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Ambient Air Pollution and Cardiovascular Malformations in Atlanta, Georgia

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An Abstract of
A dissertation submitted to the Faculty of the Graduate School of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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Abstract

Ambient Air Pollution and Cardiovascular Malformations in Atlanta, Georgia

In this dissertation I investigated temporal relationships between ambient air pollution levels during weeks three through seven of pregnancy and risk of cardiovascular malformations among the cohort of infants and fetuses conceived during January 1, 1986 through March 12, 2003 in Atlanta, Georgia. Records of infants and fetuses with cardiovascular malformations were obtained from the Metropolitan Atlanta Congenital Defects Program, which conducts active, population-based birth defects surveillance on this cohort. These surveillance records were reviewed to exclude infants with transient newborn conditions and to group infants and fetuses with similar cardiovascular malformations for analysis. Ambient air pollution measurements of 8-hour maximum ozone and 24-hour average carbon monoxide, nitrogen dioxide, particulate matter < 10 μm in diameter, and sulfur dioxide were obtained from centrally-located stationary monitors. Temporal relationships between air pollution levels and risk of cardiovascular malformations were modeled using Poisson generalized linear models. I observed a positive association between particulate matter < 10 μm in diameter and risk of patent ductus arteriosus (risk ratio for an increase in the interquartile range of the pollutant = 1.60, 95 percent confidence interval: 1.11, 2.31). No other positive associations were observed.

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Table of Contents

List of Figures and Tables

Chapter 1: Introduction

The susceptibility of human populations to ambient air pollution has been evaluated in many contexts, yet exploration of possible effects on the developing fetus has only recently emerged as a field of interest. Reviews by Maisonet et al. (2004), Glinianaia et al. (2004), Sram et al. (2005), and Lacasana et al. (2005) suggest that a small increased risk of preterm delivery and intrauterine growth retardation attributable to air pollution is plausible. Little work has been conducted on the association between ambient air pollution and birth defects; individual-level studies have been conducted in Los Angeles (Ritz et al., 2002) and Texas (Gilboa et al., 2005).

Ritz et al. (2002) reported four positive dose-response associations between ambient air pollution levels during the second month of gestation and cardiovascular malformations in Los Angeles, California. Gilboa et al. (2005) reported three positive dose-response associations between air pollution levels during weeks three through eight of gestation and cardiovascular malformations in Texas. The primary hypotheses of this dissertation, listed below, relate to these seven associations:

- High ambient carbon monoxide (CO) levels averaged over weeks three through seven of gestation are associated with an increased rate of ventricular septal defects.
- High ambient ozone (O_3) levels averaged over weeks three through seven of gestation are associated with an increased rate of aortic artery and aortic valve anomalies.
- High ambient O_3 levels averaged over weeks three through seven of gestation are associated with an increased rate of conotruncal anomalies.
- High ambient O_3 levels averaged over weeks three through seven of gestation are associated with an increased rate of pulmonary artery and pulmonary valve anomalies.

- High ambient particulate matter < 10 μg in diameter (PM_{10}) levels averaged over weeks three through seven of gestation are associated with an increased rate of atrial septal defects.
- High ambient CO levels averaged over weeks three through seven of gestation are associated with an increased rate of tetralogy of Fallot.
- High ambient sulfur dioxide (SO_2) levels averaged over weeks three through seven of gestation are associated with an increased rate of ventricular septal defects.

The cohort of births and fetal deaths recorded in metropolitan Atlanta during 1986-2003 were obtained from the Office of Health Information and Policy, Georgia Division of Public Health. Infants and fetuses with cardiovascular malformations were obtained from the Metropolitan Atlanta Congenital Defects Program (MACDP). These data were linked with data on air pollution levels obtained from the state monitoring network and several intensive air quality studies. The primary hypotheses were assessed by modeling the daily counts of cardiovascular malformations in metropolitan Atlanta during 1986-2003 using Poisson generalized linear models. The remaining air pollutant-heart defect combinations were modeled in secondary analyses using a similar analytic approach. This two-tier analytic approach was motivated by a desire to mitigate the potential for spurious associations due to multiple comparisons.

Background – laboratory studies

Deleterious effects of air pollution on the developing fetus have been documented in laboratory studies, suggesting that an association between ambient air pollution and certain birth defects may be plausible. Maternal carbon monoxide exposure has been shown to produce skeletal malformations in rabbits, mice, and chicks (Garvey & Longo, 1978; Kavlock et al., 1979; Singh et al., 1993; Loder et al., 2000; Alexander & Tuang, 2003). Singh (2003) demonstrated an increased risk of gastroschisis in the offspring of zinc deficient mice exposed to carbon monoxide during gestation. Somers et al. (2004) discovered that the DNA mutation rate of offspring of

paternal mice exposed to ambient particulate air pollution housed in an industrial setting was 2.8 times greater than in the offspring of paternal mice housed in a low pollution rural setting.

Background – ecologic studies

Relatively less attention has been devoted toward examining the possible associations between air pollution and birth defects in human populations. In the Ukraine, Antipenko & Kogut (1993) compared the frequency of selected birth defects in a town with high air pollution relative to a town with low air pollution. The highly polluted town, Mariupol, was home to approximately 520,000 people and 920,000 annual tons of air pollution; the town had numerous metallurgic industries, and emissions of benzo(a)pyrene, a polycyclic aromatic hydrocarbon, were high. The relatively clean town of Simpheropol, which contained 340,000 people and had annual air pollution emissions of 130,000 tons, was chosen for comparison. The aim of the study was to quantify the mutation rate attributable to air pollution; the investigators were particularly interested in the mutagenic effects of air pollution on various inheritance patterns, so birth defects were categorized into groups such as dominant (syndactyly, polydactyly, limb reductions), X-linked (boys' hydrocephaly), recessive (anorectal atresia, girls' hydrocephaly), and multifactorial (anencephaly, congenital pyloric stenosis, esophageal atresia). The authors reported that the frequency of multiple mutations, dominant, and X-linked congenital anomalies was approximately three times greater in Mariupol relative to Simpheropol; the overall mutation rate was 1.5-2.6 times greater in Mariupol (Antipenko & Kogut, 1993);

Smrcka & Leznarova (1998) reported that within the district of Breclav in South Moravia the most birth defects occurred in the area with the highest air pollution levels of organic solvents and phosphoric acid. In their study, Smrcka & Leznarova (1998) subdivided the district into numerous smaller areas based on the number (not the rate) of ascertained congenital defects during 1975-1990. The investigators first identified the three areas (Breclav-Postorna, Mikulov, and Velke Bilovice) with the highest number of defects and then proceeded to consider the

environmental exposures within each area. Breclav-Postorna, which had a large number of heart defects, also had factories emitting organic solvents, phosphoric acid, and ceramic dust. There was a lime factory in the area of Mikulov. The Velke Bilovice area was largely agricultural and did not include any factories. The results of Smrcka & Leznarova (1998) are purely spatial, and are based on the assumption that air pollution levels were constant throughout the 15-year follow-up. Furthermore, the investigators' use of counts rather than rates may have lead to the incorrect identification of high risk areas.

Background – individual-level studies

This dissertation was largely motivated by a publication by Ritz et al. (2002), who reported an association between ambient air pollution exposure during the second month of gestation and certain cardiovascular malformations in four Southern Californian counties. The dissertation primary hypotheses were updated to reflect the findings of Gilboa et al. (2005), who conducted a similar study in Texas. In the Ritz et al. (2002) study, outcome data were obtained from the California Birth Defects Monitoring Program during 1987-1993. Eligible cases were all fetal deaths and live births diagnosed with isolated, multiple, syndromic, or chromosomal cardiac or orofacial cleft defects who could be linked with either the birth or fetal death registry and who lived within 10 miles of an air monitoring station. Eleven birth defect groupings were created for the analysis: 1) aortic defects (n=241), 2) defects of the atrium and atrium septum (n=385), 3) endocardial and mitral valve defects (n=67), 4) pulmonary artery and valve defects (n=185), 5) conotruncal defects (n=129), 6) ventricular septal defects (n=235), 7) isolated cleft palate (n=189), 8) isolated cleft lip with or without cleft palate (n=450), 9) syndromic malformations with cardiac and/or cleft defects (n=200), 10) multiple malformations with cardiac and/or cleft defects (n=180), and 11) chromosomal malformations with cardiac and/or cleft defects (n=407). Infants and fetuses identified through birth and/or death certificates born during the same time period and living in the same zip codes as the cases were eligible as controls provided they had

complete covariate information and were not diagnosed with a birth defect by 1 year of age. A random sample of eligible controls were selected for analysis (n=9,357).

Air pollution measurements (CO, nitrogen dioxide (NO_2), O_3, and PM_{10}) were obtained from 30 monitoring stations located throughout the Southern California air basin during 1987-1993. Average pollutant levels were calculated for the first, second, and third months of gestation, as well as for the three-month period prior to conception and the second and third trimesters. Zip codes were used in conjunction with knowledge of the topography, wind direction, and air flow in the Southern California air basin to determine the most appropriate monitoring station for air pollution measurements. The analysis employed several different types of logistic models, including single-pollutant and multiple-pollutant conventional logistic regression models, polytomous logistic regression models, and hierarchical (two-stage) regression models. Odds ratios were adjusted for decade of birth, infant sex, maternal race, maternal age, singleton birth, parity, prenatal care, maternal education, and season of conception. Ritz et al. (2002) reported a strong dose-response association between ambient carbon monoxide levels during fetal heart development and risk of ventricular septal defect (odds ratio (OR) $_{4\text{th quartile}} = 2.95$, 95 percent confidence interval: 1.44, 6.05). Elevated risk of aortic artery and valve defects, pulmonary artery and valve anomalies, and conotruncal defects with increasing ambient ozone levels were also reported.

The second individual-level epidemiologic investigation of air pollution and birth defects was conducted by Gilboa et al. (2005) in Texas. Methodologically, the Gilboa et al. (2005) study design was similar to that of Ritz et al. (2002). Cases were selected from an active, population-based birth defects surveillance system, controls were selected from live birth and fetal death records, and outcome groups were defined using a nomenclature developed for birth defects surveillance. Analysis was based on contrasts in pollution levels over space and time. In addition to the outcome groups of Ritz et al. (2002), Gilboa et al (2005) examined tetralogy of Fallot, coarctation of the aorta, aortic valve stenosis, and pulmonary valve stenosis. Results from this

Texas investigation were inconsistent with the Southern California findings, although a suggestive association between ozone and pulmonary artery and valve defects was observed. The Texas investigators reported positive associations for CO and tetralogy of Fallot, PM_{10} and atrial septal defect, and SO_2 and ventricular septal defect.

An alternative method used to evaluate the health effects of ambient air pollution is residential proximity to traffic (English et al., 1999; Pearson et al., 2000; Wilhelm & Ritz, 2003). These studies used GIS software to assign daily-weighted traffic density measurements to each residence, with the rationale that households located near roadways with heavy traffic are subject to relatively more vehicle exhaust than households located father away from heavily trafficked roads. English et al. (1999) found that among children with asthma, those residing near high traffic flow areas were more likely to necessitate medical care visits for asthma. Pearson et al. (2000) reported that children living in the highest traffic areas had an elevated odds for leukemia. Wilhelm & Ritz (2003) observed an increase in the risk of preterm birth among infants whose residence was in the highest traffic density quintile. To date, the only published study that has examined the relationship between cardiovascular malformations and road traffic is an ecologic study from Cordier et al. (2004), who reported that among communities in France exposed to emissions from solid waste incinerators, the relative risk of a) conotruncal heart defects and b) other cardiac anomalies increased in a dose-response fashion with the amount of road traffic in the community. Although assignment of exposure in these studies differs from Ritz et al. (2002) and Gilboa et al. (2005), analytically these studies are similar, as the measure of association is based on spatial contrasts in pollution levels.

Many cardiovascular malformations are likely to develop near the start of the second gestational month. During the fourth and fifth weeks of gestation (gestational days 21-35), the heart loops and the septum primum, aorticopulmonary septum, endocardial cushions, aortic arches, and major veins are formed (Sadler, 2004). Formation of the mitral valve and tricuspid valve begin once the endocardial cushions have fused (Sadler, 2004). Because many parts of the

heart form during the fourth and fifth weeks of gestation, dividing gestation into the first month (days 1-30) and the second month (days 31-60), as was done by Ritz et al. (2002), may not represent the most relevant gestational time period of cardiac development. A more optimal developmental window may be gestational days 15-49 (Srivastava, 2001; Sadler, 2004).

Expected Benefits

Recently, a panel convened by the National Research Council for the purpose of identifying high-priority areas for research on health effects of particulate pollution concluded that a priority research focus should be on identification of human subpopulations that are at high risk for adverse effects of air pollution, specifically calling for research on pregnant women and infants (National Research Council, 1998, 1999, 2001). Both the inter-agency committee planning the National Children's Study (National Children's Study Interagency Coordinating Committee, 2003) and the report from a recent Environmental Protection Agency (EPA)-sponsored workshop on Risk Assessment and Children's Health (Landrigan et al., 2004) describe the need for future research on the impact of air pollution on fetal health as well. This dissertation directly addresses these needs.

Additional research should be conducted in other locations to help determine whether the associations reported by Ritz et al. (2002) and Gilboa et al. (2005) are causal or spurious. Presently, these are the only two individual-level studies in the literature that have investigated the relationship between air pollution and birth defects. This dissertation takes advantage of one of the oldest birth defects surveillance systems in the country, enabling the effects of air pollution to be modeled with ample statistical power. If this study reports associations similar to those reported by Ritz et al. (2002) or Gilboa et al. (2005) these will be important public health findings with regulatory implications. Furthermore, the review of the cardiac birth defect classification system will enhance the quality of MACDP beyond the immediate objectives of the proposal.

Chapter 2: Cardiovascular Malformations

This chapter describes the embryology of the various types of defects examined in this dissertation. The chapter contains six parts: 1) a brief overview of chromosomal abnormalities, 2) a review of the first three weeks of gestation, 3) a delineation of specific cardiovascular malformations, 4) an overview of the prevalence and mortality of cardiovascular malformations, 5) a review of environmental risk factors for birth defects, and 6) a review of risk factors specific to cardiovascular malformations.

Chromosomal abnormalities

Errors in gametogenesis, the process governing the formation of gametes, are the cause of numerous birth defects. Gametes are formed once the primary oocyte (female) or spermatocyte (male) has completed meiosis. During meiosis, the primary oocyte (or spermatocyte) divides two times. To produce normal gametes, chromosomes must separate evenly during each division. Nondisjunction occurs whenever chromosomes do not separate evenly during a division. A normal gamete has 23 single chromosomes; nondisjunction causes the gametes to have an irregular number of chromosomes. Constituting a large group of birth defects, numerical abnormalities occur whenever a gamete that does not have 23 single chromosomes completes fertilization. Down syndrome (trisomy 21), trisomy 18, and trisomy 13 are examples of birth defects resulting from an extra copy of a particular chromosome (or portion of a particular chromosome). Many chromosomal defects involve the sex chromosomes, including Klinefelter syndrome (XXY), triple X syndrome (XXX), and Turner syndrome (45, X) (Sadler, 2004).

Nondisjunction may also occur at fertilization. When the female and male gametes join to form the zygote, the two gametes combine and begin dividing. If nondisjunction occurs during the earliest cell divisions, the individual will have some cells that are normal and some cells that

are numerically abnormal. This condition is termed mosaicism. The manifestations of mosaicism are varied, depending on the number and location of the cells affected by the nondisjunction (Sadler, 2004).

Structural abnormalities constitute another group of chromosomal birth defects. Structural abnormalities typically occur from chromosome breakage or deletion, which may occur sporadically or be caused by various environmental factors such as carcinogens and viruses (Kao et al., 1993; Fortunato et al., 2000). Examples include cri-du-chat syndrome (partial deletion of chromosome 5), fragile X syndrome (breakage of the long arm of the X chromosome), and genomic imprinting (Sadler, 2004). With genomic imprinting, the characteristics of the syndrome depend on whether the structural abnormality occurs on the maternal or paternal chromosome. A well known example of genomic imprinting involves a small deletion on the long arm of chromosome 15. Angelman syndrome results when this deletion is inherited from the maternal chromosome, whereas Prader-Willi syndrome occurs if the deletion is inherited from the paternal chromosome (Sadler, 2004).

Gene mutations are another category of chromosomal birth defects and are typically inherited. A gene is comprised of two alleles (a short base sequence on a chromosome). On each paired chromosome, the maternal allele is matched with the corresponding paternal allele. A polymorphism is present whenever more than one base sequence for a given allele exists in the population. Some birth defects require both alleles to be variant (recessive), whereas others require only one of the two alleles to be variant (dominant). Examples of alleles associated with cardiovascular malformations include GATA4 (atrial septal defect secundum-type), TBX5 (Holt-Oram syndrome), CRELD1 (atrioventricular septal defects), and PTPN11 (pulmonary valve stenosis) (Gelb, 2004). Mutations can also cause inborn errors of metabolism, including phenylketonuria, homocystinuria, and galactosemia (Sadler, 2004).

Although numerical and structural abnormalities are frequent, these birth defects are not included in the outcome groups in the analyses. The focus of this dissertation is on birth defects

that arise during gestation, i.e. defects that are not predestined based on chromosomal anomalies. Although environmental insults can cause chromosomal anomalies (i.e., Hunt et al., 2003; Somers et al., 2004), the timing of this exposure period is earlier than the exposure period for teratogenesis of specific organ systems. Excluding cases with certain alleles from the analysis is not possible, however, because there is no genotype data for the majority of the birth cohort. Consequently, infants with specific alleles related to cardiovascular malformations, such as GATA4, CRELD1, and PTPN11 will be included in the cohort. If these alleles are common among infants and fetuses with cardiovascular malformations, such as with PTPN11 and Noonan Syndrome (Tartaglia et al., 2002), then inclusion of these subjects will likely lead to a loss of statistical power and may cause the estimates of the association between air pollution and birth defects to be biased towards the null value.

The first three weeks of gestation

Once a sperm has entered the oocyte, the oocyte completes its second meiotic division. The male and female pronuclei (both with 23 single chromosomes) replicate themselves, match with one another, and split into two cells (each with 23 pairs of chromosomes). This cell division is termed mitosis. In mitosis, each daughter cell has the same number of chromosomes as the parent cell. With the exception of gamete formation, cell division proceeds by mitosis.

The zygote divides for the fourth time approximately three days after fertilization. The resultant 16 cells form themselves into a ball called a morula. The inner cells of the morula will develop into the embryo, whereas the outer cells of the morula will form the trophoblast (which will later be incorporated into the placenta). Shortly thereafter fluid enters the morula and forms a cavity called a blastocele; the morula is now termed a blastocyst. The inner cell mass moves to one pole of the morula while the outer cell mass surrounds the blastocyst and forms the epithelial wall (Sadler, 2004).

At approximately eight days after fertilization, the trophoblast cells have formed two layers –an interior layer called cytotrophoblast and an outer layer called syncytiotrophoblast. The blastocyst has begun to penetrate the uterine wall, which remains intact due to human chorionic gonadotrophin (hCG) secreted by the syncytiotrophoblast. The inner cell mass has divided into two layers as well – the hypoblast layer (which borders the blastocyst cavity) and the epiblast layer. These two layers form the bilaminar germ disc. At this time the amniotic cavity begins to form within the epiblast cells.

Throughout the second week of gestation, the blastocyst continues to immerse itself more deeply into the uterine wall. The outermost layer of cells (synctiotrophoblasts) develops empty spaces, called lacunae, which will soon merge with maternal blood vessels to initiate circulation. The blastocyst cavity gives way to the formation of the primary and definitive yolk sacs. By the end of the week, the chorionic cavity has formed and the connecting stalk, which will turn into the umbilical cord, is present (Sadler, 2004).

Gastrulation occurs during the third week of gestation, transforming the bilaminar disc into the trilaminar disc. This process results in the development of the three germ layers – the ectoderm, mesoderm, and endoderm – that will be central in development of the embryo. Gastrulation begins with the formation of the primitive streak on the epiblast surface. Epiblast cells travel towards the primitive streak and then move below it in a process called invagination. Cells invaginating through the cranial end of the streak form paraxial mesoderm. Epiblast cells moving through the middle of the streak form intermediate mesoderm, whereas cells moving through the caudal end of the streak form lateral plate mesoderm. Shortly thereafter, the lateral plate mesoderm cells separate into somatic lateral plate mesoderm and splanchnic lateral plate mesoderm. Cells remaining in the epiblast form the ectoderm. There is debate as to whether the endoderm layer is comprised solely of migrating epiblast cells (Sadler, 2004) or if hypoblast cells contribute to this formation as well (Lawson & Schoenwolf, 2003).

Neurulation begins during the third week of gestation as well, with the ectoderm thickening to form the neural plate. Over the course of the third and fourth weeks of gestation, the ectoderm cells form the neural tube. During formation of the neural tube, some cells from the ectoderm break off. These migratory cells are called neural crest cells, and they will travel extensively throughout the embryo and participate in the formation of many organ systems, including the heart.

Many of the cells comprising the various germ layers are now destined for specific organ systems – the central nervous system and neural crest cells derive from ectoderm; paraxial mesoderm participates in the formation of the skeletal system; the urogenital system derives from intermediate mesoderm; somatic lateral plate mesoderm participates in the formation of the body cavities; splanchnic lateral plate mesoderm forms much of the heart and parts of the digestive system; endoderm forms the gastrointestinal tract (Sadler, 2004).

Most of the major organs form during the third through eighth week of gestation. The embryo is very sensitive during this time because an environmental insult will interfere with organ development. Prior to the third week of gestation, the embryo is not very sensitive to teratogenesis; a teratogenic insult is far more likely to kill the embryo than to cause a birth defect. After the eighth week of gestation, most (but not all) organ systems have formed, so the fetus is less susceptible to teratogens (Sadler, 2004).

Classification of cardiovascular malformations

As outlined by Ferencz et al. (1997), numerous systems have been developed to classify cardiovascular malformations according to embryological, anatomical, and/or functional characteristics. Recently implemented systems include the European Pediatric Cardiac Code (Franklin, 2002) and the International Congenital Heart Surgery Nomenclature and Database Project (Mavroudis & Jacobs, 2000). No single classification system will be optimal for all purposes; systems which focus on anatomical or functional characteristics may not be useful for

epidemiologic analyses. Clark (1996: 466) writes, "Schema that aid the pathologist and surgeon serve the epidemiologist poorly…Classification of heart defects by anatomic features may obscure developmental relationships." Werler (2001:482) echoes this sentiment, claiming "a continuing challenge among birth defects epidemiologists is the classification of congenital heart defects into etiologically meaningful groups." The classification system employed in the Baltimore-Washington Infant Study (BWIS) (Ferencz et al., 1997) was based on Clark (1987; 1996), who grouped heart defects based on their presumed embryologic mechanisms. The categories delineated by Clark (1996) form the basis of the following review of cardiovascular malformations. The outcome groups used in the dissertation are based on codes from the International Congenital Heart Surgery Nomenclature and Database Project and incorporate the embryological perspectives of Clark (1996).

Basic cardiac embryology and looping defects

The heart begins as a hollowed structure called the heart tube. Formed from splanchnic lateral plate mesoderm, the heart tube originates during the middle of the third week of gestation. During the fourth week of gestation, the heart tube grows longer. By day 28, it has bent and looped to establish its position, and the various regions of the cardiac loop have begun to form the segments of the heart (Srivastava, 2001). As it develops during this time, the splanchnic mesoderm forms three layers. The inner layer of the tube consists of endocardium, which will form the interior lining of the heart. Myocardium, situated in the middle layer, will develop into heart muscle. The outer layer of the tube is epicardium, which will form the coronary arteries (Sadler, 2004). Occasionally there are errors in cardiac looping which cause the heart to reside on the right side of the body as opposed to the left side of the body. This condition is called dextrocardia (Sadler, 2004). Other organ systems are prone to this type of reversal as well; a complete reversal of all organ systems is called situs inversus (Sadler, 2004). Clark (1996)

classifies these defects as *abnormal situs and looping defects* because they involve the regulation of genes expressing sidedness during gastrulation (Sadler, 2004).

Septum formation

The major septa (membranes dividing the chambers) of the heart develop during days 27 through 37 (Sadler, 2004). There are two manners in which septa form; one way is through deposition of extracellular matrix. Extracellular matrix is derived from myocardium (Marino & Digilio, 2000), and as the heart develops, ridges (called endocardial cushions) are formed from extracellular matrix and endothelial cells (Mjaatvedt et al., 1999). The ridges proliferate until they come in contact with each other or until they completely cross the lumen. The other way septa form is through differential growth of the heart. During development, both the atrium and the ventricle enlarge (like blowing up a balloon). A small strip of tissue from the chamber wall, however, does not grow. This forms a muscular ridge; as the rest of the chamber continues to expand, the ridge grows larger. These ridges, however, do not grow large enough to completely divide the heart, and must be closed by other tissue.

Atrioventricular septum

The atrioventricular septum divides the upper chamber of the developing heart (atrium) from the lower chamber (ventricle). The atrial septum (which divides the left and right atrium) and ventricular septum (which divides the left and right ventricle) will be considered separately.

The atrioventricular septum is formed from extracellular matrix (endocardial cushions). As these cushions grow and fuse, two canals are formed (one on the right side of the heart and one on the left side) (Sadler, 2004). The canal on the right allows blood to pass between the right atrium and the right ventricle; likewise, the canal on the left allows blood to pass between the left atrium and left ventricle. Because this septum forms from endocardial cushions, it is considered a defect of *extracellular matrix* (Clark, 1996). Once these canals have formed, various tissues

accumulate around openings. As blood flows through the canals, some of this tissue gradually dissipates, whereas other tissue remains attached to the ventricular walls. The remaining tissue forms the mitral valve in the left canal and the tricuspid valve in the right canal (Sadler, 2004).

Pulmonary veins and the mitral valve

During gestational days 27-29, the pulmonary vein, which carries oxygenated blood from the lungs to the heart, is a small outgrowth on the primitive left atrium. It is not yet connected to the respiratory system. As the pulmonary vein develops, it connects to the vasculature of the developing respiratory system and gradually displaces atrial tissue (Ward & Mullins, 1998). In a normal heart, four separate pulmonary veins (two for each lung) enter into the left atrium. Anomalous pulmonary venous return is a defect whereby the four pulmonary veins do not enter properly into the left atrium. Abnormal positioning of the primum septum (part of the atrial septum) can cause one or more of the veins to connect to the right atrium (Ward & Mullins, 1998).

In a normal heart, blood from the pulmonary veins flows into the left atrium, through the mitral valve, and into the left ventricle. Many mitral valve anomalies (such as cor triatriatum) occur because blood flow from the pulmonary veins into the left atrium is obstructed; this obstruction may be a consequence of anomalous pulmonary venous return (Grifka & Vincent, 1998). Because these defects arise from improper incorporation of the pulmonary vein into the left atrium, Clark (1996) categorizes anomalous pulmonary venous return and mitral valve defects as arising from *abnormal targeted growth*.

Atrial septum

The left and right chambers of the atrium are divided by two septa. The septum primum does not originate from extracellular matrix; rather, it forms from a ridge on the atrial wall (Colvin, 1998). This ridge grows until it comes in contact with the endocardial cushions that form

the atrioventricular septum; the space between the ridge and the endocardial cushions is called the ostium primum (Sadler, 2004). In normal development, the endocardial cushions fuse with the septum primum and close the ostium primum (Colvin, 1998). If the ostium primum does not fully close, then the result is an ostium primum atrial septal defect. This defect is categorized as a *defect of extracellular matrix* because the endocardial cushions failed to complete the septum. Prior to the closure of the ostium primum, a small hole (the ostium secundum) forms in upper portion of the septum primum (Colvin, 1998).

The other septum that participates in the formation of the atrial septum is the septum secundum. Like the septum primum, the septum secundum originates from the wall of the atrium. The septum secundum develops in an arc-like fashion (Colvin, 1998), and as it grows, a hole (called the foramen ovale) develops.

These two septa are located very close to each other. During gestation, blood from the umbilical vein travels to the heart via the inferior vena cava. Blood from the inferior vena cava enters the right atrium and passes through the foramen ovale and ostium secundum into the left atrium. When respiration commences at birth, pressure is increased in the left atrium due to increased blood volume from the lungs. At the same time, pressure is decreased in the right atrium because blood is no longer entering from the umbilical vein. This change in pressure causes the septum primum to be pressed against the septum secundum (Sadler, 2004). In normal development, the ostium secundum and foramen ovale do not overlap. Consequently, when the two septa are pressed together, complete septation of the left and right atria is achieved. Atrial septal defects occur when the development of either the ostium secundum or foramen ovale is aberrant and blood is allowed to pass between the two upper heart chambers. Clark (1996) classifies these types of atrial septal defects as *septation defects arising from abnormal cardiac blood flow*.

Ventricular septum

By day 28 of gestation, the primitive ventricles have begun to expand (Sadler, 2004). The adjacent walls of the enlarging left and right ventricles merge together to form the muscular interventricular septum. This septum extends from the base of the ventricle upwards towards the endocardial cushions that form the atrioventricular septum; the space between these structures is called the interventricular foramen (Gumbiner & Takao, 1998). Fusion of the muscular ventricular septum with the endocardial cushions partially completes septation of the ventricle. With muscular-type ventricular septal defects (VSD), the interventricular foramen remains open because the muscular interventricular septum does not sufficiently develop. This type of defect is categorized as a defect of *cell death abnormalities* (Clark, 1996). Conversely, if the interventricular foramen remains open because the endocardial cushions do not fuse with the musculature, the resulting defect is called an inlet VSD. An inlet VSD is categorized as an *extracellular matrix defect* (Clark, 1996).

Neural crest cells migrating through the conotruncal septum also participate in the fusion of the ventricular septum. This type of VSD, termed supracristal or subarterial, is classified as an *abnormality of neural crest migration* (Clark, 1996). The fourth, and most common, category of VSD is membranous VSD (Ferencz et al., 1997). Membranous VSD occurs when abnormal blood flow damages the membranous part (where the extracellular matrix fuses with the muscular interventricular septum) of the ventricular septum (Ferencz et al., 1997). Consequently, Clark (1996) classifies membranous VSD as a *septation defect arising from abnormal cardiac blood flow*.

Defects of the cardiac outflow tract

The conotruncus is a general term for the cranial segments of the heart tube. The most cranial segment is the truncus arteriosus, which will form the main pulmonary artery and the ascending aorta (also known as the great vessels). Caudal to the truncus arteriosus is the outlet

section of the heart tube; this segment will give rise to the outlet portion of both ventricles and to the inlet portion of the right ventricle (Colvin, 1998).

The outlet septum of the heart, which partially divides the aorta from the pulmonary artery, is formed largely from neural crest cells. Development of the septum begins with the formation of a pair of opposing endocardial ridges in the conotruncus. As the embryo develops, these ridges become spiral shaped. Neural crest cells, migrating from the truncus arteriosus down into the outlet segment of the heart, fuse with the endocardial cushions to complete the outlet septum. Relative to other cardiac mechanisms in the developing heart, formation of the outlet septum seems poorly understood (McQuinn & Takao, 1998), and the preceding description is a gross oversimplification. Regardless, an important concept is that the spiral shape of the outlet septum establishes the orientation of the great vessels and determines the location where the great vessels enter into the ventricles (McQuinn & Takao, 1998).

Many defects of the cardiac outflow tract arise from abnormalities in *neural crest cell migration* (Clark, 1996). The BWIS (Ferencz et al., 1997) classified cardiac outflow tracts based on the shape of the outlet septum. With some defects, such as transposition of the great vessels and parallel double-outlet right ventricle, the outlet septum is parallel rather than spiral, causing the great vessels to enter into the incorrect ventricle. These defects are classified as outflow tract defects with transposition. The other group is classified as outflow tract defects without transposition. These defects, which include Tetralogy of Fallot, spiral double-outlet right ventricle, common arterial trunk, supracristal ventricular septal defects, and aortic pulmonary window defects have a spiral shaped outlet septum. Results from the BWIS suggested that these two categories of outflow tract defects have both shared and disparate environmental risk factors (Ferencz et al., 1997).

Complete septation of the great vessels relies on both the outlet septum and the aortopulmonary septum. The aortopulmonary septum is located cranial to the outlet septum; the location and the size of aortopulmonary septal defects vary. Defects of the aortopulmonary

septum are also called aortopulmonary window defects; Clark (1996) categorizes these defects as arising from *neural crest cell migration*, although Wiggins (1998) writes that it is generally accepted that aortopulmonary window defects are not caused by abnormalities of neural crest.

Right-sided obstruction defects

Pulmonary stenosis refers to the obstruction of blood flow from the right ventricle to the pulmonary arteries. With isolated pulmonary stenosis, individuals have an intact ventricular septum, and the stenosis is caused by obstructions in the pulmonary valve, the pulmonary artery, or the right ventricle (Cheatham, 1998). The pulmonary valve is formed from endocardial cushions within the conotruncus and consists of three cusps (Cheatham, 1998). Defects of the pulmonary valve can take numerous forms, including absence, fusion, and thickening of one or more cusps. Coarctation of the pulmonary arteries distal to the pulmonary valve also causes stenosis, however the embryology of pulmonary artery stenosis is unknown (Cheatham, 1998). In addition to pulmonary valve defects and coarctation of the pulmonary arteries, fibromuscular obstructions in the right ventricle beneath the pulmonary valve can also cause pulmonary stenosis (Cheatham, 1998). Tricuspid atresia (absence) also results in pulmonary stenosis. With tricuspid atresia, the tricuspid valve is either absent or the cusps are fused together (Sadler, 2004). Clark (1996) classifies these defects of the right side of the heart, all of which result in pulmonary atresia, as *right-sided obstruction defects resulting from abnormal cardiac blood flow*.

Although Ebstein's anomaly of the tricuspid valve is also a right-sided defect that obstructs blood flow from the right ventricle to the pulmonary artery, it is considered embryologically distinct from other tricuspid valve defects (Clark, 1996). During tricuspid development, the muscular leaflets (cusps) of the tricuspid valve become fibrous; this process happens first in the anterior leaflet, with tissue replacement in the posterior and septal leaflets occurring much later (during the third gestational month) (MacLellan-Torbert & Porter, 1998). With Ebstein's anomaly, the posterior and septal leaflets fail to become fibrous and consequently

point downward into the right ventricle, obstructing blood flow into the pulmonary artery (MacLellan-Torbert & Porter, 1998). Clark (1996) categorizes Ebstein's anomaly as an *abnormality in cell death* because of the failure of the muscular tissue in the leaflets to become fibrous.

Left-sided obstruction defects

Many malformations causing aortic stenosis are analogous to those described above for pulmonary stenosis. One common cause of aortic stenosis (obstruction of blood flow from the left ventricle to the aorta) is malformation of the aortic valve. Much like the pulmonary valve, the aortic valve is comprised of three cusps derived from extracellular matrix; aortic valve defects include absence, fusion, and thickening of the cusps (Latson, 1998). Bicuspid aortic valve is a well-known deformity of the aortic valve where only two of the three cusps are fully developed. Coarctation of the aorta is another common cause of aortic stenosis. Developmentally, coarctation of the aorta results from a thickening of the aortic cell wall. Tissue protrudes into the lumen of the vessel and obstructs blood flow (Morriss & McNamara, 1998). Aortic stenosis can also occur as a consequence of narrowing of the left ventricular outflow tract (Latson, 1998). Finally, hypoplastic left heart syndrome is a term used to describe atresia or severe stenosis of the aortic and/or mitral vale that is associated with underdevelopment of the left ventricle and ascending aorta (Ferencz et al., 1997). This syndrome is developmentally heterogeneous, as any defect causing a severe reduction in either left ventricular inflow or left ventricular outflow, such as an atrioventricular septal defect, can result in hypoplastic left heart syndrome (Barber, 1998). Ferencz et al. (1997) note that hypoplastic left heart syndrome, a severe condition which frequently results in death soon after birth, does not have a right-sided analogue.

Clark (1996) categorizes aortic valve stenosis, coarctation of the aorta, bicuspid aortic valve, and hypoplastic left-heart syndrome as *left-sided obstruction defects resulting from abnormal cardiac blood flow*. Relative to other categories of defects, those included in the *left-*

and *right-sided obstruction defects* are a heterogeneous group. The Baltimore Washington Infant Study (BWIS) elected to conduct separate analyses for many of the right- and left-sided defects, deeming the categories as defined by Clark (1996) to be too broad for epidemiologic analyses (Ferencz et al., 1997).

Cardiovascular malformations – prevalence and mortality

Botto & Correa (2003) compare the prevalence of cardiovascular malformations per 10,000 live births in Atlanta during 1995-1997 (Botto et al., 2001a) with those from Baltimore-Washington during 1981-1989 (Ferencz et al., 1997). This data is presented in Table 2.1. An inspection of Table 2.1 reveals important differences and similarities between these two populations. Foremost is the difference in the overall prevalence of cardiovascular malformations (90.4 per 10,000 births in 1995-1997 Atlanta vs. 48.4 per 10,000 births in 1981-1989 Baltimore-Washington). Relatively mild anomalies, such as VSD, ASD, and patent ductus arteriosus are much higher in Atlanta, although some of this difference is due to the enrollment strategy of the Baltimore Washington Infant Study, which did not include all infants with muscular VSD (Ferencz et al., 1997). Conversely, the prevalence of severe defects such as truncus arteriosus, AV septal defects, and hypoplastic left heart are similar in the two study populations. The authors thus argue that the difference in prevalence between the two populations is largely attributed to differences in ascertainment of mild heart defects, noting that a steady temporal increase in the prevalence of mild defects has been observed in Atlanta (Botto & Correa, 2003).

The temporal increase in the ascertainment of mild heart defects is one of many issues explored by Hoffman & Kaplan (2002) in their review of the factors that may account for discrepancies in the prevalence of cardiovascular malformations from one population to another. Other factors include age at ascertainment (i.e., ascertaining cases during the first year of life vs. ascertaining cases through the first six years of life), frequency of use of echocardiography and subsequent elective abortion of severe cases, exclusion/inclusion of trivial lesions such as mild

pulmonic stenosis, and ascertainment method (i.e., relying on physician reports vs. active ascertainment) (Hoffman & Kaplan, 2002).

With respect to infant mortality, the World Health Organization estimates that approximately 10% of all infant deaths are attributable to cardiovascular malformations (Botto & Correa, 2003). Boneva et al. (2001) used multiple-cause mortality files obtained from the National Center for Health Statistics to calculate death rates for the US population during 1979-1997. Infant mortality from heart defects declined 39% from 92 deaths per 100,000 infants in 1979-1981 to 56 deaths per 100,000 infants in 1995-1997. During 1995-1997, infant mortality was 19% higher for black infants relative to white infants. Among children between one and four years of age, mortality from heart defects decreased 57% (Boneva et al., 2001).

Environmental risk factors for birth defects

In their review article, Dolk & Vrijheid (2003) examine the impact of environmental pollution on congenital anomalies. After conducting their review of articles and reports published in English, the authors conclude that there are few environmental pollution sources that have strong evidence suggesting a causal association with birth defects. One noteworthy difficulty Dolk & Vrijheid (2003) encountered was a lack of comparability between studies. Epidemiologic studies of pesticides, for example, encompass a wide range of different chemicals and are not easily compared. Another problem is the exploratory nature of much of the research on environmental pollution and birth defects. Typically dozens of statistical tests are performed on the data. Although nearly all studies report one or more statistically significant finding, some of these associations are likely attributable to type 1 error.

Numerous studies of heavy metals, nitrates, and chlorination by-products in drinking water have been conducted, and no specific associations have been consistently reported in the literature (Dolk & Vrijheid, 2003). Epidemiologic studies examining the impact of residing near landfills or waste disposal sites have suggested that these exposures may be detrimental to fetal

organ development, although most of the increases in risk are small. Residential proximity to incinerators is particularly relevant to the question of air pollution and birth defects because many of these facilities importantly contribute to air pollution levels. Dummer et al. (2003) reported an increased odds of lethal spina bifida and lethal heart defects among babies born at residences near incinerators in Cumbria, England. Conversely, in a study of municipal solid waste incinerators in France, Cordier et al. (2004) reported an increased risk of facial clefts and renal dysplasia; no increases were found for either neural tube defects or heart defects.

Dolk & Vrijheid (2003) also review the effects of specific events involving high levels of environmental pollution. One of these events occurred in Japan during the mid 1900's as a result of the Chisso Corporation dumping mercury compounds into Minamata Bay. Although the mothers exposed to this pollution did not experience symptoms, many of their children had malformations of the central nervous system. Cooking oil contaminated with polychlorinated biphenyls (PCBs) was shown to cause stillbirth, growth retardation, and malformations of the skin, nails, and teeth in Japan and Taiwan. Although the sample size has frequently been too small to draw conclusions, investigations of high-level exposure to trichlorophenol, Agent Orange, aluminum sulphate, and trichlorofon have provided evidence suggesting these compounds may be teratogenic. Reviews by Garcia (1998) and Nurminen (2001) on the epidemiology of birth defects and pesticides both conclude that there is not enough evidence to either reject or establish a relationship. Differences in the exposure periods, definition of cases, assignment of pollution exposure, and type of pesticide make combining studies difficult.

Risk factors specific to cardiovascular malformations

The Baltimore-Washington Infant Study (BWIS) provides much of our knowledge about genetic and environmental risk factors for cardiovascular malformations (Ferencz et al., 1997). A recent review by Jenkins et al. (2007), which summarizes the current knowledge about noninherited risk factors for congenital cardiovascular defects, is based largely on results from the

BWIS. The BWIS was a large, population-based case control study in Washington, D.C., Maryland, and six counties in Northern Virginia during 1981-1989. Ascertained cases and controls were interviewed to obtain information on sociodemographic, medical, reproductive, genetic, lifestyle, and environmental factors. Questions about environmental exposures included parental smoking, alcohol and caffeine intake, recreational and therapeutic drug use, diagnostic radiography, occupational history, pesticides, dyes, metals, ionizing radiation, and solvents (Ferencz et al., 1997).

Family history of cardiac malformation was found to be a prominent risk factor for 11 of the cardiac defect groups investigated in the BWIS. Of these 11 groups, bicuspid aortic valve, atrioventricular septal defect, severe pulmonic valve stenosis, and Ebstein's anomaly all had odds ratios of six or higher (Ferencz et al., 1997). Some additional results from the BWIS are presented in Table 2.2. Numerous investigations have supported the BWIS finding that uncontrolled maternal diabetes is a risk factor for cardiovascular malformations (i.e., Adams et al., 1989; Aberg et al., 2001; Cedergreen et al., 2002; Abu-Sulaiman & Subaih, 2004). Relatively less evidence exists in the literature to support the other exposure-defect associations. In their review, Mone et al. (2004) highlight maternal febrile illness and exposure to chemicals (including paints, dyes, and solvents) as potential causes of cardiovascular malformations; the authors also emphasize alcohol use as a known cause of cardiovascular defects. Although certain medications are known teratogens (i.e., retinoids, thalidomide, trimethadione), evidence is either lacking or inconsistent for many medications (Mone et al., 2004). For example, Cappon et al. (2003) demonstrated an increased risk of ventricular septal defects in rats exposed to ibuprofen during gestational days nine and ten. Epidemiologic studies, however, have yet to document a similar association in human populations.

Maternal micronutrient intake has also been connected with cardiovascular malformations (Mone et al., 2004). Folic acid deficiency has been consistently associated with an increased risk of cardiovascular malformations in laboratory experiments (Mone et al., 2004).

Human studies of periconceptional multivitamin consumption and cardiovascular malformations, however, have been inconsistent. These studies tend to show either a protective effect or no association of multivitamin use with cardiovascular malformations. Although Shaw et al. (2000) reported that periconceptional multivitamin use was associated with an increased risk of multiple malformations (encompassing all organ systems) in California, a recent publication by Czeizel & Medveczky (2003) from Hungary reported a null result for this association.

Certain forms of vitamin A, including retinol and many of its metabolites, are known, powerful teratogens when maternal exposure is high (Lammer et al., 1985; Botto et al., 2001). Insufficient intake of vitamin A and/or retinoids also causes cardiovascular defects (Mone et al., 2004). These chemicals bind to transcription factors which regulate gene expression (Mone et al., 2004). Mesenschymal tissue development (i.e., neural crest cell formation) can be disrupted by either an excess or a deficiency of vitamin A. Botto et al. (2001b) provide epidemiological evidence for this mechanism. Using BWIS data, the investigators reported a nine-fold increase in the odds of transposition of the great arteries among women consuming more than 10,000 IU of retinol per day during the year prior to conception versus women consuming less than 10,000 IU of retinol per day. Botto et al. (2001b) also report no association between retinol intake and outflow tract defects with normally related great arteries. These findings make biological sense because neural crest cells participate in the spiral formation of the aortopulmonary septum. Consequently, congenital anomalies such as outflow tract defects with normally related great arteries should not be associated with a teratogen that hinders neural crest development because in these cases the aortopulmonary septum has spiraled properly.

In summary, there are few known strong teratogens for cardiovascular malformations. Certain medications, maternal diabetes, alcohol consumption, infections, and specific forms of vitamin A are known risk factors (Jenkins et al., 2007). The exploratory nature of much of the epidemiologic research makes interpretation of study findings difficuly. Without additional epidemiologic and/or laboratory studies to confirm the findings of the BWIS, it is impossible to

know whether the associations in Table 2.2 are causal relationships or spurious findings attributable to type 1 error.

Table 2.1. Prevalence (per 10,000 births) of major heart anomalies in Atlanta during 1995-1997 and Baltimore-Washington during 1981-1989.

Cardiac defect	Atlanta, 1995-1997	BWIS, 1981-1989
Heterotaxias and L-transposition	1.6	1.4
Outflow tract defects		
Tetralogy of Fallot	4.7	3.3
D-transposition of the great arteries	2.4	2.3
Double outlet right ventricle	2.2	0.7
Truncus arteriosus	0.6	0.5
Atrioventricular septal defects		
With Down syndrome	2.4	2.3
Without Down syndrome	1.0	1.0
Total anomalous pulmonary venous return	0.6	0.7
Ebstein anomaly	0.6	0.6
Right-sided obstructive defects		
Tricuspid atresia	0.3	0.4
Pulmonary atresia, intact septum	0.6	0.6
Pulmonic stenosis, atresia	5.9	5.4
Peripheral pulmonary stenosis	7.0	-
Left-sided obstructive defects		
Hypoplastic left heart	2.1	1.8
Coarctation of the aorta	3.5	1.4
Aortic arch atresia or hypoplasia	0.6	-
Aortic valve stenosis	0.8	0.8
Ventricular septal defect	24.9	11.2
Atrial septal defect	10.1	3.2
Patent ductus arteriosus	8.1	0.9
Other major heart defects	9.7	-
Total	90.2	48.4

Table 2.2. A selection of risk factors from the Baltimore-Washington Infant Study.

Exposure	Heart Defect	Adjusted Odds Ratio
Diabetes	Laterality & looping defects	8.3
	Membranous VSD	2.9
	Spiral outflow tract defects	5.4
Fever	Pulmonic stenosis	2.9
Influenza	D-transposition of great arteries	2.2
Solvents	Hypoplastic left heart	3.4
	Coarctation of aorta	3.2
Ibuprofen	Bicuspid aortic valve	4.1
	Transposition of great arteries	2.5
Corticosteroids	Atrial septal defect	4.8
Hair dyes	Severe pulmonic stenosis	3.7
Paints (maternal exposure)	Total anomalous pulmonary venous return	2.0
Lead (solder)	Total anomalous pulmonary venous return	6.8

Chapter 3: Ambient Air Pollution

The composition and concentration of pollutants in the air is the product of many interrelated processes. Meteorological conditions, such as atmospheric stability, temperature, rainfall, and wind have an important role in the severity and duration of an air pollution episode (McGregor, 1999). The pollutants themselves derive from a variety of sources both natural and anthropogenic. Primary pollutants are those emitted directly from a source whereas secondary pollutants are formed by atmospheric reactions between the various gases and particles in the air. Generally speaking, because secondary pollutants are formed in the atmosphere, these pollutants tend to be more homogeneously distributed throughout Atlanta than primary pollutants (Wade et al., 2004). Many of the important air pollutants in Atlanta are described below.

Carbon monoxide

Most carbon monoxide (CO) is emitted as a primary pollutant from internal combustion engines (Holman, 1999). A byproduct of the combustion process, CO tends to be found in high concentrations near busy roadways (Holman, 1999). In Atlanta, CO levels tend to be highest during the winter (Tolbert et al., 2000). Although CO is not an important contributor to the atmospheric reactions that create secondary particles, it is of particular interest for this dissertation, as Ritz et al. (2002) reported that the relationship between CO and ventricular septal defects was the strongest pollutant-cardiac defect association.

Nitrogen oxides and ozone

Nitrogen oxide (NO) is a primary pollutant emitted into the atmosphere predominantly by motor vehicles, power stations, and home and industrial processes (Derwent, 1999). NO easily reacts with ozone (O_3), which is ubiquitous in the lower atmosphere, to produce nitrogen dioxide

(NO_2) and oxygen (O_2) (Derwent, 1999). This reaction is very common during the summer months when ambient ozone is plentiful. Ultraviolet radiation causes these newly formed NO_2 molecules to divide into NO and an oxygen radical; this highly reactive oxygen radical quickly combines with O_2 to form O_3. These reactions occur rapidly and are governed by a constant relationship which involves the concentrations of NO, NO_2, and ozone in sunlight (Derwent, 1999). Because NO and NO_2 exist together in the air as a mixture, NO_x (defined as NO + NO_2) is commonly used to as a measurement of nitrogen oxides. Total nitrogen species (NO_y) is also frequently used; it is defined as the sum of NO_x and all other oxidized forms of nitrogen excluding N_2O (Derwent, 1999).

The above reactions, however, do not explain the dramatic increase in ozone seen in Atlanta during the summer (Tolbert et al., 2000). The high levels of summertime ozone are caused by reactions involving peroxide radicals (Derwent, 1999). These peroxide radicals, which are formed frequently during the summertime when a hydroxyl radical (OH) reacts with an organic compound, react with both NO_2 and O_2 to form ozone. The hydroxyl radicals may also react directly with NO_2 to form nitric acid (Derwent, 1999). Ozone is an important pollutant in Atlanta; the city has been classified as a serious ozone non-attainment area according to the National Ambient Air Quality Standards (NAAQS) set in response to the 1990 amendment to the Clean Air Act (GA Department of Natural Resources, 2001). Ozone is of added interest because Ritz et al. (2003) reported that the association between ozone and a) aortic artery and valve defects, b) conotruncal defects, and c) pulmonary artery and valve anomalies increased in a positive dose-response relationship.

Sulfur dioxide

The primary pollutant sulfur dioxide (SO_2) is emitted from the combustion of fossil fuels, such as coal, that contain sulfur (Holman, 1999). Health consequences of both SO_2 and its secondary particles (sulfate and sulfuric acid) have been implicated in toxicological and

epidemiological studies (Schlesinger, 1999; Speizer, 1999). These secondary particles can form when an oxygen radical combines with SO_2 to form SO_3; SO_3 subsequently reacts with a water molecule to form sulfuric acid (Boubel et al., 1994). The other important pathway resulting in sulfuric acid formation involves peroxide radicals, which are abundant during the summer in Atlanta (Brimblecombe, 1986). This latter reaction involving peroxide radicals is very important in the Southeast (NARSTO, 2003), as secondary sulfate particles are the largest summertime contributor to $PM_{2.5}$ (particles of diameter less than 2.5 micrometers) mass (Zheng et al., 2002). The concentration of SO_2, conversely, tends to be highest in the winter (Tolbert et al., 2000).

Ammonia

Ammonia is a gas released primarily from agricultural processes (Derwent, 1999). Although ammonia is not an important primary pollutant, it is an active participant in secondary particle formation. Both sulfuric acid and nitric acid readily form salts with ammonia (Derwent, 1999). In Atlanta, summertime concentrations of secondary ammonium tend to be somewhat higher than concentrations of secondary nitrate (Zheng et al., 2002).

Particulate Matter

There are many sources of primary particles in the air. Some primary particles, such as dust, pollen, and insect parts, are components of the natural environment. Other sources of particles are anthropogenic, such as combustion of fossil fuels from cars and trucks. Many particles, such as ammonium sulfate and ammonium nitrate, result from chemical reactions in the atmosphere. Particles are generally characterized with respect to size; PM_{10} is defined as all suspended particles with diameter less than or equal to 10 microns. $PM_{2.5}$ consists of all particles less than or equal to 2.5 microns. $PM_{2.5}$ is a subset of PM_{10}; in Atlanta during 1/1/1993 through 8/31/2000 median levels of PM_{10} were 26.3 $\mu g/m^3$ and median levels of $PM_{2.5}$ were 17.8 $\mu g/m^3$ (Metzger et al., 2004). Thus, on a typical day, $PM_{2.5}$ (also referred to as fine PM) comprises

approximately 65-70% of the total PM_{10} mass in Atlanta. The remaining mass (particles between 2.5 microns and 10 microns) are often called coarse particles (PM_{coarse}). $PM_{2.5}$ is generally thought to be more harmful (particularly with respect to mortality) than larger sized particles such as PM_{coarse} (Pope & Dockery, 1999).

Conceptually, the key atmospheric process driving the production of $PM_{2.5}$ in the Southeastern United States is the formation of sulfate from atmospheric reactions involving peroxides (NARSTO, 2003). Secondary formation of organic carbon is also likely to be an important atmospheric process, as the Southeast tends to have a significant amount of organic $PM_{2.5}$ as well (NARSTO, 2003). $PM_{2.5}$ levels tend to peak during summer months, when high temperatures cause stagnant conditions (NARSTO, 2003).

The sources of $PM_{2.5}$ in the Southeastern United States have been characterized by Zheng et al. (2002) using a chemical mass balance receptor model. These receptor models combine the chemical and physical characteristics of the particles measured at the various sources and at the monitoring station to quantify the source contributions to the monitor. This study included the Jefferson Street monitor in downtown Atlanta and the rural Yorkville monitor sited west of downtown.

Although $PM_{2.5}$ mass tends to be higher at Jefferson Street than at Yorkville, the sources of the $PM_{2.5}$ mass are similar. Atmospheric formation of sulfate particles accounts for the largest percentage of $PM_{2.5}$, particularly during summer (Zheng et al., 2002). Diesel exhaust is an important year-round contributor to $PM_{2.5}$ mass, especially at Jefferson Street, where traffic is heavier. During the winter months wood burning contributes heavily to $PM_{2.5}$ mass. Atmospheric formation of nitrate and ammonium contribute to $PM_{2.5}$ mass to a lesser extent (Zheng et al., 2002). Median levels of $PM_{2.5}$ mass are approximately twice as high in the summer as in the winter (Tolbert et al., 2000)

Ultrafine Particles

Ultrafine particles are those with a diameter less than 0.1 micron (100 nm) (Woo et al., 2001). The toxicity of ultrafine particles is thought to be extremely high. This toxicity was demonstrated by Ferin et al. (1992), who administered particles of TiO_2 at a concentration of 23 mg/m^3 to two sets of rats. The size of the TiO_2 particles was 250 nm for one group of rats and 20 nm for the other group. The lungs of the rats exposed to the ultrafine (20 nm) particles showed a substantial inflammatory response whereas the rats exposed to the fine particles experienced little pulmonary inflammation. Li et al. (1996) showed a similar result using carbon particles. These studies suggest that toxicity may be more strongly related to particle size than particle composition.

Woo et al. (2001) describe the characteristics of ultrafine particles in Atlanta from August 1998 through August 1999. Of the total number of particles less than 2000 nm, 89% are less than 100 nm and 26% are less than 10 nm. Particles less than 100 nm comprise 17% of the total volume of particles less than 2000 nm (Woo et al., 2001). Woo et al. (2001) report that ultrafine particles tend to be more numerous on weekdays than on weekends. Within a given day levels tend to increase in accord with traffic density. Although the number of ultrafine particles tends to be higher in the winter than in summer, both periods are marked by periodic episodes where the number of ultrafine particles rises sharply. These peaks tend to be confined to the very small particles. Woo et al. (2001) describe three types of events – "3-10 nm events," "10-35 nm events," and "35-45 nm events." The 3-10 nm events occur during the spring and summer. A typical event is characterized by a 50-fold increase in the number of particles around midday, when solar radiation is most intense. The authors believe a photochemical process is responsible for this sharp increase in particles, many of which are thought to be below the 3 nm detection limit (Woo et al., 2001). Relatively less detail is provided about the 10-35 nm and 35-45 nm events. The 10-35 nm events occur during winter and result in a doubling in the concentration of particles. Ambient levels of SO_2, NO, NO_2, NO_x, and NO_y increase during the 10-35 nm events (Woo et al., 2001). Finally, the authors report three days in September and five days in April

when the number of 35-45 nm particles rose sharply (the observed range was a 26- to 350-fold increase). Similar to the 10-35 nm events, levels of SO_2, NO, NO_2, NO_x, and NO_y peaked during the 35-45 nm events. Some of the 35-45 nm events were observed during the late evening (Woo et al., 2001).

Water-soluble metals and trace elements

Metals can often be found in ambient $PM_{2.5}$, albeit in low concentrations (Pooley & Mille, 1999; Metzger et al., 2004). Water-soluble metals, comprised of Fe, Mn, Cr, Cu, Ni, and V, are recorded daily at the Jefferson Street station in Atlanta. Trace/crustal elements are also collected daily at Jefferson Street and include elements such as Pb, Al, and K in addition to the fraction of Fe, Mn, Cr, Cu, Ni, and V that is not water soluble. These trace elements are very important for conducting source apportionment of $PM_{2.5}$ (Zheng et al., 2002). The source of most ambient $PM_{2.5}$ metal is anthropogenic processes such as combustion of fuel and waste incineration (Pooley & Mille, 1999); concentrations tend to be highest in Atlanta during the summer (Tolbert et al., 2000).

Volatile Organic Compounds

Like particulate matter, volatile organic compounds (VOCs) are a diverse group of compounds. Many anthropogenic sources contribute to ambient VOC levels, although solvent use, mobile source emissions, and industrial processes predominate (Holman, 1999). Some VOCs, such as benzene and 1,3-butadiene, are known to be toxic to human health (Holman, 1999). These compounds may be harmful to the developing fetus, as Lehmann et al. (2002) report that mothers exposed to certain VOCs during pregnancy were more likely than unexposed mothers to deliver infants with compromised immune systems. The Jefferson Street station records levels of oxygenated hydrocarbons, which are a measure of polar VOCs,

Temporal changes in pollution concentrations

All air pollutants exhibit annual variation in addition to the seasonal variation previously described. Most pollutants exhibit day-of-week and intraday variation as well. This short-term variation is nicely illustrated by elemental carbon, a component of $PM_{2.5}$ that is a byproduct of combustion (particularly for diesel engines) (Lim & Turpin, 2002). Typically, a sharp peak in the concentration of elemental carbon is observed between 6 AM and 9 AM in Atlanta (presumably due to traffic). Concentrations of elemental carbon decline until early evening, when a modest rise is observed around 8 PM (Lim & Turpin, 2002). During the week, the mean concentration of elemental carbon tends to increase gradually from Monday to Thursday; following the peak on Thursday, concentrations begin to decline until a minimum is reached on Sunday (Lim & Turpin, 2002).

Most time-series studies of air pollution and health statistically control for the long-term (annual and seasonal) trends in pollution. The intraday variation in air pollution levels is frequently ignored because the temporal unit of analysis is the day. Averaging times used to characterize daily air quality levels tend to be pollutant-specific and frequently conform to those used by NAAQS (i.e., Metzger et al. (2004) use 24-hour averages for particles, 1-hour maximum for NO_2, CO, and SO_2, and 8-hour maximum for O_3). Conversely, the day-of-week trend is an important source of variation in time-series studies with acute outcomes (i.e. Levy et al., 2001; Metzger et al., 2004). In this dissertation air pollution levels will be averaged over a 35-day period, eliminating the concern about day-of-week variation.

Spatial variability in pollution concentration

Wade et al. (2004) use an approach based on semivariograms to describe the spatial autocorrelation of air pollution in Atlanta. Air pollution is spatially correlated; two monitors located close to one another should have more similar measurements than two monitors located far apart. The semivariograms provide information about how the correlation between two

monitors changes as a function of distance. The Wade et al. (2004) semivariograms have the properties of both stationarity (the process is invariant to translation) and isotropy (the process is invariant to rotation) (Waller & Gotway, 2004). Thus, the correlation between two monitors sited 20 km apart is assumed to be the same everywhere in Atlanta, regardless of geographic location of the monitors. Of the various air pollutant measures, SO_2 is the most spatially heterogeneous and O_3 is the most spatially homogenous. In addition to ozone, the secondary components of $PM_{2.5}$ such as sulfate, nitrate, and ammonium tended to be highly correlated throughout Atlanta. The primary gases (SO_2, CO, and NO) displayed substantial spatial heterogeneity, as did elemental carbon, which is a primary $PM_{2.5}$ component (Wade et al., 2004).

Chapter 4: Methods

Overall Study Design

In this dissertation I will model associations between ambient air pollution levels during pregnancy and risk cardiovascular malformations in the five-county Atlanta metropolitan area using a retrospective cohort study design. The retrospective cohort will consist of all births and fetal deaths in the five-county Atlanta metropolitan area (Clayton, Cobb, DeKalb, Fulton, and Gwinnett counties) conceived during January 1, 1986 through March 12, 2003. Birth defect cases are ascertained by the Metropolitan Atlanta Congenital Defects Program (MACDP), a population-based surveillance system of birth defects, operated by the Centers for Disease Control and Prevention (CDC) since 1967 (Correa-Villaseñor et al., 2003; Correa et al., 2007). Records of births and fetal deaths are maintained by the Georgia Office of Health Information and Policy.

For the 18-year study period, data on air pollution levels will be obtained from the state monitoring network and several intensive air quality studies. Average air pollution levels for the gestational time period of interest will be calculated based on these measurements.

Primary hypotheses relate to the seven air pollutant-heart defect associations reported by Ritz et al. (2002) and Gilboa et al. (2005): 1) ambient CO levels averaged over gestational days 15-49 and ventricular septal defects, 2) ambient O_3 levels averaged over gestational days 15-49 and aortic anomalies, 3) ambient O_3 levels averaged over gestational days 15-49 and conotruncal anomalies, 4) ambient O_3 levels averaged over gestational days 15-49 and pulmonary artery and pulmonary valve anomalies, 5) ambient PM_{10} levels averaged over gestational days 15-49 and atrial septal defects, 6) ambient CO levels averaged over gestational days 15-49 and tetralogy of Fallot, and 7) ambient sulfur dioxide levels averaged over gestational days 15-49 and ventricular septal defects. Poisson generalized linear models will be created to test the primary hypotheses.

The remaining air pollutant-heart defect combinations will be modeled in secondary analyses using an identical analytic approach.

Births records from the Georgia Office of Health Information and Policy

A retrospective cohort will be constructed from individual-level electronic databases of all births and fetal deaths from 1986-2003 in the five-county Atlanta metropolitan area. Data will be obtained from the Georgia Office of Health Information and Policy and will be linked to the MACDP database using a method developed by CDC. Covariate information to be obtained includes date of birth or fetal death, gestational age, date of last menstrual period, maternal age, maternal ethnicity, infant gender, parity, plurality, liveborn/stillborn status, previous preterm delivery, diabetes, pregnancy complications, alcohol use and smoking. Gestational age may be estimated either from the date of last menstrual period (LMP) or from subtracting the estimated gestational age from the date of birth.

Metropolitan Atlanta Congenital Defects Program (MACDP)

The inclusion criteria used for case ascertainment by MACDP are as follows: 1) residence of birth mother in the five-county Atlanta metropolitan area (Clayton, Cobb, DeKalb, Fulton, and Gwinnett counties); 2) presence of serious or major structural defects that can have adverse effects on health or development; 3) defect must be noted by six years of age; and 4) gestational age of 20 weeks or more. Whenever possible, MACDP ascertains affected pregnancies that are prenatally diagnosed and terminated prior to 20 weeks of gestation. Cases that might not be ascertained include: 1) some affected pregnancies prenatally diagnosed and terminated; 2) infants with birth defects that migrate or receive follow-up medical care outside of the five-county study area; 3) infants with birth defects undiagnosed or diagnosed after 6 years of age; and 4) cases born to residents of the study but outside of the study area. Cases in MACDP

are identified on an ongoing basis by trained abstractors who actively search newborn hospitals, pediatric hospitals, and other sources.

Coding and classification

For each affected infant, information is collected on up to 24 individual defects using the Reproductive Outcome Case Record. These defects are coded using modified British Pediatric Association (BPA) six-digit codes (BPA, 1979). Substantial effort is being devoted to the creation of a classification system that groups cardiac lesions based on the presumed embryological timing of the individual heart defects. This activity will produce groupings of defects appropriate for epidemiologic analyses (Clark, 1996; Ferencz et al., 1997). Cases with chromosomal defects and known syndromes will be excluded from the analysis. The major categories of cardiac lesions, as delineated by Clark (1996), are provided in Table 4.1.

Conceptually, infants with one or more cardiac defect can be thought of as falling into one of six broad groups (listed with increasing complexity): 1) isolated cardiovascular malformations and no other anomalies, 2) isolated cardiovascular malformations and other anomalies, 3) known patterns of cardiovascular malformations and no other anomalies, 4) known patterns of cardiovascular malformations and other anomalies, 5) complex cardiovascular malformations and no other anomalies, and 6) complex cardiovascular malformations and other anomalies. Generally, cardiovascular malformations with other anomalies are more complicated because of the concern that a gene mutation may be the cause of the cardiac defect. To convey this idea, Clark (2004) describes the concept of the "developmental field." Rather than concentrate on the role of one cell, Clark (2004) argues that for complex organisms focus should be on the developmental field, the region of the embryo that controls spatial orientation and temporal development. In mammals, the developmental field of the heart lies adjacent to those of the forelimb, face, brain, lungs, and midline structures of the pharyngeal pouches. Because of their proximity, a single gene mutation may impact the development of other organs with nearby

developmental fields. Consequently, it is common to find brain, immune system, limb, lung, and kidney abnormalities in conjunction with heart defects (Clark, 2004). Many well-known syndromes, such as Williams syndrome, Holt-Oram syndrome, CHARGE association, and DiGeorge syndrome, involve infants with congenital defects of multiple organ systems with adjacent developmental fields (Clark, 2004). Although known syndromes will be excluded from the analysis, some infants not classified as having a syndrome will have defects to multiple organ systems, thus increasing the complexity of the case and making classification more difficult.

Cases with multiple cardiovascular malformations are likewise more complicated than cases with a single cardiac defect. Classifying infants with a single cardiac defect will be relatively straightforward, although temporal issues in classification and ascertainment will need to be addressed. The group of ventricular septal defects (VSD) highlights this issue. A VSD has four general variations – perimembranous, muscular, supracristal, and inlet – each of which has a distinct embryology. The ability to distinguish between these four types of VSDs, however, requires appropriate technology that has likely to changed over time (Botto et al., 2001a). Infants with known patterns of cardiovascular malformations and complex defects will be more difficult to classify. For example, an infant with underdevelopment of the aorta, aortic valve, left ventricle, and mitral valve will be easily classified as a case of hypoplastic left heart syndrome because these four defects are part of a recognized pattern. However, an infant with these four codes plus an atrioventricular septal defect (AVSD) will be much more difficult to classify. The AV septal defect presumably occurs because of a defect in extracellular matrix, whereas the hypoplastic left heart syndrome is caused by improper blood flow though the left side of the heart (Clark, 1996). The reviewer must rely upon their expertise to determine whether the hypoplastic left heart syndrome could have been caused by the AV septal defect. Should this case be grouped with other AV septal defects that do not have accompanying hypoplastic left hearts? Should the case be grouped with other hypoplastic left hearts that do not have AV septal defects? Should it be classified as a “complex” or “multiple” defect? These kinds of questions will be encountered

frequently when an infant has multiple cardiovascular malformations that are not part of a recognized pattern.

Overview of air quality data

Air quality data for this study will be obtained from three main sources: a) the ARIES monitoring station, b) the Georgia Tech $PM_{2.5}$ network, and c) the state network of ambient air quality monitoring stations. Past air pollution and meteorological measurements will be obtained from the various networks so that the database extends back to 1968. A map with the locations of the current monitoring stations is presented in Figure 4.1.

ARIES monitoring site

The Electric Power Research Institute (EPRI) launched an innovative air quality monitoring station located at Jefferson Street 2.6 miles northwest of downtown Atlanta on August 1, 1998, as part of the ARIES study. Numerous measurements of PM are being conducted (Van Loy et al., 2000), including total mass of PM_{10}, $PM_{2.5}$, and PM_{coarse} (particles between 10- and 2.5- micrometers in diameter), mass measurements of the chemical components of $PM_{2.5}$, and particle counts of ultrafine PM (particles with diameter less than 0.1 micron). Several different instruments used to measure $PM_{2.5}$ are deployed at the ARIES site: the federal reference method (FRM), particle composition monitor (PCM), and tapered element oscillating microbalance (TEOM). A dichotomous, filter-based sampler is used to measure daily PM_{coarse}. Components of $PM_{2.5}$, which are measured by PCM instruments, include sulfates, organic carbon, elemental carbon, acidity, volatile organic compounds (VOCs) and a water-soluble metal index (sum of chromium, copper, iron, manganese, nickel, and vanadium). Continuous measurement of O_3, CO, SO_2, and NO_2 is conducted with standard instrumentation. Meteorological data, including daily temperature (mean, maximum, minimum), mean dew point temperature, relative humidity, barometric pressure, solar radiation flux, and pollen and mold counts are also recorded daily.

Georgia Tech $PM_{2.5}$ network

Researchers at Georgia Tech have been measuring $PM_{2.5}$ mass and composition at three Atlanta locations since March 1999. These three sites are located approximately 7 miles southwest (Fort McPherson Army Base), 12 miles northeast (Tucker), and 9 miles southeast (South DeKalb College) of downtown Atlanta. Particulate mass is measured continuously (resolved at one-minute intervals) using TEOM instruments. In addition to the $PM_{2.5}$ continuous mass measurements, PCM instruments have been sited to gain integrated, 24-hour composition data. The species collected on various filter media include ammonium, sulfate, and nitrate ions, elemental and organic carbon, and metals (magnesium through lead) (Butler et al., 2003).

State network of ambient air quality monitors

The air quality database will be augmented by several existing networks in the state of Georgia. Sources of data include EPA's Air Quality System (AQS), the Southeastern Consortium for Intensive Oxidant and Nitrogen measurements (SCION), the Clean Air Status and Trends Network (CASTNet), the Metro Atlanta Index (MAI) of the Georgia Department of Natural Resources, and the National Climatic Data Center (NCDC).

Since 1968 there have been substantial changes in the air pollutants being monitored the locations of the monitoring sites, and the methods and frequency of measurements. Air monitoring stations provide measurements of SO_2 and total suspended particles since 1968, CO and NO_2 since 1972, ozone since 1974, PM_{10} since 1986, and $PM_{2.5}$ since 1999. Although the methods used to monitor CO, NO_2, and TSP were constant during the study period, multiple methods have been employed to measure SO_2, O_3, PM_{10}, and $PM_{2.5}$. All of the pollutants have had changes in the number and location of monitoring stations.

Estimate PM_{10} measurements from total suspended particle measurements

In the Atlanta area, PM_{10} was not routinely monitored until 1986. Total suspended particles (of which PM_{10} is a component) were monitored prior to 1986. Two AQS monitoring stations collected PM_{10} and total suspended particle measurements simultaneously – Doraville Health Center (1987-1990) and Fulton County Health Dept (1993-1997). Using measurements from these two stations, in conjunction with meteorological data, statistical models will be created to estimate PM_{10} measurements from total suspended particle measurements. These models will then be used to estimate PM_{10} levels for 1968-1985. Although Lipfert (1994) argues that measured levels of TSP are inflated because HNO_3 is spuriously measured by the high-volume TSP samplers as a particle, this source of measurement bias will not impact our results provided that the PM_{10} samplers are accurate.

The relationship between PM_{10} and TSP varies substantially depending upon the measurement method and the geographic location of interest. Berico et al. (1997) compared collocated measurements of TSP and PM_{10} in Bologna, Italy, in March, 1995. A total filter of cellulose triacetate was used to measure TSP. Two different methods were used to measure PM_{10} – an Anderson dichotomous sampler using Teflon filters and a LPI Berner Hauke 11-stage cascade using aluminum impactor plates. The TSP mass was, on average, 22% higher than the PM_{10} mass recorded by the Anderson sampler and 37% higher than the PM_{10} mass recorded by the LPI Berner Hauke impactor (Berico et al., 1997). In India, at the port of Jawaharlal Nehru, PM_{10} was determined to comprise only 47% of TSP; both PM_{10} and TSP measurements were recorded with respirable dust samplers (Gupta et al., 2004). Keywood et al. (1999) report that for six cities in Australia, 86% of the TSP mass was PM_{10} (range 81%-92%). These measurements were recorded using a micro-orifice uniform deposit impactor (MOUDI) instrument.

Construct air pollution variables for analysis

A variety of air quality variables will be created for the analyses. One set of air quality variables will be based on measurements from a centralized, representative monitor following the

method employed by Metzger et al. (2004). The representative monitor is specific to each pollutant. Metzger et al. (2004) used the DeKalb Tech monitor for CO levels (available 1981-2003), the Confederate Avenue monitor for ozone levels (available 1991-2003), the Jefferson Street monitor for total $PM_{2.5}$ concentrations and $PM_{2.5}$ component concentrations (available 8/1998-2003), and the Georgia Tech monitor for NO_2 levels (available 1973-2003) and SO_2 levels (available 1982-2003). Ambient PM_{10} levels were based on the Metropolitan Atlanta Index (MAI), which records 24-hour average PM_{10} concentrations from 8AM–8AM. Daily (midnight–midnight) PM_{10} concentrations were modeled from the two MAI measurements that cover the corresponding calendar day (Metzger et al., 2004). When data from the central monitoring stations are missing, daily pollution levels are imputed using measurements from surrounding monitors, meteorology, and time variables. These variables will be used in the temporal Poisson generalized linear model analyses (see below).

Both the location of the central monitoring station and the measurement method may have changed during the follow-up of the retrospective cohort (1968-2003). An alternative ozone monitor, such as South DeKalb (available 1974-2003) or Conyers Monastery (available 1978-2003), is likely to replace Confederate Avenue (available 1991-2003) as the central monitor to provide greater continuity over the course of the study period. When a measurement method or monitor location changes, an indicator variable will be included in the statistical model to account for this change. Furthermore, new models to impute missing values will need to be created to account for these changes. Metzger et al. (2004) encountered this issue for PM_{10}, as the measurement method changed from the federal reference method (FRM) to the tapered element oscillating microbalance (TEOM) method in January 1996.

Estimate date of conception and average pollution measurements

Date of last menstrual period (LMP) will be obtained from vital records; when missing, the date of LMP will be estimated from birth date and gestational age. For each individual,

conception is presumed to occur 14 days after the date of LMP. Average air pollution levels for the gestational time period of interest will be calculated by taking an average of the available measurements. This method will be applied to all of the air pollution variables. The a priori gestational period of interest is days 15-49 (Srivastava, 2001; Sadler, 2004).

Describe seasonal and spatial trends in the data

The temporal and spatial trends for both air pollution and birth defects will be described with frequency tables, histograms, and plots with smoothers. A thorough descriptive analysis will aid in the understanding of confounding by time trends and spatial heterogeneity. Alternative methods will be used in sensitivity analyses to account for temporal, seasonal, and spatial trends in both birth defects and air pollution.

Develop temporal models for count data

Daily counts of cardiovascular malformations, births, and fetal deaths will be utilized in generalized linear models (Zeger & Liang, 1986) using the Poisson distribution. Although pollution values are averaged over a 35-day period, the unit of observation for the outcome variable is the day. The number of birth defects on each day of conception is a count. This count is a rare event and is presumed to follow the Poisson distribution. The corresponding denominator is the number of infants and fetuses conceived on each day.

The data will be modeled using generalized linear models (Zeger & Liang, 1986). A scale factor will be used to adjust for underdispersion and/or overdispersion in the models. Overdispersion occurs whenever the variance of a distribution of counts is larger than its mean; if this additional variance is not accounted for in the models, the estimated p-values and confidence intervals will be too small (Zeger & Liang, 1986). One way overdispersion can occur is if important predictor variables are missing from the model. This situation is common in air

pollution epidemiology, as information on these variables is often unavailable. These temporal Poisson models will use data from 1986-2003. The basic model has the following form:

$\log(E(Y_t)) = \text{offset}_t + \alpha + \beta * \text{pollutant}_t + g(\gamma_1,\ldots,\gamma_N; \text{time}) + \delta^*(\text{temporally varying covariates}_t)$,

where: $g(\gamma_1, \gamma_2, ...\gamma_N; x) = \gamma_1 x + \gamma_2 x^2 + \gamma_3 x^3 + \sum_{j=4}^{N} \gamma_j w_j(x)$

and where $w_j(x) = (x-\tau_j)^3$ if $x \geq \tau_j$, and $w_j(x) = 0$ otherwise

Y corresponds to the number of defects in the outcome group aggregated over the five-county Atlanta area for date of conception t. An offset term representing the log of the count of all births and fetal deaths for date of conception t will be included to control for daily variation in the outcome variable. The pollutant term refers to the average pollution measurement during the gestational period of interest.

Cubic splines, $g(\gamma_1,\ldots,\gamma_N; \text{time})$, will be used to control for temporal variation in both pollution levels and birth defects. In this study, both the outcome and the exposure vary seasonally (Ferencz et al., 1997; Tolbert et al., 2000). Furthermore, there are important long-term time trends in both air pollution and birth defects. These long-term and seasonal trends can confound the short-term (35-day) association of interest. Although indicator variables could be used to control for these time trends, cubic splines offer the added advantage of modeling the trends as smooth functions. An assumption of this Poisson process is that in the short-term the baseline risk (absent of risk factors) is constant. Thus, from one day to the next, the baseline risk is presumed to be constant. However, slow changes in the baseline risk over time will be accounted for in the cubic splines.

The covariate term is included in the model to incorporate additional terms if needed. A key advantage of this approach is that individual-level factors, such as smoking, can only be

confounders if they are related to short-term changes in air pollution, which is generally unlikely, since the long-term time trend and seasonality will be controlled.

Develop spatio-temporal models for geocoded data

These analyses will use logistic models to further investigate the pollutant-defect combinations. Logistic models will use the retrospective cohort of all recorded birth defects of interest, fetal deaths, and live births in the five-county Atlanta metropolitan area during 1994-2003 (the time period when electronic vital records are available). Assignment of pollution levels during the a priori gestational period of interest will be based on both date of conception and geocoded maternal address.

$$\text{logit}(Y_t) = \alpha + \beta^{*}\text{pollutant}_t + g(\gamma_1,\ldots,\gamma_N; \text{time}) + \delta^{*}(\text{temporally varying covariates}_t) + \eta^{*}(\text{spatially varying covariates}_t)$$

Here Y_t represents a dichotomous variable indicating the presence or absence of a specified birth defect on date of conception t. The pollutant term refers to the average pollution measurement during the a priori gestational period. Relative to the Poisson models, a larger number of knots in the cubic spline will be included in the logistic models because the individual-level data allow differential classification of average pollution levels for pregnant mothers residing in different geographic areas. Incorporating spatially varying risk factors for birth defects in the model is essential because these factors will confound the relationship between air pollution and birth defects if they are correlated with pollution levels. Although some important risk factors, such as age and ethnicity, are available on the birth records, information on many potential confounders is not available. Because of this concern, the spatio-temporal models are conceived as being secondary to the Poisson temporal models.

Apply the Ritz et al. (2002) methodology to the study data

This sensitivity analysis will use the same cardiac birth defect categories, gestational time periods, method of selecting cases and controls, method of assigning air pollution levels, and statistical analysis as Ritz et al. (2002). Cases will be eligible for the analysis if the defect was diagnosed within the first year of life and maternal residence was within ten miles of an air monitoring station. A random sample of infants who have non-missing information on their vital records, lived within ten miles of an air monitoring station, and were undiagnosed with a birth defect by their first birthday will be used as the control group. Average air pollution levels will be calculated from available measurements recorded at the most relevant monitoring station. Both conventional logistic models and hierarchical (two-stage) semi-Bayes regression models will be employed in the analysis. The semi-Bayes models specified a prior variance of $\tau^2 = 0.5$.

Ritz et al. (2002) direct the reader to Greenland (1994) for a more thorough explanation of the hierarchical regression methods used in their analyses. Greenland (1994) describes various methods for constructing Bayesian hierarchical models. The first stage of the two-stage model is:

$$g[E(y|x,w)] = \alpha + \beta*x + \gamma*w$$

In this model g is a link function (i.e., a logit link function for logistic regression). The x is a matrix of exposures and w is a matrix of control variables. The β and γ are parameter estimates for the exposures and control variables. The outcome, y, is assumed to be randomly sampled from a distribution conditional on x and w (Greenland, 1994). This first-stage model is identical to a general linear model used in traditional statistics. The second stage of the hierarchical models incorporates prior information about the mean and variance of the β parameters.

$\beta_i = \pi^* z_i + \delta_i,\ i= 1,\ldots,n \qquad$ i.e., $\beta = \pi^* Z + \delta = \mu + \delta$

In this second-stage model, z_i is a row vector of known prior covariates, π is a column vector of possibly unknown prior coefficients, and the δ_i are independent normal variables with mean zero and possibly unknown variance τ^2 (Greenland, 1994). The model assumes a common (possibly unknown) mean μ and variance τ^2 for β. The parameters μ and τ^2 are termed the prior mean and prior variance, respectively.

Greenland (1994) outlines various methods for constructing Bayesian hierarchical models. These methods differ on the treatment of the prior mean and prior variance parameters. One method, which Greenland (1994) terms "empirical Bayes," estimates the unknown μ and τ^2 from the data. Although numerous options exist for estimating these prior parameters, Greenland (1994) suggests using Monte Carlo methods. The resulting estimates are then used as the prior parameters and a standard Bayesian analysis is run.

A related method is termed "semi-Bayes" by Greenland (1994). In this approach the prior variance, τ^2, is specified a priori. The unknown prior mean is subsequently estimated from the data. The semi-Bayes approach will underestimate standard errors whenever the τ^2 prior is too small; however, Greenland (1992) has demonstrated that model results are robust to over-specification of τ^2, such that there is little penalty in specifying an a priori variance that is too large. Therefore, it is prudent to specify a sufficiently large value of τ^2 to guard against variance underestimation. The semi-Bayes approach has the advantage of being computationally simpler than the empirical Bayes approach and produces smaller standard error estimates in very small studies (Greenland, 1994). For large studies such as Ritz et al. (2002), the empirical Bayes and semi-Bayes approaches would be expected to give similar results (Greenland, 1994).

Assess relationships between distance-weighted traffic density (DWTD) values and the birth defect groups of interest

Annual average daily traffic (AADT) counts will be obtained from the Georgia Department of Transportation to assign a traffic density to each roadway. Calculation of a distance-weighted traffic density (DWTD) begins with the construction of a 500-foot radius around the geocoded location of each residence. For each roadway intersecting this radius, the dispersion of motor vehicle exhaust from the roadway within the radius is estimated by a model developed by Pearson et al. (2000). This model assumes 1) 96% of motor vehicle exhaust pollutants disperse at 500 feet, 2) wind has no influence, and 3) pollutants are inert. The model has the form:

$$Y = \left(\frac{1}{0.4\sqrt{2\pi}} \right) e^{-\left(\frac{0.5(D/500)^2}{(0.4)^2} \right)}$$

Where D is the shortest distance from the residence to the roadway and Y is the value used to weight the AADT count for the roadway. The DWTD for the residence is calculated by summing the weighted AADT counts for each road intersecting the 500-foot radius (English et al., 1999; Pearson et al., 2000; Wilhelm & Ritz, 2003).

Conduct source apportionment analyses

Results of source apportionment work being conducted by co-investigators at Georgia Tech will be applied to this dissertation (Marmur et al., 2005). The source apportionment is based on the Chemical Mass Balance (CMB) model and aims to determine which sources of pollution (i.e. mobile sources, power plants, etc.) are the main contributors to measured concentrations of pollutants at the ARIES site. Results from the source apportionment work will be used in models predicting specified birth defects as a function of air pollution sources rather than the individual pollutants.

To identify the main sources of $PM_{2.5}$ at a given site (Jefferson Street), a system of equations is created. These CMB equations have the following form (Marmur et al., 2005):

$$C_i = \sum_{j=1}^{n} f_{i,j} S_j$$

Where: C_i = ambient concentration of specie i ($\mu g/m^3$)

$f_{i,j}$ = fraction of specie i in emissions from source j

S_j = contribution of source j ($\mu g/m^3$)

n = total number of sources

In CMB models, the source emissions (the S_j) are known, constant values. All major sources of $PM_{2.5}$ need to be accounted for in the model or results will not be valid. Marmur et al. (2005) used ten source profiles ($f_{i,j}$) for the Jefferson Street monitor: gasoline vehicles, diesel vehicles, fugitive soil dust, vegetative burning, coal-fired power plants, cement kilns, secondary ammonium sulfate, secondary ammonium bisulfate, secondary ammonium nitrate, and secondary/other organic carbon. Additional assumptions of the model are: 1) chemical species do not react with each other, 2) measurement uncertainties are random, uncorrelated, and normally distributed, and 3) the source profiles are not collinear (Marmur et al., 2005). The assumption about collinearity is difficult to achieve because in practice the $PM_{2.5}$ source profiles of many sources are correlated. To decrease collinearity and improve model specification, Zheng et al. (2002) elected to use particle-phase organic compounds in conjunction with $PM_{2.5}$ components in the CMB models. Marmur et al. (2005) chose to incorporate gas-phase data (SO_2, CO, and NO_y) into the CMB models to decrease collinearity; a global optimization approach was employed to estimate the fraction of the total $PM_{2.5}$ contributed by each source.

Once the source apportionment is finalized, a database will be created containing daily $PM_{2.5}$ concentrations for each source of pollution. These source apportioned $PM_{2.5}$ concentrations will vary temporally on a day-to-day basis. Analogous to the single-pollutant Poisson GEE

models described earlier for the temporal analyses of reclassified cardiovascular malformations, single-source Poisson GEE models will be created for each source of pollution. Multi-source models will contain a term for the overall $PM_{2.5}$ concentration and additional terms corresponding to the percentage of total $PM_{2.5}$ contributed by each source. The multi-pollutant models will allow for simultaneous assessment of both the overall effect of $PM_{2.5}$ and the influence of individual sources.

Conduct sensitivity analyses

Numerous additional analyses will be conducted that use alternative modeling strategies to assess the sensitivity of results to the modeling strategies employed and to potential confounding due to seasonal and temporal trends in birth defects and air pollution. Variation in air pollution is either temporal or spatial; as such, tight control of both temporal and spatial variables can reduce the remaining variation to an extent that the effect of air pollution can not be evaluated. However, tight control for geographic region reduces potential confounding by geographic area, whereas tight control for temporal trends diminishes the possibility of confounding by time.

One set of sensitivity analyses will be based on the use of splines with additional knots to more fully account for seasonal and temporal trends. In these analyses, we will evaluate potential associations in geographic areas with higher air pollution levels that may also have a higher prevalence of the fetal outcome of interest. Adjustment for covariates such as maternal education and prenatal care will be particularly important, as geographic variation will drive the results. Another set of sensitivity analyses will control more tightly for geographic variation, allowing temporal variation to drive the analyses. A third set of sensitivity analyses will evaluate the effect of restricting the dataset to those defects diagnosed by the second year of life. In this sensitivity analysis, results will be compared to those obtained from a dataset limited to infants born prior to 1998 (who have been followed for the full six-year MACDP case ascertainment period). We will

also assess whether statistically controlling for additional risk factors available on Georgia vital records (including smoking, alcohol consumption, and obesity correlates such as hypertension and eclampsia) affect the results obtained from individual-level spatio-temporal models. Because the validity of the covariate information on vital records is suspected to be poor their use will be reserved for sensitivity analyses.

Assess measurement error

Several sources of measurement error can potentially bias results when ambient measurements are used to characterize air pollution in an epidemiologic study, including instrument error, error from local sources, error from using a limited number of monitors in the presence of spatial heterogeneity, and error from using ambient air measurements as a surrogate for personal exposure. The objective of this dissertation is to study and characterize the association between ambient levels of air pollution and the occurrence of heart defects. This is a worthy objective because knowledge of the associations between ambient pollution levels and health are relevant for regulatory decisions, since it is the ambient levels which will be monitored and regulated.

To assess the consequences of measurement error encountered by using the ambient pollution level measured at a central monitor at time t (z_t) as a proxy for the average personal exposure for at-risk individuals at time t ($\bar{x}_t$) requires information about personal exposure (Zeger et al., 2000). If $\bar{x}_t$ were available for the entire study period, a straightforward approach would be to create two Poisson time-series models – one predicting the daily count of heart defects from z_t and a second predicting the daily count of heart defects from $\bar{x}_t$. The regression coefficients and standard errors from these two models could then be compared to assess measurement error. Since $\bar{x}_t$ is not available for the entire follow-up period this approach can not

be implemented. However, data on $\bar{x}_t$ are available for metropolitan Atlanta for short periods of time (Reid et al., 2002; Wheeler et al., 2002), and this data can be used to estimate the relationship between $\bar{x}_t$ and z_t. Zeger et al. (2000) suggest the following regression calibration approach:

$$\bar{x}_t = \Theta_0 + \Theta_1 z_t + \varepsilon_t$$

The parameters Θ_0 and Θ_1 can be estimated from the personal exposure studies. Once Θ_0 and Θ_1 have been estimated these values can be used to predict $\bar{x}_t$ for time periods when $\bar{x}_t$ is unknown. The model can be extended if multiple sources of information about the relationship between $\bar{x}_t$ and z_t are available. Zeger et al. (2000) recommend using simulated values for $\bar{x}_t$ rather than predicted values of $\bar{x}_t$ in this situation because the simulations easily incorporate additional information. The simulated series of $\bar{x}_t$ provides estimates of the regression coefficient and its variance. The mean of the simulated distribution of coefficients can be compared to the coefficient estimated from z_t (β_z) to estimate the magnitude and the direction of the bias. Similarly, the distribution of the simulated coefficients can be compared with the distribution of β_z to assess the effect of measurement error on confidence interval width (Zeger et al., 2000).

Power Calculations

A major strength of the dissertation is the large number of cases ascertained by MACDP. Relative to the Ritz et al. (2002) study, the 1968-2003 database has over six times as many individuals with heart defects that do not have diagnosed chromosomal anomalies (10,691 vs. 1,691). In practice, this disparity in sample size is somewhat larger. The spatio-temporal

methodology employed by Ritz et al. (2002) necessitated complete data on a variety of covariates. Consequently, nearly 26% of the cardiac defect cases (n=419) were excluded from the analysis due to missing covariate data. Conversely, the temporal analyses that will be employed in this dissertation only necessitate an estimate of the date of conception, so a much larger percentage of the cases will be eligible for the analyses.

Power calculations are presented in Table 4.2. Power was calculated for Poisson regression models using PASS 7.0 software (NCSS Statistical Software, 2002), with the probability of a type 1 error fixed at α=0.05 and the coefficient of multiple determination for the covariates regressed on the air pollution measurement fixed at R^2=0.50. The power estimates are highly sensitive to the choice of R^2. Sample sizes were based on the number of birth defects in the MACDP registry and on the number of births and fetal deaths in the vital statistics registries for the appropriate time periods. The rate ratios presented correspond to a 1-standard deviation increase in the value of the pollution measurement, which was assumed to be Gaussian. Table 4.2 displays power for the estimated number of birth defects during 1968-2003 (for temporal analyses) and 1994-2003 (for spatio-temporal analyses).

The power calculations in Table 4.2 are for relatively broad groupings of birth defects (similar to those used by Ritz et al., 2002). A larger number of cardiac birth defect categories are likely to result from the reclassification of the cardiovascular malformations (thus reducing the sample size for each category). The power calculations presented in Table 4.2, however, are for rate ratios substantially smaller than the odds ratios reported by Ritz et al. (2002). Furthermore, the six categories of cardiovascular malformations presented are not comprehensive, as numerous cardiovascular malformations do not fit into one of these categories. Indeed, according to Table 4.2 the sum of the number of heart defects during 1968-2003 is 9,742 whereas the actual number of cardiac defect cases ascertained by MACDP during this time is 10,691.

Four of the six cardiac birth defect categories have power of 70% or better to detect a rate ratio of 1.10 for 1968-2003 data. The a) conotruncal defect and b) endocardial cushion and mitral

valve defect categories have approximately 55% power to detect a rate ratio of 1.10. The ability to detect a rate ratio of 1.05 is substantially lower, although power for the ventricular septal defects category is 50%. Removal of cardiovascular malformations prior to 1994 results in a fairly substantial loss of power, particularly for the group of conotruncal defects.

Prepare manuscripts based on dissertation results

Three manuscripts will be prepared based on the work conducted for this dissertation. One manuscript will compare the newly developed classifications for cardiovascular malformations with the preexisting codes in the MACDP database. This manuscript will describe and quantify the value added to the MACDP database attributable to the classification of cardiovascular malformations that were conducted as part of this dissertation (see dissertation Chapter 5). The second manuscript will be a methodological paper on the issue of confounding in epidemiologic studies of air pollution and adverse pregnancy outcomes. This manuscript will discuss the plausibility of confounding across the three main analytic approaches (spatial, temporal, and spatial-temporal) that can be used to analyze this type of data (see dissertation Chapter 6). The third manuscript will contain the results of the models of temporal associations between ambient pollution levels during weeks three through seven of pregnancy and risk of cardiovascular malformations. This manuscript will address the primary dissertation hypotheses as well as the secondary models based on the temporal analytic approach (see dissertation Chapter 7).

Table 4.1. Groupings of cardiac lesions based on developmental mechanisms and etiologic origins as specified in Clark (1996).

Defect group
Abnormal Situs and Looping 1. Complex cardiovascular malformations with atrial-situs abnormalities (situs ambiguous, inversus) 2. Single Ventricle with L-malposition of the aorta 3. Ventricular inversion (corrected transposition) with L-transposition (TGA) 4. Dextrocardia with situs inversus
Malformations of Ventricular Outlets and Arterial Trunks (conotruncal defects) I. Abnormalities of Ectomesenchymal (neural crest) tissue migration 1. Tetralogy of Fallot: Pulmonary atresia with VSD is often included in this category and likely has similar etiologic cause 2. Double-outlet right ventricle (DORV) 3. Truncus arteriosus 4. Supracristal (subarterial or conoseptal) type Ventricular Septal Defect (VSD) 5. Aortic-pulmonary window 6. Interrupted aortic arch (type B) 7. Double aortic arch II. Complete transposition (D-TGA): Appears to be etiologically different from the above defects associated with neural crest migration
Extracellular Matrix Defects 1. Atrial-ventricular septal defects (Endocardial cushion defect) 2. Ostium primum ASD (partial defect) 3. Inlet type VSD
Abnormal Cardiac Blood Flow I. Left-sided Obstruction Defects (aortic anomalies) 1. Bicuspid aortic valve 2. Aortic stenosis 3. Coarctation of the aorta 4. Hypoplastic left heart syndrome II. Right-sided Obstruction Defects (pulmonary valve and artery anomalies) 1. Pulmonary valve stenosis 2. Pulmonary atresia with intact ventricular septum 3. Tricuspid atresia with normally related great vessels 4. Pulmonary artery stenosis III. Septation defects and Patent Ductus 1. Membranous type VSD: Etiology is distinct from supracristal, inlet, and muscular VSDs. 2. Secundum atrial septal defect (ASD) 3. Patent ductus arteriosus (PDA): Would exclude infants born prematurely
Cell Death Abnormalities 1. Muscular type VSD: More commonly diagnosed in the current echocardiographic era 2. Ebstein's malformation of the tricuspid valve
Abnormal Targeted Growth 1. Anomalous pulmonary venous return

Table 4.2. Power calculations for cardiovascular malformations – 1968-2003 (for temporal analyses) and 1994-2003 (for spatio-temporal analyses).

Defect	Number (1968-2003)	Number (1994-2003)	Rate Ratio	Power	
				1968-2003*	1994-2003†
Atrial septal defect	1510	855	1.05	.268	.171
			1.10	.746	.504
			1.20	.999	.966
			1.30	.999	.999
Pulmonary valve and outflow tract anomalies	1693	952	1.05	.295	.185
			1.10	.793	.548
			1.20	.999	.979
			1.30	.999	.999
Aortic anomalies	1341	596	1.05	.243	.132
			1.10	.695	.376
			1.20	.997	.884
			1.30	.999	.996
Conotruncal anomalies	962	352	1.05	.187	.094
			1.10	.552	.243
			1.20	.980	.678
			1.30	.999	.939
Ventricular septal defect	3250	1467	1.05	.504	.261
			1.10	.971	.733
			1.20	.999	.999
			1.30	.999	.999
Endocardial and mitral valve defects	986	525	1.05	.190	.121
			1.10	.563	.338
			1.20	.982	.842
			1.30	.999	.990

*Number in cohort = 1,180,500 † Number in cohort = 407,200

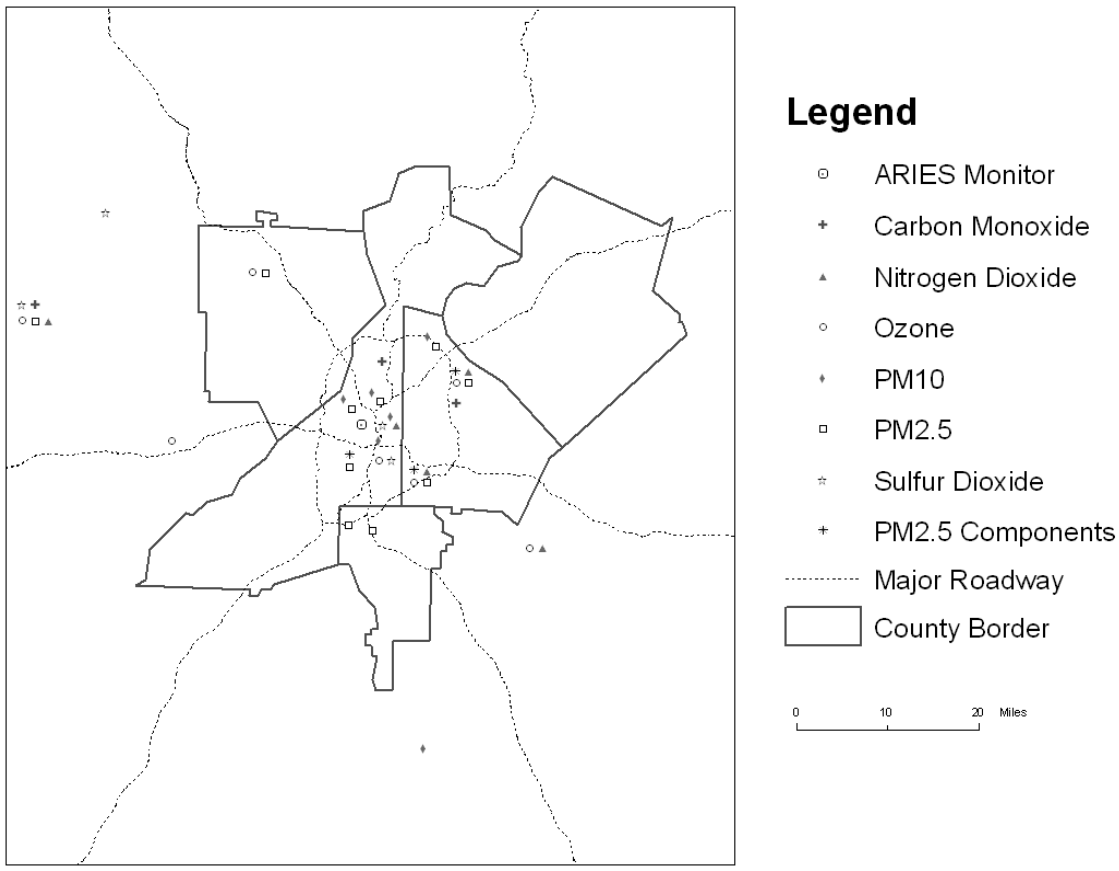

Figure 4.1. Locations of current air monitoring stations in the Atlanta metropolitan area

Chapter 5: The Importance of Nomenclature for Congenital Heart Disease: Implications for Research and Evaluation

[Formatted for submission to *Cardiology in the Young*]

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Abstract

Background: Administrative databases are often used for congenital heart disease research and evaluation, with little validation of the accuracy of the diagnostic codes.

Methods: Metropolitan Atlanta Congenital Defects Program surveillance records were reviewed and classified using a version of the International Pediatric and Congenital Cardiac Code. Using this clinical nomenclature as the referent, we report the sensitivity and false positive fraction (1 – positive predictive value) of the International Classification of Diseases, Ninth Revision, Clinical Modification diagnosis codes for tetralogy of Fallot, transposition of the great arteries, and hypoplastic left heart syndrome.

Results: We identified 4918 infants and foetuses with congenital heart disease from the surveillance records. Using only the International Classification of Diseases diagnosis codes, there were 280 records with tetralogy, 317 records with transposition, and 192 records with hypoplastic left heart syndrome. Based on the International Pediatric and Congenital Cardiac Code, 330 records were classified as tetralogy, 163 records as transposition, and 179 records as hypoplastic left heart syndrome. The sensitivity of International Classification of Diseases diagnosis codes was 83% for tetralogy, 100% for transposition, and 95% for hypoplastic left heart syndrome. The false positive fraction was 2% for tetralogy, 49% for transposition, and 11% for hypoplastic left heart syndrome.

Conclusions: Analyses based on International Classification of Diseases diagnosis codes may have substantial misclassification of congenital heart disease. Isolating the major defect is difficult, and certain codes do not differentiate between variants that are clinically and developmentally different.

Introduction

Administrative databases are often the basis for congenital heart disease research and evaluation[1-9]. In the United States of America, these databases use the International Classification of Diseases, Ninth Revision, Clinical Modification[10] to describe cardiac lesions. Evidence from two recent investigations suggests that the accuracy of the International Classification of Diseases diagnosis codes for congenital heart defects is likely to be poor[11,12]. Cronk and colleagues reported that only 52% of the congenital heart defect diagnoses contained in medical records had corresponding diagnosis codes in the hospital discharge database[11]. Frohnert and colleagues reviewed a series of medical records and were able to confirm only 41% of the diagnosis codes for congenital heart defects that were present in the administrative database[12]. The investigators offer several possible reasons for the poor diagnostic accuracy of the administrative codes, including accidental miscoding, contradictory or poorly described information in the medical record, and inadequately trained medical coders[11,12].

These two studies suggest that administrative databases fail to identify a substantial fraction of true cases of heart defects, identify many false positives, and that the heart defects studied using such databases may be unrepresentative of heart defects in the general population. Furthermore, some members of the paediatric cardiology and cardiac surgery community have argued that the International Classification of Diseases nomenclature used in administrative databases lacks sufficient detail to adequately describe the spectrum of congenital heart defects and have voiced the need for an improved nomenclature[13,14].

During the 1990s, both The Society of Thoracic Surgeons and The European Association for Cardio-Thoracic Surgery created databases to assess the outcomes of congenital cardiac surgery[13]. In 1998 these organizations collaborated to create the International Congenital Heart Surgery Nomenclature and Database Project, and in 2000 a common nomenclature and core minimal dataset was adopted by both The Society of Thoracic Surgeons and The European Association for Cardio-Thoracic Surgery[13]. By 2005, the International Working Group for

Mapping and Coding of Nomenclatures for Paediatric and Congenital Heart Disease had crossmapped the nomenclature of the International Congenital Heart Surgery Nomenclature and Database Project with the European Paediatric Cardiac Code of the Association for European Paediatric Cardiology, thereby creating the International Pediatric and Congenital Cardiac Code, which is freely available [http://www.IPCCC.NET][15].

Two commonly used versions of the International Pediatric and Congenital Cardiac Code exist[16-19]:

- The version derived from the European Paediatric Cardiac Code of The Association for European Paediatric Cardiology.
- The version derived from the International Congenital Heart Surgery Nomenclature and Database Project of The European Association for Cardio-Thoracic Surgery and The Society of Thoracic Surgeons.

Recently, the Metropolitan Atlanta Congenital Defects Program used the version of the International Pediatric and Congenital Cardiac Code derived from the International Congenital Heart Surgery Nomenclature and Database Project (hereafter referred to as the "clinical nomenclature") to classify all of its surveillance records with congenital heart disease. This was the first application of this clinical nomenclature to routinely collected birth defects surveillance data. Our objective was to evaluate the diagnostic accuracy of the administrative nomenclature in the International Classification of Diseases relative to this clinical nomenclature for the cohort of infants and foetuses with congenital heart defects born to mothers residing in metropolitan Atlanta during 1988–2003.

Materials and Methods

The Metropolitan Atlanta Congenital Defects Program is an active, population-based birth defects surveillance system administered by the Centers for Disease Control and Prevention of the United States of America since 1967[20]. Cases in the Metropolitan Atlanta Congenital

Defects Program include infants and foetuses of at least 20 weeks gestation whose mothers resided in one of five central metropolitan Atlanta counties at delivery. Major structural defects, chromosomal abnormalities, and clinical syndromes diagnosed within six years of delivery are included in Metropolitan Atlanta Congenital Defects Program. Trained abstractors access hospital medical records directly and record information on infant and foetal diagnoses and procedures. A nomenclature developed by the Centers for Disease Control and Prevention[21], based on the International Classification of Diseases, Ninth Revision, Clinical Modification[10] and the British Paediatric Association Classification of Diseases[22], is used to code birth defects, and hereafter is referred to as the "administrative nomenclature." The codes in the administrative nomenclature, while more detailed, can be mapped directly to the codes in the International Classification of Diseases via a computer algorithm if the extra detail is ignored. In the present study we ignored this extra detail and treated the codes in this administrative nomenclature as if they were codes in the International Classification of Diseases, Ninth Revision, Clinical Modification. Emory University and Centers for Disease Control and Prevention Institutional Review Boards granted waivers of informed consent for this study on July 24, 2004 and February 1, 2005, respectively.

We identified all surveillance records in the Metropolitan Atlanta Congenital Defects Program with congenital heart defects and a delivery date during 1988–2003, inclusive. Each record was manually reviewed by paediatric cardiologists: Mark D Reller, William T Mahle, Lorenzo D Botto, and Tiffany J Riehle-Colarusso. All records were coded using the clinical nomenclature as published by The Society of Thoracic Surgeons Congenital Heart Surgery Database version 2.30[23]. This activity was an enrichment of pre-existing surveillance data, based on analysis of the abstracted text and expert opinion, using a standard clinical nomenclature that enables reviewers to accurately describe congenital cardiac lesions. Reviewers determined the anatomical diagnosis based on data from surveillance records in the Metropolitan Atlanta Congenital Defects Program, which included details from echocardiographic reports and the catheterization report, if performed. Comments from the operative note regarding anatomical

features were also included in surveillance records. After the review, records with similar clinical nomenclature classifications were grouped to facilitate analysis. For records with several defects, prioritization was based on presumed developmental mechanisms[24,25]. For example, all records with isomerism of the atrial appendages were grouped into heterotaxy, regardless of other associated defects. Similarly, a constellation of defects might be placed into the "single ventricle/complex group." Although records could be placed into one or more of 35 different aggregation groups, we focus on just three of these groups: tetralogy of Fallot, transposition of the great arteries with concordant atrioventricular connections and discordant ventriculo-arterial connections, and hypoplastic left heart syndrome. We focus on these severe, commonly occurring lesions because they are frequently used as benchmarks for surgeon and programmatic performance[26-32]. The administrative nomenclature and clinical nomenclature diagnosis codes that comprise these three groups are presented in Table 5.1.

In this analysis, the clinical nomenclature-based groups were treated as the referent. The sensitivity and the false positive fraction of the administrative nomenclature codes are reported for each defect group listed in Table 5.1. Sensitivity is the probability that a case has an appropriate code from the administrative nomenclature, given its membership in a particular group from the clinical nomenclature. The false positive fraction is the probability that a case is not in the group from the clinical nomenclature, given that it has the code from the administrative nomenclature for that diagnosis. The false positive fraction is equivalent to 1 – positive predictive value. If sensitivity = 1.00, this indicates that all records in the group from the clinical nomenclature have an appropriate code from the administrative nomenclature, whereas if sensitivity = 0.00 then no records in the group from the clinical nomenclature have an appropriate code from the administrative nomenclature. A sensitivity = 1.00 does not indicate perfect agreement; excess records not contained in the group from the clinical nomenclature may be present in the group from the administrative nomenclature. The false positive fraction is this proportion of excess, or "false positive," records.

Results

During 1988–2003, there were 691,099 infants born to mothers residing in Atlanta; 4,918 infants and foetuses ascertained by the Metropolitan Atlanta Congenital Defects Program during this period had structural heart defects (0.7%). Using only the codes from the administrative nomenclature, there were 280 records with tetralogy, 317 records with transposition, and 192 records with hypoplastic left heart syndrome. Based on the review using the clinical nomenclature, 330 records were classified as tetralogy, 163 records as transposition, and 179 records as hypoplastic left heart syndrome. The sensitivity and false positive fraction for these three defect groups are presented in Table 5.2.

Tetralogy of Fallot

Of the 330 records classified as tetralogy of Fallot by the review using the clinical nomenclature, 55 did not have a code for tetralogy from the administrative nomenclature (Table 5.2, sensitivity = 0.83). Many of these hearts had pulmonary atresia and ventricular septal defect (n=36), which is often the extreme end of the anatomical spectrum of tetralogy of Fallot. However, because of limitations in the administrative nomenclature, pulmonary valve atresia cannot be distinguished from congenital absence of the pulmonary valve. Even more problematic is the fact that pulmonary artery atresia, stenosis, agenesis, and hypoplasia are all lumped under one code in the administrative nomenclature. Thus, one cannot reliably identify records with both pulmonary atresia and ventricular septal defect using codes from the administrative nomenclature.

The coding of records with double outlet right ventricle with the administrative nomenclature also decreased the sensitivity for tetralogy. Clinically, double outlet right ventricle has several phenotypes:

- Double outlet right ventricle of the transposition type
- Double outlet right ventricle of the tetralogy type

- Double outlet right ventricle of the ventricular septal defect type
- Double outlet right ventricle with uncommitted ventricular septal defect type
- Double outlet right ventricle with intact ventricular septum

The administrative nomenclature forces all patients with any double outlet right ventricle phenotype into a single code that is a subtype of transposition. The clinical nomenclature, conversely, distinguishes among these phenotypes. The patients with double outlet right ventricle of the tetralogy type can be grouped with tetralogy and the patients with double outlet right ventricle of the transposition type can be grouped with transposition (Table 5.1). Fourteen of the 55 records that did not have a code from the administrative nomenclature for tetralogy were classified by the review using the clinical nomenclature as double outlet right ventricle of the tetralogy type.

There were five additional records in which the code from the administrative nomenclature did not agree with the classification of tetralogy by the clinical nomenclature. Three records had codes from the administrative nomenclature for both atrioventricular canal defect and pulmonary artery anomaly. One record had a code from the administrative nomenclature for "pulmonary valve anomaly, other," and the fifth record used a code from the administrative nomenclature for "unspecified anomaly of the heart."

The false positive fraction for tetralogy was very low (false positive fraction = 0.02); only five of 280 records were false positives. These false positives were classified by the review using the clinical nomenclature as heterotaxy (n = 1), double outlet right ventricle of the transposition type (n = 1), perimembranous ventricular septal defect (n = 1), and perimembranous ventricular septal defect with secundum atrial septal defect (n = 1). The fifth record had insufficient information to confirm a diagnosis of tetralogy.

Transposition of the great arteries

The sensitivity was 1.00 for transposition of the great arteries (Table 5.2); all records classified as transposition by the review using the clinical nomenclature had an appropriate code from the administrative nomenclature. However, 154 of the 317 records with a code for transposition from the administrative nomenclature were false positives (false positive fraction = 0.49). These records were placed into various groups following the review using the clinical nomenclature, the most frequent being "single ventricle/complex" (n = 38), heterotaxy (n = 38), double outlet right ventricle (n = 32), and tetralogy (n = 27). Other groups include hypoplastic left heart syndrome (n = 6), congenitally corrected transposition of the great arteries (n = 5), ventricular septal defect (n = 3), and atrioventricular septal defect (n = 3). Two surveillance records had insufficient detail to support a diagnosis of transposition.

The majority of false positives in the analysis of transposition were caused by one of two issues. First, complex cardiac lesions frequently include transposed great arteries as part of the anatomical description. The hierarchy used in the clinical aggregation process tended to place these records into the "single ventricle/complex group" or the heterotaxy group rather than the transposition group. Accordingly, nearly half of the false positive records for transposition were classified by the review using the clinical nomenclature as either "single ventricle/complex" (n = 38) or heterotaxy (n = 38).

Second, the single code in the administrative nomenclature used to describe all patients with any of the double outlet right ventricle phenotypes resulted in many false positives. The administrative nomenclature considers all double outlet right ventricle phenotypes to be a subtype of transposition. Our aggregation process grouped double outlet right ventricle of the transposition type with transposition and double outlet right ventricle of the tetralogy type with tetralogy. All other phenotypes were classified as double outlet right ventricle. Records with the code from the administrative nomenclature for double outlet right ventricle classified by the review using the clinical nomenclature as either tetralogy (n = 27) or double outlet right ventricle (n = 32) were therefore counted as false positives.

We conducted a secondary analysis to evaluate whether concordance could be improved by excluding records that had codes from the administrative nomenclature for both transposition and one or more of the following: malposition of the heart and cardiac apex, common ventricle, "situs inversus", or "spleen anomaly". Doing so reduced the number of false positives from 154 to 99. The false positive fraction decreased from 0.49 to 0.38, while the sensitivity remained at 1.00.

Hypoplastic left heart syndrome

Most records classified as hypoplastic left heart syndrome after the review using the clinical nomenclature had the corresponding code from the administrative nomenclature for hypoplastic left heart syndrome (170 of 179 records, sensitivity = 0.95). Six discrepant records had a code from the administrative nomenclature for hypoplastic left ventricle, two had a code from the administrative nomenclature for "single ventricle", and one had codes from the administrative nomenclature for mitral valve stenosis and aortic valve stenosis. This finding is not attributable to a limitation in the administrative nomenclature; rather, it is the result of the medical coder or abstractor coding one or more of the component defects of hypoplastic left heart syndrome but failing to recognize the overall syndrome.

Eleven percent of the records with hypoplastic left heart syndrome were false positives (22 of 192 records). The 22 false positives had the code from the administrative nomenclature for hypoplastic left heart syndrome but were classified by the review as "single ventricle/complex" (n = 10), interrupted aortic arch (n = 4), double outlet right ventricle (n = 2), heterotaxy (n = 2), and coarctation of the aorta (n = 2). Two surveillance records had insufficient detail to confirm a diagnosis of hypoplastic left heart syndrome.

Some records were false positives because the code from the administrative nomenclature for hypoplastic left heart syndrome was used to describe hearts with only coarctation of the aorta or interrupted aortic arch. These hearts were miscoded by the medical coder or the abstractor.

False positives also occur when records with the component defects for hypoplastic left heart syndrome have additional defects that merit classification as “single ventricle/complex” or heterotaxy. For example, we elected to classify records with “Single ventricle, Unbalanced atrioventricular canal (left),” with “single ventricle/complex” defects rather than with hypoplastic left heart syndrome.

Discussion

The frequency of misclassification in reporting of tetralogy, transposition, and hypoplastic left heart syndrome suggests caution is needed when administrative diagnosis codes are used to classify congenital heart lesions. Misclassification can occur because of errors on the part of the coder, because of limitations inherent to the administrative nomenclature, or because of failure to distinguish less complicated forms of a lesion from those with heterotaxy or other complex arrangements.

In the tetralogy analysis, coding with the administrative nomenclature missed 17% of tetralogy records. Using codes from the administrative nomenclature, records with pulmonary atresia and ventricular septal defect could not be distinguished from records with pulmonary artery stenosis or hypoplasia. These pulmonary atresia and ventricular septal defect cases represent the extreme end of the anatomic spectrum of tetralogy and bear little or no relationship to simple branch pulmonary artery or valvar stenosis. Estimates of post-surgery mortality for tetralogy based on administrative databases may be overly optimistic if the most severe form of tetralogy, pulmonary atresia with ventricular septal defect, is not included in the evaluation. Additionally, the administrative nomenclature collapses all double outlet right ventricle phenotypes into a single code that is a subtype of transposition. In reality, only a fraction of DORV cases have features fundamentally related to transposition from an anatomic, physiologic, or prognostic standpoint[33].

Outcomes after surgery for transposition are often used as a benchmark for surgeon and programmatic performance[26-32]. The transposition analysis revealed that 49% of records classified as transposition by coding with the administrative nomenclature did not actually have transposition as the fundamental problem. Although excluding transposition records with codes from the administrative nomenclature for malposition of the heart and cardiac apex, common ventricle, "situs inversus," or spleen anomaly reduced the false positive fraction from 0.49 to 0.38, this level of misclassification remains high. Patients with heterotaxy syndrome and/or functionally univentricular hearts tend to have poor survival[34,35]. If these records are included in the transposition subgroup, then this will lead to a severely flawed and misleading analysis, effectively penalizing surgeons who routinely treat patients with very complex congenital heart disease.

In the hypoplastic left heart syndrome analysis, one in every nine records coded with the administrative nomenclature as hypoplastic left heart syndrome was a false positive. Coding errors were the cause of many false positives, highlighting the value of systematic record review. Hypoplastic left heart syndrome is another benchmark lesion where surgeon and programmatic performance is often measured[28-32]. Use of administrative databases that might include several false positive hypoplastic left heart syndrome records could result in inaccurate estimates of surgical outcomes.

The generalization of our findings to studies based on hospital billing databases, and other administrative databases based on the International Classification of Diseases, may be limited because of differences between these databases and the Metropolitan Atlanta Congenital Defects Program. Abstractors from the Metropolitan Atlanta Congenital Defects Program receive specialized training in birth defects coding and travel to hospitals and access medical records directly. Nine of the 11 abstractors have nursing degrees and can draw from their clinical background when reviewing medical records. Defects are coded using an enhanced International Classification of Diseases–based nomenclature[21], and additional information, such as

echocardiography report details, is often recorded on the surveillance records. After abstraction, surveillance records are further reviewed by in-house staff to reduce the frequency of coding errors. The common hospital administrative database does not have this level of quality control, and unlike Metropolitan Atlanta Congenital Defects Program abstractors, many hospital-based medical coders do not have a clinical background nor do they receive specialized training in birth defects coding. Consequently, the validity of diagnosis codes from the International Classification of Diseases for congenital heart lesions in administrative databases may be significantly worse than reported in this manuscript, and our results may represent a "best case scenario" with respect to the quality of the administrative diagnosis codes. The extremely poor agreement between the administrative codes and the medical records documented in previous studies further supports this notion[11,12].

Unlike hospital billing databases, however, the Metropolitan Atlanta Congenital Defects Program does not abstract International Classification of Diseases procedural codes or other similar codes such as the American Medical Association Current Procedure Terminology codes[36]. These codes describe medical, surgical, and diagnostic services. Many hospital billing departments record both diagnosis and procedural codes. Although many large, widely used administrative datasets, such as those of the Agency for Healthcare Research and Quality of the U.S. Department of Health and Human Services, do not have access to Current Procedure Terminology codes, analyses of these datasets typically incorporate both International Classification of Diseases diagnosis and procedural codes[37].

Unlike the United States, much of the world has already transitioned to the International Classification of Diseases, Tenth Revision, Clinical Modification. Many problems in the Ninth Revision of the nomenclature persist in the Tenth Revision. The nomenclature of the Tenth Revision continues to collapse all double outlet right ventricle phenotypes into a single code and cannot distinguish less complicated forms of lesions from those with heterotaxy or other complex arrangements. Some problems with the Ninth Revision have been addressed in the Tenth

Revision; for example, pulmonary artery stenosis and pulmonary artery atresia have unique codes in the Tenth Revision. Although we cannot quantify the impact that the transition from the Ninth Revision to the Tenth Revision has had on the accuracy of coding patients with congenital heart disease, we would speculate that misclassification continues to be a concern.

Our use of the International Pediatric and Congenital Cardiac Code classifications as a referent requires qualification. A true "gold standard" would require complete echocardiography reports for each infant and foetus included in the analysis, whereas this project relies on information contained in the surveillance records of the Metropolitan Atlanta Congenital Defects Program. As such, some diagnoses could not be confirmed because important details were missing from the surveillance records. We are unable to evaluate the extent to which our findings would differ if complete echocardiography reports were available for every surveillance record. Nevertheless, even without a true gold standard, our results reveal limitations in several codes within the nomenclature system of the International Classification of Diseases.

Our demonstration of the weaknesses of the administrative nomenclature in comparison to the clinical nomenclature, based on surveillance data from Atlanta, Georgia, United States of America, is relevant to the relationship between administrative and clinical databases worldwide. The international scope of this challenge is the driving force behind the ongoing international collaborative efforts to create and maintain the International Pediatric and Congenital Cardiac Code[15,39-43]. Reconciling differences between administrative and clinical databases is truly a challenge with global impact in our field, and this challenge will only be met with continued global collaboration[39].

Ultimately, the optimal classification and coding system will be based on clear, precise definitions of the cardiac phenotype. Our current study documents how an improved classification scheme based on the clinical coding of the International Pediatric and Congenital Cardiac Code is more precise and accurate relative to coding based on administrative nomenclature. Applying standardized definitions of cardiac phenotypes should lead to further

improvement. To this end, the International Working Group for Mapping and Coding of Nomenclatures for Paediatric and Congenital Heart Disease has provided unified nomenclature and definitions for several complex congenital cardiac malformations, including the functionally univentricular heart[40], hypoplastic left heart syndrome[41], congenitally corrected transposition[42], and heterotaxy[42]. Recently, the International Society for Nomenclature of Paediatric and Congenital Heart Disease created two new committees to further the definitions of cardiac phenotypes:

- The International Working Group for Defining the Nomenclatures for Paediatric and Congenital Heart Disease, which will write definitions for the terms used in the International Pediatric and Congenital Cardiac Code.
- The International Working Group for Archiving and Cataloguing the Images and Videos of the Nomenclatures for Paediatric and Congenital Heart Disease, which will link images and videos to the International Pediatric and Congenital Cardiac Code, and create an archive of these images which will be linked to The Cardiothoracic Surgery Network.

Conclusions

The Metropolitan Atlanta Congenital Defects Program is an active birth defects surveillance system committed to excellence in diagnostic accuracy. Numerous quality control procedures have been implemented to enhance data quality. Many common sources of error in administrative databases, including accidental miscodes, poorly trained medical coders, and other similar errors, have been greatly reduced in the Metropolitan Atlanta Congenital Defects Program. Even so, the diagnostic accuracy of certain codes for cardiac defects in the International Classification of Diseases was found to be poor.

Analyses of outcomes from paediatric cardiac surgery based on diagnosis codes from the International Classification of Diseases in administrative databases are likely to be limited by

substantial misclassification of cases of congenital heart defects. Although evaluation of surgical outcomes for children with congenital heart disease is critically important for health care quality assessment, evaluations that base lesion classification on codes from the International Classification of Diseases risk generating inaccurate results that are potentially misleading. We encourage the use of a more accurate and current nomenclature, such as the International Pediatric and Congenital Cardiac Code, for classification of congenital heart disease prior to evaluation of surgical outcomes.

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Table 5.1. Composition of the aggregate cardiac defect groups according to the clinical nomenclature and administrative nomenclature.

Aggregate cardiac defect group	Clinical nomenclature†	Administrative nomenclature‡
Tetralogy of Fallot	Tetralogy of Fallot Tetralogy of Fallot with absent pulmonary valve Tetralogy of Fallot with atrioventricular septal defect Double outlet right ventricle, tetralogy of Fallot type Pulmonary atresia with ventricular septal defect§ Pulmonary atresia with ventricular septal defect and major aortopulmonary collateral artery(ies)	745.2 – Tetralogy of Fallot
Transposition of the great arteries	Transposition with intact ventricular septum Transposition with intact ventricular spetum and left ventricular outflow tract obstruction Transposition with ventricular septal defect Transposition with ventricular septal defect and left ventricular outflow tract obstruction Transposition, not otherwise specified Double outlet right ventricle, transposition-type	745.10 – Transposition of the great arteries 745.11 – Double outlet right ventricle 745.19 – Other transposition
Hypoplastic left heart syndrome	Hypoplastic left heart syndrome Hypoplastic left heart syndrome with ventricular septal defect	746.7 – Hypoplastic left heart syndrome

† "The Clinical nomenclature" is derived from the International Congenital Heart Surgery Nomenclature and Database Project of The European Association for Cardio-Thoracic Surgery and The Society of Thoracic Surgeons.

‡ The "Administrative nomenclature" is derived from the International Classification of Diseases, Ninth Revision, Clinical Modification diagnosis codes.

§ For the clinical nomenclature, hearts with discordant ventriculo-arterial connections, pulmonary atresia, and ventricular septal defect are grouped with Transposition of the great arteries, not with Tetralogy of Fallot.

Table 5.2. Sensitivity and false positive fraction of the administrative nomenclature codes for tetralogy of Fallot, transposition of the great arteries, and hypoplastic left heart syndrome, using the clinical nomenclature codes as the referent.

Aggregate cardiac defect group			Sensitivity†	False Positive Fraction‡
Tetralogy of Fallot			275/330 (0.83)	5/280 (0.02)
	Clinical code +	Clinical code -		
Administrative code +	275	5		
Administrative code -	55	---		
Transposition of the great arteries			163/163 (1.00)	154/317 (0.49)
	Clinical code +	Clinical code -		
Administrative code +	163	154		
Administrative code -	0	---		
Hypoplastic left heart syndrome			170/179 (0.95)	22/192 (0.11)
	Clinical code +	Clinical code -		
Administrative code +	170	22		
Administrative code -	9	---		

† Sensitivity is the probability that a case has an appropriate administrative nomenclature code given the presence of the clinical nomenclature code for that diagnosis.

‡ False Positive Fraction is the probability that a case does not have the clinical nomenclature code given the presence of the administrative nomenclature code ICD for that diagnosis.

Chapter 6: On the Issue of Confounding in Epidemiological Studies of Ambient Air Pollution and Pregnancy Outcomes

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Abstract

Several recent epidemiologic studies have been conducted to investigate relationships between ambient air pollution levels during pregnancy and adverse pregnancy outcomes such as preterm delivery, low birth weight, intrauterine growth retardation, spontaneous abortion, and congenital malformations. In these studies, interest typically centers on ambient air pollution levels averaged over a few weeks or months of pregnancy. Investigators have three viable options for statistical analysis; air pollution levels can be contrasted over space, time, or both space and time. Confounding, which is always a concern in observational research, is perhaps of particular relevance in this setting because the effects of ambient air pollution on pregnancy outcomes, if they exist, are likely to be small. We highlight concerns about confounding in spatial, temporal, and spatial-temporal analyses of air pollution and pregnancy outcomes through the use of straightforward counterfactual effect definitions and the related notion of exchangeability.

Introduction

The health effects of ambient air pollution have been the topic of epidemiological investigation for over 30 years. When short-term (acute) exposure to a pollutant is of interest, temporal methods, such as a time-series or case-crossover analysis, are used most often.[1,2] On the other hand, studies of long-term (chronic) exposure to air pollution are usually based on spatial contrasts, with disease risks in highly polluted areas compared to those in less polluted areas.[3,4]

Several recent studies have linked birth certificate data with ambient air pollution measurements to examine associations between air pollution and adverse pregnancy outcomes including preterm delivery, low birth weight, intrauterine growth restriction, spontaneous abortion, and congenital malformations.[5-8] In these studies ambient pollution levels are averaged over a few weeks or months of pregnancy. Statistical analysis is challenging because the length of the pregnancy window is longer than what is typical for an acute analysis and shorter than what is typical for a chronic analysis. Some investigators have elected to analyze such data with temporal methods commonly used in acute air pollution studies;[9-11] most have opted for spatial (or spatial-temporal) approaches typically used in chronic air pollution studies.[5-8,12-15]

Although information about the relationship between ambient air pollution levels and adverse pregnancy outcomes is important from a regulatory perspective, ultimately it is the dose of the pollutant that reaches susceptible tissues that affects risk. Even if the effect of dose is large, associations will be severely dampened when the ambient pollution level is used as a proxy for dose, so long as the measurement error incurred is non-differential with respect to the pregnancy outcome.[16] Generally, estimated longitudinal correlations between personal exposure and ambient air quality measurements from stationary monitors are fairly strong for particulate matter ≤ 2.5 μm in aerodynamic diameter (correlation coefficients between 0.5 and 0.7).[17-20] Personal-ambient correlations for gaseous pollutants (carbon monoxide, nitrogen dioxide, ozone, and sulfur

dioxide) tend to be weakly correlated, although error in the measurement of personal exposures could be contributing to the weak observed correlations.[17-20]

So long as the exposure measurement error is nondifferential, substantial attenuation of the relative risk (RR) will occur when ambient pollution levels are used in place of personal exposure measurements.[16,21] Presently, there is limited information about associations between personal exposure to air pollution during pregnancy and the risks of adverse pregnancy outcomes; the significant effects reported have been small.[22,23] In addition to the small number of personal exposure studies, analogy can be used to anticipate the size of the RR for personal exposure to air pollution. For example, maternal self-reported exposure to environmental tobacco smoke has consistently been associated with an elevated risk of low birth weight; a meta-analysis estimated this RR as 1.2.[24] Although the specific particles comprising environmental tobacco smoke differ from those in the ambient urban environment, the modest effects of environmental tobacco smoke on pregnancy outcomes suggest that RRs for ambient air pollution are likely to be small.

Uncontrolled confounding, which is always a concern in observational research, is perhaps of particular relevance in this setting because the effects of ambient air pollution on pregnancy outcomes, if they exist, are likely to be small. In this paper we highlight concerns about confounding in spatial, temporal, and spatial-temporal analyses of air pollution and pregnancy outcomes through the use of counterfactual effect definitions and the related notion of exchangeability.

Counterfactual definition of a causal effect

To examine the issue of confounding in epidemiologic studies of air pollution and adverse pregnancy outcomes, we begin by reviewing the counterfactual definition of a causal effect. Consider a cohort of pregnant women for whom we wish to estimate the causal effect of a change

in ambient air pollution levels on the risk of an adverse pregnancy outcome; we refer to this cohort of pregnant women as the "target population."[25] We denote the underlying risk of the adverse pregnancy outcome in the target population as *Risk* (Table 6.1). The specific value of *Risk* is the result of all the environmental exposures relevant to the adverse pregnancy outcome as well as the genetic composition of the target population. One of these environmental exposures is the ambient air pollution level, which we denote as *Pollution* (Table 6.1). Inherent to *Risk* is the effect of *Pollution*; if we were to keep all other environmental exposures constant while altering the ambient air pollution level so it were *Pollution** instead of *Pollution* (*Pollution** ≠ *Pollution*), then the underlying risk in the target population would become *Risk** instead of *Risk* (Table 1). A counterfactual definition for the causal effect of this difference in pollution levels (*Pollution – Pollution**) is the difference in risks in the target population under the two different exposure scenarios, i.e., *Risk – Risk**.[25,26] Note that if *Pollution** = *Pollution* then *Risk** = *Risk*.

To determine the causal effect of this difference in pollution levels (*Pollution – Pollution**) on the risk of the adverse pregnancy outcome in the target population therefore requires knowledge of both *Risk* and *Risk**. However, *Risk** cannot be observed; *Risk** is a "counterfactual risk" because it describes the risk in the target population under a hypothetical alternative condition that does not occur.[25] We are thus faced with an intractable situation; both *Risk* and *Risk** must be known to estimate the causal effect of interest, yet *Risk** cannot be observed. If we wish to estimate *Risk** then we must bring in data external to the target population. One possibility is to identify a cohort of pregnant women exposed to ambient air pollution level *Pollution**. The underlying risk in this cohort could then be used to estimate *Risk**, the counterfactual risk in the target population. We refer to this second cohort of pregnant women as the "substitute population," because we use the underlying risk in this cohort to substitute for the parameter of interest (*Risk**).[25] Confounding occurs when the substitute population imperfectly represents (i.e., is not

exchangeable with) the target population with respect to the underlying risk apart from the causal effect of pollution.[25,26]

A framework to discuss confounding

Table 6.2 presents the underlying risks of an adverse pregnancy outcome for four hypothetical, mutually exclusive cohorts of pregnant women. These four cohorts are defined with respect to location and time. $Risk_{11}$ and $Risk_{21}$ are the underlying risks for two cohorts at different locations at one point in time. Likewise, $Risk_{11}$ and $Risk_{12}$ are the underlying risks for two cohorts at the same location at different points in time. The underlying risks for two cohorts that differ with respect to both location and time are denoted by $Risk_{11}$ and $Risk_{22}$.

Assume the cohort at Location 1 and Time 1 is the target population in a study of air pollution and an adverse pregnancy outcome. Because ambient air pollution levels vary across space and time, any of the other cohorts in Table 6.2 could be used as the substitute population, and $Risk_{21}$, $Risk_{12}$, or $Risk_{22}$ could be used to estimate the counterfactual risk in the target population. Unfortunately, none of these cohorts will be a perfect substitute for the target, and confounding will likely be present regardless of which substitute cohort is selected. Therefore, the choice of $Risk_{21}$, $Risk_{12}$, or $Risk_{22}$ as the estimate of the counterfactual risk should be based on the investigator's ability to compensate for differences between the two cohorts in the analysis. For each scenario we evaluate the plausibility of the exchangeability assumption, describe the challenges encountered when analytic techniques are used to compensate for differences between the cohorts, and discuss how the presumably small effect of ambient air pollution on the pregnancy outcome influences interpretation of the effect estimate.

Confounding in spatial analyses

A spatial analysis contrasts pollution levels between two cohorts in different locations at a given point in time by using $Risk_{21}$ as the estimate of the counterfactual risk (Table 6.2). Confounding occurs when the underlying risks in the two cohorts are not exchangeable, e.g., if socioeconomic status, which affects the risk of an adverse outcome, differs between the two locations of interest. To compensate for the lack of exchangeability, factors affecting risk that differ between the two cohorts must be controlled for in the analysis. Confounding can occur if these risk factors are measured with error or unavailable.

Several studies of air pollution and pregnancy outcomes have used birth certificate information to compensate for differences between the target and substitute cohorts in the analysis.[5-8] Much birth certificate data is imperfect, and the validity of the birth certificate information on tobacco use, alcohol use, prenatal care, maternal risk factors, pregnancy complications, and delivery method is generally considered to be poor.[27] Many of these adverse pregnancy outcome risk factors are not uniformly distributed across space. Given the documented disparities with respect to health status and proximity to environmental hazards within most U.S. urban areas according to factors such as race, socioeconomic status, education, and health insurance status as reported in the epidemiologic and environmental justice literature,[28] one should be concerned about confounding by these risk factors. Residual confounding will occur if variables on birth certificates that are measured with error are used to compensate for differences between the target and substitute cohorts in the analysis. The impact of residual confounding has been well described,[29,30] e.g., if the sensitivity and specificity of a dichotomous confounder are 0.95, then only 80% of confounding is removed. When sensitivity and specificity equal 0.90, only 64% of confounding is removed. These results are not affected by the strength of the exposure-disease association, the confounder-exposure association, or the confounder-disease association.[29]

When birth certificate data are missing, the mechanism by which such missing data arise may be non-random with respect to the outcome of interest. For example, very low birthweight infants and infants born to young, unmarried women with less than a high school education tend to have more missing data than their counterparts.[29] To the extent that the degree and pattern of missing data are similar across locations, the measure of the potential confounder will be subject to random error and the association between air pollution and pregnancy outcome is likely to be affected by residual confounding. However, systematic variation in missing data on potential confounders across space could give rise to biased estimates (away from, towards, or across the null).

Another concern is the overall lack of information on potential confounders in routinely collected health records, of which socioeconomic status is perhaps the most obvious example. Commonly available individual-level variables, such as race and education, do not capture the full construct of socioeconomic status.[31] Controlling for neighborhood-level socioeconomic status variables (e.g., information from a Census) will not capture potentially important within-neighborhood variation (e.g., in many U.S. urban neighborhoods houses located on highly trafficked roads tend to have lower resale values than similar houses on roads with low traffic volume).[32,33] In addition to socioeconomic status, one can imagine numerous unmeasured risk factors that could vary between the target and substitute cohorts and weakly confound the estimated effect of air pollution in a spatial analysis. For example, in the United States, health-conscious individuals may be more averse to living near highly visible environmental hazards such as automobile traffic, high tensile wires, factories, and smokestacks. These individuals could be more likely to engage in other behaviors (e.g., exercise, diet, vitamin use, etc.) that reduce their risk of the adverse pregnancy outcome.

Although residual confounding and weak uncontrolled confounding are common concerns in observational research, we believe they are of particular importance in analyses of air pollution and pregnancy outcomes. Consider a true null association for which the observed RR is 1.05 (comparing the fourth quartile with the first quartile of ambient pollution) because of confounding. In most observational settings, a bias of this magnitude is negligible;[34] however, given the ubiquity of ambient air pollution, a 5% increase in risk would have important public health implications. Since record-based studies tend to have tremendous statistical power to detect very small RRs, this biased estimate may have a small corresponding p-value.

Admittedly, the previous arguments about unmeasured spatial confounding are speculative. However, it is their speculative nature that makes drawing causal conclusions from spatial analyses difficult. Consider a spatial analysis with zero bias from confounding where the investigators estimate a small elevated risk attributable to ambient pollution. In practice, it is impossible to know whether the estimated association is confounded, as this would require accurate measurements of all possible confounders, and these data are not available. Although the observed association is compatible with a true effect of ambient pollution, it is also compatible with a null association biased away from the null because of residual confounding or weak uncontrolled confounding. It seems prudent to be concerned about confounding in this setting; the imperfect validity of data on birth records is well known, and perfect control of socioeconomic status is unlikely. Consistency of results does not necessarily rule out confounding as a plausible explanation, as confounding may persist across studies.

Confounding in temporal analyses

Temporal analyses contrast pollution levels between two cohorts at a particular location at different points in time by using $Risk_{12}$ as the estimate of the counterfactual risk (Table 6.2). Lack of exchangeability occurs when the prevalence of risk factors change over time.

As alluded to earlier, pregnancy outcome studies do not fit comfortably into the mold of acute air pollution studies, because the gestational window of interest usually spans a few weeks or months during pregnancy, whereas acute studies focus on air pollution levels over a period of days. In acute analyses, a parametric spline or nonparametric smoother is commonly used to control for confounding by factors that change gradually over time. Risk factors with short-term variation are not captured by splines or smoothers and should be explicitly controlled for in the analysis. Therefore, concerns about confounding usually center on acute, unmeasured changes in the underlying risk. Residual confounding can occur if the regression spline does not fully account for the long-term and seasonal variation in risk or if short-term variations are measured with error.

A risk factor with short-term temporal variation must be systematically associated with air pollution levels to confound a temporal analysis of air pollution and pregnancy outcomes. Otherwise, the risk factor will simply create "statistical noise" and reduce the power to detect an association. Infectious diseases are perhaps the most likely short-term change that could affect the risk of adverse pregnancy outcomes. Some infections are known to increase the risk of specific outcomes (e.g., cytomegalovirus causes intrauterine growth retardation, developmental disabilities, and certain congenital malformations),[35] whereas others are plausibly related to the outcomes (e.g., influenza and respiratory illness cause inflammation, which is a known risk factor for preterm delivery).[36] A disaster of any type could potentially change the risk of adverse pregnancy outcomes as well, e.g., because of changes in maternal stress, exposure to toxic agents, or disruptions in the availability of food, water, or medical services. Interestingly, these risk factors could also confound spatial analyses, e.g., if the event disproportionately affects people in study areas that have relatively high (or low) air pollution levels.

Whereas acute studies smoothly control for confounding by factors with seasonal and long-term variation, in pregnancy outcome studies seasonal control intrudes upon the gestational window of interest (i.e., one pregnancy trimester is the same length of time as one season). This is problematic, because both air pollution levels and the underlying risk of adverse pregnancy outcomes vary by season. For example, in the 1994–2004 Atlanta birth cohort, seasonal patterns in births differ according to maternal race, marital status and education. Because these maternal characteristics are strong predictors of preterm delivery, the underlying risk of preterm delivery in the cohort varies by season. Target and substitute cohorts in Atlanta that differ with respect to season are therefore not exchangeable; a temporal analysis of preterm delivery would need to account for this lack of exchangeability.

To improve exchangeability, a cohort identical to the target with respect to location and season from a different calendar year could be used as the substitute cohort. This approach accounts for confounding by risk factors with seasonal variation (although risk factors with long-term variation must still be addressed). Because ambient air pollution levels vary with season, selection of a substitute cohort from the same season as the target will reduce variation in pollution levels. This is perhaps a drawback of temporal analyses; the most satisfying substitute cohort (from the perspective of exchangeability) will likely be similar to the target cohort with respect to pollution levels, thus reducing the statistical power to detect an association.

Confounding in spatial-temporal analyses

Among published studies that examine the relationship between ambient air pollution and pregnancy outcomes, the most common choice has been to use $Risk_{22}$ as the estimate of the counterfactual risk (Table 6.2).[5-8] In this analysis the target and substitute cohorts differ with respect to both location and time. We speculate that spatial-temporal analyses are popular because statistical power is likely improved relative to a spatial or temporal analysis. Since the estimated

effect of air pollution is based on both spatial and temporal contrasts in pollution levels, the total exposure variability in a spatial-temporal analysis is greater than in a spatial or temporal analysis. Although the increase in exposure variability may translate to improved statistical power, it perhaps comes at the expense of additional confounding, as all of the previously described concerns about confounding for spatial and temporal analyses pertain to spatial-temporal analyses.

Discussion

Through the use of counterfactual effect definitions and the related notion of exchangeability, we have described concerns about confounding for spatial, temporal, and spatial-temporal analyses of air pollution and adverse pregnancy outcomes. None of the designs preclude or ensure confounding. In practice it is impossible to know if a particular association (or lack thereof) is confounded; the best one can do is to evaluate the plausibility of confounding and interpret the result accordingly. Compensating for lack of exchangeability between the target and substitute cohort is difficult in all three settings, and given the presumably small true effect size of ambient air pollution on the risk of adverse pregnancy outcomes, concerns about study results based on arguments of confounding should be anticipated.

In temporal analyses, smooth functions of time are commonly used to control for confounding by risk factors with long-term and seasonal variation. Many risk factors are likely to change smoothly over time, e.g., demographics, medical practices, use of assisted reproductive technologies, and prenatal vitamin use. Although residual confounding is a concern, smooth functions of time seem well suited to account for these trends. Ideally, risk factors with short-term variability should be measured and controlled for in the analysis; if these fluctuations are systematically associated with air pollution levels then the risk factor will confound the association of interest.

Spatial analyses usually rely on measured risk factors to compensate for lack of exchangeability between the target and substitute cohort. Confounding can occur if risk factors that are correlated with air pollution levels are unmeasured or measured with error. Many studies of air pollution and pregnancy outcomes rely on birth certificates, which contain information on a limited number of risk factors, some of which are likely measured with error. Given the presumably small true effect of ambient air pollution on pregnancy outcomes, unmeasured and residual confounding could be plausible explanations for an observed effect. Supplementary data collection should enable investigators to better compensate for lack of exchangeability in the analysis;[37] however, even with additional information, full control for the effects of many plausible confounders, such as socioeconomic status or maternal health consciousness, may be difficult.

As an alternative to measured risk factors, a smooth function of location could be used to control for confounding in spatial analyses. This approach is the spatial analog to control of temporal variation with a smooth function of time. As in temporal analyses, the adequacy of this approach depends upon the smoothness of the variation in the underlying risk; residual confounding may be present if the risk changes abruptly from one location to next. Whereas overall differences between U.S. urban neighborhoods might be well characterized using a smooth function of location, it would be very difficult to capture potentially important within neighborhood differences, such as abrupt changes in socioeconomic status according to residential proximity to traffic.[32,33] Thus, even when similar techniques are used to smoothly control for confounding, residual confounding perhaps seems more plausible in spatial analyses than in temporal analyses.

Ultimately it is impossible to know if a particular association is confounded. Given that the effect of ambient air pollution levels on adverse pregnancy outcomes, if real, is likely to be small, we believe that concerns about study results based on arguments of confounding are likely to persist.

We encourage investigators planning future studies of air pollution and pregnancy outcomes to implement the analytic approach they believe minimizes concerns about confounding.

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Table 6.1. Risk of an adverse pregnancy outcome in the target population under the actual ambient air pollution level (*Pollution*) and under a hypothetical alternative ambient air pollution level (*Pollution**).

Observed	*Pollution*	$\longrightarrow$	*Risk*
Hypothetical Alternative	*Pollution**	$\longrightarrow$	*Risk**

Table 6.2. Risks of an adverse pregnancy outcome for four mutually exclusive cohorts of pregnant women.

	Time 1	Time 2
Location 1	$Risk_{11}$	$Risk_{12}$
Location 2	$Risk_{21}$	$Risk_{22}$

Chapter 7: Ambient Air Pollution and Cardiovascular Malformations in Atlanta, Georgia, 1986–2003

[Formatted for submission to *American Journal of Epidemiology*]

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Abstract

The authors investigated temporal relationships between ambient air pollution levels during weeks three through seven of pregnancy and risk of cardiovascular malformations among the cohort of infants and fetuses conceived during January 1, 1986 through March 12, 2003 in Atlanta, Georgia. Records of infants and fetuses with cardiovascular malformations were obtained from the Metropolitan Atlanta Congenital Defects Program, which conducts active, population-based birth defects surveillance on this cohort. These surveillance records were reviewed to exclude infants with transient newborn conditions and to group infants and fetuses with similar cardiovascular malformations for analysis. Ambient air pollution measurements of 8-hour maximum ozone and 24-hour average carbon monoxide, nitrogen dioxide, particulate matter < 10 μm in diameter, and sulfur dioxide were obtained from centrally-located stationary monitors. Temporal relationships between air pollution levels and risk of cardiovascular malformations were modeled using Poisson generalized linear models. The authors observed a positive association between particulate matter < 10 μm in diameter and risk of patent ductus arteriosus (risk ratio for an increase in the interquartile range of the pollutant = 1.60, 95 percent confidence interval: 1.11, 2.31). No other positive associations were observed.

A growing body of epidemiological evidence suggests associations between ambient air pollution and adverse pregnancy outcomes such as preterm birth, fetal mortality, low birth weight, and intrauterine growth retardation (1-6). Two population-based case-control studies have reported associations between ambient air pollution levels during pregnancy and cardiovascular malformations in the offspring (7, 8). These two studies were similar in design; cases were selected from active, population-based birth defects surveillance systems, controls were selected from live birth and fetal death records, and outcome groups were defined using a nomenclature developed for birth defects surveillance (9, 10). Analyses were based on contrasts in pollution levels over space and time.

In the first study, conducted in Southern California, investigators reported a strong dose-response association between ambient carbon monoxide levels and risk of ventricular septal defect (odds ratio $(OR)_{\text{4th quartile vs. 1st quartile}} = 2.95$, 95 percent confidence interval (CI): 1.44, 6.05). Elevated risk of aortic artery and valve defects, pulmonary artery and valve anomalies, and conotruncal defects with increasing ambient ozone levels were also reported (7). Results from the second investigation, conducted in Texas, did not corroborate the Southern California findings, although a suggestive association between ozone and pulmonary artery and valve defects was observed. The Texas investigators reported positive associations for carbon monoxide and tetralogy of Fallot, particulate matter < 10 μm in diameter (PM_{10}) and atrial septal defect, and sulfur dioxide and ventricular septal defect (8).

We conducted a retrospective cohort study to investigate temporal relationships between ambient air pollution levels during pregnancy and cardiovascular malformations in Atlanta during 1986–2003. In Atlanta, birth defects surveillance is conducted by the Metropolitan Atlanta Congenital Defects Program (MACDP). The version of the International Pediatric and Congenital Cardiac Code implemented in the Society of Thoracic Surgeons Congenital Heart Surgery Database was used to classify cardiovascular malformations in MACDP and create outcome groups for analysis (11-15).

MATERIALS AND METHODS

Study population

We obtained vital records for the cohort of infants and fetuses of at least 20 weeks gestation whose mothers resided in one of five central Atlanta counties at delivery from the Office of Health Information and Policy, Georgia Division of Public Health. Records of infants and fetuses with cardiovascular malformations were obtained from MACDP, which conducts active, population-based birth defects surveillance on this cohort (16). MACDP ascertains infants with major structural defects, chromosomal abnormalities, and clinical syndromes diagnosed by six years of age. Abstractors code malformations using a nomenclature developed for birth defects surveillance based on the International Classification of Diseases, Ninth Revision, Clinical Modification and the British Paediatric Association Classification of Diseases (9, 17, 18). When available, details from echocardiography, catheterization, and surgical reports are included in the surveillance records; general pregnancy information such as gestational age and birth weight is also collected.

Analyses were performed on the cohort of infants and fetuses with an estimated date of conception between January 1, 1986 and March 12, 2003. Records with missing/implausible gestational age information were excluded. For each cohort member we estimated the date of conception (assuming that conception occurred 14 days after the last menstrual period date) using vital records data; however, a strong day-of-month pattern was observed in these estimates, and we deemed them unreliable.

Instead, gestational age information from MACDP surveillance records was used to estimate date of conception for infants and fetuses with cardiovascular malformations, as no day-of-month pattern was evident among these estimates. To compensate for the day-of-month pattern observed in the vital records estimates, we modeled the daily count of conceptions for the live birth and fetal death cohort. Vital records information was used to calculate the average daily

number of conceptions for each month during January, 1985 through March, 2004; we assigned these average values to each day within the month so as to create a daily time-series of monthly averages. A cubic spline with six knots per year was fit to this time-series dataset. We used the predicted values from this model as daily estimates of the number of conceptions during January 1, 1986 through March 12, 2003 (n = 715,500 total conceptions).

Cardiovascular malformation outcome groups

Recently, MACDP surveillance records were reviewed by pediatric cardiologists (reviewers were M.D.R., W.T.M., L.D.B, and T.J.R.C.) and classified using the Society of Thoracic Surgeons Congenital Heart Surgery Database v2.30 nomenclature (13-15, 19). This nomenclature is specific to cardiovascular malformations and is more detailed than the nomenclature typically used in birth defects surveillance (10, 20). Our review was an enrichment of the preexisting surveillance data based on analysis of defect codes and the abstracted text in the surveillance records. Our overarching goal was to combine current knowledge of embryology with an established, international cardiovascular malformation nomenclature to create outcome groups appropriate for surveillance and research. In this review, we classified infants with transient newborn cardiac conditions as physiologically normal, and infants and fetuses with multiple congenital heart defect codes were placed in multiple outcome groups only when these malformations were thought to be embryologically independent; otherwise only the major cardiovascular malformation was coded. Further details about this activity are available (14).

For this analysis, we excluded infants and fetuses with identified trisomies, evidence of heterotaxy syndrome, and complex single ventricle lesions. Results are presented for 12 outcome groups; three of these are aggregate groupings of cardiovascular malformations (Table 7.1).

Ambient air quality data

Ambient air quality measurements of daily 8-hour maximum ozone and 24-hour average carbon monoxide, nitrogen dioxide, PM_{10}, and sulfur dioxide were obtained from several monitoring networks in Atlanta, including the U.S. Environmental Protection Agency Air Quality System, Georgia Department of Natural Resources, and the Metro Atlanta Index. For each pollutant we selected a central monitoring station for use in analyses. Central station measurements of carbon monoxide, nitrogen dioxide, and sulfur dioxide were available on ≥ 90 percent of days; when these measurements were missing, pollution levels at the central station were modeled using measurements from nearby monitoring stations. The central station for ozone did not operate during winter months (November through February). During 1986–1992, wintertime ozone levels were modeled using maximum temperature and 1-hour maximum nitrogen dioxide measurements from a nearby monitor. During 1993–2003, wintertime ozone levels were modeled using ozone measurements from a nearby monitor. Measurements of PM_{10} were available every sixth day during 1986–1992, Sunday through Thursday during 1993–1995, and daily during 1996–2003; linear interpolation between measurements was used to estimate missing PM_{10} levels. The location of the PM_{10} central monitoring station changed on January 1, 1993 and on January 1, 1998; on January 1, 1998 the measurement method changed from the federal reference method to the tapered element oscillating microbalance method.

Statistical analyses

We began with an estimate of the number of conceptions with a particular cardiovascular malformation (numerator) and a model-based estimate of total conceptions (denominator) for each day of follow-up (January 1, 1986 through March 12, 2003). All conceptions on a given day of follow-up were assigned the same pollutant metric, which was a weighted average of the 35 daily ambient air pollution measurements during weeks three through seven of pregnancy (a period when the four chambers, inflow tract, and outflow tract of the heart develop). Relative weights were 0.7 for measurements during weeks three and seven, 0.9 for measurements during

weeks four and six, and 1.0 for measurements during week five. We chose this weighting scheme, which emphasizes pollution levels during the center of the five week window, due to uncertainty in the date of conception estimates.

We then created 52 strata representing week-of-year as follows: across all calendar years we grouped January 1 through January 7 in the first week of the year, January 8 through January 14 in the second week of the year, etc. When present, we included February 29 in the ninth week of the year (February 26 through March 4). The fifty-second week of the year was always eight days long (December 24 through December 31).

We modeled temporal associations between ambient air pollution and risk of cardiovascular malformations using Poisson generalized linear models with a log link and scaled variance estimates. We modeled the pollution metric as a continuous variable and used the natural logarithm of the daily estimates of total conceptions as the offset. We included indicator variables for the 52 strata representing week-of-year to control for potential confounding by factors with seasonal variation, and we included a cubic spline for day of follow-up with one knot per year to control for long-term trends. The risk ratios (RR) and confidence intervals correspond to an increase in the interquartile range of the ambient pollutant metric. All models were created using R statistical software, version 2.5.0 (21).

We performed sensitivity analyses to assess the robustness of results. In one sensitivity analysis we relaxed the seasonal and long-term temporal control. In this analysis, we replaced the week-of-year dummy variables with a cubic spline for day-of-year that had three knots; instead of including yearly knots in the cubic spline for day of follow-up we placed knots once every three years. We also investigated the effect of limiting the analysis to single gestation pregnancies and the effect of limiting the analysis to infants and fetuses with only one cardiovascular malformation.

RESULTS

The prevalence of cardiovascular malformations, shown in Table 7.2 by season and year of conception, suggest some seasonal variation across outcome groups (e.g., the spring prevalence is never larger than the winter prevalence). Whereas the observed prevalence of the relatively severe lesions (hypoplastic left heart syndrome, transposition of the great arteries, and tetralogy of Fallot) has remained stable over time, the observed prevalence of the less severe lesions (secundum atrial septal defect, valvar pulmonary stenosis, muscular ventricular septal defect, and perimembranous ventricular septal defect) increased markedly over time.

Descriptive statistics for the ambient air pollution metric, which is a weighted average of daily ambient pollution levels during weeks three through seven of pregnancy and presented in Table 7.3, indicate that all of the pollutants vary by season. Further, the ambient levels of PM_{10}, nitrogen dioxide, and sulfur dioxide declined over time.

As shown in Table 7.4, we observed a positive association between PM_{10} and patent ductus arteriosus (RR = 1.60, 95 percent CI: 1.11, 2.31). The 95 percent confidence intervals for all other associations, presented in Table 7.4, included the null value.

Results of the three sensitivity analyses are available in the online supplement (see Tables 7.5, 7.6, and 7.7). We observed a positive association between PM_{10} and patent ductus arteriosus in all three sensitivity analyses (analyses with less stringent seasonal and long-term temporal control RR = 1.40, 95 percent CI: 1.01, 1.95; analyses limited to single gestation pregnancies RR = 1.57, 95 percent CI: 1.07, 2.28; analyses limited to infants and fetuses with only one cardiovascular malformation RR = 1.70, 95 percent CI: 1.12, 2.56). Additionally, in analyses with less stringent seasonal and long-term temporal control, we observed a positive association between nitrogen dioxide and patent ductus arteriosus (RR = 1.40, 95 percent CI: 1.07, 1.83). In analyses limited to infants and fetuses with only one cardiovascular malformation we observed a negative association between ozone and right ventricular outflow tract defects (RR = 0.52, 95 percent CI: 0.29, 0.93).

DISCUSSION

We investigated temporal associations between ambient air pollution levels during weeks three through seven of pregnancy and cardiovascular malformations for five air pollutants and 12 outcome groups. Except for the association between PM_{10} and patent ductus arteriosus, all 95 percent confidence intervals were consistent with no association. Some confidence intervals, particularly those for ozone, were wide and are therefore compatible with both no effect as well as a harmful effect of air pollution. The observed association between PM_{10} and patent ductus arteriosus, which was consistent across sensitivity analyses, is novel; previous studies did not examine patent ductus arteriosus as an outcome group (7, 8). In our study we used strict criteria to exclude patent ductus arteriosus in premature and newborn infants and when it occurred as an obligate shunt lesion in the presence of other cardiovascular malformations. Of the 2,273 surveillance records reviewed during follow-up that contained a code for patent ductus arteriosus, only 219 infants met our criteria.

Similar to our study, the two previous studies of ambient air pollution and cardiovascular malformations reported results consistent with little or no association (7, 8). In Southern California, investigators reported four significant positive dose-response associations from 144 models. Twenty-four of these models averaged pollution levels over weeks five through eight of pregnancy; the four positive dose-response associations were observed during this window (7). The Texas study investigators observed three positive dose-response associations from 75 models of air pollution levels during weeks three through eight of pregnancy and cardiovascular malformations (8). None of the significant associations reported from the Southern California study were replicated by the Texas study.

Because of our classification and review of surveillance records, direct comparison of specific results from our study with those from the Southern California and Texas studies (7, 8) is not possible. Through our review we excluded infants and fetuses with structurally normal hearts and transient newborn conditions; thirty-four percent of the surveillance records reviewed during

follow-up were classified as "structurally normal." We created outcome groups based on embryologic considerations, and infants and fetuses with multiple congenital heart defect codes were included in multiple outcome groups only when the malformations were thought to be embryologically independent. Consequently, the specific cardiovascular malformations that comprise our outcome groups differ from those in the previous studies (7, 8). For example, in the Southern California study (7), a strong association was observed between carbon monoxide and ventricular septal defects ($OR_{4th\ quartile\ vs.\ 1st\ quartile}$ = 2.95, 95 percent CI: 1.44, 6.05). This outcome group consisted of the four major types of ventricular septal defects as well as pulmonary atresia with ventricular septal defect. In our study we distinguished among the four types of ventricular septal defects because each is thought to develop through a separate embryologic mechanism (22, 23). We analyzed perimembranous and muscular ventricular septal defects as distinct outcome groups, and we placed subarterial ventricular septal defects in the conotruncal defect outcome group (Table 7.1). We did not analyze inlet ventricular septal defects because of the small number of cases. We grouped infants with pulmonary atresia and ventricular septal defect, which is the extreme end of the anatomic spectrum of tetralogy of Fallot (24), in the tetralogy of Fallot outcome group. Although we believe our review and classification of surveillance records is a methodological advancement, our approach obscures comparisons of results across studies.

Another important difference across studies is the analytic approach. Whereas the Southern California and Texas study investigators implemented spatio-temporal analyses (7, 8), we opted for a temporal approach that relied on measurements from centrally-located monitors. Several considerations influenced this decision. Spatially-referenced data were only available back to 1994, whereas data with permissible temporal resolution were available back to 1986. Maternal mobility was also a consideration; in Atlanta at least 20% of women change residences during pregnancy (25, 26). Presumably, the relevant period of exposure is during early gestation, and assigning pollution levels according to maternal residence at delivery could result in substantial exposure misclassification. This issue is pertinent to the spatio-temporal approaches of

the Southern California and Texas studies (7, 8, 27, 28), wherein pollution levels were assigned based on the location of the maternal residence at delivery. Perhaps the most influential consideration, however, was our desire to preclude concerns about our study results based on arguments of spatial confounding. Since our results are based solely on temporal contrasts in pollution levels, plausible risk factors for cardiovascular malformations that may vary across locations (e.g., prenatal vitamin use, prenatal screening and terminations, demographics, socioeconomic status, exposure to toxic environmental agents, prenatal care) are not likely to confound our results.

A limitation of our study, also a limitation in previous studies (7, 8), is that results are based on the cohort of infants and fetuses who achieved 20 weeks gestation. Given that our gestational window of interest spanned weeks three through seven of pregnancy, we would have preferred our cohort to consist of all live fetuses at developmental week three. Unfortunately, data are not available for intrauterine fetal deaths prior to gestational week 20. The consequence of this limitation may depend on the causal effect of air pollution on cardiovascular malformations. For example, atrioventricular septal defect, Ebstein's anomaly, and tricuspid valve dysplasia can all cause intrauterine congestive heart failure, increasing the risk of intrauterine fetal death (29). If air pollution were to cause one of these specific malformations, in turn increasing the risk fetal loss prior to gestational week 20, then our study would be unable to detect this harmful effect of pollution.

Measurement error, which is present in the air quality, vital records, and surveillance data, is another limitation our study shares with previous studies (7, 8). Our use of ambient air pollution measurements from stationary monitors as proxies for personal exposure is likely the largest component of measurement error in our study. Perhaps this source of measurement error is nondifferential, which if true should bias effect estimates towards the null and could explain our predominance of null results (30). Measurement error in the vital records data was evident when we observed a strong day-of-month pattern in the date of conception estimates. Although a

statistical model was created to remove this day-of-month pattern, uncertainty in the estimates remained. The date of conception estimates from surveillance records, although lacking any obvious day-of-month pattern, likewise had uncertainty. Although we selected our exposure window (weeks three through seven of pregnancy) to coincide with the period of cardiac morphogenesis, it is possible that exposures later in pregnancy could impact the development of certain malformations, as some lesions continue to evolve and others may develop during later gestational windows (31). Lastly, some surveillance records had inadequate information to permit precise classification of cardiovascular malformations (e.g., "unspecified" ventricular septal defect). This measurement error was differential over time; relative to older surveillance records, recent records tended to be more detailed. Since this source of measurement error is unlikely to be correlated with ambient air pollution levels (apart from the long-term trend, which is accounted for in the analysis via the cubic spline), we do not believe it would bias results away from the null.

Our investigation is the third epidemiological study of air pollution and cardiovascular malformations conducted to date (7, 8). Although study-specific differences obscure comparisons of results across studies, there appears to be little consistency of results across studies. This lack of consistency could be due to the absence of a true association between air pollution and cardiovascular malformations; it could also be due to differences in populations, pollution levels, outcome definitions, and/or analytic approaches across studies. Although we caution that it may be a spurious association possibly resulting from the conduct of multiple statistical tests, the observed positive association between PM_{10} and patent ductus arteriosus is a novel finding.

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Table 7.1. Cardiovascular malformation outcome group definitions.

Outcome group	Definition
Atrial septal defect, secundum (*n* = 379)	Includes secundum-type atrial septal defect.
Coarctation of the aorta (*n* = 275)	Includes coarctation of the aorta, aortic arch hypoplasia, and interrupted aortic arch type A.
Hypoplastic left heart syndrome (*n* = 175)	Includes hypoplastic left heart syndrome with or without ventricular septal defect.
Patent ductus arteriosus (*n* = 219)	Includes only full term infants (≥ 37 weeks gestation) with patent ductus arteriosus persisting for ≥ 6 weeks following delivery. Excluded if the patent ductus arteriosus was an obligatory shunt lesion or if patency was maintained by prostaglandin infusion.
Pulmonary stenosis, valvar (*n* = 312)	Includes valvar and unspecified pulmonary stenosis as well as dysplastic pulmonary valve.
Tetralogy of Fallot (*n* = 299)	Includes typical tetralogy of Fallot, tetralogy of Fallot with absent pulmonary valve, pulmonary atresia with ventricular septal defect, pulmonary atresia with major aortopulmonary collateral arteries, and tetralogy of Fallot-type double outlet right ventricle.
Transposition of the great arteries (*n* = 165)	Includes all types of transposition with concordant atrioventricular connections and discordant ventricular arterial connections, with or without ventricular septal defect or left ventricular outflow tract obstruction. Also includes double outlet right ventricle with malpositioned great arteries.
Ventricular septal defect, muscular (*n* = 1,108)	Includes muscular-type ventricular septal defect.
Ventricular septal defect, perimembranous (*n* = 546)	Includes perimembranous-type ventricular septal defect.
Conotruncal defects (*n* = 661)*	Includes all cardiovascular malformations in the *Tetralogy of Fallot* and *Transposition of the great arteries* outcome groups. Also includes aortopulmonary window defect, all other double outlet right ventricle variants, interrupted aortic arch type B, unspecified interrupted aortic arch, vascular rings, and subarterial type ventricular septal defect.
Left ventricular outflow tract defects (*n* = 558)*	Includes all cardiovascular malformations in the *Coarctation of the aorta* and *Hypoplastic left heart syndrome* outcome groups. Also includes stenosis/atresia of the aortic valve and isolated bicuspid aortic valve.
Right ventricular outflow tract defects (*n* = 421)*	Includes all cardiovascular malformations in the *Pulmonary stenosis, valvar* outcome group. Also includes pulmonary valve atresia with intact ventricular septum, tricuspid valve atresia, double chambered right ventricle, and isolated supravalvar pulmonary artery stenosis.

* Aggregate grouping of cardiovascular malformations.

Table 7.2. Prevalence of cardiovascular malformations (per 10,000 conceptions), by season and year of conception, for the cohort of births and fetal deaths in Atlanta, Georgia, during January 1, 1986 through March 12, 2003.*

	Prevalence†, by season of conception				Prevalence†, by year of conception			Prevalence †
	Mar–May	Jul–Aug	Sep–Nov	Dec–Feb	1986–1991	1992–1997	1998–2003	Overall
Atrial septal defect, secundum (*n* = 379)	4.2	5.4	5.9	5.6	2.8	4.7	7.9	5.3
Coarctation of the aorta (*n* = 275)	3.6	4.0	3.1	4.6	3.9	3.8	3.8	3.8
Hypoplastic left heart syndrome (*n* = 175)	2.0	2.2	3.1	2.4	2.9	2.3	2.3	2.5
Patent ductus arteriosus (*n* = 219)	2.8	3.1	2.9	3.5	3.6	3.1	2.7	3.1
Pulmonary stenosis, valvar (*n* = 312)	3.6	4.6	4.8	4.4	2.7	4.6	5.4	4.4
Tetralogy of Fallot (*n* = 299)	4.4	3.8	4.0	4.5	4.2	4.0	4.3	4.2
Transposition of the great arteries (*n* = 165)	2.3	2.2	2.2	2.5	2.2	2.2	2.5	2.3
Ventricular septal defect, muscular (*n* = 1,108)	14.1	14.8	17.1	15.8	5.3	14.3	24.9	15.5
Ventricular septal defect, perimembranous (*n* = 546)	6.6	7.8	8.4	7.7	4.8	8.1	9.5	7.6
Conotruncal defects (*n* = 661)‡	9.1	8.3	9.1	10.4	8.4	9.0	10.2	9.2
Left ventricular outflow tract defects (*n* = 558)‡	6.4	7.5	8.6	8.7	7.9	7.6	7.9	7.8
Right ventricular outflow tract defects (*n* = 421)‡	5.0	6.2	6.1	6.2	3.9	6.9	6.5	5.9

* Daily counts of total conceptions are model-based estimates. To create this model, we calculated the average daily number of conceptions during each month using vital records information and created a daily time-series dataset of these monthly averages. A cubic spline with six knots per year was fit to this time-series dataset. The predicted values from this model are the daily estimates of total conceptions.

† Prevalence per 10,000 conceptions.

‡ Aggregate grouping of cardiovascular malformations.

Table 7.3. Interquartile range and mean values, by season and year of conception, for the 5-week air pollution metric* assigned to the cohort of births and fetal deaths in Atlanta, Georgia, conceived during January 1, 1986 through March 12, 2003.

	8-hour ozone (ppb)†,§	24-hour PM_{10} ($\mu g/m^3$)‡,§	24-hour nitrogen dioxide (ppb)§	24-hour carbon monoxide (ppm)§	24-hour sulfur dioxide (ppb)§
Interquartile range	29.9	14.2	5.7	0.3	4.0
Mean value, by season of conception					
Mar – May	54.6	36.0	24.2	0.6	5.4
Jun – Aug	56.5	38.7	22.6	0.8	5.4
Sep – Nov	25.4	31.2	26.9	0.9	6.9
Dec – Feb	29.2	27.3	26.5	0.7	7.1
Mean value, by year of conception					
1986 – 1991	43.3	43.2	28.0	0.7	8.7
1992 – 1997	39.8	30.0	24.3	0.8	5.5
1998 – 2003	41.2	25.8	22.5	0.7	4.0

* The air pollution metric is a 5-week weighted average of daily ambient air pollution levels during weeks 3–7 of pregnancy. Relative weights are 0.7, 0.9, and 1.0 for pollution levels during the first and last week, the second and fourth week, and the middle week of the window, respectively.

† The central station for ozone did not operate during winter months (November–February).

‡ PM_{10} (particulate matter with an average aerodynamic diameter of less than 10 μm) was measured every sixth day during 1986–1992, Sunday–Thursday during 1993–1995, and daily during 1996–2003. The location of the PM_{10} central monitoring station changed on January 1, 1993 and on January 1, 1998; on January 1, 1998 the measurement method changed from the federal reference method to tapered element oscillating microbalance.

§ Daily central monitoring station measurements available: 67 percent (4,251 of 6,315 days) for ozone, 41 percent (2,563 of 6,315 days) for PM_{10}, 90 percent (5,670 of 6,315 days) for nitrogen dioxide, 92 percent (5,804 of 6,315 days) for carbon monoxide, 94 percent (5,966 of 6,315 days) for sulfur dioxide. When feasible, missing daily measurements were modeled: 33 percent (2,064 of 6,315 days) for ozone, 59 percent (3,735 of 6,315 days) for PM_{10}, 10 percent (609 of 6,315 days) for nitrogen dioxide, 6 percent (388 of 6,315 days) for carbon monoxide, 5 percent (311 of 6,315 days) for sulfur dioxide.

Table 7.4. Risk ratios and 95 percent confidence intervals* for associations between the 5-week air pollution metric and cardiovascular malformation outcomes among the cohort of births and fetal deaths in Atlanta, Georgia, conceived during January 1, 1986 through March 12, 2003.

	8-hour ozone (ppb)	24-hour PM_{10} ($\mu g/m^3$)†	24-hour nitrogen dioxide (ppb)	24-hour carbon monoxide (ppm)	24-hour sulfur dioxide (ppb)
Atrial septal defect, secundum (*n* = 379)	1.16 (0.67, 2.00)	1.12 (0.82, 1.53)	1.15 (0.92, 1.43)	0.92 (0.74, 1.15)	1.00 (0.72, 1.38)
Coarctation of the aorta (*n* = 275)	1.15 (0.65, 2.06)	1.15 (0.84, 1.58)	1.11 (0.87, 1.41)	0.99 (0.79, 1.24)	1.04 (0.75, 1.43)
Hypoplastic left heart syndrome (*n* = 175)	0.82 (0.37, 1.84)	0.89 (0.60, 1.31)	0.91 (0.66, 1.24)	0.80 (0.60, 1.06)	0.77 (0.50, 1.18)
Patent ductus arteriosus (*n* = 219)	1.39 (0.72, 2.68)	1.60 (1.11, 2.31)	1.27 (0.96, 1.70)	1.18 (0.92, 1.51)	1.22 (0.86, 1.74)
Pulmonary stenosis, valvar (*n* = 312)	0.97 (0.53, 1.75)	0.87 (0.63, 1.21)	1.01 (0.80, 1.28)	1.06 (0.86, 1.32)	0.70 (0.49, 1.00)
Tetralogy of Fallot (*n* = 299)	1.09 (0.59, 2.00)	0.88 (0.65, 1.20)	0.94 (0.74, 1.20)	1.13 (0.91, 1.40)	0.85 (0.61, 1.17)
Transposition of the great arteries (*n* = 165)	1.29 (0.58, 2.85)	1.12 (0.74, 1.72)	0.80 (0.57, 1.11)	0.94 (0.68, 1.30)	1.13 (0.75, 1.71)
Ventricular septal defect, muscular (*n* = 1,108)	1.08 (0.77, 1.50)	1.01 (0.83, 1.23)	1.09 (0.96, 1.24)	0.99 (0.85, 1.14)	0.95 (0.77, 1.17)
Ventricular septal defect, perimembranous (*n* = 546)	1.06 (0.67, 1.68)	0.94 (0.73, 1.22)	1.12 (0.94, 1.33)	0.96 (0.81, 1.14)	0.99 (0.76, 1.28)
Conotruncal defects (*n* = 661)	1.22 (0.81, 1.85)	0.99 (0.80, 1.22)	0.95 (0.81, 1.12)	1.04 (0.89, 1.21)	1.06 (0.86, 1.31)
Left ventricular outflow tract defects (*n* = 558)	1.09 (0.70, 1.68)	1.03 (0.83, 1.29)	1.01 (0.85, 1.20)	0.97 (0.82, 1.13)	0.97 (0.76, 1.22)
Right ventricular outflow tract defects (*n* = 421)	0.73 (0.44, 1.22)	0.85 (0.64, 1.12)	1.02 (0.84, 1.25)	1.16 (0.96, 1.40)	0.74 (0.55, 1.00)

* Risk ratios and 95 percent confidence intervals correspond to an increase in the interquartile range of the 5-week air pollutant metric (which is a weighted average of the 35 daily ambient air pollution levels during weeks three through seven of pregnancy). The interquartile ranges were 29.9 ppb for ozone, 14.2 $\mu g/m^3$ for PM_{10}, 5.7 ppb for nitrogen dioxide, 0.3 for carbon monoxide, and 4.0 for sulfur dioxide.

† PM_{10} (particulate matter with an average aerodynamic diameter of less than 10 μm).

Table 7.5. Sensitivity analysis with less stringent control of seasonal and long-term temporal variation. Risk ratios and 95 percent confidence intervals for associations between the 5-week air pollution metric and cardiovascular malformation outcomes among the cohort of births and fetal deaths in Atlanta, Georgia, conceived during January 1, 1986 through March 12, 2003.*

	8-hour ozone (ppb)	24-hour PM_{10} ($\mu g/m^3$)†	24-hour nitrogen dioxide (ppb)	24-hour carbon monoxide (ppm)	24-hour sulfur dioxide (ppb)
Atrial septal defect, secundum (*n* = 379)	1.25 (0.74, 2.09)	1.12 (0.82, 1.52)	1.16 (0.94, 1.43)	0.97 (0.80, 1.18)	1.05 (0.77, 1.43)
Coarctation of the aorta (*n* = 275)	1.21 (0.68, 2.16)	1.21 (0.90, 1.63)	1.03 (0.82, 1.30)	1.00 (0.82, 1.22)	1.02 (0.75, 1.40)
Hypoplastic left heart syndrome (*n* = 175)	1.37 (0.63, 3.00)	1.12 (0.75, 1.66)	0.92 (0.68, 1.25)	0.93 (0.73, 1.20)	0.84 (0.55, 1.27)
Patent ductus arteriosus (*n* = 219)	1.26 (0.65, 2.43)	1.40 (1.01, 1.95)	1.40 (1.07, 1.83)	1.19 (0.96, 1.47)	1.27 (0.90, 1.79)
Pulmonary stenosis, valvar (*n* = 312)	1.23 (0.70, 2.15)	1.00 (0.73, 1.37)	1.07 (0.86, 1.33)	1.09 (0.90, 1.32)	0.80 (0.57, 1.13)
Tetralogy of Fallot (*n* = 299)	1.24 (0.72, 2.14)	0.98 (0.73, 1.30)	1.02 (0.82, 1.26)	1.10 (0.92, 1.30)	0.82 (0.61, 1.10)
Transposition of the great arteries (*n* = 165)	1.70 (0.83, 3.48)	1.01 (0.69, 1.49)	0.77 (0.58, 1.03)	0.94 (0.72, 1.23)	0.98 (0.67, 1.44)
Ventricular septal defect, muscular (*n* = 1,108)	1.17 (0.87, 1.56)	1.02 (0.85, 1.23)	1.05 (0.94, 1.18)	1.01 (0.90, 1.13)	0.93 (0.77, 1.13)
Ventricular septal defect, perimembranous (*n* = 546)	1.11 (0.73, 1.69)	0.92 (0.72, 1.17)	1.05 (0.89, 1.23)	1.00 (0.86, 1.17)	0.98 (0.76, 1.25)
Conotruncal defects (*n* = 661)	1.34 (0.93, 1.93)	1.01 (0.73, 1.30)	1.00 (0.87, 1.15)	1.05 (0.93, 1.19)	0.96 (0.79, 1.17)
Left ventricular outflow tract defects (*n* = 558)	1.37 (0.91, 2.06)	1.17 (0.95, 1.44)	0.99 (0.84, 1.16)	1.02 (0.90, 1.17)	0.99 (0.79, 1.23)
Right ventricular outflow tract defects (*n* = 421)	0.94 (0.58, 1.52)	0.94 (0.72, 1.23)	1.00 (0.83, 1.20)	1.16 (0.98, 1.36)	0.85 (0.65, 1.13)

* Risk ratios and 95 percent confidence intervals correspond to an increase in the interquartile range of the 5-week air pollutant metric (which is a weighted average of the 35 daily ambient air pollution levels during weeks three through seven of pregnancy). The interquartile ranges were 29.9 ppb for ozone, 14.2 $\mu g/m^3$ for PM_{10}, 5.7 ppb for nitrogen dioxide, 0.3 for carbon monoxide, and 4.0 for sulfur dioxide.

† PM_{10} (particulate matter with an average aerodynamic diameter of less than 10 μm).

Table 7.6. Sensitivity analysis limited to single gestation pregnancies. Risk ratios and 95 percent confidence intervals for associations between the 5-week air pollution metric and cardiovascular malformation outcomes among the cohort of births and fetal deaths in Atlanta, Georgia, conceived during January 1, 1986 through March 12, 2003.*

	8-hour ozone (ppb)	24-hour PM_{10} ($\mu g/m^3$)†	24-hour nitrogen dioxide (ppb)	24-hour carbon monoxide (ppm)	24-hour sulfur dioxide (ppb)
Atrial septal defect, secundum (*n* = 347)	1.19 (0.67, 2.11)	1.10 (0.79, 1.51)	1.12 (0.89, 1.41)	0.86 (0.68, 1.09)	1.02 (0.73, 1.43)
Coarctation of the aorta (*n* = 254)	1.11 (0.61, 2.01)	1.24 (0.89, 1.72)	1.14 (0.89, 1.46)	1.02 (0.81, 1.29)	1.09 (0.78, 1.53)
Hypoplastic left heart syndrome (*n* = 166)	0.92 (0.40, 2.11)	0.90 (0.60, 1.35)	0.89 (0.65, 1.23)	0.79 (0.59, 1.06)	0.78 (0.50, 1.21)
Patent ductus arteriosus (*n* = 215)	1.25 (0.64, 2.46)	1.57 (1.07, 2.28)	1.23 (0.92, 1.65)	1.19 (0.92, 1.52)	1.17 (0.81, 1.67)
Pulmonary stenosis, valvar (*n* = 286)	0.95 (0.51, 1.78)	0.87 (0.62, 1.23)	1.00 (0.78, 1.27)	1.07 (0.85, 1.34)	0.73 (0.51, 1.05)
Tetralogy of Fallot (*n* = 284)	1.04 (0.55, 1.96)	0.87 (0.63, 1.20)	0.94 (0.73, 1.21)	1.15 (0.93, 1.44)	0.85 (0.61, 1.18)
Transposition of the great arteries (*n* = 160)	1.15 (0.51, 2.60)	1.13 (0.74, 1.72)	0.79 (0.56, 1.10)	0.91 (0.65, 1.27)	1.09 (0.72, 1.66)
Ventricular septal defect, muscular (*n* = 1,027)	1.08 (0.76, 1.51)	0.99 (0.81, 1.22)	1.13 (0.99, 1.29)	1.00 (0.86, 1.16)	0.94 (0.75, 1.17)
Ventricular septal defect, perimembranous (*n* = 514)	1.06 (0.66, 1.70)	0.95 (0.72, 1.23)	1.11 (0.93, 1.33)	0.96 (0.81, 1.14)	0.94 (0.72, 1.23)
Conotruncal defects (*n* = 629)	1.16 (0.76, 1.78)	0.98 (0.63, 1.20)	0.93 (0.79, 1.10)	1.04 (0.89, 1.21)	1.03 (0.83, 1.28)
Left ventricular outflow tract defects (*n* = 523)	1.09 (0.70, 1.71)	1.08 (0.85, 1.35)	1.00 (0.84, 1.20)	0.97 (0.82, 1.14)	1.00 (0.79, 1.27)
Right ventricular outflow tract defects (*n* = 389)	0.68 (0.40, 1.16)	0.83 (0.62, 1.12)	1.00 (0.81, 1.23)	1.16 (0.96, 1.41)	0.76 (0.56, 1.03)

* Risk ratios and 95 percent confidence intervals correspond to an increase in the interquartile range of the 5-week air pollutant metric (which is a weighted average of the 35 daily ambient air pollution levels during weeks three through seven of pregnancy). The interquartile ranges were 29.9 ppb for ozone, 14.2 $\mu g/m^3$ for PM_{10}, 5.7 ppb for nitrogen dioxide, 0.3 for carbon monoxide, and 4.0 for sulfur dioxide.

† PM_{10} (particulate matter with an average aerodynamic diameter of less than 10 μm).

Table 7.7. Sensitivity analysis limited to infants and fetuses with only one cardiovascular malformation. Risk ratios and 95 percent confidence intervals for associations between the 5-week air pollution metric and cardiovascular malformation outcomes among the cohort of births and fetal deaths in Atlanta, Georgia, conceived during January 1, 1986 through March 12, 2003.*

	8-hour ozone (ppb)	24-hour PM_{10} ($\mu g/m^3$)†	24-hour nitrogen dioxide (ppb)	24-hour carbon monoxide (ppm)	24-hour sulfur dioxide (ppb)
Atrial septal defect, secundum (*n* = 202)	0.80 (0.36, 1.73)	1.03 (0.67, 1.57)	1.04 (0.77, 1.41)	1.08 (0.80, 1.46)	1.16 (0.75, 1.79)
Coarctation of the aorta (*n* = 145)	1.09 (0.51, 2.32)	1.16 (0.75, 1.79)	1.08 (0.78, 1.50)	1.04 (0.76, 1.43)	0.89 (0.56, 1.39)
Hypoplastic left heart syndrome (*n* = 167)	0.80 (0.35, 1.82)	0.90 (0.61, 1.34)	0.91 (0.66, 1.24)	0.83 (0.62, 1.10)	0.82 (0.53, 1.28)
Patent ductus arteriosus (*n* = 171)	1.20 (0.56, 2.59)	1.70 (1.12, 2.56)	1.31 (0.95, 1.81)	1.25 (0.95, 1.64)	1.37 (0.92, 2.04)
Pulmonary stenosis, valvar (*n* = 225)	0.76 (0.37, 1.56)	0.88 (0.61, 1.27)	1.01 (0.77, 1.33)	1.13 (0.88, 1.45)	0.74 (0.49, 1.11)
Tetralogy of Fallot (*n* = 279)	0.96 (0.50, 1.81)	0.85 (0.62, 1.18)	0.89 (0.69, 1.15)	1.17 (0.94, 1.46)	0.87 (0.62, 1.22)
Transposition of the great arteries (*n* = 140)	1.28 (0.54, 3.03)	1.12 (0.71, 1.78)	0.79 (0.55, 1.13)	0.93 (0.65, 1.31)	1.19 (0.76, 1.87)
Ventricular septal defect, muscular (*n* = 976)	0.90 (0.64, 1.28)	0.98 (0.79, 1.20)	1.06 (0.92, 1.21)	1.00 (0.85, 1.16)	0.91 (0.73, 1.15)
Ventricular septal defect, perimembranous (*n* = 388)	1.22 (0.71, 2.11)	0.93 (0.68, 1.26)	1.19 (0.97, 1.46)	0.97 (0.79, 1.18)	1.08 (0.80, 1.47)
Conotruncal defects (*n* = 571)	1.12 (0.72, 1.76)	0.96 (0.76, 1.21)	0.91 (0.76, 1.09)	1.07 (0.91, 1.27)	1.05 (0.83, 1.32)
Left ventricular outflow tract defects (*n* = 406)	1.00 (0.61, 1.66)	0.99 (0.76, 1.28)	0.94 (0.77, 1.14)	0.97 (0.80, 1.16)	0.89 (0.67, 1.18)
Right ventricular outflow tract defects (*n* = 331)	0.52 (0.29, 0.93)	0.81 (0.59, 1.10)	1.02 (0.81, 1.28)	1.23 (1.00, 1.51)	0.77 (0.56, 1.07)

* Risk ratios and 95 percent confidence intervals correspond to an increase in the interquartile range of the 5-week air pollutant metric (which is a weighted average of the 35 daily ambient air pollution levels during weeks three through seven of pregnancy). The interquartile ranges were 29.9 ppb for ozone, 14.2 $\mu g/m^3$ for PM_{10}, 5.7 ppb for nitrogen dioxide, 0.3 for carbon monoxide, and 4.0 for sulfur dioxide.

† PM_{10} (particulate matter with an average aerodynamic diameter of less than 10 μm).

Chapter 8: Conclusion

In this dissertation I investigated temporal relationships between ambient air pollutant levels during weeks three through seven of pregnancy and risk of cardiovascular malformations among the cohort of infants and fetuses conceived during January 1, 1986 through March 12, 2003 in Atlanta, Georgia. Across the 60 models (five air pollutants by 12 outcome groups) the only positive association observed was between PM_{10} and patent ductus arteriosus (see dissertation Chapter 7). All other 95 percent confidence intervals included the null value. Some 95 percent confidence intervals, particularly those for ozone, were wide and were therefore compatible with both no effect as well as a harmful effect of air pollution. The observed association between PM_{10} and patent ductus arteriosus, which was consistent across sensitivity analyses, is novel; previous studies did not examine patent ductus arteriosus as an outcome group (Ritz et al., 2002; Gilboa et al., 2005). There was little evidence to support any of the seven primary dissertation hypotheses.

A major component of this dissertation was the review of MACDP surveillance records to classify infants and fetuses with cardiovascular malformations. Benefits from this effort extend beyond the scope of this dissertation. For example, the fortieth anniversary MACDP surveillance report reported trends in the prevalence of cardiovascular malformations in Atlanta during 1980-2002 using the classifications developed for this dissertation project (Correa et al., 2007). Work is being conducted to describe the racial and ethnic variation in the prevalence of cardiovascular malformations, to characterize the survival of infants and fetuses with cardiovascular malformations, and to evaluate whether children with cardiovascular malformations suffer comorbidities, such as cancer or developmental disabilities, later in life. The cardiovascular malformation classifications also offer possibilities for the conduct of future environmental epidemiology projects. A manuscript detailing the methodology of the review and classification will appear in the December, 2007 issue of *Birth Defects Research (Part A)* (Riehle-Colarusso et

al., in press). Although the review improves the validity of the cardiovascular malformation classification relative to the preexisting codes (see dissertation Chapter 5), the review has an important limitation in that it does not consider non-cardiac birth defects. In the review the heart is classified in isolation from the other organ systems; further review by a clinical geneticist would be needed to discern whether the cardiovascular malformation is an isolated defect or part of a known association. Isolated malformations and multiple malformations may have different etiologies, and if this additional review were conducted, then analyses could be performed that distinguish isolated cardiovascular malformations from multiple cardiovascular malformations.

For this dissertation I elected to analyze temporal relationships between ambient air pollution levels during pregnancy and risk of cardiovascular malformations. This approach differs from that of Ritz et al. (2002) and Gilboa et al. (2005), who implemented spatio-temporal analyses. As discussed in dissertation Chapter 6, since ambient air pollution levels vary over both space and time, in a spatio-temporal approach the investigator needs to account for risk factors systematically associated with ambient air pollution levels over space or time. Since the dissertation results are based solely on temporal contrasts in pollution levels, plausible risk factors for cardiovascular malformations that may vary across locations (e.g., prenatal vitamin use, prenatal screening and terminations, demographics, socioeconomic status, exposure to toxic environmental agents, prenatal care) are not likely to confound the results. Risk factors with temporal variation that are systematically associated with ambient air pollution levels must be adequately controlled if the analysis is to be valid. Consequently, in this dissertation I tightly controlled for confounding by risk factors with seasonal and long-term temporal variation in the Poisson generalized linear models through the use of week-of-year indicator variables and a cubic spline with one knot per year (see dissertation Chapter 7).

This dissertation is the third epidemiological study of air pollution and cardiovascular malformations conducted to date. Although study-specific differences obscure comparisons of results across studies, there appears to be little consistency of non-null results across studies. This

lack of consistency could be due to the absence of a true association between air pollution and cardiovascular malformations; it could also be due to differences in populations, pollution levels, outcome definitions, and/or analytic approaches across studies. Although it may possibly be a spurious association resulting from the conduct of multiple statistical tests, the observed positive association between PM_{10} and patent ductus arteriosus is a novel finding.

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