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MATERNAL OCCUPATIONAL EXPOSURE TO ORGANIC SOLVENTS DURING EARLY PREGNANCY AND SELECTED CONGENITAL ANOMALIES

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology, Gillings School of Global Public Health.

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ABSTRACT

Tania Alejandra Desrosiers. Maternal occupational exposure to organic solvents during early pregnancy and selected congenital anomalies
(Under the direction of Andrew F. Olshan, Ph.D.)

Background: As more women enter the labor force, there is increased epidemiologic interest in the possible effects of employment and occupational exposures on adverse pregnancy outcomes. Using data from the National Birth Defects Prevention Study, we examined the prevalence and patterns of maternal employment before and during pregnancy, and examined the relation between maternal occupational exposure to organic solvents during the periconceptional period (first trimester and month before conception) and neural tube defects (NTDs) and orofacial clefts (OFCs), which toxicological data suggest may be susceptible to oxidative stressors like solvents.

Methods: Cases of NTDs (anencephaly; spina bifida; encephalocele) and OFCs (cleft lip ± cleft palate; cleft palate) delivered between 1997 and 2002 were identified by birth defect surveillance registries in 8 states; non-malformed control infants were selected using birth certificates or hospital records. Exposure to aromatic, chlorinated and Stoddard solvents were estimated by industrial hygienist review of self-reported occupational histories in combination with a literature-derived exposure database. We used employment dates to examine variability in employment status and estimated exposure prevalence to any solvent across different time periods before and during pregnancy among controls. Odds ratios (OR) and 95% confidence intervals (CI) for the association between solvent class and each birth

defect group and component phenotype were estimated using logistic regression, adjusting for maternal age, race/ethnicity, education, pre-pregnancy body mass index, folic acid supplement use and smoking.

Results: Over 70% of mothers worked at some point 3 months before and during pregnancy; employment status was not constant throughout pregnancy for 25% of these women. The prevalence of estimated exposure to any solvent during the periconceptional period among mothers of NTD cases (n=511), OFC cases (n=1163) and controls (n=2997) was 13.1%, 9.6% and 8.2%, respectively. No solvent class was associated with OFCs in these data. Exposure to chlorinated solvents was associated with increased odds of NTDs (OR=1.96; CI=1.34, 2.87), particularly spina bifida (OR=2.26; CI=1.44, 3.53).

Conclusions: Future studies of maternal employment should focus on the biologically relevant critical exposure window to reduce misclassification. Maternal occupational exposure to chlorinated solvents during early pregnancy may be associated with NTDs and merits further research.

This work is dedicated to mothers.

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ABBREVIATIONS

BMI	body mass index
BPA	British Pediatric Association
CAS	Chemical Abstracts Services
CATI	computer-assisted telephone interview
CBDRP	Center for Birth Defects Research and Prevention
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CL	cleft lip
CL/P	cleft lip with or without cleft palate
CNS	central nervous system
CP	cleft palate
DOB	date of birth
EDC	estimated date of conception
ICD	International Classification of Diseases
IH	industrial hygiene/hygienist
IRB	institutional review board
JEM	job exposure matrix
LMP	(date of) last menstrual period
NAICS	North American Industry Classification System
NBDPN	National Birth Defects Prevention Network
NBDPS	National Birth Defects Prevention Study
NIOSH	National Institute for Occupational Safety and Health

NTD	neural tube defect
OFC	orofacial cleft
OR	odds ratio
OS	oxidative stress
OSHA	Occupational Safety and Health Administration
PLR	polytomous logistic regression
ppm	parts per million
ROS	reactive oxygen species
SES	socio-economic status
SOC	Standard Occupation Classification

CHAPTER 1: BACKGROUND

1.1 Organic solvents

Organic solvents are a group of liquid, hydrocarbon-based chemicals able to extract, dissolve, or suspend fats, oils, and waxes. They are commonly used in industrial, commercial, and household settings as a cleaner, degreaser, chemical thinner or dissolver, and as an intermediate or reagent during synthesis of other chemicals. They are also a major component of paints and paint thinners, stripping agents, dry cleaning solutions, printing inks, dyes, adhesives, pesticides, and gasoline. Solvents are most often used in mixtures; hundreds make up over 30,000 industrial solvent formulations.[1]

Organic solvents are classified into subgroups primarily by molecular structure or functional group, including aliphatic, alicyclic, aromatic, and halogenated (e.g. chlorinated) hydrocarbons, as well as alcohols, ketones, aldehydes, esters, petroleum distillates, and glycol ethers. This dissertation research focuses on six chlorinated hydrocarbons (carbon tetrachloride, chloroform, methylene chloride, perchloroethylene, trichloroethylene, 1,1,1-trichloroethane), three aromatic hydrocarbons (benzene, toluene, xylene), and a petroleum mixture known as Stoddard solvent.

1.1.1 Human exposure

Most people experience low-level exposure to solvents on a daily basis while using household cleaners or glue, painting, going to the dry cleaners, or filling their car at a gasoline station. Higher doses are delivered by cigarette smoking or exposure to

environmental tobacco smoke. Some organic solvents are also common air pollutants (e.g. benzene) and drinking water contaminants (e.g. trichloroethylene). Another source of exposure is intentional solvent abuse (known as "huffing"), which is increasing in prevalence.[2] Occupational exposure, however, is among the most important exposure sources to consider for two reasons: first, solvents are exceedingly common in the workplace across multiple industries; and second, exposure levels in the occupational setting are usually substantially higher than those encountered during casual daily exposure. Occupations commonly exposed to organic solvent mixtures include painters, artists, laboratory workers, mechanics and machinists, tile setters, plumbers and carpenters, shoe and leather production and repair workers, photographic processing workers, dry cleaning workers, those who work with glues, some cosmetologists and hairdressers, and chemical manufacturing and petroleum workers.[3-12] For many occupations, exposure concentrations in the air as well as in workers' blood are well-characterized using personal air sampling and biomonitoring. Table 1 summarizes common industrial applications and occupations with potential exposure to each of the 10 organic solvents of interest in this dissertation.

The Occupational Health and Safety Administration (OSHA) estimates that 10 million people are exposed to organic solvents in the workplace.[13] The most recent national data for women specifically come from the National Occupational Exposure Survey conducted in the early 1980's which estimated that approximately 400,000 women were exposed to toluene; 800,000 to trichlorethane; 400,000 to xylene; 150,000 to benzene; and 230,000 to Stoddard solvent;[12,13] these estimates are thought to underestimate current exposure prevalence since participation of women of reproductive age and mothers with young children in the paid labor force has steadily increased over recent decades.[14] Two

thirds of mothers age 15 to 64 years with a first birth between 2001 and 2003 worked for pay during pregnancy, up from approximately 44 percent among those with a first birth between 1961 and 1965.[15]

The exact proportion of women in the United States or elsewhere who are occupationally exposed to any organic solvent during pregnancy is unknown. In recent population-based case-control studies of maternal occupational exposure and birth defects, estimated exposure prevalence to any organic solvent at different points throughout pregnancy ranged widely from zero to nearly 40 percent among controls (Section 1.4). Approximately 4 percent of controls in a case-control study of fetal death in California self-reported occupational exposure to solvents or degreasers at some point during pregnancy; women reported a higher exposure prevalence in the first trimester (2.8%) compared to subsequent trimesters (2.0% and 1.2% in 2nd and 3rd trimesters, respectively).[16]

1.1.2 General toxicity

Given that organic solvents share many physical and chemical properties, their toxicity profiles are often described collectively. However, mechanisms of toxicity are thought to differ to some degree between agents and more information is known about some organic solvents than others. In the following sections, we describe solvent toxicity in general terms with attention to important known differences between solvent classes or individual solvents.

Organic solvents are volatile and lipophilic, which together with small molecular size and lack of charge contribute directly to their enhanced ability to be absorbed into the body. Inhalation is the most common route of exposure in humans using solvents at home or work, though dermal and oral absorption following direct contact is also possible. Once inhaled,

organic solvents are readily absorbed across the alveolar-capillary membrane in the lung and are then widely distributed throughout the body. These chemicals subsequently concentrate in lipid-rich tissues such as the brain (this accounts for observed central nervous system effects following acute exposure). Multiple metabolic pathways are involved in detoxification and also bioactivation depending on the specific solvent and route of exposure. In general, organic solvents are rapidly metabolized and excreted, and do not tend to persist in the body more than a few days after exposure ceases. However, constant exposure at any level results in a measurable body burden during the exposed time period and shortly thereafter.[17]

Health effects associated with organic solvent exposure have been investigated in both animal and epidemiological studies. In general, long-term exposure to organic solvents is thought to be neurotoxic, hepatotoxic, hematotoxic, and potentially carcinogenic. Acute effects following short-term exposure include fatigue, concentration disorder, dizziness, headache, and vomiting. Exposure to higher doses, such as those experienced during intentional solvent abuse, result in euphoria, delusions, hallucination, loss of consciousness and death.[13]

The various mechanisms by which organic solvents exert toxicity are unclear and assumed to vary from one solvent to another. Further, the toxicity of solvents within the same class can vary, whereby subtle differences in chemical structure translate into significant differences in toxicity. For example, halogenated hydrocarbons are known to be mutagenic by generating free radicals, while aromatic hydrocarbons seem to disrupt polyribosomes.[13] Other solvents are also thought to damage lipid membranes through lipid peroxidation.[18] In some cases, a solvent exhibits toxicity in its primary form; in other cases,

metabolites formed during activation of certain detoxification pathways have more potential to cause harm (e.g. trichloroacetic and dichloroacetic acids, the metabolites of trichloroethylene and perchloroethylene). Adding further complexity is the fact that most solvent exposures involve multiple chemicals, and less is known about the toxicity of solvent mixtures relative to individual compounds. The toxic effects of multiple solvents are often assumed to be additive, though solvents may also interact synergistically or antagonistically. For example, repetitive alcohol consumption induces the P450 enzyme system, which may subsequently result in metabolic activation of other solvents to cytotoxic metabolites.[13] Concurrent exposure to benzene and toluene is thought to reduce the genotoxicity and erythropoietic toxicity caused by benzene exposure alone.[19]

1.1.3 Developmental toxicity

A fundamental principle of teratology is that developmental toxicity may be manifested in several ways: embryonic or fetal death, malformation, growth retardation, or functional deficit.[20,21] For some compounds, these four endpoints correspond to a continuum of increasing toxicity whereby low doses cause growth restriction and higher doses cause malformations or death. However, any given compound can produce one outcome and not another under certain conditions and the primary outcome can change depending on the animal species or strain. It is often the case that concordant defects are not induced by the same teratogen in laboratory animals and humans,[17] though it has been demonstrated that humans may sometimes be up to 10 times more sensitive than lab animals to known teratogens given optimal conditions.[20] Therefore, an indication of developmental toxicity in animal models points to a possible effect in humans that warrants further investigation. Approximately one third of solvents tested for teratogenicity in animal models have been

positive,[13] though only ethanol and toluene (at high doses) are considered known human teratogens.[22] Of the 10 organic solvents included in this research project, all have been investigated with varying intensity with regard to developmental toxicity. Most exhibit teratogenic and fetotoxic effects (less evidence for methylene chloride and Stoddard solvent), and the developing nervous system appears to be a particularly sensitive target for specific compounds including xylene, toluene and perchloroethylene.[1,3-13,17]

In humans, there is not much information on the toxicity of organic solvents in-utero, though it is known that organic solvents cross the placental barrier. Changes in maternal toxicology during pregnancy, such as enhanced blood flow to the lungs and increased cardiac output, improve absorption of organic solvents after inhalation exposure.[1] Further, an increase in body fat during pregnancy allows for a higher body burden of solvents and reduces elimination time.[13] Factors related to the placental-fetal compartment also play a role in the absorption of these compounds. Approximately half of the fetal blood circulation reaches the fetal heart and brain directly,[18] and organic solvents are known to concentrate in the lipid-rich brain. Fetal capacity to "store" solvents may thus effectively increase the maternal-fetal body burden. Perchloroethylene, for example, has been shown to accumulate in the fetus at concentrations higher than those measured in the mother.[6] Since fetal metabolic pathways for most solvents do not exist, metabolism is conducted primarily by maternal systems.[2]

The specific mechanisms of teratogenesis by which organic solvents exert developmental toxicity are not well understood. A leading hypothesis is that these compounds create reactive oxygen species (ROS) and subsequently produce oxidative stress (OS) to which early embryonic development is strongly susceptible.[23-26] The role of OS in

best described for ethanol-induced toxicity, though the literature is growing on other organic solvents. Animal models suggest that alcohol exposure during early gestation decreases neural crest cell proliferation and promotes excessive cell death, leading to facial and brain abnormalities as well as reductions in certain antioxidant enzyme activity.[27,28] In general, oxidant-induced or redox misregulation of cellular components responsible for developmental signals may result in a decrease in cell proliferation, alter cytodifferentiation, or promote apoptosis. The effects of generalized OS on multiple specific signal transduction pathways leading to teratogenesis has been recently described in the literature.[24] Early organogenesis is identified as a particularly sensitive time period to changes in the redox environment since antioxidant defenses are still immature,[24] though OS could pose a significant threat to normal growth and development throughout gestation since oxidation diminishes the capacity of fetal tissues to biotransform xenobiotics.[26] Antioxidants have been shown to ameliorate the effects of excessive cell death in selected cell populations and subsequent malformations associated with exposure to teratogenic concentrations of ethanol.[28,29]

Several pharmaceuticals, pesticides, metals, and environmental contaminants are capable of generating ROS and subsequently inducing OS.[24,25,30] The capacity of a number of organic solvents to induce the ROS-OS mechanism has been documented, including carbon tetrachloride, chloroform, methylene chloride, trichloroethylene, perchloroethylene, and benzene.[24,30,31] There is also some evidence that neural tissue may be particularly vulnerable to oxidative stress caused by solvents. Following exposure, the early expression of CYP2E1 (a mixed function oxidase pathway primarily involved in the metabolism of some organic solvents) in the fetal brain suggests that this tissue may be particularly

vulnerable to OS as a result of solvent metabolism.[7] Several other factors, including a high rate of oxygen consumption, contribute to the unique sensitivity of CNS cells to OS.[30] The role of ROS in the targeted cytoxicity of neural crest cells following ethanol exposure is also well-documented and supports the hypothesis that solvents as a chemical class may exert preferential toxicity on the developing central nervous system.

The body of literature investigating the association between maternal solvent exposure and various reproductive and developmental outcomes in humans is slowly growing. Several adverse outcomes have been considered, including infertility and delayed conception, spontaneous abortion, preeclampsia, preterm birth, growth retardation, congenital malformation, and developmental delay.[3-13,17,32] Intentional solvent abuse has also been studied and is known to cause a spectrum of defects and developmental abnormalities known as Fetal Solvent Syndrome, which resembles the well-documented Fetal Alcohol Syndrome caused by alcohol consumption during early pregnancy. With a growing yet inconsistent body of evidence from experimental studies of animal models indicating that organic solvents exert developmental toxicity, these compounds can be reasonably expected to have the capacity to induce congenital malformations under certain conditions in humans as well.

Table 1. Description of the 10 organic solvents of interest in this dissertation

Solvent (CAS) *synonyms*	**Common industrial uses**	**Primarily exposed occupations**	**OSHA PEL (ppm)**
CHLORINATED SOLVENTS			
carbon tetrachloride (56-23-5) *carbona, carbon chloride, carbon tt, benzinoform, methane tetrachloride, perchloromethane, tetrachloromethane*	Used in manufacturing of: refrigerants aerosol propellants	Chemical manufacturing workers	10
chloroform (67-66-3) *trichloromethane, methyltrichloride*	Used in manufacturing of: fluorocarbons and refrigerants aerosol propellants plastics paper products Purification of antibiotics Photographic processing Dry cleaning agent Research chemistry	Water treatment plant workers Paper and pulp mill workers Waste management and incineration workers Pool or spa workers (including lifeguards and swimming coaches)	50
methylene chloride (75-09-2) *dichloromethane, methylene dichloride, methylene bichloride*	Used in manufacturing of: pharmaceuticals photographic film aerosol propellants Component of paint remover and floor stripping solution, spray paint, and automotive cleaner	Painters and paint industry workers Aerosol packing workers Metal cleaners	25
perchloroethylene (127-18-4) *PERC, tetrachloroethylene, ethylene tetra-chlorid, pert, perclene, perchlor*	Dry cleaning agent Degreasing agent Component of water repellent, silicone lubricant, fabric finisher, spot remover, adhesives, wood cleaner, printing ink, and rust removers Used as a textile-processing solvent	Dry clearners Metal cleaners	100
trichloroethylene (79-01-6) *TCE, 1,1,2-trichloroethylene, trichloroethene, 1,1-dicloro-2-chloroethylene, acetylene trichloride, ethylene trichloride*	Dry cleaning agent Degreasing agent Intermediate in chemical synthesis of other agents including organic solvents Component of adhesives, and lubricants Component of consumer cleaning agents including strippers, stain removers and rug-cleaning fluids	Degreasing operation workers Wood processing workers Plastics manufacturing workers Gas furnace operators and repair workers Laboratory technicians	100

1,1,1-trichloroethane (71-55-6) *TRI, methyl chloroform,1,1,1-TCE, α-trichloroethane, chloroethane*	Common industrial solvent found in consumer degreasing and cleaning agents Component of adhesives, aerosol sprays, and paint Used in microelectronics industry	*Currently, there are no highly exposed occupations because domestic production and use was phased out (effective 2002). Since 2005, no 1,1,1-trichloroethane was used in the U.S.*	350
AROMATIC HYDROCARBONS			
benzene (71-43-2) *benzyl,benzol, cyclohexatriene*	Used in manufacture of: detergents pesticides other solvents paint removers rubber lubricants dyes Component of gasoline Intermediate in chemical synthesis of other agents including styrene	Benzene production, storage, and transport workers (especially in petrochemical, petroleum refining, coke, coal, and chemical manufacturing industries) Rubber tire manufacturing workers Printing workers Rubber workers Shoe makers Laboratory technicians Firefighters Gasoline station employees Janitors and dry cleaning workers	1
toluene (108-88-3) *toluol, methylbenzene*	Used in manufacture of: benzene rubber Component of paints, inks, dyes, lacquers, fingernail polish, adhesives, and gasoline Used in the printing and leather tanning industries	Painters and paint industry workers Artists and printing workers Petroleum, fuel, and gasoline station workers Floor and carpet installation workers Automotive workers Cosmetologists	200
xylene (1330-20-7) *Xylol, dimethylbenzene*	Used in manufacture of: other organic solvents plastic and rubber leather and shoes coated fabric and paper Component of paints, wood finishers, and gasoline Carrier for insecticide application Used in the printing industry	Painters and paint industry workers Biomedical laboratory workers Wood processing plant workers Automobile garage workers Metal workers Furniture refinishers	100
PETROLEUM DISTILLATE			
Stoddard solvent (8052-41-3) *dry cleaning safety solvent, naphtha safety solvent, petroleum solvent, PD-680, varnoline, spotting naphtha*	Component of paint thinner, photocopier toner, printing ink, and adhesives Used as a dry cleaning solvent Used as a general cleaner/degreaser for engine parts in machine and automotive repair shops	Janitors and dry cleaning workers Printing workers	500

CAS: A unique chemical identification number designated in the Chemical Abstracts Services database.
OSHA PEL: "Permissible exposure limit," enforceable maximum concentration in workroom air allowable during an 8-hr workday in a 40-hr workweek.

1.2 Epidemiology of neural tube defects

This section provides a brief review of the epidemiology of neural tube defects, including etiology and classification, prevalence, risk factors and public health impact.

1.2.1 Etiology and classification of NTDs

Neural tube defects are a group of heterogeneous congenital anomalies affecting the central nervous system that result from failure of the neural tube to close at either the cranial or caudal neuropore during the fourth week of embryogenesis. Though primary closure is usually implicated, clinical and experimental evidence support the rare possibility that a closed neural tube can subsequently re-open under certain conditions.[33] During normal embryonic development, closure of the anterior (i.e. cranial) neuropore occurs on the 26th day of gestation and closure of the posterior (i.e. caudal) neuropore occurs on the 28th day. Defects resulting from secondary re-opening of the neural tube are thought to occur over an extended period of time later in development.

Neural tube defects affect either the spine or cranium, and are classified as open when neural tissue is exposed (open NTDs often involve both the spine and cranium) or closed when neural tissue is not exposed (closed NTDs usually affect the spine only).[33] Cranial NTDs include anencephaly and encephalocele. Anencephaly is a lethal defect defined by the absence of a large part of the brain, skull, and scalp due to failure of the cephalic portion of the neural tube to close. Spina bifida is the primary group of malformations of the spinal cord, and is defined by incomplete closure of the neural tube along the spinal column, typically in the lumbar region.

In epidemiologic investigations, NTDs are commonly grouped together and studied as one outcome to improve sample size. Although NTDs occur as a result of similar

embryologic processes, there is sufficient epidemiologic and biologic evidence supporting their etiologic heterogeneity.[34-36] For example, some teratogens are strongly associated with spina bifida but not anencephaly. These findings imply that defects of the brain and spinal cord should be considered individually when methodologically feasible.

1.2.2 Prevalence of NTDs

Neural tube defects are relatively common birth defects, affecting approximately one in 1000 pregnancies in the United States.[37] The true incidence of NTDs is difficult to estimate, since many cases do not progress to live birth. At least one third of all known cases of NTDs end in spontaneous or elective abortion.[35] However, the proportion of terminated pregnancies varies by geographic location, type of NTD, and gestational age at prenatal diagnosis.[38,39] In a study of 6 state surveillance programs from 1985 to 1994, between 10 and 40 percent of prenatally diagnosed cases were electively terminated.[38] Estimates in Europe are substantially higher.[40] Cases of anencephaly are more likely to be terminated than cases of spina bifida.

The fact that methods for case identification and ascertainment vary across surveillance programs complicates the estimation of national birth defect rates. Active surveillance programs usually yield more cases than passive systems, as do programs that seek cases among fetal deaths and electively terminated pregnancies. In 2007, population-based surveillance data for 45 specific defects from 32 states were published. However, differences in surveillance methodology precluded calculation of prevalence estimates for all states combined.[41] The National Birth Defects Prevention Network (NBDPN) published national estimates of select birth defects using pooled data from active population-based surveillance programs in 11 states from 1999 to 2001; this sample is thought to represent 22

percent of U.S. live births.[42] All of the surveillance programs included fetal deaths; all but one included elective abortions.[43] Table 2 presents the estimated prevalence of NTDs for births of all race/ethnicity. Note that the numerator of the estimates includes known cases among live births, fetal deaths and elected abortions, and the denominator includes live births only.

Table 2. Estimated prevalence of NTDs among U.S. births, 1999-2001, NBDPN.

Defect	Estimated prevalence per 10,000 live births* (95% CI)	Estimated annual no. of cases (95% CI)	Race/ethnicity trends compared to non-Hispanic whites
Anencephalus	2.51 (2.31, 2.70)	1,009 (931 – 1,088)	↓ BL; ↑ HISP
Spina bifida	3.68 (3.45, 3.92)	1,477 (1,383 – 1,572)	↑ HISP
Encephalocele	0.93 (0.82, 1.05)	376 (328 – 423)	↑ BL; ↑ HISP

* Estimates adjusted for maternal age and maternal race/ethnicity
Abbreviations: NTD = neural tube defect; NBDPN = National Birth Defect Prevention Network; CI = confidence interval; BL = non-Hispanic black, HISP = Hispanic
Source: Canfield (2006) [42]

These national estimates were recently updated using data from 2004-2006.[44] Though the estimated prevalence of NTDs were similar to that in 1999-2001, this updated analysis showed that the estimated prevalence varies significantly by type of surveillance system (active vs. passive with follow-up vs. passive) and pregnancy outcomes included (live births; stillbirths; terminations). Active surveillance systems that include all pregnancy outcomes ascertained the most cases.

As indicated in Table 2, the prevalence of NTDs varies by maternal race/ethnicity; infants of Hispanic origin born in the U.S. have a higher prevalence of NTDs as well at higher risk of mortality due to the defect than infants born to non-Hispanic white mothers.[42,45] Geographic and temporal variation in the prevalence of NTDs is also well-documented.[34,41]

1.2.3 Factors associated with NTDs

It is generally accepted that most cases of NTDs have a multifactorial etiology, with a significant genetic component that likely interacts with a number of environmental factors. To date, no single gene has been implicated as a direct causal agent. Chromosomal abnormalities, single gene mutations, and teratogenic causes are identified in less than 10 percent of cases.[35] Fortunately, the discovery of dietary folate as a protective factor has dramatically reduced the incidence of NTDs. Maternal B-vitamin folic acid intake of at least 0.4 mg/day before conception and during early pregnancy reduces the incidence of NTDs by up to 70 percent.[35,37] Folic acid is the greatest known modifier of NTD risk to date. Table 3 presents a comprehensive list of factors known or suspected of being associated with NTDs; factors indicated in *italics* are those considered to be known risk factors.

Table 3. Factors associated with neural tube defects

Maternal and fetal factors	**Environmental factors**
Alcohol use	Androgenic hormones
BMI (>29)	Chlorination disinfection byproducts
Demographic factors	Fumonisin-contaminated food
maternal age	Metals
maternal race/ethnicity	Nitrates
Folic acid	Pesticides
Hyperthermia and febrile illness	Proximity to landfills
Infant sex (female)	Some industrial chemicals
Parity	anesthetic agents
Previous history of SAB	organic solvents
Previous history of NTD	paints
Maternal metabolic conditions[1]	vinyl chloride
Maternal infections[2]	Some occupations
Serum glucose concentration	X-irradiation
diabetes	
hyperinsulinemia & hyperglycemia	
Smoking	
Stress	
Therapeutic drug use[3]	

[1] Maternal infections include: cytomegalovirus, rubella, syphilis, and toxoplasmosis
[2] Maternal metabolic conditions include diabetes, endemic cretinism, and phehylketonuria
[3] Therapeutic drugs include anticonvulsants, antihistamines, folic acid antagonists, diuretics, and sulfonamides
Abbreviations: BMI = body mass index; SAB = spontaneous abortion
Sources: Cabrera (2004); Detrait (2005); Hwang (2003); Mitchell (2005); Sever (1995); [34,36,37,46,47]

It has been recommended that the potential roles of environmental and occupational agents in the etiology of NTDs be more rigorously investigated.[36] Human susceptibility to environmental teratogens may hinge on a complex interaction of genetic susceptibility, appropriate timing, exposure characteristics, and the availability of protective factors.[37] The pathogenesis of NTDs is thought to involve a failure in cellular proliferation, alterations in the shape of the developing neuroectoderm, or abnormal changes in the supporting vasculature.[37] These events can be caused by a number of endogenous and exogenous factors that alter gene expression or damage cellular activity directly. It is possible that maternal exposure to organic solvents during early pregnancy could affect normal neurulation by altering gene expression or by inducing targeted cell death or damage. Refer to Section 1.1.3 for a description of the hypothesized biologic mechanisms by which organic solvent exposure may be associated with an increased risk of neural tube defects.

1.2.4 Public health impact

Infants born with a NTD have an increased risk of death in the first year and also in adult years; survival rates vary by phenotype and severity.[34,45,46] On average, survival among individuals born with spina bifida is approximately 87 percent at year one, and 78 percent by 18 years.[34] Anencephaly is uniformly lethal by the end of the first year; most affected infants are stillborn or die shortly after birth.[35]

Depending on the severity of the defect, affected infants also suffer significant morbidity ranging from mild physical dysmorphology to severe physical and developmental disabilities requiring lifelong management. For example, spina bifida often results in lack of neural function below the level of the defect and is associated with a range of negative sequelae including reduced ability to walk or paralysis, hydrocephalus, endocrine

abnormalities, deformation of the limbs or spine, learning disabilities, and bladder, bowel, and sexual dysfunction.[35,46] In addition, approximately 20 percent of NTD-affected infants are diagnosed with at least one other congenital anomaly.[35] The resulting physical, emotional and financial burden makes the reduction of these defects an important public health effort.

1.3 Epidemiology of orofacial clefts

This section provides a brief review of the epidemiology of orofacial clefts, including etiology and classification, prevalence, risk factors and public health impact.

1.3.1 Etiology and classification of OFCs

Orofacial clefts include cleft lip (CL) and cleft palate (CP) and result from incomplete fusion between any of the embryonic facial swellings destined to become part of the craniofacial area. Orofacial development in the embryo initiates with the appearance of the prechordal plate at the cranial end of the embryonic disk on the 14th day of gestation, and is fairly complete by the 48th day when the upper jaw and lip components fuse.[48] In general, the critical time window for OFCs is considered to be between the 6th and 10th week post-conception, though the period of development most sensitive to teratogens is day 36 for CL and weeks 8 through 9 for CP.[17,33] Cleft lip defects result from failure of the maxillary swelling to fuse with the intermaxillary process. These defects range in length (e.g. from a minor notch in the vermilion border of the upper lip to a cleft that completely separates the lateral lip from the philtrum and nasal cavity), depth (e.g. from involving just soft tissue to dividing the primary palate completely), and can be unilateral or bilateral. Cleft palate results from failure of the palatine shelves to fuse. These craniofacial abnormalities often occur together, though they are generally considered to be etiologically distinct. Very severe

cleft lip defects may induce clefting of the palate; therefore, cleft lip with and without cleft palate (CL/P) are often grouped together and considered distinct from isolated CP.[33,49]

1.3.2 Prevalence of OFCs

Orofacial clefts are the second most common congenital anomaly among live births. It is often cited that approximately one in 1,000 live births is affected with an OFC, which translates to 4,000 infants a year in the United States.[50] More recent estimates suggest that one in 850 and one in 1500 births per year are affected by CL/P and CP, respectively.[51,52] As described previously in Section 1.2.2, the NBDPN recently published national population-based prevalence estimates for select defects using data collected from active surveillance programs in 11 states from 1999 to 2001.[42] Table 4 summarizes select results for OFCs.

Table 4. Estimated prevalence of OFCs among U.S. births, 1999-2001, NBDPN.

Defect	Estimated prevalence per 10,000 live births* (95% CI)	Estimated annual no. of cases (95% CI)	Race/ethnicity trends compared to non-Hispanic whites
CL/P	10.47 (10.08, 10.87)	4,209 (4,050 – 4,367)	↓ BL
CP only	6.39 (6.08, 6.71)	2,567 (2,445 – 2,689)	↓ BL; ↓ HISP

* Estimates adjusted for maternal age and maternal race/ethnicity
Abbreviations: OFC = orofacial cleft; NBDPN = National Birth Defect Prevention Network; CL/P = cleft lip with or without cleft palate; CP = cleft palate; CI = confidence interval; BL = non-Hispanic black, HISP = Hispanic
Source: Canfield (2006) [42]

These national estimates were recently updated using data from 2004-2006.[44] Though the estimated prevalence of OFCs were similar to that in 1999-2001, this updated analysis showed that the estimated prevalence varies slightly by type of surveillance system (active vs. passive with follow-up vs. passive).

The distribution of CL/P varies by race/ethnicity, infant sex, geographic distribution, and demographic factors such as SES, whereas the distribution of CP is relatively uniform.[53] In general, Native Americans have the highest incidence of CL/P, followed by Asian-

Americans, non-Hispanic whites, and Hispanics. African-Americans have the lowest risk of both CL/P and CP. Interestingly, females are more likely to have CP (sex ratio = 3:2) and males are more likely to have CL/P (sex ratio = 2:1) in white populations, though this pattern is inconsistent across different race/ethnicities.[48]

1.3.3 Factors associated with OFCs

Like NTDs, the causes of OFCs are likely multifactorial. Though there is a strong pattern of familial aggregation, few modifiable risk factors are consistently and strongly associated with clefts other than alcohol consumption and cigarette smoking. Periconceptional smoking is consistently associated with a modest increase in orofacial clefts, particularly CL/P.[52,54] Though linear dose-response trends across levels of smoking have not been observed, heavy smoking (>25 cigarettes per day) is most strongly associated with having an OFC-affected pregnancy (OR = 1.8; 95% CI = 1.1 to 2.9).[52] This observation could be explained by misclassification of smoking at lower levels, or it could indicate a threshold effect whereby the risk of OFCs is impacted only by maternal smoking at higher levels of exposure. Several studies have also found maternal alcohol consumption to be associated with OFCs, though estimates vary by amount, timing, and type of alcohol.[55-60] Low-level consumption, for example, does not seem to be as strongly associated.[61] Interestingly, a recent NBDPS study reported an interaction between the type of alcohol consumed and folic acid intake as risk factors for CP.[62] Folic acid antagonists, such as alcohol, have been previously shown to be associated with an increased risk of OFCs.[63] Table 5 lists several factors suspected of being associated with OFCs; those indicated in *italics* are considered known risk factors.

Table 5. Factors associated with orofacial clefts

Maternal and fetal factors	Environmental factors
Alcohol use	Air pollution
Birth order	Altitude
BMI (>29)	Chlorination disinfection byproducts
Diabetes	Environmental estrogens
Folic acid	Ionizing radiation
Hyperthermia and febrile illness	Organic solvents
Infant sex	Pesticides and herbicides
Nutritional status	Proximity to landfills
Family history of OFCs	Some occupations
Race/ethnicity	
Smoking	
Stress	
Therapeutic drug use[1]	

[1] Therapeutic drugs include anticonvulsants, corticosteroids, folic acid antagonists, and vitamin-A formulas such as Accutane®

Abbreviations: BMI = body mass index; OFCs = orofacial clefts

Sources: Hayes (2006); Honein (2007); Larsen (2001); Murray (2002); Shaw (2006) [33,52,64-66]

Several pathogenic processes are thought to affect OFCs.[33] Cleft lip can be caused by inadequate migration or proliferation of neural crest cells that contribute to the development of the face, or it can be caused by excessive or targeted cell death during development of the craniofacial features. Cleft palate can be the result of inadequate growth of the palatine shelves, failure of the shelves to fuse, or secondary rupture after fusion. Animal models suggest that toluene and other organic solvents may induce OFCs through mechanisms similar to those observed with alcohol.[2,30,67] Refer to Section 1.1.3 for a description of the proposed biologic mechanisms by which organic solvent exposure may be associated with an increased risk of orofacial clefts.

1.3.4 Public health impact

Nearly a half billion dollars is spent each year on medical care for infants born with an OFC in the United States.[50] Individuals with orofacial clefts require significant medical attention as well as nutritional, dental, speech, and behavioral interventions.[65,68] Most cases of CL and CP can be repaired to some degree, but affected infants often require special

feeding intervention until surgeries can be performed. Typically, the lip is repaired by 3 months and the palate by 1 year. Affected individuals may face other adverse medical issues since approximately 70 percent of CL/P and 50 percent of CP cases are syndromic,[65] and more than 25 percent of all OFC cases are affected by multiple birth defects.[69]

Relatively little is known about the long-term effects of OFCs; however, there is mounting evidence that cases have increased all-cause mortality as adults, as well as increased risk for cancer, cardiovascular events, and suicide.[51] The physical, psychosocial and economic burden associated with orofacial clefts makes the reduction of these congenital anomalies an important public health effort.

1.4 Review of the epidemiological literature

This section describes and summarizes the body of epidemiologic studies investigating the association between maternal organic solvent exposure and NTDs and OFCs. Interest in adverse perinatal effects due to solvent exposure during pregnancy dates back several decades, with perhaps the most seminal studies of neural tube and orofacial clefts being conducted in the early 1980's by Holmberg *et al.* in Finland.[70-72] In general, most early studies observed a moderate positive relationship between solvent exposure and birth defects.[70-75] However, the collection of early studies is less methodologically sophisticated than recent investigations and interpretations of their results are thus subject to various limitations including confounding, recall bias, and exposure misclassification. Therefore, the summary presented here is limited to relevant studies published after 1990 with the intention of focusing on the most valid investigations and sound results. Further exclusion criteria (with citations for select examples) are as follows:

- Environmental (i.e. non-occupational) exposure [76-78]

- Studies of other solvents, such as glycol ethers [79,80]
- Studies where outcome is "any major malformation" [81-83]
- Studies of birth defects other than NTDs or OFCs [84]
- Studies of solvent-exposed occupations (e.g. "laboratory workers") unless organic solvents are considered to be the primary chemical exposure [83,85]
- Studies of paternal occupational exposure to organic solvents [86,87]
- Studies of highly selective non-representative populations, such as studies of women who self-identified to occupational health clinics for suspected solvent exposure [88,89]
- Case reports or case series (i.e. non-analytic studies)

Though many of the studies in the review presented here have been previously summarized in formal reviews of the literature,[67,90-92] we include additional studies published thereafter. The studies summarized in the following sections share some important study characteristics that help to inform an assessment of the quality of the research as well as help to potentially explain inconsistencies across study results. The primary study characteristics to be considered are study population, outcome classification, exposure assessment, exposure window, and exposure prevalence. Refer to Tables 6-7 for additional study details.

1.4.1 Studies of NTDs

Five studies meeting the aforementioned inclusion criteria for this review have examined the association between maternal occupational exposure to organic solvents and neural tube defects (Table 6).[93-97] This section briefly highlights their study characteristics and results.

Study population*:* Two studies were conducted in France (Cordier 1992; Garlantezec 2009), one in Mexico (Aguilar-Garduno 2010), one in California (Shaw 1999) and one in Texas (Brender 2002). The latter study focused exclusively on births to Mexican-American

women along the Texan-Mexican border. Three studies obtained cases from hospitals; one from a population-based birth defect registry, and one observed cases that occurred within a population-based prospective cohort of pregnant women. Outcome ascertainment: All studies grouped isolated cases of any NTD together except Aguilar-Garduno *et al.*, who exclusively focused on anencephaly. Shaw *et al.* reported that effects were also estimated for spina bifida and anencephaly individually. Exposure assessment: All studies employed an industrial hygienist to classify exposure to "any solvent" based on self-reported occupational histories. The two studies conducted in the U.S. also classified exposure to solvents resulting from hobby activities. Garlantezec *et al.* also considered self-reported exposure to multiple products considered to contain solvents. Cordier *et al.* distinguished between exposure to solvents in pure form and exposure to solvent-containing products. Exposure window: The critical period for teratogenic induction of NTDs is considered to be the 4th week of gestation. Both Brender *et al.* and Shaw *et al.* considered exposure during the perinatal period, defined as 3 months prior to conception to 3 months after conception. Cordier *et al.* and Garlantezec *et al.* restricted their analysis to mothers with jobs held "at the beginning of pregnancy". Aguilar-Garduno *et al.* focused on exposure that occurred 3 months before and one month after the last menstrual period. Exposure prevalence: Shaw *et al.* and Cordier *et al.* found a similar proportion of exposed controls: 38 and 32 percent, respectively. In the study by Garlantezec *et al.*, 47% of controls self-reported occasional or regular exposure to solvents, whereas the JEM-estimated exposure prevalence among controls was approximately 20%. In the other studies, no controls were estimated to be exposed. Results: Brender *et al.* estimated the odds ratio (OR) for occupational exposure only and any NTD to be infinite (95% CI = 1.8 to ∞; 7 exposed cases) since no controls were exposed. For any

solvent exposure (occupational or hobby), the OR was 2.5 (98% CI = 1.3 to 4.7; 36 exposed cases). Notably, furniture stripping and refinishing was the hobby most strongly associated with NTD-affected pregnancies (OR = 4.4; 95% CI = 0.8 to 31.1). Shaw *et al.* did not find an association between NTDs and either occupational or hobby exposure to organic solvents in general; the OR for combined exposure was 0.89 (95% CI = 0.69 to 1.1; 211 exposed controls). Cordier *et al.* also did not find an association between occupational exposure to any organic solvent (OR for frequency >50% of workday = 1.2; 90% CI = 0.4 to 4.4; 5 exposed cases). However, effect estimates were elevated for exposure to solvent-containing products on the job (OR for frequency >50% of workday = 2.0; 90% CI = 0.7 to 6.7; 8 exposed cases). Garlantezec *et al.* observed only 1 exposed NTD case; the OR for self-reported and JEM-estimated exposure was 6.58 (95% CI = 0.7 to 63.9) and 1.30 (95% CI = 0.1 to 12.5), respectively. Eight cases of anencephaly (5.5% of all cases) were estimated to be exposed to solvents in the study by Aguilar-Garduno *et al.*,but no OR was estimated because no controls were considered exposed.

1.4.2 Studies of OFCs

This section briefly highlights the study characteristics and results of five studies meeting the aforementioned inclusion criteria for this review that have examined the association between maternal occupational exposure to organic solvents and orofacial clefts (Table 7) [93,96,98-100]. Note that Cordier *et al.* (1992) and Garlantezec *et al.* (2009) investigated both OFCs and NTDs.

Study population: Three of five studies were conducted using cases obtained in hospitals or surgical centers in France (Chevrier 2006; Laumon 1996; Cordier 1992), one was conducted within a prospective cohort of pregnant women in France (Garlantezec 2009) and

one was conducted using cases across Europe identified through a population-based birth defect registry (Lorente 2000). Outcome ascertainment: Three studies combined all cases of cleft lip and palate, whereas the other two examined CP and CL/P individually. Exposure assessment: All studies employed an industrial hygienist to classify exposure to solvents based on self-reported occupational histories. Garlantezec *et al.* also considered self-reported exposure to multiple products considered to contain solvents. Cordier *et al.* distinguished between exposure to solvents in pure form and exposure to solvent-containing products. The other three studies estimated exposure to specific solvents or solvent classes. Exposure window: For OFCs, the critical period of development is considered to be from the 6th to 10th week of gestation. Each of the five studies estimated exposure at different time periods ranging from the first two months of pregnancy to anytime during pregnancy. Exposure prevalence: The exposure prevalence among controls varied substantially across these case-control studies: Chevrier *et al.* estimated 39 percent of controls to be exposed to any solvent. In the study by Garlantezec *et al.*, 47% of controls self-reported occasional or regular exposure to solvents, whereas the JEM-estimated exposure prevalence among controls was approximately 20%. In the population-based European study, the estimated exposure prevalence for toluene, aromatic hydrocarbons, and trichloroethane was 1 percent, 4 percent, and less than 1 percent, respectively. Cordier *et al.* estimated that 11 and 21 percent were exposed to pure solvents and solvent-containing products, respectively. Laumon *et al.* did not report exposure prevalence. Results*:* Chevrier *et al.* reported elevated odds of both CP (OR = 3.78; 95% CI = 0.7 to 20.7 3 exposed cases) and CL/P (OR = 9.45; 95% CI = 2.5 to 35.3; 14 exposed cases) with any exposure to chlorinated solvents. Significantly elevated odds of OFCs were also observed for petroleum solvents, for which a positive trend was

observed for increasing exposure score (based on intensity, frequency, and rater reliability) and CL/P ($p<0.01$). Generally, higher estimates were observed in this study for CL/P than CP alone for any solvent. In contrast, Lorente *et al.* found larger estimates for CP alone than CL/P, though odds ratios for both defect groups were elevated. The OR for CP alone was 3.02 (95% CI = 0.93 to 9.84; 4 exposed cases) for exposure to aromatic hydrocarbons, 6.47 (95% CI = 1.02 to 40.9; 2 exposed cases) for trichloroethylene, and 6.73 (95% CI = 1.19 to 38.0; 2 exposed cases) for toluene. Laumon *et al.* combined all cases of orofacial clefts and reported moderately elevated estimates for exposure to any solvent (OR = 1.62; 95% CI = 1.04 to 2.52), aromatic solvents (OR = 1.78; 95% CI = 0.89 to 3.54), and halogenated solvents (OR = 4.40; 95% CI = 1.41 to 16.15). However, these estimates were unadjusted for any potential confounding factors. Cordier *et al.* also combined all cases and observed highly elevated odds of any OFC with exposure to solvents in pure form (OR = 7.9; 90% CI = 1.8 to 44.9; 7 exposed cases) or to solvent-containing products (OR = 6.8; 90% CI = 0.2 to 40.1; 4 exposed cases). Garlantezec *et al.* observed an increased odds for combined all cases of OFCs combined (n=8) associated with both self-reported exposure to any solvent-containing product (OR = 3.60; 95% CI = 0.8 to 16.0; 5 exposed cases) and JEM-assessed exposure to any organic solvent based on job title (OR = 12.85; 95% CI = 2.6 to 64.7; 6 exposed cases).

1.4.3 Overall summary and limitations of previous research

In general, evidence supporting an association between maternal occupational exposure to organic solvents and NTDs and OFCs is inconsistent. The observed relationship seems to be strongest for OFCs and less so for NTDs. Inconsistency across studies could be explained by differences in study population, exposure assessment, or outcome

ascertainment. Most studies, however, report positive findings; it is unclear whether this is due to publication bias or whether these studies reveal a true underlying etiologic association. As previously discussed in this chapter, the role of organic solvent exposure in the development of defects originating from neural crest cells is biologically plausible, though potential mechanisms of toxicity are not well understood.

The recent epidemiologic studies investigating the relationship between maternal occupational organic solvent exposure and congenital anomalies reviewed in Section 1.4 are superior to previous work in that they apply improved methods for study design, exposure assessment, and data analysis. For example, most studies employed industrial hygienists to carefully review self-reported occupational histories and classify exposure by probability or frequency. This method is preferable to using self-reported exposure directly since it can reduce recall bias as well as exposure misclassification. Also, results from recent studies are adjusted for potentially confounding factors such as maternal age, BMI, and smoking. Despite these strengths, this collection of studies also shares limitations.

Exposure assessment is arguably the study characteristic with the most potential to directly influence observed results. In the absence of personal monitoring data, occupational exposure assessment methods are particularly sensitive to misclassification that can bias study results in either direction (i.e. toward or away from the null) to various degrees. Assessment by expert review is perhaps the optimal method available to most epidemiologic studies that must rely on indirect retrospective assessment.[101] Evaluations of generic job-exposure matrices (JEMs) in studies of organic solvent exposure as well as studies of birth defects suggest that sensitivity and specificity are often unsatisfactory and that hybrid JEMs that are study-specific and informed by expert review perform better.[101-103]

Even with a perfect method of exposure assessment, studies of organic solvent exposure are challenged by the fact that most occupational exposure is to solvent mixtures and not to individual compounds. This fact limits the ability of studies to isolate specific putative solvent exposures from others that may have no effect. Chevrier *et al.* (2006) attempted to compare women exposed to one solvent class with women unexposed to any solvent; the intended analysis could not be implemented given that all women exposed to chlorinated solvents in their study population were additionally exposed to either petroleum or oxygenated solvents.[100] Grouping exposure to "any solvent" is also problematic since concurrent exposure can have additive, synergistic, or antagonistic joint effects on the risk of adverse outcomes.

Choice of study population differed across these studies. Some studies included singleton births only, or restricted their sample to liveborn infants, which may introduce selection bias. Given the range of study populations, exposure assessment methods and exposure windows, it is not surprising that the exposure prevalence also ranged widely across these studies. However, there does not seem to be a pattern between exposure prevalence and magnitude of the observed effect estimates in this collection of studies.

Choice of referent group for analysis also differed across these studies. Some studies restricted eligible participants only to women who were working during the exposure period of interest; in these studies, exposed working women were compared to non-exposed working women. Other studies, however, included non-working women in the referent group. This latter approach can introduce confounding by factors such as SES.

Only the most salient issues specifically pertinent to studies of maternal occupational organic solvent exposure and NTDs and OFCs have been highlighted in this section.

Numerous other methodological considerations specific to epidemiologic studies of chemical exposures and birth defects have been discussed at length in the literature.[104,105] A recent review of occupational exposure to glycol ethers and congenital malformations developed a series of sensitivity analyses to show that both positive and null findings in the literature are quite sensitive to several methodological problems including selection bias due to unrecognized SAB, case non-response, or non-random control selection, as well as exposure misclassification and residual confounding.[106] For example, given a plausible range of sensitivity and specificity for exposure classification, the sensitivity analysis suggested that odds ratios observed by Cordier *et al.* (1997) for glycol ether exposure and CL/P could be 1.1 to 1.8 times the "corrected" OR resulting from perfect exposure classification. Similarly, given a plausible range of case response proportions, the same odds could be 0.7 to 1.6 times the "corrected" OR resulting from complete case response. Despite the sensitivity of results to such errors, weak or moderate associations that consistently recur across studies with different methodological characteristics may reflect true underlying mechanisms of teratogenesis and warrant further investigation.[107]

Table 6. Summary of epidemiologic studies investigating maternal occupational organic solvent exposure and neural tube defects

Author (year) ***Location***	**Study Characteristics**	**Exposure Assessment**	**Adjustment Covariates**	**Results** **OR (95% CI); # exposed cases**
Aguilar-Garduno *et al.* (2010) *Mexico*	*Design:* case-control *Study population:* Births identified by the Mexican Epidemiologic Surveillance System (2000-2001) *Outcome:* anencephaly (n=151), and 151 controls	*Exposure period:* between 3 months prior to LMP and 1 month after LMP *Exposure assessment:* IH review of self-reported occupational history *Exposure prevalence in controls:* no controls were exposed to solvents	Frequency matched by date of birth and delivery facility Models adjusted for maternal age, SES, adverse reproductive history, folic acid intake, caloric intake, cooking with wood, coal or tires	**Any solvent** *8 cases (5.5%) exposed &* *0 controls exposed;* *effect estimate not reported*
Garlantezec *et al.* (2009) *France*	*Design:* prospective cohort *Study population:* 3,421 pregnant women in Brittany (2002- 2005) *Outcome:* any CNS defect combined (n=4)	*Exposure period:* early pregnancy based on employment at 19 weeks *Exposure assessment:* self-reported exposure and job-exposure matrix *Exposure prevalence in controls:* 47% based on self-report; 20% by JEM	Models adjusted for tobacco and alcohol consumption Other factors considered but not adjusted for included maternal age and education	**Any solvent** *Based on self-reported exposure* 6.58 (0.7 – 63.9); 3 *Based on JEM-estimated exposure* 1.30 (0.1 – 12.5); 1
Brender *et al.* (2002) *Texas*	*Design:* case-control *Study population:* Births among Mexican-American women (1995- 2000) *Outcome:* any isolated NTD combined (n=225), and 378 controls	*Exposure period:* between 3 months prior to conception and 3 months after conception *Exposure assessment:* IH review of self-reported occupational history *Exposure prevalence in controls:* no controls were exposed to solvents	Frequency matched by year of birth and delivery facility Models adjusted by BMI and maternal age Maternal smoking was not found to be a confounder	**Any solvent** *Occupational exposure only* ∞ (1.8 – ∞); 7 *Hobby exposure only* 1.9 (1.0 – 3.6); 27 *Combined exposure* 2.5 (1.3 – 4.7); 36

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Shaw *et al.* (1999) *California*	*Design:* case-control *Study population:* Singleton births (liveborn, stillborn, or electively terminated) at hospitals in CA (1989-1991) *Outcome:* any isolated NTD (n=538), and 539 non-defect controls	*Exposure period:* between 3 months prior to conception and 3 months after conception *Exposure assessment:* IH review of self-reported occupational history and job-exposure linkage for task-specific exposures *Exposure prevalence in controls:* 38% for occupational exposure only	Unadjusted Maternal education, race/ethnicity, and multivitamin use were not found to be confounders	**Any solvent** *Occupational exposure only* 0.97 (0.71 – 1.3); 158 *Hobby exposure only* 1.1 (0.66 – 1.7); 45 *Combined exposure* 0.89 (0.69 – 1.1); 211
Cordier *et al.** (1992) *France*	*Design:* case-control *Study population:* Births at hospitals in Paris or Marseille (1984-1987) *Outcome:* any isolated CNS defect combined (n=83), and 83 controls	*Exposure period:* restricted to jobs held "at beginning of pregnancy" *Exposure assessment:* IH review of self-reported occupational history; classified by frequency *Exposure prevalence in controls:* 32% for pure solvents; 36% for solvent-containing products	Individually matched by delivery hospital Models adjusted for residential area, maternal age and SES	**Any solvent** *Any frequency* 1.0 (0.4 – 2.4); 12 *Frequency >50% of workday* 1.2 (0.4 – 4.4); 5 **Any solvent-containing product** *Any frequency* 1.4 (0.6 – 3.2); 15 *Frequency >50% of workday* 2.0 (0.7 – 6.7); 8

* 90% CIs in this study

Table 7. Summary of epidemiologic studies investigating maternal occupational organic solvent exposure and orofacial clefts

Author (year) ***Location***	**Study Characteristics**	**Exposure Assessment**	**Adjustment Covariates**	**Results** **OR (95% CI); # exposed cases**
Garlantezec *et al.* (2009) *France*	*Design:* prospective cohort *Study population:* 3,421 pregnant women in Brittany (2002- 2005) *Outcome:* any orofacial cleft defect combined (n=8)	*Exposure period:* early pregnancy based on employment at 19 weeks *Exposure assessment:* self-reported exposure and job-exposure matrix *Exposure prevalence in controls:* 47% based on self-report; 20% by JEM	Models adjusted for maternal age, education level, tobacco and alcohol consumption	**Any solvent** self-reported exposure 3.60 (0.8 – 16.0); 5 JEM-estimated exposure 12.85 (2.6 – 64.7); 6
Chevrier *et al.* (2006) *France*	*Design:* case-control *Study population:* Infants hospitalized at 9 hospitals (1998-2001) whose mothers all worked during the first trimester *Outcome:* CP (n=76), CL/P (n=164), and 236 controls	*Exposure period:* 1st trimester *Exposure assessment:* IH review of self-reported occupational history; classified by intensity, frequency, and reliability *Exposure prevalence in controls:* 39% to any solvent	Frequency matched by sex, age, geographic origin and residence The following factors were not found to be confounders: maternal smoking, alcohol intake, and first trimester dietary folate intake	**Chlorinated solvents** any vs. none CL/P: 9.45 (2.5 – 35.3); 14 CP: 3.78 (0.7 – 20.7); 3 **Petroleum solvents** any vs. none CL/P: 3.64 (1.5 – 8.8); 17 CP: 1.21 (0.3 – 20.7); 3 very low-low vs. none CL/P: 3.21 (1.1 – 9.3); 10 medium-high vs. none CL/P: 4.60 (1.1 – 19.2); 7 $p_{trend} < 0.01$ for CL/P
Lorente *et al.* (2000) *Europe*	*Design:* case-control *Study population:* Births identified by the European Registration of Congenital Anomalies (1989-1992) *Outcome:* CL/P (n=64), CP (n=36), and 751 controls	*Exposure period:* any time during pregnancy *Exposure assessment:* IH review of self-reported occupational history; classified by probability and frequency *Exposure prevalence in controls:* 1% for toluene; 4% for aromatic hydrocarbons; <1% for trichloroethane	Models adjusted for center, maternal age, SES, urbanization, and country of origin	**Aromatic hydrocarbons** any vs. none CL/P: 1.79 (0.62 – 5.16); 5 CP: 3.02 (0.93 – 9.84); 4 **Trichloroethylene** any vs. none CL/P: 3.21 (0.49 – 20.9); 2 CP: 6.47 (1.02 – 40.9); 2 **Toluene** any vs. none CL/P: 1.61 (0.15 – 17.7); 1 CP: 6.73 (1.19 – 38.0); 2

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Author (year) *Location*	Study Characteristics	Exposure Assessment	Adjustment Covariates	Results OR (95% CI); # exposed cases
Laumon *et al.* (1996) *France*	*Design:* case-control *Study population:* Infants presenting for outpatient facial surgery at 6 centers in Rhone-Alpes (1985-1989) *Outcome:* non-syndromic CL/P and CL combined (n=200), and 400 controls	*Exposure period:* 1st two months of pregnancy *Exposure assessment:* IH classification of self-reported exposure to solvents *Exposure prevalence in controls:* not reported	Unadjusted	**Any solvent** 1.62 (1.04 – 2.52) **Aromatic solvents** 1.78 (0.89 – 3.54) **Halogenated solvents** 4.40 (1.41 – 16.15)
Cordier *et al.** (1992) *France*	*Design:* case-control *Study population:* Births at hospitals in Paris or Marseille (1984-1987) *Outcome:* isolated CL and CP combined (n=29), and 29 non-defect controls	*Exposure period:* restricted to jobs held "at beginning of pregnancy" *Exposure assessment:* IH review of self-reported occupational history; classified by frequency *Exposure prevalence in controls:* 11% for pure solvents; 21% for solvent-containing products	Individually matched by delivery hospital Models adjusted for residential area, maternal age and SES	**Any pure solvent** *Any frequency* 7.9 (1.8 – 44.9); 7 *Frequency >50% of workday* ∞ (0.4 – ∞); 3 **Any solvent-containing product** *Any frequency* 6.8 (0.7 – 128.3); 8 *Frequency >50% of workday* 2.2 (0.2 – 40.1); 4

* 90% CIs in this study

CHAPTER 2: STATEMENT OF SPECIFIC AIMS

Toxicologic and epidemiologic evidence suggest a possible association between maternal occupational exposure to organic solvents and the risk of congenital anomalies. Though findings in recent investigations are inconsistent due to methodological differences and other factors, this potential association warrants further inquiry since many women work during early pregnancy and organic solvents are commonly used in various workplaces. The primary purpose of this research was to advance our knowledge about the potential relation between maternal occupational exposure to organic solvents during pregnancy and the risk of neural tube defects and orofacial clefts in offspring. The National Birth Defects Prevention Study (NBDPS), one of the largest ongoing population-based case-control studies of risk factors for major structural congenital anomalies, was for many reasons a notable framework in which to examine this research question and improve upon the methods of previous work. The available study population, for example, consisted of a large, population-based sample of demographically diverse mothers of carefully classified cases and controls delivered relatively recently in 8 states across the United States, including cases among fetal deaths and terminations from the majority of study sites. In addition, self-reported occupational histories and expert-assessed determinations of exposure to 10 organic solvents were available for up to 6 jobs that mothers reported having three months before and during pregnancy.

Particularly for studies of pregnancy outcomes that are most vulnerable during a specific period of development, like congenital anomalies, accurate assessment of prenatal

exposures requires evaluation of exposure during the appropriate time window. The effects of exposure misclassification due to exposure variability across critical time windows in studies of pregnancy outcomes have been described in the literature [16,108]. In short, greater variability in exposure across different time periods results in greater exposure misclassification when exposure is considered "anytime during pregnancy." However, if exposure is known to be invariable over the course of pregnancy, then the collection of timing-specific exposure may not be necessary. Little is known about the timing, pattern, and prevalence of occupational exposure to organic solvents during pregnancy. Therefore, we were also interested in exploring the prevalence and patterns of maternal employment and estimated solvent exposure during pregnancy. The specific aims of this dissertation project are as follows:

AIM 1: to explore the prevalence and pattern of occupational exposure to organic solvents among women before and during pregnancy

Specifically, to:

- determine estimated solvent exposure prevalence across different time periods before and during pregnancy
- describe within-woman variability in exposure status across different time periods before and during pregnancy
- estimate the magnitude and direction of bias resulting from misspecification of the critical window of exposure

AIM 2: to evaluate the impact of maternal occupational organic solvent exposure on selected major structural birth defects

Specifically, to:

- estimate the effect of estimated occupational exposure during the periconceptional period on the prevalence of NTDs and OFCs
- evaluate effect heterogeneity across component phenotypes of NTDs and OFCs

CHAPTER 3: METHODS

Chapter 3 describes the general analytic framework and methods for this research project. Additional descriptions of the methods are included in the manuscripts (Sections 4.2.2 and 4.3.2).

3.1 Study population

The National Birth Defects Prevention Study is an ongoing, multi-center, population-based case-control study designed to investigate genetic and environmental factors associated with over 30 major congenital defects.[109] It began in 1997, and is among the largest collaborative birth defect case-control studies in the United States. The study is sponsored by the CDC, which coordinates a group of Centers for Birth Defects Research and Prevention (CBDRP) that contribute to the study using data from local population-based birth defect surveillance systems. The annual birth population covered by the CBDRP (i.e. the sampling frame for cases and controls) represents approximately 10 percent of all U.S. births. Currently, there are 9 participating centers including Arkansas, California, Iowa, Massachusetts, New York, North Carolina, Texas, Utah, and a CDC-based center in Atlanta, Georgia; participating centers have changed over time.

3.1.1 Case and control ascertainment

Each center contributes approximately 300 cases and 100 controls to the study annually. Methods for case and control ascertainment vary by center. Potentially eligible cases are identified from each participating state's birth defect surveillance system. Some centers ascertain cases statewide (AR, IA, MA, NJ, UT), and others cover only selected areas

of the state (CA, NC, NY, TX, CDC). Cases include live births (all centers), fetal deaths greater than 20 weeks gestation (AR, CA, IA, MA, NC, TX, UT, CDC), and prenatally diagnosed elective terminations (AR, CA, IA, NC, TX, UT, CDC) with estimated or actual dates of delivery (EDD) on or after 01 October 1997 (01 January 1998 for AR and NJ; 01 January 2003 for NC and UT) who were diagnosed with at least one eligible birth defect of interest within the first year of life. New Jersey contributed cases and controls through 31 December 2002.

Controls include live births without a major defect with an EDD during the same time frame as cases. Controls are randomly selected from hospital delivery records (AK, CA, NY, TX, and CDC through 2000) or birth certificates (IA, MA, NC, NJ, UT and CDC since 2001). Though small differences exist between controls selected from hospital *vs.* controls selected from birth certificates, the combined population of controls is similar to target populations with regard to demographic and health factors including maternal age, race/ethnicity, and timing of entry into prenatal care.[110]

Additional eligibility criteria apply. Case and control infants must be in the custody of and reside with the birth mother to be eligible for the study. Birth mothers must be alive at the time of enrollment and speak either English or Spanish to be eligible.

3.1.2 Case classification

Case classification is standardized across all contributing centers. Clinical geneticists at each center review pertinent case information abstracted from medical records to determine eligibility for study inclusion. Eligible cases are then reviewed again by a team of NBDPS clinicians to confirm eligibility and to distinguish whether a case has the defect of interest as an isolated defect (e.g. no additional major, unrelated defects), as one of multiple

congenital anomalies (e.g. two or more major, unrelated defects), or as a component of a known syndrome, sequence or association. Cases with defects of known etiology (e.g. single-gene disorders and chromosomal anomalies) are excluded. An important purpose of the uniform case classification process is to apply what is known about embryologic and pathogenetic mechanisms to make case groups for analysis more comparable while respecting important etiologic heterogeneity between defects.[111]

3.2 Data collection

NBDPS collects information about participants from multiple sources: (1) medical and hospital records for case infants are reviewed for classification purposes; (2) mothers of cases and controls are interviewed; and (3) parents of cases and controls are asked to collect buccal cells from themselves and their infants as a source of DNA. Standard procedures are used for contacting, recruiting, and enrolling mothers of case and control infants, as well as for obtaining informed consent for all data collection procedures.[109] Contact with mothers is first established no earlier than 6 weeks after the infant's EDD. Monetary incentives are offered for completed participation in both the interview and collection of biologics.

3.2.1 Maternal interview

A structured computer-assisted telephone interview (CATI) Mother Questionnaire is administered in English or Spanish by female interviewers between 6 weeks and 24 months after the EDD. The average infant age at interview was 10 months for NTD cases, 10 months for OFC cases and 8 months for controls. The CATI takes approximately one hour and covers a wide range of health and environmental topics including demographic, physical, behavioral, nutritional, and chemical factors. Mothers are asked to report pre-conceptional and post-conceptional illness, medication use, vitamin use, residence, occupation, substance

abuse, information on the index pregnancy, and family history of birth defects. See Section 3.3 for a description of the occupational history section of the maternal interview.

3.2.2 Participation rates

Participation rates are calculated by case-control status, race/ethnicity, and defect group. The following participation rates are calculated by the NBDPS and are specific to the study population of cases included in this research project (i.e. EDD from study start date through 12/31/2002). The overall study participation rate (i.e. participation in the interview) was 72 percent for cases and 69 percent for controls. Among cases, non-Hispanic whites were more likely to participate (75%) than Hispanics or non-Hispanic blacks (65 and 63%, respectively). The same pattern held among controls. Defect-specific rates also varied slightly. Seventy percent of NTD cases participated; mothers of cases with spina bifida were more likely to participate than cases of anencephaly (74 *vs*. 63%, respectively). The participation rate among cases of orofacial clefts (76%) did not vary by phenotype.

3.3 Exposure assessment

The occupational history section of the maternal interview (Appendix A) identified mothers who were employed for at least one month duration from three months preceding the EDC through the end of pregnancy. Employment was defined as compensated, volunteer or military service, including part-time work and work performed at home. For each reported job, mothers were asked about the employer, job title, primary tasks and duties, chemicals and machines handled on the job, dates of employment, and hours and days worked per week; up to 6 jobs could be recorded. Jobs were then coded by occupation and industry according to the Standard Occupational Classification Manual (2000) [112] and North American Industry Classification System (1997).[113]

The National Institute for Occupational Safety and Health (NIOSH) led an initiative to perform a comprehensive occupational exposure assessments for women enrolled in the NBDPS through 2002. Study investigators identified specific substances to include in the assessments based on published estimates of exposure prevalence in the workplace and evidence in the scientific literature relating these agents to birth defects and other adverse health outcomes. Agents of interest included: chlorinated solvents, aromatic solvents , Stoddard solvent, glycol ethers, oil mist, polycyclic aromatic hydrocarbons (PAHs), pesticides, and metals. This dissertation research includes exposure data from assessments performed for 10 organic solvents including 3 aromatic solvents (benzene, xylene, toluene), 6 chlorinated solvents (carbon tetrachloride, chloroform, methylene chloride, perchloroethylene, trichloroethylene, 1,1,1-trichloroethane), and the petroleum-based mixture Stoddard solvent (also known as mineral or white spirits).

3.3.1 Assessment strategy

Comprehensive era-specific (1997-1999; 2000-2002) and solvent-specific job-exposure databases were developed for NBDPS by a team of occupational epidemiologists and industrial hygienists (IH) contracted by NIOSH. These job-exposure databases, based on extensive literature reviews of published papers reporting direct measurements and determinants of exposure for various occupations and industries, were then used in combination with IH review of self-reported job information to estimate the probability, intensity, and frequency of exposure for each reported job. *Probability* was defined as the likelihood that a specific job within an industry within a given era had any exposure to the solvent; each job was assigned one of the following categories for exposure probability: 0 (unexposed), <10%, 10-49%, 50-89% and ≥90%. *Intensity* was defined as the expected

concentration (ppm or mg/m^3) of solvent in the woman's breathing zone. *Frequency* was defined as the expected number of hours per week of exposure to the solvent, based on a 40-hour work week.

3.3.2 Estimated exposure prevalence

Approximately 12,500 jobs among 10,528 mothers who delivered between October 1997 and December 2002 were assessed for potential exposure to 10 organic solvents. Table 8 presents the distribution of estimated exposure to each of the solvents of interest.

Table 8. Distribution of estimated exposure to organic solvents among all jobs reported by NBDPS participants anytime during pregnancy or 3 months before conception, 1997-2002.

Solvent	Unique jobs (n=12,536)				
	Unknown	Not exposed	Exposed		
	n	n	n	%	Probability score (mode)*
Aromatic solvents					
Benzene	50	12,419	66	0.5	4
Toluene	49	12,255	231	1.8	4
Xylene	49	12,247	239	1.9	4
Chlorinated solvents					
Carbon tetrachloride	49	12,455	31	0.3	1
Chloroform	49	12,180	306	2.4	1
Methylene chloride	48	11,727	760	6.1	1
Perchloroethylene	48	12,057	430	3.4	1
Trichloroethane	48	11,725	762	6.1	1
Trichloroethylene	49	12,133	353	2.8	1
Stoddard solvent	41	12,148	346	2.8	2

*Exposure probability scores: 1 = <10%; 2 = 10-49%; 3 = 50-89%; 4 = and ≥90%.

The prevalence estimates in Table 8 are among all of the jobs held for at least one month duration anytime during pregnancy or the 3 months preceding pregnancy among mothers of controls and cases of cardiovascular defects, musculoskeletal defects, neural tube defects, orofacial defects, and male reproductive tract defects with an EDD on or before 31 December 2002.

3.3.3 Exposure characterization for analysis

To determine estimated exposure at the mother level (rather the job level) for various time windows during pregnancy, employment dates were linked to pregnancy dates for each mother.

Dates of employment obtained during the interview were recorded as the month and year that each job started and ended. Therefore, we developed an algorithm to assign complete job dates consisting of day, month and year. First, each job was assumed to begin on the first day of the reported starting month and end on the last day of the reported ending month. Second, for mothers with multiple jobs, jobs overlapping by exactly one month were assumed to have been held consecutively and job dates were further modified such that the overlapping month was approximately evenly divided between jobs (i.e., Job 1 was modified to end on mm/14/yy and Job 2 was modified to start on mm/15/yy). Any jobs overlapping by more than one month were assumed to have been held concurrently and job dates were not further modified. Complete job dates were set to missing if the starting month, starting year, ending month or ending year was unknown, or if the starting and ending dates were inconsistent; these jobs were later reviewed manually to determine whether partial job dates were informative.

Pregnancy dates reported during the interview included the date of the last menstrual period (LMP) and the infant's date of birth (DOB). The estimated date of conception (EDC) was calculated as the DOB-266 days, or LMP+14 days if the DOB was missing. We constructed five pregnancy time windows of interest: (1) before pregnancy, defined as 90 days preceding the EDC; (2) the first trimester, defined as the time between the EDC and 89 days after the EDC; the periconceptional period, defined as the time between 30 days preceding the EDC and the end of the first trimester; (4) the second trimester, defined as the

time between 90 days after the EDC and 179 days after the EDC (or the DOB, whichever came first); and (5) the third trimester, defined as the time between 180 days after the EDC and the DOB.

Job-level information was then summarized across women to obtain summary estimates of exposure for each mother for each time window of interest. Thus, for each solvent, a mother was considered *exposed* if any of her jobs during the time window was rated as exposed (i.e., probability of exposure > 0 for any job). She was considered *unexposed* if she did not have a job during the time window or if all her jobs during that window were rated as unexposed (i.e., exposure probability = 0 for all jobs).

3.4 Data analysis

All data management and analyses were conducted using SAS 9.1 (SAS Institute, Inc., Cary, NC) and Stata 9.2 (Statcorp, College Station, TX).

3.4.1 Analytic plan for Aim 1: Prevalence of solvent exposure during pregnancy

Aim 1 was to evaluate variability in occupational organic solvent exposure prevalence across different time periods of pregnancy.

Study population: The sample population included for this analysis included all mothers of NBDPS controls through 2002 who reported having at least one job ($n \approx 3{,}000$). We focused exclusively on controls under the assumption that the control population (and therefore the distribution of estimated exposure among controls) is a representative sample of the general study population.

Data analysis strategy: First, self-reported pregnancy dates were linked with self-reported employment dates to determine jobs that were held during different time periods before and throughout pregnancy. Then, jobs were linked with exposure data to determine

whether mothers were estimated to be exposed to any solvent in the different time windows. Exposure was characterized dichotomously (i.e. any/none) to any solvent. No further refinement in exposure (e.g. by solvent class, etc.) was considered under the assumption that variability in exposure is independent of the type of solvent to which a participant is primarily exposed. However, the average duration of exposure within each window was calculated to evaluate whether exposures during certain windows were more likely to be transient.

The next step was to evaluate exposure variability between windows. For this analysis, we compared mutually exclusive windows using the Kappa statistic, which indicates the degree of concordance above and beyond what would be expected by chance alone. We also constructed another measure of variability, which is the ratio of overall exposure prevalence to time-window-specific exposure prevalence (overall:time window [OTW] ratio).[16] This measure was developed for a previous investigation of exposures during pregnancy to characterize variability across time and to make inferences about subsequent misclassification. Assuming the distribution of exposure across pregnancy is known for all participants, the OTW ratio is defined as follows:

OTW ratio = p / p_i

$p = e/n$, where: p = overall prevalence

e = number exposed out of n participants

$pi = e_i/(n - d_i)$, where: e_i = number exposed in time interval i

d_i = number who did not survive to time interval i

As indicated in the formula above, the denominator of the time-specific prevalence excludes participants whose pregnancies ended prior to the start of the time window; this exclusion addresses survivor bias and is important since our study population includes fetal deaths, elective terminations, and live births of various gestational ages. Interpretation of the

OTW ratio is straightforward: the higher the ratio, the greater the variability in terms of different participants being exposed across different time windows; high ratios that do not change across pregnancy imply that prevalence is similar across windows, but different people are exposed at different windows. A consistently low OTW ratio implies that the same people are being exposed across time windows. The advantage of the OTW ratio over other measures of concordance is that it can be used to evaluate how well a crude definition of exposure (i.e. "anytime during pregnancy") performs relative to a more refined definition (i.e. "1st trimester only"), which is crucial when investigating the effect of a prenatal exposure on an outcome where the critically relevant exposure window may be narrow. Further, the OTW ratio has a direct relationship with exposure misclassification: the higher the ratio, the more misclassification is expected if exposure were considered anytime during pregnancy rather than during the narrower etiologically relevant window.

Additional analyses: Originally, we hypothesized that we would observe variability in solvent exposure status over the course of pregnancy, and thus we intended to conduct additional sensitivity analyses to empirically demonstrate the effect of misclassifying exposure during the biologically relevant critical window for congenital anomalies. In actuality, very little variability in exposure status over the course of pregnancy was observed in these data (Section 4.1) and therefore further analyses were not conducted. We then hypothesized that we did not observe variability in estimated exposure status over the course of pregnancy because exposure status was based on employment histories, which must have been very stable (i.e. no variability in employment status or occupation) over the course of pregnancy. Using the same strategy of linking job dates with pregnancy dates described earlier in this Chapter, we examined the assumption that employment status was constant (i.e.

women who worked *anytime* during pregnancy worked for *all* of their pregnancy). We considered a mother to be employed in a given time window if she worked at least one job during any portion of that window. We calculated the prevalence of maternal employment anytime before and during pregnancy and for each time window of interest. Among mothers employed anytime before and during pregnancy, we determined the proportion who experienced a change in employment status during pregnancy (e.g., from unemployed to employed) and the proportion who remained employed for the entire duration of their pregnancy. We also examined the distribution of major occupations held by women with different employment patterns. For these analyses, we extended our sample through December 2005 (n ≈ 7,000).

3.4.2 Analytic plan for Aim 2: Solvent exposure and NTDs

Aim 2 was to estimate the effect of maternal occupational solvent exposure during pregnancy on the prevalence of neural tube defects.

Study population: The study population included employed mothers of cases of NTDs (n=521) and non-malformed controls (n=2997) delivered between 01 October 1997 and 31 December 2002. These mothers had participated in the NBDPS interview, reported having at least one job during the time between the 3 months before the EDC through delivery (67% of participating cases were employed; 72% of controls), and were from the following NBDPS sites: Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York and Texas. We further excluded women with pregestational diabetes (7 NTD cases; 20 controls), and 3 cases and 5 controls with a first degree family history of NTDs.

Outcome assessment: We considered all NTDs combined as an outcome of interest as well as the following mutually exclusive NTD phenotypes: anencephaly and

craniorachischisis (BPA modification of ICD-9 [114] 740.0; 740.1), spina bifida (741.0; 741.9) and encephalocele (742.0).

Exposure window: We restricted the exposure period of interest to the periconceptional period, defined as one month preceding the EDC through the end of the first trimester.

Adjustment for confounding: Covariates of interest included those considered in the literature to be moderate to strong risk factors for NTDs that may be associated with maternal occupational solvent exposure but are not on the causal pathway between exposure and NTD incidence. The distribution of each covariate was examined and categorized appropriately. All covariates were included in adjusted models. Table 9 presents the categorization of each covariate.

Table 9. Covariates for analyses of maternal solvent exposure and congenital anomalies

Covariate	**Categorization**
Maternal age at delivery	<20 yrs 20-25 yrs (REF) 26-35 yrs ≥36 yrs
Maternal race/ethnicity	Non-Hispanic white (REF) Non-Hispanic black Hispanic Other
Maternal education	<12 yrs 12 yrs >12 yrs
Smoking	*During periconceptional period:* Any None
Folic acid supplementation	*During periconceptional period* Little/no use (≤30 days) Some use (>30 days, <daily) Daily use (REF)
Pre-pregnancy BMI	*Categorized according to NIH standard, with overweight and obese categories combined:* Thin/normal weight (<25) (REF) Overweight (25 ≤ BMI <30) Obese (≥30)

Descriptive analysis: Data analysis began with standard variable description, data cleaning, variable (re)coding, and univariate and bivariate graphical and tabular analyses for all outcome and exposure variables as well as covariates of interest. Disjoint indicator variables were constructed for categorical variables not meeting the assumption of linearity on the log scale. The amount of missing data was evaluated for each variable; no variable was missing ≥5% of the total sample. A series of pairwise associations between exposures, outcome of interest, and covariates were examined to gain familiarity with the underlying data structure, to examine the strength and pattern of associations between variables, and to evaluate correlation between primary exposures and covariates.

The crude association between exposure to any solvent and NTDs was stratified by each covariate of interest to assess effect measure modification using the Breslow-Day test for homogeneity (*a priori* α-level = 0.20). No effect measure modifying covariates were identified.

Modeling strategy: Three sets of models were conducted for all NTDs combined using unconditional logistic regression. Three additional sets of models were conducted for each series of component phenotypes (anencephaly, spina bifida, encephalocele) using polytomous logistic regression (PLR). In the first set of models, we estimated unadjusted odds ratios (OR) and 95% confidence intervals (CI) to examine the association between exposure to each solvent class and all NTDs combined and component phenotypes. In the second set of models, we estimated the *independent* effects of each solvent class by simultaneously including terms for each class in the models. The final set of models included terms for each solvent class as well as for the following maternal characteristics reported during the maternal interview: age at delivery, race/ethnicity, education, pre-

pregnancy body mass index, folic acid supplement use, and smoking. Within each PLR model, we evaluated heterogeneity in the estimated exposure effects across component phenotypes using likelihood ratio tests (*alpha*-level = 0.20).[115]

To account for the varying levels of estimated exposure probability in the exposure assessment, we repeated the primary exposure-defect analyses restricting the exposed group to women with at least one job with an estimated probability of exposure greater than or equal to 10% for any individual solvent within each solvent class. This strategy was used to sharpen the exposure contrast by excluding women less likely to be exposed. We also repeated analyses restricting all cases to only those with an isolated NTD, since cases of isolated congenital anomalies may differ etiologically from those presenting with multiple defects.

Beta-estimates from the logistic models estimated the log-odds of having an NTD-affected pregnancy among women estimated to be occupationally exposed to an organic solvent class during the periconceptional period of the index pregnancy, adjusting for all other covariates in the model.

Effect size calculation: *A priori* effect size calculations were performed assuming 500 NTD cases and 3000 controls for a range of distribution of exposure to solvents among all NBDPS participants presented in Table 8. All effect size calculations were performed assuming an alpha-level of 0.05, 80 percent power, and unadjusted dichotomous exposure contrasts. Table 10 presents the smallest detectable OR for all OFCs and a range of solvent exposure prevalence.

Table 10. Preliminary effect size calculations, NTDs.

Exposure prevalence (%)	NTD cases (n≈500)	Controls (n≈3000)	Minimum detectable OR
1.0	5	30	2.8
2.0	10	60	2.2
5.0	25	150	1.7
8.0	**40**	**240**	**1.6**
10.0	50	3000	1.5

This analysis had 80% power to detect an OR of approximately 1.6 for the association between estimated exposure to "any solvent" during the periconceptional period (observed prevalence ~ 8% among controls) and all NTDs combined. Actual estimable effect sizes were likely larger given loss of sample size due to sample restrictions, missing data, and multivariate adjustment.

3.4.3 Analytic plan for Aim 3: Solvent exposure and OFCs

Aim 3 was to estimate the effect of maternal occupational solvent exposure during pregnancy on the prevalence of orofacial clefts. Most features of the analytic framework and approach to data analysis (exposure window, adjustment for confounding, descriptive analysis, and modeling strategy) were the same as described for Aim 2 (NTDs). Therefore we describe only the differences below.

Study population: The study population included employed mothers of cases of OFCs (n=1249) and non-malformed controls (n=2997) delivered between 01 October 1997 and 31 December 2002. These mothers had participated in the NBDPS interview, reported having at least one job during the time between the 3 months before the EDC through delivery (67% of participating cases were employed; 72% of controls), and were from the following NBDPS sites: Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York and Texas. We further excluded women with pregestational diabetes (17 OFC cases; 20 controls), and 69 cases and 8 controls with a first degree family history of OFCs.

Outcome assessment: We considered all OFCs combined as an outcome of interest as well as the following two mutually exclusive OFC phenotypes: cleft palate alone (749.0 except 749.08) and cleft lip with or without cleft palate (749.1 except 749.19; 749.2).

Effect size calculation: *A priori* effect size calculations were performed assuming 1250 OFC cases and 3000 controls for a range of distribution of exposure to solvents among all NBDPS participants presented in Table 8. All effect size calculations were performed assuming an alpha-level of 0.05, 80 percent power, and unadjusted dichotomous exposure contrasts. Table 11 presents the smallest detectable OR for all OFCs and a range of solvent exposure prevalence.

Table 11. Preliminary effect size calculations, OFCs.

Exposure prevalence (%)	OFC cases (n≈1250)	Controls (n≈3000)	Minimum detectable OR
1.0	12.5	30	2.2
2.0	25	60	1.8
5.0	62.5	150	1.5
8.0	**100**	**240**	**1.4**
10.0	125	3000	1.3

This analysis had 80% power to detect an OR of approximately 1.4 for the association between estimated exposure to "any solvent" during the periconceptional period (observed prevalence ~ 8% among controls) and all OFCs combined.

3.5 Approvals

The Public Health Institutional Review Board (IRB) at the University of North Carolina, Chapel Hill, determined that this dissertation does not constitute human subjects research as defined by federal regulations and therefore does not require IRB approval (study no. 08-1875; 10/31/2008).

The National Birth Defects Prevention Study is approved by the IRB of the CDC as well as by all participating CBDRP. Additional approvals for this project required by the NBDPS, including approval of the research proposal by the internal Data Sharing Committee, data use agreement, and declaration of confidentiality and data security have all been satisfactorily met.

CHAPTER 4: RESULTS

4.1 Prevalence of solvent exposure during pregnancy

As originally proposed, Specific Aim 1 of this dissertation was to evaluate variability in occupational organic solvent exposure prevalence across different time periods of pregnancy. The results of the associated analyses are summarized below in Table 12. Approximately 8.7% of mothers were considered to be occupationally exposed to one or more organic solvents "anytime" during pregnancy (i.e., between 3 months before the EDC through delivery). Between different time windows, the exposure prevalence varied slightly from 6.2% in the third trimester to 8.0% in the first trimester and before conception. The series of low OTW ratios (close to 1.0) across all time windows suggests little-to-no within-woman variability in estimated exposure. In other words, the same women were considered exposed in each window. The interpretation of low OTW ratios is that there would be little exposure misclassification introduced by an anytime-during-pregnancy measure of solvent exposure status in these data even if the biologically relevant critical window for exposure was a narrower time window like a particular trimester. Kappa coefficients ranged from 0.77 to 0.95, also indicating high concordance in exposure status across mutually exclusive time windows.

Table 12. Estimated occupational solvent exposure prevalence, exposure duration, and OTW ratios for different pregnancy time windows among employed mothers of controls (n=2,997), NBDPS, 1997-2002.

	Anytime	**BEFORE**	**T1**	**T2**	**T3**
Exposure prevalence					
Exposed	261	239	240	211	185
Unexposed	2713	2736	2734	2768	2794
Unknown	23	22	23	18	18
Percent exposed among all mothers	8.7%	8.0%	8.0%	7.0%	6.2%
Length of time window (days)	388	90	90	90	118
Duration of exposure among exposed					
Mean	272	84	81	83	75
Min, Max	29, 378	2, 90	2, 90	4, 90	2, 108
OTW ratio	--	1.09	1.09	1.24	1.41

BEFORE, conception – 3 months; T1, first trimester; T2, second trimester; T3, third trimester; OTW, overall-to-window

Originally, we hypothesized that we would observe variability in solvent exposure status over the course of pregnancy, and thus we intended to conduct additional sensitivity analyses to empirically demonstrate the effect of misclassifying exposure during the biologically relevant critical window for congenital anomalies. In actuality, very little variability in exposure status over the course of pregnancy was observed in these data and therefore further analyses were not conducted.

We hypothesized that an explanation for this lack of variability in estimated exposure status over the course of pregnancy was because exposure status was based on employment histories, which must have likewise been very stable (i.e. no variability in employment status or occupation) over the course of pregnancy. Using the same strategy of linking job dates with pregnancy dates described earlier (Section 3.3.3), we examined the assumption that employment status was constant throughout pregnancy (i.e. women who worked *anytime* during pregnancy worked for *all* of their pregnancy).

Manuscript 1 (Section 4.2) is the resulting brief report that presents the rationale, methods and results of our examination of the prevalence and patterns of employment before and during pregnancy among mothers of controls in the NBDPS, as well as a discussion of

the implications of our findings for future studies of prenatal exposures and adverse pregnancy outcomes.

4.2 Manuscript 1: Patterns of maternal employment before and during pregnancy

4.2.1 Introduction

In epidemiologic studies of prenatal exposures and pregnancy outcomes, exposure variability over the course of pregnancy is important to consider since the *timing* of the exposure frequently determines the nature and magnitude of its effect.[108,116] Thalidomide, for example, is associated with a spectrum of human embryopathies depending on the timing of exposure: 20-23 days after conception causes external ear malformations, whereas exposure 24-31 and 27-33 days after conception causes upper and lower limb defects, respectively.[117] Another example is tobacco use, which is more strongly associated with restricted fetal growth as gestational age increases, such that no effect on fetal growth is observed among women who stop smoking earlier in pregnancy.[118] Identifying critical windows for prenatal exposures can advance hypotheses about biologic mechanisms, inform exposure assessment, and help identify susceptible populations for public health intervention. In practice, this pursuit requires use of time-dependent exposure measures that capture exposure variability over the course of pregnancy.

Collection of timing-specific occupational information (e.g., by month of pregnancy) is resource intensive and particularly challenging in retrospective studies. Often, studies of maternal employment obtain only one measure of employment status, such as employment at delivery or "anytime" during pregnancy, and assume that employment status is constant throughout pregnancy. If employment status is *not* constant, this practice can lead to biased

measures of association between employment and adverse pregnancy outcomes due to misclassification of employment during the critical window of exposure.[16,107]

There is little evidence in the literature to support – or refute – the assumption that maternal employment status is constant throughout pregnancy. Published reports on patterns of maternal employment have generally focused on duration of employment (*What proportion of employed women work into their second [third] trimester?*) rather than changes in employment status (*What proportion of women start [stop, remain] working during pregnancy?).*[15,119] Our objective was to explore the latter questions. In the following brief report, we present a description of the prevalence and patterns of maternal employment before and during pregnancy in a population-based sample of mothers of infants delivered between 1997 and 2005 who participated in the National Birth Defects Prevention Study (NBDPS).

4.2.2 Methods

The NBDPS is an ongoing case-control study of risk factors for structural congenital anomalies in the United States.[109] The study population for this analysis consisted of mothers of live born infants with no major birth defects (controls) delivered between October 1997 and December 2005 who were randomly selected from birth certificates or hospital delivery records in 10 participating states (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas and Utah). Approximately 66% (n=6,807) of eligible mothers participated in the NBDPS during this time.

Mothers were interviewed in English or Spanish using a structured telephone questionnaire within 24 months after delivery; median infant age at interview was 8 months. During the interview, mothers were asked whether they had been employed at any time

between the three months before they became pregnant and their infant's date of birth. Employment was defined as any compensated or volunteer work lasting at least one month, including military service. Dates of employment for up to six jobs were obtained and recorded as the month and year that each job started and ended.

We developed an algorithm to assign complete job dates consisting of day, month and year. First, each job was assumed to begin on the first day of the reported starting month and end on the last day of the reported ending month. Second, for mothers with multiple jobs, jobs overlapping by exactly one month were assumed to have been held consecutively and job dates were further modified such that the overlapping month was approximately evenly divided between jobs (i.e., Job 1 was modified to end on mm/14/yy and Job 2 was modified to start on mm/15/yy). Any jobs overlapping by more than one month were assumed to have been held concurrently and job dates were not further modified.

Pregnancy dates reported during the interview included the date of the last menstrual period (LMP) and the infant's date of birth (DOB). The estimated date of conception (EDC) was calculated as the DOB-266 days, or LMP+14 days if the DOB was missing. We constructed four mutually exclusive pregnancy time windows: (1) before pregnancy, defined as 90 days preceding the EDC; (2) the first trimester, defined as the time between the EDC and 89 days after the EDC; (3) the second trimester, defined as the time between 90 days after the EDC and 179 days after the EDC (or the DOB, whichever came first); and (4) the third trimester, defined as the time between 180 days after the EDC and the DOB.

We considered a mother to be employed in a given time window if she worked at least one job during any portion of that window. We calculated the prevalence of maternal employment anytime before and during pregnancy and for each time window of interest.

Among mothers employed anytime before and during pregnancy, we determined the proportion who experienced a change in employment status during pregnancy (e.g., from unemployed to employed) and the proportion who remained employed for the entire duration of their pregnancy.

Of the 6,807 eligible mothers, 98 were excluded from all analyses because they did not provide any employment information during the interview. We further excluded 96 women from all time window-specific analyses: 2 women who reported having worked but did not provide any additional employment information, 63 women with missing employment dates that could not be reconciled by manual review, and 31 women whose reported employment dates were inconsistent with the time period of interest (i.e., between 3 months before conception and delivery).

4.2.3 Results

Seventy-two percent of women (n=4,832) reported having been employed anytime before or during pregnancy (Table 13). The highest prevalence of employment was observed during the first trimester (67.8%) and the lowest during the third trimester (58.7%), indicating that fewer women were employed later in pregnancy.

Further examination of within-woman patterns of employment among mothers employed anytime before and during pregnancy revealed that approximately 75% (n=3,569) of women were consistently employed across all time windows (Table 14). The remaining women experienced a change in employment status at some point during pregnancy. Among these women, the most common patterns represented women employed before pregnancy who ceased employment during the first or second trimester and remained unemployed

thereafter (n=710; 15.0%). It was less common for women to be unemployed before conception and initiate employment sometime during pregnancy (n=344; 7.3%).

Table 13. Prevalence of employment across time windows of pregnancy among mothers of non-malformed liveborn controls, National Birth Defects Prevention Study, 1997-2005 (n=6,709).

Anytime[a]		Pregnancy time windows							
		Before conception[b]		First trimester		Second trimester		Third trimester	
n	(%)	n	(%[c])	n	(%[c])	n	(%[c])	n	(%[c])
4832	(72.0)	4392	(66.4)	4482	(67.8)	4262	(64.4)	3870	(58.7)

[a] Defined as the 3 months before the estimated conception date through the infant's date of birth.
[b] Defined as the 3 months before the estimated conception date.
[c] Window-specific estimates are based on all mothers who reported working anytime during pregnancy for whom information was available about the timing of all jobs relative to each pregnancy time window (n=4736), plus non-employed mothers (n=1877), with the exception of the third trimester, for which mothers who delivered in the second trimester were also excluded (n=21).

Table 14. Patterns of employment status before and during pregnancy among mothers of non-malformed liveborn controls, in descending order by frequency, National Birth Defects Prevention Study, 1997-2005.

Time windows (**x** = employed during window)				Number of women	Percent of employed women only (n=4736)[a]	Percent of all women (n=6709)
Before conception[b]	First trimester	Second trimester	Third trimester			
x	**x**	**x**	**x**	3,569	75.4	53.2
x	**x**	**x**		370	7.8	5.5
x	**x**			340	7.2	5.1
	x	**x**	**x**	152	3.2	2.3
		x	**x**	110	2.3	1.6
x				98	2.1	1.5
	x	**x**		39	0.8	0.6
			x	24	0.5	0.4
Other patterns[c]				*34*		

[a] All mothers who reported working anytime during pregnancy for whom information was available about the timing of all jobs relative to each pregnancy time window
[b] Defined as the 3 months before the estimated date of conception.
[c] Other observed patterns: first trimester only (n=10); second trimester only (n=9); all windows except first trimester (n=13); all windows except second trimester (n=2).

4.2.4 Discussion

Recent data on the prevalence and patterns of maternal employment during pregnancy are limited. The U.S. Census Bureau reports that 67% of women worked for pay at some point during the pregnancy leading to their first birth between 2001 and 2003, and that 87% of these employed women worked into their last trimester.[15] In contrast to the Census report, our study included both primi- and multiparous women who worked with or without pay for at least one month duration. Despite differences in design, our results were generally consistent with the Census report. We found that 72% of women worked anytime before and during pregnancy, and that approximately 80% worked into their last trimester. Unique to our study, we further examined patterns of employment status, revealing that three fourths of employed women were consistently employed in one or more jobs before pregnancy through their last trimester, whereas one fourth of women changed employment status during pregnancy. Among women who changed employment status, more than twice as many were employed before pregnancy and later stopped working (15%) than started working after their pregnancy began (~7%).

The fact that employment status is not constant over the course of pregnancy for a large proportion of women (25%) means that single measures of employment status may not accurately characterize maternal occupational exposure occurring in different time windows during pregnancy. Use of anytime-during-pregnancy measures will include women employed during irrelevant time periods and introduce misclassification that, in general, will bias effect measure estimates toward the null.[16,107] The effect of this misclassification could be magnified in a study in which the exposure of interest is not employment *per se* but rather some other related factor like job title or chemical exposure that further varies *within* levels of employment status. Results from studies using an anytime-during-pregnancy measure of

employment status should therefore be interpreted cautiously as null findings may reflect attenuation due to exposure misclassification rather than evidence of no effect. However, since 95% of women who were employed anytime before and during pregnancy were employed in the first trimester, misclassification resulting from use of an anytime-during-pregnancy measure may be less of a concern for studies in which the critical window is known to be around the time of conception or early pregnancy.

Use of a single-point-in-time measure of employment status, such as "employment at delivery," will likewise introduce exposure misclassification if the point in time does not correspond to the critical window. Consider a study in which the biologically relevant window is shortly after conception, but employment information is limited to employment status at the time of delivery. Given the employment patterns observed in our sample, this hypothetical study would incorrectly classify 16% of employed women as non-employed (i.e., women employed in the first trimester but not the third) and 3% of non-employed women as employed (i.e., women employed in the third trimester but not the first). This potential for misclassification may be of greater concern in studies that obtain employment information from birth certificates, on which it's often unclear whether employment corresponds to usual occupation, most recent occupation, current occupation at delivery or occupation at some other time before or during pregnancy. Studies investigating the validity of parental occupation information on birth certificates suggest that employment is generally underreported.[120,121] In one such study by Brender et al. (2002) of nearly 650 women in Texas who participated in NBDPS,[121] approximately one third of women who reported via interview that they were employed during the first trimester were documented on the birth certificate as unemployed (JD Brender, written communication of unpublished data, 2/2011).

A number of factors make the NBDPS sample of controls a useful framework in which to explore patterns of maternal employment during pregnancy. Our population-based sample of nearly 7,000 primi- and multiparous women was geographically and demographically diverse and spanned over 8 recent years. Detailed occupational histories were carefully collected via interview, allowing for employment status to be determined for different time periods before and during pregnancy. Additionally, because mothers were interviewed soon after delivery, we minimized the potential for recall error in employment dates.

Despite the short recall period, error in self-reported employment dates or in our assignment of complete job dates may have resulted in misclassification of employment status in any of the four pregnancy time windows, thereby influencing the observed patterns of employment. Another limitation of our study is that mothers participating as controls in the NBDPS may not be representative of other populations of women with different distributions of factors related to employment. Participants were all mothers of live born non-malformed infants, and were more likely than women in their base population to be white and have more years of education.[110] Though participants were employed in a wide variety of occupations,[122] other differences in demographic, behavioral and obstetric factors associated with self-selection into (and out of) employment likely influenced the prevalence and patterns of employment observed in our study, and thus the ability to generalize our findings to other populations of pregnant women.[123,124]

In conclusion, we found that employment status is not constant over the course of pregnancy for a substantial proportion of women. Our findings underscore the importance of using a time-dependent assessment of employment status that corresponds as closely as

possible to the biologically relevant critical window to reduce exposure misclassification. We encourage investigators to consider the impact of within-woman variability in employment status in the design and analysis of future studies of maternal employment and adverse pregnancy outcomes.

4.3 Manuscript 2: Maternal occupational exposure to organic solvents during early pregnancy and selected congenital anomalies

4.3.1 Introduction

Organic solvents are a group of volatile carbon-based chemicals common in occupational settings due to their wide application as cleaners, degreasers and reagents in varied industrial processes. These solvents are commercially available in thousands of industrial formulations and are used in the production of paints, adhesives, inks and dyes, dry cleaning solutions, pesticides, fuels, cosmetics and pharmaceuticals. Millions of workers in the United States are potentially exposed to organic solvents,[125] but the current prevalence of occupational exposure among pregnant women is unknown.

A number of organic solvents are recognized reproductive toxins, although the specific mechanisms by which they exert developmental toxicity and teratogenesis in particular are not well understood.[1,13] One leading hypothesis is that these compounds produce oxidative stress (OS) to which early embryonic development is strongly susceptible.[24,25] The capacity to induce embryonic OS has been demonstrated for several organic solvents including benzene, carbon tetrachloride, chloroform, methylene chloride, perchloroethylene and trichloroethylene.[30,31] Animal models of ethanol-induced OS suggest that OS causes alterations in gene expression and interferes with normal cellular activity of the neural crest cell population, ultimately leading to brain and facial abnormalities.[27,28,126,127] Neural tube defects (NTDs) and orofacial clefts (OFCs) are two major groups of congenital

anomalies thought to result from abnormal embryological development of neural crest cells, and thus may be particularly susceptible to oxidative stressors.

Though a number of epidemiologic studies have investigated the potential association between maternal occupational exposure to organic solvents and NTDs or OFCs, inconsistent results between studies are difficult to interpret given important limitations in study design and exposure assessment.[80,94-97,99,100,105,106] For example, some studies have combined major malformations that are embryologically or pathogenetically distinct into one outcome group of interest; this practice may dilute effect measure estimates by masking etiological heterogeneity between phenotypes.[128] Another limitation common to retrospective studies is exclusive use of job title (e.g., "nurse") as a surrogate for exposure; this strategy is less able to discriminate exposure profiles within groups of occupation and industry than more detailed assessments incorporating expert review of occupational histories.[101] The resulting bias is of special concern in studies where the overall prevalence of exposure is low, since misclassification of even a few unexposed individuals as exposed can lead to substantial attenuation of observed effect estimates.[129]

Given the prevalent use of organic solvents in the workplace and their suspected capacity to exert developmental toxicity in humans, the potential effects in offspring among women exposed during pregnancy warrant further investigation in studies designed to minimize both exposure and outcome misclassification. We investigated the association between maternal occupational exposure to organic solvents during early pregnancy and the prevalence of NTDs and OFCs in a large, population-based sample of women for whom exposure was assigned using a comprehensive job-exposure database and expert review of self-reported occupational histories.

4.3.2 Methods

The National Birth Defects Prevention Study (NBDPS) is an ongoing, multi-site, population-based case-control study designed to investigate a range of risk factors for major congenital anomalies.[109] Participating birth defect surveillance programs identified cases of NTDs and OFCs among live births, fetal deaths greater than 20 weeks gestation, and prenatally diagnosed elective terminations. Non-malformed live birth controls were randomly selected using either birth certificates or hospital records from the same base population as cases in each state. Mothers of cases and controls were interviewed by telephone in either English or Spanish up to 24 months after the date of delivery. Using pregnancy calendars to aid recall, interviewers elicited information about demographic, environmental, nutritional, behavioral and clinical factors before and during pregnancy. The NBDPS is approved by the institutional review boards of the Centers for Disease Control and Prevention and all participating sites.

Our study population included employed mothers of cases of NTDs (n=521), OFCs (n=1249) and non-malformed controls (n=2997) delivered between 01 October 1997 and 31 December 2002. These mothers had participated in the NBDPS interview (71% of cases participated; 68% of controls), reported having at least one job during the time between the 3 months before the estimated date of conception (EDC) through delivery (67% of participating cases were employed; 72% of controls), and were from the following NBDPS sites: Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York and Texas. The average infant age at interview was 10 months for NTD cases, 10 months for OFC cases, and 8 months for controls.

We excluded women with pregestational diabetes (7 NTD cases; 17 OFC cases; 20 controls). For analyses of NTDs, we further excluded 3 cases and 5 controls with a first

degree family history of NTDs; for analyses of OFCs, we excluded 69 cases and 8 controls with a positive family history.

Outcome classification

Clinical geneticists at each site performed a standardized review of abstracted medical records to confirm eligibility of cases for the NBDPS.[111] Eligible cases were then further classified by NBDPS clinicians as having one isolated major congenital anomaly, multiple major anomalies, or a pattern of anomalies representing a complex developmental syndrome. Cases with anomalies of known etiology (e.g., single-gene disorders and chromosomal abnormalities) were excluded from the NBDPS. Neural tube defects were further classified by major component phenotype: anencephaly and craniorachischisis (BPA modification of ICD-9 [114] 740.0; 740.1), spina bifida (741.0; 741.9) and encephalocele (742.0). Orofacial clefts were further classified into two component phenotypes: cleft palate alone (749.0 except 749.08) and cleft lip with or without cleft palate (749.1 except 749.19; 749.2).

Exposure characterization

The occupational history section of the maternal interview identified mothers who were employed for at least one month duration from three months preceding the EDC through the end of pregnancy. Employment was defined as compensated, volunteer or military service, including part-time work and work performed at home. For each reported job, mothers were asked about the employer, job title, primary tasks and duties, chemicals and machines handled on the job, dates of employment, and hours and days worked per

week; up to 6 jobs could be recorded. Jobs were then coded by occupation and industry according to the Standard Occupational Classification Manual (2000)[112] and North American Industry Classification System (1997),[113] and assessed for exposure to 10 organic solvents including 3 aromatic solvents (benzene, xylene, toluene), 6 chlorinated solvents (carbon tetrachloride, chloroform, methylene chloride, perchloroethylene, trichloroethylene, 1,1,1-trichloroethane), and the petroleum-based mixture Stoddard solvent (also known as mineral or white spirits). Comprehensive era-specific (1997-1999; 2000-2002) and solvent-specific job-exposure databases were developed for NBDPS by a team of occupational epidemiologists and industrial hygienists (IH). These job-exposure databases, based on extensive literature reviews of published papers reporting direct measurements and determinants of exposure for various occupations and industries, were then used in combination with IH review of self-reported job information to estimate the probability of exposure for each reported job. Probability was defined as the likelihood that a specific job within an industry within a given era had any exposure to the solvent; each job was assigned one of the following categories for exposure probability: 0 (unexposed), <10%, 10-49%, 50-89% and ≥90%.

Using self-reported job dates, we restricted the exposure period of interest to the periconceptional period, defined as one month preceding the EDC through the end of the first trimester. The periconceptional period corresponds to the critical window in embryologic development during which NTDs and OFCs are thought to occur.[116] Thus, for each solvent, a mother was considered *exposed* if any of her jobs during the periconceptional period were rated as exposed (i.e., probability of exposure > 0 for any job). She was considered

unexposed if she did not have a job during the perioconceptional period or if all her jobs during that time were rated as unexposed (i.e., exposure probability = 0 for all jobs).

Statistical analysis

Using the dichotomous exposure variable previously described (exposed/unexposed), we examined the prevalence of estimated exposure to each solvent and solvent class (aromatic; chlorinated; Stoddard solvent) among mothers by case-control status. We then explored correlation in assigned exposure status within and between solvent classes among all exposed mothers of controls to determine the best modeling strategy. Exposure status was strongly correlated between individual solvents within solvent class. For example, 98% of women exposed to methylene chloride were also exposed to trichloroethane. Exposure correlation between solvent classes was substantially lower than within classes. Given strong exposure correlation among individual solvents within solvent class, in multivariable modeling analyses we considered exposure to solvent class only.

Three sets of models were conducted for each composite defect group (e.g., NTDs) using unconditional logistic regression, and for each series of component phenotypes (e.g., anencephaly, spina bifida, encephalocele) using polytomous logistic regression (PLR). In the first set of models, we estimated unadjusted odds ratios (OR) and 95% confidence intervals (CI) to examine the association between exposure to each solvent class and each composite or component outcome. In the second set of models, we estimated the *independent* effects of each solvent class by simultaneously including terms for each class in the models. The final set of models included terms for each solvent class as well as for the following maternal characteristics reported during the maternal interview: age at delivery, race/ethnicity,

education, pre-pregnancy body mass index, folic acid supplement use, and smoking. Within each PLR model, we evaluated heterogeneity in the estimated exposure effects across component phenotypes using likelihood ratio tests (*alpha*-level = 0.20).[115]

To account for the varying levels of estimated exposure probability in the exposure assessment, we repeated the primary exposure-defect analyses restricting the exposed group to women with at least one job with an estimated probability of exposure greater than or equal to 10% for any individual solvent within each solvent class. This strategy was used to sharpen the exposure contrast by excluding women less likely to be exposed. We also repeated analyses restricting all cases to only those with an isolated NTD or OFC, since cases of isolated congenital anomalies may differ etiologically from those presenting with multiple defects.

4.3.3 Results

Analyses consisted of mothers of 511 NTD cases (and 2972 corresponding controls) and 1163 OFC cases (and 2969 corresponding controls) who were employed for at least one month duration from three months preceding the EDC through the date of infant delivery. Table 15 summarizes the distribution of maternal characteristics in this sample.

Among all women rated as exposed to any solvent during the periconceptional period, approximately 85% were exposed to more than one solvent (data not shown). The prevalence of estimated occupational exposure to any organic solvent during the periconceptional period was 8.2% among mothers of controls, 13.1% among mothers of all NTD cases and 9.6% among mothers of all OFC cases (Table 16). The prevalence of any solvent exposure was higher among mothers of spina bifida (14.4%) and encephalocele

(16.4%) cases than anencephaly (8.4%); exposure prevalence did not vary across OFC component phenotypes.

Across all case and control mothers, exposure prevalence was highest for the chlorinated solvent class (e.g., 6.9% among controls) and lowest for the aromatic solvent class (e.g., 2.0% among controls). The distribution of probability of exposure also varied between solvent classes (data not shown). For Stoddard solvent and aromatic solvents, over 90% of exposed mothers worked in at least one job with an estimated exposure probability of at least 10%. However, for chlorinated solvents, only 30% of exposed mothers had an exposure probability of at least 10%. Within solvent class, exposure prevalence to individual solvents varied considerably. For example, within the chlorinated solvent class, exposure prevalence among controls ranged from 0.3% for carbon tetrachloride to 6.0% for both methylene chloride and trichloroethane.

In analyses of neural tube defects (Table 17), we observed a positive association with maternal exposure to chlorinated solvents (adjusted OR=1.96 [95%CI = 1.34, 2.87]) but not with aromatic solvents (0.75 [0.36, 1.55]) or Stoddard solvent (0.63 [0.33, 1.23]) after adjusting for solvent class and potential confounders. The magnitude of the effect measure was stronger for spina bifida (2.26 [1.44, 3.53]) and encephalocele (2.22 [0.84, 5.82]) than for anencephaly (1.25 [0.58, 2.71]). However, these observed differences in effect across NTD phenotypes were not statistically significant (p=0.36). Results were nearly identical when restricting cases to only those with an isolated NTD (n=448; 88%). In the secondary analysis restricting the exposed group to women with an estimated exposure probability ≥10%, results were similar to the observed effect measure estimates for all exposed women

for both Stoddard and aromatic solvents. For chlorinated solvents, the unadjusted OR was closer to the null and considerably less precise (1.32 [0.77, 2.29]; 16 exposed cases).

In analyses of orofacial clefts (Table 18), we did not observe a strong association with maternal exposure to any solvent class. Effect measure point estimates for Stoddard solvent were slightly elevated in general, but the associated confidence intervals were wide. Restriction to isolated cases of OFCs (n=997; 86%) as well as to women with an estimated exposure probability ≥10% yielded similar results.

Table 15. Distribution of select demographic and behavioral factors among employed[a] mothers of cases of neural tube defects, orofacial clefts and non-malformed controls, National Birth Defects Prevention Study, United States, 1997-2002.

Covariate	Controls[b] (n = 2977)		All NTDs (n = 511)		All OFCs (n= 1163)	
	n	(%)	n	(%)	n	(%)
Maternal age at delivery						
<20 years	240	(8.1)	47	(9.2)	109	(9.4)
20-25 years (R)	798	(26.8)	140	(27.4)	339	(29.2)
26-35 years	1600	(53.8)	259	(50.7)	578	(49.7)
≥ 36 years	339	(11.4)	65	(12.7)	137	(11.8)
Maternal race/ethnicity						
White, non-Hispanic (R)	1929	(65.0)	304	(59.5)	807	(69.5)
Black, non-Hispanic	376	(12.7)	58	(11.4)	74	(6.4)
Hispanic	525	(17.7)	123	(24.1)	214	(18.4)
Other	140	(4.7)	26	(5.1)	67	(5.8)
Missing	*7*		*0*		*1*	
Maternal education						
<12 years	294	(9.9)	72	(14.1)	152	(13.1)
12 years	736	(24.8)	153	(30.0)	306	(26.3)
>12 years (R)	1942	(65.3)	285	(55.9)	704	(60.6)
Missing	*5*		*1*		*1*	
Pre-pregnancy BMI						
Thin/normal weight (<25) (R)	1824	(62.6)	272	(55.2)	698	(61.5)
Overweight (25 ≤ BMI <30)	662	(22.7)	105	(21.3)	246	(21.7)
Obese (≥30)	430	(14.8)	116	(23.5)	191	(16.8)
Missing	*61*		*18*		*28*	
Folic acid supplement use[c]						
Little/no use (≤30 days)	638	(21.8)	123	(34.4)	251	(21.8)
Some use (>30 days, <daily)	1498	(51.5)	243	(48.2)	593	(51.6)
Daily use (R)	795	(27.1)	138	(27.4)	306	(26.6)
Missing	*46*		*7*		*13*	
Maternal smoking[c]						
Any	607	(20.4)	95	(18.6)	300	(25.8)
None (R)	2370	(79.6)	416	(81.4)	863	(74.2)

NTD, neural tube defect; OFC, orofacial cleft; BMI, body mass index; R, referent category

[a] Employed in at least one job for at least one month duration between three months preceding the estimated date of conception through the date of infant delivery.

[b] The control group for analyses of neural tube defects further excluded 5 controls with a family of history of neural tube defects; the control group for analyses of orofacial clefts further excluded 8 controls with a family history of orofacial clefts.

[c] During the periconceptional period, from one month preceding the estimated date of conception through the first three months of pregnancy.

Table 16. Prevalence of estimated occupational exposure to organic solvents during the periconceptional period[a] among employed mothers of cases of neural tube defects, orofacial clefts and non-malformed controls, National Birth Defects Prevention Study, United States, 1997-2002.

	Controls[b] (n = 2977)	All NTDs (n = 511)	Anencephaly (N = 134)	Spina bifida (n = 316)	Encephalocele (n = 61)	All OFCs (n = 1163)	Cleft palate (n = 414)	Cleft lip ± cleft palate (n = 749)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Any solvent	**242 (8.2)**	**66 (13.1)**	**11 (8.4)**	**45 (14.4)**	**10 (16.4)**	**111 (9.6)**	**39 (9.5)**	**72 (9.7)**
Missing	*23*	*7*				*9*		
Chlorinated solvents	**205 (6.9)**	**61 (12.1)**	**11 (8.4)**	**40 (12.8)**	**10 (16.4)**	**88 (7.6)**	**29 (7.0)**	**59 (8.0)**
Carbon tetrachloride	8 (0.3)	0	0	0	0	3 (0.3)	2 (0.5)	1 (0.1)
Chloroform	84 (2.8)	18 (3.5)	2 (1.5)	11 (3.5)	5 (8.2)	34 (2.9)	11 (2.7)	23 (3.1)
Methylene chloride	179 (6.0)	56 (11.0)	10 (7.5)	37 (11.8)	9 (14.8)	80 (6.9)	27 (6.5)	53 (7.1)
Perchloroethylene	111 (3.7)	27 (5.3)	5 (3.7)	16 (5.1)	6 (9.8)	44 (3.8)	15 (3.6)	29 (3.9)
Trichloroethane	177 (6.0)	57 (11.2)	11 (8.2)	37 (11.8)	9 (14.8)	80 (6.9)	26 (6.3)	54 (7.2)
Trichloroethylene	97 (3.3)	23 (4.5)	3 (2.2)	15 (4.8)	5 (8.2)	39 (3.4)	12 (2.9)	27 (3.6)
Stoddard solvent	**79 (2.7)**	**18 (3.5)**	**4 (3.0)**	**11 (3.5)**	**3 (4.9)**	**41 (3.5)**	**16 (3.9)**	**25 (3.4)**
Aromatic solvents	**60 (2.0)**	**11 (2.2)**	**3 (2.3)**	**6 (1.9)**	**2 (3.3)**	**24 (2.1)**	**10 (2.4)**	**14 (1.9)**
Benzene	15 (0.5)	3 (0.6)	2 (1.5)	1 (0.3)	0	6 (0.5)	2 (0.5)	4 (0.5)
Toluene	58 (2.0)	11 (2.2)	3 (2.2)	6 (1.9)	2 (3.3)	22 (1.9)	9 (2.2)	13 (1.7)
Xylene	59 (2.0)	11 (2.2)	3 (2.2)	6 (1.9)	2 (3.3)	23 (2.0)	9 (2.2)	14 (1.9)

NTD, neural tube defect; OFC, orofacial cleft

[a] One month preceding the estimated date of conception through the end of the third month of pregnancy.

[b] The control group for analyses of neural tube defects further excluded 5 controls with a family of history of neural tube defects; the control group for analyses of orofacial clefts further excluded 8 controls with a family history of orofacial clefts.

Table 17. Association between maternal occupational exposure during the periconceptional period[a] to organic solvents and neural tube defects, National Birth Defects Prevention Study, United States, 1997-2002.

	Any NTD[b]		Anencephaly[c]		Spina bifida[c]		Encephalocele[c]		
Solvent class	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)	p[d]
Unadjusted									
Chlorinated	1.85	(1.37, 2.51)	1.23	(0.66, 2.33)	1.98	(1.38, 2.84)	2.64	(1.32, 5.28)	0.23
Stoddard	1.35	(0.80, 2.28)	1.14	(0.41, 3.16)	1.34	(0.70, 2.54)	1.91	(0.59, 6.24)	0.81
Aromatic	1.07	(0.56, 2.06)	1.13	(0.35, 3.65)	0.94	(0.40, 2.20)	1.66	(0.40, 6.96)	0.80
Adjusted for solvent class									
Chlorinated	2.02	(1.42, 2.88)	1.25	(0.59, 2.64)	2.30	(1.52, 3.48)	2.43	(1.03, 5.70)	0.29
Stoddard	0.86	(0.47, 1.55)	0.99	(0.31, 3.20)	0.79	(0.38, 1.63)	1.00	(0.26, 3.90)	0.92
Aromatic	0.72	(0.36, 1.44)	0.99	(0.28, 3.45)	0.59	(0.24, 1.45)	0.94	(0.20, 4.37)	0.76
Adjusted for solvent class and covariates[e]									
Chlorinated	1.96	(1.34, 2.87)	1.25	(0.58, 2.71)	2.26	(1.44, 3.53)	2.22	(0.84, 5.82)	0.36
Stoddard	0.63	(0.33, 1.23)	0.66	(0.18, 2.43)	0.66	(0.31, 1.43)	0.38	(0.04, 3.21)	0.87
Aromatic	0.75	(0.36, 1.55)	1.12	(0.32, 3.94)	0.65	(0.26, 1.61)	0.67	(0.08, 5.41)	0.78

OR, odds ratio; CI, confidence interval; NTD, neural tube defect

[a] One month preceding the estimated date of conception through the end of the third month of pregnancy.

[b] Effect measure estimates for all NTDs combined estimated using unconditional logistic regression.

[c] Effect measure estimates for NTD phenotypes estimated using polytomous logistic regression.

[d] P-value for Likelihood Ratio test of homogeneity across neural tube defect phenotypes.

[e] Covariates include maternal age, race/ethnicity, education, pre-pregnancy BMI, folic acid and smoking.

Table 18. Association between maternal occupational exposure during the periconceptional period[a] to organic solvents and orofacial clefts, National Birth Defects Prevention Study, United States, 1997-2002.

Solvent class	Any OFC[b] OR	(95%CI)	Cleft palate[c] OR	(95%CI)	Cleft lip ± cleft palate[c] OR	(95%CI)	p[d]
Unadjusted							
Chlorinated	1.11	(0.86, 1.44)	1.02	(0.68, 1.52)	1.16	(0.86, 1.57)	0.57
Stoddard	1.34	(0.91, 1.96)	1.47	(0.85, 2.54)	1.26	(0.78, 1.99)	0.64
Aromatic	1.02	(0.63, 1.65)	1.20	(0.61, 2.36)	0.93	(0.51, 1.66)	0.54
Adjusted for solvent class							
Chlorinated	1.03	(0.76, 1.40)	0.85	(0.52, 1.38)	1.14	(0.80, 1.62)	0.30
Stoddard	1.35	(0.86, 2.11)	1.63	(0.85, 3.14)	1.22	(0.72, 2.06)	0.45
Aromatic	0.92	(0.55, 1.52)	1.11	(0.54, 2.29)	0.81	(0.44, 1.51)	0.49
Adjusted for solvent class and covariates[e]							
Chlorinated	0.96	(0.70, 1.33)	0.83	(0.50, 1.38)	1.04	(0.72, 1.51)	0.45
Stoddard	1.25	(0.78, 1.99)	1.45	(0.72, 2.87)	1.15	(0.67, 2.00)	0.59
Aromatic	0.88	(0.52, 1.49)	1.03	(0.49, 2.20)	0.80	(0.42, 1.51)	0.58

OR, odds ratio; CI, confidence interval; OFC, orofacial cleft

[a] One month preceding the estimated date of conception through the end of the third month of pregnancy.

[b] Effect measure estimates for all OFCs combined estimated using unconditional logistic regression.

[c] Effect measure estimates for OFC phenotypes estimated using polytomous logistic regression.

[d] P-value for Likelihood Ratio test of homogeneity across OFC phenotypes.

[e] Covariates include maternal age, race/ethnicity, education, pre-pregnancy BMI, folic acid and smoking.

4.3.4 Discussion

We observed an increased prevalence of neural tube defects among offspring of women exposed to chlorinated solvents during the periconceptional period. The observed association remained after restriction to only isolated cases of NTDs, and after adjusting for several potential confounding factors. Though effect measure estimates were stronger in magnitude for encephalocele and spina bifida than for anencephaly, formal homogeneity testing did not indicate statistically significant differences in the exposure effect across component phenotypes.

Previous studies with comparable exposure assessment and outcome classification have not consistently reported an association between occupational solvent exposure and NTDs. In a California study of occupational risk factors for NTDs, Shaw *et al.* (1999) found no association between organic solvent exposure during the periconceptional period and all NTDs combined (0.97 [0.71, 1.3]).[95] However, a study of maternal occupation among Mexican-American women in Texas found evidence that women with exposure to glycol ethers and other solvents were more likely to have an NTD-affected pregnancy.[94] To our knowledge, our study is the first to investigate maternal occupational exposure to specific classes of organic solvents and NTD phenotypes.

We did not observe a positive association between maternal occupational exposure to organic solvents and orofacial clefts. This finding is not consistent with a number of recent studies, all of which have reported large effect estimates for OFC phenotypes and various solvent classes including aromatic, chlorinated and petroleum solvents.[93,96,98-100] Given that all but one of these studies were conducted in France, it is possible that the exposure profiles between study populations differed with respect to other parameters (intensity, frequency,

etc.) not assessed in this study that are relevant to the potential etiologic relationship between solvent exposure and OFC risk.

We caution against the interpretation of null findings as evidence of no association between solvent exposure and OFCs or NTDs, since various sources of bias, such as exposure misclassification, could lead to the masking of effects in our study.[107] In the absence of direct quantitative exposure measurements for each woman from workplace or biologic monitoring, our retrospective exposure assessment was limited to estimation of exposure status based on published measurements from similar occupations within the same industry and era. Our estimation of exposure therefore was unlikely to capture relevant within-job variability related to exposure status as well as other potentially critical factors, such as dose and timing.

Our study was also limited by small sample size, driven primarily by the low prevalence of estimated solvent exposure in our study population. Though our study had larger numbers of both NTD and OFC cases than most previous investigations, the results from the multivariable logistic models adjusting for multiple potential confounders were based on small numbers and often imprecise, especially for encephalocele. However, effect measure sizes in both unadjusted and adjusted analyses were similar for all exposure-defect combinations. A further consequence of small sample size is that if the effect of exposure truly varied across NTD or OFC phenotypes, the likelihood ratio tests of homogeneity may have been underpowered to detect such heterogeneity.

The majority of exposed women in our study population were judged to be exposed to multiple solvents, and the observed exposure correlation was highest within solvent classes. Though correlation in exposure status was expected since mixtures of individual solvents are

frequently used in the workplace, the observed correlation was also a function of the exposure assessment method. For example, a number of organic solvents were used for spot treatment in dry cleaning operations from 1997 to 2002, making it challenging if not impossible to identify the specific solvent(s) to which any given woman with a dry cleaning job was exposed. In such scenarios, the job would be assigned a non-zero probability of exposure to all solvent(s) potentially used in that occupation and industry. Therefore, exposure ratings in our study were likely more sensitive than specific, and the observed correlation in exposure status was thus high among solvents that were used simultaneously or were otherwise mutually prevalent in a given job. Given this exposure correlation, another limitation of our study was that we were unable to examine the potential effect of exposure to each of the 10 organic solvents *individually*. Grouping solvents by major chemical class addressed some of the challenges of within-class correlation. However, the toxicity of solvents is known to vary across individual solvents within class, and analyses by solvent class in our study may be biased in an unpredictable direction if exposure effects of individual solvents were not additive but rather synergistic or antagonistic.[17,19]

In case-control studies with a low prevalence of exposure, suboptimal specificity in the exposure assessment despite good sensitivity can lead to substantial attenuation of effect estimates.[129] We attempted to refine the exposure contrasts in our study and reduce misclassification by restricting exposed women in a secondary analysis to those with at least one job with an estimated probability of exposure greater than or equal to 10% for any individual solvent within each solvent class. This strategy did not change the observed results for Stoddard solvent and aromatic solvents since the vast majority of mothers rated as exposed to these solvents had a job with an estimated exposure probability ≥10%. In

contrast, only one third of mothers rated as exposed to chlorinated solvents had a job with an estimated exposure probability ≥10%. The unadjusted OR for chlorinated solvents and NTDs in this restricted sample was closer to the null (1.32 *vs*. 1.85) but also considerably less precise given the loss in sample size. We note that the association we observed between chlorinated solvents and NTDs was therefore based on a sample of women with jobs generally estimated to have a low probability of exposure. This might imply that chlorinated solvent exposure has a strong effect on NTD risk, though a more likely explanation may be that the assigned exposure probabilities based on expected prevalence of exposure to chlorinated solvents in a given occupation and industry did not accurately reflect individual probability of exposure or another more relevant exposure measure (e.g., peak internal dose) in our study population.

Despite its limitations, our study also has several notable strengths. The NBDPS is a geographically and ethnically diverse population-based study with a relatively large number of controls and carefully classified cases, including stillbirths and electively terminated pregnancies. We obtained extensive data from the maternal interview about occupational history and potential confounders including maternal age at delivery, race/ethnicity, education, pre-pregnancy BMI, and periconceptional folic acid supplement use and smoking. The relatively short recall period (on average within 1 year of delivery) minimized the potential for recall error in these self-reported data. Our exposure assessment process utilized comprehensive literature-based job-exposure databases to estimate probability of exposure to 10 organic solvents for every reported job held during the critical window of developmental susceptibility for NTDs and OFCs. Though resource intensive, this strategy avoids recall bias associated with exclusive use of self-reported exposure in case-control

studies. Finally, by restricting eligibility to women who reported having at least one job shortly before conception and during pregnancy, we attempted to mitigate residual confounding by socio-economic status and other factors related to employment status.

In summary, we observed a positive association between maternal occupational exposure to chlorinated solvents during the periconceptional period and the prevalence of NTDs in offspring. Though not consistently reported in previous epidemiologic studies, this finding is biologically plausible given that NTDs may be particularly susceptible to oxidative stressors like organic solvents. Recurring weak associations observed in epidemiologic studies of suspected teratogens may reflect true underlying causal mechanisms and merit further attention.[107] To establish (or refute) causality, future studies should ideally be designed to improve upon previous limitations in exposure assessment and outcome classification in an effort to produce unbiased estimates. Additional experimental research is also needed to advance our understanding of the possible biologic mechanisms by which organic solvents may cause congenital anomalies.

CHAPTER 5: DISCUSSION

The primary purpose of this research was to advance our knowledge about the potential relation between maternal occupational exposure to organic solvents during pregnancy and the risk of neural tube defects (NTDs) and orofacial clefts (OFCs) in offspring. During the process of evaluating this research question, we also explored the prevalence and patterns of maternal employment during pregnancy to evaluate the presence of within-woman variability in employment status over the course of pregnancy. In addition to the strengths, limitations and conclusions that were addressed in detail with respect to each research objective in preceding chapters, the following chapter highlights key issues influencing the results, discusses the broader interpretation of this research, and provides recommendations for future work.

5.1 Summary of findings, strengths and limitations

5.1.1 Patterns of maternal employment before and during pregnancy

As more women enter the labor force, there is increased epidemiologic interest in the possible effects of employment on adverse pregnancy outcomes. Given that the *timing* of prenatal exposures during pregnancy frequently determines the nature and magnitude of observed effects, variability in the timing of maternal employment and employment-related exposures should be considered when investigating whether such exposures are risk factors for adverse pregnancy outcomes. Yet studies of maternal employment during pregnancy often obtain only one measure of employment status and assume that employment status (and

other related factors of interest, like usual working hours per week) is constant throughout pregnancy. If employment status is *not* constant, this practice can lead to biased measures of association between employment and adverse pregnancy outcomes due to misclassification of employment status during the critical window of exposure. Although there are published reports in the literature that examine duration of employment during pregnancy, none to our knowledge have examined patterns of employment change to determine, for example, the proportion of women who *start* working during pregnancy.

In the first manuscript of this dissertation, we report the prevalence and patterns of maternal employment before and during pregnancy among mothers who participated as controls in the NDBPS between 1997 and 2005. Consistent with recent Census data, we found that 72% of women worked at some point 3 months before and during pregnancy, and that approximately 80% of these women worked into their last trimester. Unique to our study, we further examined within-woman patterns of employment status, revealing that 75% of employed women were consistently employed in one or more jobs before pregnancy through their last trimester, whereas 25% of women changed employment status during pregnancy. Among this latter group of women, twice as many were employed before pregnancy and later stopped working (15%) than started working after their pregnancy began (~7%). The observation that employment status is not constant over the course of pregnancy for a large proportion of women implies that single measures of employment status may not accurately characterize maternal occupational exposure occurring during the biologically relevant time window of susceptibility to exposure. Therefore, we remind investigators to use a time-dependent assessment of employment status when possible to reduce exposure misclassification.

While many studies focus on employment status as the primary "exposure" of interest, others focus on job title/occupation or job-related exposure (physical exertion, chemical use, etc.). Our study examined within-woman variability in employment status but not in occupation. Approximately 15% of NBDPS control mothers reported having multiple jobs before and during pregnancy,[122] and thus an examination of the patterns of job change would build on our study and further illustrate the potential for exposure misclassification when measures of employment do not correspond to the critical window of exposure. We also recognize that the allocation of women into different patterns of employment likely represents a non-random function of demographic, behavioral and obstetric factors associated with self-selection into (and out of) employment at different times during pregnancy. Though we do not know the reasons why women changed employment status (prescribed bed rest, need for more income, no need for more income, etc.), it would be valuable to describe heterogeneity in the exposure profiles between working women that have differing patterns of employment during pregnancy, as differences in sociodemographic and clinical characteristics may have implications for the consideration of residual confounding and selection bias. We intend to explore the possibility of expanding our analysis to examine patterns of job change as well as to examine factors associated with initiation and termination of employment during pregnancy.

5.1.2 Maternal occupational exposure to organic solvents during early pregnancy and selected congenital anomalies

In the second manuscript of this dissertation, we investigated the relation between maternal occupational exposure to organic solvents during the periconceptional period and NTDs and OFCs (corresponding to Specific Aims 2 and 3). We observed an increased prevalence of NTDs among offspring of women estimated to be exposed to chlorinated

solvents. The observed association remained after restriction to only isolated cases of NTDs, and after adjusting for several potential confounding factors. Though odds ratio estimates were larger for encephalocele and spina bifida than for anencephaly, formal homogeneity testing did not indicate statistically significant differences in the exposure effect across specific NTD phenotypes. We did not observe an association between NTDs and estimated exposure to Stoddard or aromatic solvents. For OFCs, we did not observe a positive association with estimated exposure to any solvent class.

Numerous epidemiologic studies have investigated whether maternal exposure to organic solvents is associated with congenital anomalies in offspring. In general, positive associations are frequently but not consistently reported. Inconsistent findings are likely explained by differences and limitations in study population, outcome classification and exposure assessment. Our objective was to improve upon the methods of previous studies in several ways. First, rather than use the etiologically heterogeneous outcome of all major malformations combined, we focused exclusively on specific congenital anomalies of interest. Toxicological data suggest that NTDs and OFCs may be particularly susceptible during early embryonic development to oxidative stressors like organic solvents. The precise classification of defects by clinical geneticists, an asset of the NBDPS, allowed us to examine potential differences in observed effects across multiple distinct phenotypes of both NTDs and OFCs using polytomous logistic regression. Second, we attempted to reduce exposure misclassification by using an exposure assessment process that combined expert review of detailed self-reported occupational histories with era-specific job-exposure databases compiled using published data on direct measurements of occupational solvent exposure in various occupations and industries. Although not as ideal as having individual-level

exposure data from biologic or environmental monitoring, this strategy minimized the potential for recall bias associated with self-reported exposure, and likely resulted in more accurate exposure assignment than by using only job title to infer potential workplace exposure to solvents. Third, we accounted for several potentially confounding factors, such as folic acid supplementation and smoking, for which extensive timing-specific data were obtained during the maternal interview. Lastly, a number of advantageous features of the NBDPS strengthened our analysis, such as the study population, which consisted of a large, population-based sample of demographically diverse mothers of cases and controls delivered relatively recently in 8 states across the United States, including cases among fetal deaths and terminations.

Despite the strengths of our analysis, our results must be interpreted cautiously in the context of our study's primary limitation: exposure misclassification. As previously discussed in Section 4.3.4, the exposure assessment was likely sensitive but not highly specific, meaning that the women truly exposed to a given solvent were likely to be rated as exposed, whereas women who were truly unexposed were less likely to be correctly rated as unexposed. This inaccuracy in assignment of exposure status would have resulted in misclassification of truly unexposed women into the exposed group for analysis, which generally leads to severe attenuation of effects when the prevalence of exposure is low and exposure is dichotomized, as was the situation in our study. Even if our exposure assessment could perfectly distinguish between exposed and unexposed jobs, exposure misclassification would still be introduced by within-job and within-woman variability in other exposure parameters (timing, frequency, intensity, etc.) not assessed in this study. The expected impact of exposure misclassification in our study is attenuation of the observed effect

estimates toward the null, though we cannot know with certainty the degree to which or the direction in which our results are biased since there are likely other factors influencing our results, such as selection bias related to the overall NBDPS participation rate.

Given the assumption that our results are influenced by substantial exposure misclassification, we do not interpret the "null" results we observed for estimated solvent exposure and OFCs to be *evidence of no association*. Rather, we admit that our study was unable to detect an association if one truly exists. Yet despite the presumed attenuation of effect measure estimates, we consistently observed a moderate association between estimated exposure to chlorinated solvents and NTDs, even after adjustment for several potentially confounding factors such as race/ethnicity. This suggests either that the effect of chlorinated solvent exposure on NTDs is particularly strong, such that our study was able to detect it, or that there's another unknown source of bias inflating the effect measure estimates. What this source(s) of bias could be is difficult to conceive, as it would have to be specifically affecting analyses of NTDs (because we didn't observe elevated estimates for OFCs) and further, analyses of NTDs and chlorinated solvents (because we didn't observe elevated estimates for Stoddard or aromatic solvents). We believe that the observed association between exposure to chlorinated solvents and NTDs – particularly spina bifida and encephalocele – may be indicative of an underlying relationship that merits further investigation.

5.2 Direction for future research

Over two decades of epidemiologic research have been devoted to the investigation of maternal solvent exposure during pregnancy and the occurrence of various congenital anomalies. Despite advances in study design, exposure assessment and analytic methods over the years, findings are collectively summarized as "inconsistent" and the scientific community remains uncertain about the true effect of solvent exposure during pregnancy. Perhaps we've reached the limit of what can be learned about solvents and congenital anomalies with the suite of epidemiologic tools currently at our disposal. There are significant challenges encountered when studying this particular exposure and outcome of interest: congenital anomalies are rare, the prevalence of occupational exposure is low, the critical window of exposure is narrow, exposure assessment is indirect, etc. Thus, one or even 10 additional studies constrained by the same practical limitations will not definitively prove – or disprove – that solvent exposure causes birth defects.

To meaningfully advance our understanding of the relation between occupational solvent exposure and the risk of birth defects, future research efforts should focus on three major areas: First, we need validation studies of indirect measures of occupational solvent exposure during pregnancy, including probability and intensity, so that data are available for sensitivity analyses or correction of misclassification error in larger population-based studies that must rely on indirect assessment. Second, we need further laboratory research to determine the specific pathway(s) by which individual solvents exert developmental toxicity, which will help to inform population-based investigators about relevant exposure parameters such as timing, dose, and genetic susceptibility that may be critical to the etiologic relationship between solvent exposure and NTDs or other congenital anomalies. Lastly, we

encourage development of novel exposure biomarkers in media that could be reasonably obtained for retrospective epidemiologic studies, like maternal serum or newborn blood spots, which would present a distinct opportunity to revisit this research question with an independent source of exposure assessment.

Until we have a better understanding of the potential teratogenic effects of organic solvent exposure during pregnancy, it's reasonable for women to avoid using organic solvents or products containing high concentrations of organic solvents, or to take precautions to minimize exposure (such as using personal protective equipment) during early pregnancy. Occupational physicians and other health care providers should discuss potential exposure to organic solvents with their patients when evaluating other occupational risk factors during preconception counseling and early prenatal care.

APPENDIX A: NBDPS OCCUPATIONAL HISTORY QUESTIONNAIRE

ID #.....|_|_|_|_|_|_|_|_|_|_|_|_|

FORM: 02 VERSION: 02

FORM APPROVED: OMB # 0920-0010

OMB EXPIRATION DATE: 05/31/2009

National Birth Defects Prevention Study

Mother Questionnaire CATI Version 4.1

Centers for Disease Control and Prevention
U.S. Department of Health and Human Services
Public Health Service

January 26, 2007

Information contained on this form which could permit identification of any individual or establishment has been collected with an assurance that it will be held in strict confidence by the contractor and CDC, will be used only for purposes stated in this study, and will not be disclosed or released to anyone other than authorized staff of CDC without the consent of the participant in accordance with Section 301(d) of the Public Health Service Act (42 U.S.C. 241d).

Public reporting burden of this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. An agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to CDC/ATSDR Reports Clearance Officer; 1600 Clifton Rd NE, MS D-74, Atlanta, GA 30333; ATTN: PRA (0920-0010).

SECTION H: MOTHER'S OCCUPATION

H1. The next section is a series of questions about your work experiences—paid, volunteer, or military service. This includes part-time and full-time jobs, jobs at home, and jobs on a farm or outside your home that lasted one month or more. From 3 months before you became pregnant to the end of your pregnancy, did you have a job?

YES.....(SKIP TO H3) 1
NO 2
DK.....-1
RF .....-2

H2. Were you (READ CHOICES) or did you do something else?

A homemaker/parent.....(SKIP TO H12) 1
A student..... (SKIP TO H3) 2
Disabled .....(SKIP TO H12) 3
Unemployed/in between Jobs.....(SKIP TO H12) 4
OTHER ..... (SPECIFY THEN SKIP TO H12) .....-5
DK.....(SKIP TO H12) .....-1
RF .....(SKIP TO H12) .....-2

SPECIFY: _____ DK❑

H3. What were the names of the companies or organizations you worked for between (B3) and ([DOIB]/[DOPT])? / What other companies did you work for? LIST ALL EMPLOYERS, INCLUDING "SELF-EMPLOYED." IF STUDENT, CATI FILLS IN "SCHOOL" HERE.
COMPANY/ORGANIZATION: _____

DK❑ ASK H4 – H11 RF❑ SKIP TO H12

H4. What was your job title there? IF STUDENT, CATI FILLS IN "STUDENT" HERE AND SKIPS H5 & H6.

JOB TITLE: _____ DK❑ RF❑

H5. What did they make or do? IF CONGLOMERATE: What did your division make or do?

SPECIFY: _____
_____ DK❑ RF❑

H6. Describe what you did and how you did it. What were your main activities or duties? Anything else?

MAIN ACTIVITIES/DUTIES: _____
_____
_____ DK❑ RF❑

H7. Describe any chemicals or substances you handled or machines that you used or worked in the same room with. Anything else?

CHEMICALS/SUBSTANCES/MACHINES USED: _____
_____ NONE❑ DK❑ RF❑ IF NONE, DK OR RF, SKIP TO H8.

H8. What month and year did you start that job/school?

DATE: ..... MM YYYY
DK❑ DK❑

H9. What month and year did you end that job/school?

DATE: ..... MM YYYY
DK❑ DK❑
CURRENTLY WORKING = DATE OF INTERVIEW

H10. How many days per week did you usually work?
IF STUDENT: How many days per week did you go to school?

DAYS PER WEEK..... ❑❑
DK❑ RF❑

H11. How many hours per day did you usually work?
IF STUDENT: How many hours per day did you spend either at school or studying?

HOURS PER DAY..... ❑❑
DK❑ RF❑

PAPER COPY INTERVIEWER INSTRUCTION: IF RESPONDENT HAS HAD MORE THAN ONE JOB BETWEEN (B3) AND ([DOIB]/[DOPT]), USE SUPPLEMENT SHEET FOR EACH ADDITIONAL JOB. (REPEAT H3 –H11.)

MOTHER'S OCCUPATION-MILITARY

H12. Have you served in active duty in the U.S. armed forces since 1990?

YES.....1
NO ..... (SKIP TO I1) 2
DK..... (SKIP TO I1) .....-1
RF ..... (SKIP TO I1) .....-2

H13. In which country did you serve? Any other?	H14. From which month and year?	H15. To which month and year? (IF STILL SERVING ENTER CURRENT DATE)
A. _____ DK❑ ASK H14 & H15 RF❑ SKIP TO I1	FROM: MM YYYY DK❑ DK❑	TO: MM YYYY DK❑ DK❑
B. _____ DK❑ ASK H14 & H15 RF❑ SKIP TO I1	FROM: MM YYYY DK❑ DK❑	TO: MM YYYY DK❑ DK❑

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