | 1.20 | [0.80, 1.79] | 1.19 | [0.78, 1.80] |
| | | Secondary amines | 21 | 20.8 | 222 | 17.9 | 1.20 | [0.74, 1.93] | 1.19 | [0.73, 1.94] |
| | | Tertiary amines | 14 | 14.9 | 150 | 12.8 | 1.17 | [0.67, 2.07] | 1.09 | [0.61, 1.94] |
| | | Amides | 10 | 11.1 | 88 | 7.9 | 1.39 | [0.72, 2.68] | 1.46 | [0.75, 2.85] |

**Table 11** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | 0.46-0.71 | No nitrosatable drug exposure | 68 | 68.7 | 963 | 71.2 | 1.00 | Referent | 1.00 | Referent] |
| | | Any nitrosatable | 31 | 31.3 | 389 | 28.8 | 1.12 | [0.73, 1.71] | 1.12 | [0.72, 1.74] |
| | | Secondary amines | 20 | 22.7 | 222 | 18.7 | 1.26 | [0.77, 2.08] | 1.36 | [0.81, 2.28] |
| | | Tertiary amines | 11 | 13.9 | 180 | 15.8 | 0.87 | [0.46, 1.64] | 0.88 | [0.46, 1.71] |
| | | Amides | 11 | 13.9 | 111 | 10.3 | 1.38 | [0.73, 2.60] | 1.33 | [0.69, 2.59] |
| | > 0.71 | No nitrosatable drug exposure | 64 | 68.8 | 1156 | 82.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 29 | 31.2 | 244 | 17.4 | **2.08** | [**1.34, 3.23**] | **2.09** | [**1.30, 3.35**] |
| | | Secondary amines | 17 | 21.0 | 141 | 10.9 | **2.12** | [**1.24, 3.62**] | **2.25** | [**1.27, 4.00**] |
| | | Tertiary amines | 11 | 14.7 | 96 | 7.7 | **2.00** | [**1.06, 3.79**] | **2.16** | [**1.09, 4.31**] |
| | | Amides | 8 | 11.1 | 80 | 6.5 | 1.81 | [0.87, 3.76] | 1.71 | [0.80, 3.66] |
| Total Nitrite[c] P1P3 | < 3.04 | No nitrosatable drug exposure | 86 | 72.3 | 1033 | 71.3 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 33 | 27.7 | 416 | 28.7 | 0.95 | [0.64, 1.42] | 1.00 | [0.66, 1.52] |
| | | Secondary amines | 19 | 18.1 | 217 | 17.4 | 1.05 | [0.64, 1.72] | 1.05 | [0.63, 1.76] |
| | | Tertiary amines | 17 | 16.5 | 218 | 17.4 | 0.94 | [0.56, 1.57] | 0.95 | [0.55, 1.64] |
| | | Amides | 14 | 14.0 | 120 | 10.4 | 1.37 | [0.78, 2.41] | 1.51 | [0.84, 2.71] |
| | 3.04-4.57 | No nitrosatable drug exposure | 60 | 63.2 | 1003 | 70.0 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 35 | 36.8 | 429 | 30.0 | 1.36 | [0.90, 2.06] | 1.30 | [0.84, 2.02] |
| | | Secondary amines | 20 | 25.0 | 230 | 18.7 | 1.45 | [0.87, 2.40] | 1.38 | [0.81, 2.35] |
| | | Tertiary amines | 16 | 21.1 | 226 | 18.4 | 1.19 | [0.68, 2.06] | 1.00 | [0.55, 1.81] |
| | | Amides | 10 | 14.3 | 148 | 12.9 | 1.12 | [0.58, 2.20] | 1.15 | [0.58, 2.28] |
| | > 4.57 | No nitrosatable drug exposure | 66 | 66.7 | 1086 | 75.9 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 33 | 33.3 | 345 | 24.1 | **1.54** | [**1.02, 2.35**] | **1.60** | [**1.03, 2.50**] |
| | | Secondary amines | 18 | 21.4 | 181 | 14.3 | 1.62 | [0.96, 2.73] | 1.68 | [0.96, 2.92] |
| | | Tertiary amines | 13 | 16.5 | 186 | 14.6 | 1.15 | [0.63, 2.08] | 1.22 | [0.65, 2.31] |
| | | Amides | 14 | 17.5 | 113 | 9.4 | **1.96** | [**1.10, 3.50**] | **1.97** | [**1.07, 3.62**] |

**Table 11** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| P4P6 | < 3.04 | No nitrosatable drug exposure | 86 | 70.5 | 1033 | 73.7 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 36 | 29.5 | 368 | 26.3 | 1.16 | [0.79, 1.72] | 1.26 | [0.84, 1.90] |
| | | Secondary amines | 21 | 19.6 | 217 | 17.4 | 1.16 | [0.72, 1.87] | 1.28 | [0.78, 2.09] |
| | | Tertiary amines | 14 | 14.0 | 162 | 13.6 | 1.03 | [0.59, 1.82] | 1.02 | [0.57, 1.83] |
| | | Amides | 13 | 13.1 | 106 | 9.3 | 1.41 | [0.79, 2.53] | 1.51 | [0.82, 2.76] |
| | 3.04-4.57 | No nitrosatable drug exposure | 60 | 64.5 | 1003 | 71.9 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 33 | 35.5 | 392 | 28.1 | 1.40 | [0.91, 2.14] | 1.35 | [0.87, 2.11] |
| | | Secondary amines | 19 | 24.1 | 228 | 18.5 | 1.38 | [0.82, 2.31] | 1.38 | [0.81, 2.35] |
| | | Tertiary amines | 20 | 25.0 | 186 | 15.6 | **1.75** | [**1.06, 2.91**] | 1.68 | [0.99, 2.86] |
| | | Amides | 7 | 10.5 | 131 | 11.6 | 0.91 | [0.42, 1.99] | 0.89 | [0.40, 1.98] |
| | > 4.57 | No nitrosatable drug exposure | 66 | 64.1 | 1086 | 76.6 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 37 | 35.9 | 331 | 23.4 | **1.79** | [**1.19, 2.67**] | **2.02** | [**1.32, 3.10**] |
| | | Secondary amines | 20 | 23.3 | 200 | 15.6 | 1.62 | [0.98, 2.67] | **1.91** | [**1.12, 3.26**] |
| | | Tertiary amines | 15 | 18.5 | 158 | 12.7 | 1.53 | [0.87, 2.67] | **1.87** | [**1.03, 3.39**] |
| | | Amides | 15 | 18.5 | 97 | 8.2 | **2.40** | [**1.37, 4.21**] | **2.35** | [**1.30, 4.23**] |
| P7P9 | < 3.04 | No nitrosatable drug exposure | 86 | 71.7 | 1033 | 75.1 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 34 | 28.3 | 342 | 24.9 | 1.18 | [0.79, 1.76] | 1.26 | [0.83, 1.90] |
| | | Secondary amines | 22 | 20.4 | 205 | 16.6 | 1.27 | [0.80, 2.03] | 1.39 | [0.85, 2.25] |
| | | Tertiary amines | 12 | 12.2 | 148 | 12.5 | 0.97 | [0.53, 1.77] | 0.96 | [0.51, 1.79] |
| | | Amides | 12 | 12.2 | 95 | 8.4 | 1.46 | [0.80, 2.67] | 1.48 | [0.80, 2.75] |
| | 3.04-4.57 | No nitrosatable drug exposure | 60 | 71.4 | 1003 | 74.0 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 24 | 28.6 | 353 | 26.0 | 1.14 | [0.71, 1.82] | 1.02 | [0.62, 1.68] |
| | | Secondary amines | 15 | 20.0 | 215 | 17.7 | 1.17 | [0.66, 2.06] | 1.12 | [0.63, 2.02] |
| | | Tertiary amines | 11 | 15.5 | 163 | 14.0 | 1.13 | [0.59, 2.14] | 1.03 | [0.52, 2.02] |
| | | Amides | 7 | 10.5 | 92 | 8.4 | 1.26 | [0.58, 2.76] | 0.98 | [0.43, 2.24] |

**Table 11** (continued)

| Period of Gestation | Dietary Intake (mg/day) | Type of drug exposure | Cases | | Controls | | Unadjusted HR[a] | 95% CI | Adjusted HR[b] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | | | | |
| | > 4.57 | No nitrosatable drug exposure | 66 | 65.4 | 1086 | 79.2 | 1.00 | Referent | 1.00 | Referent |
| | | Any nitrosatable | 35 | 34.6 | 285 | 20.8 | **1.95** | [**1.29, 2.93**] | **2.07** | [**1.33, 3.22**] |
| | | Secondary amines | 21 | 24.1 | 164 | 13.1 | **2.03** | [**1.24, 3.31**] | **2.18** | [**1.28, 3.71**] |
| | | Tertiary amines | 13 | 16.5 | 115 | 9.6 | 1.79 | [0.99, 3.25] | **1.94** | [**1.03, 3.66**] |
| | | Amides | 10 | 13.2 | 92 | 7.8 | 1.75 | [0.90, 3.41] | 1.86 | [0.93, 3.70] |

Abbreviations: HR, odds ratio; CI, confidence interval; P1P3, first trimester; P4P6, second trimester; P7P9, third trimester.

[a] Crude and adjusted hazard ratios include only cases and controls with complete information for dietary contaminants and covariates, and who had a daily caloric intake between 500-5000 kcal.

[b] Adjusted for caloric intake, study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.

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

[d] Significant additive interaction (95% CIs for RERI and/or AP exclude 0).

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

preterm births, we observed similar patterns of increasing risk with higher levels of intake among secondary and tertiary amine exposure during the first trimester. Additive interaction was noted between secondary amine exposure during the first trimester and dietary animal nitrite (AP 0.59, [95% CI 0.22, 0.96]). The highest risk among women with secondary amines during the last trimester was found with the upper tertile of animal nitrite (aHR 1.97, [95% CI 1.16, 3.35]). For amide usage, however, risk of delivering a moderately preterm infant was highest among women with the lowest levels of animal nitrite during the first and second trimester (aHR 2.31, [95% CI 1.35, 3.94] and aHR 2.44, [95% CI 1.42, 4.19], respectively).

For the most part, stratifying nitrostable drugs by levels of plant nitrite yielded HRs which would decline in the second tertile and increase in the third. With secondary and tertiary amine exposure during the last two trimesters, a higher adjusted HR was detected in the upper tertile of plant nitrite intake. Estimated risk of having a moderately preterm birth was 2.45 [95% CI 1.45, 4.15] and 1.97 [95% CI 1.03, 3.78] for secondary and tertiary amine usage in conjunction with high levels of plant nitrite, respectively, during the second trimester. Similar findings were noted during the third trimester with high levels of plant nitrite and secondary (aHR 2.25, [95% CI 1.27, 4.00]) and tertiary amine usage (aHR 2.16, [95% CI 1.09, 4.31]). A pattern of increasing risk was observed with total nitrite intake in conjunction with secondary and tertiary amine usage during the first two trimesters of pregnancy. Positive associations were

detected among high levels of total nitrite during the second trimester of pregnancy for secondary (aHR 1.91, [95% CI 1.12, 3.26]) and tertiary amine usage (aHR 1.87, [95% CI 1.03, 3.39]). Exposure to nitrosatable amides and levels of total nitrite >4.57 mg/day also yielded the highest risk for moderately preterm births during the first (aHR 1.97, [95% CI 1.07, 3.62]) and second (aHR 2.35, [95% CI 1.30, 4.23]) trimester of pregnancy. Adjusted HRs for moderately preterm births in relation to tertiary amine usage during the third trimester from the lowest tertile to the highest were 0.96 [95% CI 0.51, 1.79], 1.03 [95% CI 0.52, 2.02], and 1.94 [95% CI 1.03, 3.66]. Risk associated with nitrosatable drug exposure was also highest in the upper tertile of total nitrite intake among women who reported taking secondary amines during the last trimester (aHR 2.18, [95% CI 1.28, 3.71]). All graphical depictions based on the Cox-Snell residual analysis yielded results that support a reasonably well-fit model. Additionally, the link test and global test based on Schoenfeld residuals suggest no violation of the proportional hazards assumption.

**Comment**

This study examined the relation between maternal consumption of dietary nitrites (animal, plant, and total) and preterm births as well as the relation between prenatal exposure to nitrosatable drugs in conjunction with dietary intake of nitrites and preterm births. Utilizing data from control women with EDDs between 1997 and 2003 within the NBDPS, we found insufficient evidence to

suggest a positive association between dietary intake of nitrates or nitrites (including animal and total nitrite) and preterm births. However, high levels of nitrite from plant sources were observed to have a protective effect for preterm and moderately preterm births compared to the lowest tertile of plant nitrite. This finding may be due to other nutrients and vitamins that are contained within grain products, vegetables, and fruit which may have contributed to the reduction in risk that was detected. Also, a significant source of dietary nitrites from plant sources is from cereals, which often are fortified with vitamins.

This is the first study, to our knowledge, to examine the independent association between dietary levels of nitrite and preterm births. Studies regarding dietary intake have focused mainly on different types of diets rather than exposure to nitrite levels. A Mediterranean-type diet consists of vegetables, fruits, whole grains, nuts, legumes, fish, and use of olive oil. Consumption of red meat, full-fat dairy products, and eggs are limited. While Haugen *et al*.[266] noted no association, a significantly lower incidence of preterm births was reported among mothers who adopted a Mediterranean-type diet from gestational week 17-20 to birth.[267] Mikkelsen *et al*.[268] reported a similar finding utilizing the Danish National Birth Cohort. In addition, Scholl *et al*.[269] examined the association of high-sensitivity C-reactive protein (hsCRP) and preterm births. Higher hsCRP concentrations are associated with a Western diet, which consists of high quantities of red meat and high cholesterol food items. A significant increase in

risk for early preterm delivery (<34 weeks) was observed with the highest tertile of hsCRP (7.06-137.41 mg/L).

In the present study, exposure to prenatal nitrosatable drug use and high levels of nitrite intake showed a positive association with preterm births as well as moderately preterm births. An increasing pattern was noted with secondary amine exposure and tertiles of dietary nitrite (first and second trimester), animal nitrite (every trimester), plant nitrite (first trimester), and total nitrite (first and second trimester) in relation to preterm births. Similar patterns were found with tertiary amine use and levels of dietary nitrite (every trimester), animal nitrite (every trimester), and total nitrite (second trimester). Nitrosatable drug exposures showed little to no association with preterm births among women in the lowest tertiles of dietary nitrite (including animal, plant, and total), with the exception of second trimester amide use and animal nitrite intake. Analyses of moderately preterm births yielded similar conclusions, though associations were weaker. Although previous studies have not examined the association between prenatal exposure to nitrosatable drugs in conjunction with dietary intake of nitrite, a number have indicated an association between drugs classified as nitrosatable and preterm births. In addition, formation of *N*-nitroso compounds in the presence of a nitrosatable compound is greater if nitrite concentration is high,[286] lending support for our findings of an increased risk among women with higher levels of nitrite who were concomitantly exposed to nitrosatable drugs.

One of the strengths of the present study is the relatively large sample of preterm delivery cases and controls. In addition, we utilized control data from the NBDPS, one of the largest population-based studies of birth defects in the U.S., which has its advantages. First, NBDPS control women were those who delivered infants without any birth defects. By analyzing this population of control mothers, we were able to eliminate the confounding effects of birth defects since preterm births are more likely to occur if congenital malformations are present.[301] Further, an association was reported between nitrosatable drugs and dietary nitrite and total nitrite intake in relation to selected birth defects in two previous studies.[274, 294] Second, despite slight differences in terms of maternal race/ethnicity and education, NBDPS control women have been found to be representative of their base populations with regard to maternal age, smoking status, and prevalence of diabetes mellitus.[282] Time to interview is also consistent between mothers with preterm and full term deliveries, with both having a 7.7 month median length of time from the EDD to the interview.

With studies implicating different time periods, it is not known when the critical window of susceptibility is for preterm births. Though this question has been addressed numerous times, the weight of evidence does not lend itself towards any one time period. It is likely that the critical period of susceptibility would depend, partially, on the pathway which the exposure initiates its action.[3] Since prenatal exposures vary in their chemical structure and biological activities, the critical period of susceptibility for preterm births may differ and be

contingent upon the exposure itself. In the present study, we were able to examine different periods of exposure for nitrosatable drugs by trimester.

Our study has several limitations, including the potential maternal recall bias of drug exposures. In the original study birth defects was the primary outcome of interest, and there was a concern that mothers of infants with birth defects may more likely recall prenatal drug exposures compared to mothers of infants without birth defects. Our study population, however, consists solely of NBDPS control women. In addition, little evidence has been found on the differential recall of drugs classified as nitrosatable, particularly antibiotics,[302, 303] antinauseants,[302] analgesics,[303] and benzodiazepines.[303] However, a 20% higher sensitivity was reported for antibiotics among case-mothers than in control-mothers.[304] NBPDS utilized a two-level approach for drug assessment to reduce recall bias by asking about drug use by indication and then prompting participants with medication lists. This method of drug assessment has been shown to be more accurate compared to asking either type of question individually.[305] Medications were classified based on their nitrosatability and further categorized based on their functional groups (secondary amines, tertiary amines, or amides) after the interview was completed. Therefore, recall bias would be unlikely since participants were not questioned directly about nitrosatable drug exposure. Nevertheless, some sub-types of nitrosatable drugs may have been recalled differentially. In addition, despite utilizing extensive reviews[160, 288] that were not available in previous studies, some exposures may

have been missed since some components may not have been tested for its nitrosatability or results from such tests may not have been available.

Measurement error in estimates of dietary nitrates and nitrites is a potential concern.[321] Since levels were based on a FFQ, not all dietary sources of these contaminants may have been captured. Recall inaccuracy is also an issue; because frequency of foods eaten during the year prior to conception was collected, some misclassification of foods consumed during pregnancy may have resulted. However, any misclassification would be nondifferential since mothers were not aware of the nitrate and nitrite content in the foods they ingested when the FFQ was completed. In addition, the same time period of dietary assessment was used for all participants. To evaluate the effects of measurement error within the NBDPS FFQ, Huber *et al.*[322] used the simulation extrapolation algorithm, varying the amount of measurement error included in the model from zero to an additional 60%. No substantive differences were found in terms of statistical significance or magnitude of effect size. Furthermore, Cuco *et al*.[323] found that average consumption patterns of meats and vegetables do not significantly differ before and during pregnancy.

Secondly, information on several risk factors previously reported to influence the risk of preterm births were not collected by the NBDPS. Of particular concern is prior history of preterm births since reoccurrence is estimated to range from 15 to over 50%, depending on the number and gestational age of previous deliveries.[2, 306] Other risk factors include intrauterine

infections,[15, 16, 18, 19] marital status,[29-31, 34, 36] and psychological or social stress.[307, 308] Significant associations within the present study may be due to the lack of adjustment for previously reported risk factors. And lastly, the NBDPS does not collect information on subtypes of preterm births. Because of which, preterm births were treated as a single entity within the present study. Evidence has suggested that preterm deliveries consist of three clinical subtypes with partially heterogeneous etiologies, including spontaneous preterm delivery after preterm labor, medically indicated preterm delivery, and spontaneous preterm delivery after PPROM.[309, 310] Examining preterm births as a group versus splitting them into their respective subsets is still debated.[311, 312] Some researchers support splitting preterm births by clinical presentation since preterm delivery can result from diverse clinical pathways. For instance, rupturing of the membranes and spontaneous onset of labor is quite distinctive from fetal distress, which is recommended to be managed with early delivery. On the other hand, researchers who support examining preterm births as a group argue that conditions prompting medical intervention for early delivery, such as preeclampsia and fetal growth restriction, have similar mechanisms as the pathways resulting in spontaneous preterm delivery.[311, 313] Spontaneous preterm births are also motivated by the same predictors of medically indicated preterm births, including placenta abruption,[314] preeclampsia,[315] and restricted fetal growth.[316, 317] Therefore, since etiologies are shared grouping preterm births would offer an increase in statistical power.

Multiple analyses and comparisons were involved in this study, mainly with respect to nitrosatable drug exposures stratified by dietary nitrite intake. Twenty statistical tests were conducted to assess the association between dietary nitrate/nitrite and preterm and moderately preterm births. One statistically significant finding was observed; however, one is expected by chance alone. To assess interaction between nitrosatable drugs and dietary nitrite, including animal and plant sources and total nitrite, with preterm and moderately preterm births, 96 tests were conducted. Fifteen statistically significant interactions were detected, while only 5 would have been expected by chance.

In conclusion, we found insufficient evidence to suggest a positive relationship between dietary intake of nitrates, nitrites, animal nitrite, and total nitrites and preterm births. However, high levels of nitrite from plant sources were observed to have a protective effect for preterm and moderately preterm births. Prenatal exposure to nitrosatable drugs, particularly secondary and tertiary amines, in conjunction with higher levels of dietary nitrites (including animal nitrites, plant nitrites, and total nitrites) may increase the risk of preterm births. Further research is needed to confirm the findings related between prenatal nitrosatable drug use and dietary nitrite intake levels in relation to preterm births.

# CHAPTER IV

# PRENATAL EXPOSURE TO NITROSATABLE DRUGS, VITAMIN C, AND RISK OF PRETERM BIRTHS

## Overview

Nitrosatable drugs react with nitrite in the stomach to form *N*-nitroso compounds, observed to result in adverse pregnancy outcomes in animal models. An increased risk of preterm births has been detected with prenatal exposure to medications classified as nitrosatable. Vitamin C is a known nitrosation inhibitor.

Using data from mothers of babies without major birth defects from the National Birth Defects Prevention Study, we assessed nitrosatable drug exposure and vitamin C intake among 496 mothers of preterm infants and 5398 mothers with full term deliveries between 1997 and 2005. Daily intake of vitamin C was estimated from maternal interviews that collected information on supplemental and dietary intake.

Lower hazard ratios were observed among women with combined exposures to nitrosatable drugs and dietary vitamin C ≥85 mg/day during the second trimester compared to those with lower levels. Most notably, a reduced risk of moderately preterm births was observed among women with amide use who also had higher levels of dietary vitamin C (adjusted hazard ratio (aHR)

1.14, [95% confidence interval (CI) 0.66, 1.98]) compared to those with <85 mg/day (aHR 2.08, [95% CI 1.25, 3.47]). Inconsistent patterns were found with nitrosatable drug exposure and vitamin C supplementation in relation to preterm births.

Dietary vitamin C intake ≥85 mg/day may attenuate the association between nitrosatable drug use during the second trimester and preterm and moderately preterm births. Daily vitamin C supplementation did not diminish the association between prenatal nitrosatable drug use and preterm births in this study population.

**Background**

Preterm birth, defined as delivery of a liveborn infant before 37 weeks gestation, is the leading cause of early neonatal death and neonatal morbidity, including respiratory distress, infections, and hypoglycemia.[21] Worldwide, preterm deliveries complicate 5-12.7% of all deliveries,[324] and in the United States the proportion of infants born preterm has increased 31% between 1981 and 2003.[4] It is hypothesized that survival limits for preterm infants has been reached using the current methods of neonatal intensive care.[298] Despite numerous studies, the causes and mechanisms of preterm birth are not fully understood.

*N*-nitroso compounds, including nitrosamines and nitrosamides, are formed when nitrosatable amines or amides react with nitrosating agents, such

as nitrite, in the acidic environment of the stomach.[11] While endogenous formation constitutes approximately 45-75% of total levels, exogenous sources are responsible for some *N*-nitroso compound exposure.[299] Certain nitrosatable drugs, relegated as secondary amines, tertiary amines, and amides, contribute to levels of *N*-nitroso compounds by reacting with nitrosating agents. Within the National Birth Defects Prevention Study (NBDPS), 24% of control mothers reported taking drugs classified as nitrosatable during the first trimester of pregnancy.[10] In the NBDPS study population, first trimester exposure to nitrosatable drugs was associated with several birth defects, including neural tube defects, limb deficiencies, cleft lip with cleft palate, cleft palate alone, single ventricle heart defects, atrioventricular septal defects, and hypoplastic left heart syndrome.[274, 294] Further, animal models have found a positive association between *N*-nitroso compounds and adverse pregnancy outcomes, such as reduced fetal weight and birth defects in mice.[12-14] The effects of these compounds on gestational age are not known as no study has been published to date on this relationship.

Vitamin C is a known nitrosation inhibitor, shown to block endogenous formation of *N*-nitroso compounds. Ascorbic acid inhibits the formation of *N*-nitroso compounds by rapidly reducing nitrite to nitrous oxide, followed by the production of dehydroascorbic acid.[270] Animal models have further demonstrated the ability of vitamin C to suppress nitrosation. In particular, a reduced risk of peripheral nervous system tumors in the offspring of pregnant

hamsters was observed when ascorbic acid was given in conjunction with ethylurea and nitrite.[271, 272] In a clinical trial of human volunteers, increased doses of ascorbic acid, starting from 1.76 mg to 1,000 mg, were administered along with combined exposures of nitrate and a nitrosatable precursor, proline.[273] A significant 44% reduction in *N*-nitroso compound excretion was noted among individuals who were given vitamin C in conjunction with nitrate and proline compared to those without concomitant administration of vitamin C. In a recent study of NBDPS mothers, daily vitamin C supplementation along with tertiary or secondary amine drug exposure resulted in lower odds of having anencephalic births compared to taking these drugs without vitamin C supplementation.[274] A reduction in risk was also noted for transverse limb deficiency in conjunction with secondary amine drug exposure, cleft lip without cleft palate with tertiary amine exposure, and several congenital heart defects in conjunction with tertiary amine and amide drug exposures with daily use of supplements with vitamin C.[275] Given previous animal and human data on the impact of vitamin C on ameliorating the effects of *N*-nitroso compounds, we examined the: 1) effects of dietary vitamin C intake on the relation between drugs classified as nitrosatable such as secondary amines, tertiary amines, or amides and preterm births (including moderately preterm births); and the 2) effects of vitamin C supplementation on the association between nitrosatable drugs and preterm births (including moderately preterm births).

## Methods

*Study Population*

The NBDPS is an ongoing population-based, case-control study of major structural birth defects in the United States. Ten sites have participated since the study's inception in 1997, including: Arkansas, California, Georgia, Iowa, Massachusetts, New York, and Texas (from 1998 to present); New Jersey (from 1998 to 2002); and North Carolina and Utah (from 2003 to present). Case-infants within the NBDPS are identified from live births (all centers), stillbirths (all centers except New Jersey and New York from 1997 to 1999), and elective pregnancy terminations (all centers except Massachusetts, New Jersey, and New York before 2000) from birth defect surveillance programs. For the present study, data from control mothers of babies without major birth defects from the NBDPS were used to examine the effects of dietary and supplemental vitamin C intake on the relation between prenatal exposure to nitrosatable drugs and preterm births.

Control-infants within the NBDPS were live born without major birth defects, who were delivered in the same time frame and study area as the case births with major birth defects. Birth certificates (Arkansas and Georgia, for estimated delivery dates (EDDs) after 2000; Iowa; Massachusetts; New Jersey; North Carolina; and Utah) and hospital records (Arkansas, for EDDs before 2001; California; Georgia, for EDDs before 2001; New York; and Texas) were used as sources for random sampling.[282] Control-infants were excluded if the

infant was either adopted or in foster care or if the mother possessed at least one of the following characteristics: did not speak English or Spanish, previously participated in the NBDPS, was incarcerated, was a donor or a surrogate parent, was unable to answer questions, or was deceased.

NBDPS control-infants with EDDs between October 1, 1997 and December 31, 2005 were included in the present study. Our study population was further restricted to singletons since multiple births are a major risk factor for preterm deliveries. Small for gestational age infants were also excluded since *N*-nitroso compounds have been reported to affect fetal weight.[12] Case-infants in the present study were defined as preterm births, infants with a gestational age less than 37 weeks. Medical records and birth certificates were utilized to obtain gestational age at delivery. If these documents could not provide gestational age, then it was calculated using the reported EDD from the interview. Additional methods used to calculate gestational age (in order of descending preference) included: ultrasound <14 weeks, last menstrual period, ultrasound >14 weeks, and standard neonatal exam. Infants were further categorized as moderately preterm if their gestational age was between 33 and 36 weeks. Infants with 37 to 41 weeks of gestation served as the control-infants. The institutional review boards in each state and the Centers for Disease Control and Prevention approved the NBDPS study protocol, and the institutional review board of Texas A&M University also approved this project on nitrosatable drugs, vitamin C, and preterm births.

*Data Collection*

The NBDPS interviews took about one hour to complete and were conducted by trained female interviewers in either English or Spanish using a computer-assisted telephone interview following informed consent.[283] Interviews were conducted 6 weeks to 24 months after the EDDs (or delivery of a full-term infant) and targeted for completion within 6 months of the EDD. Time to interview was comparable between mothers of preterm and full term infants, with both having a 7.7 month median length of time from the EDD to the interview. The maternal interview included several sections, such as maternal health during the index pregnancy (including medication usage), diet (food consumption in the year before pregnancy, and vitamin supplementation from three months preconception to the end of pregnancy), infections, and behavioral factors.

*Classification of Nitrosatable Drugs*

Information on prescription and nonprescription medication use, including medication name, frequency of use, and corresponding dates of usage, from three months preconception to the end of pregnancy were collected during the NBDPS interview. The Slone Epidemiology Center Drug Dictionary system was used to link reported medications to their active ingredients.[287] Categorization methods of drugs by their nitrosatability, functional groups, and indication have been described in detail by Brender *et al*.[10] Briefly, all reported orally

administered medications and their active ingredients were identified and cross-referenced against comprehensive nitrosatable medicinal compounds lists.[160, 288] These drugs were further classified by their chemical structure, i.e. whether an amine (secondary or tertiary) or amide functional group was present. Medline and internet sources were used to evaluate the presence of amine or amide functional groups of all remaining active ingredients. Lastly, each component was grouped by primary indication or therapeutic use and pharmacologic class. This study focuses on drugs reported to have been taken during pregnancy, concentrating on periods of exposure by trimester.

*Assessment of Vitamin C Intake*

The NBDPS collected information on average food consumption throughout the year before conception using a 58-item food frequency questionnaire (FFQ) that was adapted from the short Willett FFQ. The Willett FFQ has been validated and reproduced and provides useful information about nutrient intake in women during pregnancy.[284, 318] Information on cereal intake three months preconception to the end of pregnancy was also collected. The USDA National Nutrient Database for Standard Reference 19 served as the basis for nutrient calculations, such as dietary intake of vitamin C.[325] Complete data for any nitrosatable drug use stratified by dietary vitamin C intake were available for mothers of 477 (96.2%) preterm infants, 392 (95.8%) moderately preterm infants, and 5193 (96.2%) full-term infants (controls).

Vitamin C supplementation was assessed using the NBDPS questionnaire, which contained questions regarding start and stop dates, duration of use, and frequency of vitamin use (single, prenatal, and multivitamins) from three months preconception to the end of pregnancy. Vitamin C supplementation was categorized into less than daily and daily, depending on frequency of intake for the first and second trimester. Due to insufficient numbers, vitamin C supplementation during the third trimester could not be examined. Women who reported using a daily vitamin C supplement during a specified period were classified as “daily” while those who reported taking a vitamin C supplement less than 90 days in a given trimester or less than every day in a given period were categorized as “less than daily.” Complete data for any nitrosatable drug use stratified by supplemental vitamin C intake during the first trimester were available for 472 (95.2%), 389 (95.1%), and 5130 (95.0%) mothers of preterm, moderately preterm, and full term infants, respectively.

*Covariates*

Covariate selection was based on factors previously reported to be associated with preterm births and/or nitrosatable drug use.[10] Potential confounders assessed included maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, other), educational level (<12 years, 12 years, 13-15 years, >15 years), age (<18, 18-19, 20-24, 25-29,

30-34, ≥35 years), and smoking status (yes/no); body mass index (BMI) based on self-reported height and weight ($kg/m^2$), categorized according to NIH guidelines (underweight, normal, overweight, and obese); study site; infant gender (male/female); parity (nulliparous, primiparous, and multiparous); pre-pregnancy diabetes (yes/no); gestational diabetes (yes/no); and pre-pregnancy hypertension (yes/no). Nonsignificant covariates as well as those that did not change the hazard ratio by 10% or more were eliminated from the final model using forward selection.

*Statistical Analysis*

Descriptive analyses were performed to examine the distribution of several covariates among case- and control-mothers. Since preterm birth is a time-based outcome that depends on gestational age, Cox proportional hazards model was used to estimate hazard ratios (HR) and corresponding 95% confidence intervals (CI) for preterm and moderately preterm births in relation to nitrosatable drug use by supplemental and dietary intake of vitamin C. Gestational age at birth, measured in weeks, was the underlying time variable. Each woman remained in the risk set of giving birth to a preterm infant until delivery or gestational age of 37 weeks, whichever occurred first. In other words, women with term or post-term deliveries were censored at 37 weeks. Maternal race/ethnicity, educational level, and age; study center; pre-pregnancy diabetes; and pre-pregnancy hypertension were included in the regression models as possible confounders. Analyses were restricted to singleton pregnancies with

complete information on all covariates included in the final model. Statistical tests were two-sided, and findings were considered statistically significant at the 5% level if the CI did not include 1.00. Cox-Snell residuals analysis, link test, and a global test based on Schoenfeld residuals was utilized to assess violation of the proportional hazards assumption.[300]

Nitrosatable drug exposure (secondary amines, tertiary amines, and amides) was stratified by vitamin C supplementation (less than daily, daily) by trimester of pregnancy for preterm births, and HRs and 95% CIs were estimated for preterm births and moderately preterm births for each stratum. Women who reported no nitrosatable drug use during pregnancy served as the referent group in all analyses. Nitrosatable drugs were also stratified by dietary vitamin C intake (<85 mg/day or ≥85 mg/day). Cut points were based on the recommended dietary allowance for pregnant women over 18 years of age,[326] which corresponds to the 41st percentile for control participants. The median cut-point for dietary vitamin C among our control-mothers was 101.35 mg/day. Dietary vitamin C analyses were restricted to women who had an estimated daily caloric intake between 500 and 5000 kcal. Models examining the effect of dietary vitamin C on the relation between nitrosatable drugs and preterm births included covariates previously listed in addition to daily caloric intake.

Additive and multiplicative interaction was assessed for the associations of preterm births, as well as moderately preterm births, with nitrosatable drugs by supplemental and dietary vitamin C. Measures of relative excess risk due to

interaction (RERI) and attributable proportion due to interaction (AP) were utilized to determine whether significant additive interaction was present.[295] If the 95% CIs of either or both measures excluded 0, additive interaction was considered present, indicating that the risk of preterm births attributable to the two risk factors in combination is greater than the sum of risks associated with each risk factor separately. Multiplicative interaction was assessed with the inclusion of product terms of nitrosatable drug groups with supplemental and dietary vitamin C in the Cox proportional hazards models and was considered significant if the *P* value was less than 0.05. STATA version 12.0 was used for all analyses.

**Results**

The maternal participation rate among NBDPS controls mothers was 66%. A total of 496 eligible case-mothers who delivered a preterm infant (409 who delivered a moderately preterm infant) and 5398 control-mothers with an EDD between 1997 and 2005 participated in the study. Case-mothers were significantly less likely to be non-Hispanic white compared to control-mothers (Table 12). They were also significantly more likely to have pre-pregnancy hypertension, pre-pregnancy diabetes, reside in Arkansas and Texas, and were somewhat younger at time of delivery. The proportion of case- and control-mothers with an estimated vitamin C intake less than 85 mg/day was

**Table 12.** Selected Maternal Characteristics of Preterm Cases and Controls in the National Birth Defects Prevention Study, 1997-2005

| Characteristics of Participants | Controls[a] (%) n=5398 | Preterm Cases[a] (%) n=496 |
|---|---|---|
| Race/ethnicity* | | |
| Non-Hispanic white | 3252 (60.2) | 277 (55.9) |
| Non-Hispanic black | 605 (11.2) | 74 (14.9) |
| Hispanic | 1180 (21.9) | 107 (21.6) |
| Asian/Pacific Islander | 155 (2.9) | 9 (1.8) |
| All others | 206 (3.8) | 29 (5.9) |
| Education (years) | | |
| <12 | 852 (15.8) | 91 (18.4) |
| 12 | 1288 (23.9) | 137 (27.6) |
| 13-15 | 1462 (27.1) | 124 (25.0) |
| >15 | 1723 (31.9) | 136 (27.4) |
| Missing | 73 (1.4) | 8 (1.6) |
| Age at delivery (years)* | | |
| <18 | 193 (3.6) | 24 (4.8) |
| 18-19 | 370 (6.9) | 40 (8.1) |
| 20-24 | 1223 (22.7) | 119 (24.0) |
| 25-29 | 1433 (26.6) | 142 (28.6) |
| 30-34 | 1459 (27.0) | 96 (19.4) |
| >34 | 720 (13.3) | 75 (15.1) |
| Study center* | | |
| Arkansas | 650 (12.0) | 88 (17.7) |
| California | 697 (12.9) | 57 (11.5) |
| Georgia | 597 (11.1) | 44 (8.9) |
| Iowa | 607 (11.2) | 56 (11.3) |
| Massachusetts | 672 (12.5) | 58 (11.7) |
| North Carolina | 321 (6.0) | 34 (6.9) |
| New Jersey | 449 (8.3) | 32 (6.5) |
| New York | 499 (9.2) | 33 (6.7) |
| Texas | 602 (11.2) | 68 (13.7) |
| Utah | 304 (5.6) | 26 (5.2) |
| Body mass index (kg/m$^2$) | | |
| <18.5 | 257 (4.8) | 29 (5.9) |
| 18.5–24.9 | 2904 (53.8) | 268 (54.0) |
| 25.0–29.9 | 1190 (22.1) | 99 (20.0) |
| >29.9 | 847 (15.7) | 86 (17.3) |
| Missing | 200 (3.7) | 14 (2.8) |
| Smoking | | |
| No | 4371 (81.0) | 382 (77.0) |
| Yes | 969 (18.0) | 107 (21.6) |
| Missing | 58 (1.1) | 7 (1.4) |
| Pre-pregnancy diabetes* | | |
| No | 5244 (97.2) | 475 (95.8) |
| Yes | 26 (0.5) | 10 (2.0) |
| Missing | 128 (2.4) | 11 (2.2) |
| Pre-pregnancy hypertension* | | |
| No | 4723 (87.5) | 393 (79.2) |
| Yes | 668 (12.4) | 102 (20.6) |
| Missing | 7 (0.1) | 1 (0.2) |
| Infant gender | | |
| Male | 2702 (50.1) | 243 (49.0) |
| Female | 2696 (49.9) | 253 (51.0) |

**Table 12** (continued)

| Characteristics of Participants | Controls[a] (%) n=5398 | Preterm Cases[a] (%) n=496 |
|---|---|---|
| Parity | | |
| Nulliparous | 2150 (39.8) | 207 (41.7) |
| Primiparous | 1816 (33.6) | 154 (31.1) |
| Multiparous | 1432 (26.5) | 135 (27.2) |
| Dietary Vitamin C | | |
| < 85 mg/day | 2191 (40.6) | 217 (43.8) |
| ≥ 85 mg/day | 3181 (58.9) | 275 (55.4) |
| Missing | 26 (0.5) | 4 (0.8) |
| Vitamin C supplements | | |
| First Trimester | | |
| <Daily | 3601 (66.7) | 328 (66.1) |
| Daily | 1710 (31.7) | 159 (31.1) |
| Missing | 87 (1.6) | 9 (1.8) |
| Second Trimester* | | |
| <Daily | 944 (17.5) | 120 (24.2) |
| Daily | 4368 (80.9) | 367 (74.0) |
| Missing | 86 (1.6) | 9 (1.8) |

[a] NBDPS control women who gave birth to preterm infants (cases) and women who had full term infants without SGA (controls)

* Statistically significant differences in the distribution between cases and controls at $P < 0.05$.

comparable. Frequency of supplemental vitamin C intake was similar across cases and controls during the first trimester, but during the second trimester control-mothers were significantly more likely to report taking supplements containing vitamin C.

Dietary vitamin C intake above 85 mg/day in conjunction with nitrosatable drug exposure during the first trimester of pregnancy resulted in higher hazard ratios for preterm delivery (Table 13). Among women who reported taking drugs classified as amides, a higher point estimate was observed among those who had higher daily vitamin C intake (aHR 1.52, [95% CI 0.99, 2.32]) compared to those with lower levels of vitamin C intake (aHR 1.20, [95% CI 0.73, 1.96]). In contrast, lower hazard ratios for nitrosatable drug use combined with higher

**Table 13.** Maternal Nitrosatable Drug Exposure by Gestational Period and Preterm Births Stratified by Dietary Vitamin C, National Birth Defects Prevention Study, 1997-2005

| Gestational Period | Dietary Vitamin C mg/day | Type of drug exposure | Cases | | Controls | | Unadjusted | | Adjusted | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | %[a] | No. | %[a] | HR[b] | 95% CI | HR[b,c] | 95% CI |
| P1P3 | <85 | No nitrosatable drug exposure | 105 | 64.8 | 1181 | 67.6 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 36 | 25.5 | 298 | 20.2 | 1.32 | [0.91, 1.93] | 1.27 | [0.86, 1.89] |
| | | Tertiary amines | 30 | 22.2 | 318 | 21.2 | 1.05 | [0.70, 1.58] | 0.99 | [0.65, 1.52] |
| | | Amides | 20 | 16.0 | 182 | 13.4 | 1.20 | [0.75, 1.94] | 1.20 | [0.73, 1.96] |
| | ≥85 | No nitrosatable drug exposure | 162 | 70.4 | 1989 | 75.8 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 36 | 18.2 | 335 | 14.4 | 1.32 | [0.92, 1.90] | 1.31 | [0.90, 1.92] |
| | | Tertiary amines | 30 | 15.6 | 318 | 13.8 | 1.16 | [0.79, 1.71] | 1.11 | [0.74, 1.68] |
| | | Amides | 26 | 13.8 | 201 | 9.2 | **1.56** | [**1.03, 2.36**] | 1.52 | [0.99, 2.32] |
| P4P6 | <85 | No nitrosatable drug exposure | 105 | 62.5 | 1181 | 70.2 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 35 | 25.0 | 294 | 19.9 | 1.31 | [0.89, 1.92] | 1.39 | [0.94, 2.06] |
| | | Tertiary amines | 33 | 23.9 | 248 | 17.4 | 1.44 | [0.98, 2.13] | 1.49 | [0.99, 2.24] |
| | | Amides | 22 | 17.3 | 145 | 10.9 | **1.61** | [**1.02, 2.55**] | **1.71** | [**1.06, 2.75**] |
| | ≥85 | No nitrosatable drug exposure | 162 | 71.4 | 1989 | 76.9 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 38 | 19.0 | 356 | 15.2 | 1.31 | [0.92, 1.86] | 1.35 | [0.93, 1.94] |
| | | Tertiary amines | 29 | 15.2 | 262 | 11.6 | 1.34 | [0.91, 2.00] | 1.33 | [0.88, 2.00] |
| | | Amides | 20 | 11.0 | 192 | 8.8 | 1.28 | [0.80, 2.03] | 1.23 | [0.76, 1.97] |
| P7P9 | <85 | No nitrosatable drug exposure | 105 | 66.5 | 1181 | 72.0 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 31 | 22.8 | 272 | 18.7 | 1.25 | [0.84, 1.87] | 1.25 | [0.83, 1.89] |
| | | Tertiary amines | 26 | 19.9 | 216 | 15.5 | 1.31 | [0.85, 2.01] | 1.37 | [0.88, 2.14] |
| | | Amides | 14 | 11.8 | 136 | 10.3 | 1.13 | [0.65, 1.97] | 1.17 | [0.66, 2.06] |

**Table 13** (continued)

| Gestational Period | Dietary Vitamin C mg/day | Type of drug exposure | Cases | | Controls | | Unadjusted | | Adjusted | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | %[a] | No. | %[a] | HR[b] | 95% CI | HR[b,c] | 95% CI |
| | ≥85 | No nitrosatable drug exposure | 162 | 74.7 | 1989 | 79.2 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 36 | 18.2 | 314 | 13.6 | 1.39 | [0.97, 1.99] | 1.40 | [0.97, 2.04] |
| | | Tertiary amines | 19 | 10.5 | 211 | 9.6 | 1.11 | [0.69, 1.79] | 1.12 | [0.69, 1.84] |
| | | Amides | 18 | 10.0 | 143 | 6.7 | 1.51 | [0.93, 2.46] | 1.37 | [0.83, 2.25] |

Abbreviations: HR, hazard ratio; CI, confidence interval; P1P3, first trimester; P4P6, second trimester; P7P9, third trimester.

[a] Percentages for no nitrosatable drug exposure are based on total participants with complete information while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[b] Crude and adjusted hazard ratios include only cases and controls with complete information for drug exposures and covariates and who had a daily caloric intake between 500-5000 kcal.

[c] Adjusted for daily caloric intake, study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.

levels of dietary vitamin C were noted during the second trimester. The beneficial effects of dietary vitamin C were seen across all broad classifications of nitrosatable drugs. The difference was most notable with amide exposure, with a smaller effect observed among women who had an estimated dietary intake of vitamin C ≥85 mg/day (aHR 1.23, [95% CI 0.76, 1.97]) compared to less than 85 mg/day (aHR 1.71, [95% CI 1.06, 2.75]). During the third trimester of pregnancy, a slightly lower risk of delivering a preterm infant was noted among women with tertiary amine use in conjunction with higher levels of dietary vitamin C (aHR 1.12, [95% CI 0.69, 1.84]) compared to women with less than 85 mg/day (aHR 1.37, [95% CI 0.88, 2.14]). However, secondary amine or amide exposure during the same gestational period and vitamin C intake ≥85 mg/day resulted in higher hazard ratios than dietary vitamin C levels less than 85 mg/day. No significant additive or multiplicative interaction was observed for preterm births by dietary vitamin C in relation to nitrosatable drug exposure.

Table 14 displays the results of the Cox proportional hazard models for prenatal nitrosatable drug use and moderately preterm births stratified by dietary vitamin C. Higher intake of dietary vitamin C did not appear to make a difference with nitrosatable drug exposure during the first trimester of pregnancy, except for secondary amines. Exposure to secondary amines and dietary vitamin C ≥85 mg/day resulted in lower risk of having a moderately preterm birth (aHR 1.16, [95% CI 0.75, 1.79]) compared to dietary vitamin C levels under 85 mg/day (aHR 1.45, [95% CI 0.94, 2.24]). Higher dietary vitamin C appeared to provide more

**Table 14.** Maternal Nitrosatable Drug Exposure by Gestational Period and Moderately Preterm Births Stratified by Dietary Vitamin C, National Birth Defects Prevention Study, 1997-2005

| Gestational Period | Dietary Vitamin C mg/day | Type of drug exposure | Cases | | Controls | | Unadjusted | | Adjusted | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | %[a] | No. | %[a] | HR[b] | 95% CI | HR[b,c] | 95% CI |
| P1P3 | <85 | No nitrosatable drug exposure | 78 | 61.4 | 1181 | 67.6 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 32 | 29.1 | 298 | 20.2 | **1.59** | [**1.05, 2.39**] | 1.45 | [0.94, 2.24] |
| | | Tertiary amines | 24 | 23.5 | 318 | 21.2 | 1.13 | [0.72, 1.79] | 1.01 | [0.62, 1.65] |
| | | Amides | 18 | 18.8 | 182 | 13.4 | 1.46 | [0.87, 2.43] | 1.40 | [0.82, 2.37] |
| | ≥85 | No nitrosatable drug exposure | 135 | 71.8 | 1989 | 75.8 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 26 | 16.2 | 335 | 14.4 | 1.15 | [0.76, 1.75] | 1.16 | [0.75, 1.79] |
| | | Tertiary amines | 22 | 14.0 | 318 | 13.8 | 1.02 | [0.65, 1.61] | 1.02 | [0.64, 1.64] |
| | | Amides | 20 | 12.9 | 201 | 9.2 | 1.45 | [0.90, 2.31] | 1.46 | [0.90, 2.36] |
| P4P6 | <85 | No nitrosatable drug exposure | 78 | 58.2 | 1181 | 70.2 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 30 | 27.8 | 294 | 19.9 | 1.51 | [0.99, 2.31] | **1.58** | [**1.02, 2.44**] |
| | | Tertiary amines | 29 | 27.1 | 248 | 17.4 | **1.71** | [**1.12, 2.62**] | **1.74** | [**1.12, 2.72**] |
| | | Amides | 20 | 20.4 | 145 | 10.9 | **1.97** | [**1.20, 3.22**] | **2.08** | [**1.25, 3.47**] |
| | ≥85 | No nitrosatable drug exposure | 135 | 72.6 | 1989 | 76.9 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 31 | 18.7 | 356 | 15.2 | 1.28 | [0.87, 1.90] | 1.34 | [0.89, 2.00] |
| | | Tertiary amines | 21 | 13.5 | 262 | 11.6 | 1.17 | [0.74, 1.85] | 1.19 | [0.74, 1.92] |
| | | Amides | 15 | 10.0 | 192 | 8.8 | 1.16 | [0.68, 1.97] | 1.14 | [0.66, 1.97] |
| P7P9 | <85 | No nitrosatable drug exposure | 78 | 61.9 | 1181 | 72.0 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 28 | 26.4 | 272 | 18.7 | 1.52 | [0.99, 2.34] | 1.47 | [0.94, 2.30] |
| | | Tertiary amines | 23 | 22.8 | 216 | 15.5 | 1.56 | [0.98, 2.48] | 1.58 | [0.97, 2.56] |
| | | Amides | 13 | 14.3 | 136 | 10.3 | 1.40 | [0.78, 2.52] | 1.44 | [0.79, 2.63] |

**Table 14** (continued)

| Gestational Period | Dietary Vitamin C mg/day | Type of drug exposure | Cases No. | Cases %[a] | Controls No. | Controls %[a] | Unadjusted HR[b] | 95% CI | Adjusted HR[b,c] | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | ≥85 | No nitrosatable drug exposure | 135 | 74.6 | 1989 | 79.2 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 31 | 18.7 | 314 | 13.6 | 1.44 | [0.97, 2.13] | 1.46 | [0.98, 2.19] |
| | | Tertiary amines | 13 | 8.8 | 211 | 9.6 | 0.92 | [0.52, 1.62] | 0.96 | [0.54, 1.73] |
| | | Amides | 16 | 10.6 | 143 | 6.7 | 1.62 | [0.97, 2.72] | 1.49 | [0.88, 2.54] |

Abbreviations: HR, hazard ratio; CI, confidence interval; P1P3, first trimester; P4P6, second trimester; P7P9, third trimester.

[a] Percentages for no nitrosatable drug exposure are based on total participants with complete information while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[b] Crude and adjusted hazard ratios include only cases and controls with complete information for drug exposures and covariates and who had a daily caloric intake between 500-5000 kcal.

[c] Adjusted for daily caloric intake, study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension

beneficial effects against moderately preterm births than preterm births overall during the second trimester of pregnancy. The largest reduction was observed with amide usage in combination with a vitamin C intake ≥85 mg/day (aHR 1.14, [95% CI 0.66, 1.97]) in comparison to an intake less than 85 mg/day (aHR 2.08, 95% CI 1.25, 3.47]). We observed comparable point estimates in the last trimester of pregnancy across nitrosatable drug groups between the two levels of dietary vitamin C, with the exception of tertiary amines. A reduction in point estimates among women with tertiary amine exposure and high levels of dietary vitamin C (≥85 mg/day) (aHR 0.96, [95% CI 0.54, 1.73]) compared to those with lower levels (aHR 1.58, [95% CI 0.97, 2.56]) was detected. No significant additive or multiplicative interactions were observed for moderately preterm births by dietary vitamin C in relation to prenatal nitrosatable drug use.

The effects of prenatal nitrosatable drug exposures on preterm births stratified by vitamin C supplementation are shown in Table 15. We observed conflicting results regarding the effects of daily vitamin C supplementation on nitrosatable drug use during the first trimester. A higher hazard ratio was detected among women with amide usage who reported taking a daily vitamin C supplement (aHR 2.02, [95% CI 1.25, 3.26]) compared to those with less than daily supplementation (aHR 0.99, [95% CI 0.64, 1.55]); multiplicative interaction was noted between amide exposure and vitamin C supplementation ($P$ = 0.04). However, a reduction in the point estimate was noted with secondary amine use and daily supplementation of vitamin C (aHR 1.03, [95% CI 0.63, 1.70]) when

**Table 15.** Maternal Nitrosatable Drug Exposure by Gestational Period and Preterm Births Stratified by Vitamin C Supplementation, National Birth Defects Prevention Study, 1997-2005

| Gestational Period | Frequency of Vitamin supplement | Type of drug exposure | Cases | | Controls | | Unadjusted | | Adjusted | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | %[a] | No. | %[a] | HR[b] | 95% CI | HR[b,c] | 95% CI |
| P1P3 | Less than daily | No nitrosatable drug exposure | 188 | 69.9 | 2213 | 74.1 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 52 | 21.7 | 392 | 15.1 | **1.54** | [**1.13, 2.09**] | **1.45** | [**1.05, 2.00**] |
| | | Tertiary amines | 39 | 17.2 | 421 | 16.0 | 1.09 | [0.77, 1.54] | 0.99 | [0.69, 1.43] |
| | | Amides | 23 | 10.9 | 253 | 10.3 | 1.07 | [0.69, 1.65] | 0.99[d] | [0.64, 1.55] |
| | Daily | No nitrosatable drug exposure | 80 | 64.0 | 958 | 68.8 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 21 | 20.8 | 241 | 20.1 | 1.03 | [0.64, 1.67] | 1.03 | [0.63, 1.70] |
| | | Tertiary amines | 21 | 20.8 | 218 | 18.5 | 1.15 | [0.71, 1.85] | 1.14 | [0.70, 1.87] |
| | | Amides | 23 | 22.3 | 134 | 12.3 | **1.94** | [**1.22, 3.09**] | **2.02**[d] | [**1.25, 3.26**] |
| P4P6 | Less than daily | No nitrosatable drug exposure | 71 | 71.0 | 596 | 77.2 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 13 | 15.5 | 89 | 13.0 | 1.19 | [0.66, 2.15] | 1.11 | [0.58, 2.11] |
| | | Tertiary amines | 18 | 20.2 | 91 | 13.3 | 1.57 | [0.94, 2.63] | 1.41 | [0.79, 2.51] |
| | | Amides | 8 | 10.1 | 56 | 8.6 | 1.18 | [0.57, 2.46] | 1.11 | [0.51, 2.39] |
| | Daily | No nitrosatable drug exposure | 197 | 66.3 | 2575 | 73.6 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 61 | 23.6 | 562 | 17.9 | **1.40** | [**1.05, 1.87**] | **1.47** | [**1.10, 1.98**] |
| | | Tertiary amines | 45 | 18.6 | 420 | 14.0 | 1.38 | [1.00, 1.91] | 1.37 | [0.98, 1.91] |
| | | Amides | 33 | 14.4 | 281 | 9.8 | **1.49** | [**1.03, 2.16**] | 1.44 | [0.99, 2.10] |

Abbreviations: HR, hazard ratio; CI, confidence interval; P1P3, first trimester; P4P6, second trimester.

[a] Percentages for no nitrosatable drug exposure are based on total participants with complete information while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

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

[c] Adjusted for study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.

[d] Significant multiplicative interaction ($P < 0.05$)

compared to less than daily (aHR 1.45, [95% CI 1.05, 2.00]). Daily vitamin C supplementation did not appear to lower the effects of nitrosatable drugs on the risk of preterm delivery during the second trimester. Taking a vitamin C supplement every day in conjunction with secondary amines or amides were observed to have higher risk estimates than if vitamin C supplementation was taken less than daily.

Similar conclusions regarding the effects of vitamin C supplementation on nitrosatable drug use and moderately preterm births were observed. Women who took a daily vitamin C supplement during the first trimester of pregnancy in conjunction with nitrosatable drugs appeared to have higher risk of delivering a moderately preterm infant compared to those with less than daily supplementation, especially among amide users (aHR 2.17, [95% CI 1.29, 3.66] versus aHR 1.00, [95% CI 0.61, 1.65]) (Table 16); significant multiplicative interaction ($P$ = 0.03) was detected between first trimester amide exposure and supplemental vitamin C. A diminished association, however, was noted with daily supplementation and secondary amine usage, with a hazard ratio of 1.02 [95% CI 0.59, 1.77] compared to 1.46 [95% CI 1.02, 2.09] with less than daily supplement use. Exposures to secondary amines or amides and daily supplementation resulted in higher point estimates during the last two gestational periods. Though, for the second trimester of pregnancy, daily vitamin C supplements did appear to provide some benefits among women with tertiary amine exposures. A lower risk (aHR 1.33, [95% CI 0.92, 1.93]) for moderately

**Table 16.** Maternal Nitrosatable Drug Exposure by Gestational Period and Moderately Preterm Births Stratified by Vitamin C Supplementation, National Birth Defects Prevention Study, 1997-2005

| Gestational Period | Frequency of Vitamin supplement | Type of drug exposure | Cases | | Controls | | Unadjusted | | Adjusted | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | %[a] | No. | %[a] | HR[b] | 95% CI | HR[b,c] | 95% CI |
| P1P3 | Less than daily | No nitrosatable drug exposure | 152 | 70.1 | 2213 | 74.1 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 42 | 21.7 | 392 | 15.1 | **1.55** | [**1.10, 2.18**] | **1.46** | [**1.02, 2.09**] |
| | | Tertiary amines | 29 | 16.0 | 421 | 16.0 | 1.00 | [0.67, 1.49] | 0.93 | [0.61, 1.42] |
| | | Amides | 18 | 10.6 | 253 | 10.3 | 1.03 | [0.63, 1.68] | 1.00[d] | [0.61, 1.65] |
| | Daily | No nitrosatable drug exposure | 63 | 62.4 | 958 | 68.8 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 17 | 21.3 | 241 | 20.1 | 1.06 | [0.62, 1.81] | 1.02 | [0.59, 1.77] |
| | | Tertiary amines | 17 | 21.3 | 218 | 18.5 | 1.18 | [0.69, 2.02] | 1.17 | [0.67, 2.02] |
| | | Amides | 20 | 24.1 | 134 | 12.3 | **2.16** | [**1.30, 3.56**] | **2.17**[d] | [**1.29, 3.66**] |
| P4P6 | Less than daily | No nitrosatable drug exposure | 50 | 68.5 | 596 | 77.2 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 11 | 18.0 | 89 | 13.0 | 1.43 | [0.74, 2.75] | 1.38 | [0.68, 2.82] |
| | | Tertiary amines | 15 | 23.1 | 91 | 13.3 | **1.86** | [**1.05, 3.32**] | 1.82 | [0.94, 3.53] |
| | | Amides | 5 | 9.1 | 56 | 8.6 | 1.05 | [0.42, 2.64] | 1.04 | [0.40, 2.73] |
| | Daily | No nitrosatable drug exposure | 165 | 66.0 | 2575 | 73.6 | 1.00 | Referent | 1.00 | Referent |
| | | Secondary amines | 51 | 23.6 | 562 | 17.9 | **1.41** | [**1.03, 1.93**] | **1.48** | [**1.07, 2.04**] |
| | | Tertiary amines | 36 | 17.9 | 420 | 14.0 | 1.32 | [0.92, 1.89] | 1.33 | [0.92, 1.93] |
| | | Amides | 29 | 15.0 | 281 | 9.8 | **1.57** | [**1.06, 2.33**] | **1.55** | [**1.04, 2.31**] |

Abbreviations: HR, hazard ratio; CI, confidence interval; P1P3, first trimester; P4P6, second trimester.

[a] Percentages for no nitrosatable drug exposure are based on total participants with complete information while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

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

[c] Adjusted for study center, maternal age, race/ethnicity, education, pre-pregnancy diabetes, and pre-pregnancy hypertension.

[d] Significant multiplicative interaction ($P < 0.05$).

preterm births was detected among mothers with daily supplementation compared to less than daily (aHR 1.82, [95% CI 0.94, 3.53]).

No clear pattern was observed when examining total vitamin C intake (dietary intake and supplementation) and its effects on prenatal nitrosatable drug use (data not shown). A smaller HR was noted for preterm births in relation to secondary amine exposure during the first trimester of pregnancy among women with ≥85 mg/day of dietary vitamin C and daily vitamin C supplementation (aHR 1.04, [95% CI 0.51, 2.13]) compared with those with lower dietary vitamin C and less than daily supplement use (aHR 1.42, [95% CI 0.88, 2.30]). Conversely, an increase in risk was detected with high dietary vitamin C and daily supplementation for both tertiary amine and amide exposure and preterm births. The difference was most notable among women who reported taking amide drugs during the first trimester, with higher risk of preterm delivery among those with ≥85 mg/day of dietary vitamin C and daily supplementation (aHR 2.14, [95% CI 1.08, 4.23]) compared to <85 mg/day and less than daily supplementation (aHR 0.73, [95% CI 0.35, 1.51]). Conflicting results were also found with regard to exposures during the second trimester. Associations were only diminished between tertiary amine exposure and preterm births among women with higher dietary vitamin C intake coupled with daily vitamin C supplementation (aHR 1.27, [95% CI 0.79, 2.04]) compared to less than daily (aHR 1.62, [95% CI 0.74, 3.58]). All graphical depictions based on the Cox-Snell residual analysis yielded results that support a reasonably well-fit model. Additionally, the link test and

global test based on Schoenfeld residuals suggest no violation of the proportional hazards assumption.

**Comment**

In this large, population-based case-control study, we found that higher levels of dietary vitamin C (≥85 mg/day) reduced associations between nitrosatable drug exposure during the second trimester of pregnancy and both preterm and moderately preterm births. Women with dietary vitamin C levels ≥85 mg/day who also reported taking nitrosatable drugs during the first trimester, however, had higher risk of delivering a preterm infant. Conflicting results were noted with third trimester exposures, as higher risk of preterm births was observed among mothers who had ≥85 mg/day of dietary vitamin C intake in conjunction with secondary amine or amide usage while the opposite was detected with tertiary amines.

Daily supplementation with preparations containing vitamin C did not appear to diminish the association between nitrosatable drugs and preterm births or moderately preterm births. Inconsistent patterns were found with every gestational period of exposure for both outcomes. For instance, a reduction in risk was observed during the first trimester for preterm births among women with secondary amine usage and daily vitamin C supplementation compared to less than daily; however, the opposite was noted among mothers reporting to have taken drugs classified as either tertiary amines or amides. The second trimester

yielded similar contradictory results for both preterm and moderately preterm births. Vitamin C supplementation does not modulate the association between prenatal nitrosatable drug use and preterm births or moderately preterm births in the expected direction. Previous studies utilizing data from the NBDPS have found that prenatal dietary and supplemental vitamin C may diminish the association between nitrosatable drugs and selected birth defects.[274, 275] Results from the present study do not align with what has formerly been reported. Though dietary intake may confer some benefit in reducing risk of preterm births among women with nitrosatable drug use, particularly in the second trimester, vitamin C supplementation did not appear to have an ameliorating effect.

In a prospective cohort of pregnant women in North Carolina, total vitamin C intake preconceptionally and during the second trimester was examined for its relation with preterm births.[276] Although no association was noted between women with either preconception or second trimester total vitamin C intake less than the 10$^{th}$ percentile and overall preterm births, there was an increased risk of preterm births due to preterm premature rupture of the membrane (PPROM) (relative risk 2.2, [95% CI 1.1, 4.5]) among women with total vitamin C intake less than the 10$^{th}$ percentile preconceptionally. Other studies that have also reported a higher incidence of PPROM among women with low vitamin C levels (ascertained from serum or leucocytes).[277-279] However, when we examined the main effects of dietary vitamin C, we did not observe higher risk of preterm births (aHR 1.13, [95% CI 0.92, 1.39]) or moderately preterm births (aHR 1.10, [95%

CI 0.88, 1.38]) among women with less than 85 mg/day compared to those with ≥85 mg/day. In contrast, a significantly higher risk of preterm delivery was noted among women who did not take a supplement containing vitamin C during the second trimester of pregnancy (aHR 1.41, [95% CI 1.13, 1.75]). Mothers with less than daily vitamin C supplementation during the second trimester also had higher risk of delivering a moderately preterm infant (aHR 1.31, [95% CI 1.02, 1.67]), but to a lesser extent. Two previous studies have stated conclusions which differed from that of our own. Steyn *et al*.[280] noted no difference in preterm births between women who received 250 mg of vitamin C and women who were given a matching placebo daily until 34 weeks of gestation. Further, maternal supplementation with vitamin C and E beginning at 9 to 16 weeks gestation did not reduce the risk of preterm births among nulliparous women in a randomized controlled trial.[281]

Our study has a number of strengths, including the fairly large sample size of preterm delivery cases and controls. In addition, our study population consists primarily of control women from the NBDPS, one of the largest population-based, case-control studies of birth defects in the United States. Utilizing control data from the NBDPS is advantageous since control-mothers were those who delivered infants without any birth defects. Analyzing this population eliminates the confounding effects of birth defects since infants are more likely to be born preterm if congenital malformations are present.[301] An association was also observed with nitrosatable drugs and dietary nitrite and

total nitrite intake with selected birth defects in two previous studies.[274, 294] Second, NBDPS control data has been found to be representative of their base populations with respect to maternal age, smoking status, and prevalence of diabetes mellitus, though slight differences were observed in terms of maternal race/ethnicity and education.[282] Time to interview is also consistent between mothers of preterm and full term infants, with mothers of preterm and full term infants having both a 7.7 month median length of time from the EDD to the interview.

At this time it is not known when the critical window of susceptibility for preterm births is. Though this question has been addressed by numerous studies, no clear time frame has been pinpointed. It is reasonable to believe that the critical period of susceptibility would depend, at least partially, on the exposure and the pathway it initiates its action.[3] Because prenatal exposures vary in their chemical structure and biological activities, there may not be one particular period of susceptibility for preterm births, but would differ depending upon the exposure. One of the strengths of the present study is that various gestational periods of nitrosatable exposure were examined.

The present study has several limitations. Because we did not have information on several risk factors previously reported to influence the risk of preterm births, we were unable to account for factors such as prior history of preterm births,[2, 306] intrauterine infections,[15, 16, 18, 19] marital status,[29-31, 34, 36] and psychological or social stress.[307, 308] Results must be interpreted with caution as

many confounding maternal behavioral factors were not controlled for, suggesting that the measure of effect may be overestimated. In addition, NBDPS does not collect information on the clinical subtypes of preterm births, such as spontaneous preterm delivery after preterm labor, medically indicated preterm delivery, and spontaneous preterm delivery after PPROM. Examining preterm births as a group versus splitting them into their respective subsets remains controversial.[311, 312] Preterm delivery can result from diverse clinical pathways. For instance, rupturing of the membranes and spontaneous onset of labor is quite distinctive from fetal distress, which is managed with early delivery. Therefore, some have argued that separating preterm births by clinical presentation would be more appropriate. On the other hand, researchers who support examining preterm births as a group reason that conditions prompting medical intervention for early delivery, such as preeclampsia and fetal growth restriction, have similar mechanisms as the pathways resulting in spontaneous preterm delivery.[311, 313] The same predictors of medically indicated preterm births, including placenta abruption,[314] preeclampsia,[315] and restricted fetal growth[316, 317] also give rise to spontaneous preterm births. Consequently, since etiologies are shared, grouping preterm births is acceptable and would also offer an increase in statistical power.

Another limitation concerns potential maternal recall bias of drug exposures. In the original study, NBDPS focused on birth defects. Because of which, there was a concern that prenatal drug exposures would more likely be

recalled by mothers of infants with birth defects than mothers of infants without birth defects. It is unlikely that recall bias would be an issue in the present study, however, since our population consists solely of NBDPS control-mothers. In addition, little evidence of differential recall of drugs classified as nitrosatable, particularly antibiotics,[302, 303] antinauseants,[302] analgesics,[303] and benzodiazepines[303] have been reported. However, Werler *et al*.[304] noted that antibiotics had a 20% higher sensitivity among case-mothers than in control-mothers. To reduce recall bias, NBPDS utilized a two-level approach for drug assessment by first asking participants about drug use by indication and then prompting them with medication lists. This method of drug assessment has been shown to be more accurate than asking either type of question separately.[305] Medications were classified based on their nitrosatability and further categorized based on their functional groups (secondary amines, tertiary amines, or amides) after the interview. Because participants were not directly questioned about nitrosatable drug use, recall bias is not likely to be a major concern. Nevertheless, it may be possible that certain types of nitrosatable drugs have been recalled differently. Furthermore, possible exposure misclassification may have occurred even though extensive reviews[160, 288] were utilized since some components' nitrosatability may not have been tested or results from such tests may not have been published, resulting in exposures which would have been missed.

Assessment of dietary nitrates and nitrites were restricted to a FFQ that collected information on foods consumed a year prior to conception. Measurement error is a potential concern since not all dietary sources of nitrates and nitrites may have been captured.[321] Since estimates were based on food consumption a year prior to conception and not during the actual period of pregnancy, recall inaccuracy is an issue. However, any misclassification would be nondifferential since participants were not aware of nitrate and nitrite levels in foods reported to have been consumed at the time of the interview. In addition, Huber *et al*.[322] evaluated the effects of measurement error of the NBDPS FFQ using the simulation extrapolation algorithm by varying the amount of measurement error included in the model from zero to an additional 60% variability in 0.10 increments and found no substantive differences with regard to statistical significance or magnitude of effect size. Furthermore, a previous study found that the average consumption of vegetables and meats did not significantly differ before and during pregnancy.[323]

In the NBDPS, women were interviewed about their medications and supplement use from three months preconception to the end of pregnancy. And, as mentioned earlier, questions concerning diet were collected during the year prior to pregnancy. The specific timing of dietary or supplemental vitamin C intake in relation to nitrosatable drug use was not collected. Vitamin C is known to inhibit *N*-nitroso compound formation when administered concurrently with a nitrosatable precursor. Given that the precise timing of vitamin C intake could

not be ascertained, the reduced risk observed for preterm births may not be due to the effect of dietary vitamin C itself, but could be due to other factors or healthy behaviors correlated with higher dietary intake of vitamin C. In addition, we were unable to examine the effects of vitamin C supplementation during the third trimester of pregnancy on nitrosatable drug usage and preterm births due to insufficient numbers.

Multiple analyses and comparisons were involved in this study. Eighteen statistical tests were conducted to assess the interaction between dietary vitamin C and preterm and moderately preterm births. No statistically significant interactions were observed. To assess interaction between nitrosatable drugs and supplemental vitamin C with preterm and moderately preterm births, 12 tests were conducted. Two statistically significant interactions were detected, while only one would have been expected by chance.

In conclusion, we found that dietary vitamin C intake greater than 85 mg/day reduced the associations between nitrosatable drug exposures during the second trimester of pregnancy and both preterm and moderately preterm births. However, dietary vitamin C did not appear to confer the same benefits in the first or third trimester. Conflicting patterns for supplemental vitamin C were observed with every trimester of nitrosatable drug exposure and preterm births. No evidence has been observed within this study indicating that daily vitamin C supplementation reduces the association between prenatal nitrosatable drug use and preterm births or moderately preterm births. Further research is needed to

examine the role of vitamin C in reducing the potential risks of preterm births in relation to nitrosatable drugs, especially with respect to timing of supplement use in conjunction with nitrosatable drugs.

# CHAPTER V

# CONCLUSIONS

## Discussion

In this large, population-based case-control study based on NBDPS mothers (controls) of babies without major birth defects, who had EDDs between 1997 and 2003, we examined: 1) prenatal nitrosatable drug use and its relation to preterm births; 2) the association between dietary intake of nitrites and nitrates and preterm births; 3) the joint effects of nitrosatable drug use during pregnancy and dietary nitrate/nitrate intake on risk of preterm births; and 4) the role of dietary and supplemental vitamin C on the relation between nitrosatable drug usage and preterm births.

Exposure to nitrosatable drugs during the first trimester of pregnancy was associated with higher risk of preterm delivery. The strongest relationship was detected among nitrosatable drug use during the second trimester of pregnancy. Secondary amines were the most notable among the nitrosatable functional groups, with women who reported taking drugs classified as secondary amines during the second and third trimester of pregnancy having an increased risk of delivering a preterm infant. When timing of nitrosatable drug exposure was further examined by month of gestation, the strongest associations were observed during the sixth and seventh month. However, exposure during the

ninth gestational month yielded conflicting results. A reduction in risk for preterm delivery was observed among women with nitrosatable drug usage during the last month of gestation. Since length of gestation was taken into account, we have no explanation for the protective effect which was observed with nitrosatable drug use and preterm births during the ninth month of gestation. Similar results were found between nitrosatable drug exposure by trimester and month of gestation and moderately preterm births. These associations were generally stronger than that observed with all preterm births combined. Prenatal exposure to nitrosatable drugs during the second and third trimester of pregnancy, particularly secondary amines, might increase the risk of having a preterm delivery.

Insufficient evidence was found that would suggest a positive association exists between dietary intake of nitrates or nitrites (including animal and total nitrite) and preterm births. We did, however, find that higher levels of nitrite consumption from plant sources reduced the risk of delivering a preterm or moderately preterm infant when compared to the women with the lowest level of plant nitrite intake. This protective effect may be due to other nutrients and vitamins contained within grain products, vegetables, and fruit. In addition, a large portion of plant nitrite intake comes from cereals, which are often fortified with vitamins.

Prenatal nitrosatable drug use in conjunction with high levels of nitrite intake had a positive association with both preterm and moderately preterm

births. An increasing pattern was noted with secondary amine exposure and tertiles of dietary nitrite (first and second trimester), animal nitrite (every trimester), plant nitrite (first trimester), and total nitrite (first and second trimester) in relation to preterm births. Similar patterns were found with tertiary amine use and levels of dietary nitrite (every trimester), animal nitrite (every trimester), and total nitrite (second trimester). Nitrosatable drug exposures showed little to no association with preterm births among women in the lowest tertiles of dietary nitrite (including animal, plant, and total), with the exception of second trimester amide use and animal nitrite intake. Analyses of moderately preterm births yielded similar conclusions, though associations were weaker. Prenatal exposure to nitrosatable drugs, particularly secondary and tertiary amines, in conjunction with higher levels of dietary nitrites (including animal nitrites, plant nitrites, and total nitrites) may increase the risk of preterm births.

Higher levels of dietary vitamin C intake (≥85 mg/day) did not appear to lower the risk of delivering a preterm infant among women who reported taking nitrosatable drugs during the first trimester of pregnancy. However, during the second trimester, we observed that dietary vitamin C levels ≥85 mg/day reduced the association between nitrosatable drug exposure and preterm births. Dietary vitamin C conferred similar beneficial effects when combined with nitrosatable drug exposure and moderately preterm births. Conflicting results, however, were noted with exposure to nitrosatable drugs and dietary vitamin C during the third trimester. Though a lower risk of delivering a preterm infant was detected among

mothers with concomitant exposure to levels of dietary vitamin C ≥85 mg/day and tertiary amines, an increased risk was found with secondary amines and amides.

Daily supplementation with preparations containing vitamin C does not appear to diminish the association between nitrosatable drugs and preterm or moderately preterm births. Conflicting patterns were observed with every trimester of exposure for both outcomes. For instance, during the first trimester, a lower hazard ratio was observed for preterm births among women with secondary amine exposure who also took a daily vitamin C supplement compared to less than daily. In contrast, mothers who reported taking drugs classified as either tertiary amines or amides had higher risk if they also took a daily vitamin C supplement compared to those with less than daily. The second trimester yielded similar contradictory results for both preterm and moderately preterm births. This study has found no evidence that would indicate that daily vitamin C supplementation reduces the association between prenatal nitrosatable drug use and preterm births or moderately preterm births.

**Implications**

Prenatal nitrosatable drug usage, particularly during the second and third trimester of pregnancy, should be avoided as it has been observed to be associated with higher risk of preterm delivery. Women who are considering taking medications during pregnancy should seek the guidance of their

healthcare professional prior to usage. Non-nitrosatable drugs with similar therapeutic indications as possible alternatives during pregnancy are recommended. Among women who are prescribed nitrosatable drugs, such as anti-epileptics, prenatal exposure may be unavoidable. Consuming higher levels of vitamin C (≥85 mg/day) may attenuate the association between nitrosatable drugs and preterm births. Future studies should examine the role of vitamin C in reducing the potential risks of preterm births in relation to nitrosatable drugs, especially with respect to timing of supplement use in conjunction with nitrosatable drugs.

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