EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

Sulfur Dioxide

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Reproductive and Cancer Hazard Assessment Branch
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PREFACE

Proposition 65[1] requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity. It specifies that "a chemical is known to the state to cause reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity ...." The "state's qualified experts" regarding findings of reproductive toxicity are the members of the Developmental and Reproductive Toxicant Identification Committee (DART IC) of the Office of Environmental Health Hazard Assessment (OEHHA) Science Advisory Board[2]. OEHHA, a department within the California Environmental Protection Agency, is the lead agency for implementing Proposition 65.

After consultation with the DART IC, OEHHA selected sulfur dioxide (SO_2) as a chemical for consideration for listing by the DART IC. Upon selection, the public was given the opportunity to submit information relevant to the assessment of the evidence on the reproductive toxicity of SO_2. One submission was received, and relevant information in the submission was considered during preparation of this document.

OEHHA developed this document to provide the DART IC with comprehensive information on the reproductive toxicity of SO_2 for use in its deliberations on whether or not the chemical should be listed under Proposition 65.

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code section 25249.5 *et seq*.)
[2] Title 27 Cal. Code of Regs. section 25302

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ACRONYMS AND ABBREVIATIONS

AC	abdominal circumference
ANOVA	analysis of variance
AOR	adjusted odds ratio
AQMS	Air Quality Monitoring Station
AQS	Air Quality System
ATSDR	Agency for Toxic Substances and Disease Registry
avg.	average
AVSD	atrial ventricular septal defect
BC	black carbon
BPD	biparietal diameter
BS	black smoke
CA	chromosomal aberrations
CARB	California Air Resources Board
CHD	congenital heart disease
CI	95% confidence interval(s)
CLP	cleft lip with or without cleft palate
CO	carbon monoxide
COHb	carboxyhemoglobin
conc.	concentration
COPD	chronic obstructive pulmonary disease
CSD	Census subdivision (Canada)
CRL	crown-rump length
d	day(s)
DA	dissemination area
df	degrees of freedom
DFI	DNA fragmentation index
ED	exposure day
ETS	environmental tobacco smoke
FDA	(U.S.) Food and Drug Administration
FISH	fluorescence in situ hybridization
FL	femur length
FUMC	first unprotected menstrual cycle
g	gram(s)
G6-PD	glucose 6 phosphate dehydrogenase
GD	gestation day
GHCD9	Georgia Health Care District 9
GPx	glutathione peroxidase
GRAS	generally recognized as safe
GSH	glutathione
GST	glutathione s-transferase
hr	hour(s)
HC	head circumference
hCG	human chorionic gonadotropin
HDS	high DNA stainability

HID	hazard identification document
IARC	International Agency for Research on Cancer
ICSI	intracytoplasmic sperm injection
IDW	inverse distance weighting
IOP	index of overall pollution
i.p.	intraperitoneal
IQR	interquartile range
i.v.	intravenous (-ly)
IVF	in vitro fertilization
IUGR	intrauterine growth retardation
km	kilometer(s)
LBW	low birthweight
LMP	last menstrual period
m	meter(s)
MDA	malondialdehyde
MI	mitotic index
ml	milliliter(s)
MDC	Midwives Data Collection
µg	microgram(s)
$\mu g/m^3$	microgram per cubic meter
µm	micrometer(s)
mM	millimolar
NA	National Academies
NAAQS	National Ambient Air Quality Standards
NIOSH	National Institute for Occupational Safety and Health
nM	nanomolar
NO	nitrogen monoxide
NO_2	nitrogen dioxide
NOAEL	no observed adverse effect level
NorCAS	Northern Congenital Anomaly Survey
NO_x	refers to NO and NO_2
O_3	ozone
O/E	observed/expected ratio
OEHHA	Office of Environmental Health Hazard Assessment
OR	odds ratio(s)
ppb	parts per billion
ppm	parts per million
PAH	polycyclic aromatic hydrocarbon
PM	particulate matter
$PM_{2.5}$	particulate matter less than 2.5 µm in size
PM_{10}	particulate matter less than 10 µm in size
PMSG	pregnant mare's serum gonadotropin
PND	postnatal day(s)
PTB	preterm birth
QEPA	Queensland Environmental Protection Agency
RADS	reactive airways dysfunction syndrome

RER	rough endoplasmic reticulum
RH	relative humidity
ROS	reactive oxygen species
RR	relative risk(s)
SCE	sister chromatid exchanges
SCSA	sperm chromatin structure assay
SCOGS	Select Committee on GRAS Substances
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SES	socioeconomic status
SGA	small for gestational age
SLA	statistical local area
SO_2	sulfur (sulphur) dioxide
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances
TSP	total suspended particles
U.S. EPA	U.S. Environmental Protection Agency
VLBW	very low birthweight
W	tungsten
WHO	World Health Organization
wk	week(s)
yr	year(s)

A. EXECUTIVE SUMMARY

Sulfur dioxide (SO_2) is a colorless, nonflammable gas with a pungent odor. As a component of air pollution, SO_2 is found in combination with sulfuric acid, sulfur trioxide, ozone, nitrogen dioxide, and particulates. Its presence in ambient air occurs primarily as a result of fossil fuel consumption at power generation and other industrial facilities.

Male Reproductive Toxicity

There are human, animal and other relevant data that provide information on the relationship between SO_2 exposures and male reproductive toxicity. There are four epidemiologic publications. One cohort study evaluated the effects of SO_2 exposure on fecundability. Three other studies evaluated the effects of exposure to air pollution on sperm quality, with some using SO_2 as an index measure of air pollution.

- The cohort study was well-conducted and showed that exposure to relatively high levels of SO_2 resulted in decreased fecundability in humans. Evidence strongly supporting a cause and effect association between exposure to SO_2 and decreased fecundability included:
    - The odds of conception in the first unprotected menstrual cycle (FUMC) were significantly and consistently reduced in couples exposed to SO_2 levels above 15.3 parts per billion (ppb) in the second month before conception.
    - The timing of the effect coincided with the sperm maturation period.
    - A dose-response association was seen with increasing levels of SO_2 exposure.

Three studies in mice investigated the effects of exposure to SO_2 by inhalation on male reproductive parameters including testicular histology and biochemistry and effects on sperm. One additional study investigated the effects of SO_2 exposure on lipid peroxidation and redox status in mouse organs.

- Mice exposed to SO_2 by inhalation showed toxic effects in testis as well as other organs:
    - SO_2 inhalation altered testis basement membranes, and damaged Sertoli cells and spermatids in one study.
    - In two studies, SO_2 inhalation altered several testicular biochemical parameters, leading study authors to conclude that SO_2 is toxic to the male reproductive system.
    - SO_2 inhalation increased levels of lipid peroxidation and altered intracellular redox status in mouse organs, including testes.

Numerous studies in humans and animals provide evidence that exposure to episodes of relatively high air pollution causes male reproductive toxicity manifested as adverse effects on semen quality, sperm chromatin integrity and biochemical parameters in the testis. In addition to direct evidence of male reproductive toxicity,

the studies on semen quality and sperm chromatin integrity also provide mechanistic evidence consistent with decreased fecundability in males:

- Exposure to SO_2 is associated with increased DNA damage:
    - Increased DNA damage occurs in sperm cells of humans and animals exposed to SO_2.
    - Three occupational studies reporting an association between exposure specifically to SO_2 and increased DNA damage in lymphocytes of workers provide supporting evidence.

Female Reproductive Toxicity

Few studies of the relationship between SO_2 exposure and female reproductive toxicity have been conducted.

- One epidemiologic retrospective cohort study of females undergoing their first cycle of in vitro fertilization (IVF) found that SO_2 concentrations were consistently, though not statistically significantly, associated with decreased odds of live birth. Associations with other pollutants were stronger.

- One study of potential female reproductive toxicity of SO_2 in animals was conducted in rats exposed via inhalation. The authors reported effects on:
    - Estrous cycle length,
    - Pregnancy frequency and duration
    - Offspring growth.

- Additional information on female reproductive toxicity comes from a study of meiosis in oocytes following exposure to sodium sulfite. *In vitro* exposure of sheep or cow oocytes to sodium sulfite resulted in fragmentation of chromosomes, with or without rearrangement, but these effects were not seen in mouse oocytes exposed either *in vitro* or *in vivo*.

Developmental Toxicity

Numerous epidemiologic studies have evaluated the effects of SO_2 exposure on endpoints including preterm birth, fetal growth, pregnancy loss, and congenital malformations. Three animal studies looked at postnatal physical and behavioral development following prenatal exposures to SO_2. One other animal study evaluated external and internal malformations in two species.

Ten human epidemiologic studies examined the association between SO_2 exposure and preterm births:

- Seven studies reported a statistically significant higher risk of preterm birth associated with exposure to SO_2 and one reported a marginally significant association.
- Studies with higher levels of exposure were more likely to report significantly increased risk of preterm birth.
- A significant dose response relationship was observed between SO_2 and total suspended particulates (TSP) and gestational age in a prospective cohort study that examined the highest exposure levels. In this study, the gestation age distribution curve was more skewed toward the left tail (shorter gestation) on high pollution days, suggesting that pregnancies at high risk for preterm delivery may be particularly susceptible to adverse effects of air pollution.

Twenty-two human epidemiologic studies evaluated fetal growth in association with SO_2 exposure:

- Thirteen studies reported ambient SO_2 exposure was statistically significantly associated with indicators of fetal growth restriction or increased risk of low birthweight (LBW).
- Two studies reported mixed findings for fetal growth, i.e., SO_2 exposure was significantly associated with indicators of both reduced and increased birthweight, depending on the exposure levels, exposure periods, and populations.
- Five studies found no significant associations with birthweight.
- Two studies reported SO_2 was associated with decreased risk of LBW or increased birthweight.

Overall, the majority of studies that had measured SO_2 concentrations well above limits of detection found that SO_2 was associated with increased risk of LBW or other measures of fetal growth restriction. The studies found different susceptible exposure periods. The role of co-pollutants in relation to the potential effects of SO_2 on birthweight is unclear, but several well-conducted studies suggest that SO_2 is associated with reduced birthweight, independent of co-pollutants.

Four epidemiologic studies examined the association between exposure to SO_2 and pregnancy loss:

- Three studies looked at the frequency of stillbirth. One study reported a positive correlation between spontaneous fetal death and SO_2, though the increase in risk associated with SO_2 exposure (e.g., relative risk) was not estimated.
- The single study that specifically examined the association between SO_2 and spontaneous abortion found no association.

Seven epidemiologic studies examined exposure to ambient SO_2 as a risk factor for congenital malformations. The studies examined groupings of chromosomal and non-chromosomal congenital malformations, heart defects, and oral clefts, and reported increases in risk, decreases in risk (protective effects), and no changes in risk of malformations associated with SO_2 exposure. The findings varied widely between and within studies, yielding no discernible patterns for any type of defect. Common limitations of these studies included:

- Lack of control for important potential confounders, including use of nutritional supplements such as folate, or use of alcohol or tobacco
- Lack of specificity of timing of exposure relative to the specific malformation
- Very low SO_2 concentrations and lack of variability in SO_2

Three animal studies focused on evaluating the effects of prenatal exposure to SO_2 on social behavior or the acquisition of physical and behavioral developmental landmarks. An additional study evaluated physical effects in both mice and rabbits.

- A study in mice reported significant effects of prenatal SO_2 exposure on male-male social behavior in offspring.
- Of two studies looking at acquisition of developmental landmarks in mice, one identified significant delays in the appearance of certain reflexes as well as significantly decreased birth weights associated with prenatal exposure to SO_2.
- A study in mice and rabbits did not find SO_2 exposure to be associated with specific or aggregate malformations in either species. There was some suggestion of an increase in minor skeletal variations in rabbits. Fetal weights were significantly reduced in treated mice, though rabbit fetal weights were not affected.
- An additional study of reproductive toxicity, conducted in rats by the inhalation route of exposure, reported increased litter sizes in SO_2-exposed animals.

Overall, two studies reported that SO_2 exposure resulted in reduced fetal/birth weights in mice; no such effect was found in rabbits. Where evaluated, litter sizes were not found to be decreased by SO_2 exposure, and major malformations were not increased in frequency.

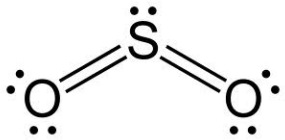
B. INTRODUCTION

This document reviews the reproductive toxicity of sulfur dioxide (SO_2). It begins with a brief discussion, in this section, of the physical characteristics, exposures and uses of SO_2. This is followed by sections on each of the major endpoints – developmental, male reproductive and female reproductive toxicity. Each section reviews effects seen in studies of humans who were exposed to SO_2 as an air pollutant, effects seen in whole animal studies and other data relevant to reproductive toxicity. The discussion of each endpoint concludes with an integrative evaluation of the entirety of evidence on that endpoint.

For ease of comparison, concentrations of SO_2 are generally expressed as parts per billion (ppb) throughout the document. In some cases where concentration was expressed in different units in source documents, both values may be given. Also, statements throughout the document that effects, associations or correlations were significant means that statistical significance, such as $p<0.05$ or a confidence interval (CI) that does not include 1 was reported, unless otherwise indicated. The findings of significant effects of SO_2, as reported by the authors, appear in bold.

B.1. Chemical Structure and Characteristics

SO_2 has the chemical structure given below and a molecular weight of 64.06 g/mol.



The Chemical Abstracts Service registry number for SO_2 is 7446-09-5. Chemical synonyms for the compound include: sulfur oxide, sulfurous anhydride, sulfurous oxide.

At room temperature, SO_2 is a colorless, nonflammable gas with a pungent odor (ATSDR, 1998, 2007). As a gas, it is heavier than air; in liquid form, it is heavier than water.

SO_2 in contact with water readily produces sulfurous acid (Cosmetic Ingredient Review Expert Panel (CIREP), 2003). At a pH of 7.4 and temperature of 37°C (physiological conditions), a mixture of sulfite ions and bisulfate ions will predominate. More acidic conditions liberate SO_2 vapor. With more alkaline conditions, sulfites, bisulfites and metabisulfites are produced.

B.2. Use and Exposure

Exposure to SO_2 in California results from the combustion of sulfur-containing fuel by mobile sources, such as locomotives, ships, and off-road diesel equipment. Exposure can also result from emissions from several industrial processes, such as petroleum refining, the smelting of sulfide ores, the manufacture of hydrosulfites and other sulfur-containing chemicals as well as in the bleaching of wood pulp and paper and food bleaching and processing. Pesticidal and sterilant applications, waste and water treatment, the use of SO_2 in manufacturing, as well as volcanic emissions and other natural sources can also result in SO_2 exposure (IARC, 1997; ATSDR, 1998; WHO, 2006; ATSDR, 2007; CDPR, 2007; U.S. EPA, 2008; CARB, 2009; CDPR, 2009a).

As a component of air pollution, SO_2 is found in combination with sulfuric acid, sulfur trioxide, ozone, nitrogen dioxide (NO_2), and particulates. SO_2 is also an important precursor for the formation of particulate matter (PM) (U.S. EPA, 2006).

SO_2 is one of the six criteria air pollutants identified by the Clean Air Act of 1970 that the U.S. Environmental Protection Agency (U.S. EPA) regulates through the establishment of air quality standards. A new 1-hour SO_2 standard of 75 ppb, based on the 3-year average of the annual 99th percentile of 1-hour daily maximum concentrations, has recently been established (U.S. EPA, 2010). U.S. EPA stated that it specifically replaced the previous annual and 24-hour primary SO_2 standards with the new 1-hour standard "to better protect public health by reducing people's exposure to high short-term (5-minutes to 24 hours) concentrations of SO_2." The standard is based on adverse respiratory effects including bronchoconstriction and increased asthma symptoms.

OEHHA has established an inhalation reference exposure level (REL) of 660 µg/m^3 (250 ppb) for acute exposure, based on impairment of airway function, especially in asthmatics (OEHHA, 1999).

In California, electricity generation from coal is far less than the U.S. average (1% of total electricity generation in California versus 50% in 2005 nationally (DOE, 2008)). Within the transportation sector, diesel-burning commercial marine engines are a growing source of SO_2 (CARB, 2004). According to the National Emission Inventory, SO_2 levels decreased 34 percent from 1990 to 2002 as a result of emission controls on electric generating units (U.S. EPA, 2006). For California in 2010, emissions from the commercial marine sector were expected to contribute 28% of the state's sulfur oxides. Many large ships docked at deep water ports burn "bunker" fuel, which is especially polluting (Thurston et al., 2007). Locomotives and some non-road diesel equipment still burn high-sulfur fuel and emit large quantities of SO_2.

SO_2 is a component of residential wood smoke, which has been a significant contributor to ambient air pollution in parts of California (Lipsett et al., 1997). Back-drafted residential wood smoke can also be a significant contributor to SO_2 indoors. SO_2 is also in wildfire smoke (Viswanathan et al., 2006). For example, in the San Diego area fires

of 2003, 2518 tons of SO_2 were emitted into the atmosphere by the combined Paradise and Cedar fires.

SO_2 has pesticidal uses (CDPR, 2007, 2008, 2009a, b). SO_2 was ranked 82nd out of the top 100 pesticides used statewide in California in 2007 (CDPR, 2007). In 2008, 187,535 pounds of SO_2 were reported as having been used in California (CDPR, 2009a). Specific uses include sanitizing equipment for wine production and storage, and post-harvest commodity fumigation of fruit including wine grapes (CDPR, 2007, 2008, 2009a).

While inhalation is by far the most significant route of exposure (ATSDR, 1998; NA, 2004; ATSDR, 2007), dermal and oral exposures are also possible. Endogenous or added sulfites, which release SO_2, can be consumed in food or drink. Sulfites are also found in some cosmetic and personal care products, raising the potential for dermal as well as aerosol exposures from the use of these products (Cosmetic Ingredient Review Expert Panel (CIREP), 2003).

Sulfites can be produced as a result of fermentation in some foods and beverages, such as beer and wine (Institute of Food Technologists Expert Panel on Food Safety and Nutrition and the Committee on Public Information (IFT), 1976; Grotheer et al., 2005). The U.S. Food and Drug Administration (FDA) has estimated average per capita SO_2 consumption at about 0.2 mg/kg-day, with estimates of up to 2 mg/kg-day for individuals consuming foods and beverages relatively high in SO_2 content (FDA, 1976) (FDA, 1982).

SO_2 was among the sulfiting agents listed as chemical preservatives for food “Generally Recognized as Safe” (GRAS) by (FDA, 1976). Other inorganic sulfites that liberate SO_2 with use were also listed as GRAS: potassium bisulfate, potassium metabisulfite, sodium bisulfate, and sodium metabisulfite. With certain restrictions, these sulfiting agents are approved for use as sanitizing agents, inhibitors of undesirable microbial growth, and to prevent oxidative browning of foods (FDA, 1982) (Grotheer et al., 2005). Residual sulfites in treated foods usually do not exceed several hundred parts per million (ppm), but may approach 1,000 ppm (i.e., 0.1%) in some products (Grotheer et al., 2005). Theoretical SO_2 yields of various sulfiting agents are listed in Table B1.

Table B1. Theoretical Sulfur Dioxide Yield
(Cosmetic Ingredient Review Expert Panel (CIREP), 2003)

Sulfiting agent	Theoretical yield of SO_2 (%)
Sulfur Dioxide	100.00
Sodium Sulfite, Anhydrous	50.82
Sodium Sulfite, Heptahydrate	25.41
Sodium Bisulfite	61.56
Potassium Bisulfite	53.32
Sodium Metabisulfite	67.39
Potassium Metabisulfite	57.60

B.3. Metabolism and Pharmacokinetics

Absorption

Inhalation of gaseous SO_2 is the primary route of exposure (ATSDR, 1998; NA, 2004; ATSDR, 2007). Gaseous SO_2 is rapidly absorbed through mucous membranes of the upper and lower respiratory tract (NA, 2004; WHO, 2006). Absorption of SO_2 usually occurs in the upper part of the lungs (ATS, 1996). It immediately passes through the mucous membranes into the blood where it becomes associated with the alpha-globulin fraction of plasma (Petruzzi et al., 1994).

At low air concentrations, a smaller percentage of the SO_2 may be absorbed than at high concentrations (e.g. 100 ppm) (NA, 2004; WHO, 2006). For example, a rabbit study reported by ATSDR (1998) found absorption of SO_2 at concentrations >100 ppm to be >90%, but about 40% for concentrations <1 ppm.

Data pertaining to absorption of SO_2 by the oral and dermal routes of exposure appear to be lacking (NA, 2004).

Distribution, Metabolism and Excretion

In the moist environment of the lungs, SO_2 is hydrolyzed to produce a mixture of sulfite, bisulfate, and hydrogen ions (ATSDR, 1998; WHO, 2006). Distribution throughout the body is rapid (ATSDR, 1998; NA, 2004).

An estimated 12–15% of inhaled and absorbed SO_2 is desorbed from mucous membranes and exhaled (ATSDR, 1998). Absorbed bisulfate is converted to sulfate by molybdenum-dependent sulfite oxidase (WHO, 2006). Circulating sulfite is metabolized primarily in the liver by sulfite oxidase, or sulfites can react with plasma proteins to form S-sulfonate (ATSDR, 1998; NA, 2004).

Further metabolism and eventual elimination of S-sulfonates is not well understood (WHO, 1974; ATSDR, 1998). The metabolites of sulfite oxidase reactions, sulfate esters and sulfate, are eliminated through the urine (ATSDR, 1998).

B.4. Non-DART Toxicities

B.4.1. Human Studies

Inhalation exposure

SO_2 exposures in the range of 100–400 ppm in air pose an immediate danger of death (ATSDR, 1998; NA, 2004). Lower concentrations (<40 ppm), however, have been associated with death in specific incidents (ATSDR, 1998). Increased morbidity and mortality have been associated with chronic SO_2 exposure, usually in the context of generally high levels of air pollution (NA, 2004; WHO, 2006). Changes in lung function were observed in some workers exposed to 0.4–3.0 ppm SO_2 for 20 years or more (ATSDR, 1998).

As noted above, SO_2 is highly soluble and is a reactive gas that quickly dissolves in the aqueous lining of the respiratory epithelium to form acidic species such as the severe irritant sulfurous acid (H_2SO_3) that react with and injure epithelial cells (Frampton et al., 2007). Other compounds formed affect the smooth muscles and nerves involved in bronchoconstriction (NA, 2004). Inhalation of 4–6 ppm SO_2 for 10 minutes by seven healthy adults decreased airway conductance (mean -39%, $p<0.001$) (Nadel et al., 1964). The onset of changes in airway conductance varied from 10 seconds to 4 minutes; usually airway conductance decreased maximally within the first minute (Nadel et al., 1964). Lung function changes such as these have been examined experimentally in human subjects exposed to SO_2 by oro-nasal, nose-only, or mouth breathing (ATSDR, 1998).

Asthmatics, particularly during exercise, are a sensitive population, showing respiratory effects at concentrations as low as 0.25 ppm (ATSDR, 1998; WHO, 2006; U.S. EPA, 2008). Exercise can enhance sensitivity even in non-asthmatic individuals. Asthmatics doing strenuous physical activity can show significant respiratory changes and asthmatic attacks with exposure to SO_2 at concentrations as low as 0.1 ppm for 10 minutes or longer (NA, 2004). Children may be more sensitive than adults to the same SO_2 concentrations due to relative greater lung surface area and higher minute volumes (ATSDR, 2007; U.S. EPA, 2008).

In addition to lung effects, cardiovascular, gastrointestinal, neurological, and hematological effects have been observed in humans exposed to SO_2 by inhalation (ATSDR, 1998; NA, 2004). For example, human, non-asthmatic subjects exposed to 1–8 ppm SO_2 showed increased pulse rate (ATSDR, 1998). One workplace study found

hematological effects such as polymorphonuclear leukocytes and lymphocytes with SO_2 exposure, while another found increased blood methemoglobin.

Workers in a sulfuric acid factory were found to show increased frequencies of micronuclei in their lymphocytes, as well as evidence of clastogenesis and inhibited DNA synthesis (ATSDR, 1998; NA, 2004). Increased chromosome aberrations and sister chromatid exchanges were reported for lymphocytes from workers in a fertilizer plant exposed to an average of 15.92 ppm SO_2 (ATSDR, 1998). Chromosomal aberrations were also reported for workers in a sulfite pulp factory (ATSDR, 1998).

Both ATSDR (1998) and U.S. EPA (2008) concluded that there was no definitive evidence for increased cancer potential with SO_2 exposure of humans. The International Agency for Research on Cancer (IARC) concluded: "SO_2, sulfites, bisulfites and metabisulfites *are not classifiable as to their carcinogenicity to humans*." On the other hand, IARC concluded that “Occupational exposure to strong-inorganic-acid mists containing sulfuric acid is carcinogenic to humans.” (IARC, 1997).

Dermal and ocular exposure

SO_2 is handled and transported in a compressed, liquefied form (ATSDR, 1998, 2007). Exposure to liquid SO_2 can produce severe injuries to the eyes and frostbite-like damage to skin.

The sulfurous acid (H_2SO_3) formed when SO_2 reacts with water is a severe irritant to the eyes, skin, and mucous membranes, and may be formed *in situ* on moist tissues (ATSDR, 1998; NA, 2004; NIOSH, 2005). Chemical burns are considered the primary cause of corneal burns and opacification, resulting in loss of vision.

Oral exposure

Endogenous or added sulfites, which release SO_2, can be consumed in food or drink. Approximately 1% of the population is sensitive to sulfites and can have allergic reactions ranging from mild to quite severe anaphylaxis (ATSDR, 1998; Grotheer et al., 2005). It is not clear if, or to what extent, effects of orally consumed sulfite might be due to inhalation of off-gassed SO_2 (ATSDR, 1998).

B.4.2. Animal Studies

Inhalation exposure

According to ATSDR (1998) and U.S. EPA (2008) animal studies support the findings of human studies, particularly on the pulmonary effects of inhaled SO_2. For short-term exposure to SO_2, concentrations associated with effects observed in various studies for different species include (ATSDR, 1998):

- **0.9–1 ppm,** 24 hrs, rats – oxidative effects on erythrocytes
- **2.6 ppm,** 1–3 hrs, guinea pigs – respiratory effects
- **20 ppm,** 120 mins, mice – degenerative changes in respiratory epithelium
- **1.1–141 ppm,** 20–40 mins, dogs – respiratory effects
- **200–300 ppm,** 10–20 mins, rabbits – respiratory effects
- **230, 800 ppm,** 5 and 8 hrs, respectively, rats – increased polymorphonuclear lymphocytes in the trachea, loss of cilia and cell necrosis in the trachea and main bronchus
- **2,350 ppm** (176 mins)**, 50,000 ppm** (<10 mins)**, 500,000 ppm** (< 2 mins) – rats, seizures and prostration prior to death

Adverse effects of repeated or chronic SO_2 exposure have been documented in animal studies for multiple organ systems, including: brain, lung, heart, liver, stomach, intestine, spleen, kidney, and testis (NA, 2004). Increased sensitivity (asthma reaction) to low levels of SO_2 has been documented in guinea pigs. Effects reported following repeated exposures for different species at various concentrations of SO_2 included (ATSDR, 1998):

- **0.1 ppm**, 5 hrs/d for 5 days, guinea pigs – increased sensitivity (asthma reaction).
- **5 ppm,** 6 hrs/d for 5 days, guinea pigs – severe pulmonary effects
- **10 ppm,** 1 hr/d, 30 days, guinea pigs – nasopharyngitis and increased lipid peroxidation of lung tissue
- **10 ppm,** 1 hr/d for 45 days or 8 wks, rats – increased ratios of methemoglobin and sulfhemoglobin, lipid peroxidation, increased fragility of erythrocytes, altered antioxidant enzyme activities
- **25 ppm,** up to 5 days, rats – nasal epithelial metaplasia and basal cell hyperplasia
- **30–40 ppm,** 1 hr/d, 5 d/wk for 12 wks, rats – biochemical and cellular alterations in bronchial tissues
- **70–300 ppm,** 6 wks, rabbits – respiratory effects, rhinitis, bronchopneumonia
- **400 ppm,** 3 hrs/d, 5 d/wk, for 3 wks or up to 42 days, rats – adverse airway effects
- **650 ppm,** 4 hrs/d , 5 d/wk, 19–74 days, hamsters – mild bronchitic lesions

SO_2 increases lipid peroxidation of cell membranes and interferes with antioxidative processes by decreasing the levels of superoxide dismutase, catalase and glutathione peroxidase (Meng et al., 2003; NA, 2004). U.S. EPA (2008) interpreted the animal data as indicating high concentrations of SO_2 "may cause DNA damage."

There is one study investigating the potential carcinogenicity of inhaled SO_2 in mice. The incidence of benign and malignant lung tumors was significantly increased in females. Based on this study, IARC (1992) concluded that "There is *limited evidence*" for the carcinogenicity in experimental animals of SO_2.

SO_2 has also been implicated as a co-carcinogen by inhalation in rats and mice (NA, 2004), although the studies are of limited design or poorly reported (IARC, 1992). U.S. EPA (2008) was not convinced that SO_2 had been shown to induce carcinogenesis, co-carcinogenesis, or tumor promotion.

Dermal and ocular exposure

Ocular irritation has been demonstrated in rabbits after 4 hrs of exposure to SO_2 at a concentration of 6 ppm in air, with permanent damage occurring only at higher, near-fatal concentrations (NA, 2004). Direct application of pure SO_2 gas for five seconds to the eyes of rabbits produced severe and lasting damage to the cornea and conjunctiva (ATSDR, 1998).

Oral exposure

According to the FDA's "Database of Select Committee on GRAS Substances (SCOGS) Reviews," the no observed adverse effect level (NOAEL) for chronic oral SO_2 exposure of laboratory animals is in the range of 30–100 mg/kg-d (Select Committee on GRAS Substances Reviews (SCOGS), 1976, last updated 5/4/09).

B.5. Issues Specific to Epidemiologic Studies

As part of its review of the evidence on the reproductive toxicity of SO_2, this document reviews air pollutant studies. In addition to the usual factors that need to be addressed in epidemiologic research, the area of air pollution presents its own specific issues and challenges. Issues particularly pertinent to evaluating the epidemiologic research presented in this document are highlighted below.

Exposure Assessment

Measurements from ambient air pollution monitors, usually for regulatory purposes, have long been used as a surrogate for individual exposures in epidemiologic studies. Many studies in this review used this approach. The advantage is that the available data cover a large spatial area and time frame. The disadvantage is that, due to the

spatial and temporal variations, the use of air pollution monitoring data in epidemiologic studies results in exposure misclassification.

The assumption inherent in the use of these data is that all individuals within the community will experience the same exposure. This assumption, however, disregards the heterogeneity in individual exposures from daily activity patterns such as movement to work, differences in indoor and outdoor concentrations, and from spatial heterogeneity in pollutant concentrations (Samet et al., 2007). Therefore, exposure measurement error in these studies includes:

- The difference between true and measured ambient concentrations.
- The difference between average personal exposure to ambient pollutants and ambient concentrations at central monitoring sites.
- The use of average population exposure rather than individual exposure estimates (U.S. EPA, 2008).

Exposure misclassification due to the use of ambient monitors as surrogates for individual exposures is likely to underestimate, rather than overestimate, the effect of air pollution on birth outcomes (Zeger et al., 2000). In general, if the misclassification affects the subjects equally then the bias will reduce the effect estimates towards the null; that is, toward not finding an effect. As the degree of random misclassification increases in a study, the statistical capability to detect an effect of exposure declines sharply (Samet et al., 2007). Various methods can reduce the degree of misclassification, such as including a factor for the distance from the air monitor to the mother's residence, or employing methods such as kriging, a spatial interpolation technique. Some studies reviewed in this document have used methods to reduce misclassification error.

Other factors in this paradigm that could contribute to measurement error include: distance from the monitor; timing of sampling; number of measures; and the sensitivity of the monitor. The analytical (chemical/physical) measurement errors are generally considered small provided that the concentration levels are well above the detection limits (Ito et al., 2007). Greater relative error is most often observed at the lower ambient concentrations compared to the less frequent higher concentration exposures from local point sources. Measurements from regulatory ambient monitoring systems are considered inadequate for accurate and precise measurement at or near the current mean 24-h average ambient concentration of 4 ppb (U.S. EPA, 2008). Since typical ambient SO_2 concentrations in the contiguous U.S. are at or below the detection limit of the monitors used in the regulatory network, there is likely considerable measurement error.

Measurement error can be greater when meteorological factors are not considered in study design. Meteorological factors are particularly important when monitoring data is used to assess a wide area. Wind (direction and velocity), precipitation, and varying geographical elevations (mountains, valleys) can affect the dispersal of SO_2 in the air. Unimpeded wind and precipitation may reduce SO_2 concentration in the air (Cheng,

2001). Mountains and valleys can act as natural barriers that prevent dissipation of air pollutants.

In time series air pollution data, exposure errors may also be associated with temporal fluctuations in pollutant concentrations between locations within an area. This would tend to bias results toward the null. Differences in absolute concentrations across an area could lead to bias either toward or away from null (Ito et al., 2007).

Since SO_2 is primarily a pollutant with few indoor sources in most countries, the presence of SO_2 indoors is dependent upon the penetration rate of outdoor air into the indoors (Thurston et al., 2007). However, some studies presented in this document have important indoor sources of SO_2, such as coal burning stoves (Xu et al., 1995; Wang et al., 1997). In this case, personal exposures to SO_2 may be more accurately assessed, as illustrated by (Xu et al., 1993).

Exposure to SO_2 may be characterized using different measures such as: average exposure, peak exposure, or an integrated measure of exposure. The time periods over which these measures are taken can be long or short. These distinctions are important because health effects caused by long-term exposure to low average levels may differ from those caused by large peak exposures over a short period. However, it is not currently known which exposure may be most important to a specific health outcome.

Many of the studies presented in this document divided the study population into exposure quantiles (such as quartiles and quintiles), with those in the lowest quantile serving as the reference group. No studies identified a truly unexposed control group. If the lowest exposure quantile does carry some risk, then demonstrating added risk to the highest quantile by comparison to this group may be difficult, particularly if the levels in these quantiles are not substantially different.

Window of exposure

A major issue in studying the environmental exposures and reproductive outcomes is identifying the exposure period of any susceptibility or enhanced susceptibility (Bobak, 2000). For preterm birth, exposures proximate to the birth may be most relevant if exposure causes an acute effect. However, exposures in early gestation might affect the formation and growth of the placenta, with results observable later in pregnancy, or cumulative exposure during pregnancy might actually be the most important determinant.

The studies presented in this document examined many different time periods during pregnancy including the first or last month, each trimester, the whole pregnancy, or several days before birth. Exposure windows were often selected by study authors based on the outcome of interest.

Study Design

As discussed above, exposure measurements were almost always based on ambient pollutant concentrations, usually from air quality monitors. No study measured exposures of individuals; all exposures were assessed on a group or geographic basis. Moreover, many studies were designed to evaluate the effects of air pollution – not only SO_2 – and therefore examined numerous pollutants.

However, there were important differences among these exposure assessments. For example, in some studies very few air quality monitors provided data for a wide area, while in other studies, measurements from many monitors were available. Studies used various methods to improve exposure assessment such as kriging, or limiting the study population to those living close to monitors. Moreover, most studies also assessed pollutant exposures at periods relevant to outcomes of interest, e.g. during the gestation period for each individual birth, or during the time of spermatogenesis. Some studies sought to identify more specific windows of vulnerability within the gestational period as mentioned above.

Although the studies described in this document assessed exposures on a group basis, most studies had individual-level outcomes, typically from birth certificates, and analytic study designs characteristic of cohort or case-control studies. Therefore, the studies are classified according to their overall study designs.

Possible associations of SO_2 and health outcomes

The many ways SO_2 may be causally related or associated with adverse reproductive outcomes are discussed below and illustrated in Figure B1.

SO_2 may be associated with reproductive outcomes in the following ways:

- As a direct effect;
    - Exposure to SO_2 itself causes the effect.
- As a surrogate;
    - It may be an indicator for other air pollutants coming from the same source. The other air pollutants may cause the effect but since SO_2 is highly correlated with them, increases in SO_2 will appear to be associated with increased risk.
- As a mediated effect;
    - SO_2 may cause the increase of another pollutant that causes the effect.
- As an interaction;
    - Occurs when the joint effect of two exposures differs from that expected on the basis of the independent effects of either factor. In the example illustrated below, the observed joint effect of SO_2 and PM is greater than that expected on the basis of summing the independent effects of SO_2 and

PM. Although in Figure B1 below, the joint effects are synergistic (augmenting the effect of one another), this interaction can also be antagonistic (reducing the effect of one another). This form of interaction refers to a biologic mechanism of interaction, not to a statistical interaction.

- As a confounding effect;
    - "In confounding, the apparent effect of the exposure of interest is distorted because the effect of an extraneous factor is mistaken for, or mixed with, the actual exposure effect (which may be null)" (Rothman et al., 1998). In more general terms, *a factor must be associated with both the exposure and the disease to be a confounder* (Rothman, 1986).
    - Although confounding is always a possibility in epidemiologic studies, most covariates would not be correlated with daily air pollutant levels, making confounding by these factors unlikely (Schwartz et al., 1995). For example, gestational age is difficult to measure accurately. However, the error in gestational age is probably not associated with air pollution. Therefore, the misclassification of gestational age is likely to be non-differential or random with respect to exposure and would most likely result in an underestimation of the true effects of air pollution on pregnancy outcome. Co-pollutants were controlled for in some of the studies. Even though it is not possible to completely rule out an air pollutant acting as a surrogate for some other components of air pollution, or a synergistic effect between two air pollutants, individual air pollutants are nonetheless identified by agencies as hazards for various health outcomes.

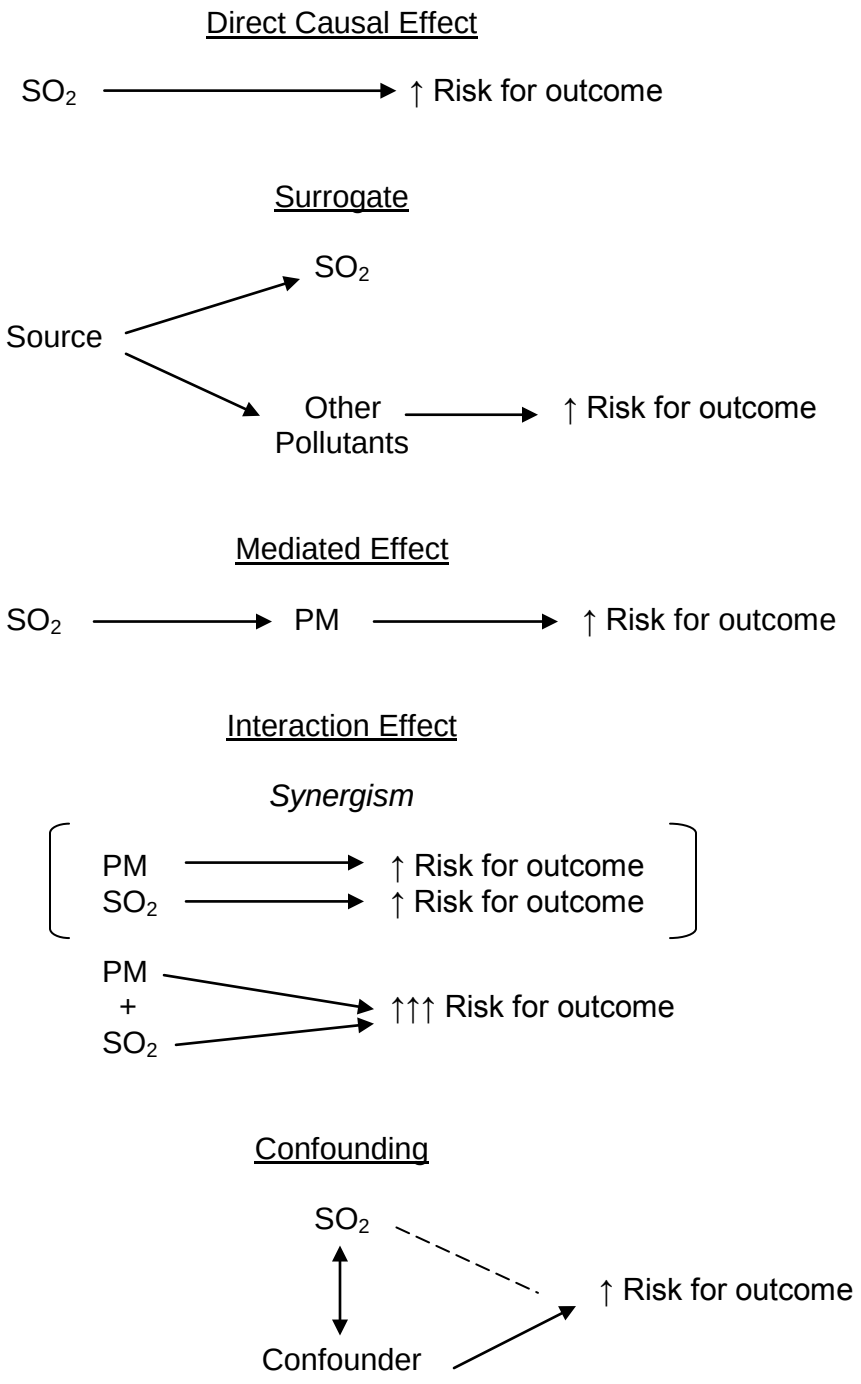


Figure B1. Potential relationships of SO_2 with adverse health effects (adapted from (U.S. EPA, 2008)).

In epidemiologic studies, multivariate models are used to address confounding. However, such models are not always easily interpreted when assessing effects of co-varying pollutants such as PM, ozone (O_3), and NO_2 and can violate the assumptions

inherent in the mathematical models. (Such as the assumption of little or no multicollinearity in multiple linear regression models, in this case meaning that two or more air pollutants are not highly correlated.)

Little attention has been given to possible synergistic effects of air pollutants. Research in this area is just now beginning to address this issue. No studies of reproductive outcomes were found that examined synergy, although SO_2 has been reported to act synergistically with NO_2 in an asthma study in adults (Rusznak et al., 1996). Rusznak et al. (1996) studied mild atopic asthmatic subjects in relation to allergen responsiveness. Exposure to NO_2 plus SO_2 decreased the dose of allergen required to produce a 20% fall in forced expiratory volume in 1 second at all of the post-exposure time points. The findings suggest that environmental peaks of SO_2 exposure, in combination with NO_2, may enhance allergen responsiveness in asthmatics (Frampton et al., 2007).

Studies have identified individuals, such as asthmatics, who are more sensitive to SO_2 levels than are healthy individuals relative to respiratory function. However, Amdur et al. noted that even in healthy individuals some subjects became tolerant of even the highest concentrations of SO_2 (8 ppm), whereas others could only tolerate the lowest levels (1–2 ppm) (Amdur et al., 1953). Unlike for well-studied respiratory endpoints, no studies have identified individuals more sensitive to SO_2 in relation to reproductive outcomes. In epidemiologic studies, if there is a sensitive subpopulation that is not accounted for in the analyses the result would likely be a bias towards the null.

The shape of the exposure – response curve may also be an important issue for SO_2 especially since, as mentioned above, less SO_2 is absorbed at low concentrations than at high concentrations (e.g. 100 ppm).

B.6. Standardization of SO_2 Exposure in Epidemiology Studies

For all human studies, SO_2 concentrations reported in micrograms per cubic meter ($\mu g/m^3$) were converted to ppb using the following formula:

ppb = 24.45 x concentration ($\mu g/m^3$) ÷ molecular weight

(molecular weight of SO_2 = 64.1g/mol)

SO_2 concentrations reported in ppm were multiplied by 1,000 to obtain ppb.

C. Male Reproductive Toxicology Studies

This section describes the data available for judging relationships between SO_2 exposure and male reproductive outcomes. It includes an air pollution epidemiologic study of SO_2 impacts on fecundability, three studies of sperm morphology and chromatin integrity, and three occupational studies linking SO_2 to DNA damage in lymphocytes of workers. The section then presents four studies in animals of SO_2 exposure and changes in ultrastructural morphology in the testes, concentration-dependent oxidative damage in the testes and DNA damage in sperm.

The mechanism by which SO_2 exposure may exert effects on reproductive outcomes such as decreased fecundability or fertility is illustrated below in Figure C1. Studies reporting effects of SO_2 exposure on DNA damage, and other studies showing effects of DNA damage in sperm cells on fecundability or fertility, may explain observed effects of SO_2 exposure on decreased fecundability or fertility. This will be discussed further in the integrative evaluation (section C.3.).

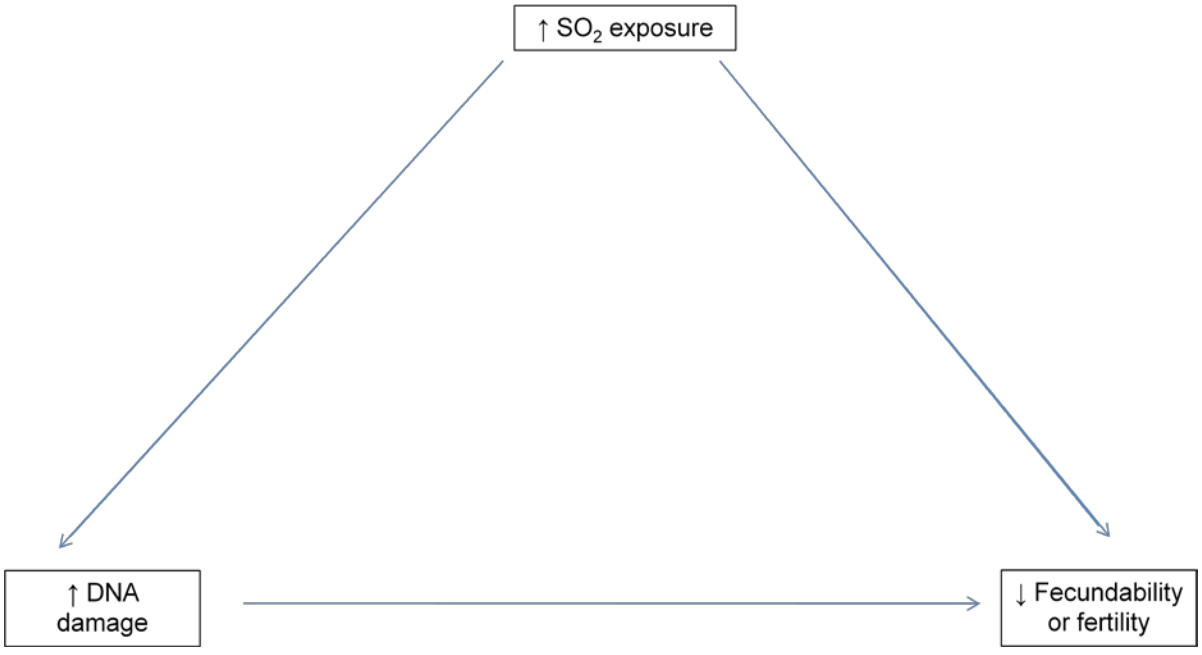


Figure C1. The diagram illustrates the possible pathways through which exposure to SO_2, may affect male fecundability or fertility.

All of the human and animal studies of the effects of SO_2 are described in more detail in the Appendices.

C.1. Human Male Reproductive Studies

The primary human studies on male reproductive toxicity and SO_2 exposure were conducted in the Teplice region of the Czech Republic as part of a large scientific effort. As a result of community concern about the potential health effects of air pollution, the Ministry of Environment of the Czech Republic, in collaboration with the U.S. EPA, designed the Teplice Program (1991–1999) to study environmental health problems in the Northern Bohemian basin area of the Czech Republic (Sram, 2001). This region is bordered by the Ore Mountains to the north and Central Bohemian Highlands to the south, creating a basin in which air pollution emissions are trapped during meteorological conditions that result in inversions leading to high concentrations of air pollutants, particularly in the winter.

At the time of the Teplice Program, half of the SO_2 and nitrogen oxides emitted in the Czech Republic originated from the mining districts of Northern Bohemia. This region also contributed about one-quarter of the total air particulate matter emissions in the country. Teplice, one of the mining districts in Northern Bohemia, had a high population density of 285 per km^2 (Sram et al., 1996). Prachatice is a region in the southern part of the country that had some of the cleanest air in the country and thus was chosen as a comparison district. It has no mining aside from several stone quarries, and had a low population density (39.6 per km^2).

The concentrations of all measured pollutants were significantly higher in the winter than in the spring and summer in both districts, with intermittent peaks due to thermal inversions. During these peaks, values occasionally exceeded the U.S. and Czech 24 hr air quality standards for SO_2 and particulate matter less than 10 μm in size (PM_{10}). The Teplice district had more severe pollution episodes than those in Prachatice. "While the fine particle mass, trace elements and polycyclic aromatic hydrocarbons (PAH) in Teplice were on average 2–3 fold higher than those in Prachatice, the average SO_2 concentrations were generally at least 5-fold higher in Teplice than in Prachatice" (Figure C2) (Sram et al., 1996). Seasonal differences in air pollutant concentration, even within the same district, were greater than the differences between districts.

The monitoring data from the early 1990s motivated the Czech government to take actions to ameliorate the air pollution. As a consequence, pollution levels significantly decreased between 1993 and 1994 and thereafter (Selevan et al., 2000). The concentrations of SO_2 were substantially decreased and by 1999 "they were ~20% of those seen in the winter of 1989. Surprisingly, the concentrations of PM_{10} did not substantially change in the period 1994–1998." (Sram et al., 1999).

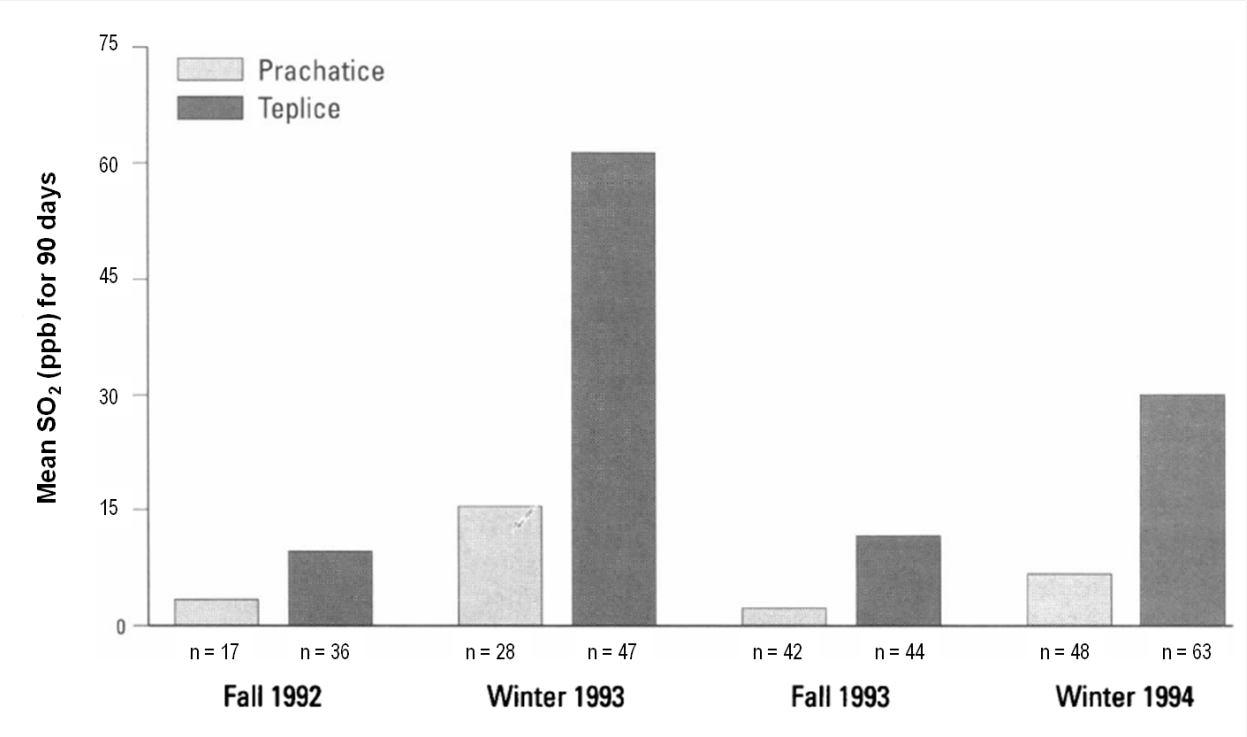


Figure C2. (Sram et al., 1996): Mean SO_2 levels (units converted by OEHHA from µg/m^3 to ppb) in Teplice and Prachatice calculated for the 90-day interval preceding early fall or late winter semen sampling period. The number of men sampled in each survey is shown for the appropriate date and place of sampling.

The primary studies reviewed in this section include Robbins et al. (1999), Dejmek et al. (2000), Selevan et al. (2000), Rubes et al. (2005), Rubes et al. (2007). Figure C3 shows how the studies are related. Additional data from these studies, as presented by Sram et al. (1996 and 2001), have also been included in this section.

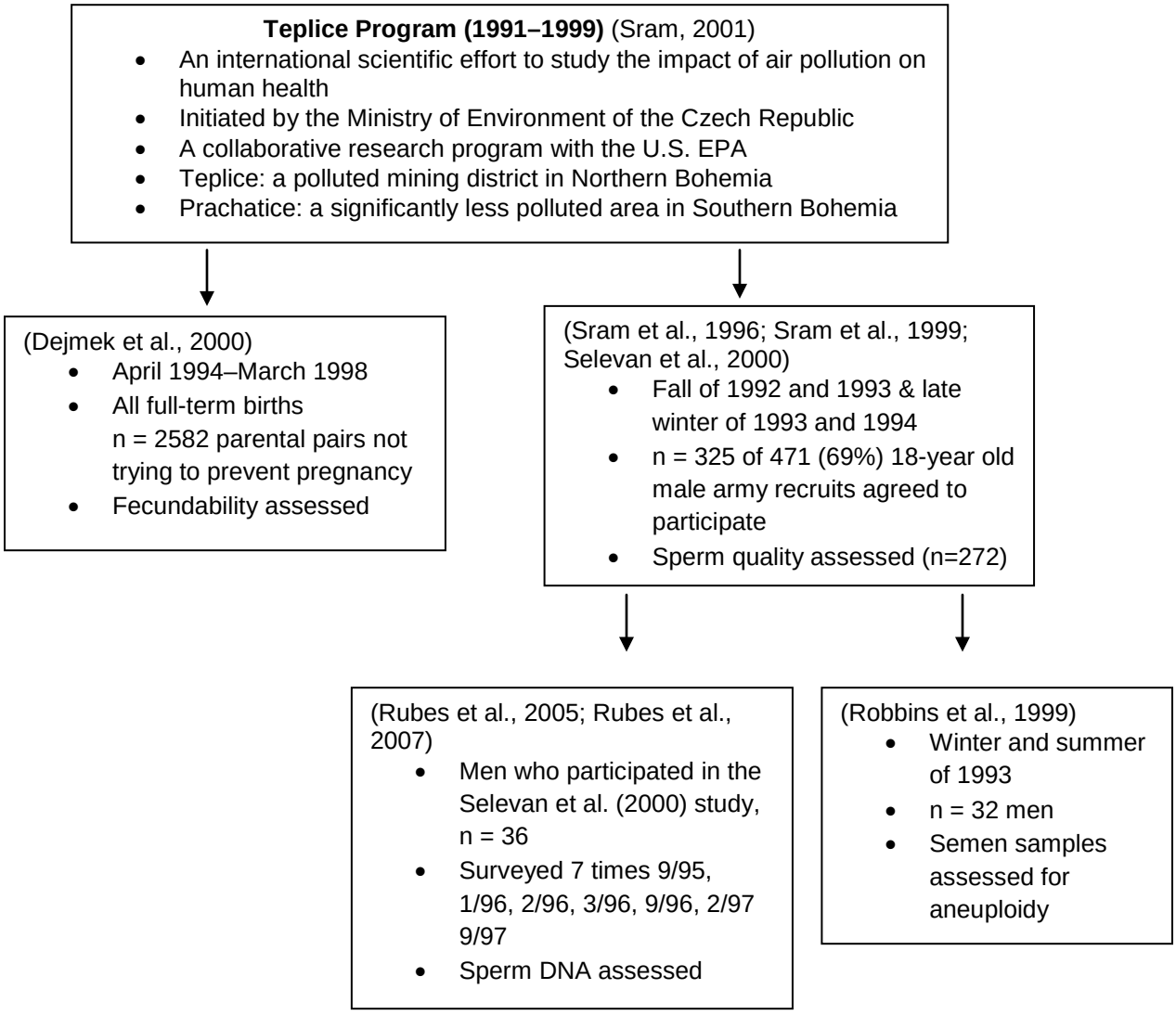


Figure C3. Teplice Program study subjects and published studies that assessed these samples.

Fecundability

The retrospective cohort study by Dejmek et al. examined the association between a measure of fecundability and exposure to ambient SO_2 between April 1994 and March 1998 (Dejmek et al., 2000). This study used conception in the FUMC as a measure of fecundability, and found that SO_2 exposure above the first tertile 30–60 days before FUMC was associated with reduced odds of conceiving. SO_2 was highly correlated with other pollutants, but exposures to those other pollutants were not found to be associated with fecundability.

Semen Quality

Three studies examined the association between exposure to air pollution and semen quality (Selevan et al., 2000; Rubes et al., 2005), with additional information published in Rubes et al. (2007). The original sample population for the studies included 18-year old men who were being examined and interviewed to determine fitness for military service. The cross-sectional study by Selevan et al. (2000) analyzed semen quality in samples from 272 men (154 from Teplice and 118 from Prachatice) collected between 1993 and 1994. This study found decrements in sperm chromatin integrity, reductions in the proportion of sperm with normal morphology, and mixed effects on other sperm parameters, including sperm motility, progression, and swimming patterns.

Subsequent to the first evaluation of semen quality, a longitudinal study by Rubes et al. (2005) evaluated a small group of men over time. Only men from the study by Selevan et al. (2000) were contacted; thus, of the eligible men who participated, each served as his own control (n = 36). Rubes et al. (2005, 2007) found high air pollution was associated with poor sperm chromatin integrity.

A third study (Robbins et al., 1999), examined sperm aneuploidy in a subset of samples (n=32) collected among non-smoking men in the original sample of Selevan et al. (2000). This study found an association between YY8 disomy and exposure to high air pollution levels.

Thus, all three studies reported associations between air pollution and semen quality.

Table C1 summarizes the epidemiologic studies of SO_2 as a risk factor for male reproductive toxicity. This table provides some detail about the study methods, including exposure assessment, study population, consideration of co-pollutants and other covariates, statistical analyses, and study strengths and limitations. Results include measures of association and 95% CI when available. The findings of significant effects of SO_2, as reported by the authors, appear in bold. More detailed summaries of the human studies are available in Appendix 1.

Related Male Reproductive Studies

Three occupational studies investigated the frequency of chromosomal aberrations in lymphocytes in workers exposed to SO_2 (Nordenson et al., 1980; Meng et al., 1990; Yadav et al., 1996). Each study reported significantly increased frequencies of chromosomal damage in exposed workers compared to controls. In Nordenson et al. (1980), workers at a sulfite pulp factory in Sweden exposed to SO_2 had significantly increased frequency of chromosomal aberrations in lymphocytes. In a study conducted in China, Meng et al. (1990) observed similar findings of significantly increased frequencies of chromosomal-type and chromatid-type aberrations in lymphocytes of workers in a sulfuric acid factory (SO_2 concentrations ranged from 100 ppb to 4,600 ppb). Additionally, Yadav et al. reported that workers in a fertilizer plant in India had

significantly higher frequencies of chromosomal aberrations and sister-chromatid exchanges (average SO_2 concentration = 15,900 ppb) (Yadav et al., 1996). All associations remained significant when smoking status was considered (similarly when alcohol consumption was considered). These studies therefore concluded that SO_2 is clastogenic and genotoxic in humans.

Additional study

In addition to these studies, a study by Beckman and Nordstrom examined adverse birth outcomes among families of men who worked at a smelter (Beckman et al., 1982). They found significant increases in the rates of spontaneous abortions among men who had greater exposure to the smelting process and worked at the smelter before the pregnancy. SO_2 exposures were not measured. As noted by the authors, the results suggest the increased rate of fetal deaths is caused by germ cell damage through the occupational exposure of the husbands.

Table C1. Highlights of male reproductive outcome studies (significant SO_2 findings in bold).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Dejmek et al,, 2000 Teplice district, Czech Republic April 1994–March 1998	2,585 parental pairs from Teplice region; 587 (22.7%) conceived in the first unprotected menstrual cycle (FUMC), resulting in live birth. Retrospective cohort Logistic regression	Daily ambient air pollution concentrations from a single monitoring station in the center of the town of Teplice. Mean 30-day averages of SO_2 levels in each of 4 months before the estimated date of conception, grouped into 3 levels.	Annual means: 1994-95: 20.7 1995-96: 19.3 1996-97: 18.7 1997-98: 14.5 Categories based roughly on tertiles in the first 2 years: Low <15.3 Medium 15.3–30.5 High >30.5	Co-pollutants : - NO_x - PM - PM_{10} (r=0.83) - PAHs Not included in final analyses due to high correlations (correlations other than for PM_{10} were not reported) Models adjusted for: - maternal age - parity - year and season - marital status - temperature (avg & max) - periods of extreme pollution; public was advised on reducing exposure - epidemic respiratory infections in prior month - 10-year patterns in conceptions	SO_2 exposure in the second month (30-60 days) before conception was associated with reduced fecundability in the FUMC. AORs (95% CI) for conception in FUMC, by month before conception Medium SO_2 exposure: Month prior to conception AOR (95% CI) 4 1.32 (0.90, 1.91) 3 0.95 (0.63, 1.48) **2 0.57 (0.37, 0.88)** 1 1.01 (0.68, 1.51) High SO_2 exposure: Month prior to conception AOR (95% CI) 4 0.93 (0.57, 1.51) 3 0.90 (0.55, 1.48) **2 0.49 (0.29, 0.81)** 1 0.96 (0.58, 1.58) By distance from monitor: Medium SO_2 exposure: < 3.5 km: 0.56 (0.31, 1.00) p=0.05) >3.5 km: 0.58 (0.31, 1.08) High SO_2 exposure: **< 3.5 km: 0.36 (0.17, 0.73)** >3.5 km: 0.70 (0.34, 1.45)	Authors note that in previous studies of fecundability, SO_2, NO_x, PM_{10}, $PM_{2.5}$, PAHs were highly correlated, with coefficients ranging from 0.55-0.83. However, in single pollutant models in this study, *only* SO_2 was consistently associated with fecundability. High correlation between SO_2 and PM_{10} explained a weaker association between fecundability and PM_{10}. Including both SO_2 and PM_{10} in one model yielded a stronger SO_2 effect and eliminated the association for PM_{10}. The authors point out the consistency of their findings with data concerning the most probable stage of sperm maturation damage associated with SO_2 exposure (unpublished).

Table C1. Highlights of male reproductive outcome studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Rubes et al., 2005 Rubes et al., 2007 Teplice District, Czech Republic 1995–1997	36 men, age 19–21 years, completed a questionnaire and provided semen samples up to seven times over 2 years. Prospective, longitudinal Regression for mixed models to allow for repeated measures and participants with fewer than seven semen samples.	Pollution monitoring system is described in Pinto et al., 1998. Data on air pollutants were collected for 24-hour periods daily (except that PM_{10} was measured once or twice weekly during summer). For statistical analysis, air pollution was categorized simply as high (winter) or low (summer).	(Reported as a graph, without exact numbers) Winter means: ~29 to > 34. Summer means: ~6 to 11 ppb. Winter was the high exposure period due to the use of coal for heating and frequent temperature inversions.	Measured but not analyzed separately: PM_{10} and constituent PAH, NO_x Factors considered: - sexual abstinence interval - high fever in the last three months - wearing briefs vs. boxers - alcohol use - cigarette smoking - caffeine consumption - working with solvents or metals	Semen volume, sperm concentration, total sperm count, % motile sperm, straight line velocity, curvilinear velocity, linearity, % normal morphology, % normal head morphology, total aneuploidy were measured. None of them was significantly associated with pollution. **Poor sperm chromatin integrity, as indicated by increased percentage of DNA fragmentation index (%DFI) through Sperm Chromatin Structure Analysis (SCSA), was associated with increased SO_2 when adjusted for smoking and wearing briefs: β=0.19, CI (0.02, 0.36); p<0.05. The mean baseline SCSA-%DFI was 12-15%, and increased to 15-20% with higher pollution exposure.**	Blood levels of lead, cadmium, and mercury were measured and did not differ significantly from the reference sample to mid-winter samples. Although SO_2, PM_{10}, and NO_x were measured separately, exposure was modeled simply as high or low exposure, based on season.

Table C1. Highlights of male reproductive outcome studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Selevan et al., 2000 Teplice and Prachatice Districts, Czech Republic 1993–1994	Authors collected questionnaire data and semen samples from, and conducted physical examinations on 18-year old men in early fall or late winter. Semen samples were analyzed for semen volume, sperm concentration and counts, motility, morphology, and chromatin integrity n=272 Cross-sectional Multivariate regression	Pollution monitoring system is described in Pinto et al., 1998. Data on air pollutants were collected for 24-hour periods daily (except that PM_{10} was measured once or twice weekly during summer). For statistical analysis, exposure to air pollution as a whole was categorized as: - High (winter 1993 in Teplice) - Medium (winter 1994 in Teplice) - Low (summer in Teplice and winter and summer in Prachatice).	<u>High</u> Mean (SD) 62.6 (61.4) Median 40.8 Range 5.5–266.2 <u>Medium</u> Mean (SD) 30.4 (15.2) Median 31.0 Range 4.3–88.0 <u>Low</u> (values shown are for summer in Teplice only) Mean (SD) 11.7 (5.7) Median 9.6 Range 4.0–26.7	Measured but not analyzed separately (only correlations with PM_{10} were reported): PM_{10} (r=0.81) TSP NO_x CO Factors considered: - sexual abstinence interval - high fever in the last three months - wearing briefs vs. loose-fitting underwear - alcohol consumption - cigarette smoking - caffeine consumption - hobby or work with solvents or metals	Semen volume, sperm conc., and total sperm counts were not significantly associated with pollution levels. **Medium air pollution was significantly associated with decreased % motile sperm, lower numbers of motile and progressive sperm.** The associations between pollution and measures of sperm motion were mixed: - **Average point-to-point velocity was significantly lower after medium pollution exposure and significantly higher with high pollution exposure** - **Swimming pattern linearity was significantly higher after medium pollution exposure and significantly lower after high exposure** - **% with normal morphology and morphologically normal heads were lower in the medium and high air pollution groups** - **% of sperm with poor chromatin integrity was higher after high exposures ($p<0.05$)**	The specific components of the air pollution that may account for observed adverse outcomes were not identified. This study used only one semen sample from each participant. Therefore, adverse semen characteristics may have preceded exposures. The authors acknowledge that if the important exposures are peak levels, error may have been introduced given the timing of these peaks within the 90-day periods. Season is associated with some measures of sperm motility and morphology, but season is also causally associated with use of coal for residential heat and temperature inversions; therefore, adjusting for season may not be appropriate.

Table C1. Highlights of male reproductive outcome studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Robbins et al., 1999 Teplice District, Czech Republic 1993	18-year old healthy nonsmokers n=32 Questionnaire data and semen samples were collected from the subjects. Semen samples were analyzed using fluorescence in situ hybridization (FISH) to detect damage at the chromosome level. Cross-sectional Kendall's tau, Spearman rank correlation, Poisson and linear regression	Season was a proxy for exposure. Semen samples collected in March were considered highly exposed during the previous 90 days of winter (when more coal is burned for home heating and industry, and temperature inversions lead to extremely high pollution levels). October represented summer or low exposure in the past 90 days. The 90-day lag was intended to allow a complete cycle of spermatogenesis.	Average ± SD: Winter: 75.1 ± 75.1 Summer: 12.2 ± 5.2	Co-pollutant levels were not reported. Covariates (evaluated but not associated with sperm cytogenetic endpoints): - sperm count (per ml or total count) - sexual abstinence prior to semen sampling - recent high fever - viscosity - volume - sperm head morphology Multivariate analyses adjusted for: - date slide was scored - caffeine intake - alcohol intake semen parameter morphology	**YY8 aneuploidy was significantly associated with season: summer frequency=0.6/10,000 cells and winter frequency =3.5/10,000 cells (p=0.001).** **Controlling for the date the slide was scored, the incidence rate ratio for YY8 and winter vs. summer exposure was 5.25, CI (2.5, 11.0).**	SO_2 was used as an index of air pollution mixture. The specific components of air pollution that may account for the higher frequency of YY8 were not identified. Although the authors had information on other exposures, such as occupational or hobby-related exposures to metals or solvents, it is not clear whether these covariates were included in analyses.

C.2. Male Animal Reproductive Toxicology Studies

No studies of reproductive outcome in male animals exposed to SO_2 were identified. Three studies in mice investigated the effects of exposure to SO_2 by inhalation on testicular histology and biochemistry, including effects on sperm (Meng et al., 2004; Zhang et al., 2005; Meng et al., 2007). One additional study investigated the potential of SO_2 to increase levels of lipid peroxidation and alter intracellular redox status in mouse organs including testes (Meng et al., 2003). The protocols and findings of these four studies are presented, then summarized in Table C5 below; complete study summaries are provided in Appendix 2.

Related information consists of two studies investigating the results of exposure to sodium bisulfate (Bhattacharjee et al., 1980) and sodium metabisulfite (Til et al., 1972), respectively. As described in Sections B.1. and B.2. above, SO_2 equilibrates with sulfites, bisulfites, and metabisulfites, depending on conditions. No adverse effects on testes were reported in these studies.

C.2.1. Male Animal Reproductive Toxicology Studies of SO_2

Meng and Liu studied the effects of inhaled SO_2 on the ultrastructure of organs, including testes, from male mice (Meng et al., 2007). Compared to control mice, pathological changes were observed in the testes of male mice exposed to 28.00 ± 1.98 mg SO_2/m^3 (~11,000 ± 710 ppb), or 56.00 ± 3.11 mg SO_2/m^3 (~21,000 ± 1,100 ppb). Effects included alterations in basement membranes, as well as damage to Sertoli cells and spermatids. Severity increased with increasing dose. Based on their findings that inhaled SO_2 adversely affected ultrastructural morphology of multiple organs in mice, the authors concluded that the toxic effects of inhaled SO_2 were not limited to the respiratory systems.

Zhang et al. conducted biochemical analyses on testicular tissues taken from mice that had been exposed to SO_2 for 7 d, 4hr/d, at a concentration of 0, 28, 56, or 112 mg/m^3 (0, ~11,000, ~21,000, or ~43,000 ppb) (Zhang et al., 2005). Treatment had no demonstrable effect on food intake or body weight gain. Statistically significant differences from controls were found in SO_2-exposed animals for testicular biochemical parameters as follows:

- Malondialdehyde (MDA) content (nmol/g prot) – at all concentrations, concentration-related
- Glucose-6-phosphate dehydrogenase (G6-PD) activity (nmol/(mg min prot)) – at 56 and 112 mg/m^3 (~21,000 and ~43,000 ppb), concentration-related
- Glutathione S-transferase (GST) activity (µmol/(mg min prot)) – at 112 mg/m^3 (~43,000 ppb), concentration-related
- Glutathione (GSH) content (µmol/g prot) – at 56 and 112 mg/m^3 (~21,000 and ~43,000 ppb), concentration-related

In addition, assays of "comet tails" following electrophoresis of DNA from cells collected from seminiferous tubules indicated increasing DNA damage with increasing concentration of SO_2. The authors concluded that SO_2 exposure can influence GSH oxidation/reduction and damage spermatocyte DNA. Based on these mechanistic findings, they further concluded that SO_2 can damage the mouse male reproductive system.

Meng and Bai also studied the effects of inhalation exposure to SO_2 on testicular biochemistry in mice (Meng et al., 2004). Concentrations of 22 ± 2 mg/m^3 (~8,400 ± 760 ppb), 56 ± 3 mg/m^3 (~21,000 ± 1,100 ppb), or 112 ± 8 mg/m^3 (~43,000 ± 3,100 ppb) were not associated with increases in mortality or morbidity. Nor did mean body weights differ between any of the treated groups and their corresponding controls. These concentrations were associated with significant changes in levels and activities of several key testicular biochemical markers (Table C4 below).

Table C4. Effect of SO_2 on testicular biochemistry in mice (Meng et al., 2004).

Endpoint	control 22 ± 2 mg/m^3 (~8,400 ± 760 ppb)	control 56 ± 3 mg/m^3 (~21,000 ± 1,100 ppb)	control 112 ± 8 mg/m^3 (~43,000 ± 3,100 ppb)
TBARS Thiobarbituric acid reactive substances levels	0.170 ± 0.036 ---------- 0.223 ± 0.028*** (+31.18%)	0.214 ± 0.055 ---------- 0.296 ± 0.075*** (+38.32%)	0.192 ± 0.045 ---------- 0.297 ± 0.068*** (+54.69%)
GSH Reduced glutathione levels	58.15 ± 10.59 ---------- 56.03 ± 15.73 (-3.65%)	53.86 ± 6.36 ---------- 40.96 ± 16.34* (-23.95%)	54.93 ± 11.78 ---------- 47.36 ± 5.36* (-13.78%)
SOD Cu, Zn-superoxide dismutase activities	979.4 ± 102.3 ---------- 863.8 ± 95.4 (-11.80%)	1515.2 ± 146.9 ---------- 1197.4 ± 220.5 (-20.97%)	2437.2 ± 785.3 ---------- 1703.9 ± 227.2* (-30.09%)
GPx Glutathione peroxidase activity	4.30 ± 0.88 ---------- 4.00 ± 0.91 (-6.98%)	4.23 ± 0.93 ---------- 3.27 ± 0.62* (-22.70%)	4.11 ± 0.52 ---------- 3.29 ± 0.46** (-19.95%)
CAT Catalase activity	0.895 ± 0.217 ---------- 1.140 ± 0.453* (+27.37%)	0.510 ± 0.067 ---------- 0.700 ± 0.164* (+37.25%)	0.726 ± 0.064 ---------- 0.829 ± 0.116 (+14.19%)

Data expressed as mean ± standard deviation (n=10)
Levels of TBARS and GSH are expressed as nM/mg of tissue proteins
Activities of SOD, GPx and CAT are expressed as defined units (U) of activity/mg of tissue proteins
Changed percentages of enzyme activities are expressed in parentheses
Significant differences from controls by t-test at: *p<0.05, **p<0.01, ***p<0.001

Overall, the Meng and Bai (2004) study results were taken to demonstrate a SO_2-induced increase in lipid peroxidation in mouse testicles, with accompanying changes in testicular SOD and GPx activities, as well as GSH levels. TBARS, in particular, was found to be significantly increased at all tested concentrations of SO_2, which was interpreted as indicative of endogenous lipid peroxidation. The authors concluded that exposure to SO_2 caused oxidative damage to the testicles of male mice. By extension, they further concluded that SO_2 is toxic to the mammalian male reproductive system.

The potential of SO_2 to increase levels of lipid peroxidation and alter intracellular redox status was studied in multiple organs of Kunming albino mice, including testis, by Meng (Meng et al., 2003). While all animals gained weight over the seven-day treatment period, body weight gain did not differ between treated and control groups. Biochemical data were taken to indicate that at a concentration of 20 mg/m^3 (~7,600 ppb) four hours/day for seven days, SO_2 caused systemic oxidative damage in all tissues tested, including testis. Exposure resulted in significant increases in lipid peroxidation accompanied by concurrent changes in antioxidant status. Therefore, the author suggests that the oxidative damage produced by inhalation of SO_2 may contribute to toxicological damage to many organs, including testis, and not only to the respiratory system.

Studies of male reproductive toxicity in animals are summarized in Table C5.

Table C5. Animal studies of male reproductive toxicity of SO_2.

Reference	Study design	Systemic toxicity	Male reproductive toxicity
Meng and Liu, 2007	Mice, inhalation, 6 ♂/group, 4 hr/day for 7 days 0, 28, 56 mg/ m^3 (as calculated by OEHHA: 0, ~11,000, ~21,000 ppb) *	Histopathological changes in multiple organ systems No effect on body weight gain	Histopathological effects on testes at both concentrations Frequency and severity increasing with concentration
Zhang et al., 2005	Mice, inhalation, 10 ♂/group, 4 hr/day for 7 days 0, 28, 56, 112 mg/m^3 (0, ~11,000, ~21,000, ~43,000 ppb)	Not discussed	Effects on testicular biochemistry at all 3 concentrations Increased frequency of comet tails with increasing SO_2 concentration taken to indicate increasing DNA damage
Meng and Bai, 2004	Mice, inhalation, 10 ♂/group, 30 controls, 6 hr/day for 7 days 0, 22, 56, 112 mg/m^3 (0, ~8,400, ~21,000, ~43,000 ppb)	No deaths, morbidity, or clinical signs of toxicity No effect on body weight gain	Effects on testicular biochemistry at all 3 concentrations
Meng, 2003	Mice, inhalation, 10 ♂/group, 6 hr/day for 7 days 0, 20 mg/m^3 (0, ~7,600 ppb)	Not discussed	Increases in lipid peroxidation and changes in antioxidant status in all organs including testes of treated mice

* Reported by authors to be 0, 10, and 20 ppm.

C.2.2. Related Studies Relevant to Male Reproductive Toxicity of SO_2

Bhattacharjee et al. investigated the effects of sodium bisulfite on differentiating spermatogonia in male Swiss mice (Bhattacharjee et al., 1980). Treatment was by intraperitoneal (i.p.) injection, and given either one time (doses up to 1000 mg/kg) or repeated (doses up to 400 mg/kg). No mortality was observed with even the highest repeated dose, or with single doses of up to 700 mg/kg. Mortality was observed at the higher single doses, reaching 80% at 1000 mg/kg. Histological examination of testes from treated animals revealed no effects on the numbers of various types of spermatogonia, regardless of the dosing level and repetition.

The Til et al. (1972) study is also discussed in Section D.2.2., "Other Animal Studies Relevant to Female Reproductive Toxicity of SO_2" below. No evidence was presented for adverse effects of sodium metabisulfite on male fertility or male reproductive organs.

C.3. Integrative Evaluation for Male Reproductive Toxicity

Ambient exposure to SO_2 levels >15.3 ppb resulted in decreased fecundability in humans as reported by Dejmek et al. (2000). Three occupational studies also report an association between exposure specifically to SO_2 and increased DNA damage in lymphocytes of workers. Human studies have demonstrated a dose-response relationship between higher levels of DNA damage in sperm and infertility both *in vivo* and *in vitro*, as discussed below.

Many aspects of the study by Dejmek et al. (2000) provide strong evidence of a cause and effect association between exposure to SO_2 and decreased fecundability:

1) The odds of conception in the FUMC were significantly and consistently reduced in couples exposed to SO_2 levels >15.3 ppb in the second month before conception.
2) The timing of the effect coincides with the critical period of sperm maturation.
3) A dose-response association was seen with increasing levels of SO_2 exposure. In addition, the association was weaker in the second two years of the study, suggested by the authors to be probably due to the gradual decrease in SO_2 levels in the region.
4) The dose-response association was strengthened by the exclusion of couples living farther away from the monitoring stations.
5) An association with decreased fecundability was found only with SO_2 exposure and not with exposure to other air pollutants.
6) The effects on sperm motility and morphology seemed to be reversible since the men evaluated six months after episodes of elevated air pollution had improved semen quality.

Other studies from the Teplice Program showed evidence that exposure to episodes of elevated air pollution have adverse effects on semen quality and sperm chromatin integrity (Selevan et al., 2000), while exposure to SO_2 was also associated with increased DNA damage (Rubes et al., 2005; Rubes et al., 2007). The association between higher air pollution levels and decreased normal sperm morphology and decreased sperm motility that Selevan et al. (2000) reported was not evident in the follow-up longitudinal study by Rubes et al. (2005). However, as noted by Rubes et al. (2007), this may be a result of decreasing SO_2 levels over time. Selevan et al. (2000) collected samples during periods of low and high pollution in 1993 and 1994 while samples in Rubes et al. (2005) were collected years later at seven time points, including during periods of lower and higher air pollution, over a two-year period from September 1995 to September 1997. It has been noted that during those years the concentrations of SO_2 were decreasing and by 1998 the levels were approximately 20% of those seen in the winter of 1989 (Sram et al., 1999). The effects of exposure to air pollution on sperm motility and morphology seemed to be reversible since men evaluated six months after episodes of higher pollution had improved semen quality (Rubes et al., 2007). In addition, the study by Robbins et al. from the Teplice program, using a different method, fluorescence *in situ* hybridization (FISH), for detecting chromosomal damage in human sperm, found the risk of aneuploidy in sperm was increased in association with exposure to higher levels of air pollution, as indicated by SO_2 levels (Robbins et al., 1999). As the authors note, aneuploidy is a plausible contributor to the trend in male subfertility that has been proposed to be associated with declining semen quality worldwide (Rogers et al., 1999).

Sperm DNA damage and pregnancy

Epidemiological evidence has linked paternal exposure to occupational or environmental agents with an increased risk of abnormal reproductive outcomes. Embryos receive part of their genetic composition from sperm. During the later part of spermatogenesis, when the nucleus undergoes major chromatin restructuring and epigenetic reprogramming, elongated spermatids are deficient in DNA-repair capabilities and genetic lesions induced during this period may accumulate in sperm and persist until fertilization (Marchetti et al., 2005). Oocytes and early embryos have been shown to repair some sperm DNA damage; however, the capacity of an oocyte to repair pre-existing damage in sperm is not unlimited (Matsuda et al., 1988; Genesca et al., 1992). Paternally-transmitted chromosomal damage has been associated with pregnancy loss, developmental and morphological defects, infant mortality, infertility, and genetic diseases in the offspring, including cancer (Marchetti et al., 2005). Little is known about the etiology, transmission, and early embryonic consequences of paternally-derived chromosomal abnormalities. However, sperm chromatin integrity has been identified as a more sensitive marker of male fertility compared with basic sperm parameters such as sperm count, morphology, and motility (Rubes et al., 2005).

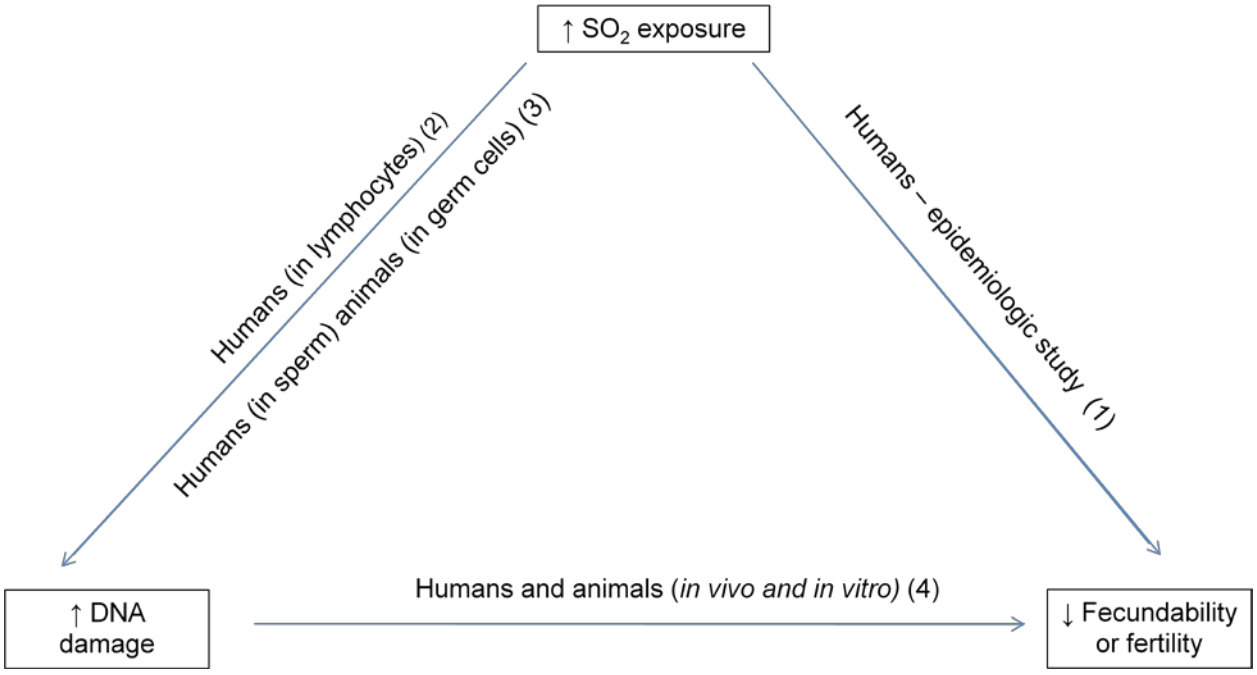


Figure C4. The diagram above illustrates the relationships between the studies presented in this section examining SO_2 exposure, DNA integrity and fecundability or fertility in animals and humans, as summarized below:

1. Higher SO_2 exposure was associated with decreased fecundability in humans, as shown in the epidemiologic study by Dejmek et al. (2000).
2. Increased DNA damage in lymphocytes of workers exposed to SO_2 was seen in occupational studies (Nordenson et al., 1980; Meng et al., 1990; Yadav et al., 1996).
3. Increased DNA damage was seen in sperm cells of humans and animals exposed to SO_2 (Robbins et al., 1999; Zhang et al., 2005; Rubes et al., 2007).
4. Increased DNA damage in humans and animals, *in vivo* and *in vitro*, has been associated with decreased reproductive performance, including infertility and decreased fecundability, in numerous studies (Evenson et al., 1999; Spano et al., 2000; Virro et al., 2004; Evenson et al., 2005; Boe-Hansen et al., 2006; Evenson, 2006; Bungum et al., 2007; Zini et al., 2008; Rybar et al., 2009; Giwercman et al., 2010).

Sperm Chromatin Structure Analysis (SCSA) uses a fluorescence staining technique to detect DNA strand breaks in sperm that are thought to be potential precursors to gross chromosomal damage. The percentage of sperm in a semen sample with fragmented DNA is reported as the DNA Fragmentation Index (DFI (%)). High SCSA–%DFI has been associated with clinical infertility and increased risk of spontaneous abortion in many studies (Evenson et al., 1999; Virro et al., 2004; Boe-Hansen et al., 2006; Bungum et al., 2007; Zini et al., 2008). Similarly, DNA damage, as measured by SCSA, has been shown to be a predictor of infertility in population-based cohort studies (Spano et al., 2000; Evenson et al., 2005) and in a case-control study (Giwercman et al., 2010). In a population of Czech men, Rybar et al. (2009) found sperm chromatin damage was

significantly higher in men with unexplained infertility compared to men with no experiences of infertility. In a recent meta-analysis, couples with no known infertility problems were seven times more likely to achieve a pregnancy/delivery via *in vivo* fertilization if the DFI <30 (Evenson et al., 2005). The meta-analysis of 11 studies by Zini et al. (2008) reported a combined odds ratio (OR) of 2.48 (CI 1.52, 4.04), indicating that sperm DNA damage was predictive of pregnancy loss after in vitro fertilization or intracytoplasmic sperm injection (IVF or ICSI). Even in the absence of significant changes in classic measures of sperm quality, increases in sperm DNA fragmentation (as detected by SCSA) has shown associations with decreases in fertility (Evenson et al., 2005).

The SCSA has been shown to be sensitive to mutagen dose, and to be a biomarker of fertility in humans and other mammals (Evenson et al., 2005). In addition, the SCSA parameters have been found not to vary by season and therefore any changes in SCSA associated with exposure to air pollution are not likely to be confounded by season of sampling (Sram, 2001).

Environmental stress, gene mutations, and chromosomal abnormalities can all disturb biochemical events that occur during spermatogenesis; this can ultimately cause abnormal chromatin structure that is incompatible with fertility (Evenson et al., 2002). The mechanisms by which this may occur have been hypothesized to include apoptosis and possibly reactive oxygen species (ROS) activity (Evenson et al., 2005; Erenpreiss et al., 2006; Schulte et al., 2010).

As stated in Schulte et al. (2010):

> Sperm DNA damage has also been associated with high levels of ROS. At low levels, ROS play an important role in sperm maturation and functions such as capacitation and the acrosome reaction. Seminal plasma contains antioxidants which help protect sperm DNA. However, when an excessive amount of ROS is produced beyond the antioxidant capacity of seminal plasma and male reproductive tract, the pathogenic result is often cellular and DNA damage. Increased levels of ROS have been reported in the semen of approximately 25% of infertile men. Additionally, a positive correlation was reported between sperm DNA fragmentation and ROS. Major sources of ROS in semen are leukocytes and the sperm themselves, particularly immature sperm with cytoplasmic retention and abnormal head morphology characterized by retention of residual cytoplasm. Both leukocytospermia and retention of residual cytoplasm within sperm have been associated with increased sperm DNA damage, likely secondary to increased level of ROS produced by these cells.

The animal studies, as discussed above in section C.2., reported adverse effects in mice exposed to SO_2 including: changes in ultrastructural morphology in the testes (Meng et al., 2007); concentration dependent oxidation damage in the testes (Meng et al., 2004); and DNA damage in germ cells (Zhang et al., 2005). These results support

the results of the studies in humans. In addition, the findings of oxidative damage are consistent with the proposed mechanism of DNA damage in sperm.

Summary

There is considerable evidence that air pollution (with SO_2 used as an index measure in some studies) induces DNA damage in human sperm (Selevan et al., 2000; Rubes et al., 2005; Rubes et al., 2007) as well as other cell types (Nordenson et al., 1980; Meng et al., 1990; Yadav et al., 1996). The data from animal studies are also indicative of oxidative damage, including DNA damage in the testes caused by exposure to SO_2. The association between DNA damage in sperm and reduced fertility is well established (Evenson et al., 1999; Spano et al., 2000; Virro et al., 2004; Boe-Hansen et al., 2006; Evenson, 2006; Bungum et al., 2007; Zini et al., 2008; Rybar et al., 2009; Giwercman et al., 2010) and is consistent with the decrease in fecundability associated with SO_2 exposure reported by Dejmek et al. (2000). Thus, these data, taken together, provide important evidence of the association between exposure to SO_2 and a decrease in fecundability.

D. Female Reproductive Toxicology Studies

This section describes the human and animal data available for judging relationships between SO_2 exposure and female reproductive outcomes. One human study evaluated the effects of air pollutants on women receiving IVF treatments. Human data from air pollution studies on stillbirths and spontaneous abortions, discussed in section E, are also available, but from studies of very limited design.

In animals, there is a female fertility study in rats exposed to SO_2 through the inhalation route. Additional animal studies related to the female reproductive toxicity of SO_2 include oral studies of sulfites. As described in Sections B.1. and B.2. above, SO_2 equilibrates with sulfites, bisulfites, and metabisulfites, depending on conditions. In particular there is a mechanistic study that used sodium sulfite to evaluate the impact of SO_2 on oocytes of the mouse, sheep and cow.

All of the human and animal studies discussed are described in more detail in Appendices 1 and 2.

D.1. Human Female Reproductive Studies

The human data on female reproductive toxicity of SO_2 are sparse. One retrospective cohort study was identified, which assessed pregnancy outcomes among women who were undergoing their first cycle of IVF (Legro et al., 2010). The study is summarized in Table D1 and, in greater detail, in Appendix 1. The study covered 7,403 female patients of three centers in Hershey, Pennsylvania; Rockville, Maryland; and New York, New York for seven years in 2000–2007. SO_2 concentrations were associated with

decreased odds of live birth, but none of the associations were statistically significant. Associations with other pollutants were stronger. Exposure to either NO_2 or SO_2 appeared to decrease the odds of live birth. Information on smoking history, prior IVF cycles in other centers, and infertility diagnoses was lacking in this study.

In addition to the Legro et al. (2010) study, there are the epidemiologic studies of pregnancy loss described in the developmental toxicology section of this document. Endpoints such as fetal death, spontaneous abortion, and stillbirth may be manifestations of direct toxicity to the conceptus, but may also be mediated in part or in whole through toxicity to the female reproductive system of the mother. That is, the effect may be directly on the conceptus, on the female reproductive system, or both. Mechanistic data can clarify this, but such data are not available for SO_2. Thus, the studies reviewed in the developmental toxicity sections E.1.3. and E.1.4. can also be considered in the context of identifying female reproductive toxicity.

Table D1. Highlights of the assisted reproduction study.

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Legro et al., 2010 Hershey, PA Rockville, MD New York, NY 2000–2007	7,403 females undergoing their first cycle of *in vitro* fertilization (IVF) at one of three IVF centers. Outcomes were pregnancy and live births Retrospective cohort Logistic regression	Ambient air pollution concentrations were obtained from the U.S. EPA. Air pollution data were used to fit national-scale, log-normal kriging with a spherical model for spatial interpolations to produce daily mean concentrations at patients' residential zip code and IVF centers for the entire IVF cycle and pregnancy of each patient.	Average daily mean (SD): Date of retrieval at clinic: 63 (30) Embryo transfer to pregnancy outcome date: 57 (12)	Pearson correlation coefficients during oocyte retrieval to embryo transfer at clinics (all $p<0.001$): $PM_{2.5}$ r=0.17 PM_{10} r=0.09 NO_2 r=0.74 O_3 r=-0.40 SO_2 was analyzed singly. Controlled for: - maternal age - IVF center - year and season of oocyte retrieval	AOR (CI) for live birth and 30 ppb increase in SO_2 exposure during: Gonadotropin start to oocyte retrieval 1.00 (0.91, 1.10) Oocyte retrieval to embryo transfer 0.94 (0.87, 1.02) Embryo transfer to pregnancy test (14 days) 0.94 (0.86, 1.04) Embryo transfer to date of live birth 0.96 (0.73, 1.27) NO_2 exposure during IVF phases was significantly associated with decreased odds of live birth (except OR for exposure from embryo transfer to live birth, which was almost significant). O_3 exposures in different IVF phases were associated with increases and decreases in odds of live birth.	Authors lacked information on smoking history, prior IVF cycles in other centers, and infertility diagnoses. The strongest correlation was between SO_2 and NO_2, and exposure to each of these pollutants appeared to decrease the odds of live birth. It is possible that some of the effects observed in the model with only SO_2 could be explained by NO_2.

D.2. Female Animal Reproductive Toxicology Studies

One female fertility study examined rats exposed to SO_2 through the inhalation route (Mamatsashvili, 1970). Other information from animal studies related to the female reproductive toxicity of SO_2 is also presented. It includes oral studies of sulfites, compounds that can release SO_2 under conditions of use as food additives (Til et al., 1972; Hugot et al., 1978; Dulak et al., 1984). Additionally, there is a mechanistic study that evaluated the impact of SO_2, given as sodium sulfite (Na_2SO_3), on oocytes of the mouse, sheep and cow (Jagiello et al., 1975). Brief discussions of these studies are provided in sections D.2.1. and D.2.2. below, while complete study summaries are provided in Appendix 2.

D.2.1. Animal Study of Direct Exposure to SO_2 on Female Fertility

Mamatsashvili exposed female rats to SO_2 by inhalation at concentrations of 0.15 (~57 ppb) or 4 mg/m^3 (~1,500 ppb) (Mamatsashvili, 1970). Additional groups were exposed to CO, with or without concurrent SO_2. The treatment period lasted 72 days, but the duration of exposure per day was not reported.

Effects observed at 4 mg/m^3 (~1,500 ppb) included alterations in various stages of the estrous cycle, changes in pregnancy frequency and duration, as well as changes in offspring growth as measured by body weight. Significant changes in the estrous cycle were also seen in the F_1 female offspring of animals exposed to 4 mg/m^3 (~1,500 ppb) SO_2. None of these effects were seen at the lower concentration of 0.15 mg/m^3 (~57 ppb) SO_2, and normal histopathology was reported for this group. Litter size was considered to have been increased in all treated groups.

No actual data or statistical analyses are presented in the paper, but the authors suggest that SO_2 may have had "a stimulating effect on the endocrine system."

D.2.2. Other Animal Studies Relevant to Female Reproductive Toxicity of SO_2

Dulak et al. studied sulfite-induced reproductive toxicity in female Wistar rats (Dulak et al., 1984). Some of the test animals were rendered sulfite oxidase-deficient by means of a diet low in molybdenum and drinking water high in sodium tungstate. Doses of 0, 25, or 50 mM sulfite (as sodium metabisulfite) were provided in drinking water.

No effect of sodium sulfite was found on maternal mortality or symptoms of toxicity. Sulfite exposure was not found to be associated with any concentration-dependent changes in reproductive performance. The only effect of exposure on pregnancy or offspring endpoints was a slight increase in the mean number of corpora lutea/dam with 50 mM sulfite (18.1 ± 3.2, compared to 14.9 ± 2.2 for controls), which the authors presumed was responsible for the significant ($p < 0.05$) increase in pre-implantation loss for this group (20.8%, compared to 6.7% for controls).

In a French language paper with an English abstract, Hugot and Causeret reported on female reproductive effects in rats fed a diet containing 1% potassium metabisulfite (Hugot et al., 1978). The study is not well-reported; exposure of the females began seven weeks prior to mating, but it is not clear when exposure ended. The discussion states that potassium metabisulfite did not affect pup birthweight, but did depress later growth. The linear phase of postnatal growth was said to have been particularly affected, resulting in a mean weight at weaning that was decreased by an average of 7% with treatment relative to controls.

In the Til et al. study, sodium metabisulfite was added to the diets of Wistar-derived rats for periods of up to two years and over three generations (Til et al., 1972). Concentrations ranged from 0–2%. To avoid confounding by thiamine deficiency due to the known destructive effects of sulfite on thiamine, the diet was enriched with 50 ppm thiamine.

The overall condition of rats in all groups, for all generations studied, was considered to be good for the first 72 wks of observations. After that time, symptoms of aging became evident in many rats, and mortality increased. Treated groups generally showed higher survival rates than controls. As for growth data, there appears to have been marginal reductions in weight gain for F_1 and F_2 rats exposed to 2% sodium metabisulfite in the diet, although statistical significance was not mentioned. Treatment had no effect on fertility at any concentration of sodium metabisulfite, for any generation.

Mean litter size was affected only for the first mating of the F_2 generation. Relative to controls, significant ($p < 0.01$ or $p < 0.001$) decreases were seen in litter sizes with exposure to 0.5, 1.0, or 2.0% sodium metabisulfite. Effects of treatment on offspring weights were generally more profound at postnatal day (PND) 21, as compared to PND 1 or 8. Weight deficits were also more evident in offspring of F_2 animals, as opposed to offspring of the F_0 or F_1 generations. Significant effects on offspring mortality at birth or weaning appeared to be sporadic across concentrations and generations, with no clear concentration-response effect.

Measures of thiamine content in urine and liver were taken to indicate that the added dietary thiamine had succeeded in preventing thiamine deficiency, even at the highest intake of 2% sodium metabisulfite. Hematology parameters were considered to be generally normal. Findings of occult blood in feces, indicating intestinal bleeding, were common for rats of the highest concentration group in all generations.

No effects of sodium metabisulfite exposure were detected on relative weights of testes or ovaries. No treatment-related neoplastic or non-neoplastic lesions were identified in organs including testes, ovaries, mammary glands, uterus, prostate, seminal vesicles, or coagulating glands.

Jagiello et al. used *in vivo* and *in vitro* techniques to evaluate the capacity of SO_2 to damage meiotic chromosomes in oocytes of the mouse, ewe, and cow (Jagiello et al.,

1975). Based on earlier reports in rats and guinea pigs that inhaled SO_2 appeared rapidly in the bloodstream as the potassium or sodium salt, sodium sulfite (Na_2SO_3) was used in these studies.

Results for mouse oocytes included the following findings for oocytes cultured for up to 15 hours with Na_2SO_3 at concentrations ranging from 10 to 10,000 µg/cc of culture media:

- inhibition of entry into meiosis to the stage of M_1 ($p<0.05$)
- manifestations of nuclear damage
- more severe damage described as “overt atresia”
- complete failure of meiosis at higher concentrations

Similar experiments with ewe and cow oocytes resulted in:

- Ewe
    - No effects of sodium sulfite on meiotic division
    - Atresia and chromosome breaks at 250 µg/cc, more severe effects at higher concentrations
    - Anaphase lagging at 350 and 1250 µg/cc
- Cow
    - Sensitive to meiotic inhibition by sodium sulfite at $\geq$ 500 µg/cc ($p<0.05$)
    - Damage to oocyte nuclei progression at $\geq$ 250 µg/cc
    - One anaphase lag at 350 µg/cc

Additional experiments involved *in vivo* treatment of female Camm mice with subsequent evaluation of oocytes. These experiments did not reveal any effects of intravenous (i.v.) sodium sulfite on subsequently cultured oocytes.

D.3. Integrative Evaluation for Female Reproductive Toxicity

Pregnancy loss

The data on pregnancy loss or fetal death, including spontaneous abortion and stillbirth in humans and some animal species, and resorptions in other animal species, are presented and reviewed under Developmental Toxicity in section E. As indicated in that section, epidemiologic and experimental animal data do not provide evidence for an association between gestational exposure to SO_2 and pregnancy loss.

Assisted Reproduction

One retrospective cohort study of females who were undergoing their first cycle of IVF and pregnancy outcome found that SO_2 concentrations were associated with decreased odds of live birth (0.94–0.96), but none of the findings were statistically significant (Legro et al., 2010). Associations with NO_2 and O_3 were stronger.

Estrous cycle effects

One study conducted in rats by the inhalation route of exposure over a test period of 72 days specifically investigated the effects of SO_2 on female reproduction (Mamatsashvili, 1970). Exposure to SO_2 at a concentration of 4 mg/m^3 (~1500 ppb) affected estrous cycle length, pregnancy frequency and duration, and offspring growth. Estrous cycles were also affected in F_1 female offspring of the 4 mg/m^3 (~1500 ppb) group. None of these effects were seen with exposure to SO_2 at a lower concentration of 0.15 mg/m^3 (~57 ppb).

Effects on chromosomes

Jagiello et al. (1975) studied meiosis in oocytes from mice, sheep, and cows following *in vivo* or *in vitro* exposure to sodium sulfite. Fragmentation of chromosomes, with or without rearrangement, seen in ewe and cow oocytes was taken to indicate the potential for sodium sulfite to "contribute to a variety of transmissible chromosomal disorders in progeny." Also observed were lags in anaphase, which can lead to aneuploidy in offspring if fertilization occurs. The authors concluded that despite the absence of such effects in mouse ova, their occurrence in ewe and cow ova implicate SO_2 and its metabolites as potential contributors to fetal abnormality and loss in livestock in contaminated areas.

E. Developmental Toxicology Studies

This section presents the evidence on the developmental toxicity of SO_2 from human, animal, and other relevant studies. The potential associations between SO_2 exposure and various developmental outcomes have been investigated in over 50 human epidemiologic studies. Forty-three of these studies included SO_2 measurements and are described in this section. These studies, as well as other studies that did not specifically measure SO_2, are described in more detail in Appendix 1.

Four studies investigated physical and functional developmental effects of SO_2 exposure in laboratory animals. These studies are discussed below, and in more detail in Appendix 2. This section concludes with an integrative evaluation that incorporates the human and animal data (Section E.2).

E.1. Human Developmental Studies

Ten studies of the association between SO_2 and preterm birth (PTB) are presented, followed by presentation of 22 studies that examined the association between fetal growth and exposure to SO_2. Studies of pregnancy loss – three that examined the association between SO_2 and stillbirth, and one that examined spontaneous abortion – are then described. Seven studies that explicitly examined associations between

congenital malformations and SO_2 are then presented. The discussion of the human developmental studies concludes with a description of the one study that examined the association between *in utero* SO_2 exposure and development of asthma in early childhood.

E.1.1. Preterm Birth

Ten studies examined the association between SO_2 and PTB. These are individually summarized in Table E1, ordered by SO_2 concentration, starting with the lowest SO_2 concentrations.

Seven of the 10 studies reported a statistically significant association and one reported a marginally significant association (CI 1.0, 1.32) between SO_2 exposure and PTB (Sagiv et al., 2005). These studies evaluated different exposure windows, ranging from single days to the entire pregnancy. Various studies found PTB to be significantly associated with exposure to SO_2 late in pregnancy, such as seven days before delivery (Xu et al., 1995), or the last month (Liu et al., 2003; Jalaludin et al., 2007), last two months (Jiang et al., 2007), or last three months, of pregnancy (Jalaludin et al., 2007).

Studies also found significant associations between PTB and SO_2 exposure in the first trimester (Leem et al., 2006; Jalaludin et al., 2007), and with exposure in each trimester (Bobak, 2000). In addition, Jalaludin et al. (2007) found that the association between SO_2 and PTB depended on the season in which the infant was conceived. Three studies mentioned evidence for an exposure-response relationship between SO_2 and PTB (Xu et al., 1995; Sagiv et al., 2005; Leem et al., 2006), two of which were statistically significant (Xu et al., 1995; Leem et al., 2006).

As noted previously, exposure assessment is a challenge and may explain some of the disparities among findings of epidemiologic studies of SO_2, for example:

- Jalaludin et al. (2007) found that when analyzing data for all of Sydney, there was evidence for a protective association between SO_2 exposure and PTB. However, when the analyses were limited to mothers within 5 km of monitoring stations (thereby likely increasing the accuracy of the exposure assessment), higher SO_2 levels in both early and late pregnancy were significantly associated with an increased risk of PTB.
- In Brauer et al. (2008), the mean SO_2 concentrations were at the limit of detection and varied too little to provide an exposure gradient. This study found no association between SO_2 exposure and PTB.
- Darrow et al. (2009) had higher exposure levels; however, their main analyses used data from five monitors to assign exposure over an area of 1,752 square miles. The authors did conduct a “capture-area analysis” intended to provide better exposure assessment by limiting the analysis to subjects within four miles of a monitor. No significant associations were observed between SO_2 and PTB.

Figure E1 is a chart of the exposure levels reported in each of the studies. The studies reported SO_2 concentrations using various parameters, including mean, median, interquartile range (IQR), and range, as noted in the chart.

The summaries of the preterm birth studies in Table E1 provide more detail about the study methods, including exposure assessment, study population, consideration of co-pollutants and other covariates, statistical analyses, and study strengths and limitations. Results include measures of association and CI, if available. The findings of significant effects of SO_2, as reported by the authors, appear in bold.

Figure E1. Reported SO_2 exposures (ppb) in preterm birth studies.

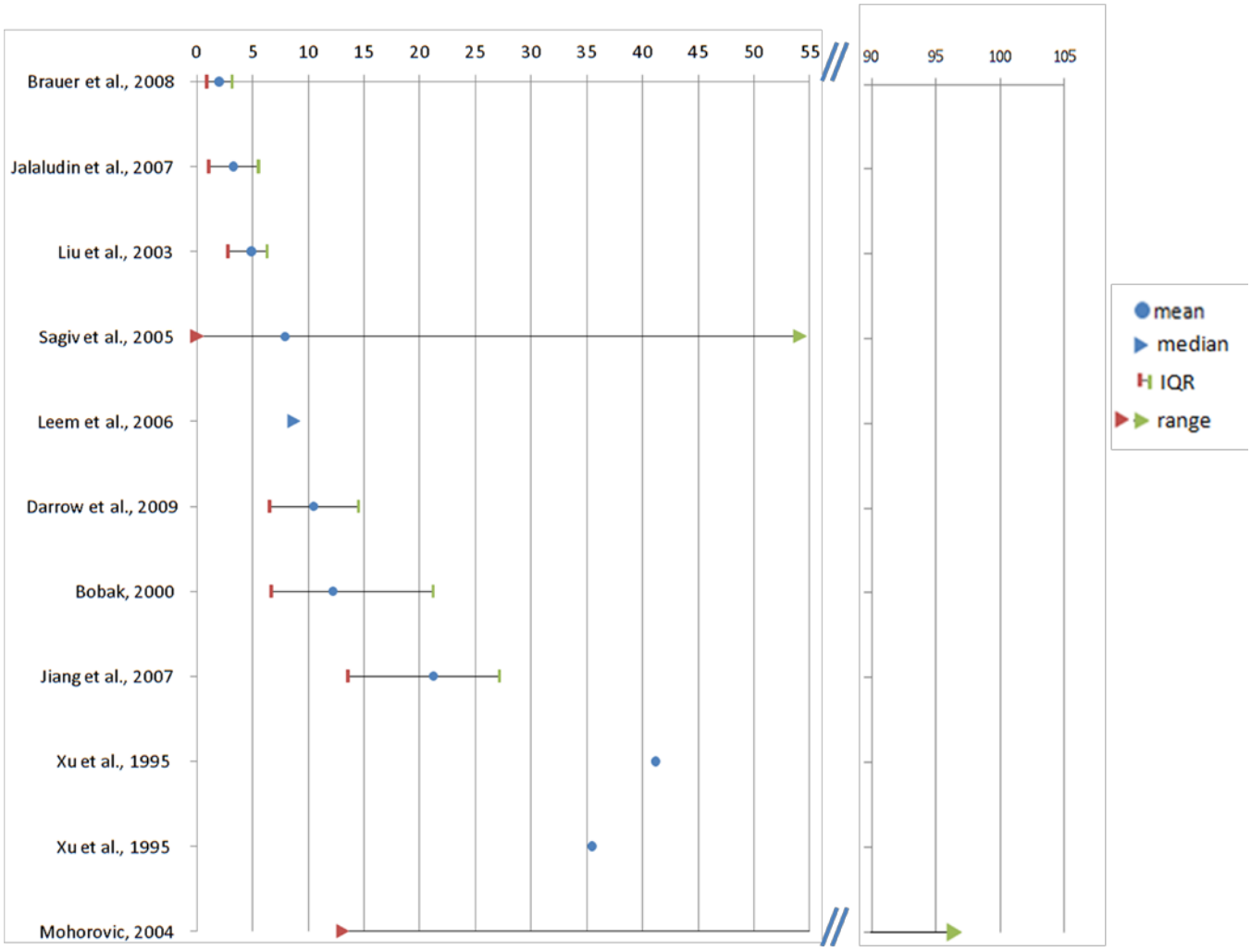


Table E1. Highlights of preterm birth studies (significant SO_2 findings in bold).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Brauer et al., 2008 Vancouver, Canada 1999–2002	Singleton births n = 70,249 3,748 (5.3%) preterm (<37 weeks) 241 (0.3%) preterm (< 30 weeks) Population-based cohort Logistic regression	Data from regulatory monitoring network, including 14 monitors for SO_2 3 approaches to assigning SO_2 exposure: • Nearest monitor to residential postal code (~ 1 block in urban areas) and w/in 10 km; calculated monthly mean • Inverse distance weighting (IDW) by 3 monitors nearest to postal code w/in 50 km; calculated monthly mean • Proximity to major roads (not specific to SO_2)	Mean exposures for entire pregnancy, first and last month and first and last three months. Modeled by nearest monitor: Mean =2.17 Min=0.00 Max=9.50 IQR, difference between 75th and 25th percentiles=1.34 Modeled by IDW: Mean=2.02 Min=0.11 Max=6.79 IQR=1.14	Analyzed separately: $PM_{2.5}$ PM_{10} NO NO_2 O_3 Black carbon (BC) CO Correlations among IDW estimates for CO, NO, NO_2, and SO_2 were all > 0.8. (correlations between SO_2 and $PM_{2.5}$ and PM_{10} were not reported) Controlled for: - gender - First Nations status - parity - maternal age - maternal smoking during pregnancy - month-year of birth - income (quintile-census) - education (quartile-census)	Using IDW, for births < 30 weeks and 0.38 ppb increase in SO_2 for entire pregnancy: Crude OR 1.02 (0.97,1.07) Adjusted OR 1.01 (0.95,1.06) OR for births <37 weeks were also not significant.	7.4% of the cohort reported maternal smoking during pregnancy. Air pollution concentrations were low relative to air quality standards and international guidelines. Limitation: entire pregnancy exposure averaging periods will be different for cases and non-cases. OR for late and early pregnancy windows were similar to those for full duration of pregnancy. Data on education and income are based on census at neighborhood-level.

Table E1. Highlights of preterm birth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Jalaludin et al., 2007 Sydney, Australia 1998–2000	Singleton births n = 123,840 6,011 (4.9%) preterm Cohort Logistic regression	14 monitoring stations w/in Sydney 1-hour max. concentrations of SO_2, averaged over potentially relevant exposure windows (e.g., 1st month, 1st trimester, final month before birth). Levels were averaged for all of metropolitan Sydney. Additional analyses of 65,814 births to mothers within 5 km of 11 monitoring stations.	All year daily mean (SD): 3.6 (1.95) Median (IQR): 3.3 (2.24) Summer daily mean (SD): 3.6 (2.37) Median (IQR): 3.1 (2.58) Autumn daily mean (SD): 3.6 (1.93) Median (IQR): 3.3 (2.16) Winter daily mean (SD): 3.8 (1.69) Median (IQR): 3.6 (1.88) Spring daily mean (SD): 3.6 (1.74) Median (IQR): 3.2 (2.30)	Correlations w/ SO_2 PM_{10} (r = 0.42) $PM_{2.5}$ (r = 0.43) NO_2 (r = 0.46) O_3 (r = 0.36) CO (r = 0.24) Models included one pollutant at a time. Two-pollutant models were also discussed, but not reported. Controlled for - gender - maternal age - maternal smoking during pregnancy - gestational age at first prenatal visit - socioeconomic status (SES; based on postcode, in quintiles) - indigenous status - parity - season Relative humidity (RH) and temperature at exposure were evaluated but were not significant and were excluded from multivariate models.	Adjusted OR (CI) for 1 ppb increase in SO_2 during specified intervals: **W/in 5 km of monitor:** 1st month 1.38 (0.88, 2.17) **1st trimester 2.31 (1.29, 4.15)** **Final month before birth: 1.56 (1.02, 2.38)** **Final 3 months: 2.33 (1.34, 4.04)** By season of conception, for exposure in 1st trimester (all Sydney): **Autumn: 6.49 (4.37, 9.65)** **Winter: 1.32 (1.03, 1.70)** Spring: 1.29 (0.96, 1.73) **Summer: 0.83 (0.76, 0.90)** Other results for all Sydney were non-significant	Season of conception was a significant effect modifier. Results are inconsistent; authors note multiple comparisons and suggest results should be interpreted with caution.

Table E1. Highlights of preterm birth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Liu et al., 2003 Vancouver, Canada 1985–1998	Singleton live births n = 229,085 ~12,142 (5.3%) preterm (< 37 weeks gestation) Retrospective cohort Multiple logistic regression using single and multiple pollutant models.	Continuous sampling measures from 13 census subdivisions in the Vancouver area Daily average and daily 1-hr conc. of SO_2, CO, NO_2, and O_3 5 years of PM_{10} data analyzed	Daily average SO_2 Mean: 4.9 25th percentile: 2.8 75th percentile: 6.3 95th percentile: 10.5 Mean daily maximum (1-hr conc.): 13.4 Gestational exposure windows evaluated: • 1st, 2nd, and 3rd month; • last and next-to-last month; and, • 1st, 2nd, and 3rd trimesters.	Correlations with co-pollutants: NO_2 (r = 0.61) CO (r = 0.64) O_3 (r = -0.35) Controlled for - maternal age - parity, - infant sex - gestational age (LBW analyses only) - birth weight (preterm analyses only) - season of birth	Exposure to SO_2 (in 5 ppb increments) was associated with: Exposure in: • 1st month AOR = 0.95 (0.88,1.03) • **Last month AOR = 1.09 (1.01, 1.19)** Adjusted for gaseous co-pollutants, exposure in last month **AOR = 1.09 (1.01, 1.20)**	Results reported for single pollutant models and selected exposure windows. Exposures were averaged over the entire study area. No data on maternal smoking, environmental tobacco smoke (ETS), and SES Limited data on PM_{10}, not reported in most results.
Sagiv et al., 2005 Pennsylvania 1997–2001	Singleton births in 4 counties n = 187,997 21,450 (11.4%) preterm Time series	U.S. EPA Air Quality System (AQS) 3 counties had more than 1 monitoring station	6-week Mean: 7.9 ± 3.5 Median: 8.1 Range: 0.8–17.0 Daily Mean: 7.9 ± 6.2 Median: 6.4 Range: 0–54.1	Co-pollutants Included in analyses: PM_{10} (r=0.46) CO NO_2 O_3 Controlled for - temperature - dew point temperature (humidity) - long- term and seasonal trends in preterm birth. Acute (1-day) exposure analyses also adjusted for d of the wk.	RR for preterm delivery, per 15 ppb increase in avg. SO_2 in the 6 wks before birth= 1.15 (1.00, 1.32) (reported as an effect by the authors) The data suggested a dose-response between SO_2 and risk of preterm delivery. Acute effect of SO_2 exposure 3 d before birth (RR = 1.07; CI 0.99, 1.15), adjusted for temperature, dew point temperature, and d of wk.	Only late pregnancy exposure examined. No information on location and number of monitors and size of counties.

Table E1. Highlights of preterm birth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Leem et al., 2006 Incheon, Republic of Korea 2001–2002	Singleton births n = 52,113 2,082 (4.0%) preterm Retrospective cohort Log-binomial regression	27 monitoring stations in or around Incheon, from the Korean National Institute of Environmental Research Monthly pollutant levels were predicted for each dong (unit of location) using monitoring data and block kriging*, and predictions were validated. Each birth was assigned exposure values based on the dong of maternal residence. *Block kriging is a statistical mapping technique that allowed prediction of values over a region from data collected at point locations. In this case, the values are pollutant concentrations, the regions are dongs, and the point locations are monitors.	Exposure quartiles among full-term infants; first trimester: 4th: 17.5 - 39.7 3rd: 8.7 - 17.5 2nd: 6.7 - 8.7 1st: 3.0 - 6.7 3rd trimester exposure levels were comparable.	Correlations with co-pollutants (analyzed separately): NO_2 (r=0.54*) CO (r=0.27*) PM_{10} (r=0.13*) *p<0.001 Controlled for - sex - parity - season of birth - maternal age - education level of each parent	Adjusted RR for preterm delivery in the highest quartile compared to lowest quartile of exposure: **1st trimester 1.21 (CI 1.04, 1.42)** **There was a dose-response relationship between exposure to SO_2 in the first trimester and risk of preterm delivery (p<0.02 for trend).** 3rd trimester 1.11 (0.94, 1.31)	Did not control for smoking or ETS

Table E1. Highlights of preterm birth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Darrow et al., 2009 5 counties in Metro Atlanta 1994–2004	Singleton births without major structural defects n=476,489 48,843 (10.3%) preterm Time-series analysis Additional "Capture-area analysis" of the subset of births with residential geocodes within 4 miles of each monitor; n=45,974 4,001 (8.7%) preterm	5 monitors provided daily air metrics, including 1-hour maximum SO_2, (including U.S. EPA AQS) Daily pollutant values were averaged over the exposure windows of interest as follows: • Late pregnancy: pollution levels in the 6 wks or last wk leading up to the study day (date of preterm birth) were averaged. The 6-wk or 1-wk average values were assigned to each day in the exposure window. • Early pregnancy: each study day was assigned the average pollution level in the 28 days after the estimated conception date.	Daily 1-hour maximum exposures for first month, final week, and final 6 wks of pregnancy First month: Mean (SD)= 10.5 (3.1) Min=3.9 Max=22.7 IQR=4.0 Final wk: Mean (SD)=10.3 (4.7) Min=1.4 Max=30.7 IQR=6.0 Final 6 wks: Mean (SD)=10.3 (2.6) Min=4.2 Max=18.8 IQR=3.0	Analyzed separately (correlation coefficients): $PM_{2.5}$ (r=-0.12) PM_{10} (r=-0.17) $PM_{2.5-10}$ (r=-0.17) $PM_{2.5}$ species (r=-0.22 - 0.66 [NO_3]) NO NO_2 (r=0.37) O_3 (r=-0.32) BC CO (r=0.44) Controlled for - long-term and seasonal trends in preterm birth - race/ethnicity - marital status - education - gestational week (late pregnancy exposures only) Checked for interactions between gestational age and socio-demographic variables	Small negative (protective) to no association between SO_2 and preterm birth RR for 1 IQR increase in SO_2 levels: Full cohort (5 counties): 1st month RR 0.97 (0.96, 0.99) Final wk RR 0.99 (0.98,1.01) Final 6 wks RR 0.99 (0.97,1.01) Capture-area analysis: 1st month RR 1.00 (0.97,1.03) Final wk RR 0.99 (0.96,1.02) Final 6 wks RR 0.98 (0.95,1.02)	SO_2 measurements were taken at 5 monitors in an area of 1,752 square miles. For the capture-area analysis, SO_2 data came from 3 monitors.

Table E1. Highlights of preterm birth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Bobak, 2000 Czech Republic 1990–1991	Singleton live births in 67 districts with air monitoring data n = 108,173 ~5,192 (4.8%) preterm (< 37 weeks) Cohort Robust logistic regression with districts as clusters	Air pollution monitoring system overseen by the Hydrometeorological Institute, Prague Mean of all 24-hr measurements by all monitors in the district in which an infant was born	Median trimester exposure: 12.2 25^{th} percentile: 6.7 75^{th} percentile: 21.2 Effects reported per 19.1 ppb (50 µg/m^3)	Analyzed separately (1^{st} trimester correlations): NO_x (r= 0.53) TSP (r=0.71) Controlled for - sex - parity - maternal age group - education - marital status and - nationality - month of birth	**AOR per 19.1 ppb increase in SO_2, by trimester:** **1^{st}: 1.27 (1.16, 1.39)** **2^{nd}: 1.25 (1.14, 1.38)** **3^{rd}: 1.24 (1.13, 1.36)**	No data on maternal smoking or ETS Author does not describe how large districts are Possible misclassification of exposure during pregnancy based on residence at date of delivery
Jiang et al., 2007 Shanghai, China 2004	3,346 preterm births (<37 weeks) Total number of births not reported Time series	6 monitoring stations; Shanghai Environmental Monitoring Center Used 24-hour averages for SO_2, PM_{10}, NO_2 and 8-hour averages for O_3 from all monitors to compute average exposures across the study area. <u>2 lag structures</u>: 1) Mean pollutant concentrations for 4, 6, and 8-wk periods preceding birth. 2) Single-day exposure window with lags of 0-6 days before birth.	Daily mean (SD)=21.25 (0.53) Minimum 4.31 25^{th} percentile 13.54 Median 19.64 75^{th} percentile 27.16 Maximum 62.25	Co-pollutants PM_{10} NO_2 O_3 Not clear whether co-pollutants were included in models with SO_2. Controlled for - time - temperature - humidity	↑ preterm birth rates associated with an ↑ of 3.81 ppb avg. SO_2 conc.: • 4-wk period before birth: 2.44% (-1.01%, 5.89%) • 6-wk period before birth: 0.90% (-3.26%, 5.06%) • **8-wk period before birth: 11.89% (6.69%, 17.09%)** Associations for 1-day exposure 0–6 day preceding birth were not statistically significant	Pollution data from all monitoring stations were averaged over the entire area of 279 km^2

Table E1. Highlights of preterm birth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Xu et al., 1995 Beijing, China	All singleton live first births to women over 20 years old in 4 areas in Beijing n = 25,370 ~819 (3.2%) preterm Prospective cohort Linear and logistic regression	WHO Global Environmental Monitoring System sites (2 monitors; all subjects lived within 5 km of a monitor) Daily measurements from each monitor 2 wks of pollutant data analyzed for each month	Annual mean concentrations at the 2 monitors: 41.2 and 35.5	TSP (analyzed separately and in multi-pollutant models) Controlled for - outdoor temperature -humidity - day of the week - season - gender - maternal age - residential area	**For each 38.1 ppb increase in SO_2 (7-day lagged moving average), duration of gestation was reduced 0.075 week (12.6 h) ($p < 0.01$)** Including TSP in model reduced effect by 32% **There was a clear dose-dependent relationship between gestational age and SO_2.** **AOR for preterm delivery = 1.21 (1.01, 1.46) for each $\log_e(\mu g/m^3)$ increase in SO_2**	Stable residential population, no major industries in the areas, bicycles were the primary mode of transportation Coal stoves were used in 97% of residences and were the major source of air pollution A random sample showed that the trend of indoor particulate concentration was similar to that of outdoor, regardless of the presence of coal stoves Early pregnancy exposures were not evaluated

Table E1. Highlights of preterm birth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Mohorovic, 2004 Labin, Croatia 1/1/1987–12/31/1989	704 women living near Plomin 1, a coal power plant n = 5 (0.7%) ≤28 wks n =14 (2%) 29–32 wks n =51 (7.2%) 32–37 wks Retrospective cohort Correlations (study design, analytic methods, and the exposures are not described in detail)	Calculated based on daily coal consumption at power plant	Monthly ground-level values in 3 locations in 1989, while plant was operating: 13.01– 96.46	No co-pollutants measured Collected data on relevant weather conditions (temperature, wind, precipitation) but did not report their inclusion in analyses No information on social and behavioral risk factors, such as education level and smoking	SO_2 exposure was associated with shorter gestation. Correlation between gestation length and prenatal exposure to SO_2 at: **End of the first month: -0.0914 (p=0.008)** **End of the second month: -0.0806 (p=0.016)** **Whole pregnancy: -0.0932 (p=0.007)** RR for prematurity (not defined) in 1987 (power plant operating all year) vs. 1988 and 1989 (plant not operated full capacity): 1.76	The coal used at the Plomin 1 power plant had especially high sulfur content and radioactivity Although the author reports emissions of multiple toxic products of coal combustion, only SO_2 was measured for a limited part of the study period, and effects are attributed to SO_2 alone Due to the unconventional methods used, this study is difficult to compare with other studies The author received funding from PP Plomin, the management company for Plomin 2

E.1.2. Fetal Growth

Twenty-two studies examined the association between fetal growth and exposure to SO_2 in some manner. These studies are summarized in Table E2. Outcomes examined include birthweight, LBW (defined as birthweight less than 2,500 g), very low birthweight (VLBW, defined as birthweight less than 1,500 g), fetal ultrasound measurements (e.g., femur length, abdominal circumference), and intrauterine growth restriction (IUGR) or small for gestational age (SGA). IUGR and SGA were defined as birthweight less than the 10^{th} percentile for gender and gestational age. In order to distinguish fetal growth restriction from prematurity, most fetal growth analyses were limited to full-term infants, defined as infants born at 37 wks gestation or later. In some studies there were also upper limits on gestational age, and some authors adjusted for gestational age even after limiting the study subjects to full-term LBW infants.

Fifteen of the 22 studies found significant associations between SO_2 and LBW or other measures of restricted fetal growth (Wang et al., 1997; Bobak et al., 1999; Ha et al., 2001; Maisonet et al., 2001; Lee et al., 2003; Liu et al., 2003; Yang et al., 2003; Lin et al., 2004a; Lin et al., 2004b; Mohorovic, 2004; Medeiros et al., 2005; Dugandzic et al., 2006; Williams et al., 2007; Hansen et al., 2008; Nascimento et al., 2009). One of these studies also reported that SO_2 exposure was associated with increased fetal growth for some exposure periods (Medeiros et al., 2005), while another reported decreased risk of LBW at some exposure levels in some populations (Maisonet et al., 2001).

Two studies reported decreased risk of IUGR or LBW, or increased birthweight (Gouveia et al., 2004; Liu et al., 2007). Five studies that assessed SO_2 exposures reported no statistically significant associations with fetal growth (Sakai, 1984; Bobak, 2000; Rogers et al., 2000; Bell et al., 2007; Brauer et al., 2008); among these, Brauer et al. (2008) had very low SO_2 levels and Rogers et al. (2000) found a non-significant association between SO_2 alone and VLBW, but had a small number of subjects. For their main analyses, Rogers et al. combined SO_2 concentrations with TSP and found that exposure to TSP and SO_2 *together* was statistically significantly associated with VLBW (Rogers et al., 2000).

As with studies of preterm birth, most studies of fetal growth were retrospective cohort studies that relied on birth certificate data to ascertain birthweight and gestational age. Many factors could be associated with both SO_2 exposure and birthweight, and thus, could confound the analyses. These potential confounders include pre-pregnancy weight, weight gain during pregnancy, maternal nutrition, exposure to active and passive smoking, history of adverse pregnancy outcomes, and occupational exposures; however, these factors often could not be studied if they were not included in the birth certificate data.

Three studies assessed *spatial* variation in SO_2 exposure without assessing temporal variation (Sakai, 1984; Bobak et al., 1999; Rogers et al., 2000). In these studies, if some of the unmeasured factors associated with birthweight, such as SES or access to prenatal care, vary over a geographic area, confounding could be an important problem.

For instance, mothers who have inadequate prenatal care might live in different areas than mothers who have good prenatal care. In this situation, the unmeasured factor (inadequate prenatal care) could confound the observed association between SO_2 and birthweight, with the potential for bias in either direction (toward or away from the null).

Most of the fetal growth studies assessed *temporal* variation in SO_2 exposure to some degree (many of these also assessed spatial variation). In studies that assess temporal variation in SO_2 exposure, such as the Lee et al. (2003) and the Nascimento et al. (2009) studies, there is somewhat less concern about confounding by unmeasured factors because these factors typically do not vary greatly over the gestational period.

The main disadvantage of measuring temporal variation in SO_2 exposure without also measuring spatial variation (e.g., (Ha et al., 2001; Medeiros et al., 2005)) is the loss of information inherent in assuming all people in an area have the same exposure at a given time. For example, people living very close to an SO_2 source might have greater exposure to SO_2, but if the study only measures changes in SO_2 exposure over time, the greater exposure due to their location would not captured. The study would be less likely to find an association between fetal growth restriction and SO_2, even if a true association exists.

Five of the six studies that clearly assessed exposure both temporally and spatially found SO_2 exposure was associated with poorer fetal growth (Yang et al., 2003; Lin et al., 2004b; Dugandzic et al., 2006; Williams et al., 2007; Hansen et al., 2008). The exception was the study by Brauer et al. (2008), which had a mean SO_2 concentration at the limit of detection. Studies that assessed only temporal or spatial variation in SO_2 exposure were less likely to find that SO_2 exposure was associated with restricted fetal growth.

Table E2 summarizes the epidemiologic studies of SO_2 as a risk factor for fetal growth restriction. This table provides more detail about the study methods, including exposure assessment, study population, consideration of co-pollutants and other covariates, statistical analyses, and study strengths and limitations. Results include measures of association and 95% CI if available. The findings of significant effects of SO_2, as reported by the authors, appear in bold.

Other Relevant Studies

Four additional studies reported SO_2 as a pollutant but had no measurements of SO_2 and were therefore not included in Table E2 (Nordstrom et al., 1978a, 1979a; Bhopal et al., 1994; Dolk et al., 2000). Summaries of these studies are available in Appendix 1.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Hansen et al., 2008 Brisbane, Australia 1993–2003	120 fetal ultrasound scans w/ the following measurements: - femur length (FL) - biparietal diameter (BPD) - head circumference (HC) - abdominal circumference (AC) Measurements were taken at 13–26 wks gestation from women referred to a private clinic for "routine ultrasound" and whose postcode centroid was within 2 km of an air pollution monitoring station. 84% of pregnancies had 1 ultrasound scan. Retrospective cohort Multi-stage regression to control for confounding and collinearity; generalized estimating equations w/ exchangeable correlation	18 monitoring stations (4 for SO_2) in and around Brisbane. Daily averages were calculated from hourly readings. Pollutant exposures and temperature for each day of gestation were assigned using the monitoring site closest to the mother's residential postcode. Avg. exposures were calculated for the first four 30-day periods of gestation	Mean IQR Summer 1.06 (0.84) Fall 1.01 (0.88) Winter 1.29 (1.06) Spring 1.46 (1.03) All 1.19 (1.00)	Modeled separately and in 2-pollutant models: PM_{10} NO_2 O_3 Models adjusted for: - fetal gender - gestational age - mother's age - temperature - seasonality - long-term trends - index of SES (derived from area- level attributes such as income, educational attainment, unemployment, and occupations)	**0.8 ppb ↑ in SO_2 exposure during days 0–30 was associated w/ ↓ in BPD -0.68 (-1.09, -0.27) mm** **SO_2 exposure during days 61–90 was associated w/ ↓ in mean fetal AC -1.67 (-2.94, -0.40) mm** Including co-pollutants in models did not change results for SO_2	The least complete data were for SO_2, with missing data for 61% of days. No individual data on maternal SES Multiple comparisons Whether the reductions observed in the study persisted until birth and had clinical relevance is unknown. SO_2 concentrations and variability in this study were very low.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Rogers et al., 2000 24 counties in Georgia that make up Georgia Health Care District 9 April 1986–March 1988	143 very low birthweight (VLBW; <1,500 g) cases 202 controls (≥2,500 g) born in the same area and time period Population-based case-control Linear and logistic regression	$TSPSO_2$ was defined as the sum of SO_2 and TSP, in $\mu g/m^3$. A Gaussian plume atmospheric transport model was used to estimate annualized ground-level TSP and SO_2 concentrations at the home of each study subject, using the following: 1) annual $TSPSO_2$ release data from 32 industrial facilities accounting for ~95% of releases, 2) annual meteorologic data, 3) geocodes of release points and birth residences, 4) distances between release points and birth residences. The model was validated against measurements taken by SO_2 and TSP monitors.	<u>Annual SO_2 (ppb)</u> Median: 1.45 75th percentile: 3.10 95th percentile: 5.49 <u>Annual $TSPSO_2$ ($\mu g/m^3$)</u> Median: 9.94 75th percentile: 25.18 95th percentile: 56.75	TSP (r=0.93) Combined with SO_2 as a single variable; also analyzed separately. Analyses adjusted for: - gender - race - toxemia during pregnancy - active and passive smoking - income - pre-pregnancy weight - maternal weight gain - maternal age - inadequate prenatal care - income - maternal and paternal education - illicit drug use - alcohol use - stress	AOR for VLBW and SO_2 exposure above the 95th percentile, compared to exposure < median, 1.49 (0.77, 2.89) AOR for $TSPSO_2$ exposure > 95th percentile, compared to exposure < median, 2.88 (1.16, 7.13)	Mean gestational age was 28.8 wk for cases and 39.5 wk for controls. However, gestational age seems to have been excluded from the final model, in which case the model is likely mixing prematurity and growth restriction. Because TSP and SO_2 were combined as a single exposure, this study is difficult to compare with other studies. The exposure assessment in this study does not take into account other pollutants or seasonal or other temporal variation in exposure.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Brauer et al., 2008 Vancouver, Canada 1999–2002	Singleton births n = 70,249 897 (1.3%) term LBW 7,217 (10.3%) SGA Population-based cohort Logistic regression	Data from the regulatory monitoring network (British Columbia Ministry of Environment and Metro Vancouver), including 14 monitors for SO_2 3 approaches to assigning SO_2 exposure: Nearest monitor to residence postal code (~ 1 block face in urban areas) and w/in 10 km IDW by 3 monitors nearest to postal code w/in 50 km Proximity to major roads (not specific to SO_2) Exposure windows: - entire pregnancy - 1st month - last month - 1st 3 months - final 3 months	Monthly mean exposures, modeled by IDW: Mean=2.02 Min=0.11 Max=6.79 IQR=1.14	Analyzed separately: $PM_{2.5}$ PM_{10} NO NO_2 O_3 BC CO Correlations among IDW estimates for CO, NO, NO_2, and SO_2 were all > 0.8. IDW correlations between SO_2 and $PM_{2.5}$ and PM_{10} were not reported Controlled for - gender - First Nations status - parity - maternal age - maternal smoking during pregnancy - month-year of birth - income (quintile-census) - education (quartile-census)	AORs using IDW, per 0.38 ppb increase in SO_2 for entire pregnancy: LBW: 0.98 (0.96, 1.01) SGA: 1.01 (1.00, 1.02)	Limit of detection for the method used to measure SO_2 was 2 ppb. 7.4% of the cohort reported maternal smoking during pregnancy. Air pollution concentrations were low relative to air quality standards and international guidelines ORs for late and early pregnancy windows were similar to those for full duration of pregnancy Data on education and income are based on census at neighborhood-level

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Nascimento et al., 2009 São José dos Campos, Brazil 2001	Inclusion criteria: - singleton birth - vaginal delivery - 37–41 wks' gestation - age 20–34 years - complete high school education - at least seven prenatal visits n=2,529 births 99 (3.95%) LBW Retrospective cohort Logistic regression	Daily pollutant data from two monitoring stations located 3 km apart and inside the city limits. Pollutant data were collected for 24-hour periods, summed for the 90 days preceding each birth, then grouped by quartile.	90-day sums for SO_2: Mean (SD) 204.3 (39.6) Min 139.1 Max 284.1 (Corresponding with daily avg. of 2.27)	Co-pollutants: - O_3 (negatively correlated with SO_2) - PM_{10} (r=0.88) PM_{10} was not significant in multi-pollutant models and was therefore dropped. No adjustment for other factors reported; authors defined a restricted cohort instead.	ORs for LBW and SO_2, by exposure quartile (1^{st} quartile is referent): 2^{nd}: **3.39 (1.54, 7.53)** 3^{rd}: **2.89 (1.33, 6.34)** 4^{th}: 2.14 (0.96, 4.82) **AOR for exposure > 1^{st} quartile, adjusted for O_3: 1.30 (1.02, 1.65)**	Exposure assessment in this study is unlike that of other studies and is not reported in great detail. Ambient pollutant levels are not reported. Data from the two pollution monitors represented an area of 1,100 km^2 with many industrial establishments and an oil refinery. Although the authors restricted the cohort to births at 37–41 wks gestation, gestation length could still account for differences in birthweight.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Williams, et al., 2010 Tennessee 2002	13,559 (95%) of 14,194 births whose address could be geocoded Retrospective cohort Hierarchical linear modeling; - Level 1 was individual level - Level 2 unit was defined as the residential area within a census tract that shares the same 3 monitors for lead, SO_2, and $PM_{2.5}$	Ambient SO_2 from the nearest monitor, averaged over 1st trimester	1st trimester average SO_2: Average: 3.4 SD 1.1 99th percentile: 5.1 Maximum: 8.0	Analyzed together: - Lead - $PM_{2.5}$ (dropped) Level 1 variables: - multiple birth - gender - prior preterm infant - prior infant >4000 g - hypertension (pregnancy) - chronic hypertension - oligohydramnios - pre-eclampsia - pregnancies w/o live births - education - smoking - race/ethnicity - other maternal risk factors - # hazardous waste sites w/in 5k Level 2 variables - % below poverty in the census tract - Interaction of maternal risk factors & poverty	**Adjusted estimated ↓ in birthweight was 61 g per 1.1 ppb SO_2.** **For the highest SO_2 levels, compared to no SO_2 exposure, the ↓ in birth weight would be 304-440 g.** A smaller but significant ↓ was also observed for lead, but no result was reported for $PM_{2.5}$.	Observed effect is extremely large. Exposure assessment is not reported in detail, e.g., authors did not address distances between residences and monitors. If preterm births were included, some of the observed effects on birth weight may be attributable to effects on prematurity. O_3, NO_2, CO, and PM_{10} were not included in analyses.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Liu et al., 2007 Calgary, Edmonton, and Montreal, Canada January 1986–June 2000	Full-term (37–42 weeks), singleton live births n=386,202 ~42,096 (10.9%) IUGR (birthweight < 10th percentile, by sex and gestational week) Retrospective cohort Logistic regression	Concentrations of pollutants were collected from monitoring stations (4 in Calgary, 2 in Edmonton, 8 in Montreal). 24-hour avg. pollutant concentrations were matched with birth dates and gestation length to estimate air pollutant concentrations in each month, trimester, and the whole pregnancy for each birth.	Daily avg. SO_2 concentrations: Mean 3.9 25th percentile 2.0 Median 3.0 75th percentile 5.0 95th percentile 10.0. Daily 1-hour maximum SO_2 concentrations: Mean 10.8 25th percentile 5.0 Median 8.6 75th percentile 14.0 95th percentile 28.0.	Correlation coefficients: NO_2 (r=0.34) CO (r=0.21) O_3 (r=–0.30) $PM_{2.5}$ (r=0.44) Analyzed separately and in multi-pollutant models (but not with SO_2) Analyses controlled for: - maternal age - parity - infant gender - season of birth - city of residence	**3 ppb ↑ in SO_2 in the 1st trimester was associated with a ↓ in risk of IUGR; AOR ≅ 0.96, statistically significant.** **Risk of IUGR ↓ slightly with a 3.0 ppb ↑ in SO_2 exposure during each of the 1st 3 months of pregnancy; AORs ≅ 0.96 to 0.98, statistically significant.** ORs increased with later trimester exposures, but all remained < 1.	Information on risk factors for IUGR, such as race, education, smoking, caloric intake, alcohol use, paternal height, and socioeconomic status were not available. However, these factors are not likely to vary with changes in ambient pollutant levels and therefore should not confound associations between pollution and fetal growth restriction. Edmonton and Calgary had few monitoring stations but cover relatively large areas, so the assumption that all mothers in these cities had the same exposure may have caused substantial exposure misclassification.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Bell et al, 2007 Massachusetts and Connecticut 1999–2002	All registered births of 32-44 weeks' gestation in 15 counties (7 for SO_2), with adequate data on gestation, exposure, etc. n = 358,504 ~14,340 (4.0%) LBW Retrospective cohort Linear and logistic regression	U.S. EPA monitoring data Exposure was assigned as the avg. daily county-level conc. over gestation and each trimester based on mother's residence. Each county's daily avg. was based on measurements from a single monitor or averaged from multiple monitors within a county.	Mean (SD) 4.7 (1.2) IQR = 1.6	Analyzed separately and in 2 pollutant models (but not with SO_2): $PM_{2.5}$ PM_{10} NO_2 CO Correlations w/ SO_2 were not reported. Models adjusted for: - gestational length - month prenatal care began - child's gender - birth order - year - mother's race - education - marital status - mother's age - tobacco use during pregnancy - apparent temperature	1 IQR ↑ in SO_2 exposure over gestational period was associated with: - ↓ birthweight 0.9 (-4.4, 2.6) - OR for LBW = 1.00 (0.96, 1.05) Other pollutants were significantly associated with ↓ birthweight and/or LBW.	Avg. conc. for all pollutants in the study were below the U.S. EPA health-based National Ambient Air Quality Standards (NAAQS), and all counties in the study were in compliance with the NAAQS for SO_2, NO_2, CO, and PM_{10}. In addition to low levels, the SO_2 levels showed little variability, thereby decreasing the chance of observing an effect. The study does not provide any information on the distance between monitors and residences.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Liu et al., 2003 Vancouver, Canada Study period: 1985–1998	Singleton live births n = 229,085 ~9,163 (4.0%) LBW (<2500 g) ~21,534 (9.4%) IUGR (<10th percentile for sex and gestational week) Retrospective Cohort Multiple logistic regression	Continuous sampling measures from 13 census subdivisions in the Vancouver area Daily avg. conc. of SO_2, CO, NO_2, and O_3 5 years of PM_{10} data analyzed	Daily average SO_2: Mean: 4.9 25th percentile: 2.8 75th percentile: 6.3 95th percentile: 10.5 Mean daily maximum (1-hr concentration): 13.4 Gestational exposure windows evaluated: - 1st, 2nd, and 3rd month; - the last and next to last month; and, - 1st, 2nd, and 3rd trimesters	Correlations with co-pollutants: NO_2 (r = 0.61) CO (r = 0.64) O_3 (r = -0.35) Analyzed in single and multi-pollutant models. Controlled for - maternal age - parity, - infant sex - gestational age - season of birth	AOR for each 5 ppb increase in SO_2 exposure in: <u>LBW</u> - **1st month: 1.11 (1.01, 1.22)** - Last month: 0.98 (0.89, 1.08) - **1st month, adjusting for gaseous co-pollutants 1.29 (1.12, 1.50)** <u>IUGR</u> - **1st trimester:** - **1.07 (1.00, 1.14)** - 2nd trimester: 0.98 (0.91, 1.04) - 3rd trimester: 1.03 (0.96, 1.10) - **1st trimester, adjusting for gaseous co-pollutants, significance "tended to persist" (no ORs were reported)**	Results were reported for single pollutant models and selected exposure windows. Summary results were reported for multiple pollutant models. Exposures were averaged over the entire study area. No data on maternal smoking, ETS, and SES Limited data and analyses on PM_{10}

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Lin et al., 2004(a) Taipei and Kaohsiung, Taiwan 1995–1997	92,288 full-term births to mothers living within 3 km of an air quality monitoring station (AQMS) 2,069 (2.2%) LBW Retrospective cohort Multivariate linear and logistic regression	10 AQMS (5 in each city) Exposure to each pollutant was estimated as the mean of all daily measurements taken in each trimester and the entire pregnancy by the AQMS nearest to the mother's district of residence at the time of birth.	Mean SO_2 level, all AQMSs in Taipei: 4.9 Mean SO_2 level, all AQMSs in Kaohsiung: 17.0 Mean exposure levels for each trimester and the entire pregnancy were categorized as - *low* (<25th percentile) - *medium* (25th – <75th percentiles - *high* (>75th percentile) Medium exposure categories (25th – 75th percentiles), by trimester: 1st 6.8 – 11.5 2nd 6.9 – 11.9 3rd 6.8 – 12.4 entire pregnancy: 7.1 – 11.4	Analyzed in multi-pollutant models: CO PM_{10} NO_2 O_3 Covariates: - gestational age - gender - birth order - maternal education - season of birth - maternal occupation - maternal residence	ORs for LBW andexposure periods and levels (low exposure is referent): <u>Whole pregnancy</u> **Medium 1.16 (1.02, 1.33)** **High 1.26 (1.04, 1.53)** <u>1st trimester</u> Medium 1.02 (0.90, 1.16) High 1.11 (0.94, 1.33) <u>2nd trimester</u> Medium 1.09 (0.96, 1.24) High 1.17 (0.99, 1.37) <u>3rd trimester</u> Medium 1.13 (0.99, 1.28) **High 1.20 (1.01, 1.41)** Medium and high exposures to CO were associated with lower risk of LBW. PM_{10}, NO_2, and O_3 generally did not have statistically significant associations with risk of LBW.	Authors noted unadjusted risk factors such as maternal nutrition, maternal weight gain during pregnancy, maternal height, smoking, intensity of prenatal care, and maternal occupational exposure. Correlations among pollutants were not reported.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Gouveia et al., 2004 São Paulo, Brazil, 1997	Singleton, full term (≥ 37 weeks) live births with birthweight 1,000 g – 5,500 g. n=179,460 ~8,973 (5.0%) LBW (<2,500 g) Mean birthweight = 3,186 g Retrospective cohort Generalized additive models, linear and logistic regression	Daily means of SO_2 from 12 monitoring sites Pollutant levels were avg. across all sites for daily citywide levels.	Annual mean (SD) 7.5 (3.9) Annual minimum 1.3 ppb Annual maximum 21.7 ppb.	Analyzed separately and in multi-pollutant models: NO_2 CO O_3 PM_{10} (correlations were not reported) Analyses adjusted for: - infant gender - gestational age - month of birth - maternal age - maternal education - prenatal care - type of delivery	**2nd trimester SO_2 exposure was associated with ↑ birthweight: 33.7 g (1.6, 65.8) per 3.8 ppb ↑ in SO_2.** Slightly ↓ risk of LBW associated with SO_2 exposure in the 1st trimester: **OR 0.90 (0.84, 0.97) for the 2nd quartile compared to the 1st**	The linear and logistic regression findings are inconsistent.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Dugandzic et al., 2006 Nova Scotia, 1988–2000	All term (≥37 weeks) singleton births ≥500 g, with adequate exposure data n=74,284 1,193 (1.6%) LBW Retrospective cohort Logistic regression	18 monitors measured SO_2 and O_3 levels daily and PM_{10} every 6 days (only 3 monitored more than one pollutant). There were no SO_2 data for 1997. Authors developed individual exposure profiles for each mother by geocoding the postal code, town or village place name, or municipal code for the residence at the time of delivery. Only mothers living within 25 km of a monitoring station were included in the analysis. Daily pollutant data were averaged over trimesters for which ≥75% of the daily values for a period were available.	Trimester SO_2 exposure levels: mean=10 25th percentile=7 median=10 75th percentile=14 max=38 IQR=7	Modeled separately: O_3 PM_{10} Models adjusted for: - maternal age - parity - prior fetal death - prior neonatal death - prior LBW infant - smoking during pregnancy - infant gender - gestational age - weight change - year of birth - neighborhood family income	The largest difference in mean birthweight between the bottom and top quartiles of exposure was for 1st trimester SO_2: 3,467 g vs. 3,428 g. **Adjusted RR for LBW and highest quartile of SO_2 exposure in the 1st trimester (w/o adjustment for year): 1.36 (1.04, 1.78)** Adjusted RR (w/ year): 1.26 (0.96, 1.66) Adjusted RR for SO_2 in 1st trimester, per 1IQR increase: 1.15 (1.00, 1.31) A dose response for 1st trimester SO_2 and ↓ birthweight was suggested. The highest quartile of first trimester PM_{10} exposure was associated w/ LBW (w/o year).	Did not analyze the possible effects of several other potentially important pollutants Correlations among pollutants were not reported. There was a statistically significant trend for decreasing LBW rates over time ($p<0.001$). Authors also reported a statistically significant trend in SO_2 levels.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Yang et al., 2003 Kaohsiung, Taiwan 1995–1997	13,396 singleton, full-term (≥ 37 gestation weeks) live births to first-time mothers residing within ~2 km of a monitoring station. ~308 (2.3%) LBW (<2,500g) Mean birthweight = 3,185 g Retrospective cohort Linear regression	Daily air pollutant concentrations came from 6 air monitors maintained by the Taiwan EPA. Pollutant readings were averaged over each trimester of each pregnancy based on the birth date and gestational age of each child. Trimester pollutant averages were divided into 3 tertiles of exposure levels: - "low" (≤ 33rd percentile; comparison group) - "moderate" (33rd - 67th percentile) - "high" (> 67th percentile)	<u>1st trimester</u> 33rd percentile 9.92 67th percentile 13.76 <u>2nd trimester</u> 33rd percentile 9.83 67th percentile 13.59 <u>3rd trimester</u> 33rd percentile 9.68 67th percentile 14.10	PM_{10} (analyzed separately. Correlations between SO_2 and PM_{10} were 0.45 - 0.46 in the three trimesters). Models adjusted for: - maternal age - season - marital status - maternal education - gender of baby - gestational age	High vs. low SO_2 exposure during the 1st trimester was associated with **adjusted 18.11 (1.88, 34.34) ↓ in birthweight** Associations were weaker and non-significant for the 2nd and 3rd trimesters. **0.38 ppb ↑ in SO_2 (continuous variable) in the 1st trimester was associated with a 0.52 g (0.09, 2.63) ↓ in mean birthweight.** PM_{10} exposures in the 1st and 3rd trimesters were also associated with ↓ birthweight.	Analyses with both SO_2 and PM_{10} were not reported. No attempt to account for the correlation among trimester exposures for each pollutant is reported. The authors lacked information on maternal nutrition, pre-pregnancy weight and weight gain, cigarette smoking, occupational exposures, and income. However, 3-4% of Taiwanese women smoke, so smoking was not likely to be a significant confounder. The unmeasured factors were unlikely to be associated with pollution levels and therefore probably did not confound the study estimates.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Lee et al., 2003 Seoul, Korea 1996–1998	Full-term (37–44 weeks gestation) singletons n = 388,105 ~11,255 (2.9%) LBW (<2500g) Retrospective cohort Generalized additive logistic regression	20 monitoring stations covered nearly all areas of the city. Hourly measurements across all monitoring stations were combined to calculate 24-hour means. These means were used to estimate exposures during the entire pregnancy, each trimester, and each month of pregnancy.	Mean: 12.1 Min: 3.0 25th percentile: 6.8 Median: 9.8 75th percentile: 15.6 Max: 46.0 IQR: 8.8	Pearson correlation coefficients for SO_2 and each co-pollutant in 1st, 2nd, and 3rd trimesters, respectively: CO 0.79, 0.86, 0.86 PM_{10} 0.78, 0.82, 0.85 NO_2 0.75, 0.77, 0.76 Co-pollutants were analyzed separately. Covariates - infant gender - birth order - maternal age - parental education - time trend - gestational age - birth year (season and parental occupation were available but did not improve models)	LBW was significantly associated with exposure to each of the pollutants in the first two trimesters. Adjusted ORs for LBW per 8.8 ppb SO_2, by trimester: 1st 1.02 (0.99, 1.06) **2nd 1.06 (1.02, 1.11)** 3rd 0.96 (0.91, 1.00) **All trimesters 1.14 (1.04, 1.24)** **For each 8 ppb ↑SO_2 in the 2nd trimester, birthweight ↓ 14.6 g** (p-value not reported) There was a positive dose response relationship between LBW and SO_2 exposure in the 2nd trimester (p-value not reported). Risks of LBW tended to ↑ with SO_2 exposure in months 3 to 5.	The major source of air pollution was automobile exhaust. The authors report that 2nd trimester exposures to SO_2, PM_{10}, and NO_2 were associated with LBW after controlling for CO, although the results are difficult to interpret because of collinearity among pollutants. No other multipollutant analyses were reported.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Bobak and Leon, 1999 45 districts in the Czech Republic 1986-1988	n=223,929 births in monitored districts ~13,212 (5.9%) LBW (<2500 g) Ecologic Conditional logistic regression for grouped data (outcome and exposure data were aggregated by district)	National Public Health Service monitored air pollution and provided geometric means of all daily readings in all stations in each district. Most districts had at least three monitoring stations, generally in the principal towns of the districts.	Annual geometric mean SO_2 conc: Mean: 12.2 ppb Min: 1.7 Max: 41.0 25th percentile: 5.3 75th percentile: 20.1	TSP NO_x Modeled separately and together (correlations were not reported). District-level socioeconomic factors included: - mean income - car ownership - divorce rate - mean savings - % births outside marriage - induced abortion rate - % gypsies in the population	AORs for LBW per 19.1 ppb ↑ SO_2: **Adjusted for socioeconomic factors: 1.10 (1.02, 1.17)** **Adjusted for socioeconomic factors and copollutants: 1.10 (1.01, 1.20)**	Wide variation in SO_2 levels Use of annual means caused exposure misclassification by assigning exposure data incorrectly to births early in the year. The Czech Republic had some of the highest levels of air pollution in Europe during the study period.
Bobak, 2000 Czech Republic 1990–1991	108,173 singleton live births in 67 districts with air monitoring data. ~5,625 (5.2%) LBW Cohort Robust logistic regression with districts as clusters	Air pollution monitoring system (Hydrometeorological Institute, Prague) 24-hour measurements from all monitors in a district were averaged for each trimester	Median trimester exposure: 12.2 25th percentile: 6.7 75th percentile: 21.2	Analyzed separately (correlations in 1st trimester): NO_x (r= 0.53) TSP (r=0.71) Controlled for - gender - parity - maternal age group - education - marital status - nationality - month of birth - gestational age	AOR for LBW per 19.1 ppb ↑ in SO_2: 1st trimester 1.01 (0.88,1.17) 2nd trimester 0.95 (0.82, 1.10) 3rd trimester 0.97 (0.85, 1.10)	No data on maternal smoking or ETS. Author does not describe how large districts are. Author states SO_2 may be an indirect measure of small respirable particles, which were not monitored and may underlie the observed association between SO_2 and LBW.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Ha, 2001 Seoul, Korea 1996–1997	Full-term (37–44 wks gestation) singleton births n= 276,763 ~7,749 (2.8%) LBW Mean birthweight 3,310 g Retrospective cohort Generalized additive logistic regression	21 monitoring stations covered 84% of the area. Hourly measurements across all monitoring stations were combined to calculate 24-hour means. These means were used to estimate exposures during the first and third trimester of pregnancy.	1st trimester 25th percentile 10.0 Median 13.2 75th percentile 16.2 2nd -3rd trimester 25th percentile 8.4 Median 12.2 75th percentile 16.3	Correlations between SO_2 and co-pollutants: - CO 0.83 - NO_2 0.70 - TSP 0.67 - O_3 -0.29 Co-pollutants were analyzed separately. Covariates - gestational age - maternal age - parental education level - parity - infant gender	**For each IQR (6.2 ppb) SO_2 during the 1st trimester, RR 1.06 (1.02, 1.10)** When both 1st and 3rd trimester exposures were included in the models, the ↑ risk for LBW for SO_2, CO, NO_2, and TSP persisted for the 1st trimester, while the 3rd trimester risk changed toward the null for all pollutants: 1st trimester RR 1.07 (0.98, 1.17) and 3rd trimester RR 1.03 (0.90, 1.17) **6.2 ppb ↑ in SO_2 during 1st trimester was associated with 8.06 (5.59, 10.53) g ↓ birthweight**	Ha et al. note that the pollutants were highly correlated, that secondary particles are formed in the atmosphere by chemical reactions involving SO_2 and NO_2, and that it is therefore reasonable to consider these pollutants together rather than separately. However, multi-pollutant models are not reported in this study. The authors lacked information to adjust for individual risk factors such as smoking and alcohol consumption; however, they assert that such risk factors would not be associated with ambient air pollution levels.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Lin et al., 2004(b) Taipei and Kaohsiung, Taiwan 1995–1997	92,288 full-term births to mothers living w/in 3 km of an AQMS 1,276 (2.1%) LBW in Taipei 757 (2.4%) LBW in Kaohsiung Retrospective cohort Exposure of interest was residence in Kaohsiung, a petrochemical industrial city) Multivariate logistic regression	10 AQMS (5 in each city) Exposure to each pollutant was estimated as the mean of all daily measurements taken in each trimester and the entire pregnancy by the AQMS nearest to the mother's district of residence at the time of birth. However, these measurements were not used in analyses.	<u>Kaohsiung</u>: Mean 17.0 Range 10.07– 25.36 <u>Taipei</u>: Mean 4.9 Range 5.65–9.33	Measured but not included in analyses: CO PM_{10} NO_2 O_3 Covariates: - gestational age - gender - birth order - maternal education - season of birth - maternal residence	**LBW risk in Kaohsiung was 13% higher than in Taipei, AOR 1.13 (1.03, 1.24)**	Authors state they have no evidence regarding which specific air pollutant was responsible for the higher risk of LBW for births in Kaohsiung. Few women living in Taipei were exposed to high levels of SO_2 or PM_{10}; in Kaohsiung, almost all women were exposed to high levels of SO_2 and PM_{10}.
Sakai, 1984 4 districts near Tokyo Bay, Japan 1975–1977	LBW rate = 5.73 n=2,767 LBW births Ecologic Correlations	Air Pollution Monitoring System in 3 districts	Annual district means: <u>1975</u> Tsurumi 23 Kanagawa 12 Totsuka 11 <u>1976</u> Tsurumi 17 Kanagawa 14 Totsuka 12 <u>1977</u> Tsurumi 15 Kanagawa 13 Totsuka 9	Analyzed separately: NO_2 NO Dust Correlations among co-pollutants were not reported No covariates were considered	Correlation coefficient for SO_2 and LBW rate: $r = 0.627$, $p < 0.10$	The number of monitors is not reported. For births earlier in the year, use of annual mean pollutant concentrations causes misclassification because much of the exposure of those births occurred In the prior year.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Wang et al., 1997 4 areas in Beijing, China (area covered 45 km^2) 1988–1991	74,671 live, first-parity, full term (37-44 weeks), single births weighing > 1000 g. Mothers were at least 20 years old at the start of pregnancy. ~2,195 (2.94%) LBW (<2500 g) Mean birthweight 3,318 g Retrospective cohort Multiple linear and logistic regression	World Health Organization (WHO) monitors in 2 of the areas. Mean pollutant conc. measured at the 2 monitors were used as the daily air pollution levels. Authors analyzed 2–3 wks of daily air samples each month, starting in the 2nd wk of the month. Measurements were highly correlated between the 2 monitors for each pollutant (0.92 for SO_2 and 0.93 for TSP). Several exposure variables for SO_2 and TSP were evaluated: 1) entire pregnancy mean; 2) trimester mean; and 3) lagged moving averages. The best-fitting models included the mean exposure during the 3rd trimester.	Mean 3rd trimester SO_2 concentrations, in quintiles: 1st 3.4–6.9 (referent) 2nd 6.9–21.0 3rd 21.0–55.7 4th 55.7–91.2 5th 91.2–117.5	Analyzed separately: TSP (highly correlated with SO_2; coefficient not reported) Models adjusted for: gestational age season (some analyses) residential area maternal age (20–24, 25–29, 30+ years) gender year of birth	<u>For 3rd trimester exposure:</u> ↑ risk of LBW associated with high SO_2 exposure (4th and 5th quintiles) compared to low exposure (1st quintile). AORs for quintiles: 2nd: 1.09 (0.94, 1.26) 3rd: 1.12 (0.97, 1.29) **4th: 1.16 (1.01, 1.34)** **5th: 1.39 (1.22, 1.60)** **AOR per 38 ppb SO_2: 1.11 (1.06, 1.16)** **Adjusted ↓ in birthweight was 7.3 g per 38 ppb SO_2.** Test for linear trend (dose -response) was highly significant (p-value not reported). Results were similar for TSP (AOR=1.10 per 100 µg/m^3 TSP).	Coal is used for heating or cooking in 97% of households and is the major source of air pollution. Authors note that due to high correlation between SO_2 and TSP, it is difficult to determine the relative importance of each pollutant. The authors were unable to examine factors such as maternal nutrition, weight gain, and smoking. However, such factors are unlikely to be related to daily pollution exposures. Authors state the proportion of LBW that was attributable to air pollution was 13%, which is among the highest attributable risks ever reported for known risk factors of LBW.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Mohorovic, 2004 Labin, Croatia 1/1/1987 – 12/31/1989	704 women living near Plomin 1, a coal power plant 0.9% < 999 g 0.1% 1000 –1499 g 6.1% 1500 –2499 g 92.9% >2500 g Retrospective cohort Correlations	Calculated based on daily coal consumption at power plant	Monthly ground-level values in 3 locations in 1989, while plant was operating: 13.01–96.46	No co-pollutants measured. Collected data on relevant weather conditions (temperature, wind, precipitation) but did not report their inclusion in analyses. No information on social and behavioral risk factors, such as education level and smoking.	Early pregnancy exposure to SO_2 was negatively correlated with birthweight (i.e., higher SO_2 exposure was correlated with lower birthweight): **End of 1st month r = –0.0807 (p=0.016)** **End of 2nd month r = –0.0733 (p=0.026)**	Study design, analytic methods, and the exposures are not described in detail. The coal used at the Plomin 1 power plant had especially high sulfur content and radioactivity. Although the author reports emissions of multiple toxic products of coal combustion, only SO_2 was measured for a limited part of the study period, and effects are attributed to SO_2 alone. The author received funding from PP Plomin, the management company for Plomin 2.

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Maisonet et al., 2001 1994–1996 Boston, MA; Hartford, CT; Philadelphia, PA; Pittsburgh, PA; Springfield, MA; and Washington, D.C	Singleton, term (37–44 wks gestation), live births n = 89,557 3.7% LBW (<2,500 g) Retrospective cohort Linear and logistic regression, with multistage regression models to adjust for clustering by study area.	Hourly monitor readings of SO_2 and CO for each day and 24-hr readings of PM_{10} for every 6 days, from U.S. EPA. For SO_2, there was 1 monitor in Boston and Hartford, 2 in Philadelphia and Springfield, 3 in Washington, and 4 in Pittsburgh. Monitoring data were used to estimate average ambient pollutant levels for each trimester. Exposure level was categorized by percentile: A: ≤25 percentile (referent) B: 25 to <50 percentile C: 50 –75 percentile D: 75 to <95 percentile E: ≥95 percentile	<u>1st trimester</u> 25th percentile 7.1 Median 8.9 75th percentile 12.0 <u>2nd trimester</u> 25th percentile 6.6 Median 8.9 75th percentile 12.0 <u>3rd trimester</u> 25th percentile 5.8 Median 8.5 75th percentile 11.8 (Corrected SO_2 concentrations were provided by the author through personal communication.)	Analyzed separately: CO PM_{10} Adjusted for: - gestational age - gender - birth order - maternal age - race/ethnicity, - education - marital status - adequacy of prenatal care - previous induced or spontaneous abortions - weight gain during pregnancy - maternal prenatal smoking and alcohol consumption	For all races combined, SO_2 was associated with both increases and a decrease in risk: AORs for LBW and 2nd trimester SO_2 compared to Level A: **B 1.18 (1.12, 1.25)** **C 1.12 (1.07, 1.17)** **D 1.13 (1.05, 1.22)** **E 0.87 (0.80, 0.95)** <u>For White mothers,</u> a **10 ppb ↑ in SO_2 was associated with ↑ risk of LBW in <u>each</u> trimester (AORs 1.18-1.20, all significant).** <u>For African American mothers,</u> **SO_2 was associated with: Overall ↓ risk of LBW for 1st trimester SO_2; AOR per 10 ppb SO_2 0.91 (0.85, 0.98)** **However, LBW risk ↑ with 2nd trimester SO_2 Levels B & C. AORs:** **B 1.22 (1.14, 1.32)** **C 1.07 (1.04, 1.11)**	The authors state that correlations among SO_2 readings for any two monitors within a city ranged from 0.43 to 0.91, suggesting low heterogeneity within study areas. SO_2 levels reported are high, although the authors state that the concentrations of the pollutants were "well below the established standards"

Table E2. Highlights of fetal growth studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Medeiros and Gouveia, 2005 Sao Paulo, Brazil 1998–2000	311,735 singleton, full-term (37–41 wks gestation), live births with birthweight >1,000 g and <5,500 g, to mothers residing in the more central districts. ~14,388 (4.6%) LBW Retrospective cohort Multivariate linear and logistic regression	14 monitoring stations (6 for SO_2) Daily mean concentrations were used to calculate mean concentrations of each pollutant in the three trimesters of pregnancy, based on the date of birth of each child. For logistic regression models, pollutant levels were categorized into quartiles based on trimester means	Not reported	Analyzed separately: PM_{10} CO O_3 NO_2 Covariates: - infant gender - prenatal care - type of delivery - year of birth - maternal age - maternal education - number of living children - number of deceased children	SO_2 exposure in the 1st trimester was associated with lower birthweight, while later exposures were associated with increased birthweight (per 0.38 ppb increase in SO_2): **1st trimester: -1.3 g (-1.7, -0.8)** **2nd trimester: 0.7 g (0.2, 1.3)** **3rd trimester: 2.0 g (1.5, 2.6)** Other pollutants were also associated with reduced birthweight; 1 ppm increase in CO was associated with 11.9 g reduction No statistically significant associations between LBW and SO_2 or other pollutants were found by logistic regression.	Although correlations among the pollutants were not reported, the authors state that it is difficult to isolate the effects of each individual pollutant due to strong correlations between the different pollutants. Maternal risk factors for LBW such as smoking and malnutrition were not included in the analysis. Pollutant concentrations might vary considerably across the city, but the authors appear to have averaged the concentrations from all monitors for each pollutant. Authors conclude that CO is more relevant to birthweight.

E.1.3. Pregnancy Loss (Stillbirth and Spontaneous Abortion)

Three ecologic studies examined the association between SO_2 and stillbirth, and one cross-sectional study examined spontaneous abortion. Of the stillbirth studies, only one assessed temporal variation in SO_2 concentrations (Pereira et al., 1998); the others used district-wide annual SO_2 levels (Sakai, 1984). Two of the stillbirth studies reported no significant associations with SO_2. Sakai (1984) reported a significant positive correlation between spontaneous fetal death (presumably stillbirth) and SO_2, but did not estimate the increase in risk associated with SO_2 exposure, and NO_2 was more strongly correlated with spontaneous fetal death.

The spontaneous abortion study was an occupational study in Finland which used pollution maps for SO_2 exposure assessment; this study reported no association between SO_2 and spontaneous abortion (Hemminki et al., 1982).

Table E3 summarizes the epidemiologic studies of SO_2 as a risk factor for stillbirth and spontaneous abortion.

Other Relevant Studies

Four additional studies that examined spontaneous abortion (Nordstrom et al., 1978b, 1979a; Beckman et al., 1982; Smrcka et al., 1998) and three studies that examined stillbirth (Beckman et al., 1982; Bhopal et al., 1994; Dolk et al., 2000) are not discussed above or in Table E3 because they lacked measurement of SO_2 exposures. These studies are summarized in Appendix 1.

Table E3. Highlights from studies of pregnancy loss (stillbirth and spontaneous abortion) (significant SO_2 findings in bold).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Pereira et al., 1998 São Paulo, Brazil 1991–1992	Stillbirth (death after 28 wks gestation) The total number of subjects was not reported, but the mean (SD) was 8.36 (3.08) stillbirths per day, or approximately 6,111 stillbirths over the study period. Ecologic Time series	The city of São Paulo's air pollution control agency provided daily pollution conc.; 14 stations collected SO_2 data Daily values were averaged to arrive at single citywide measures for each pollutant	Mean ± SD= 7.2 ± 3.3 ppb Minimum= 1.4 Maximum= 22.8	Correlation coefficients with SO_2: NO_2: 0.41 CO: 0.34 O_3: 0.15 PM_{10}: 0.74 Pollutants were modeled separately and together. Covariates - lowest daily temperature - humidity (as both continuous and indicator variables) - month of study - day of week	Adjusted RR for intrauterine death (model w/ SO_2 only) =1.00 (1.00, 1.01) per 0.38 ppb change in SO_2 Fully adjusted model with all co-pollutants and other covariates: Adjusted RR = 1.00 (1.00, 1.01) per 0.38 ppb change in SO_2 Adjusted RR = 1.05 (0.95, 1.17) for a 2 SD (6.5 ppb) increase in SO_2, adjusting for co-pollutants	Assumed all pregnancies had the same exposure, regardless of location within a large area. As noted in summary, author provided corrections to the paper.

Table E3. Highlights from studies of pregnancy loss (stillbirth and spontaneous abortion) (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Bobak et al., 1999 45 districts in the Czech Republic 1986–1988	971 (0.42%) stillbirths (fetal death after 28 wks' gestation or weighing >1000 g) Ecologic Conditional logistic regression for grouped data (outcome and exposure data were aggregated by district)	National Public Health Service monitored air pollution & provided geometric means of all daily readings in all stations in a given district Most districts had at least 3 monitoring stations, generally in principle towns of the districts	Annual geometric mean SO_2 concentrations: Mean 12.2 ppb Minimum 1.7 Maximum 41.0 25 percentile 5.3 75 percentile 20.1 19.1 ppb=50 µg/m^3	Modeled separately & together: TSP NO_x Correlations among pollutants were not reported. District-level socioeconomic factors: - income - car ownership - divorce rate - savings - births outside marriage - induced abortion rate - % of gypsies in population	ORs for stillbirth and 19.1 ppb increase in SO_2: SO_2 only: 1.07 (0.91, 1.26) Adjusted for socioeconomic factors: 0.98 (0.80, 1.20) Adjusted for socioeconomic factors and co-pollutants: 0.90 (0.70, 1.16)	Possibility of auto-correlation because each district could have been included up to 3 times & neighboring districts may also be correlated. Use of annual means caused exposure misclassification by, e.g., assigning exposure data incorrectly to births earlier in the year. The Czech Republic had some of the highest levels of air pollution in Europe during the study period.
Sakai, 1984 4 districts near Tokyo Bay, Japan 1975–1977	1,710 (3.47%) spontaneous fetal deaths (not defined) Ecologic Correlations	Air Pollution Monitoring System in 3 districts for 1975–1977.	Annual means (not reported for Kawasaki): 1975 Tsurumi 23 Kanagawa 12 Totsuka (comparison) 11 1976 Tsurumi 17 Kanagawa 14 Totsuka 12 1977 Tsurumi 15 Kanagawa 13 Totsuka 9	Analyzed separately: NO_2, NO, Dust Correlations among co-pollutants were not reported. No covariates were considered.	**Correlation coefficient for SO_2 and spontaneous fetal death rate = 0.704 ($p \leq 0.05$)**	The number of monitors was not reported. For births earlier in the year, use of annual mean pollutant concentrations causes more misclassification because much of the exposure of those births will have occurred In the prior year.

Table E3. Highlights from studies of pregnancy loss (stillbirth and spontaneous abortion) (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Hemminki and Niemi, 1982 Valkeakoski, Finland 1974–1977	1,792 spontaneous abortions (gestational age not defined) among women 20–39 years, with one child or less. Cross-sectional	Authors matched woman's residence with pollution maps of SO_2 conc. in 1977–1979, which had been prepared by the Institute of Meteorological Sciences. Woman's and husband's occupation and industry were also considered exposures, but not for SO_2.	3 exposure levels: < 1.9 1.9-5.7 > 5.7 ppb	Hydrogen sulfide, carbon disulfide were also pollutants of interest, but were not analyzed with SO_2 Information was collected from 1975 census data on: - the women's & their husband's occupations - places of work - number of children - places of residence Occupation group was a proxy for socioeconomic status	The frequency of spontaneous abortions was lowest in the intermediate pollution zone (7.5% of pregnancies) and highest in the most polluted zone (9.3% of pregnancies). However, the authors state that the higher rate of spontaneous abortions in the most polluted zone was entirely due to higher rates among employers and higher officials (individuals with high socioeconomic status).	Data on spontaneous abortions were of uncertain quality. The number of spontaneous abortions was small. Possible bias introduced by missing data. Cases for which they could not get census data had higher spontaneous abortion rates. Pollution maps were for 1977–1979, whereas the spontaneous abortions and births occurred in 1974–1977.

E.1.4. Congenital Malformations

Seven studies explicitly examined associations between congenital malformations and SO_2. All of the studies that had SO_2 exposure estimates were published in the past five years, and three of them studied populations in the U.S. (New Jersey, Texas, and metropolitan Atlanta). The studies examined groupings such as chromosomal and non-chromosomal anomalies (Rankin et al., 2009; Dolk et al., 2010), types of heart defects (Gilboa et al., 2005; Hansen et al., 2009; Rankin et al., 2009; Strickland et al., 2009; Dolk et al., 2010), and/or oral clefts (Gilboa et al., 2005; Hwang et al., 2008; Hansen et al., 2009).

Studies of associations between birth defects and SO_2 are subject to the same concerns as epidemiologic studies of SO_2 and other reproductive and developmental outcomes, such as susceptible periods of exposure, exposure misclassification, and separation of the possible effects of SO_2 from those of other pollutants. A challenge that is particular to studies of congenital heart malformations is the numerous ways different authors group the different malformations. In addition, defining cases with heart defects may be less clear than defining a birth as preterm or less than 2,500 g. For example, Strickland et al. (2009) found that upon review and classification of birth defect surveillance records with an indication of cardiovascular malformation, the authors deemed only 65% to have cardiovascular malformations. Furthermore, birth defects may occur as part of a syndrome, rather than in isolation. Syndromes may have a known genetic cause, or familial association, which complicates detection of an environmental contribution. Hence, how groups of defects are classified could affect the ability to observe associations. Finally, most of the studies of birth defects examined possible associations between multiple defects and multiple pollutants, resulting in substantially more comparisons than among studies of other reproductive outcomes.

Common limitations of these studies included lack of control for important potential confounders, such as maternal nutrition or use of nutritional supplements such as folate; use of alcohol, tobacco, or other drugs; occupational and other exposures (such as pesticides); and history of defects (especially oral clefts) among siblings. Further, the studies generally considered average exposures during specific weeks, months, or the entire first trimester. The timing of an insult in causing a particular congenital malformation is very precise. Exposure assessment relative to the timing of specific malformations may not have been sufficiently specific for each type of malformation, and may not have captured peak levels of exposure, which might be important. The SO_2 concentrations were also very low in some studies, and may not have been variable enough to reveal effects of SO_2.

Overall, the studies examining exposure to ambient SO_2 as a risk factor for congenital malformations had inconsistent findings. Hansen et al. (2009) found both adverse and protective associations with cleft lip with or without cleft palate (CLP) and heart defects that were statistically significant, and concluded that the results of their primary and sensitivity analyses were mixed, with no consistent patterns. The SO_2 concentrations in this study were very low and <0.1% of the sample lived within 2 km of air monitors,

suggesting misclassification of exposure was likely. In the 2009 study of heart defects by Strickland et al., the authors were particularly careful with exposure assessment and classification of cases with multiple defects, but the only statistically significant effects were for PM_{10}. Dolk et al. (2010) applied pollutant data for 1996 to estimate exposure in 1991–2000, although the correlation between 2001 and 1996 SO_2 values was 0.54. In the Dolk study, only tetralogy of Fallot was significantly associated with SO_2, and the relative risks for tetralogy of Fallot were stronger for PM_{10} and NO_2. Hwang et al. (2008) used IDW methods to estimate exposures to multiple pollutants as risk factors for CLP, and found SO_2 was associated with non-significant *reductions* in risk of CLP. Similarly, Rankin et al. (2009) found numerous protective associations between SO_2 and various defects. In contrast, Marshall et al. found the highest quartile of SO_2 exposure was associated with increased risk of CLP, and some evidence of a possible dose-response relationship, despite relatively large distances between monitors and residences (Marshall et al., 2010).

Gilboa et al. (2005) had information on alcohol and tobacco use (though these factors did not appear to confound associations), checked multi-pollutant models, and evaluated possible effect modification, making this one of the more thorough studies of SO_2 and birth defects. Among Gilboa et al.'s 255 comparisons, they observed a positive association between SO_2 exposure and isolated ventricular septal defects and all ventricular septal defects combined, though they also found *inverse* associations between SO_2 and isolated atrial septal defects and multiple conotruncal defects. Gilboa et al. conclude their study does not provide strong evidence that air pollution increases the risk of cardiac defects or oral clefts.

Table E4 summarizes the epidemiologic studies of SO_2 as a risk factor for congenital malformations. This table provides highlights of each study's results and information about the methods, including exposure assessment, study population, consideration of co-pollutants and other covariates, statistical analyses, and strengths and limitations. The studies in the table are ordered by SO_2 concentration, starting with the lowest SO_2 concentrations. To help identify potential patterns among the findings of studies that examined heart defects, an additional table, Table E5 shows results from these studies, classified into major groups of heart defects.

Additional Studies

Several earlier studies considered the associations between congenital malformations and more general exposures that may have included SO_2, among other pollutants (Nordstrom et al., 1978a, b, 1979a, b; Smrcka et al., 1998). These studies considered all birth defects as a general outcome and are summarized in Appendix 1 but are not discussed here or in the table due to lack of specific information on exposures, methods, and results.

Table E4. Highlights of congenital malformations studies (significant SO_2 findings in bold).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Hansen et al., 2009 Brisbane, Australia 1998–2004	Aortic artery and valve defects, n=63 Atrial septal defects, n=127 Pulmonary artery and valve defects, n=64 Ventricular septal defects, n=222 Conotruncal defects, n=63 Endocardial cushion and mitral valve defects, n=33 Cleft lip, n=57 Cleft palate, n=100 Cleft lip w/ or w/o cleft palate (CLP; includes isolated cleft lip and cleft lip w/ cleft palate), n=145 Case-control with 1:5 matching Conditional logistic regression	18 monitoring stations (7 for SO_2) in and around Brisbane Daily averages were calculated from hourly readings. Exposure assignment was based on the monitor closest to the mother's statistical local area (SLA) at time of delivery. (In Brisbane most SLAs are smaller than postal areas) SO_2 levels were averaged over wks 3–8 of gestation (post-LMP).	Mean 24-hour average SO_2 levels: All seasons: mean: 1.5 (min=0, max=7.1) Summer: 1.5 Autumn: 1.6 Winter: 1.4 Spring: 1.4 IQR = 0.6	Included in models as continuous covariates: PM_{10} NO_2 O_3 CO Controls were matched to cases on: - mother's age - marital status - indigenous status - # previous pregnancies - month of LMP - area-level SES - distance to monitor All analyses were adjusted for gender.	For 0.6 ppb increase in SO_2 during weeks 3-8: <u>Primary analysis</u> - **CLP AOR 1.27 (1.01, 1.62)** - No statistically significant associations for other defects. <u>Sensitivity Analysis</u> (women w/in 6 km of monitor) - **Aortic artery and valve defects AOR 10.76 (1.50, 179.83)** - **Conotruncal defects AOR 0.27 (0.07, 0.81)** - No statistically significant associations for other defects.	Study area appears to be about 50 x 80 km. Number of cases w/in 6 or 12 km of monitors was not reported, but <0.1% of the sample lived within 2 km of monitors. No data on smoking, drug and alcohol use, or occupational exposures. People in semi-rural areas might have agricultural pesticide exposures. Authors note inconsistent results and multiple comparisons; suggest caution in interpreting results.

Table E4. Highlights of congenital malformations studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Rankin et al., 2009 Northern England 1985–1999	Cases: all infants and fetuses with congenital anomalies reported to the Northern Congenital Anomaly Survey (NorCAS), including late miscarriages (≥ 20 weeks), pregnancy terminations for fetal anomaly, and registered live births and stillbirths n=3,197 (2,714 with non-chromosomal and 483 with chromosomal anomalies) Controls: live and stillbirths from national data on all registered births. Case-control Unconditional logistic regression	62 ambient air pollution monitoring stations (UK National Air Quality Information Archive); substantially fewer monitors were recording at any given time Daily pollutant levels from all monitors within 10 km of maternal residence were summed over the 1st trimester.	1st trimester sums Controls: Median: 1390 ppb 1st quartile: 1035 3rd quartile: 1710 Cases: Median: 1376 ppb 1st quartile: 1020 3rd quartile: 1691	Black smoke (PM w/ aerodynamic diameter < 4µg/m^3), analyzed separately from SO_2. Models adjusted for: - birthweight - gender - material deprivation (based on census data for maternal residence postcodes).	SO_2 exposure was associated w/ significant ↓ risk of congenital anomalies. AORs for 2nd vs. 1st quartile of exposure: **Non-chromosomal 0.65 (0.56, 0.73)** **Nervous system 0.64 (0.44, 0.94)** **Congenital heart disease overall 0.60 (0.50, 0.72)** **Coarctation of aorta 0.50 (0.26, 0.94)** **Patent ductus arteriosus 0.45 (0.27, 0.76)** **Ventricular septal defect 0.67, (0.51, 0.88)** **Digestive system 0.51 (0.34, 0.78)** **Musculoskeletal 0.63 (0.46, 0.87)** No significant associations for: - all congenital - other heart defects - respiratory tract - cleft lip and palate - eye, ear, face, neck - internal urogenital system - chromosomal - miscellaneous	Maternal and gestational age were not available for controls. Authors state that because maternal residential mobility among mothers notified to NorCAS was 9% and most moved locally, mobility was unlikely a major concern. Mean birthweight was higher for controls (3.32 kg vs. 2.93 kg). Cases were more likely to be male (53% vs. 51%). Mean deprivation scores were higher (more deprived) for cases than controls (1.30 vs. 0.91). Exposures were extremely high compared to other studies.

Table E4. Highlights of congenital malformations studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Strickland et al., 2009 Metropolitan Atlanta 1986–2003	Cardiovascular malformations ≥ 20 weeks' gestation, conceived 1/1/1986-3/12/2003, n=715,500 (3,338 cases) Retrospective cohort Time-series analysis, Poisson generalized linear models	Measurements of ambient daily 8-hour maximum O_3 and 24-hour avg. CO, NO_2, PM_{10}, and SO_2 (U.S. EPA ,Georgia Department of Natural Resources, and Metro Atlanta Index) For each pollutant, 1 central monitoring station was used in the analyses. A weighted average of daily air pollution measurements during gestation wks 3–7 was assigned to each pregnancy. Weights were 0.7 for measurements in wks 3 and 7, 0.9 for wks 4 and 6, and 1.0 for wk 5.	Pollution metric (weighted 5-wk averages): IQR: 4.0 Seasonal means March-May: 5.4 June-August: 5.4 September-November: 6.9 December-February 7.1 Means by year: 1986-1991: 8.7 1992-1997: 5.5 1998-2003: 4.0	Correlations with SO_2: (analyzed separately) O_3: 0.30 CO: 0.23 NO_2: 0.39 PM_{10}: 0.41 Controlled for seasonal variation and long-term trends.	Adjusted RRs for one IQR (4 ppb) increase in SO_2: Valvar pulmonary stenosis 0.70 (0.49, 1.00) Right ventricular outflow tract defect 0.74 (0.55, 1.00) No statistically significant associations for: - Atrial septal defect, secundum - Coarctation of the aorta - Hypoplastic left heart syndrome - Patent ductus arteriosus Tetralogy of Fallot - Transposition of the great arteries - Muscular ventricular septal defect - Perimembranous ventricular septal defect - Conotruncal defect Left ventricular outflow tract defect	60 associations examined in the primary analysis. All pregnancies with the same conception date were assigned SO_2 exposure from a single monitor, so this study did not measure spatial variation in exposure. Authors conducted several sensitivity analyses. The authors noted misclassification in the vital records data.

Table E4. Highlights of congenital malformations studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Dolk et al., 2010 Four regions of England 1991–1999	6,136 non-chromosomal anomalies 2,949 chromosomal anomalies Ecologic Poisson regression	Mapped maternal census wards to pollutant maps (maps of estimated mean background SO_2, NO_2, and PM_{10}), at a 1 km x 1 km grid resolution for 1996 Pollutant map values are based on regional background air pollutant concentrations from data monitors and local low-level emissions	10^{th} percentile = 1.48 Median =3.00 90^{th} percentile = 5.72 10^{th} to 90^{th} percentile range = 4.24	Correlations with co-pollutants (analyzed separately): NO_2 (r=0.60) PM_{10} (r=0.54) Models adjusted for: - maternal age groups - Index of deprivation (based on census data)	Adjusted RR comparing 90^{th} to 10^{th} percentile of exposure (4.24 ppb) **Tetralogy of Fallot: 1.38 (1.07, 1.79).** Non-significant associations for: - non-chromosomal anomalies overall - chromosomal anomalies overall - all other congenital anomaly subgroups and congenital heart disease subgroups (Increased risks for Tetralogy of Fallot were also observed with both PM_{10} and NO_2, though they were not statistically significant.)	Considered only spatial variation based on data for one year; authors noted that SO_2 levels declined markedly during the study period. Multiple comparisons could easily result in positive associations by chance.

Table E4. Highlights of congenital malformations studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Hwang et al., 2008 Taiwan 2001–2003	653 cases of cleft lip w/ or w/o cleft palate (CLP) 6,530 randomly selected controls Population-based case-control Logistic regression	72 monitoring stations on Taiwan's main island (Taiwan Environmental Protection Agency) Hourly pollutant reports were integrated into monthly pollutant data and interpolated using inverse distance weighting.	Mean ± SD: Spring: 4.22 ± 2.59 Summer: 3.35 ± 2.00 Fall: 3.41 ± 2.07 Winter: 4.88 ± 3.71 All year: 3.96 ± 2.36	Correlations with SO_2 (analyzed separately and in co-pollutant models): O_3 (r=0.23) CO (r=0.24) NO_x (r=0.45*) PM_{10} (r=0.50*) *p<0.05 Analyses adjusted for: - gender - maternal age - gestational age (<37 weeks vs. ≥ 37 weeks)	AOR per 10 ppb change in SO_2: 1st month: SO_2 alone: 0.92 (0.63, 1.35) SO_2, O_3: 0.82 (0.55, 1.23) SO_2, O_3, NO_x: 0.85 (0.55, 1.31) 2nd month: SO_2 alone: 0.84 (0.57, 1.25) SO_2, O_3: 0.75 (0.50, 1.13) SO_2, O_3, NO_x: 0.70 (0.44, 1.11) 3rd month: SO_2 alone: 0.72 (0.47, 1.08) SO_2, O_3: 0.67 (0.44, 1.03) SO_2, O_3, NO_x: 0.68 (0.43, 1.07)	No data on smoking, drug and alcohol use, occupational exposures, socioeconomic factors. Authors note that presence of more premature infants in the case group may indicate other exposures that might mediate risk for CLP. Findings were consistent with other research suggesting O_3 is a risk factor for CLP.

Table E4. Highlights of congenital malformations studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Marshall et al., 2010 New Jersey February 1998 – 2003	414 Cleft lip w/ or w/o cleft palate (CLP) 303 Cleft palate only (CPO) 12,925 controls Population-based case-control Logistic regression	Hourly conc. of SO_2, CO, O_3, NO_2, and daily conc. of $PM_{2.5}$ and PM_{10} were averaged for gestational wks 3–8. Pollutant values for each birth came from the nearest air monitor w/in 40 km with at least 70% of the expected values. Numbers of monitors for each pollutant varied; there were 11 monitoring sites for SO_2 in 2002. Authors also evaluated the subset of births within 10 km of monitors.	Quartiles of exposure among controls: 1st : < 3 (referent) 2nd: 3– <5 3rd: 5– <7 4th: ≥ 7	Analyzed separately (and in multi-pollutant models; correlations not reported): - CO - O_3 - NO_2 - PM_{10} - $PM_{2.5}$ SO_2 models adjusted for: - mother's age - race, ethnicity - smoking during pregnancy - alcohol use - season of conception Authors also evaluated smoking for effect modification	AORs and CIs for SO_2 exposure in gestational wks 3–8, compared to the lowest exposure quartile: CLP 2nd: 1.2 (0.9, 1.7) 3rd: 1.3 (0.9, 1.9) **4th: 1.6 (1.1, 2.2)** CPO 2nd: 1.2 (0.8, 1.6) 3rd: 1.0 (0.7, 1.4) 4th: 0.7 (0.5, 1.1) The highest quartile of CO was negatively associated with CPO.	No data on occupational exposures, nutrition, or economic factors. There could be considerable exposure misclassification due to distances to monitors.

Table E4. Highlights of congenital malformations studies (significant SO_2 findings in bold) (continued).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Gilboa et al., 2005 Seven counties in Texas 1997–2000	114 cleft palate 317 CLP 45 aortic artery and valve defects 192 atrial septal defects 80 pulmonary artery and valve defects 503 ventricular septal defects 300 conotruncal defects 168 endocardial cushion and mitral valve defects 4,580 controls Population-based case-control Logistic regression	Hourly measurements from EPA monitors; including SO_2 monitors in 4 counties. Pollutant values were averaged for gestational wks 3–8. Values from the nearest monitor with at least 70% of the expected total number of daily means or hourly values were assigned to each pregnant woman.	Quartiles of exposure among controls: 1^{st}: <1.3 ppb 2^{nd}: 1.3– <1.9 3^{rd}: 1.9– <2.7 4^{th}: ≥ 2.7	Reported separately (also examined in multi-pollutant models) CO O_3 NO_2 PM_{10} Controls were frequency matched to cases on: - year of birth - vital status - maternal county of residence SO_2 models adjusted for: - season of conception - parity Other factors (alcohol, tobacco, prenatal care, maternal age, etc) were considered but did not act as confounders	AOR for highest vs. lowest quartile of SO_2 exposure in gestational wks 3–8 <u>Isolated cardiac defects</u> **Atrial septal defects: 0.42 (0.22, 0.78)** **Ventricular septal defects: 2.16 (1.51, 3.09)** Non-significant associations for pulmonary artery and valve defects, and aortic artery and valve defects <u>Multiple cardiac defects</u> **Conotruncal defects 0.58: (0.37, 0.91)** Non-significant associations for endocardial cushion and mitral valve defects Non-significant associations for Isolated oral clefts (CLP and cleft palate)	The authors did not incorporate weather conditions or use dispersion models in their estimates of exposure. The authors acknowledge presenting multiple (255) associations and that some of their statistically significant findings were likely the result of chance. SO_2 levels were relatively low and variation in exposure to SO_2 was small.

Table E5. Summaries of septal defects, conotruncal defects, defects of the great vessels, and other cardiac defects (significant SO_2 findings in bold).

Study	Septal defects (including atrial and ventricular)			
	Atrial septal defects	**Ventricular septal defects**	**Malformations of cardiac septa**	**Endocardial cushion and mitral valve defects**
Dolk, 2010[a]			1.03 (0.89, 1.19)	0.98 (0.68, 1.42)
Hansen, 2009[b]	1.61 (0.84, 3.08)	0.64, (0.35, 1.08)		No OR
Rankin, 2009[c]		**0.67, (0.51, 0.88)**		0.87 (0.39, 1.98)
Strickland, 2009[d]	1.00 (0.72, 1.38)	Muscular 0.95, (0.77, 1.17) Perimembranous 0.99 (0.76, 1.28)		
Gilboa, 2005[e]	**0.42 (0.22, 0.78)**	**2.16 (1.51, 3.09)**		(includes hypoplastic left heart) 1.18 (0.68, 2.06)

Study	Conotruncal defects				
	Conotruncal Defects	**Tetralogy of Fallot**	**Transposition of the great arteries**	**Left ventricular outflow tract defect**	**Right ventricular outflow tract defect**
Dolk, 2010[a]		**1.38 (1.07, 1.79)**	("transpositions of great vessels") 0.94 (0.71, 1.25)		
Hansen, 2009[b]	**0.27 (0.07, 0.81)**				
Rankin, 2009[c]		0.67 (0.30, 1.47)			
Strickland, 2009[d]	1.06 (0.86, 1.31)	0.85 (0.61, 1.17)	1.13 (0.75, 1.71)	0.97 (0.76, 1.22)	0.74 (0.55, 1.00)
Gilboa, 2005[e]	(incl Tetralogy of Fallot, transpositions of great vessels) **0.58: (0.37, 0.91)**				

[a] Adjusted relative risk for 10th to 90th percentile change in SO_2 exposure
[b] AORs from sensitivity analysis using births with ≤ 6 km to monitor
[c] AORs for 2nd vs. 1st quartile of SO_2 exposure, because authors noted a U-shaped response curve
[d] Adjusted risk ratios for an IQR increase in SO_2 exposure in gestational wks 3–7
[e] AORs for highest vs. lowest quartile of SO_2 exposure in gestational wks 3–8

Study	Defects of the great vessels (other than conotruncal)				
	Aortic artery and valve defects	**Coarctation of the aorta**	**Pulmonary artery and valve defects**	**Patent ductus arteriosus**	**Malformations of great arteries and veins**
Dolk, 2010[a]		1.15 (0.90, 1.47)			0.98 (0.836, 1.15)
Hansen, 2009[b]	**10.76 (1.50, 179.83)**		0.70 (0.16, 1.96)		
Rankin, 2009[c]		**0.50 (0.26, 0.94)**		**0.45 (0.27, 0.76)**	
Strickland, 2009[d]		1.04 (0.75, 1.43)	(pulmonary stenosis, valvar) 0.70, (0.49, 1.00)	1.22 (0.86, 1.74)	
Gilboa, 2005[e]	(including coarctation of the aorta) 0.83 (0.26, 2.68)		1.07 (0.43, 2.69)		

Study	Other cardiac defects			All cardiac defects
	Anomalies of cardiac chambers	**Malformations of valves**	**Hypoplastic left heart**	
Dolk, 2010[a]	0.99 (0.81, 1.20)	1.02 (0.90, 1.16)	1.07 (0.80, 1.44)	1.00 (0.91, 1.10)
Hansen, 2009[b]				
Rankin, 2009[c]			0.43 (0.14, 1.31)	**0.60 (0.50, 0.72)**
Strickland, 2009[d]			0.77 (0.50, 1.18)	
Gilboa, 2005[e]			(included in endocardial cushion & mitral valve)	

[a] Adjusted relative risk for 10th to 90th percentile change in SO_2 exposure
[b] AORs from sensitivity analysis using births with ≤ 6 km to monitor
[c] AORs for 2nd vs. 1st quartile of SO_2 exposure, because authors noted a U-shaped response curve
[d] Adjusted risk ratios for an interquartile range increase in SO_2 exposure in gestational wks 3–7
[e] AORs for highest vs. lowest quartile of SO_2 exposure in gestational wks 3–8

E.1.5. *In Utero* Exposures and Childhood Asthma

One study examined the association between *in utero* SO_2 exposure and development of asthma in early childhood, and found *in utero* SO_2 exposure was significantly associated with later development of asthma. Exposure to SO_2 in the first year of life was also associated with asthma, as were exposures to numerous other air pollutants. Therefore, it is not possible to discern the importance of exposure during pregnancy versus the first year of life, or the importance of SO_2 versus other pollutants.

Table E9. Highlights of a developmental study on asthma (significant SO_2 findings in bold).

Study	Methods	Exposure Assessment	SO_2 levels (ppb)	Covariates	Results	Comments
Clark et al., 2010 Southwestern British Columbia, Canada 1999–2003	Singleton term births weighing > 2,500 g 3,394 cases diagnosed with asthma 16,970 randomly selected controls Nested case-control Logistic regression	24-hr averages from the regulatory monitoring network, including 14 monitors for SO_2 The authors estimated each subject's SO_2 exposure for the duration of pregnancy and 1st year of life based on postal code at each contact with the health system and using daily SO_2 values from the 3 nearest monitors w/in 50 km, weighted by inverse distance to subject's postal code. Also, each postal code was assigned an inverse distance weighted (IDW) sum of industrial emissions within 10 km.	*In utero* exposure <u>Cases</u> Mean: 2.00 ± 0.96 25th percentile: 1.41 Median: 1.71 75th percentile: 2.57 <u>Controls</u> Mean: 1.95 ± 0.92 25th percentile: 1.41 Median: 1.71 75th percentile: 2.36	Analyzed separately: $PM_{2.5}$ PM_{10} NO NO_2 O_3 BC CO wood smoke Correlations among pollutants were generally high, so multipollutant models were not feasible. Controlled for: - gender (separate models) - parity - intention to breast feed - birthweight - gestation length - income quintile (census) - education quartile (census) Adjustment for maternal age and smoking during pregnancy were not needed.	AOR per 0.38 ppb increase in SO_2 during pregnancy **1.03 (1.02, 1.05)** SO_2 had a non-linear association with risk of asthma. Traffic-related pollutants (NO, NO_2, CO, BC) and PM_{10} were associated with higher risk estimates	Air pollution concentrations were low. Data on education and income are based on census at neighborhood-level. Due to high correlations between pregnancy and 1st-year SO_2 exposures, the relative importance of the two exposure periods cannot be discerned. Asthma risks associated with air pollution, including SO_2, were generally higher for girls (data and statistics were not reported).

E.2. Animal Developmental Toxicity Studies

Studies in animals included evaluation of the effects of prenatal exposure to SO_2 on male-male social behavior and delayed acquisition of behavioral landmarks (Singh, 1989; Petruzzi et al., 1996; Fiore et al., 1998). Mice and rabbits were evaluated for external and internal malformations after gestational exposure to SO_2 with and without CO_2 (Murray et al., 1977; Murray et al., 1979). Brief summaries of study protocols and results are provided in Table E10, as well as the text below. Detailed summaries of all studies are provided in Appendix 2.

Adult male and female CD-1 mice were exposed to 0, 5, 12, or 30 ppm SO_2 in air from 9 d before they were caged as breeding pairs (10 pairs per treatment group) (Fiore et al., 1998). Exposure continued throughout the mating period, and up to pregnancy day 14 for females. Male offspring were cross-fostered to unexposed lactating dams, and reared to adulthood for behavioral testing. Testing consisted of placing a treated male mouse with an untreated male of the same age and body weight. The specific behaviors scored were: attacking, freezing, tail "rattling," offensive postures, defensive postures, fleeing, exploration, rearing, wall rearing, body sniffing, digging, and self-grooming.

Body sniffing and non-social activities were found to occur with greater frequency in male mice prenatally exposed to SO_2, while freezing, tail rattling, and defensive behaviors decreased in frequency. The frequencies of other behaviors were not affected by treatment. The authors note that changes in responses such as tail rattling, freezing, and defensive postures decreased in a concentration dependent manner. In an earlier study from the same laboratory (Petruzzi et al., 1996), using the same treatment protocol, prenatally exposed pups were observed postnatally for time-to-acquisition of physical and behavioral developmental landmarks. No effects of prenatal SO_2 exposure were found. Hence, Fiore et al. (1998) concluded that their findings of alterations in social behavior with prenatal SO_2 exposure occurred in the absence of general effects on physical or neurobehavioral development.

Singh (1989) examined the behavior of neonatal CD-1 mice subsequent to prenatal exposure to SO_2 at concentrations of 0, 32, or 65 ppm. Mean litter size was not significantly altered by either concentration of SO_2. Birthweights were reduced in a concentration-dependent manner, reaching statistical significance ($p < 0.05$) at the higher SO_2 concentration of 65 ppm.

Statistically significant ($p < 0.005$), concentration-dependent increases were found in the times pups required for the righting reflex on PND 1, and for negative geotaxis on PND 10. Aerial righting scores were lower for both groups of SO_2-exposed mouse pups on PND 12, but the apparent effect did not reach statistical significance.

The author concluded that chronic prenatal exposure to SO_2 at moderate levels had adverse effects on birthweight and reflex development in CD-1 mice. The finding was

taken as possible evidence for altered neuromuscular coordination of the developing offspring resulting from a toxic prenatal exposure.

Murray et al. (1977 and 1979) report on the potential of SO_2 to cause developmental toxicity in CF-1 mice (25 ppm) and New Zealand rabbits (70 ppm), with or without concurrent carbon monoxide (CO). Implantation and resorption frequencies, mean litter size, and mean fetal crown-rump lengths (CRLs) were not affected by SO_2 (without CO) exposure in either species. Mean fetal weights were significantly decreased ($p < 0.05$) in mice prenatally exposed to SO_2, whether or not CO was also present. Fetal weights of rabbits were not affected.

No specific type of malformation was found to be increased over control levels by SO_2 treatment of mice. Aggregate malformations were slightly, but not significantly ($P = 0.14$), increased with exposure to SO_2 plus CO, but not SO_2 alone. According to the text of the paper, “delayed ossification of the sternebrae and of the occipital bone, was seen more often among the litters of mice exposed to SO_2 alone or in combination….” These data, however, were not presented.

In rabbit fetuses, no evidence was found for significant effects of prenatal SO_2 exposure on the frequencies of major malformations. According to the text of the paper, the frequencies of several minor skeletal variations were significantly increased among litters of rabbits exposed to either SO_2 or SO_2 plus CO, but these data are not presented.

Overall, an effect of SO_2 on fetal weights was observed in mice, but not in rabbits. As only one concentration of SO_2 was used, the potential for a concentration-response relationship cannot be evaluated.

Table E10. Animal studies of developmental toxicity of SO_2

Reference	Study design	Maternal toxicity	Developmental toxicity
Fiore et al., 1998	Mice, inhalation, prior to mating through gd 14; 10 pairs/group 0, 5, 12, 30 ppm; "near continuous"	Not reported	Significant effects on social behavior in male offspring at 12 & 30 ppm
Petruzzi et al., 1996	As above (same animals as Fiore et al., 1988)	Not reported	No effects on acquisition of postnatal physical or behavioral developmental landmarks No effect on passive avoidance learning and retention
Singh, 1989	Mice, inhalation, gd 7-18, 14 litters/group 0, 32, 65 ppm; continuous	Not reported	Significantly ↓ birthweight at 65 ppm Significant delays in righting reflex and negative geotaxis at 32 & 65 ppm
Murray et al., 1979 and 1977 (same data)	Mice, inhalation, gd 6-15, 20-40/♀ group; 0, 25 ppm; 7 hr/day Rabbits, inhalation, gd 6-18, 20 ♀/group; 0, 70 ppm; 7 hr/day	↓ food consumption during 1st few days of exposure, both species	↓ fetal weights in mice at 25 ppm No effect in rabbits at 70 ppm

E.3. Integrative Evaluation for Developmental Toxicity

Preterm birth studies

Eight of the ten epidemiologic studies examining the association between SO_2 exposure and preterm births reported significant findings. Studies with higher levels of exposure were more likely to report significantly increased risk of preterm birth. In addition, in the prospective cohort study with the highest SO_2 exposure levels, Xu et al. (1995) found that increasing exposures to SO_2 and TSP were associated with decreasing gestational age in a dose-dependent manner. This study, conducted in Beijing, also observed that on high pollution days the gestation age distribution curve was more skewed toward the left tail compared with low pollution days (i.e. more babies were born preterm during

high pollution days). This implies that pregnancies at high risk for preterm delivery may be particularly susceptible to adverse effects of air pollution. The attributable risk calculated for women exposed to the highest levels of SO_2 (i.e. the proportion of preterm delivery attributable to air pollution) was 33.4%.

The advantages of the Xu et al. study include the high levels of SO_2 exposure and the likelihood that measured SO_2 levels were well correlated with personal SO_2 exposure, as a random sample of households examined for indoor particulate concentration suggested. Consequently, decreased measurement error in personal exposures would have resulted in decreased random noise in the estimates of association. Additional strengths of this study include:

- a large gradient of SO_2 exposure; control for TSP, an important co-pollutant;
- the homogeneous nature of the population in terms of sociodemographic characteristics, living conditions and lifestyle, which make possible confounding by these factors much less likely;
- the close proximity of the population to an air monitoring station;
- important information such as gestational age was collected prospectively;
- the advantages of time series analyses which are unlikely to be affected by differences in population demographics;
- the monitoring of seasonal changes;
- the investigation of different number of lag days (exposure windows).

Six other studies of preterm delivery were consistent in finding significant adverse effects of SO_2 on preterm birth; however, comparison across all the 10 studies is challenging as they differed in many aspects. One study reported a marginally significant adverse association. Not all studies finding an association agreed in terms of the important window of exposure, as different time periods were reported as significant. Six studies reported associations late in pregnancy and four reported associations early in pregnancy. Jalaludin et al. (2007) reported that distance from the monitoring stations was an important factor and observed significant effects only when the analyses were restricted to within 5 km of a monitoring station. Not all studies, however, accounted for or reported distance from monitoring stations. Additionally, most studies assessed SO_2 exposure based on the maternal residence reported on the birth certificate. Residential mobility has been reported to be from 12% (Bell et al., 2004) to as high as 35% (Brauer et al., 2008). In studies with high maternal residential mobility, SO_2 exposure estimates would be subject to misclassification and would most likely bias results towards the null. The study by Brauer et al. (2008) did construct a longitudinal residential history for each woman from the beginning of pregnancy. In addition, they used IDW approaches to estimate SO_2 exposure levels. However, this well-conducted study did not observe an association with adverse reproductive outcomes, most likely due to a lack of gradient in exposure levels, a small sample size, and the low levels of exposure (mean $\cong$ 2 ppb), which were at the limit of detection of 2 ppb (personal communication with Brauer confirmed limit of detection). In another study conducted in the same city, Vancouver, Canada, Liu et al. (2003) did observe a significant association between SO_2 exposure and PTB. In the Liu et al. study the exposure levels were higher (mean = 4.9 ppb), most

likely because SO_2 levels have been declining over time and this study used data from a somewhat earlier time period than the Brauer et al. study. In addition the sample size for preterm births (< 37 wks) was much larger in the Liu study, 12,142 compared with 3,748 in the Brauer et al. study.

Co-pollutants were correlated in most studies where values were reported. However, in Leem et al. (2006), PM and SO_2 were weakly correlated (r = 0.13), yet both were associated with PTB, with a dose response relationship observed for SO_2. CO and NO_2 were also observed to be associated with PTB in this study. The findings of Sagiv et al. (2005), however, reported an association between SO_2 and PTB while none was seen for NO_2 and CO.

Another important aspect to consider in PTB is the window of exposure. Studies observed associations with SO_2 during early pregnancy (first month, first trimester) (Mohorovic, 2003; Leem et al., 2006) late pregnancy (Xu et al., 1995; Liu et al., 2003; Sagiv et al., 2005; Jiang et al., 2007) or during both early and late (Bobak, 2000; Jalaludin et al., 2007). Not all studies, however, examined all the various periods of pregnancy. The possible biological mechanisms by which air pollutants could affect PTB, as presented earlier in this document, would suggest the biologic plausibility of both early and late effects.

Thus, many of these studies had limitations, which would have made it more difficult to detect an adverse effect of exposure if one were present. However, most studies did report an effect. Although confounding is always a possibility in epidemiologic studies, most covariates would not have been associated with air pollutant levels, making confounding by these factors unlikely. For example, gestational age is difficult to measure accurately. However, the error in gestational age is probably not associated with air pollution. Therefore, the misclassification is likely to be non-differential with respect to exposure and would most likely result in an under-estimation of the true effects of air pollution on preterm birth.

No studies of SO_2 and length of the gestational period in animals were identified.

Although the biological mechanisms by which exposure to air pollutants could affect reproductive outcomes have not been established, the diagram below (Figure E2) presents possible pathways for an association using IUGR and PTB as an example.

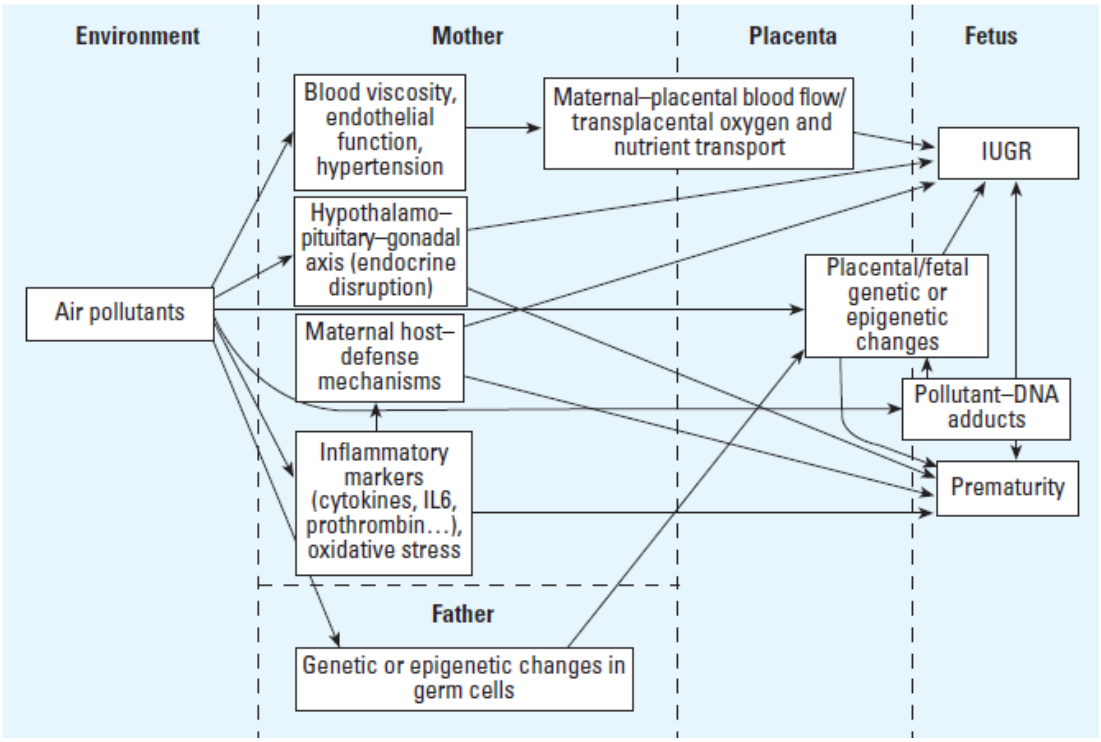


Figure E2. Possible biological mechanisms by which air pollutants could influence IUGR or prematurity (Slama et al., 2008). IL: interleukin.

Fetal Growth Studies

Among the 22 studies that used SO_2 concentrations in their analyses, 15 studies reported that ambient SO_2 exposure was significantly associated with indicators of fetal growth restriction or increased risk of LBW (Wang et al., 1997; Bobak et al., 1999; Ha et al., 2001; Maisonet et al., 2001; Lee et al., 2003; Liu et al., 2003; Yang et al., 2003; Lin et al., 2004a; Lin et al., 2004b; Mohorovic, 2004; Medeiros et al., 2005; Dugandzic et al., 2006; Williams et al., 2007; Hansen et al., 2008; Nascimento et al., 2009). Two of these 15 studies also found that for some populations and exposure periods and levels, SO_2 was also associated with decreased risk of LBW or increased birthweight (Maisonet et al., 2001; Medeiros et al., 2005).

The studies of the potential effects of SO_2 on fetal growth varied widely in the outcomes assessed, analytic methods, methods of exposure assessment, exposure levels, susceptible exposure windows, assessment of co-pollutants and other covariates, and findings. Generally, studies that analyzed temporal variation in SO_2 levels are likely more robust to confounding than studies that analyzed only spatial variation in SO_2. Five of the six studies that clearly assessed exposure both temporally and spatially found SO_2 exposure was associated with poorer fetal growth (Yang et al., 2003; Lin et al., 2004a; Dugandzic et al., 2006; Williams et al., 2007; Hansen et al., 2008).

SO_2 exposure levels in fetal growth studies

Low exposure levels and lack of variability in exposure levels is an important concern. The studies by Brauer et al. (2008) and Bell et al. (2007) had very little variability in SO_2 exposure levels. In the Brauer et al. (2008) study, the mean SO_2 concentration was at the limit of detection, and two other studies had mean daily SO_2 exposure levels below 3 ppb (Hansen et al., 2008; Nascimento et al., 2009). Three ppb is in the range of detection limits for most regulatory methods of measuring ambient SO_2 concentrations (U.S. EPA, 2008), though most studies did not report measurement methods. Exposure misclassification due to inaccurate detection or measurement of SO_2 at lower levels *decreases* the likelihood of these studies finding an association even if one exists. Rogers et al. (2000) also reported very low SO_2 concentrations, but their SO_2 exposure assessment was based on atmospheric transport modeling, rather than ambient SO_2 measurements.

Negative associations in fetal growth studies

The 2007 study by Liu et al. reported exposure to SO_2 in the first trimester (mean 3.9, IQR 3.0) was associated with small but statistically significant decreases in risk of IUGR. However, the exposure measurement in this study may have been subject to substantial misclassification due to the size of the areas covered by the monitors. Gouveia et al. (2004) also assumed the same exposures across a large city (mean ± SD 7.5 ± 3.9), and found inconsistent results. Another concern with the Gouveia et al. (2004) study is the possibility that by including month of birth, the authors over-adjusted the models, as SO_2 levels showed strong seasonal variation. Maisonet et al. (2001) also reported inconsistent results, though more of their findings suggest increased risk of LBW associated with SO_2 exposure.

Adjustment for co-pollutants in fetal growth studies

Eight studies reported results of models that included co-pollutants in SO_2 analyses. The co-pollutants varied among the studies. The Rogers et al. (2000) study is distinct in that rather than providing co-pollutant adjustment, it analyzed a combined SO_2 and TSP metric. That study suggested that both TSP and SO_2 contribute to risk of VLBW (Rogers et al., 2000). In the Gouveia et al. (2004) study, SO_2 alone was associated with *greater* birthweight in the second trimester; however, when NO_2, CO, O_3, and PM_{10} were included in the model, the association was not significant (Gouveia et al., 2004). Nascimento et al. (2009) found that late pregnancy exposure to SO_2 was significantly associated with increased risk of LBW, OR=1.30 (1.02, 1.65), adjusting for O_3. The authors did not control for PM_{10} because it was not significant in multi-pollutant models (Nascimento et al., 2009). Williams et al. (2007) controlled for ambient lead and number of hazardous waste sites, but dropped $PM_{2.5}$ from analyses, and found a very large effect of SO_2 on birthweight.

Three studies that included multi-pollutant models found that inclusion of co-pollutants did not change the associations between SO_2 and measures of fetal growth. Liu et al. (2003) found an increased risk associated with SO_2 exposure in the *last* month of pregnancy in Vancouver. After adjusting for gaseous co-pollutants (CO, O_3, NO_2), the AOR of 1.09 (CI 1.01, 1.20) for LBW and a 5 ppb increase in SO_2 was the same as the AOR of 1.09 (CI 1.01, 1.19) for SO_2 without co-pollutants, though the CI was slightly wider. The authors were unable to adjust for PM_{10}. Bobak et al. (1999) also found that adjustment for co-pollutants (TSP and NO_x) did not change the OR, but did widen the CI. The AOR for a 19 ppb increase in annual SO_2, controlling for district-level socioeconomic factors, was 1.10 (CI 1.02, 1.17); controlling for TSP and NO_x in addition to socioeconomic factors, the AOR was 1.10 (CI 1.01, 1.20). Hansen et al. (2008) found reductions in fetal AC and BPD were associated with exposure to SO_2 during gestation days 61–90 and 0–30, respectively; including PM_{10} or O_3 in two-pollutant models with SO_2 caused little to no change in the statistical significance or magnitude of these associations.

A study conducted by Lin et al. (2004a) in Kaohsiung and Taipei, Taiwan reported ORs adjusted for PM_{10}, CO, O_3, NO_2. This study found consistent and statistically significant associations between risk of LBW and SO_2 exposure over the pregnancy period: AOR for medium (second and third quartile) exposure, compared to the lowest quartile = 1.16 (CI 1.02, 1.33); AOR for high (fourth quartile) exposure = 1.26 (CI 1.04, 1.53). The associations were consistent, though smaller and non-significant for each trimester, except that the highest exposure in the last trimester was statistically significant. The correlations among the pollutants were not reported; however, CO was negatively associated with LBW, and the other pollutants were not statistically significantly associated with LBW. This study is also noteworthy because the authors limited the study to mothers living within 3 km of air pollution monitors, which probably reduces exposure misclassification. Further, this study had considerable variation in SO_2 exposures. The mean SO_2 levels were 4.9 ppb in Taipei and 17.0 ppb in Kaohsiung, and the IQRs were 4.7, 5.0, and 5.6 for the first, second, and third trimesters, respectively.

Another study in Kaohsiung and Taipei found first trimester SO_2 exposure in the highest vs. lowest tertile was associated with an 18.11 g reduction in birthweight, and that birthweight was reduced by 0.52 g per 0.38 ppb increase in SO_2 (Yang et al., 2003). SO_2 was modestly correlated with PM_{10}, which was also associated with lower birthweight, and was analyzed separately. Two studies in Seoul, Korea covered overlapping time periods and did not adjust for co-pollutants, which were highly correlated with SO_2. Both found SO_2 was associated with risk of LBW, but Lee et al. (2003) found associations for 2nd trimester and entire pregnancy exposures (AORs 1.06, CI 1.02, 1.11 and 1.14, CI 1.04, 1.24, respectively) and Ha et al. (2001) found an association for first trimester exposure (adjusted RR 1.06, CI 1.02, 1.10).

A study in Beijing by Wang et al. (1997) had some of the highest SO_2 exposures among the birthweight studies. This study found a statistically significant dose-response effect in the third trimester, and an AOR per 38 ppb SO_2 of 1.11 (CI 1.06, 1.16). The source

of pollution was burning coal for heating or cooking in 97% of households, and the authors previously found the trend of indoor particulate concentration was similar to outdoor concentrations, suggesting the measured SO_2 levels accurately represent personal exposure. However, TSP showed a similar association with LBW. Because it was highly correlated with SO_2, the authors did not report multi-pollutant models to separate the effects of TSP from those of SO_2 (Wang et al., 1997).

In experimental studies in animals, two studies examined fetal growth associated with inhaled SO_2 in mice, and both found reductions in birthweight associated with maternal SO_2 exposure. In one study, mice were continuously exposed to 32,000 ppb or 65,000 ppb SO_2 on days 7–18. Birthweights were reduced in a concentration-dependent manner, with the reduction in birthweight significant at 65,000 ppb (Singh, 1989). Another study found significant reductions in birthweight associated with gestational exposure to 25,000 ppb SO_2 for 7 hours/day during GD 6–15; however, crown-rump length was unchanged compared with controls (Murray et al., 1977; Murray et al., 1979).

Summary

In summary, the majority of studies that measured SO_2 concentrations well above limits of detection found that SO_2 was associated with increased risk of LBW or other indicators of fetal growth restriction. The studies found different susceptible exposure periods. The role of co-pollutants in relation to the potential effects of SO_2 on birthweight remains unclear, but several well-conducted studies suggest that SO_2 is associated with birthweight, independent of co-pollutants.

The majority of the 22 epidemiologic studies examining the association between gestational SO_2 exposure and fetal growth suggest that exposure to ambient SO_2 may affect fetal growth. Although the epidemiologic evidence has limitations, especially the inherent difficulty in isolating the effects of a single component of air pollution, the two studies in mice are consistent with the association between maternal inhaled SO_2 and fetal growth restriction in humans.

Pregnancy Loss (Stillbirth and Spontaneous Abortion)

Two of the three studies examining the association between exposure to SO_2 and stillbirth reported no significant findings. One study, by Sakai (1984), reported a positive correlation between spontaneous fetal death (presumably stillbirth) and SO_2, though the increase in risk associated with SO_2 exposure (e.g., relative risk) was not estimated, and NO_2 had a stronger correlation with spontaneous fetal death.

The single study that specifically examined the association between SO_2 and spontaneous abortion (Hemminki et al., 1982) found no association. The authors noted this cross-sectional study was limited by its design and the quality of the data.

Overall, the set of epidemiologic studies examining the association between SO_2 and stillbirth and spontaneous abortion was small, and no study was published after 2000. These studies generally had less sophisticated exposure assessment and analytic methods than more recent studies, most did not address the potential effects of co-pollutants, and most found no association between SO_2 and pregnancy loss.

One study in mice and rabbits included data collection on the number of live, dead, and resorbed fetuses after gestational exposure to 25 ppm SO_2 (Murray et al., 1977; Murray et al., 1979). The authors reported that treatment did not result in changes in resorption frequencies or mean litter size in either mice or rabbits. Thus, the epidemiologic evidence and experimental animal data do not provide evidence for an association between gestational exposure to SO_2 and stillbirth or spontaneous abortion.

Congenital Malformations

The human and animal data do not support an association between exposure to ambient SO_2 and birth defects. The epidemiologic studies of SO_2 as a risk factor for birth defects examined congenital malformations together, heart defects, and oral clefts, with inconsistent results. A study reported in two papers (Murray et al., 1977; Murray et al., 1979) evaluated mice and rabbits for external and internal malformations after gestational exposure to SO_2 with and without CO_2. In the mice, neither specific types of malformations nor aggregate malformations were significantly increased by exposure to SO_2. There was some suggestion of delayed ossification of certain skeletal elements in mice and minor skeletal variations in rabbits exposed to SO_2 alone, but the data were not provided.

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APPENDIX 1: Summaries of Epidemiological Studies on SO_2 and DART Outcomes

In this appendix, the epidemiological studies discussed in the main document are summarized. The study summaries provided here are arranged in alphabetical order by the first author's last name and year of publication. An individual study may assess multiple endpoints. For example, Bobak and Leon, 1999 discusses effects of air pollution (including SO_2) on stillbirth and low birthweight.

Bell et al., 2007: Ambient Air Pollution and Low Birth Weight in Connecticut and Massachusetts.

Bell et al. conducted this retrospective cohort study to examine the association between LBW (< 2,500 g) and maternal exposure to PM_{10}, $PM_{2.5}$, SO_2, NO_2, and CO, by trimester of exposure and race. The authors used birth records data for singleton births in Massachusetts and Connecticut from January 1, 1999 to December 31, 2002. The authors excluded births with missing location data, gestation >44 or <32 weeks, birthweight >5,500 g or <1,000 g, missing last menstrual period (LMP), exposure data missing for ≥75% of weeks in each trimester for a given pollutant, and specified data inconsistencies. The cohort consisted or 358,504 births, of which 4.01% (approximately 14,484) were LBW.

Monitoring data from U.S. EPA was used to estimate daily county-level concentrations for air pollutants for 1998–2002. Air pollution data for SO_2 were available for 7 of the 15 counties in the study. SO_2, NO_2, and CO were measured approximately every day, and $PM_{2.5}$ and PM_{10} were measured approximately every 2.7 and 5.5 days, respectively. Exposure was assigned as the average daily county-level concentration over gestation and each trimester based on mother's residence. Each county's daily average was based on measurements from a single monitor or averaged from multiple monitors within the county. Exposures to pollutants were correlated, though the authors did not report correlations for SO_2. The mean gestational exposure to SO_2 was 4.7 ±1.2 ppb; IQR=1.6 ppb. Supplemental information available online shows that monthly SO_2 levels ranged from approximately 3–8 ppb, with the lowest levels during summer. Daily temperatures, dew point temperatures, and calculated apparent temperatures were averaged over each pregnancy and trimesters.

The authors applied linear regression models with birthweight as a continuous variable, and logistic models comparing LBW and non-LBW births. The authors adjusted for gestational length, month prenatal care began, child's gender, birth order, year, mother's race, education, marital status, mother's age, tobacco use during pregnancy, and apparent temperature. Alcohol use was not significantly associated with birthweight, and was not included in models.

In single-pollutant models, exposure to pollutants for the gestational period was associated with decreases in birthweight and increased odds for LBW, though these associations were not significant for SO_2 (Table 1). The difference in birthweight per IQR increase in SO_2 for the gestational period was -0.9 grams (-4.4, 2.6), and the OR for LBW was 1.003 (0.961, 1.046). In linear models, every pollutant except SO_2 was associated with a decrease in birthweight. In logistic models, only NO_2 and $PM_{2.5}$ were significantly associated with LBW.

Table 1. Change in birthweight and ORs for LBW per IQR increase in pollution for the gestational period (95% CI) (adapted from (Bell et al., 2007))

Pollutant	Difference in birthweight (g)	Odds ratio for LBW
SO_2	-0.9 (-4.4, 2.6)	1.003 (0.961, 1.046)
NO_2	**-8.9 (-10.8, -7.0)**	**1.027 (1.002, 1.051)**
CO	**-16.2 (-19.7, -12.6)**	1.028 (0.983, 1.074)
PM_{10}	**-8.2 g (-11.1, -5.3)**	1.027 (0.991, 1.064)
$PM_{2.5}$	**-14.7 (-17.1, -12.3)**	**1.054 (1.022, 1.087)**

The authors applied two-pollutant linear models only to pollutants with significant associations in single-pollutant models. Results remained significant for all pollutants after adjustment using two-pollutant linear models, including those that adjusted for SO_2.

The authors also conducted sensitivity analyses to deal with covariance among the trimester exposures. The most important trimester of exposure to SO_2 was the first. Exposure to SO_2 in the first trimester was associated with lower birthweight in the range of 3.7 to 3.3 g per IQR increase in SO_2. To investigate whether air pollution has different effects by race, the authors also tested an interaction model of race and gestational exposure for each pollutant. SO_2 effect estimates were not different for infants of black vs. white mothers.

Comments

- The authors note that average concentrations for all pollutants in the study were below the U.S. EPA health-based National Ambient Air Quality Standards (NAAQS), and that all counties in the study were in compliance with the NAAQS for SO_2, NO_2, CO, and PM_{10}. Only two counties were in noncompliance for $PM_{2.5}$.
- In addition to low SO_2 levels in this study, the SO_2 levels showed little variability, thereby decreasing the chance of observing an effect.
- The study does not provide any information on the distance between monitors and residences.

Bhopal et al., 1994: Is living near a coking works harmful to health? A study of industrial air pollution.

Bhopal et al. conducted an ecologic study to determine whether there was an excess of ill health in people living near a cokeworks in England. The authors examined numerous health outcomes, including birth outcomes (gender ratios, birthweight, and stillbirths), respiratory symptoms and illnesses, standardized mortality ratios, and cancer rates. Sources of health data included routinely collected birth statistics (1982–1989), postcoded mortality (1981–1989), and cancer (1986–1989); a self-administered postal survey in the community; general practice medical records; and lung function tests from a sample of the community survey respondents.

For exposure, the authors defined an area close to the cokeworks ("perceived" higher exposure), a second area surrounding the first area ("perceived" lower exposure), and a third "control" area 6–10 km (3.7–6.2 miles) from the cokeworks. The health of the whole district was also used as a comparison for routinely collected data. For further exposure assessment, Bhopal et al. used 24-hour mean daily measures of SO_2 and smoke which had been made by the local authority at three sites, 1 km (0.6 miles) northeast, 1.5 km (0.9 miles) southeast, and 0.75 km (0.5 miles) west of the cokeworks) in 1987–1991 specifically to address concerns about the cokeworks. The authors note that the housing nearest the cokeworks is 250 m (0.2 miles) from the cokeworks, so the monitors did not provide an absolute level of exposure. The authors also used meteorological data. No routine air pollution data were available for the control area, but the authors obtained some monitoring data from about 1.6 km (1 mile) from the boundary of the control area. The authors report measured SO_2 levels before and after the cokeworks suddenly closed, but not for the study period.

In addition, a model of the dispersion of emissions, based on the US EPA's Industrial Source Complex model, 1989 emissions data for the cokeworks, published data on low level emissions, and weather data, was provided to the authors. According to the model, the highest pollutant concentrations were near the cokeworks, and there was a rapid decline in SO_2 level with distance. The maximal annual average SO_2 ground level concentration due to the cokeworks was estimated to be 15.3 ppb (40 $\mu g/m^3$) at 200 m (0.1 miles) east of the cokeworks.

Bhopal et al. report that the male:female birth ratios were similar in the perceived higher exposure (ratio=1.04), perceived lower exposure (ratio=0.97), and control areas (ratio=1.00). The proportions of LBW babies were also comparable: 8.0% in the perceived higher exposure, 6.3% in the perceived lower exposure, and 7.5% in the control areas. Although the authors reported no increased risk of stillbirth, the data were not presented.

Bobak and Leon, 1999: Pregnancy outcomes and outdoor pollution: an ecologic study in districts of the Czech Republic 1986-8.

In this ecologic study, Bobak et al. re-analyzed data from an earlier infant mortality study to investigate the association between stillbirths and LBW and outdoor air pollution in the Czech Republic, where air pollution was high. The authors examined TSP, SO_2, and the sum of oxides of nitrogen (NO_x).

Data on pregnancy outcomes were taken from routine registration of births published in printed form by the Czech Statistical Office. LBW was defined as birthweight <2500 g, and stillbirth was defined as the birth of a dead infant >28 weeks' gestation or weighing >1000 g. There were 223,929 births in monitored districts in 1986–1988; of these, 971 were stillbirths. LBW rates in the whole country and in monitored districts were 5.5%

and 5.9%, respectively; stillbirth rates were 4.18 and 4.23 per 1000 total births, respectively.

The study area included 45 of 85 administrative districts of the Czech Republic where air pollution was monitored by the National Public Health Service in 1986–1988. However, 37 districts were monitored in 1986, 39 in 1987, and 45 in 1988, resulting in 121 units of analysis with data for all three pollutants. Most districts had at least three monitoring stations, generally in the principle towns of the districts. For the study period, only annual arithmetic and geometric means of all daily readings in all stations in a given district were available; the authors used the geometric means in analyses. The mean of the annual geometric mean SO_2 concentration in monitored districts was 12.2 ppb (31.9 μg/m^3), with minimum = 1.7 ppb (4.4 μg/m^3), maximum = 41.0 ppb (107.4 μg/m^3), 25 percentile = 5.3 ppb (13.9 μg/m^3), and 75 percentile = 20.1 ppb (52.8 μg/m^3).

The Czech Statistical Office provided the following socioeconomic characteristics of each district: income, savings, number of people per car, births outside marriage, proportion of divorces to new marriages, induced abortions/100 live births, and proportion of gypsies in the population.

Bobak et al. used logistic regression on grouped data to estimate the association between air pollution and frequency of pregnancy outcomes. The unit of analysis was the annual experience of a district. The authors present results for crude single pollutant models (unadjusted), single pollutant models adjusting for socioeconomic factors, and "fully adjusted" models (with all pollutants and adjusting for socioeconomic factors).

Table 2 shows the results for SO_2. SO_2 was a statistically significant risk factor for LBW in crude and adjusted models. Adjusting for socioeconomic factors, the adjusted OR per 19.1 ppb (50 μg/m^3) increase in SO_2 was 1.10 (CI 1.02, 1.17). In the fully adjusted model, the adjusted OR for SO_2 and LBW was 1.10 (1.01, 1.20), p=0.033. The authors note that LBW and SO_2 pollution were both higher in the north western districts. TSP and NO_x were not significantly associated with LBW. Stillbirth was not statistically significantly associated with any pollutants in any of the models.

Table 2. Odds ratios and 95% CI for LBW and stillbirth associated with a 19.1 ppb (50 μg/m^3) increase in SO_2.

Outcome	Crude	Adjusted for socioeconomic factors[a]	Fully adjusted[b]
Low birthweight	1.21 (1.13, 1.30)	**1.10 (1.02, 1.17)**	**1.10 (1.01, 1.20)**
Stillbirth	1.07 (0.91, 1.26)	0.98 (0.80, 1.20)	0.90 (0.70, 1.16)

[a] Adjusted for district-level factors: income, savings, number of people/car, births outside marriage, divorces/new marriages, induced abortions/100 live births, and proportion of gypsies in the population
[b] Adjusted for socioeconomic factors and co-pollutants (TSP and NO_x)

Comments

- Wide variation in SO_2 exposure levels may have increased the ability of this study to find an association with birth outcomes.
- Correlations among the pollutants were not reported.
- The authors also note that each district could have been included in the dataset up to three times, possibly leading to serial autocorrelation. Also, neighboring districts could also be correlated, causing spatial autocorrelation. Such autocorrelation could have caused narrower confidence intervals but would not change the point estimates.
- The authors report that during the study period, the Czech Republic had some of the highest levels of air pollution in Europe and two-thirds of the monitored districts recorded annual mean concentrations of suspended particulate matter above the World Health Organization guidelines.
- The use of annual district means caused exposure misclassification in several ways, including reducing spatial variation to a district mean and reducing temporal variation to an annual mean. In addition, using annual data causes assigning exposure data incorrectly, e.g., to births that happened early in the year. This would likely bias the study toward finding no association.

Bobak, 2000: Outdoor Air Pollution, Low Birth Weight, and Prematurity.

Bobak studied the associations between adverse birth outcomes, including LBW, prematurity, and IUGR, and SO_2, TSP, and NO_x in the Czech Republic. Data for all singleton, live births registered by the Czech national birth register in 1991 in 67 districts where at least one pollutant was monitored in 1990–1991 were analyzed. Of the total 85 districts, the 67 monitored districts account for more than 85% of all births. The cohort consisted of 108,173 births in districts with SO_2 monitors; 5.2% were LBW (<2,500 g), 4.8% were premature (<37 weeks), and the prevalence of IUGR (<10^{th} percentile of birthweight for gestational age and sex) was not reported.

The birth data were linked with air pollution data compiled by the Hydrometeorological Institute and the Czech Public Health Service. Not all of the three pollutants were

measured in each district, so numbers of births with data on exposure were different for each pollutant. SO_2 data were available for most births. For each trimester of pregnancy, the author calculated mean outdoor SO_2, TSP, and NO_x levels from 24-hour measurements from all monitors in the district of each birth. For SO_2, the 25th percentile of mean trimester levels was 6.7 ppb (17.5 µg/m^3), the median was 12.2 ppb (32.0 µg/m^3), and the 75th percentile was 21.2 ppb (55.5 µg/m^3).

Odds ratios for associations between exposures and pregnancy outcomes were estimated by robust logistic regression using the Huber formula, with districts coded as clusters to account for the fact that exposures were area-based and that individuals might be more similar to each other within districts than between districts. Based on analyses of quintiles of exposure, Bobak modeled the pollutants as continuous variables and reported ORs per 19.1 ppb (50 µg/m^3). Mean pollutant concentrations were correlated within each trimester, e.g., the correlation between SO_2 and TSP in the first trimester was 0.71, 0.68 in the second trimester, and 0.73 in the third trimester (statistical significance not reported). The correlations between SO_2 and NO_x were 0.53, 0.62, and 0.63 in the first, second, and third trimesters, respectively. For single pollutants, correlations between trimesters were lower. Correlations for SO_2 concentrations between the first trimester and second trimester, the first and third trimester, and the second and third trimester were 0.25, 0.45, and 0.13, respectively. Analyses were adjusted for sex, parity, maternal age group, education, marital status and nationality, month of birth, and gestational age (for LBW analyses only).

SO_2 was associated with LBW and preterm birth, but not growth restriction. The adjusted OR for low birthweight and a 19.1 ppb increase in SO_2 in the first trimester was 1.20 (1.11, 1.30); however, adjustment for gestational age reduced the OR to 1.01 (0.88, 1.17). Similarly, restriction of the analysis to term births yielded an adjusted OR of 1.10 (0.96, 1.16). A 19.1 ppb increase in SO_2 during the first trimester was also associated with an 11.4 g (5.9, 16.9) reduction in mean birthweight. Table 3 shows the ORs for SO_2 and low birthweight and preterm birth by trimester of exposure.

Table 3. ORs and 95% CIs for maternal exposure to SO_2 (19.1 ppb increase) and risk of LBW, preterm birth, and IUGR, by trimester of pregnancy.

Outcome	Trimester	Odds Ratio, 95% CI[a]
Low birthweight (<2500 g)	1	1.01 (0.88, 1.17)
	2	0.95 (0.82, 1.10)
	3	0.97 (0.85, 1.10)
Preterm birth (<37 weeks gestation)	1	1.27 (1.16, 1.39)
	2	1.25 (1.14, 1.38)
	3	1.24 (1.13, 1.36)

[a] Adjusted for sex, parity, maternal age group, education, marital status and nationality, month of birth, and gestational age (for LBW only).

When gestational age was analyzed as a continuous variable, gestation was reduced by 0.056 weeks (0.037–0.075) per 19.1 ppb increase in SO_2 in the first trimester.

Bobak states that there were no significant associations between pollutant concentrations and IUGR.

Comments

- Bobak acknowledges he lacked data on maternal smoking, but argues that smoking is unlikely to confound the relationships examined because smoking is not likely to be associated with pollution. (Socioeconomic factors were also not associated with pollutants.) He adds that adjustment for socioeconomic factors did not change relationships, and that smoking would affect IUGR, which was not associated with pollution in this study.
- SO_2 levels in this study were much higher than would likely be seen in the U.S.
- Pollutants were highly correlated.
- The author does not report how large districts are; there could be substantial variation among pollutant levels within districts.
- Bobak states that SO_2 levels may indirectly measure small respirable particles that could underlie the observed association.

Brauer et al., 2008: A cohort study of traffic-related air pollution impacts on birth outcomes.

Brauer et al. evaluated the relationship between air pollution and reproductive outcomes, including fetal growth restriction and inadequate gestational length, in a cohort study in Vancouver, British Columbia, Canada. The authors used linked administrative data sets which combined information from the British Columbia Ministry of Health (for data on medical services and hospitalizations), the British Columbia Vital Statistics Agency, and the British Columbia Perinatal Database Registry. After excluding multiple births and births with incomplete data for birthweight, mother's residence during the pregnancy, maternal age, parity status, specific census covariates of interest, and mother's native status (ethnicity), the cohort consisted of 70,249 children born during 1999–2002 in the greater metropolitan Vancouver region.

The authors used the vital statistics birth records to identify small for gestational age (SGA) births, defined as below the 10^{th} percentile of the cohort for each week of gestation, stratified by sex. LBW at term was defined as birthweight <2,500 grams and at least 37 weeks gestation. The authors examined different definitions of preterm birth, including < 37 weeks gestation, birthweights below the 5^{th} percentile, and births of <30, 30–34, and 35–37 weeks gestation. Among the various definitions of preterm birth, only the results for births <30 weeks were reported. The numbers of births included in reported analyses are shown in Table 4.

Covariates included gender, parity, month and year of birth, First Nations ("status Indians") status, maternal age, and maternal smoking during pregnancy. However, individual-level data on maternal income and education were not available, so authors assigned income quintiles and education quartiles using Census data at the Census

dissemination area (DA) level. The DA is the smallest geographic area for which all Canadian Census data are disseminated and have target populations of 400-700 persons.

The regulatory monitoring network operated by the British Columbia Ministry of Environment and Metro Vancouver provides daily measurements at 24 monitors for ozone, 22 monitors for NO and NO_2, 14 for SO_2, 19 for CO, 19 for PM_{10}, and 7 for $PM_{2.5}$. The authors took two different approaches to assess and assign SO_2 exposures to each subject. For the first approach, residential exposures were assigned using daily concentrations from the area monitors nearest the mother's postal code of residence and within 10 km. (A postal code typically corresponds to one block-face in urban areas and larger areas where population density is low.) The authors computed the average exposure concentration for each month of pregnancy for each individual.
In the second approach to exposure assignment, the authors used inverse-distance weighting (IDW), i.e., they used an inverse distance (1/distance) weighted average of the three closest monitors within 50 km to compute a monthly mean concentration. This approach provided high temporal resolution (daily measures for most days) with less spatial resolution than the third approach. For both the nearest monitor and IDW approaches, a month was considered missing if there was a gap of more than five consecutive days of monitoring data or if there were more than 10 days missing in a month. Brauer et al. also examined proximity to major roads as residence postal code within 50 or 50–150 m of an expressway, primary or secondary highway, or major/arterial road.

After comparing the mean estimates from the nearest monitor and IDW approaches, the authors chose to focus analyses on IDW estimates, which were similar to nearest monitor estimates. SO_2 concentrations, based on IDW methods, were as follows: mean 2.02 ppb (5.3 μg/m^3), minimum 0.11 ppb (0.3 μg/m^3), maximum 6.79 ppb (17.8 μg/m^3), IQR 1.14 ppb (3.0 μg/m^3). Correlations among IDW estimates of SO_2, NO, NO_2, and CO were >0.8, while correlations with O_3 were between -0.7 and -0.8. Correlations among IDW estimates of SO_2, $PM_{2.5}$, and PM_{10} are not reported. The authors reported that exposures for various periods of pregnancy were generally highly correlated, limiting their ability to assess the importance of exposures during specific windows of pregnancy. Logistic regression was used to estimate associations between exposure to SO_2 and other air pollutants on SGA (<10^{th} percentile), term LBW (<2,500 g), and preterm birth (<30 weeks) (Table 4). In this study, exposure to SO_2 was not associated with SGA, LBW, or preterm births (regardless of the definition of preterm). The authors report ORs for preterm births defined as <30 weeks gestation, but not for other definitions of preterm.

Table 4. Crude and adjusted Odds Ratios for SGA, LBW, and preterm birth per 0.38 ppb increase in SO_2 exposure (IDW) for the entire pregnancy.

Endpoint	No. (%)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
SGA	7,138 (10.2)	1.00 (0.99, 1.02)	1.01 (1.00, 1.02)
Term LBW	891 (1.3)	0.99 (0.96, 1.02)	0.98 (0.96, 1.01)
Preterm (<30 weeks gestation)	238 (0.3)	1.02 (0.97, 1.07)	1.01 (0.95, 1.06)

[a] Adjusted for infant sex, First Nations status, parity, maternal age, maternal smoking during pregnancy, month and year of birth, income (quintile-census), maternal education (quartile-census).

Residence within 50 m of highways was associated with a 21% increase in SGA, with an OR = 1.21 (1.03, 1.42). The authors conclude their results suggest associations between pollutants dominated by traffic sources (NO_x and CO) and LBW, and $PM_{2.5}$ and preterm birth (<37 weeks). However, they also note that given high correlations between NO, NO_2, CO, and SO_2, it was not possible to differentiate impacts of specific pollutants.

Comments

- The authors note that despite the large study population, there were relatively few cases.
- This study was conducted in an area with relatively low concentrations of air pollutants, which could limit the ability of the study to observe an effect of SO_2 exposure, even if such an effect exists.
- The low concentrations of air pollutants in this study also limit its generalizability to areas with higher levels of air pollutants.
- Maternal income and education data were based on Census data from the mother's residential address, and not individual data. As a result, control for maternal income and education might have been inadequate
- For each subject, a longitudinal maternal residential history was constructed using multiple databases and was taken into account when assigning exposure values.

Clark et al., 2010: Effect of early life exposure to air pollution on development of childhood asthma.

Clark et al. conducted a case-control study nested in a population-based cohort study to examine the relationship between *in utero* and first-year ambient air pollution exposure and the risk of asthma up to three and four years of age. This summary will address the associations for *in utero* exposure only. The cohort data were obtained by linking data from the British Columbia (BC) Ministry of Health Services, BC Vital Statistics Agency, and the BC Perinatal Database Registry. The cohort comprised all births in 1999 and 2000 in southwestern BC, and included 37,401 children who were registered for the mandatory provincial medical plan and lived in the study area for the duration of

pregnancy and the first year of life. LBW (<2,500 g), preterm (<37 weeks), and multiple births were excluded. SO_2 analyses included 3,390 cases who had a minimum of two primary care physician diagnoses of asthma in 12 months or at least one hospitalization for asthma, and 16,970 age- and gender-matched controls. The mean ± SD age at the end of follow-up was 48 ± 7 months, and the range was 36 to 59 months.

The authors obtained the birth date and gender of each child, birthweight, gestational length (based on date of LMP), information on maternal smoking during pregnancy, maternal age, number of siblings, intention to breast feed, and First Nations status ("status Indians"). Individual-level data on socioeconomic factors were not available, so the authors assigned income quintiles and maternal education level quartiles at the level of Census dissemination area (DA), which is the smallest geographic area for which census data are distributed (each DA contains approximately 400–700 people).

Clark et al. calculated average pollutant exposures for the duration of pregnancy and the first year of life of each child, based on the child's and mother's residential postal codes at each contact with the health system. Exposures were assigned at the level of six-digit postal code, which corresponds to one block-face in urban areas but is larger in lower density areas. For this cohort, 92% of subjects' residences were coded at the resolution of one block-face. For pollutant values, the authors used regulatory air monitoring data, land use regression modeling (for NO, NO_2, black carbon, and $PM_{2.5}$ exposures), and proximity to stationary pollution sources. The monitoring network provided 24-hour average measurements at 14 monitors for SO_2, and different numbers of monitors for O_3, NO, NO_2, CO, PM_{10}, and $PM_{2.5}$. Daily SO_2 values at the three closest monitors within 50 km were inverse distance (1/distance) weighted (IDW) to a residence postal code. Table 5 shows the estimated exposures for cases and controls during both pregnancy and the first year. The mean ± SD ambient SO_2 levels during pregnancy were 2.00 ± 0.96 ppb (5.25 ± 2.51 μg/m^3) for cases and 1.95 ±0.92 ppb (5.11 ± 2.40 μg/m^3) for controls.

Table 5. Inverse distance weighting (IDW) estimates of SO_2 concentrations during pregnancy among cases and controls, ppb**.**

Pregnancy SO_2 estimates	Cases	Controls
Mean ± SD	2.00 ± 0.96	1.95 ± 0.92
25th percentile	1.41	1.41
Median	1.71	1.71
75th percentile	2.57	2.36

The authors conducted conditional logistic regression analyses separately for each gender. Clark et al. state that because correlations between different pollutants were generally high, multi-pollutant models were not feasible (correlations and statistics were not reported). Pregnancy and first-year ambient pollutant exposures were also highly correlated; therefore, the association of asthma with SO_2 exposure during pregnancy could not be examined while controlling for SO_2 exposure during the first year, and vice-versa (correlations and statistics were not reported).

The AOR (CI) for asthma and a 0.38 ppb (1 μg/m^3) increase in SO_2 exposure during pregnancy was 1.03 (1.02, 1.05), adjusted for parity, breast feeding, income quintile (Census DA), maternal education status (Census DA), birthweight, and gestation length. The AOR (CI) for asthma and first-year SO_2 exposure was also 1.03 (1.02, 1.05). When SO_2 exposures were grouped into quartiles, the trend across quartiles was not linear. The third quartile of *in utero* exposure was associated with a non-significant decreased asthma risk compared to the first quartile (data and statistics were not reported). However, the fourth quartile of *in utero* SO_2 exposure was associated with a statistically significant increased risk of asthma (data and statistics were not reported). Traffic-related pollutants (NO, NO_2, CO, and black carbon) and PM_{10} were associated with higher risk estimates.

The authors also used proximity to roadways and industrial point sources (e.g., power plants, waste treatment facilities, paper production plants, and shipyards) as estimates of ambient air pollution. For industrial point sources, each permitted point source was assigned a point source index value based on its contribution of pollutants ($PM_{2.5}$, sulfur oxides, nitrogen oxides, and volatile organic compounds) relative to other point sources in the region. Each postal code was then assigned a weighted sum of emissions from point sources within 10 km to estimate exposure. The AOR (CI) for an IQR increase in the point source index was 1.11 (1.04, 1.18) for *in utero* exposure.

Comments

- The authors note that LBW and gestational age may be in the causal pathway between air pollution and asthma; therefore, excluding LBW and preterm births may have resulted in bias toward the null.
- Due to high correlations between pregnancy and first-year SO_2 exposures, it is not possible to discern the relative importance of the two exposure periods.
- The study did not have information on the child or family history of atopy, which is an important risk factor for asthma development and a potential effect modifier.
- The authors report that asthma risks associated with air pollution, including SO_2, were generally higher for girls, though they do not provide data or statistics.

Darrow et al., 2009: Ambient Air Pollution and Preterm Birth: A Time-series Analysis.

Darrow et al. conducted a time-series analysis to examine the relationship between preterm birth and levels of ambient air pollution, including SO_2, CO, NO_2, O_3, PM_{10}, $PM_{2.5}$, and speciated PM.

The authors constructed a cohort using vital records data for births between 1994 and 2004 in five central counties of metropolitan Atlanta. The study area had a radius of 16 miles at its narrowest and 32 miles at its widest, and covered 1,752 square miles. The cohort included 476,489 singleton births of at least 20 weeks gestation and without major structural birth defects, with complete data on maternal marital status and education. Induced preterm births were included in the analysis. Preterm birth was

defined as live birth before 37 weeks gestation. Gestational age was based on LMP (98.5% of the cohort) or clinical estimate (1.4%). For the 0.1% of births without a valid LMP date and clinical estimates of gestational age, the authors used the gestational age estimated by the Georgia Division of Public Health based on birthweight. Risk of preterm birth was calculated for each day.

Based on previous research and current hypotheses about the mechanisms leading to preterm birth, Darrow et al. focused on three windows of exposure: the first month, the final week, and the final six weeks of gestation. They used different definitions of preterm birth, depending on the exposure window of interest:

- The authors hypothesized that disruption of the implantation and placentation process early in pregnancy could increase risk of both extreme and moderate prematurity. Therefore, they included all preterm births between 20 and 36 weeks gestation in analyses of exposure in the first month of pregnancy.
- Because the authors hypothesized that acute effects of air pollution would be unlikely to induce extreme preterm birth before 29 weeks, they included only infants who had reached at least 29 weeks' gestation for analyses of exposures in late pregnancy.

The outcome modeled was risk of preterm birth for each day, with the number of preterm births as the numerator and the number of pregnancies at risk as the denominator.

Darrow et al. obtained daily exposure data from three sources: the US EPA Air Quality System, the Georgia Institute of Technology $PM_{2.5}$ network, and the Aerosol Research and Inhalation Epidemiology Study monitor. Daily air metrics included 1-hour maximum SO_2, CO, and NO_2, 8-hour maximum O_3, and 24-hour average PM_{10}, $PM_{2.5}$, $PM_{2.5\text{-}10}$, and $PM_{2.5}$ components. Five monitors provided data on SO_2. The authors averaged the daily pollutant values over the exposure windows of interest as follows:

- For the late pregnancy exposure windows, the pollution levels in the six weeks leading up to the study day (date of preterm birth) or the one week leading up to the study day were averaged. The six-week and one-week average values were assigned to each day in the six-week and one-week late pregnancy exposure windows, respectively.
- For the early pregnancy exposure window, each study day was assigned the average pollution level in the 28 days after the estimated conception date.

Because previous research had shown seasonal variation in preterm births and the cohort showed a relationship between demographic and socioeconomic characteristics and seasonal variation, the authors controlled for seasonal trends in the analyses (using parametric cubic splines) and modeled temporal associations within racial, educational, and marital status groups. However, each pollutant was modeled separately.

Darrow et al. reported that results were generally consistent with little or no association between air pollution and preterm birth. The authors presented relative risks (RR) comparing the risk of preterm birth associated with an interquartile range increase

(difference between the 75th and 25th percentile exposure levels) in pollutant levels. The results for SO_2 are shown in Table 6.

Table 6. Relative Risks for preterm birth per 1 IQR increase in SO_2 exposure.

Exposure	RR (95% CI)	IQR (ppb)
First month	0.97 (0.96, 0.99)	4.0
Final week	0.99 (0.98, 1.01)	6.0
Final six weeks	0.99 (0.97, 1.01)	3.0

In addition to the five-county analysis, Darrow et al. also took a complementary approach in which they created spatial capture areas around each monitor that recorded daily pollutant concentrations. For the cohort of births with residential geocodes within 4 miles of each monitor, they conducted monitor-specific time-series analyses. This capture-area analysis included 45,974 births, of which 4,001 (8.7%) were preterm. Results for the capture-area analysis were similar to those for the whole cohort, suggesting little to no association between exposure to SO_2 and risk of preterm birth. Capture-area analysis results are shown in Table 7.

Table 7. Capture-area analysis: Relative Risks for preterm birth per 1 IQR increase in SO_2 exposure

Exposure	RR (95% CI)
First month	1.00 (0.97, 1.03)
Final week	0.99 (0.96, 1.02)
Final six weeks	0.99 (0.95, 1.02)

Comments

- The authors cite a recent study showing that 22% of women in Atlanta change residences during pregnancy; therefore, exposure assignment based on residence at time of birth likely results in substantial misclassification for the early pregnancy window.
- No attempts to control for other pollutants are discussed; instead, they conducted separate analyses for each pollutant.

Dejmek et al., 2000: Fecundability and Parental Exposure to Ambient Sulfur Dioxide.

Dejmek et al. conducted this retrospective cohort study in the district of Teplice, Czech Republic to investigate the effect of SO_2 on fertility. The sample of 2,585 births included all full-term singleton births in April 1994 to March 1998 if the parents were of European origin, did not try to prevent the index pregnancy, and were not treated for fertility disorders. The sample was further restricted to the mother's first delivery in the study period. The authors obtained personal and lifestyle data from medical records and self-administered questionnaires completed by mothers in the hospital after delivery. As a measure of fecundability, couples who achieved a clinically recognized pregnancy in the

first unprotected menstrual cycle (FUMC) were classified as “conceived” and all others as “non-conceived.”

Air pollution data came from a single monitoring station in the center of the town of Teplice. The authors used the mean 30-day averages of SO_2 levels in each of four months before the estimated date of conception. Based on the SO_2 exposure distribution in the population in the first two years of the study, SO_2 levels were classified near tertile cutoffs (<15.3 ppb), medium (15.3–30.5 ppb), and high (>30.5 ppb). SO_2 levels decreased each year of the study.

The authors considered as potential confounders those factors that were associated with the outcome (fertility) or with exposure, including maternal age:

- maternal and paternal education;
- marital status;
- parity;
- spontaneous abortion
- induced abortion;
- alcohol use;
- maternal and paternal smoking, and maternal passive smoking;
- employment status of each parent;
- eight types of occupational risks of each parent (e.g., exposure to radiation, chemicals, dust, infection);
- 10-year temporal patterns in conceptions;
- seasons;
- temperature (four versions);
- weekly acute respiratory infection incidence >1,500/100,000 in a given month prior to conception.

During periods of extremely high air pollution (e.g., 3-hour average SO_2 >153 ppb during a meteorological inversion), district authorities broadcast a special signal via local media and encouraged residents to reduce outdoor activities and delay or reduce airing of their homes. A variable for this signal was defined and included in some analyses, based on the assumption that the signal would cause behavior changes to reduce exposure. A long inversion episode, with 20 days of extreme SO_2 levels, occurred during the second half of the study (December 1996 to January 1997).

Although co-pollutants (NO_x, PM_{10}, $PM_{2.5}$, and PAHs) were also considered as potential confounders, the authors report that high correlations among the pollutants would cause misleading results when two pollutants were simultaneously included in a single model. Therefore, only results of models with SO_2 alone were reported.

Among parents exposed during the second pre-conceptional month, 24.5% of those exposed to mean SO_2 levels <15.3 ppb (40 μg/m^3) conceived in FUMC. Of those exposed to 15.3 to < 30.5 ppb (40 to <80 μg/m^3) and > 30.5 (>80 μg/m^3), 20% and 17%

conceived in the FUMC, respectively (Dejmek et al., 2001). The authors used logistic regression to estimate AORs for conception in the FUMC resulting in a live birth. Each month prior to pregnancy was analyzed separately. The odds of conception in the FUMC were consistently reduced with higher levels of SO_2 exposure in the second month (30–60 days) before conception. The AOR for medium SO_2 exposure in the second month before conception was 0.57, 95% CI (0.37, 0.88; p<0.011) and for high exposure was 0.49 (0.29, 0.81; p<0.006). Conception in the FUMC was not significantly associated with SO_2 exposure in any other period. Results are shown in Table 8.

Table 8. AORs (95% CI) for fecundability in the FUMC by month of pre-conception exposure to SO_2 (adapted from (Dejmek et al., 2000)).

	Medium exposure[a]		High exposure[b]	
Month prior to conception	AOR	CI	AOR	CI
4	1.32	0.90, 1.91	0.93	0.57, 1.51
3	0.95	0.63, 1.48	0.90	0.55, 1.48
2	**0.57**	**0.37, 0.88**	**0.49**	**0.29, 0.81**
1	1.01	0.68, 1.51	0.96	0.58, 1.58

[a] Comparing medium (15.3 - 30.5 ppb) to low (<15.3 ppb) exposure
[b] Comparing high (>30.5 ppb) to low (<15.3 ppb) exposure

Table 9 shows that results were somewhat weaker for the second half of the study, when SO_2 levels were generally lower. Excluding couples exposed to monthly mean concentrations >42 ppb or inversion situations in the four months before conception appeared to magnify the effect of SO_2 on fertility, but only in the second half of the study period.

Table 9. AORs (95% CI) for exposure to SO_2 in the second month before conception and fecundability in the FUMC, first (I) vs. second half of study period (II).

	Medium exposure[a]		High exposure[b]	
Period, exclusion	AOR	CI	AOR	CI
I	**0.49**	**0.25, 0.96**	**0.43**	**0.20, 0.93**
II	0.67	0.36, 1.28	0.59	0.36, 1.28
Excluding those exposed to > 42 ppb[c]				
I	**0.49**	**0.25, 0.96**	**0.43**	**0.20, 0.93**
II	0.54	0.28, 1.07	0.44	0.18, 1.09
Excluding those exposed to inversion				
I	**0.49**	**0.25, 0.96**	**0.43**	**0.19, 0.93**
II	0.54	0.27, 1.09	**0.41**	**0.17, 0.99**

[a] Comparing medium (15.3–30.5 ppb) to low (<15.3 ppb) exposure
[b] Comparing high (>30.5 ppb) to low (<15.3 ppb) exposure
[c] Mean monthly exposure > 42 ppb (all such exposures occurred only in the second half of the study)

Assuming exposure estimates would be more accurate for those living closer to the monitor, the authors also conducted separate analyses of the one-half of couples living within 3.5 km of the monitoring station, vs. farther away. Table 10 shows that the observed association between conception in the FUMC and high SO_2 exposure in the second pre-conception month was stronger for those living within 3.5 km of the monitor. For those living farther away, the association between SO_2 exposure and conception in the FUMC was weaker and non-significant. A more significant association was observed for the combined effect of excluding couples who lived farther from the monitor (> 3.5 km), and those exposed to SO_2 levels >42 ppb, when behavior may change, resulting in a reduction of personal exposure.

Table 10. AORs (CI) for exposure to SO_2 in the second month before conception and fecundability in the FUMC, by distance from monitor.

	Medium exposure[a]			High exposure[b]		
Distance	AOR	CI	*p-value*	AOR	CI	*p-value*
<3.5 km	0.56	**0.31, 1.00**	**0.05**	**0.36**	**0.17, 0.73**	**0.005**
>3.5 km	0.58	0.31, 1.08	0.09	0.70	0.34, 1.45	0.34
Total[c]	**0.57**	**0.37, 0.88**	**0.011**	**0.49**	**0.29, 0.81**	**0.006**
Combined effect[d]	**0.51**	**0.27, 0.95**	**0.034**	**0.28**	**0.13, 0.61**	**0.0012**

[a] Comparing medium (15.3 - 30.5 ppb) to low (<15.3 ppb) exposure
[b] Comparing high (>30.5 ppb) to low (<15.3 ppb) exposure
[c] Including all couples, regardless of distance from monitor
[d] Combined effect of excluding those living > 3.5 km from a monitor and those exposed to SO_2 > 42 ppb.

In this study the timing of SO_2 effects coincided with the period of sperm maturation. These results are consistent with the findings of Selevan et al. (2000) and Rubes et al. (2005).

Comments

- The authors note that conception in the FUMC allows appropriate comparisons for evaluating the effect of air pollution on fecundability, and that a previous study suggests that this measure gives an unbiased estimate of the mean fecundability in the cohort.
- Dejmek et al. note that in previous studies of fecundability, SO_2, NO_x, PM_{10}, $PM_{2.5}$, and polycyclic aromatic hydrocarbons (PAHs) were highly mutually correlated, with coefficients ranging from 0.55–0.83. However, the authors state that analyzing each pollutant in a separate model, only SO_2 was consistently associated with fecundability. A much weaker association between PM_{10} exposure and fecundability was explained by the high correlation between SO_2 and PM_{10} (r=0.83). Including both pollutants in one model yielded a stronger SO_2 effect and eliminated the association for PM_{10}. The authors point out the consistency of their findings with those of Selevan et al. (Selevan et al., 2000) concerning the most probable stage of sperm maturation damage associated with SO_2 exposure within 90 days.

Dolk et al., 2000: Perinatal and infant mortality and low birth weight among residents near cokeworks in Great Britain.

Dolk et al. conducted an ecologic study to investigate whether low birthweight, stillbirth, and death up to one year of age were more common in populations residing near cokeworks, a major source of smoke and SO_2. The outcomes were defined as stillbirths as a proportion of all births (gestational age or weight not specified); LBW (<2,500 g) as a proportion of all live births with known weight; and neonatal deaths up to one month of age, post-neonatal respiratory deaths, post-neonatal sudden infant death syndrome, and infant deaths, as a proportion of all live births. Only the stillbirth and LBW findings are described here.

The authors acquired 1981–1992 birth and death data for England, Wales, and Scotland. Due to unavailability or incomplete coding, stillbirths and birthweight were excluded for 1981 in England and Wales and for all years in Scotland. Each individual record had a postcode that referred to a small geographical area of typically 15–17 addresses. Each postcode, based on the first address in the postcode, was referenced to a grid with 100-m squares.

Deprivation scores were calculated based on census enumeration district-level data for the proportion of people without access to a car, unemployed males, overcrowded households, households headed by a person in social class 4 or 5. Deprivation scores were assigned to each birth and death based on the census enumeration districts in which the postcode was situated and grouped into quintiles, plus a category for enumeration districts for which an index could not be calculated. (There are approximately 13 postcodes and five annual births in a typical enumeration district.)

The study population was defined as all births to persons who resided within a 7.5-km radius around 22 cokeworks. The authors assumed that within the study zone, the population living within 2 km of the nearest cokeworks had the highest potential exposure levels. There were six groups of two or more sites with overlapping study zones, which they called “sitegroups,” and five sites with non-overlapping 7.5 km zones.

Dolk et al. calculated observed/expected (O/E) ratios and exact 95% CIs for the 0–2 km and 0–7.5 km zones around the cokeworks. Observed numbers were calculated as the cases (for stillbirth analysis) and births with weight <2,500 g (for birthweight analysis) with postcodes within the study zones. Expected numbers were calculated from regional rates, stratified for year, sex, and deprivation quintile. For all cokeworks combined, the O/E ratio (95% CI) within 2 km of cokeworks was 1.00 (0.95–1.06) for LBW (<2,500 g) and 0.94 (0.78–1.12) for stillbirths. The observed outcomes at 0–2 km from the plant and 0–7.5 km from the plant are shown in Table 11.

Table 11. Observed number, rate per 1000 births, and observed/expected ratios near all 22 cokeworks combined in Great Britain, 1981–1992.

	0–2 km from plant				0–7.5 km from plant				
Outcome	**O**	**Rate per 1,000**	**O/E***	**95% CI**	**O**	**Rate per 1,000**	**O/E***	**95% CI**	**P†**
LBW ‡	1,365	70.0	1.00	0.95, 1.06	15,903	69.6	0.98	0.97, 1.00	0.706
Stillbirths §	127	5.5	0.94	0.78, 1.12	1,665	6.1	1.02	0.98, 1.08	0.949

* adjusted for sex, deprivation, and region
† Stone's conditional test for trend with distance p value
‡ 1982–1992 — England and Wales only
§ 1981–1991 only

The authors also divided the study zones into 8 bands of increasing distance, up to 7.5 km from the cokeworks and calculated observed/expected ratios. Dolk et al. also used logistic models with distance, the reciprocal of distance, and a model with risk declining exponentially with distance, controlling for deprivation and site/sitegroup. There was no statistically significant decline in risk with distance from cokeworks for any of the outcomes studied, in any of the bands. The authors concluded that there was no evidence of an increased risk of low birthweight, stillbirths, and/or neonatal mortality near cokeworks, and there was no strong evidence for any association between residence near cokeworks and postneonatal mortality.

Comment

- Because this study did not include actual measurement of SO_2, the effects of SO_2 and smoke cannot be discerned using this study design.

Dolk et al., 2010: Ambient air pollution and risk of congenital anomalies in England, 1991–1999.

Dolk et al. examined associations between rates of selected congenital anomalies and geographic data on ambient concentrations of SO_2, NO_2, and PM_{10}. Data on congenital anomalies were contributed by four English congenital anomaly registries operating active ascertainment in the cities of Southampton, part of London, Oxford, and Newcastle, and surrounding areas for 1991–1999 (1994–1999 for one register). The study population was 759,993 live and stillbirths in 1,474 census wards where at least 80% of mothers delivered in hospitals covered by the registry. Cases of selected congenital anomalies included live births, fetal deaths from 20 weeks gestation, and pregnancy terminations following prenatal diagnosis at any gestational age. Dolk et al. included anomaly types that were considered well-defined and recorded by the participating registries.

Maternal age was classified into categories (<20, 20–34, 35+ for non-chromosomal and <30, 30–34, 35+ for chromosomal anomalies). For each case, the authors identified the

census enumeration district, which is a small area comprising about 1,000 residents. The Carstairs index of deprivation was calculated for each enumeration district based on 1991 census data on social class of head of household, car ownership, unemployment and overcrowding, standardized to Great Britain. Enumeration districts were divided into quintiles by Carstairs scores. The authors report that no other relevant risk factors could be obtained for both register data and all births.
Dolk et al. obtained maps of estimated mean background SO_2, NO_2, and PM_{10} at a 1 km by 1 km grid resolution for 1996. These maps had been developed with regional background air pollutant concentrations from data monitors and local low-level emissions. The distribution of SO_2 values was as follows (converted to ppb): 10^{th} percentile=1.48; median=3.00, 90^{th} percentile=5.72; 10^{th} to 90^{th} percentile range=4.24. The correlation of the modeled mean and a sample of annual mean measured concentrations was 0.73 for SO_2. In the 1996 data, PM_{10} and NO_2 were highly correlated (r=0.93); SO_2 was somewhat less correlated with PM_{10} (r=0.54) and NO_2 (r=0.60). The census ward pollution concentrations were population-weighted averages of the smallest census output areas, which had been mapped to the 1 km squares of the pollutant maps.

SO_2 levels were lower in 2001 compared with 1996. The correlation between 1996 and 2001 SO_2 levels was 0.54. Data were not available for any other years.
Dolk et al. used a Poisson regression model of maternal age-specific enumeration district counts to adjust for maternal age, registry, area deprivation, and hospital catchment (hospital catchment had been shown to be a determinant of anomaly rate, reflecting hospital diagnostic and recording practice). Due to the correlations among pollutants, only one pollutant was included in each model. Comparisons are for an increase from the 10^{th} to the 90^{th} percentile of SO_2 concentration (4.24 ppb).
The authors found no evidence of associations between any of the pollutant measures and non-chromosomal anomalies overall, and only non-significant associations for chromosomal anomalies overall: for SO_2, adjusted RR=1.06 (0.98 to 1.15). Of the nine subtypes of cardiac anomalies, the only statistically significant increased risk was that between SO_2 and tetralogy of Fallot: adjusted RR 1.38 (1.07, 1.79). Increased risks for tetralogy of Fallot were also observed with both PM_{10} and NO_2: adjusted RR 1.48 (0.57, 3.84) and 1.44 (0.71, 2.93), respectively. The authors state that given the multiple comparisons involved in testing many congenital anomaly subtypes against three pollutants, the positive associations could easily be chance associations.

Comment

- This study did not measure temporal variation, such as seasonal variation, in exposure. Also, it had only 1996 data which were used to estimate the average for 1991–2000.

Dugandzic et al., 2006: The association between low level exposures to ambient air pollution and term low birth weight: a retrospective cohort study.

Dugandzic et al. conducted a retrospective cohort study to evaluate the relationship between exposures to ambient air pollution and several birth outcomes, including LBW (<2500 g). The authors used a population-based perinatal database to identify and obtain maternal and perinatal data on singleton infants born at term (≥37 weeks gestation) between January 1, 1988 and December 31, 2000 and weighing at least 500 g in the Canadian province of Nova Scotia. The cohort included 74,284 infants for whom adequate exposure data existed in at least one trimester; 1,193 (1.6%) were classified as LBW. The annual LBW rates ranged from 1.45% to 2.38%, with a decreasing trend over time ($p<0.001$).

The authors obtained SO_2, O_3, and PM_{10} concentrations from the National Air Pollution Surveillance (NAPS) network. Eighteen monitoring stations provided daily data for SO_2 and O_3 (except there were no SO_2 data for 1997). PM_{10} was sampled every six days. Only three stations monitored more than one pollutant. The authors developed individual exposure profiles for each mother by geocoding the postal code, town or village, or municipal code for the resident address reported at the time of delivery. The authors note that though the true distance between rural residences and central postal locations is not known, levels of air pollution in these areas is homogeneous, especially given the absence of major industry. Only mothers living within 25 km of a NAPS station were included in the analysis. The authors averaged the daily pollutant data over trimesters when at least 75% of the daily values for a period were available. For addresses assigned to more than one station, the trimester exposure was estimated using distance weighting to give more weight to monitors closer to the residence. The trimester SO_2 exposure levels in ppb were as follows: mean=10, 25^{th} percentile=7, median=10, 75^{th} percentile=14, and maximum=38. The authors reported statistically significant trends in mean SO_2 and O_3 levels, though the direction of the trends was not described. O_3 appeared to be increasing, while the direction of the SO_2 trend was unclear.

Dugandzic et al. conducted logistic regression analyses with trimester exposures for each single pollutant modeled as continuous and categorical variables. Covariates included in the analyses were maternal age, parity, prior fetal death, prior neonatal death, prior LBW infant, smoking during pregnancy, neighborhood family income, infant gender, gestational age, weight change, and year of birth. Neighborhood family income was based on 1996 summary data at the enumeration area (the smallest census unit, consisting of 125–375 households).

The largest difference in mean birthweight between the bottom and top quartiles of exposure was for first trimester exposure to SO_2: 3,467 g for the bottom quartile vs. 3,428 g for the top quartile. Table 12 shows adjusted relative risks for SO_2 exposure in each trimester. Compared to the lowest quartile of SO_2 exposure in the first trimester, the highest quartile was associated with a statistically significant increase in risk of LBW (adjusted RR 1.36, CI 1.04, 1.78), with a suggested dose-response effect. However,

including birth year in the model reduced the observed effect (RR 1.26, CI 0.96, 1.66). When exposure to SO_2 was coded as a continuous variable, an IQR (7 ppb) increase in SO_2 in the first trimester was associated with a 15% increase in LBW risk (adjusted RR 1.15; 95% CI 1.00, 1.31) of borderline significance. The authors also report a linear response between increasing SO_2 and decreasing birthweight.

Table 12. Adjusted RRs for term LBW according to trimester-specific exposure to SO_2[a].

Exposure	Adjusted RR (95% CI)	
	Without birth year	Adjusted for birth year
1st Trimester		
25 – 50th percentile[b]	0.96 (0.73, 1.28)	0.98 (0.74, 1.31)
51 – 75th percentile	1.18 (0.88, 1.58)	1.12 (0.84, 1.50)
>75th percentile	**1.36 (1.04, 1.78)**	1.26 (0.96, 1.66)
Continuous[c]	**1.20 (1.05, 1.38)**	1.15 (1.00, 1.31)
2nd Trimester		
25 – 50th percentile	1.12 (0.86, 1.46)	1.14 (0.88, 1.49)
51 – 75th percentile	1.13 (0.85, 1.50)	1.09 (0.82, 1.45)
>75th percentile	1.04 (0.79, 1.37)	0.99 (0.75, 1.32)
Continuous	0.99 (0.87, 1.13)	0.97 (0.85, 1.11)
3rd Trimester		
25 – 50th percentile	1.04 (0.80, 1.34)	1.03 (0.80, 1.34)
51 – 75th percentile	0.85 (0.63, 1.15)	0.81 (0.60, 1.10)
>75th percentile	0.88 (0.67, 1.15)	0.82 (0.63, 1.08)
Continuous	0.93 (0.81, 1.06)	0.89 (0.78, 1.02)

[a] Adjusted for maternal age, parity, prior fetal death, prior neonatal death, prior LBW infant, smoking during pregnancy, neighborhood family income, infant gender, gestational age, weight change, and year of birth
[b] <25th percentile is the referent category
[c] Based on IQR (7ppb) increase in SO_2

First trimester exposure to PM_{10} in the highest quartile was also associated with increased risk of LBW before adjustment for birth year (adjusted RR 1.33, CI 1.02, 1.74). After adjustment for birth year the effect was smaller and non-significant (adjusted RR 1.11, CI 0.84, 1.48). When coded as a continuous variable, PM_{10} was not significantly associated with birthweight. No significant effects of exposure to O_3 in any trimester, or any of the pollutants in the second or third trimesters, were reported.

Comment

- The authors acknowledge that, particularly for SO_2, proximity to a monitor may have been a concern because SO_2 concentrations are known to decrease substantially as distance from the source increases. However, they cite monitor-site pair data from across Canada, indicating a strong correlation of 0.8 for SO_2 within a distance of 25 km. 70% of the mothers in the study lived within 15 km of a monitoring station.

Gilboa et al., 2005: Relation between Ambient Air Quality and Selected Birth Defects, Seven County Study, Texas, 1997–2000.

Gilboa et al. conducted this population-based case-control study to examine the association between the risk of selected cardiac birth defects and oral clefts and maternal exposure to CO, NO_2, O_3, SO_2, and PM_{10} during the 3^{rd}–8^{th} week of pregnancy.

The Texas Birth Defects Registry provided data on birth defects for 7,381 live births and fetal deaths delivered at or after 20 weeks' gestation between 1/1/1997 and 12/31/2000 to mothers residing in one of seven Texas counties with at least 10,000 births annually. Data on 5,338 cases were available for analysis after exclusions due to missing vital record, a parent less than 18 years at delivery, isolated patent ductus arteriosus or patent foramen ovale, missing gestational age, maternal diabetes, holoprosencephaly in addition to an oral cleft, and missing or post office box addresses. The authors used the same exclusion criteria to select from the state vital records database a stratified random sample of 4,580 non-malformed controls, frequency-matched to cases on year of birth, vital status and maternal county of residence at delivery. The controls were selected to ensure a ratio of approximately 2 controls per case for the largest group, and the same control group was used for all analyses.

The authors attempted to geocode maternal residence at delivery for all cases and almost all controls, and succeeded for 86% and 80%, respectively. Cases and controls who could not be geocoded were not meaningfully different from geocoded subjects on social and behavioral factors, and were excluded from the analysis. The EPA provided raw data for hourly (for gases) or daily (for PM_{10}) air pollution concentrations for the study counties for 1996–2000. SO_2 was monitored in four counties. For each pollutant, the authors calculated the distance between the pollution monitor and each maternal residence, and calculated the average exposure measured at each monitor during weeks 3–8 of pregnancy. For each pregnancy, exposures were assigned according to the closest monitor with at least 70% of possible daily means available. At least 85% of pollutant means came from either the closest or second closest monitor to the maternal residence. Median distances were 8.6–14.2 km.

Alcohol consumption during pregnancy, type of attendant of delivery (physician/nurse-midwife vs. other), gravidity, marital status, maternal age, maternal education, maternal illness, maternal race/ethnicity, parity, place of delivery, plurality, prenatal care, season of conception, and tobacco use during pregnancy were considered as potential confounders and evaluated for each model.

The authors developed logistic regression models to test for single and multipollutant associations with birth defect groupings, adjusting for available covariates. Simultaneous adjustment for co-pollutants did not result in meaningful changes in the OR, but did decrease precision. Pollutants were modeled as continuous variables and in quartiles based on the pollutant distribution among the controls. The authors also

investigated potential effect modification by sex, plurality, maternal education, maternal race, and season of conception.

Gilboa et al. observed a positive association between SO_2 exposure and isolated ventricular septal defects: the AOR for the fourth quartile of SO_2 exposure vs. the first quartile was 2.16 (1.51, 3.09). SO_2 was also associated with all ventricular septal defects combined; AOR 1.31 (1.06, 1.61; *p* trend=0.0850) for the fourth quartile of SO_2 exposure vs. the first quartile. The authors observed an inverse association for SO_2 and isolated atrial septal defects and multiple conotruncal defects.

Table 13. AOR[a] and CI for isolated and multiple clinical diagnostic groupings, by quartile of average SO_2 concentration during weeks 3-8 of pregnancy.

Congenital anomaly	**2nd: 1.3– <1.9 ppb**	**3rd: 1.9– <2.7 ppb**	**4th: ≥2.7 ppb**
Isolated cardiac defects			
Aortic artery and valve defects	NA	1.06 (0.34, 3.29)	0.83 (0.26, 2.68)
Atrial septal defects	1.22 (0.79, 1.88)	0.76 (0.47, 1.23)	**0.42 (0.22, 0.78)**
Pulmonary artery and valve defects	0.63 (0.23, 1.74)	0.93 (0.36, 2.38)	1.07 (0.43, 2.69)
Ventricular septal defects	1.02 (0.68, 1.53)	1.13 (0.76, 1.68)	**2.16 (1.51, 3.09)**
Multiple cardiac defects			
Conotruncal defects	0.71 (0.46, 1.09)	0.71 (0.46, 1.09)	**0.58 (0.37, 0.91)**
Endocardial cushion and mitral valve defects	0.89 (0.50, 1.61)	0.89 (0.49, 1.62)	1.18 (0.68, 2.06)
Isolated oral clefts			
CLP	0.79 (0.52, 1.20)	0.95 (0.64, 1.43)	0.75 (0.49, 1.15)
Cleft palate	0.89 (0.40, 1.97)	1.49 (0.72, 3.06)	1.22 (0.56, 2.66)

[a] Adjusted for season of conception and parity. Lowest quartile of SO_2 exposure is the referent category.

Gilboa et al. conclude that their study does not provide strong evidence that air pollution increases the risk of cardiac defects or oral clefts, though it does support one of the findings of a California study which it sought to corroborate.

Comments

- The authors note that they did not incorporate weather conditions or use dispersion models in their estimates of exposure.
- The authors acknowledge presenting 255 associations and that some of their statistically significant findings were likely the result of chance.
- Variation in exposure to SO_2 was relatively small.

Gouveia et al., 2004: Association between ambient air pollution and birth weight in São Paulo, Brazil.

The aim of this cross-sectional study was to examine the association between exposure to outdoor air pollution during pregnancy and birthweight in the city of São Paulo, Brazil. Gouveia et al. obtained data on all live births to mothers resident in São Paulo in 1997 from the Birth Information System of the Ministry of Health in Brazil. Only singleton, full term (≥37 weeks gestation) live births with birthweight between 1,000 g and 5,500 g were included. The cohort included 179,460 live births, of which 5.0% were LBW. The mean birthweight was 3,186 g.

Data on SO_2, NO_2, CO, O_3, and PM_{10} were obtained from the environmental protection agency in São Paulo. The data comprised daily means of SO_2 and PM_{10}, maximum hourly mean for a 24-hour period for NO_2 and O_3, and a maximum 8-hour moving average for CO. There were 12 monitoring sites for SO_2 and PM_{10}. The pollutant levels were averaged across all sites for daily citywide levels. Based on each date of birth and length of gestation, mean exposures of about three months for each pollutant were assigned to each birth. The annual mean (SD) SO_2 concentration was 7.5 (3.9) ppb, with an annual minimum of 1.3 ppb and an annual maximum of 21.7 ppb. SO_2 concentrations varied considerably by season, as shown in Table 14.

Table 14. SO_2 concentrations by period, São Paulo, Brazil, 1997.

Period	**Mean (SD), ppb**
Annual	7.5 (3.9)
January – March	8.5 (2.9)
April – June	10.7 (3.9)
July – August (September?)	6.8 (3.3)
October – December	3.9 (1.5)

The authors used generalized additive models to estimate the change in mean birthweight, and logistic regression to estimate ORs for LBW associated with exposure to air pollutants. The models adjusted for gender, gestational age, month of birth, maternal age, maternal education, prenatal care, and type of delivery. In single pollutant models, all pollutant exposures in the first trimester were associated with lower birthweight, but only the associations for PM_{10} and CO were statistically significant. Results for exposure in the second and third trimesters were inconsistent; only SO_2 exhibited a significant relation with birthweight, with SO_2 exposure in the second trimester associated with greater birthweight (Table 15). However, when other pollutants were included in the model, the association was not significant.

Table 15. Changes in birthweight for a 3.8 ppb increase in exposure to SO_2 at each trimester of pregnancy. Results from regression models adjusted for covariates.*

Trimester	Change in birthweight (g)	95% CI
First	-24.2	-55.5, 7.1
Second	33.7	1.6, 65.8
Third	9.7	-25.6, 44.9

* Adjusted for gender, gestational age, month of birth, maternal age, maternal education, prenatal care, and type of delivery

Logistic regression models had generally non-significant results. The authors state the relation between LBW and exposure to SO_2 during the second trimester exhibited an inverse pattern with higher risk of a LBW baby; however, this is not consistent with their table of logistic regression results. One of few statistically significant findings from the logistic regression analyses was a slightly reduced risk of LBW associated with SO_2 exposure in the first trimester: OR 0.90 (0.84, 0.97) for the second quartile of exposure levels, compared to the first quartile (Table 16). Third trimester SO_2 exposures were associated with modest increases in risk of LBW that were not statistically significant.

Table 16. Adjusted ORs* and 95% CIs for LBW according to quartiles of exposure in SO_2 each trimester of pregnancy.

SO_2 level (quartiles)	1st Trimester	2nd Trimester	3rd Trimester
1st	1	1	1
2nd	0.90 (0.84, 0.97)	0.99 (0.92, 1.05)	1.20 (0.86, 1.68)
3rd	0.91 (0.82, 1.01)	1.01 (0.90, 1.12)	1.23 (0.87, 1.72)
4th	0.91 (0.79, 1.04)	1.02 (0.88, 1.17)	1.15 (0.75, 1.75)

* Adjusted for gender, gestational age, month of birth, maternal age, maternal education, prenatal care, and type of delivery

Comments

- The linear and logistic regression findings are inconsistent.
- This study assumes the same exposure across a very large city.

Ha et al., 2001: Is air pollution a risk factor for low birth weight in Seoul?

In this study, Ha et al. examined the associations between air pollution exposures during the first and third trimesters of pregnancy and low birthweight (LBW; <2500 g) among full-term births (37–44 weeks gestation) for a two-year period. The authors obtained birth certificates for 276,763 full-term singleton births in the Seoul, South Korea area between January 1, 1996 and December 31, 1997 from the Korean National Birth Register. The prevalence of LBW was 2.8% and mean birthweight was 3,310 g.

The major source of air pollution in Seoul is automobile exhaust emissions. Air pollution data on SO_2, CO, NO_2, TSP and O_3 were obtained from the Department of Environment.

The 21 monitoring sites represented 84% of all administrative areas. Hourly measurements were averaged over 24-hr periods, except for O_3, for which daytime 8-hr averages were calculated. These daily averages were used to estimate first and third trimester mean exposures for each infant, based on the infant's gestational age and birth date. The 25th, 50th, and 75th percentiles of SO_2 were 10.0, 13.2, and 16.2 ppb during the first trimester, respectively, and 8.4, 12.2, and 16.3 ppb during the third trimester. The Pearson correlation coefficients for correlations between SO_2 and co-pollutants were: CO 0.83; NO_2 0.70; TSP 0.67; and O_3 -0.29.

Ha et al. evaluated applied generalized additive logistic regression, adjusting for gestational age, maternal age, parental education level, parity and infant gender. Because concentrations of pollutants showed seasonal patterns, a LOESS smooth function of time trends was used to avoid confounding by season. The RR of LBW was 1.06 (CI 1.02, 1.10) for each IQR increase in exposure to SO_2 during the first trimester of pregnancy. CO, NO_2, and TSP were also associated with an increase in LBW, while O_3 was associated with a decreased risk, in single pollutant models. The associations were reversed for pollutant exposures in the third trimester when the exposures during the two trimesters were analyzed separately. However, when the first and third trimester pollutant exposures were both included in the models, the observed increase in risk for LBW for SO_2, CO, NO_2, and TSP in the first trimester persisted, while the apparent third trimester reduction in risk changed toward the null for all pollutants. When both trimester exposures were included, the RR for LBW was 1.07 (0.98, 1.17) for first trimester exposure and 1.03 (0.90, 1.17) for third trimester exposure.

Birthweight was also analyzed as a continuous variable to estimate changes associated with IQR changes in each pollutant. The reduction of birthweight for an IQR increase of SO_2 (6.2 ppb) during the first trimester was 8.06 (5.59, 10.53) grams. The relationships between birthweight and first trimester exposure to SO_2 CO, NO_2, and TSP were relatively linear, without thresholds for pollutant concentrations.

Comments

- Ha et al. note that the pollutants were highly correlated, that secondary particles are formed in the atmosphere by chemical reactions involving SO_2 and NO_2, and that it is therefore reasonable to consider these pollutants together rather than separately. However, multipollutant models are not reported in this study.
- The authors note that they lacked information to adjust for individual risk factors such as smoking and alcohol consumption; however, they assert that such risk factors would not be associated with ambient air pollution levels.
- The authors concluded there was a clear negative relation between birthweight and concentrations of CO, NO_2, SO_2, TSP and O_3 during the first trimester (i.e., higher pollutant exposures were correlated with lower birthweight).
- Correlations between first and third trimester exposures were not reported, but might be relevant.

Hansen et al., 2008: The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy.

Hansen et al. examined possible associations between fetal ultrasonic measurements and exposure to ambient SO_2, PM_{10}, NO_2, and O_3 in the first 4 months of pregnancy.

Fetal ultrasound measurements (recorded in millimeters) were obtained from a private ultrasound clinic in Brisbane, Australia. The authors used data from a previous fetal biometry study, which included all women attending the clinic for an ultrasound scan between January 1993 and April 2003 who consented to participate. Women had been referred for "routine ultrasound" between 11 and 41 weeks gestation. The present study included all women who agreed to participate, whose postcode centroid was within 14 km of an air pollution monitoring station, and whose scans occurred during gestational weeks 13–26. Most (84%) pregnancies had one ultrasound scan in the study. The authors examined the following fetal ultrasonic measurements: femur length (FL), biparietal diameter (BPD), head circumference (HC), and abdominal circumference (AC). There were 11,475 scans within 14 km of an SO_2 monitor. When exposure to an air pollutant was associated with a measurement, the relationship decreased toward the null as distance from the monitor increased. *For this reason, the authors only presented results for the 120 ultrasound scans of women living within 2 km of a monitor.*

Covariates available for this study included gender, gestational age, and mother's age and postcode. No measure of SES was collected from the women, so the authors used the postcode to link an index of relative socioeconomic disadvantage derived from area-level attributes such as income, educational attainment, unemployment, and occupations.

Air pollution and meteorologic data for Brisbane and surrounding areas were obtained from the Queensland Environmental Protection Agency (QEPA) for 1992–2003. Pollution was monitored at 18 different sites, with the majority located within a 30-km radius of Brisbane; however, only 4 sites monitored for SO_2. Hourly readings for SO_2, O_3, NO_2, and PM_{10} were used to calculate daily averages for SO_2, NO_2, and PM_{10}, and 8-hr averages for O_3. Daily average temperature was available from five of the QEPA monitoring sites and the Brisbane airport. The authors then assigned pollutant exposures and temperature for each day of gestation using the monitoring site closest to the mother's residential postcode. Average exposures were also calculated for the first four 30-day periods of gestation. SO_2 levels were as follows [mean (IQR), ppb]: all seasons 1.19 (1.00); summer 1.06 (0.84); fall 1.01 (0.88); winter 1.29 (1.06); spring 1.46 (1.03). The least complete data were for SO_2, with missing data for 61% of days. The authors excluded observations from analysis if the exposure was missing for more than 5 of 30 days.

The authors conducted a multi-stage analysis using regression analyses to produce residuals that they could use to adjust for potential confounders such as gestational age, mother's age, SES, season, long-term trends, and temperature. Finally, the

authors used generalized estimating equations with an exchangeable correlation structure because 16% of women had repeated (non-independent) ultrasound scans.

When adjusted for fetal gender, gestational age, mother's age, SES, concurrent temperature exposures, seasonality, and long-term trend, a reduction in fetal AC was associated with exposure to SO_2 during days 61–90. The mean change in fetal AC per 0.8 ppb increase in maternal exposure during early pregnancy was -1.67 (-2.94, -0.40) mm. Exposure to SO_2 during days 0–30 was also associated with a -0.68 (-1.09, -0.27) mm change in BPD. Including PM_{10} or O_3 in two-pollutant models with SO_2 caused little to no change in the statistical significance or magnitude of these associations. However, there was evidence for effect modification by SES for AC. The association between SO_2 exposure in days 61–90 and AC was greater for women in the fourth (highest) SES quartile: mean change per 0.8 ppb increase in SO_2 in days 61–90 was -2.29 (-3.65, -0.94) mm, and somewhat lower for women in the third SES quartile: -1.33 (-2.65, -0.02) mm. The authors were unable to calculate associations in the lower SES quartiles for these two measures.

Exposure to PM_{10} was associated with statistically significant reductions in HC (exposure in fourth month of gestation), AC (fourth month), and FL (first and fourth months). Exposure to O_3 in the second month was associated with a statistically significant reduction in AC. Exposure to NO_2 was not associated with any statistically significant changes in fetal measurements.

Comments

- The authors acknowledge that given the many comparisons made, some of their findings could have occurred by chance. However, all the significant associations observed suggested exposure to pollutants was associated with reduced fetal growth.
- Lifestyle factors, such as maternal smoking during pregnancy, exposure to environmental tobacco smoke, and alcohol use were not assessed. However, Hansen et al. contend that these factors would not confound the results because they are not associated with temporal variations in air pollution.
- The authors recommend using only monitors within a 2-km radius of the subject.
- The authors note that they were unable to obtain birth outcome data for the pregnancies in the study, and therefore cannot conclude whether the reductions observed in the study persisted until birth and had clinical relevance.
- The SO_2 concentrations in this study were very low, with very little variability.

Hansen et al., 2009: Ambient Air Pollution and Birth Defects in Brisbane, Australia.

Hansen et al. conducted a case-control study to examine associations between ambient air pollution and congenital heart defects and oral clefts among births in Brisbane, Australia in 1998–2004. Birth outcome data were collected from the Queensland Health Perinatal Data Collection Unit, which routinely collects data from all public and private hospitals in Brisbane, and data submitted voluntarily from homebirths. Cases were

singleton births with cardiac, cleft lip or palate defects (see Table 17). The total number of cases was not reported.

For each case, the authors selected five controls matched on mother's age, marital status, indigenous status, number of previous pregnancies, month of LMP, area-level SES (based on deciles of an SES index), and distance to pollution monitor.

Air pollution data for Brisbane and surrounding areas were obtained from the QEPA. Eighteen different fixed sites were monitored, with the majority located within a 30-km radius of Brisbane; however, only 7 sites monitored for SO_2. Hourly readings were obtained for O_3, NO_2, SO_2, CO, and PM_{10}. Daily averages were calculated for PM_{10}, NO_2, and SO_2, and 8-hr averages were calculated for CO and O_3. The authors then calculated average exposure estimates over the days of weeks 3–8 of gestation (post LMP) based on the monitor closest to the mother's statistical local area (SLA) at time of delivery. The average daily mean SO_2 level was 1.5 ppb for all seasons combined; the seasonal means were 1.5 ppb in summer, 1.6 ppb in autumn, 1.4 ppb in winter, and 1.4 ppb in spring.

Hansen et al. used conditional logistic regression to calculate OR for an increase of one IQR for each air pollutant (0.6 ppb for SO_2). The authors note that the IQR can be thought of as the difference between a moderately good and a moderately bad exposure period. In addition, they note that this makes the changes seen with different air pollutants more comparable. Air pollutants were entered into the models as continuous covariates. To assess bias introduced by measurement error in exposure estimates, the authors also conducted sensitivity analyses that included only women who lived within 12 or 6 km of a monitor (less than 0.1% of the sample lived within 2 km of a monitor). Finally, to evaluate potential over-matching of controls, Hansen et al. also conducted analyses using five randomly selected controls per case.

All reported results for SO_2 are shown in Table 17. In the primary analysis, a 0.6 ppb increase in SO_2 was associated with reduced risks for the heart defects, except atrial septal defects, though the associations were not statistically significant. The observed associations changed with many of the sensitivity analyses. Specifically, among women living within 6 km of a monitor, SO_2 was associated with an increased risk of aortic artery and valve defects (OR 10.76, CI 1.50, 179.8) and a decreased risk of conotruncal defects (OR 0.27, CI 0.07, 0.81). For oral cleft defects, SO_2 was associated with CLP (OR 1.27, CI 1.01, 1.62) and cleft lip alone, though the latter was not statistically significant (OR 1.40, CI 0.96, 2.01).

Table 17. AOR[a] for the risk of specific congenital defects associated with ambient SO_2 by distance from monitor (adapted from (Hansen et al., 2009)).

Malformation	Cases (n)	Primary analysis	≤ 12 km from monitor	≤ 6 km from monitor	Unmatched controls
Aortic artery and valve defects	63	0.87 (0.61, 1.21)	1.42 (0.73, 2.85)	**10.76 (1.50, 179.83)**	0.83 (0.60, 1.13)
Atrial septal defects	127	1.30 (0.99, 1.74)	1.23 (0.85, 1.79)	1.61 (0.84, 3.08)	0.84 (0.69, 1.01)
Pulmonary artery and valve defects	64	0.93 (0.65, 1.31)	1.12 (0.65, 1.91)	0.70 (0.16, 1.96)	0.82 (0.60, 1.08)
Ventricular septal defects	222	0.84 (0.69, 1.02)	0.85 (0.63, 1.14)	0.64 (0.35, 1.08)	0.87 (0.74, 1.01)
Conotruncal defects	63	0.71 (0.48, 1.07)	0.58 (0.30, 1.04)	**0.27 (0.07, 0.81)**	0.83 (0.61, 1.12)
Endocardial cushion and mitral valve defects	33	0.86 (0.52, 1.45)	1.20 (0.57, 2.65)	-	0.91 (0.60, 1.40)
Cleft lip	57	1.40 (0.96, 2.01)	1.22 (0.64, 2.22)	1.24 (0.37, 4.41)	1.05 (0.79, 1.39)
Cleft palate	100	0.84 (0.64, 1.09)	0.62 (0.22, 1.60)	0.79 (0.51, 1.21)	0.90 (0.72, 1.12)
CLP	145	**1.27 (1.01, 1.62)**	1.05 (0.74, 1.46)	0.90 (0.46, 1.76)	1.07 (0.88, 1.28)

[a]Adjusted for gender. Cases and controls were matched on mother's age, marital status, indigenous status, number of previous pregnancies, month of LMP, area-level SES, and distance to pollution monitor

Given that there were several statistically significant adverse associations among the analyses of all the pollutants, as well as significant protective associations, Hansen et al. conclude their study found mixed results across all analyses and no consistent patterns with regard to adverse effects and distance to a monitor. Therefore, the authors argue, the few adverse effects need to be interpreted with caution.

Comments:

- The authors note they lacked information on various potential confounders, such as maternal smoking, drug and alcohol use, diet, and occupational exposures.
- By the authors' calculations, this study had a low power to detect small increases in risk.
- Another limitation the authors note is that exposure data was based on the closest monitoring site to the mothers' residence at the time of birth. The authors state that studies have documented that 12–22% of women change address during pregnancy; thus, residential mobility during pregnancy may have introduced misclassification.

- The authors state that their data suggest lower-than-average exposure to CO among cases, which would indicate low exposure to traffic, and therefore, residence in semi-rural areas, where pesticide exposure might be a competing cause of birth defects.
- Given the number of analyses performed, there is a possibility that the statistically significant associations occurred by chance.
- The statistical models do not appear to have included co-pollutants.

Hemminki and Niemi, 1982: Community study of spontaneous abortions: relation to occupation and air pollution by sulfur dioxide, hydrogen sulfide, and carbon disulfide.

In this cross-sectional study, Hemminki et al. used a hospital discharge registry to investigate the possible effects of occupation and environmental pollution on the frequency of spontaneous abortions compared with induced abortions and births in Valkeakoski, an industrial community in Finland, from 1974–1977. The authors examined the occupation of the mothers and their husbands, and the level of air pollution in the family's residential area. More than 60% of the town's over 20,000 inhabitants were employed in manufacturing industries, such as pulp, paper, metal, viscose rayon, and the chemical industry, and the area had a relatively high frequency of spontaneous abortion. The pulp and viscose rayon industries emit hydrogen sulfide and the viscose rayon industry also emits carbon disulfide. The occupation component of the study is not included here because the occupations were not linked to SO_2 exposure.

The authors obtained information on all the women of Valkeakoski who were treated in any hospital in Finland for spontaneous abortion, induced abortion, or childbirth. The data consisted of 1,792 cases of spontaneous abortion, including nine patients with two, and one patient with three spontaneous abortions in the study period. The authors report that according to another study in a large hospital, the mean gestational length of hospitalized spontaneous abortions was 2–3 months. Only women 20–39 years and with one child or less before the pregnancy were included in the analyses. The sample size for SO_2 analyses appeared to be 83 cases.

Information on the women's and their husband's occupations, places of work, number of children, and places of residence were collected from 1975 census data. The authors were able to find census data for 89% of the cases in the hospital registry; cases missing census data had likely moved away before the census or moved to Valkeakoski after the census. These cases had a higher spontaneous abortion rate than cases with census data (13.1% vs. 9.0% of pregnancies), though the difference was not statistically significant.

SO_2, produced mainly by heating of residential and industrial facilities, and hydrogen sulfide and carbon disulfide, both emitted from industrial facilities, had been mapped in a special field study of the air pollutants in Valkeakoski from 1977 to 1979 by the Institute of Meteorological Sciences. The authors used these maps and women's

addresses to classify subjects into groups of different exposure levels. Residential areas were divided into three groups of mean annual ambient SO_2 concentration: <1.9, 1.9–5.7, >5.7 ppb (<5, 5–15, and > 15 $\mu g/m^3$).

As shown in Table 18, the frequency of spontaneous abortions was lowest in the intermediate pollution zone (7.5% of pregnancies) and highest in the most polluted zone (9.3%). The authors state that the high rate of spontaneous abortions in the most polluted zone was entirely due to the high rates among employers and higher officials, while in other socioeconomic groups, no clear gradient was observed. The authors conclude that it is unlikely that the concentration of SO_2 is related to the frequency of spontaneous abortions.

Table 18. Rate of spontaneous abortions by mean annual concentration of SO_2 in the air and the socioeconomic status in Valkeakoski from 1974 to 1977 (women aged 20–39 with one child or less).

Socioeconomic status	Mean annual concentration of SO_2 (ppb)					
	<1.9		1.9–5.7		>5.7	
	# of spontaneous abortions	Rate of spontaneous abortions	# of spontaneous abortions	Rate of spontaneous abortions	# of spontaneous abortions	Rate of spontaneous abortions
Employers, own-account workers, managers, and higher administrative or clerical employees	1	11.1	2	11.1	4	21.1
Lower administrative or clerical employees	4	10.0	17	9.3	18	10.5
Workers	3	9.4	7	5.8	9	9.2
Others[a]	3	6.0	8	6.0	7	5.8
Total	11	8.4	34	7.5	38	9.3

[a] Includes economically inactive and family workers

Comments

- The authors report that this study is limited by its cross-sectional design and quality of data on spontaneous abortions.
- The authors also note that they were unable to check the actual time subjects spent at home, and local aberrations may be present in hospital discharge data. The authors conclude chance correlations are likely to be present.

- As the authors point out, the numbers of spontaneous abortions was small. This is especially true when the data are stratified by pollutant exposure, industry, and socioeconomic status.
- The cases with missing census data had higher rates of spontaneous abortion but could not be included in the analyses. This may have biased the results away from the ability to find associations.
- Pollution maps were for 1977–1979, whereas the spontaneous abortions and births occurred in 1974–1977.

Hwang and Jaakkola, 2008: Ozone and other air pollutants and the risk of oral clefts.

Hwang et al. conducted a nationwide population-based case-control study in Taiwan to assess exposure to ambient air pollution during the first three months of pregnancy on the risk of CLP. The authors identified 653 subjects born in 2001–2003 with CLP and sufficient information on gestational age and air pollutants through the Taiwan Local Household Registry of the Taiwan Department of Health. The 6,530 control subjects had no birth defects, were randomly selected from the source population, and had sufficient information on gestational age and air pollutants.

The authors examined traffic-related pollutants such as NO_x, CO, and O_3, and other pollutants mainly from fossil fuel combustion sources, such as SO_2 and PM_{10}. Hourly monitoring data for the air pollutants as well as daily temperature and relative humidity came from 72 Taiwan Environmental Protection Agency monitoring stations. The hourly measures were integrated into monthly point data. The authors then interpolated the pollutant data using IDW to estimate the spatial distribution of each pollutant and used the estimated pollution concentration at the center of townships or districts to assign exposure for the duration of each pregnancy from 2000 through 2003. Based on the date of birth and gestational age, the authors estimated the monthly average concentration corresponding to the first through third months of gestation, the relevant embryologic period for oral cleft. SO_2 levels were as follows (mean ± SD): spring 4.22 ±2.59 ppb, summer 3.35 ±2.00, fall 3.41 ± 2.07, winter 4.88 ± 3.71, all year 3.96 ± 2.36. The concentrations of PM_{10} and SO_2 were highly correlated (r=0.50), indicating a common source of stationary fuel combustion, although SO_2 concentrations were also correlated with NO_x (r=0.45).

Available covariates included gender of the infant, maternal age, plurality (singleton or multiple births), gestational age, season of conception, and proportion of urban population in the municipality. Final estimates adjusted for gender, maternal age, and gestational age. Hwang et al. conducted logistic regression to estimate adjusted OR for the effect of each pollutant on the risk of CLP.

The adjusted OR for a 10 ppb change in SO_2 alone was 0.92 (0.63,1.35) for exposure in the first month, 0.84 (0.57,1.25) for the second month, and 0.72 (0.47,1.08) for the third month. Including O_3 and NO_x in the model reduced the effect estimates, making the relationship appear more protective, though not statistically significant. The authors

found negative or weak associations for CO, NO_x, SO_2, and PM_{10}. However, exposure to outdoor O_3 during the first and second month of pregnancy appears to increase the risk of CLP (1.20, CI 1.02,1.29 and CI 1.03,1.52 per 10 ppb change, respectively).

Jalaludin et al., 2007: Impact of ambient air pollution on gestational age is modified by season in Sydney, Australia.

Jalaludin et al. evaluated the effect of early and late pregnancy exposure to PM_{10}, $PM_{2.5}$, O_3, NO_2, CO, SO_2 on preterm birth in the Sydney metropolitan area. The authors obtained information on 123,840 singleton births in metropolitan Sydney between January 1, 1998 and December 31, 2000; 6,011 (4.9%) were preterm (<37 weeks gestation). Information on all births was obtained from the Midwives Data Collection (MDC), New South Wales Department of Health. MDC is a population-based surveillance system of all live and stillbirths of at least 20 wks gestation and at least 400 grams birthweight. Babies born past 42 wks gestation or to women with gestational hypertension or diabetes were excluded.

The authors obtained air pollution and meteorological data from the New South Wales Department of Environment and Conservation. Air pollution data came from 14 monitoring stations and included 1-hr maximum SO_2 levels. The authors averaged pollutant levels from monitoring stations to provide an estimate for the whole metropolitan Sydney area. For each birth, exposure to each air pollutant was estimated for the first month after the estimated date of conception, the first trimester, one month and three months prior to delivery. In an additional analysis, the authors matched pollutant levels to births in postcodes if at least 50% of the postcode was within 5 km of the monitoring station. For the 5 km analysis, 11 monitoring stations had sufficient data for inclusion and there were 65,814 eligible births, including 3,596 (5.5%) preterm births.

Regression models included gender of child; maternal age; maternal smoking during pregnancy (yes/no); gestational age at first prenatal visit (at or before 20 wks vs. later); maternal indigenous status; whether first pregnancy; season of conception, and SES. SES was categorized in quintiles based on census data applied to postcode of residence. The authors also considered temperature and humidity at time of pollutant exposure, but excluded these variables from multivariate models because they were not significant in single variable models.

Preterm birth was significantly associated with maternal age, maternal smoking, male infant, indigenous status, first pregnancy, and SES. Risk of preterm delivery was lower if conception occurred in spring compared to summer. Concentrations of CO, NO_2, $PM_{2.5}$ and SO_2 were highest in winter and lowest in summer.

SO_2 results for Sydney and mothers living within 5 km of air monitors are shown in Table 19.

Table 19. Adjusted ORs[a] for preterm birth and 1 ppb increase in SO_2 exposure.

Residence	Timing of exposure to SO_2			
	First month	First trimester	Final month	Final 3 months
Sydney	**0.86 (0.82, 0.89)**	**0.83 (0.78, 0.89)**	0.99 (0.95, 1.04)	0.98 (0.90, 1.06)
Within 5 km	1.38 (0.88, 2.17)	**2.31 (1.29, 4.15)**	**1.56 (1.02, 2.38)**	**2.33 (1.34, 4.04)**

[a] Adjusted for maternal age, smoking during pregnancy, gestational age at first prenatal visit; indigenous status; parity; season of conception, and SES.

In the metropolitan Sydney cohort, higher exposure to SO_2 in the first month of gestation was associated with a slight decrease in the risk of preterm birth (OR 0.86, CI 0.82, 0.89). Similarly, exposure to SO_2 in the first trimester appeared to be slightly protective (OR 0.83, CI 0.78, 0.90). Exposure to SO_2 or any other pollutant in the final month and final three months of pregnancy showed no statistically significant effect on risk of preterm birth.

However, among infants born to mothers living within 5 km of monitors, exposure to SO_2 in the final month and final three months of pregnancy were associated with increased risk of preterm birth (OR 1.56, CI 1.02, 2.38 and OR 2.33, CI 1.34, 4.04, respectively).

The season in which an infant was conceived modified the apparent effects of some pollutants on preterm birth, e.g., a 1-ppb increase in first trimester SO_2 exposure was strongly associated with preterm birth for infants conceived in autumn (OR 6.49, CI 4.37, 9.65). Table 20 shows the ORs for preterm birth and SO_2 exposure in the first trimester of pregnancy, for Sydney.

Table 20. Adjusted ORs[a] for preterm birth and 1-ppb increase in SO_2 exposure in the first trimester of pregnancy, by season of conception, Sydney, 1998–2000.

Season of conception	OR (95% CI)
Autumn	**6.49 (4.37, 9.65)**
Winter	**1.32 (1.03, 1.70)**
Spring	1.29 (0.96,1.73)
Summer	**0.83 (0.76, 0.90)**

[a] Adjusted for maternal age, smoking during pregnancy, gestational age at first prenatal visit; indigenous status; parity; and SES.

There were significant associations between SO_2 exposure in the first trimester and preterm birth if conception occurred in autumn or winter (OR 6.49, CI 4.37, 9.65 and OR 1.32, CI 1.03, 1.70, respectively, for 1 ppb increase in SO_2). Conversely, analyses suggested a modest protective effect of SO_2 exposure in the first trimester for infants conceived in summer (OR 0.83, CI 0.76, 0.90).

The authors also constructed models for two pollutants. In two-pollutant models for the 5 km study area, SO_2 levels in the first and third trimester of pregnancy remained a significant predictor of preterm birth (ORs were between 2.30 and 3.15; CIs were not reported).

Comments

- The authors note that given the number of comparisons made in their study, many of the significant findings may be spurious. In view of inconsistent associations, both within their study and also in comparison to other published studies, the authors urge caution in interpreting the effects.
- The authors chose to limit subjects to those living within 5 km of monitors to provide a more sensitive exposure estimate. Therefore, the analyses from that subset would be expected to yield more accurate estimates of the association between pollutant exposures and gestational age.

Jiang et al., 2007: A Time Series Analysis of Outdoor Air Pollution and Preterm Birth in Shanghai, China.

Jiang et al. examined the relationship between four pollutants (SO_2, NO_2, O_3, PM_{10}) in the 4, 6, and 8 wks preceding birth in Shanghai, China. The authors obtained data on all live births in 2004 from the Shanghai Municipal Center of Disease Control and Prevention. To determine the number of preterm births (<37 weeks), the authors calculated gestational age based on the date of the LMP. If LMP date was missing, they used the clinical estimate of gestational age. The number of preterm births was tallied for each day, and ranged from 1 to 21 per day. There were 3,346 preterm births in the study period; the total number of births was not reported.

Daily air pollution data for SO_2, NO_2, O_3, and PM_{10} for the study period were obtained from the Shanghai Environmental Monitoring Center. Data on concentrations of each pollutant were collected at six monitoring stations in urban areas of Shanghai and averaged daily. The authors used 24-hr average concentrations for SO_2, NO_2, and PM_{10}. For O_3, they used 8-hr averages (from 10 AM to 6 PM), as recommended by the WHO. The mean daily concentration of SO_2 was 21.25 ppb ± 0.53 (55.7 ±1.4 μg/m^3); the minimum was 4.31 ppb (11.3 μg/m^3), maximum 62.25 ppb (163.2 μg/m^3), 25th percentile 13.54 ppb (35.5 μg/m^3), median 19.64 ppb (51.5 μg/m^3), and 75th percentile 27.16 ppb (71.2 μg/m^3). The authors also obtained daily average temperature and humidity data.

The authors first built basic statistical models for daily counts of preterm births and included time and weather conditions, day of week and other covariates (not specified). The authors then introduced pollutant variables to the models, and evaluated the models for fit and quality of predictions. The authors analyzed the effects of subchronic exposures by using daily pollutant levels to compute a mean air pollution concentration value for the 4-, 6-, and 8-wk periods preceding each day of observation. For acute

exposure effects, the authors examined pollutant levels for single-day exposure windows with lags from 0 to 6 days before birth.

Jiang et al. presented the results as percent change in daily preterm births per 3.81 ppb (10 μg/m^3) increase in concentration of each pollutant. There were no statistically significant associations between acute exposures to outdoor air pollutants and preterm birth for any of the time lags (0–6 days) examined. SO_2, NO_2, and PM_{10} were associated with non-significant effects in the 4- and 6-wk periods. However, for all pollutants examined, exposure for the 8-wk period preceding birth was significantly associated with increases in preterm births. Each 3.81 ppb increase in average SO_2 concentration during the 8 wks preceding birth was associated with an 11.89% (6.69, 17.09) increase in preterm births - the largest association observed in the study. Comparing the 75th vs. 25th percentile exposure levels, the estimated increase in risk of preterm birth at the higher exposure level would be 42%.

The authors note that only a small proportion of Shanghai residents have air conditioning; thus, the monitored ambient air pollutant data might better represent actual population exposure than similar data in more developed countries.

Comments

- No attempt to disentangle the effects of the different pollutants is mentioned; the authors do not state whether the pollutants were modeled together or in separate models. The fact that several pollutants had significant associations for the same exposure period raises concern that the study is measuring the combined effects of multiple pollutants.
- No effort to account for differences in pollutant levels among the six monitoring locations is mentioned. The likely effect of this would be a bias toward the null.

Lee et al., 2003: Exposure to air pollution during different gestational phases contributes to risks of low birth weight.

The objective of this study was to evaluate the specific timing of peak effects of SO_2, PM_{10}, CO, and NO_2 on LBW (<2500 g) throughout the gestational period. Lee et al. obtained birth certificate data for births in Seoul, Korea between January 1, 1996 and December 31, 1998 from the Korean National Birth Register. Subjects were restricted to mothers who delivered full-term (37–44 weeks gestation) singletons during the study period (n = 388,105). The prevalence of LBW in the cohort was 2.9% (~11,255 infants).

The major source of air pollution in Seoul is automobile exhaust emissions. Air pollution data for 1995–1998 were obtained from the Department of the Environment. The 20 monitoring stations measuring pollutant levels covered nearly all areas of the city. The authors calculated 24-hr means from hourly measurements across all monitoring stations. These means were used to estimate exposures during the entire pregnancy, each trimester, and each month of pregnancy, based on the gestational age and birth date of each infant. The SO_2 concentrations, in ppb, were as follows: mean (SD) 12.1

(7.4), minimum 3.0, first quartile 6.8, median 9.8, third quartile 15.6, maximum 46.0. No air pollutant exceeded the WHO recommended criteria. The pollutants were highly correlated. Pearson correlation coefficients for SO_2 and CO were 0.79, 0.86, and 0.86 in the first, second, and third trimesters, respectively. Correlation coefficients for SO_2 and PM_{10} were 0.78, 0.82, and 0.85 in the first through third trimesters, respectively; and for SO_2 and NO_2 the coefficients were 0.75, 0.77, and 0.76.

Lee et al. used a generalized additive logistic model to allow regressions that included non-parametric smoothing functions in order to control the potential non-linear effects of date and season on births. After evaluating all covariates available, the authors determined season and parental occupation did not improve the models. The final models controlled for infant gender, birth order, maternal age, parental education level, date, and gestational age.

All of the pollutants were associated with increases in risk of LBW, but only in the first two trimesters. Each IQR (8.8 ppb) increase of SO_2 in the second trimester was associated with a reduction in birthweight of 14.6 g. The adjusted odds ratios for LBW associated with maternal SO_2 exposure are shown in Table 21. The authors report a positive dose response relationship between LBW and SO_2 exposure in the second trimester. The monthly analyses suggested that the risks for LBW tended to increase with SO_2 exposure between the third and fifth months of pregnancy. Similarly, the risks for LBW tended to increase with NO_2 exposure in months 3–5. First trimester exposures to CO and second trimester exposures to PM_{10} and NO_2 were also associated with statistically significant increases in risk of LBW, with adjusted ORs of 1.03 or 1.04.

Table 21 . Odds ratios and CIs of LBW for IQR increases of SO_2 during pregnancy[a].

Trimester	OR (95% CI)
First	1.02 (0.99, 1.06)
Second	**1.06 (1.02, 1.11)**
Third	0.96 (0.91, 1.00)
All	**1.14 (1.04, 1.24)**

[a] Adjusted for date, gestational age, infant gender, birth order, maternal age, and parental education level.

Lee et al. also conducted analyses designed to clarify the specific effect period of air pollution exposure. Two subgroups were created, the first with low exposures (<25th percentile for each pollutant) during the first five months of pregnancy, and a second subgroup with low exposures in the last five months of pregnancy. These analyses suggested that if exposures to PM_{10} and CO were low during the first five months of pregnancy, the association between LBW and air pollution would be low even if there were high pollution exposures later in pregnancy.

Comments

- The authors note that they did not consider risk factors for LBW, such as parental weight and height, history of adverse pregnancy outcomes, maternal nutrition, gestational weight gain, cigarette smoking, alcohol consumption, and occupational exposures. However, they argue that these factors are not likely to have confounded the results because the factors are not expected to be correlated with daily air pollution levels.
- Lee et al. report that second trimester exposures to SO_2, PM_{10}, and NO_2 were associated with LBW after controlling for CO, although the results are difficult to interpret because of collinearity among pollutants. No other multi-pollutant analyses are reported.

Leem et al., 2006: Exposures to Air Pollutants during Pregnancy and Preterm Delivery.

Leem et al. investigated the relationship between preterm delivery (<37 weeks gestation) and exposure to air pollutants in Incheon, Republic of Korea, using spatial and temporal modeling to infer individual exposures. The study included all mainland singleton births from January 1, 2001 to December 31, 2002 for which adequate data on gestational age, parental age, and parental education level were available. The cohort consisted of 52,113 births, of which 2,082 (4.0%) were preterm.

Measurements of air pollutants were collected from 11 monitoring stations in Incheon and 16 monitoring stations in the surrounding area. The mainland Incheon area appears to be approximately 18 km at its widest and 28 km at its longest, and to have had nine monitoring stations. Data included 1-hr concentrations of SO_2, NO_2, CO, and PM_{10} and were used to calculate daily and monthly averages. Each mother's residential address at time of delivery included the *dong*, which is an administrative unit that is typically much smaller than a U.S. zip code. (There were 132 *dongs*; the mean area of a *dong* is 7.8 km^2 and the median is 1.4 km^2; for comparison, the mean U.S. Zip code is 296.6 km^2 and the median is 98.8 km^2.) The authors used the pollutant concentrations in a statistical mapping technique called ordinary block kriging to predict monthly pollutant concentrations for each *dong*. Block kriging is a statistical mapping technique that allows prediction of average pollutant concentrations over a region from data collected at point locations. In this case, the values are pollutant concentrations, the regions are *dongs*, and the point locations are monitors. The authors also statistically cross-validated the kriging technique and predictions. The monthly average pollutant concentrations were matched to each birth, and average concentrations were calculated for each trimester.

Leem et al. used univariate and multivariate log-binomial regressions corrected for over dispersion to evaluate the associations between preterm delivery and exposure to ambient air pollutants. They used the log link function to estimate risk ratios (RR) and 95% CIs for preterm delivery in relation to air pollutant exposure, controlling for maternal age (<20, 20–24, 25–29, ≥30 years), parity, sex, season of birth (see comments below), and education level of each parent. Exposure levels were categorized by quartiles of

the concentrations of each pollutant for full-term births, with the lowest quartile serving as the referent category.

SO_2, NO_2, CO, and PM_{10} were all positively correlated, with Pearson correlation coefficients ranging from 0.13 to 0.63 ($p<0.001$ for each correlation). The weakest correlation of 0.13 was for SO_2 and PM_{10}. The Pearson correlation coefficient for SO_2 and NO_2 was 0.54. Analyses appear to be for single pollutants.

The adjusted RRs for preterm delivery were 1.21 (CI 1.04, 1.42) for the highest quartile of exposure to SO_2 in the first trimester, and 1.11 (0.94, 1.31) for exposure in the third trimester. Crude and adjusted RRs for all SO_2 exposure levels are shown in Table 22. There was a dose-response relationship between exposure to SO_2 in the first trimester and risk of preterm delivery ($p<0.02$ for trend).

Table 22. Crude and adjusted RR (95% CIs) of preterm delivery attributable to maternal exposure to SO_2 during the first and third trimester of pregnancy.

Trimester	SO_2 quartiles (ppb)	Crude RR (95% CI)	Adjusted RR (95% CI)[a]	Trend[b]
First	17.5 – 39.7	**1.06 (1.03, 1.31)**	**1.21 (1.04, 1.42)**	0.02
	8.7 – 17.5	1.09 (0.97, 1.23)	1.13 (0.98, 1.30)	
	6.7 – 8.7	1.11 (0.99, 1.26)	1.13 (0.99, 1.28)	
	3.0 – 6.7	1.00	1.00	
Third	17.8 – 39.3	1.04 (0.93, 1.17)	1.11 (0.94, 1.31)	0.26
	9.8 – 17.7	0.95 (0.84, 1.07)	0.97 (0.83, 1.13)	
	6.5 – 9.8	0.87 (0.77, 1.02)	0.87 (0.76, 1.01)	
	2.5 – 6.5	1.00	1.00	

[a] Adjusted for maternal age, parity, sex, season of birth, and education levels of father and mother.
[b] P value for the trend of adjusted RRs.

Comments

- Leem et al. note that they lacked data on maternal smoking and environmental tobacco smoke. However, as noted by the authors, most Korean women are not likely to smoke during pregnancy. Therefore, omission of this variable is not likely to bias the results.
- The authors also acknowledge that their analyses, which used surrogate ambient air pollution data (based on kriging methods and air monitoring data), did not incorporate the uncertainty introduced by the use of predicted average pollution concentrations for each dong. However, they also note that this is a common limitation of most similar studies.
- Because the analyses appear to include only one pollutant at a time, the observed associations may be partly attributable to other pollutants.
- The authors defined season as eight intervals of three months duration, except the two-month period (January–February 2001) at the beginning of the study

period and the four-month referent period of September–December 2002. Bivariate analysis of the risk associated with season and preterm birth indicate that summer was the lowest risk season in 2001 and the highest risk season in 2002. Therefore, adjustment for season is not comparable to other analyses.

Legro et al., 2010: Effect of air quality on assisted human reproduction.

In this cohort study, Legro et al. examined the effects of SO_2, $PM_{2.5}$, PM_{10}, NO_2, and O_3 on the first IVF cycle of 7,403 female patients of three centers in Hershey, Pennsylvania; Rockville, Maryland; and New York, New York for seven years in 2000–2007. An IVF cycle includes the administration of gonadotropin; oocyte maturation (11.9 ± 1.9 days), retrieval (1 day), and fertilization (3.6 ± 1.2 days); embryo transfer and implantation (14 days); pregnancy; and delivery or end of pregnancy.

The coordinates of the centroid of patients' home zip codes and of IVF centers were geocoded to assign ambient air pollution concentrations which the authors obtained from the U.S. EPA. The air pollution data were used to fit national-scale, log-normal kriging with a spherical model for spatial interpolations to produce location-specific daily mean concentrations of the pollutants at the patients' homes and IVF centers for the entire study period. The authors used these data to calculate daily concentrations of each pollutant for each patient during the entire IVF cycle and pregnancy. Correlations between SO_2 and co-pollutants during the oocyte retrieval to transfer at clinic sites were all significant; the Pearson's correlations coefficients were: $PM_{2.5}$ (r=0.17), PM_{10} (r=0.09), NO_2 (r=0.74), and O_3 (r=-0.40).

Mean pollutant concentrations did not vary significantly among the intervals of an IVF cycle. Table 23 shows the mean daily concentrations of SO_2 during the phases of an IVF cycle.

Table 23. Mean daily SO_2 levels in patient home or IVF clinic, by IVF cycle interval

IVF cycle interval	Mean (SD) SO_2 level, ppb
Gonadotropin start to oocyte retrieval (home)	59 (22)
Date of retrieval (clinic)	63 (30)
Oocyte retrieval to embryo transfer (home)	59 (25)
Oocyte retrieval to embryo transfer (clinic)	63 (25)
Embryo transfer to pregnancy test (14 days; home)	59 (22)
Embryo transfer to pregnancy outcome date	57 (12)

Pollutant levels were entered as continuous variables in single pollutant logistic regression models. The authors also grouped pollutant data into quartiles and deciles and did not detect significant deviation from linear relationships. All models were adjusted for patient age, IVF center, and the year and season of oocyte retrieval. Smoking history, prior IVF cycles in other centers, and infertility diagnoses were not

available. The results for SO_2 are shown in Table 24. Although the effects were not statistically significant, the authors noted consistently poorer outcomes with increasing SO_2 exposure. The trend was nearly significant for intrauterine pregnancy rates according to the SO_2 concentration at the clinic during the period of embryo culture (between fertilization and transfer).

Table 24. Adjusted ORs* of live birth per 30 ppb increase in mean daily SO_2, by IVF cycle interval in patient home

IVF phase	**OR (95%CI) per 30 ppb SO_2**
Gonadotropin start to oocyte retrieval	1.00 (0.91, 1.10)
Oocyte retrieval to embryo transfer	0.94 (0.87, 1.02)
Embryo transfer to pregnancy test (14 days)	0.94 (0.86, 1.04)
Embryo transfer to date of live birth	0.96 (0.73, 1.27)

* Adjusted for patient age, IVF center, and the year and season of oocyte retrieval

Higher concentrations of NO_2 had consistently adverse effects on the odds of live birth, O_3 had bidirectional effects, PM_{10} had no effects, and $PM_{2.5}$ had a non-significant adverse effect on the odds of intrauterine pregnancy. The authors also built two-pollutant logistic regression models to examine the effects of NO_2 and O_3; SO_2 was not included in these models. The authors also used negative binomial estimation to examine possible effects of pollution on the number of oocytes retrieved and the number of embryos transferred, and found no effects.

Comments:

- The strongest correlation was between SO_2 and NO_2, and exposure to each of these pollutants appeared to decrease the odds of live birth. It is possible that some of the effects observed in the model with only SO_2 could be explained by NO_2.
- The authors note that air quality changes have a clinically significant effect on pregnancy outcomes (10–15% change per 1 SD fluctuation in pollutants), representing perhaps the clearest evidence to date of the potential impact of air pollution on conception and live birth in human reproduction. However, in this study, significant effects on pregnancy outcomes were observed for NO_2, O_3, and $PM_{2.5}$. No significant effects were observed for SO_2 or PM_{10}.

Lin et al., 2004a: Association between maternal exposure to elevated ambient sulfur dioxide during pregnancy and term low birth weight.

In this retrospective cohort study, Lin et al. examined whether the risk of delivering a full-term LBW (<2,500 g) infant varies with exposure to air pollutants in different trimesters. Birth data were obtained from the Taiwan birth registry. The authors included 92,288 full-term (37–44 weeks gestation) singleton births in the cities of Taipei and Kaohsiung, Taiwan between 1995 and 1997; 2,069 (2.2%) were LBW. Births

outside of hospitals, to unmarried women, or with weight <1000 g or >5000 g were excluded.

Measurements of SO_2, CO, O_3, NO_2, and PM_{10} were taken from ten air quality monitoring stations (AQMS) which were supervised by the Taiwan Environmental Protection Agency. Taipei and Kaohsiung each had five AQMS. Subjects residing in districts farther than three km from the nearest AQMS were excluded because previous research had shown that an AQMS in Taiwan can provide representative concentrations of SO_2 within a 2.1 km radius. The corresponding distances for CO, O_3, NO_2, and PM_{10} were 1.4, 3.7, 3.3, and 3.3 km, respectively. Maternal exposure to each pollutant was estimated as the arithmetic mean of all daily measurements taken in each trimester and the entire pregnancy by the AQMS nearest to the mother's district of residence at the time of birth. SO_2 concentrations were higher in the spring and summer in Taipei, but in Kaohsiung, they were higher in spring and winter. The mean concentration of SO_2 of all five AQMSs in Taipei was 4.88 ppb, and the mean concentration of SO_2 in Kaohsiung was 16.95 ppb, with much greater heterogeneity among the five AQMS's. The authors categorized the first quartile of pollutant exposure as low, the second and third quartiles as medium, and the fourth quartile as high exposure, for each trimester or over the entire pregnancy, depending on the analysis. Following are the lower and upper bounds for the medium exposure categories (which also serve to define the low and high exposure categories): entire pregnancy 7.1–11.4 ppb; first trimester 6.8–11.5 ppb; second trimester 6.9–11.9 ppb; third trimester 6.8–12.4 ppb.

Multivariable logistic regression with adjustment for gestational age, gender, maternal age and education level, season of birth, occupation, maternal residence, birth order, and concentrations of other pollutants were used to produce adjusted ORs. ORs for LBW in association with entire pregnancy and trimester-specific SO_2 exposure are shown in Table 25. The authors found a significant increase in risk of LBW associated with maternal exposure to medium (OR 1.16, CI 1.02, 1.33) and high (OR 1.26, CI 1.04, 1.53) SO_2 exposures during the entire pregnancy. Analyses of trimester-specific exposures suggested an exposure-response relationship for high exposures, according to trimester. ORs (CIs) for high exposure were as follows: first trimester exposure 1.11 (0.94, 1.33), second trimester 1.17 (0.99, 1.37), and third trimester 1.20 (1.01, 1.41). The pattern was similar for medium exposure, with increasing ORs in later trimesters, but the ORs were smaller and none was statistically significant.

Table 25. Adjusted odds ratios[a] and 95% CI of low birthweight infants in association with entire pregnancy and trimester-specific SO_2 exposure.

SO_2 exposure[b]	Entire pregnancy	First Trimester	Second Trimester	Third Trimester
Low	1.00	1.00	1.00	1.00
Medium	1.16 (1.02, 1.33)	1.02 (0.90, 1.16)	1.09 (0.96, 1.24)	1.13 (0.99, 1.28)
High	1.26 (1.04, 1.53)	1.11 (0.94, 1.33)	1.17 (0.99, 1.37)	1.20 (1.01, 1.41)

[a] Adjusted for gestational age, gender, maternal age and education level, season of birth, occupation, maternal residence, birth order, and co-pollutants

[b] Lower and upper bounds for the medium exposure categories (which also define the low and high exposure categories): entire pregnancy 7.1–11.4 ppb; first trimester 6.8–11.5 ppb; second trimester 6.9–11.9 ppb; third trimester 6.8–12.4 ppb

Medium and high exposures to CO were associated with lower risk of LBW. The negative association was statistically significant for high CO exposure over the entire pregnancy. PM_{10}, NO_2, and O_3 generally did not have statistically significant associations with risk of LBW.

Comments

- Lin et al. noted several limitations of their study, including unadjusted risk factors such as maternal nutrition, maternal weight gain during pregnancy, maternal height, smoking, intensity of prenatal care, and maternal occupational exposure. In another article (Lin et al., 2004a), several of the same authors reported that 2.2% of a sample of mothers from the same cohort reported smoking during pregnancy.
- The correlations among pollutants were not reported.

Lin et al., 2004b: Increased risks of term low birth weight infants in a petrochemical industrial city with high air pollution levels.

Lin et al. examined whether the risk of delivering a full-term LBW (<2500g) baby was associated with living in Kaohsiung, a petrochemical industrial city, compared with Taipei, a larger, less industrial city. Kaohsiung, in southern Taiwan, is 153 km^2 with a population of 1.5 million and Taipei, in northern Taiwan, is 272 km^2 with a population of 2.7 million. The authors assumed the air pollutants studied were emitted from fossil fuel combustion.

The authors obtained from the Taiwan birth registry data for full-term (37–44 weeks gestation) singleton births in the cities of Taipei and Kaohsiung, Taiwan between 1995 and 1997. Births outside of hospitals, to unmarried women, or with weight <1000 g or >5000 g were excluded. The final study population included 92,288 births, including 757 (2.4%) LBW births in Kaohsiung and 1,276 (2.1%) LBW births in Taipei.

Ambient SO_2, CO, NO_2, O_3, and PM_{10} concentrations were measured at five AQMS each in Taipei and Kaohsiung that were supervised by the Taiwan Environmental Protection Agency. Subjects residing in districts farther than three km from the nearest AQMS at the time of delivery were excluded. Maternal exposure to each pollutant during the pregnancy was estimated as the arithmetic mean of all daily measurements taken by the AQMS closest to the mother's district of residence at the time of birth. The authors classified the first quartile of pollutant exposure as low, the second and third quartiles as medium, and the fourth quartile as high exposure. The different industrial bases of the two cities was reflected in higher levels of SO_2, CO, and PM_{10} in Kaohsiung. Few women living in Taipei were exposed to high levels of SO_2 or PM_{10}; in Kaohsiung, almost all women were exposed to high levels of SO_2 and PM_{10}.

The authors conducted multiple logistic regression with adjustment for gestational age, gender, birth order, maternal age and education level, and season of birth to estimate ORs and 95% CI for LBW. Women living in Kaohsiung had significantly higher risk of term LBW than women in Taipei: OR 1.13 (1.03, 1.24). This risk was statistically significant for the following births: girls, OR 1.14 (1.01, 1.28); second or subsequent births, OR 1.17 (1.02, 1.35); maternal age >25 years, OR 1.15 (1.04, 1.28); and births during the warm season, OR 1.15 (1.02, 1.31).

Comments

- Lin et al. state they have no evidence regarding which specific air pollutant was responsible for the higher risk of LBW for births in Kaohsiung.
- It is not clear why the authors limited the data to women within three km of an AQMS if the AQMS measurements were not used in analyses.
- The authors note that they lacked information on maternal occupational exposure and maternal smoking during pregnancy. However, 409 women were interviewed by telephone for the present study, and 2.2% reported having smoked during pregnancies. The authors argue that smoking was therefore not an important influence on LBW in this study.

Lin et al., 2008: Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates.

In this review and prospective study, Lin et al. investigated the relationship between SCSA parameters, DNA fragmentation index (DFI), high DNA stainability (HDS), and outcomes of IVF and ICSI. Samples from 223 couples undergoing conventional IVF (n=137) and ICSI (n=86) were assessed between October 2003 and September 2005. All female patients were <40 years old, and freshly ejaculated sperm were prepared for IVF/ICSI procedures. Only normal sperm samples with concentration >20 x 10^6/ml, motility >50%, and normal strict morphology >14% were used for IVF. Sperm samples used for ICSI were taken from men of severe male factor infertility with at least one of the following criteria: sperm concentration <10 x 10^6/ml, motility <30%, or normal strict

morphology <4%. Embryos were scored from grades 1–5 according to the size and shape of the blastomeres and to their degree of fragmentation as follows:

Grade 1: no fragmentation with equal-sized cells
Grade 2: <20% fragmentation with equal-sized cells
Grade 3: no fragmentation with unequal-sized cells
Grade 4: >20% fragmentation with unequal-sized cells
Grade 5: >50% fragmentation

Embryos with grade 1 or 2 were considered "good" embryos; only these were selected for embryo transfer. Pregnancy was detected 2 wks after embryo transfer. Biochemical pregnancy was defined as one positive β-hCG test two wks after embryo transfer, and clinical pregnancy was defined as the presence of a gestational sac detected by ultrasound. Women with clinical pregnancies who miscarried before the 12^{th} wk were described as having a spontaneous abortion.

Data were presented as mean values ± SEM. DFI class intervals were 0–9%, 9–27%, and > 27%. HDS class intervals were 0–5%, 5–15%, and >15%. Corresponding differences in reproductive outcome between the three DFI and HDS groups were compared using the χ^2 test, independent t-test, and one way ANOVA test.

ICSI "good" embryo transfer rate in the HDS >15% group was significantly higher than in the HDS <5% group (71.1 vs. 49.7%, $p<0.05$). A trend of increased spontaneous abortion rate was noted in the DFI >27% group, but the differences between the 3 DFI groups were not significant. The abortion rate (44.4%) in the IVF group with HDS >15% was significantly higher than with HDS between 5–15% (4.0%) and with HDS <5% (5.4%). No statistically significant difference between the outcomes of IVF and ICSI with regard to fertilization rate, good embryo rate, clinical pregnancy rate, and abortion rate were observed between the 3 DFI groups and the 3 HDS groups.

The authors suggest their results demonstrate that both DFI and HDS correlate poorly with fertilization rate and embryo quality. Such results suggest that normal fertilization does not ensure high-quality DNA in the paternal genome because no relationship exists between fertilization rate and DNA fragmentation.

Liu et al., 2003: Association between gaseous ambient air pollutants and adverse pregnancy outcomes in Vancouver, Canada.

Liu et al. conducted a retrospective cohort study to examine associations between pregnancy outcomes and SO_2, NO_2, CO, and O_3, in Vancouver, Canada from January 1, 1986 through December 31, 1998. Data on PM_{10} were also available for 1994 through 1998. The outcomes examined were preterm birth, LBW, and IUGR among singleton live births.

The authors obtained data from Statistics Canada for all singleton live births in 13 census subdivisions in the greater Vancouver area between 1986 and 1998. After infants with birthweights <500 g or gestational age <22 weeks were excluded, the cohort consisted of 229,085 births. Preterm birth was defined as <37 complete weeks gestation, with gestational age determined by the responsible obstetrician based on all available information. Low birthweight was defined as <2,500 g at birth. IUGR was defined as birthweight below the 10^{th} percentile, by sex and gestational week, of all singleton live births in Canada between 1986 and 1998; only full term infants (37–42 weeks gestation) were included. The cohort included approximately 9,163 (4.0%) LBW, 12,142 (5.3%) preterm, and 20,397 (9.4% of 216,988) IUGR births. These rates varied by season of birth, infant sex, parity, maternal age, and time period of birth. The authors noted that LBW and preterm birth rates appeared to increase slightly over the study period.

The authors had continuous sampling measures for air pollutants that had been compiled for the 13 census subdivisions in the study area. The pollutant data included daily averages and daily 1-hour concentrations for the gaseous pollutants for the period April 1, 1985 through December 31, 1998, and PM_{10} measurements for the period January 1, 1994 through December 31, 1998. Daily averages of gaseous pollutant concentrations were computed from hourly measurements that were averaged across the available monitoring stations. The daily average SO_2 levels were as follows: mean=4.9 ppb, 25^{th} percentile=2.8 ppb, 75^{th} percentile=6.3 ppb, 95^{th} percentile=10.5 ppb, and 100^{th} percentile=30.5 ppb. However, the daily maximum1-hour concentrations of SO_2 had a mean of 13.4 ppb and 100^{th} percentile value of 128.5 ppb.

Liu et al. linked the birth records data temporally with the air pollutant exposure levels to determine exposure levels from conception through delivery. For each live birth, average air pollution concentrations were calculated for the first, second, and third month; the last and next to last month; and, the first, second, and third trimester.

SO_2, NO_2, and CO were highly positively correlated, with correlation coefficients ranging from 0.61 to 0.72 ($p < 0.0001$). However, O_3 was negatively correlated with the other gaseous pollutants, with coefficients of -0.35,-0.25, and -0.49 ($p < 0.0001$) for SO_2, NO_2, and CO, respectively. The authors used multiple logistic regression to estimate odds ratios and 95% CI for associations between concentrations of ambient air pollutants and adverse pregnancy outcomes, controlling for maternal age (<20, 20–24, 25–29, 30–34, ≥35 years), parity, infant sex, gestational age or birthweight, and season of birth.

Two outcome measures were associated with exposure to SO_2 during the first month of pregnancy: LBW (Adjusted OR 1.11, CI, 1.01, 1.22, for a 5.0 ppb increase) and IUGR (AOR 1.07, CI, 1.01, 1.13, for a 5.0 ppb increase). Preterm birth was associated with exposure to SO_2 during the last month of pregnancy (AOR 1.09, CI, 1.01, 1.19, for a 5.0 ppb increase). Results for the effects of SO_2 are in Table 26.

Table 26. ORs and 95% CIs for maternal exposure to SO_2 (per 5 ppb increase) and risk of LBW, preterm birth, and IUGR, by exposure window (month or trimester of pregnancy).

Outcome	Exposure window	Crude OR (95% CI)[a]	Adjusted OR (95% CI)[a,b]
LBW	First month	0.95 (0.89, 1.02)	**1.11 (1.01, 1.22)**
	Last month	0.99 (0.92, 1.07)	0.98 (0.89, 1.08)
Preterm birth	First month	1.00 (0.95, 1.06)	0.95 (0.88, 1.03)
	Last month	1.06 (0.99, 1.13)	**1.09 (1.01, 1.19)**
IUGR	First month	**1.07 (1.02, 1.12)**	**1.07 (1.01, 1.13)**
	Last month	1.01 (0.96, 1.06)	1.00 (0.94, 1.06)
	First trimester	**1.07 (1.01, 1.14)**	1.07 (1.00, 1.14)
	Second trimester	0.98 (0.92, 1.04)	0.98 (0.91, 1.04)
	Third trimester	1.02 (0.96, 1.09)	1.03 (0.96, 1.10)

[a] ORs were estimated based on a 5.0 ppb increase in SO_2.
[b] Adjusted for maternal age, parity, infant sex, birthweight (preterm only), gestational age (LBW only), and season of birth.

The authors also examined the relationship between SO_2 and adverse pregnancy outcomes, controlling for gaseous co-pollutants individually and simultaneously, and found that the observed elevated risks persisted. The association between low birthweight and SO_2 exposure during the first month of pregnancy, controlling for NO_2, CO, and O_3, as well as the maternal and other covariates, was slightly greater than when co-pollutants were not in the statistical model (AOR 1.29, CI 1.12, 1.50). The association between preterm birth and SO_2 exposure in the first last month of pregnancy, controlling for NO_2, CO, O_3, and the maternal and other covariates, was the same as when not controlling for co-pollutants, though not all adjusted ORs remained statistically significant. The authors also report that associations between exposure to SO_2 and IUGR are robust to adjustment for other pollutants, but do not provide ORs.

The authors concluded that relatively low concentrations of gaseous air pollutants are associated with adverse effects on birth outcomes in populations experiencing diverse air pollution profiles.

In the present study, the authors note associations between individual gaseous pollutants and adverse pregnancy outcomes tended to persist after adjustment for co-pollutant exposure in multiple-pollutant models. Overall, the associations among SO_2 and LBW, preterm birth, and IUGR appeared most robust when adjusted for co-pollutants. The authors suggest that the effects of air pollutants on birth outcomes are likely related to more than one component of the complex mix of air pollutants present in an urban environment.

Comments

- The authors note that they lacked information on SES; however, they also state that there is no evidence of an association between socioeconomic factors and

air pollution in Vancouver, and that another study using the same air pollution data found that SES measured at the community level did not modify the association between air pollution and mortality.

- Similarly, the authors note that they had no information on maternal smoking, but assert that smoking is unlikely to be associated with air pollution independently from maternal SES.
- As the authors note, the use of ecologic measurements of ambient air pollution and maternal residence at time of delivery to estimate individual exposure likely caused random exposure misclassification and underestimation of effects of air pollution.
- Only five years of data on PM_{10} were available; however, Liu et al. report that analyses did not indicate an association between PM_{10} and birth outcomes.
- Exposure levels observed in this study were relatively low.
- Effects of acute exposures or spikes in exposure to air pollutants would likely not be detected by this study.
- The statistical analysis included a number of multiple comparisons which increase the likelihood of a significant finding due to chance.

Liu et al., 2007: Association between maternal exposure to ambient air pollutants during pregnancy and fetal growth restriction.

Liu et al. studied the effect of exposure to NO_2, CO, SO_2, O_3, and $PM_{2.5}$ on fetal growth in the Canadian cities of Calgary, Edmonton, and Montreal. All full-term (37–42 weeks gestation), singleton live births from January 1, 1986 to June 10, 2000 in the census subdivision boundaries (CSDs) covered by air monitors were abstracted from the Canada live birth database and included in the study. Information about these births, including date of birth, birthweight, gestational age, parity, birth order, maternal and paternal age, and residence, was obtained from birth certificates. The outcome was IUGR, defined as birthweight <10th percentile, by sex and gestational week of all singleton live births in Canada between 1986 and 2000. The cohort included 386,202 births, of which 10.9% (approximately 42,096) were classified as IUGR.

Concentrations of NO_2, CO, SO_2, and O_3 were collected from each monitoring station in Calgary (four stations), Edmonton (two stations), and Montreal (eight stations) from April 1, 1985 to December 31, 1999. $PM_{2.5}$ concentrations were collected in the same cities on a 6-day schedule. The concentrations were used to calculate hourly and 24-hr average pollutant concentrations. The areas covered by the monitoring stations were defined according to CSD boundaries. The authors matched the data on air pollutant concentrations with the dates of birth and length of gestation to estimate air pollutant concentrations in each month, trimester, and the entirety of the pregnancy for each birth. The daily average SO_2 concentrations, in ppb, were distributed as follows: mean 3.9, 25th percentile 2.0, median 3.0, 75th percentile 5.0, 95th percentile 10.0. The 1-hr maximum SO_2 concentrations were distributed as follows: mean 10.8, 25th percentile 5.0, median 8.6, 75th percentile 14.0, 95th percentile 28.0. All of the co-pollutants were

statistically significantly correlated; the Pearson correlation coefficients for co-pollutants and SO_2 were: NO_2 0.34, CO 0.21, O_3 -0.30, and $PM_{2.5}$ 0.44.

Associations between IUGR and average daily concentrations of pollutants were analyzed using multiple logistic regression analysis. Analyses controlled for maternal age, parity, infant gender, season of birth, and city of residence. The risk of IUGR associated with an increase of 3.0 ppb in maternal exposure to SO_2 was not higher overall, and was slightly lower for exposure during each of the first three months of pregnancy (AORs were between 0.96 and 0.98, and were statistically significant). Similarly, a 3.0 ppb increase in SO_2 in the first trimester was associated with a decrease in risk of IUGR; the AOR was approximately 0.96 and was statistically significant. The ORs increased with later trimester exposures, but did not exceed 1. The authors also conducted logistic regression with multiple pollutants, but only included NO_2, CO, and $PM_{2.5,}$ which were consistently statistically significantly associated with increased risk of IUGR in single pollutant models.

Comments

- The authors note that they lacked information on factors known to affect fetal growth, such as maternal race, education, cigarette smoking, caloric intake, alcohol use, paternal height, and socioeconomic status. However, these factors are not likely to vary with changes in ambient pollutant levels and therefore should not confound associations between pollution and fetal growth restriction.
- The cities of Edmonton and Calgary had few monitoring stations but cover relatively large areas (684 km^2 and 726 km^2, respectively), so the assumption that all mothers in these cities had the same exposure may have caused substantial exposure misclassification.

Maisonet et al., 2001: Relation between ambient air pollution and low birth weight in the northeastern United States.

The objective of this study was to evaluate the relationship between term LBW (<2,500 g) and maternal exposures to ambient SO_2, CO, and PM_{10} in 6 northeastern cities in the US. The study population consisted of 89,557singleton, full-term (37–44 weeks gestation), live births between January 1, 1994 and December 31, 1996 in Boston, MA; Hartford, CT; Philadelphia, PA; Pittsburgh, PA; Springfield, MA; and Washington, D.C. Study infants were identified by birth data obtained from the National Center for Health Statistics Natality Data Sets. Birth certificate data on birthweight and date of the mother's LMP were used to define term LBW. Approximately 3,314 (3.7%) births were LBW.

Hourly monitor readings of SO_2 and CO for each day, and 24-hr readings of PM_{10} for every six days were obtained from the U.S. EPA, excluding monitors in industrial areas. There were several air monitors located throughout the six cities; for SO_2 monitoring, there was one monitor in Boston and Hartford, two in Philadelphia and Springfield, three in Washington, and four in Pittsburgh. For each infant, the authors estimated the dates

of the first, second, and third trimesters from the LMP and used the monitoring data to estimate average ambient levels of SO_2, CO, and PM_{10} for each of the study areas; for SO_2 and CO these were average daily values. The authors estimated the average exposure during each trimester for each subject, assuming the maternal residence reported on the birth certificate was the one where she lived throughout the pregnancy. Infants for whom ambient pollution data were available for less than 75% of the days. The range of values for exposure categories are described in Table 27.

Covariates included in the analysis include: gestational age, gender, birth order, maternal age, race or ethnicity, years of education, marital status, adequacy of prenatal care, previous induced or spontaneous abortions, weight gain during pregnancy, prenatal smoking, and alcohol consumption. Adequacy of prenatal care was based on the month prenatal care began, number of visits, and gestational age.

Logistic regression models were used to estimate adjusted odds ratios and 95% CI for term LBW, and linear regression models were constructed to assess reductions in birthweight (in grams) in relation to each air pollutant. Exposure to pollutants was applied in continuous form and as a categorical variable, as shown in Table 27. Because the authors assumed exposures were not independent within study areas, they applied multistage regression models, which adjusted for the standard errors for clustering by study area.

Table 27. SO_2 exposure categories.

Exposure period	Percentile of SO_2 concentration (ppb)				
	<25th	**25 to <50**	**50 to <75th**	**75 to <95th**	**<95th**
First Trimester	< 7.090	7.090–8.906	8.907–11.969	11.970–18.447	≥ 18.448
Second Trimester	< 6.596	6.596–8.896	8.897–11.959	11.960–18.275	≥18.276
Third Trimester	< 5.810	5.810–8.453	8.454–11.777	11.778–18.134	≥ 18.135

Infants with second trimester SO_2 exposures above the reference category (<25th percentile) were at increased risk for term LBW (Table 28). AORs for each exposure category above the referent were as follows: 25 to <50th (AOR 1.18; CI 1.12, 1.25); 50 to <75th (AOR 1.12, CI 1.07, 1.17), and 75 to <95th (AOR 1.13, CI 1.05, 1.22). In the highest exposure category, the association was reversed (AOR 0.87, CI 0.80, 0.95). First and third trimester exposures to SO_2 were not associated with LBW.

Table 28. Adjusted odds ratios[a] (95% CI) for term LBW by trimester of SO_2 exposure.

Exposure level	Trimester of Exposure		
	First	**Second**	**Third**
<25th	1.00	1.00	1.00
25 to <50th	1.04 (0.88, 1.23)	1.18 (1.12, 1.25)	1.04 (0.92, 1.18)
50 to <75th	1.04 (0.94, 1.15)	1.12 (1.07, 1.17)	1.02 (0.87, 1.18)
75 to <95th	0.98 (0.81, 1.17)	1.13 (1.05, 1.22)	1.04 (0.84, 1.28)
>95th	0.88 (0.73, 1.07)	0.87 (0.80, 0.95)	1.06 (0.76, 1.47)
Per 10,000 ppb	0.98 (0.93, 1.03)	1.01 (0.93, 1.10)	1.01 (0.86, 1.20)

[a] Alcohol consumption, smoking during pregnancy, maternal education, maternal age, maternal race/ethnicity, marital status, weight gain during pregnancy, previous terminations, gender of infant, season of birth, firstborn, prenatal care, gestational age, and other pollutants.

The authors also conducted analyses stratified by race/ethnicity, and observed that exposures to ambient SO_2 were statistically significantly associated with term LBW among whites and African Americans. Among whites, increases in risk of term LBW were associated with 10-ppm increases in ambient SO_2 in the first (AOR 1.18; CI 1.12, 1.23), second (AOR 1.18; CI 1.02, 1.35), and third (AOR 1.20; CI 1.06, 1.36) trimesters. The second trimester associations for SO_2 exposure in the 50 to <75th (AOR 1.16, CI 1.11, 1.22), and 75 to <95th percentiles (AOR 1.30, CI 1.20, 1.42) persisted when the analysis was limited to whites.

For African Americans, exposure to SO_2 in the 50 to <75th percentile in the first trimester was associated with a marginally significant increase in LBW risk (AOR 1.10, CI 1.00, 1.20) and decreases in risk for those exposed to the highest level (AOR 0.75, CI 0.64, 0.87) and per 10 ppm SO_2 (AOR 0.91, CI 0.85, 0.98). There were also increases in risk associated with second trimester exposure to SO_2 at the 25 to <50th (AOR 1.22; CI 1.14, 1.32) and 50 to <75th percentiles (AOR 1.07, CI 1.04, 1.11) for African Americans. No statistically significant associations were observed for Hispanics.

Maisonet et al. report that the magnitude of birthweight reductions in relation to air pollutants was quite small. For example, among whites, a 10-ppm increase in first trimester SO_2 exposure was associated with a mean reduction of 28.5 g (CI -4.1, 61.1).

There were no associations between PM_{10} exposures and LBW. CO exposure in the third trimester was associated with increased risk of LBW for the whole cohort. When analyses were stratified by race, a 1 ppm increase in CO in the first or third trimester was associated with increased risk of LBW for African Americans.

Comments

- The authors state that correlations among SO_2 readings for any two monitors within a city ranged from 0.43 to 0.91, suggesting low heterogeneity within study areas.
- SO_2 levels reported are extremely high, although the authors state that the concentrations of the pollutants were well below the established standards.

Marshall et al., 2010: Oral Cleft Defects and Maternal Exposure to Ambient Air Pollutants in New Jersey.

Marshall et al. conducted a case-control study of the risk of oral clefts associated with exposure to air pollutants in the first three months of gestation. The authors identified 414 cases of CLP, and 303 cases of cleft palate only born between February 1998 and December 2003 through the New Jersey Special Child Health Services Registry, a population-based birth defects registry with mandated reporting. Infants with holoprosencephaly or an additional diagnosis of a chromosomal abnormality were excluded. The authors drew 12,925 controls from all 690,000 births to New Jersey residents in 1998–2003. The following were among the exclusion criteria: births associated with pre-existing maternal insulin-dependent diabetes; birth weight <750 g; multiple births; maternal age <15 years or >45 years; gestation <20 wks; missing gestational age; and ineligible residence address. The authors matched the control sample with the birth defects registry to exclude reportable birth defects.

The authors obtained publicly available air monitoring data on CO, NO_2, O_3, SO_2, PM_{10}, and $PM_{2.5}$ from the New Jersey Department of Environmental Protection. Each year of the study, 43–47 monitors provided hourly concentrations of gases and daily average concentrations of particulates, with monitors located primarily in urban and suburban areas. In 2002, there were 11 monitoring sites for SO_2, though the numbers of monitors for each pollutant varied over time. To estimate exposure, the authors matched the geocoded mother's residence to the closest monitors. When an address could not be matched to an exact location, a centroid location for zip+4, zip+2, or zip code polygon was used to estimate distance from monitors. For the primary analyses, the air monitoring station closest to the residence served as the estimate of exposure for that birth. All births within 40 km of at least one monitoring station were included in most analyses (thus 1%–5% of case and control residences were excluded for all pollutants except PM_{10}). Mean distances to monitors varied by pollutant and year, and were not reported for SO_2.

The critical exposure period was defined as the six weeks from five to ten weeks into the gestational period (defined as the date of birth minus the gestational age). The authors calculated mean concentrations of each pollutant for the 6-wk period for the main analyses. The importance of peak values was assessed by assigning births the number of days exceeding the 90^{th} percentile for each pollutant during the critical period. Births were excluded if less than 70% of pollutant values were available during

the critical period, unless another monitor was eligible and provided data. The authors evaluated the effect of missing monitoring data for each contaminant. Table 29 shows the estimated SO_2 exposures and the proportion of missing SO_2 levels for cases and controls.

Table 29. SO_2 exposures among cases of CLP and cleft palate only, and controls (from Marshall et al.)

	SO_2 exposure		
Case status	Mean (SD), ppb	% >90th percentile	Missing (%)
CLP (n=414)	5.3 (2.4)	4.8	5.1 (2.2)
Cleft palate only (n=303)	4.8 (2.1)	10.7	0 (0.0)
Controls (n=12,925)	5.1 (2.2)	9.8	27 (0.2)

Marshall et al. included the following variables, extracted from the birth certificate data, in all logistic regression models: mother's age, race, ethnicity, smoking and drinking alcohol during pregnancy, and season of conception. The authors also evaluated smoking for potential effect modification, but did not report these results.
Among single pollutant models of CLP and cleft palate only with exposures in quartiles, only two pollutants were statistically significantly associated with case status: the highest quartile of SO_2 was associated with elevated risk of CLP: AOR 1.6, CI 1.1, 2.2 (Table 30) and the highest quartile of CO was negatively associated with cleft palate only. Only CO showed an overall statistically significant association with case cleft palate only status. In addition, Marshall et al. evaluated the effect of limiting the analyses to subjects whose residences were within 10 km of the closest monitor, which drastically reduced the sample but yielded results that were not substantially different.

Table 30. Adjusted* ORs and 95% CIs for oral clefts and SO_2 exposure in weeks 3–8 of pregnancy (from Marshall et al.)

SO_2 (ppb)**	CLP		cleft palate only	
	n	OR (CI)	n	OR (CI)
<3	79	1	76	1
3– <5	89	1.2 (0.9, 1.7)	84	1.2 (0.8, 1.6)
5– <7	88	1.3(0.9, 1.9),	67	1.0 (0.7, 1.4)
>7	96	**1.6 (1.1, 2.2)**	49	0.7 (0.5, 1.1)

*Adjusted for mother's race, age, education, gravidity, alcohol use, smoking, season of conception, and infant gender

**Quartiles were calculated based on the distribution of pollutant concentrations among controls, with the lowest quartile serving as the reference group.

When SO_2 exposure was included in the regression model as a continuous variable, the authors found a weak relationship with CLP that was consistent with the categorical (quartile) model, though statistics were not reported. The authors also conducted analyses including all or combinations of the pollutants in a single logistic regression model. In these models, the sample was reduced by approximately 40% because data

for all six pollutants must be available, and the authors report the results were not substantially changed.

The authors note that this study confirmed previously known risk factors for oral clefts, such as infant gender, maternal race, and smoking during pregnancy, but little evidence of increased risks of oral clefts associated with the estimated concentration of the six air pollutants. CO consistently appeared to be associated with decreased risk of cleft palate only, which the authors state is difficult to interpret or to attribute to confounding. The authors also note that the evidence for an increased risk of CLP associated with SO_2 exposure, and an association between O_3 and cleft palate only merit further evaluation.

Comments:

- Occupational exposures, nutrition, and economic factors were not considered in this study.
- Although the authors attempted to address potential misclassification of exposure due to distance from the monitors by conducting analyses of births within 10 km of air monitors, substantial misclassification is still possible.

Medeiros and Gouveia, 2005: Relationship between low birthweight and air pollution in the city of São Paulo, Brazil.

Medeiros and Gouveia studied the effects of maternal exposure to air pollution in the three trimesters of pregnancy in the city of São Paulo, Brazil on birthweight. The authors obtained birth data from the National Live Birth Information System for all babies born to mothers living in the Municipality of São Paulo between 1998 and 2000 with completed live birth declarations. The study included term (37–41 weeks gestation), hospital-born singletons with birthweight between 1,000 g and 5,500 g. The authors included only the more central districts of the city due to the greater concentration of air quality monitoring stations. LBW was defined as <2,500 g. The study included 311,735 births, of which 4.6% were classified as LBW.

The authors obtained daily records of the concentrations of SO_2, PM_{10}, CO, O_3, and NO_2 from 14 monitoring stations in São Paulo; six of these stations monitored SO_2. The authors used the mean daily values to calculate mean concentrations of each pollutant in the three trimesters of pregnancy, based on the date of birth of each child.

The pollutants were analyzed separately in multivariate linear and logistic regression models. In linear regression models, the exposure was the mean concentration of each pollutant in a trimester. Linear regression models adjusted for prenatal care, gender, type of delivery, year of birth, maternal age and education, and numbers of living and deceased children. First trimester exposures to all pollutants except O_3 were associated with reduced birthweight. The decrease in birthweight associated with a 0.38 ppb increase in SO_2 in the first trimester was 1.3 (CI 0.8, 1.7) g, (Table 31).

Exposures to CO and SO_2 in the second trimester were associated with 4.9 (CI 0.5, 9.3) g and 0.7 (0.2, 1.3) g increases in birthweight, respectively. Increases in each pollutant in the third trimester were associated with increases in birthweight; except for O_3, all were statistically significant.

Table 31. Change in birthweight associated with 0.38 ppb increase in SO_2 exposure, by trimester of exposure.

Trimester	Change in birthweight, g (95% CI)
1st	-1.3 (-1.7, -0.8)
2nd	0.7 (0.2, 1.3)
3rd	2.0 (1.5, 2.6)

In logistic regression models, pollutant levels were categorized into quartiles based on trimester means. Logistic regression analyses showed no statistically significant changes in the odds of LBW associated with pollutant exposures in any trimester.

Comments

- The authors state that although it is difficult to isolate the effects of individual pollutants due to the strong correlations among the pollutants, exposure to CO appeared to be more relevant to birthweight.
- Medeiros and Gouveia postulate that the associations between third trimester pollutant exposures and increased birthweight are probably due to the seasonal component of air pollution: first and second trimester exposures were almost always inversely associated with third trimester exposure.
- The authors state that one potential limitation of their study is the use of trimester exposures, when a shorter period may have been more appropriate. They also note that use of mean levels of pollutants, and CO levels may vary considerably between different areas of the city.
- The authors also acknowledge the lack of information on risk factors for LBW, such as smoking and maternal malnutrition.

Meng and Zhang, 1990: Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of workers exposed to sulfur dioxide.

In this study, Meng and Zhang examined the frequencies of chromosomal aberrations and sister-chromatid exchanges in peripheral blood lymphocytes of workers chronically exposed to SO_2 in Taiyuan City (North China). The study subjects were 40 workers exposed to SO_2 in a sulfuric acid factory, and 42 controls working and studying in Shanxi University, situated in the same city as the factory, matched according to sex, age and smoking habits. The SO_2 concentrations varied over one year in the factory; the range was 130 ppb (0.34 mg/m^3) to 4,566 ppb (11.97 mg/m^3) at the time of investigation. Occupational exposure to SO_2 had not caused clinical symptoms in the factory workers and all controls were considered healthy.

The mean per 100 metaphase frequencies of lymphocytes carrying chromosome type aberrations, or chromatid-type aberrations, or total aberrant cell number in the workers were significantly higher than in the controls ($p<0.01$). Frequencies of dicentrics, rings, fragments, chromosome gaps, translocations, chromatid breaks and chromatid interchanges were also all significantly higher in workers than in controls ($p<0.05$). The mean per 1000 metaphase frequencies of severe chromosomal aberration types (including rings, translocations, and dicentrics) in workers and controls were 9.63 and 2.27, respectively ($\chi^2 = 37.98$, df = 1, $p<0.01$). Finally, the frequencies of aberrant cells or sister-chromatid exchanges in workers were significantly higher than in controls whether amongst the smoker or non-smoker group. The authors concluded that SO_2 is a clastogenic and genotoxic agent.

Mohorovic, 2004: First two months of pregnancy – critical time for preterm delivery and low birthweight caused by adverse effects of coal combustion toxics.

Mohorovic's objective for this study was to define the most critical gestation period for effects of environmental toxins on risk of preterm delivery (<37 weeks) and LBW (<2,500 g). The study population included 704 pregnant women living in the district of Labin, Croatia and getting primary care at the Labin Primary Healthcare Center, which was the only primary obstetric-gynecologic service in the area. Term, preterm, and LBW from January 1, 1987 to December 31, 1989 were included.

Zones were defined by concentric circles around the Plomin 1 power plant. Subjects lived in the zone that was 3.5–12 km from Plomin1. Plomin 1 burns coal, and the local coal was extremely rich with sulfur (9–11%) and had about 10–15 times higher radioactivity than the average for other types of coal in the world. Mohorovic states that every hour Plomin 1 operated, it emitted about 8.5 metric tons (18,080 mg/m^3 or 6.900 ppm) of SO_2, in addition to nitrogen oxides, CO_2, CO, TSP, iron, titanium, vanadium, chromium, nickel, copper, zinc, selenium, lead, and other products of coal combustion. Labin is situated on a plateau roughly 10 km downwind from and 200–300 meters higher than Plomin 1.

Data about daily consumption of coal at Plomin 1 were used to calculate the maximum output of SO_2 emissions on a daily, weekly, and monthly basis for each woman's entire pregnancy, using software designed for this purpose. The author also calculated individual exposure at the end of the first, second, third, and sixth month of pregnancy, and the average for the whole pregnancy, beginning with the LMP.

In 1987, Plomin 1 was operating almost throughout the year (4,772 work hours). In 1988 and 1989, it did not work at full capacity (2,754 and 579 work hours, respectively). Plomin1 was closed from February 19, 1989 to September 6, 1989. The author defined within the period of closure a "clean" period, from April to July, 1989. A "dirty" period, December, 1989 to March, 1990 was also defined, during which the daily ground level of SO_2 was monitored at three locations. Monthly ground level values of SO_2 ranged from 13 ppb to 96.5 ppb (34.1$\mu g/m^3$ to 252.9 $\mu g/m^3$) in 1989. The Regional Institute of

Health Care (Pula, Croatia) performed the air quality measurements. The Labin meteorological station provided data on temperatures, wind, precipitation, and other weather conditions, but the author does not state how these data were used. The data were analyzed using multiple correlation analyses, factor analyses, and chi-square.

Of the 704 births from 1987 through 1989, 9.9% were preterm, with 5 (0.7%) at 28 weeks gestation or less, 14 (2.0%) at 29–32 weeks gestation, and 51 (7.2%) at 32–37 weeks gestation. There were 50 (7.1%) newborns with birthweight below 2500 g. As shown in Table 32, Mohorovic reports statistically significant correlations between exposure to SO_2 in end of the first and second months of pregnancy and gestation length and birthweight, and between exposure throughout the pregnancy and gestation length. The author does not provide detail on how the analysis was conducted; however, the results suggest that greater exposure to SO_2 during the first two months of pregnancy is associated with shorter gestation and lower birthweight.

Table 32. Correlation between birth outcomes and exposure to SO_2 during pregnancy.

Exposure window	Gestation length	Birthweight
End of first month	**-0.0914 (p=0.008)**	**-0.0807 (p=0.016)**
End of second month	**-0.0806 (p=0.016)**	**-0.0733 (p=0.026)**
End of third month	-0.0396 (p=0.147)	-0.0416 (p=0.135)
End of sixth month	-0.0236 (p=0.266)	-0.0390 (p=0.151)
Entire pregnancy	**-0.0932 (p=0.007)**	-0.0387 (p=0.153)
Weekly average, entire pregnancy	-0.0515 (p=0.086)	-0.0561 (p=0.069)

Mohorovic reports a statistically significant relative risk of 1.76 (p=0.026) of prematurity associated with 1987, when Plomin 1 worked almost throughout the year (4,772 hours), relative to when it did not operate at full capacity in 1988 (2,754 hours) and 1989 (579 hours).

The author also reports that ground level SO_2 was positively correlated with maternal methemoglobin concentration ($r=0.72$, $p<0.01$).

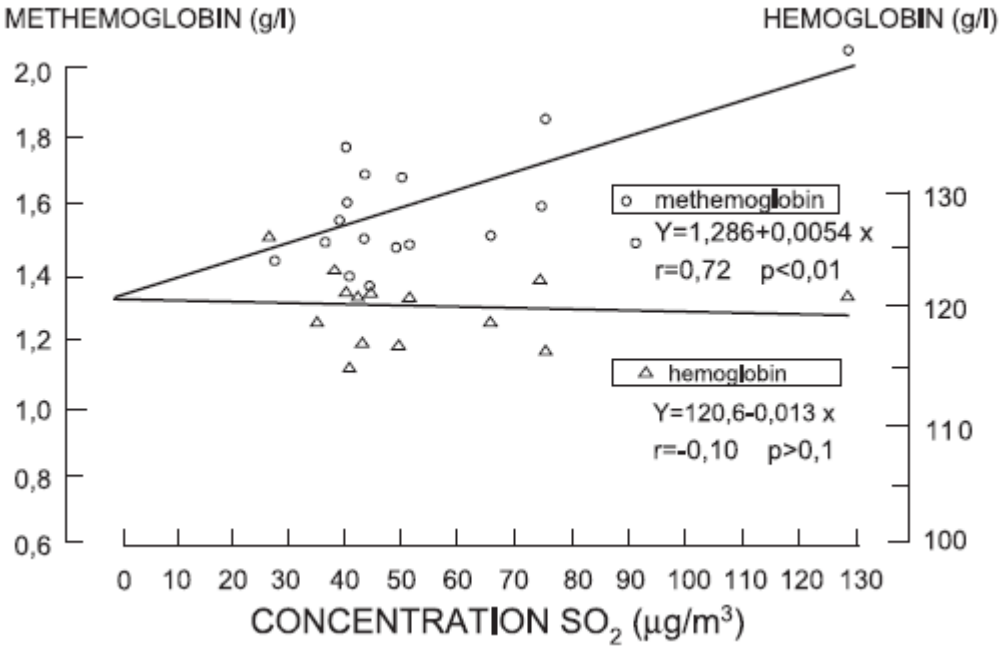

Figure 1. The correlation between ground-level concentration of SO_2 ($\mu g/m^3$) and the average value of methemoglobin and hemoglobin in blood samples of pregnant women during the dirty period when Plomin 1 was in operation (1989–1990). For methemoglobin, y=1.286+0.0054x; r=-.72; p<0.01. For hemoglobin, y=120.6-0.013x; r=0.10; p>0.1 (Mohorovic, 2004).

Mohorovic concludes that the synergism of a proposed role of SO_2 metabolites as inhibitors of enzymes and antioxidants and the adverse effects of NO_x metabolites in the early embryonic development may lead to symmetric IUGR and premature delivery or LBW.

This study reported on a unique opportunity to compare pregnancy outcomes in women in the same population during the time the power plant was in operation with when it was not.

Comments

- The study is not clearly reported. Study design, analytic methods, and the exposures are not defined in detail.
- Although the collection of data on weather is mentioned, there is no mention of inclusion of such information in analyses.
- There was no information on social and behavioral risk factors, such as education level and smoking.
- Although the author reports emissions of multiple products of coal combustion, only SO_2 was measured for a limited part of the study period, and effects are attributed to SO_2 alone.

- Due to the unconventional methods used, this study is difficult to compare with other studies.
- The author received funding for this study from PP Plomin, the management company for Plomin 2.

Nascimento and Moreira, 2009: Are environmental pollutants risk factors for low birth weight?

Nascimento et al. conducted an ecologic (retrospective cohort) study of the association between LBW (< 2500 g) and SO_2, O_3, and PM_{10} in São José dos Campos, a medium-sized city in Brazil. The authors obtained birthweight data for 2001 from the website of the São Paulo State Health Department. Only singleton newborns who had been delivered vaginally at 37 to 41 weeks of gestational age, and whose mothers were 20–34 years of age, had a complete high school education, and completed at least seven prenatal visits, were included. After applying these criteria, 2,529 births (25.6%) were included; of these, 99 (3.95%) were LBW.

Daily data on SO_2, O_3, and PM_{10} from two monitoring stations located three km apart and inside the city limits were provided by the São Paulo State Environmental Agency. Data were collected for 24-hour periods and summed for the 90 days preceding each birth. The pollutant data were then grouped by quartile. The authors did not include season or altitude in their models because the temperature variation between the hottest and coldest seasons was negligible and the city is flat. The mean (SD) 90-day value for SO_2 was 204.3 (39.6) ppb [535.6 (103.8) µg/m^3], with minimum 139.1 ppb (364.6 µg/m^3) and maximum 284.1 ppb (744.9 µg/m^3). SO_2 and PM_{10} were highly correlated (r=0.88), and both were negatively correlated with O_3.

Nascimento et al. used logistic regression to estimate the effects of each pollutant, with the lowest quartile as the referent category, on LBW. When analyzed in a model without co-pollutants, LBW was associated with SO_2; the ORs for the second and third quartiles of SO_2 levels compared to the first were statistically significant, with the odds of a LBW infant increasing two to more than three-fold (Table 33).

Table 33. Odds ratios for LBW and quartiles of 90-day SO_2 values, compared to the lowest quartile[a]

Percentile of 90-day SO_2 values	OR (95% CI)
25 – <50	**3.39 (1.54, 7.53)**
50 – <75	**2.89 (1.33, 6.34)**
≥ 75	2.14 (0.96, 4.82)

[a] adjusted for maternal age, prenatal visits, singleton pregnancy, vaginal delivery, and maternal schooling

However, when the three co-pollutants were analyzed together, PM_{10} was not statistically significant and was therefore excluded. In the final model, the OR for SO_2,

controlling for O_3, was 1.30 (1.02, 1.65) and the OR for O_3, controlling for SO_2, was 1.26 (1.00, 1.58) for exposure in the second and third quartiles, compared to the first. When levels of both SO_2 and O_3 were above the first quartile, the odds of LBW was five times as great.

Comments

- The exposure assessment in this study (90-day cumulative values) is unlike that of other studies: the authors state that pollutant data collection covered 24-hour periods beginning at midnight, and daily measurements were considered for each pollutant, but it is not clear whether the values summed into the exposure variable were 24-hour means. If 24-hour means were used, the 90-day sums would be analogous to mean exposures over the 90 days preceding delivery.
- Ambient pollutant levels are not reported.
- Pollution data came from two locations within 3 km, in an area of 1,100 km^2 with many industrial establishments and an oil refinery.
- Although the authors restricted the cohort to births at 37–41 weeks gestation, gestation length could still account for differences in birthweight.
- The study sample was quite small.
- The authors had no information on potential confounders, such as smoking during pregnancy, weight gain, income, etc.

Nordenson et al., 1980: Is exposure to sulphur dioxide clastogenic? Chromosomal aberrations among workers at a sulphite pulp factory.

Nordenson et al. conducted a preliminary study of chromosomal aberrations among workers at a sulfite pulp factory in Northern Sweden. Blood samples were collected from 19 workers who had been employed more than 15 years at a sulphite pulp factory.

The workers were selected from three work places within the factory:

1) boiling of pulp and handling of sulfuric acid (exposure to SO_2,),
2) bleaching of pulp (exposure to chlorine),
3) paper mill (exposure to dust).

Fifteen healthy males were assessed as controls. Lymphocyte cultures were prepared and chromosomal aberrations were recorded according to the WHO recommendations. Smoking was examined as a covariate.

Workers occupied with bleaching and in the paper mill had about the same frequency of chromosomal aberrations as the controls (Table 34). Nordenson et al. observed an increased frequency of all types of chromosomal aberrations only among those occupied with boiling of pulp and handling of acid.

Table 34. Chromosomal aberrations in workers at a sulphite pulp factory and in controls (adapted from (Nordenson et al., 1980)).

	Work place			
	Boiling (SO_2)	**Bleaching (chlorine)**	**Paper mill (dust)**	**Controls**
# of individuals	7	6	6	15
# of cells	1156	621	662	1500
Gaps				
#	44[a]	8	18	31[a]
Per cell	0.038	0.013	0.027	0.021
Chromatid aberrations				
#	24	4	4	9
Per cell	0.021	0.006	0.006	0.006
Chromosome aberrations				
#	19	7	4	1
Per cell	0.016	0.011	0.006	0.001
All aberrations				
#	87[b]	19	26	41[b]
Per cell	0.075	0.031	0.039	0.027

[a] χ^2 =7.21, 1 df, p<0.01
[b] p<0.0001

The results on the effects of smoking are unremarkable.

Nordstrom et al., 1978a, b, 1979a, b; Beckman and Nordstrom, 1982: Occupational and environmental risks in and around a smelter in northern Sweden. (Parts I, III, V, VI, and IX)

Nordstrom et al. published numerous studies on the occupational and environmental risks in and around Rönnskär, a smelter in the Skellefteå region of northern Sweden, in the late 1970s and early 1980s. The Rönnskär smelter processes the local ore and purchased smelting materials to produce copper, lead, and numerous other metallurgical and chemical products. Smelting processes produce emissions of a number of toxic substances into the environment, including arsenic, lead, mercury, cadmium, and SO_2. In a series of studies, Nordstrom et al. investigated the effects of pollutants from the smelter on the offspring of women, or the wives of men, who were employed at the smelter during pregnancy, or who resided near the smelter. The authors examined birthweight, fetal loss (spontaneous abortion and fetal mortality), and congenital malformations, among other outcomes. However, measurements of exposures to pollutants are not reported.

I. Variations in birth weight (Nordstrom et al., 1978a)

Nordstrom et al. studied birthweight of offspring of women working at the Rönnskär smelter and four nearby areas during 1975–1976. The employees were working at three main locations within the plant: the factory, the laboratory, and administration (office and restaurant). Four nearby areas (called A, B, C, and D) of increasing distance from Rönnskär were also included in the study, of which A and B were somewhat urbanized areas within 10 km of the smelter, and C and D were more distant parishes within the Skellefteå area. A total of 3,688 pregnancies of Rönnskär workers and the four nearby areas were included in the study. The authors used 2,700 births at the University hospital in Umeå in the years 1955, 1965, and 1975 as a control group.

Birthweights tended to be lower closer to the smelter. The mean birthweight in the offspring of female smelter employees (3391.06 g) was significantly lower than in Umeå (3460.14 g; $p<0.05$) and in areas C and D (3495.49 g; $p<0.0l$ and 3470.31 g; $p< 0.05$, respectively). Similarly, in area A, birthweights were significantly lower than in Umeå ($p<0.001$) as well as areas C ($p<0.001$) and D ($p<0.01$), and so forth. However, there were no significant differences in birthweight among smelter employees and areas A and B, and no differences among areas C, D and Umeå. Among Rönnskär workers, the mean birthweight of offspring was lower among women working at the factory ($p<0.001$) and the administration ($p<0.01$) than among women working at the laboratory.

In smelter workers and groups A and B, the decreased birthweight was mainly among women with higher parity (>2). However, parity among the groups was similar, and therefore would not explain the observed differences. The authors suggest that environmental pollutants may be affecting fetal growth and that longer maternal exposure increases the effect, or that pollution exposure shortens gestation time.

Comment: Authors did not control for gestational age, so it is unclear if the lower birthweights are due to lower gestation, vs. intrauterine growth restriction.

III. Frequencies of spontaneous abortion (Nordstrom et al., 1978b)

In the study of frequencies of spontaneous abortion, the authors considered rates of spontaneous abortion in four communities of varying distance from the smelter. The highest frequency of spontaneous abortion was found in the area closest to the smelter. The frequencies of spontaneous abortion decreased with increasing distance from the smelter for the other three areas examined. Comparing the area closest to the smelter with the most distantly located area, the difference in spontaneous abortion frequencies was highly statistically significant ($\chi^2= 10.38$; 1 df; $p< 0.005$). The area closest to the smelter also had the highest frequency of spontaneous abortion among first pregnancies ($\chi^2=5.67$, $p<0.025$).

V. Spontaneous abortion among female employees and decreased birth weight in their offspring (Nordstrom et al., 1979a)

Another study reported on spontaneous abortions and birthweight as pregnancy outcomes among female employees at the Rönnskär smelter. The mean birthweight of offspring of women occupied directly in smelting processes and cleaning operations near these processes (3086.89 g) was lower than the mean birthweight of offspring of administration and restaurant workers (3410.83 g), and laboratory workers (3409.13 g; $p < 0.05$). The relatively lower birthweight among offspring of women in cleaning or smelter work was consistent across all pregnancy orders (i.e., first, second, or later pregnancies).

An increased frequency of spontaneous abortion was found if the mother worked at the smelter during pregnancy (13.9%) or had worked at the smelter before pregnancy and lived close to (≤10 km) the smelter during pregnancy (17.0%). In contrast, women who worked at the smelter only after their pregnancy had lower spontaneous abortion rates (10.6% among those who lived >10 km from the smelter, and 8.6% among those living within 10 km). Among women who worked in close connection with smelting processes (cleaning or smelter work), the frequency of spontaneous abortion was 28.0%, which was twice as high as the frequency among women who worked in administration or the restaurant (13.5%) or laboratory work (13.7%) during pregnancy. Also, when the father was employed the abortion frequency was higher (19.4%) than if only the mother was employed (13.5%). The authors suggest that the high frequency of spontaneous abortions can probably be explained by genetic damage caused by exposure to toxic agents from the smelter.

VI. Congenital malformations (Nordstrom et al., 1979b)

For the congenital malformations study, the authors extracted data on all live births during 1955–1976 from files and case records at the Skellefteå hospital. The personnel office at the Rönnskär smelter provided information on employment at the plant (including time, duration, and kind of occupation). Information on pregnancy outcomes was obtained by employee questionnaires, and the authors checked the questionnaire data against data from the hospital.

Of 1291 live children born to female Rönnskär workers, 3.0% had malformations. Children born to mothers who had worked at the smelter during pregnancy were more likely to have congenital malformations (5.8%) compared with children whose mothers had not worked during pregnancy (2.2%). Five of the six children with multiple malformations had mothers who had worked during pregnancy. Nordstrom et al. did not find statistically significant differences in the total frequency of congenital malformations in the regions around the smelter, suggesting that emissions from the smelter were not causing congenital defects in the population close to the smelter. Nordstrom et al. do not attribute the observed effects to any particular exposures.

IX. Fetal mortality among wives of smelter workers (Beckman et al., 1982)

The authors investigated fetal mortality and congenital malformations among pregnancies of the wives of Rönnskär workers. The study population comprised male smelter workers and office workers born in 1925 or later who were employed at the smelter in 1978 and who were married or in marriage-like relationships. In this study, "exposure" appears to denote employment at Rönnskär before or during the pregnancy.

Among wives of male smelter workers, 4.3% of exposed pregnancies and 3.4% of non-exposed pregnancies, for a total of 3.8%, resulted in offspring with congenital malformations. For office workers, 3.3% of exposed pregnancies and 1.4% of non-exposed pregnancies, for a total of 2.7%, resulted in offspring with congenital malformations. For both the smelter workers and office workers, the differences in congenital malformation rates were not statistically significant.

Among smelter workers, the rate of spontaneous abortion was significantly increased in exposed pregnancies (10.8% among exposed vs. 7.0% among non-exposed pregnancies).

Smelter workers were found to have higher rates of stillbirth than office workers (2.0% vs. 0.8%), and among smelter workers the rate of stillbirths was higher in exposed pregnancies (2.8%) than in non-exposed pregnancies (1.2%), but these differences were not statistically significant. The rate of stillbirths was statistically significantly higher in families where the father used alcohol (2.1% vs. 0.3%, $p<0.05$).

In the discussion, the authors state that in pregnancies where the husband was occupationally exposed and the family lived close to the smelter, the spontaneous abortion rate was 12.0%, and when the husband was not exposed and the family lived farther away from the smelter the abortion rate was as low as 5.27%. The difference between these two extreme categories was highly significant ($p<0.005$).

Comments

- Among Rönnskär workers, those working close to smelting processes may have very different socioeconomic status compared with office workers and laboratory workers.
- In paper V, the authors report smoking rates among surveyed workers to be slightly higher than in Sweden generally (38% vs. 35%, though statistical tests were not reported). Depending on the comparison groups actually used, this and related behavioral differences, such as alcohol use, could potentially bias some findings.

Pereira et al., 1998: Association between air pollution and intrauterine mortality in São Paulo, Brazil.

In this ecologic, time series study, Pereira et al. investigated the associations between air pollutants and intrauterine mortality in São Paulo, Brazil between January 1991–December 1992. Intrauterine mortality was defined as death of a fetus over 28 weeks. Brazilian law requires late fetal losses (over 28 wks gestation) to be recorded on a death certificate. The authors obtained counts of late fetal losses from the municipal mortality information improvement program. The mean (SD) daily intrauterine mortality count was 8.36 (3.08), with minimum=1 and maximum=18.

The city of São Paulo's air pollution control agency (CETESB) monitors air quality through a network which included 14 stations that collected SO_2 data and provided data on NO_2, SO_2, CO, O_3 and PM_{10} daily concentrations. Pereira et al. averaged the values from the different stations to arrive at single citywide measures for each pollutant. The mean (SD) SO_2 concentration was 7.2 (3.3) ppb (18.90 (8.53) μg/m^3), with minimum=1.4 ppb (3.80 μg/m^3) and maximum=22.8 (59.70 μg/m^3). The correlations between SO_2 and the other pollutants and weather conditions are shown in Table 35.

Table 35. Pairwise Pearson correlation coefficients between SO_2 and co-pollutants and weather conditions.

Covariate	Pearson correlation coefficient
NO_2	0.41*
CO	0.34*
O_3	0.15*
PM_{10}	0.74*
Temperature	-0.19*
Humidity	-0.33*

*p<0.01

Pollutant concentrations were entered into Poisson regression models, with the daily number of intrauterine deaths as the dependent variable. The models also included terms for day of week, each month of the study, and the lowest temperature of the day and relative humidity (as continuous and indicator variables). The authors investigated models with different lags and moving averages in time windows from 2 to 14 days for different pollutants, but for SO_2 adopted the mean of the concurrent day.

The authors found a marginal association between SO_2 and intrauterine mortality when co-pollutants were not included in the model. After adjusting for day of week, month, and weather conditions, the regression coefficient for SO_2 was 0.0038 (standard error (SE) 0.0020), which corresponds to a RR of 1.00 (1.00, 1.01) per 0.38 ppb change in

SO_2 (RRs are not reported in the article). Figure 2 shows the RR of intrauterine death when SO_2 concentration is included as five categories, without co-pollutants.

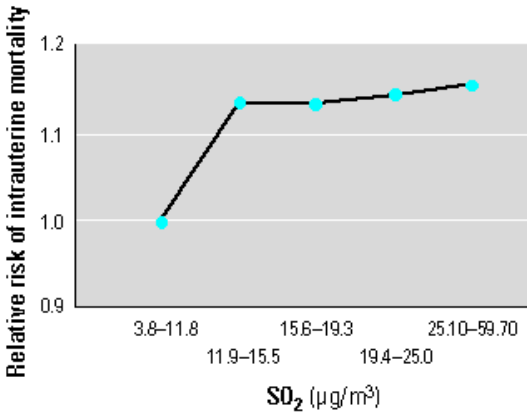

Figure 2. Relative risk of intrauterine mortality for increasing concentrations of SO_2 (quintiles of the daily mean moving average). (Figure from Pereira et al., 1998.)

When all pollutants were included in the model, the coefficient for SO_2 was 0.0029 (SE 0.0031), corresponding to a RR of 1.00 (1.00, 1.01) per 0.038 ppb (1 µg/m^3) change in SO_2. For a 2 SD (6.5 ppb) increase in SO_2, adjusting for co-pollutants and other covariates, the adjusted RR = 1.05 (0.95, 1.17), adjusting for co-pollutants and other covariates. In the multi-pollutant model, only NO_2 remained significant.

The authors also developed an index of overall pollution (IOP) which incorporated concentrations of SO_2, CO, and NO_2 into a single proxy variable of total pollution exposure. The IOP was more strongly associated with intrauterine death than any single pollutant, with the 3-day moving average providing the best signal. IOP exhibited a dose-dependent, nearly linear relationship with intrauterine death, with no evidence of a safe threshold.

The authors state that given the high degree of correlation among the pollutants, it is very difficult to ascribe the observed adverse health effect to a single pollutant.

Comments

- Although the time series analyses include different lag and moving averages, as well as temperature and humidity, the analyses assume no spatial variation in exposure in a very large city.
- The units and coefficients were reported inconsistently in this paper. In response to inquiries by OEHHA staff, the first author provided the following clarifications on May 1, 2010:
    - The SO_2 coefficient in the abstract is wrong. The correct SO_2 regression coefficient is in Table 5 of the paper.

    - All of the pollutants were measured in micrograms per cubic meter, despite reporting in mg/m^3 except CO (in ppm).
- The main source of SO_2 in São Paulo since the 1970s, has been automotive emissions. The oil used has very high levels of diesel sulfur (500 ppm).

Rankin et al., 2009: Maternal exposure to ambient air pollutants and risk of congenital anomalies.

Rankin et al. conducted a case-control study of the association between risk of congenital anomalies and exposure to black smoke (BS, specifically PM with an aerodynamic diameter of <4 μg/m^3) and SO_2 during the first trimester of pregnancy. Case data for births from January 1, 1985–December 31, 1990 were extracted from the Northern Congenital Anomaly Survey (NorCAS), a voluntary, population-based registry of all congenital anomalies in infants and fetuses delivered in the Northern Region of England. NorCAS includes late miscarriages (≥20 weeks gestation), pregnancy terminations for fetal anomaly following prenatal diagnosis, and registered live births and stillbirths to mothers residing in the Northern Region at the time of the birth. NorCAS is notified of cases from multiple sources, including antenatal ultrasound, fetal medicine records, cytogenetic laboratories, the regional cardiology center, pathology departments, and pediatric surgery, and all cases of congenital heart disease are confirmed by autopsy, surgery, echocardiography, or cardiac catheterization. NorCAS records up to six congenital anomalies per case, accepts cases diagnosed up to age 16, and excludes minor anomalies according to the European Surveillance of Congenital Anomalies. There were a total of 3,197 cases, of which 2,714 (84.9%) were non-chromosomal and 483 (15.1%) were chromosomal anomalies. The authors randomly selected 15,000 controls (live and stillbirths) from births in the national data on all registered births in 1985–1990.

Daily levels of BS and SO_2 from a network of 62 ambient air pollution monitoring stations were available from the UK National Air Quality Information Archive website (the number of monitors recording at any given time was substantially fewer). The authors summed the pollutant exposure during the first trimester of pregnancy over the daily readings from all monitors within 10 km of the mother's residence. The distributions of both BS and SO_2, as represented by the median and quartile values, indicated that exposure to the pollutants was slightly lower for all cases than for controls. SO_2 exposure data are summarized in Table 36.

Table 36. The distribution of SO_2 exposure[a] during the first trimester, 1985–1990[b] across case and control groups.

	n	Median (Q1, Q3) in ppb
Controls [c]	176,187	1390 (1035, 1714)
Controls [d]	158,186	1365 (1035, 1710)
Controls (random sample)	11,969	1357 (1086, 1655)
Cases [c]	2,781	1376 (1020, 1691)
Cases [d]	2,515	1337 (1017, 1667)

[a] Individual exposures were the sum of daily readings in the first trimester.
[b] Numbers of cases and controls with data on each comparison differ due to missing data.
[c] Missing exposure data replaced where possible.
[d] Subset for which no exposure data was missing.

Unconditional logistic regression models were used to estimate the associations between each congenital anomaly group and subtype, and BS and SO_2 separately. Models were adjusted for birthweight, gender, and maternal deprivation (measured using Townsend deprivation scores, which are based on postcode and census data). The relationship between the odds of being a case and SO_2 exposure was nonlinear, so the authors categorized SO_2 exposure into quartiles. The authors considered for inclusion in the analyses the variables that were available and reliable for both cases and controls. For example, maternal age and gestational age were available for cases but not controls, and were therefore not used. Table 37 shows results for SO_2.

Table 37. AOR[a] and CI for congenital anomalies and quartiles of first trimester SO_2 exposure.

Congenital anomaly	**2nd quartile**	**3rd quartile**	**4th quartile**
All	1.01 (0.90, 1.13)	0.98 (0.87, 1.09)	0.97 (0.86, 1.08)
All non-chromosomal	0.64 (0.56, 0.73)	0.77 (0.67, 0.89)	0.94 (0.82, 1.08)
Nervous system	0.64 (0.44, 0.94)	0.90 (0.62, 1.31)	1.27 (0.89, 1.82)
Congenital heart disease (overall)	0.60 (0.50, 0.72)	0.73 (0.61, 0.88)	0.82 (0.68, 0.98)
Atrio-ventricular septal defect	0.87 (0.39, 1.98)	0.97 (0.42, 2.26)	1.35 (0.60, 3.05)
Tetralogy of Fallot	0.67 (0.30, 1.47)	0.67 (0.29, 1.56)	0.87 (0.38, 1.99)
Hypoplastic left heart	0.43 (0.14, 1.31)	0.66 (0.23, 1.91)	0.94 (0.35, 2.54)
Coarctation of aorta	0.50 (0.26, 0.94)	0.30 (0.13, 0.68)	0.99 (0.55, 1.80)
Patent ductus arteriosus	0.45 (0.27, 0.76)	0.49 (0.28, 0.85)	0.36 (0.19, 0.69)
Ventricular septal defect	0.67 (0.51, 0.88)	0.80 (0.60, 1.05)	0.78 (0.58, 1.04)
Respiratory tract	1.25 (0.61, 2.56)	1.63 (0.78, 3.39)	1.30 (0.60, 2.83)
Cleft lip and palate	0.79 (0.52, 1.21)	0.85 (0.55, 1.33)	1.09 (0.71, 1.67)
Eye, ear, face and neck	1.28 (0.44, 3.76)	1.64 (0.56, 4.83)	1.76 (0.59, 5.29)
Digestive system	0.51 (0.34, 0.78)	0.82 (0.55, 1.21)	0.97 (0.65, 1.43)
Internal urogenital system	0.68 (0.46, 1.00)	0.85 (0.58, 1.26)	1.17 (0.81, 1.71)
Musculoskeletal	0.63 (0.46, 0.87)	0.84 (0.61, 1.16)	1.02 (0.74, 1.40)
Miscellaneous	1.08 (0.63, 1.85)	1.09 (0.62, 1.92)	1.38 (0.79, 2.40)
Chromosomal	0.73 (0.53, 1.01)	1.14 (0.84, 1.56)	0.86 (0.61, 1.22)

[a] Adjusted for birthweight, sex, and maternal deprivation (measured using Townsend deprivation scores, which are based on postcode and census data). Lowest quartile of SO_2 exposure is the referent category.

The distributions of both BS and SO_2, as represented by the median and quartile values, show that exposure to the pollutants was slightly lower for all cases than for controls. The small case group of eye, ear, face, and neck anomalies and the congenital heart disease (CHD) subtype atrial ventricular septal defect (AVSD) are the only cases where the distribution of both BS and SO_2 is higher than controls. Rankin et al. found a statistically significant negative association across all quartiles relative to the lowest quartile of SO_2 exposure for CHD combined and patent ductus arteriosus. The relationship between SO_2 levels and other anomaly subtypes were less clear; there were either no significant associations or a suggestion of a U-shaped relationship. The authors stated the U-shaped/non-linear association with SO_2 noted for certain congenital anomaly groups/subtypes is difficult to explain and that overall, maternal exposure to BS and SO_2 in the Northern region had limited impact on congenital anomaly risk.

Comments

- The authors view all their findings with caution given that they analyzed 17 congenital anomaly groups/subtypes and two air pollutants, one of which was divided into quartiles, and the number of cases in some groups was small. With such a large number of comparisons, they expect some significant associations to occur by chance.
- The authors state that because maternal residential mobility among mothers who delivered a baby with a reportable defect was 9%, and most moved locally, mobility was unlikely a major concern.
- Potentially important variables such as maternal age, income, education, and alcohol use were not available. Gestational age was only available for cases.
- Each control was assigned a gestational age of 40 wks. Therefore, pollutant exposure for the controls may have been incorrectly estimated for the first trimester.
- Because the exposure levels in this study were summed over the first trimester, they are not comparable with other studies.
- Routine data was not available for other pollutants for the study period.

Robbins et al., 1999: Air pollution and sperm aneuploidy in healthy young men.

This study of air pollution and male reproductive health sponsored by the Czech Ministry of the Environment and U.S. EPA (Sram et al., 1996), is from the same program as the other sperm studies reviewed in this document, with the semen specimens being a subset of samples collected in a larger cross-sectional study (Selevan et al., 2000; Rubes et al., 2005; Rubes et al., 2007). Men were invited to participate in the larger study shortly after turning 18 years of age. Two trained Czech interviewers used a questionnaire to solicit information about exposures through occupation and hobbies (e.g., metals, solvents, pesticides), reproductive history, and health and lifestyle factors pertinent to reproductive health, such as recent fever, active and passive smoking, alcohol consumption, and medications. The participants were also given a physical examination, including a detailed examination of the urogenital system. Semen samples were collected for the larger study over a two-year period, ending in March 1994. The authors conducted conventional semen analysis and evaluated sperm morphology, and aliquots of remaining semen were stored frozen for later analyses or archiving.

The present study included only samples from residents of the Teplice district of the Czech Republic who donated semen during March or October 1993, because these months represent a high air pollution period (winter 1993) and a low air pollution period (summer 1993) with 90 days to allow completion of one cycle of spermatogenesis. Air pollution was severe during winter 1993 as a result of increased use of coal for industry and home heating during the especially cold winter, and temperature inversions. The average SO_2 level was 75.1 ppb (196.9 µg/m^3) during winter 1993 and 12.2 ppb (32.0 µg/m^3) during summer 1993.

Because smoking might share common causal pathways with air pollution, only nonsmokers were included in the sample for the current study. Nonsmoking status was confirmed by cotinine analysis on urine collected at the time of semen sampling. Finally, the samples had to have a sperm count >40 million/sample or 20 million/ml, and have adequate frozen sample remaining for the analysis. Of 471 men interviewed, 325 donated a semen specimen, and 32 (19 winter and 13 summer) met the other inclusion criteria.

The authors used FISH, which they describe as the most common molecular genetic technique in use at the time to detect damage in human sperm at the chromosome level. Denatured sperm were stained for three chromosomes: X in green, Y in red, and 8 in yellow. Disomic cells in the form of XX8, YY8, XY8, X or Y-88 per 10,000 cells were scored. One scorer scored all sperm-FISH slides over an eight-month period. The authors conducted analyses to rule out temporal effects on laboratory procedures, and found no significant temporal correlations between dates of procedures and cytogenetic endpoints and most covariates of interest (XX8 and YY8 disomies were associated with the date slides were scored). To be conservative, the authors included the date slides were scored in regression models.

Statistical analyses (Kendall's tau or Spearman rank correlation) revealed no significant associations between sperm cytogenetic endpoints and the following covariates: sperm count (per ml or total count), sexual abstinence prior to semen sampling, high fever, viscosity, volume, or sperm head morphology. The authors used Poisson regression modeling and linear regression with normal transformations of the cytogenetic endpoints to evaluate relationships between cytogenetic endpoints and other variables of interest, including caffeine and alcohol intake and semen parameter morphology. YY8 disomy was significantly associated with season, with frequency=0.6/10,000 cells in summer and 3.5/10,000 cells in winter (Wilcoxon rank sum, p=0.001). Controlling for date, the incidence rate ratio for YY8 and winter vs. summer exposure was 5.25, (CI 2.5, 11.0). Robbins et al. also observed a small, nonsignificant difference between frequencies of XX8 disomy (2.8/10,000 cells in summer and 3.7/10,000 cells in winter). No other cytogenetic endpoints varied by season. The results suggest that exposure to high levels of SO_2 in the winter season may be associated with increased aneuploidy in the subjects studied. The authors state that the specific components of air pollution which may be associated with an increase in sperm aneuploidy cannot be determined from the present study.

Rogers et al., 2000: Association of very low birth weight with exposures to environmental sulfur dioxide and total suspended particulates.

Rogers et al. conducted a population-based case-control study of the association between risk of having a VLBW baby and maternal exposures to ambient TSP and SO_2 ("$TSPSO_2$"). The data were obtained from a VLBW study which examined five counties in metropolitan Atlanta, and 24 contiguous counties that make up Georgia Health Care District 9 (GHCD9). For the present study, subjects were drawn from GHCD9, including

the city of Savannah, Georgia. The cases were all live-born, singleton, VLBW (<1,500 g) infants born between April 1, 1986 and March 30, 1988. The medical data were collected from labor and delivery logbooks and medical records. Controls were selected from a pool consisting of a 3% sample of infants who met the same time frame and residency criteria as cases, and excluded LBW babies (1,500 g to 2,499 g). Approximately 25% of cases and controls eligible for interview refused, could not be located, or were not allowed by their physicians to participate. Of case and control mothers who completed the interview, additional subjects were lost to analyses because of missing data, leaving 143 cases and 202 controls for analyses.

The authors used a Gaussian plume atmospheric transport model to estimate annualized ground-level $TSPSO_2$ concentrations through a combination of 1) annual $TSPSO_2$ release data from 32 industrial facilities that account for approximately 95% of releases in GHCD9, 2) annual meteorologic data that includes wind speed and direction, 3) geocodes of the release points and birth residences, and 4) distances between release points and birth residences. This model provided estimates of TSP and SO_2 concentrations which were summed to estimate individualized overall ground-level exposure at the home of each eligible study subject. The model is designed to estimate ground-level concentrations over a long period, such as a season or year. The authors checked the accuracy of the model estimates against measurements taken by pollutant monitors at five locations for SO_2 and seven locations for TSP. The model-predicted levels of $TSPSO_2$ correlated well with the observed concentrations. TSP and SO_2 exposures were highly correlated (r=0.93).

Annual $TSPSO_2$ exposure estimates for all study subjects ranged from 1.93–123.82 $\mu g/m^3$ (because the metric includes TSP, $TSPSO_2$ measurements have not been converted to ppb). Table 38 shows the exposure levels used to categorize $TSPSO_2$ levels into low, medium, and high exposures, as well as separate SO_2 and TSP levels. $TSPSO_2$ levels below the median level for controls, or 9.94 $\mu g/m^3$, were considered unexposed and served as the comparison group. $TSPSO_2$ levels between the median and 75th percentile were considered “low” exposure; levels between the 75th and 95th percentiles were considered “moderate” exposure, and levels above the 95th percentile were considered “high” exposure.

Table 38. Individual and combined TSP and SO_2 levels for controls in Georgia Health Care District 9, 1986–1988

Pollutant level	**$TSPSO_2$**[a], $\mu g/m^3$	**SO_2, ppb**	**SO_2,** $\mu g/m^3$	**TSP,** $\mu g/m^3$
Median (low)	9.94	1.45	3.80	5.93
75th percentile (moderate)	25.18	3.10	8.12	16.98
95th percentile (high)	56.75	5.49	14.38	43.60

[a] $TSPSO_2$ is the sum of TSP and SO_2

Rogers et al. examined 17 variables associated with VLBW and used unconditional logistic regression to estimate ORs, adjusting for race, toxemia during pregnancy, active and passive smoking, income, pre-pregnancy weight, maternal weight gain, maternal age, inadequate prenatal care, income (<$10,000 vs. ≥$10,000 per year), maternal and paternal education, illicit drug use, gender, alcohol use, and stress. Table 39 shows AORs and 95% CIs for the association between VLBW and low, moderate, and high $TSPSO_2$ exposures. High exposure to $TSPSO_2$ was significantly associated with VLBW, with an AOR of 2.88 (1.16, 7.13), and the authors concluded the trend in the ORs suggests an association between VLBW and maternal exposures to high levels of air pollution.

The authors also analyzed the contribution of each pollutant to the risk of VLBW in separate models. The OR for women exposed to the SO_2 above the 95th percentile (5.5 ppb or 14.38 μg/m^3) was 1.49 (0.77, 2.89); for TSP above the 95th percentile, the OR was 2.36 (0.88, 6.28).

Table 39. Adjusted odds ratios[a] (95% CIs) for exposure to SO_2, TSP, and $TSPSO_2$

Pollutant level	$TSPSO_2$ [b]	SO_2	TSP
Median (low)	0.99 (0.51, 1.72)	Not reported	Not reported
75th percentile (moderate)	1.27 (0.68, 2.37)	Not reported	Not reported
95th percentile (high)	**2.88 (1.16, 7.13)**	1.49 (0.77, 2.89)	2.36 (0.88, 6.28)

[a] Adjusted for race, toxemia during pregnancy, active and passive smoking, income, prepregnancy weight, maternal weight gain, maternal age, inadequate prenatal care, income, maternal and paternal education, illicit drug use, gender, alcohol use, stress
[b] $TSPSO_2$ is the sum of total suspended particulates and SO_2

Comments

- The mean gestational age was 28.8 weeks for cases and 39.5 weeks for controls, and is strongly associated with birthweight. However, gestational age is not listed among the variables analyzed in the bivariate analyses or the final logistic regression model. If gestational age was actually excluded from the model, the model is likely combining the two distinct outcomes of prematurity and growth restriction.
- The authors note that the meteorologic data were based on annual summaries of wind speed and direction from a single weather station, and that this presents a limitation in modeling exposures to environmental pollutants in a large study area such as GHCD9. Ideally, daily emission data from the release points and daily meteorologic data would allow the authors to identify peak exposures or a critical time of exposure.
- The authors assessed selection bias that may have resulted from the low response rate. Lacking data from interviews, the authors were not able to adjust for the same potential confounders as in the main analysis, but still found ORs of 2.20 (1.01, 4.76) in the sampled group and 2.16 (1.06, 4.36) in the eligible group, after controlling for race and gender.

- Because TSP and SO_2 were combined as a single exposure, this study is difficult to compare with other studies. In addition, the authors do not discuss the possible contribution of other air pollutants in the area to risk of VLBW.
- The exposure assessment in this study does not take into account seasonal or other temporal variation in exposure.

Rubes et al, 2005: Episodic air pollution is associated with increased DNA fragmentation in human sperm without other changes in semen quality, and Rubes et al., 2007: Impact of air pollution on reproductive health in Northern Bohemia.

Rubes et al. conducted this study in the Teplice district of the Czech Republic to examine the potential associations between changes in semen quality and exposures to SO_2, NO_x, and PM_{10} and its constituent PAHs. This study was designed to overcome the limitations of a previous study in the same district (Selevan et al., 2000), which had found associations between some indicators of sperm quality and exposure to air pollution.

Eligibility requirements for study subjects included age (born 1974–1976; age 19–21 years in 1995) and residence in the Teplice district for at least three months before the study began, with intention of remaining for at least a year. Only men who had participated in the previous study, (Selevan et al., 2000), were contacted; of 89 men contacted, 48 enrolled and 36 completed the study. Participants passed a routine physical examination and completed questionnaires to provide information about each participant's reproductive and general health, use of medications and vitamins, lifestyle, and relevant exposures through work or hobby, in the 90 days preceding semen sampling. The cohort was sampled seven times during low and high pollution periods over two years (September 1995–September 1997). Brief questionnaires were subsequently administered at each sampling to update information. Blood samples were obtained with samples 1, 3, 5, and 6 for analysis of lead, mercury, and cadmium.

Late summer semen samples were the reference samples, because air pollution was consistently lower in the summer and higher in winter, when coal is burned for residential heating and temperature inversions trap pollution in the valleys. Samples were collected on-site and processed within one hour of collection. Samples were videotaped for motility analyses. One trained technician using computer-aided sperm analysis analyzed the videotapes for motility and various motion characteristics. Sperm were also processed and examined for morphology (% of sperm with normal morphology and % of sperm with normal head morphology), sperm chromatin structure analysis (DNA fragmentation index through sperm chromatin structure analysis, SCSA-%DFI), and aneuploidy (numbers of disomies and diploidies per subject). The authors describe the processing procedures and assays in detail.

Data on air pollutants were collected for 24-hour periods daily except in summer months, when PM_{10} was measured only once or twice weekly. The winter 90-day

means for SO_2 ranged from approximately 29 to >34 ppb (75 to >90 µg/m^3) and the summer means ranged from approximately 6 to 11 ppb (15 to <30 µg/m^3). However, the authors report results with pollutant exposure classified as high (winter) or low (summer). NO_x and PM_{10} were also highest in winter and lowest in summer, though variation was less than for SO_2.

The authors conducted regression for mixed models to allow for repeated measures and inclusion of participants with fewer than seven semen samples. The following factors were considered as potential confounders: sexual abstinence interval, high fever in the last three months, wearing briefs vs. loose-fitting underwear, alcohol consumption, cigarette smoking, caffeine consumption, and working with solvents or metals. Because cigarette smoke contains chemicals found in air pollution and has been associated with altered semen quality, smoking was included in all models tested. The authors found no significant associations between air pollution exposure levels and any of the routine semen measures (volume, concentration, total sperm count, percent motile, percent normal morphology).

The authors did report a statistically significant association between high pollution levels and poor sperm chromatin integrity, indicated by the SCSA-%DFI, when adjusted for smoking and wearing briefs: β (for log SCSA-%DFI)=0.19, 95% CI (0.02, 0.36); $p<0.05$. The mean baseline SCSA-%DFI was 12–15%, and increased to 15–20% with higher exposure. The authors report that the latter is still considered indicative of good fertility potential, but as SCSA-%DFI approaches and exceeds 30%, the risk of infertility and spontaneous abortion is considerable. A positive association between SO_2 exposure and SCSA-%DFI was also significant: β (for log SCSA-%DFI)=0.026, 95% CI (0.001, 0.053). The authors reported that correlations between DFI and either PM_{10} or PAH were of borderline significance.

Comments

- The authors noted that although the change in average SCSA-%DFI observed in the study may not have affected the fertility potential of these individual men, changes of similar magnitude could impact fertility of men in the general population who have higher baseline SCSA-%DFI.
- There was a lack of consistency of findings for associations with sperm parameters between this longitudinal study and the cross-sectional study by Selevan et al. (2000) presented above. However, the authors proposed that this may be related to the remedial actions by the Czech government that resulted in a decline in air pollution between 1993 and subsequent years.

  As specifically described in Rubes et al. (2007):

  "With specific reference to the two semen studies described above, mean SO_2 levels for comparable 90 days intervals (late December to late March) were notably higher in 1993 (164.0 µg/m^3 (62.6 ppb), Selevan et al., 2000) compared with 1996 (78.5 µg/m^3 (29.9 ppb)). The same was true for PM_{10} where the

comparable 1993 mean was 184.7 µg/m^3 (70.5 ppb) (Selevan et al., 2000) compared with 67.8 µg/m^3 (25.9 ppb) for 1996. In a subsequent study conducted in 1998, another group of 50 eighteen-year old men were similarly examined (unpublished data). Comparison of mean values for the first group of men sampled in spring 1993 to this group sampled in 1998 showed that sperm concentration and percentage of motile spermatozoa were significantly higher (60.1 vs. 102.3 mil/ml and 32.5 vs. 62% motile, respectively) in the 1998 group. Taken together, the three studies provide evidence that exposure to high levels of air pollution is associated with decrements in semen quality, and that these appear to be reversible once the pollution is lowered".

Rybar et al., 2009: Sperm chromatin integrity in young men with no experiences of infertility and men from idiopathic infertility couples.

In this study, Rybar et al. compared semen quality in men assigned to two defined groups: men from couples with unexplained infertility (A), and young men with no experiences of infertility (B). The objective of this study was not stated, but appeared to be an assessment of whether sperm chromatin disorders may be associated with subfertility or infertility in men with unexplained infertility. The sample included a total of 177 men; 65 were from couples with unexplained infertility (group A) (20 of 65, i.e. 30.1%, were smokers) and 112 were young fertile men (group B) (26 of 112, i.e. 23.2%, were smokers). The men with unexplained infertility were selected from couples attending an infertility clinic who had tried for 12 months or more to achieve pregnancy without success. The group of young fertile men consisted of university students with no experience of infertility. A detailed medical history, including reproductive history and infertility evaluation of the female partner, was obtained from all participants.

Semen samples were collected by masturbation after at least 2 days of abstinence, and were examined by standard ejaculate analysis and SCSA. DNA damage can be assessed by SCSA. Each semen sample contained a percentage of mature cells with non-detectable (main population of spermatozoa in semen) and detectable DFI (percentage of mature spermatozoa with increased chromatin damage). The percentage of immature cells (HDS; cells with high DNA stainability) was also evaluated. Smoking was assessed as a covariate.

Sperm samples from men in group A had lower concentrations and lower motility compared with group B ($p<0.01$), but semen volume was comparable. Sperm chromatin damage was significantly higher in men from group A than in those from group B.

Evidence of a relationship between chromatin disorders and standard parameters of ejaculates were not found. Nor was a relationship between smoking and sperm quality in men from both groups found.

Sagiv et al., 2004: A Time Series Analysis of Air Pollution and Preterm Birth in Pennsylvania, 1997–2001.

Sagiv et al. investigated the associations of risk of preterm delivery with outdoor SO_2 and PM_{10} in four Pennsylvania counties. The authors used birth certificate data to construct a study population of all 187,997 live singleton births in 1997 through 2001, of gestational age 20 to 44 weeks, whose mothers resided in Allegheny, Beaver, Lackawanna, or Philadelphia Counties. The authors selected these counties for their contrasting SO_2 and PM_{10} concentrations (and "diverse mix"). Gestational age was calculated using the LMP, and where LMP was missing, the clinical estimate of gestational age was used. There were 21,450 (11.4%) preterm births.

The authors obtained ambient outdoor air pollution data from the US EPA Air Quality System and computed daily mean values for SO_2 and PM_{10}, as well as co-pollutants CO and NO_2 for each county. Three of the counties had multiple monitoring stations for each pollutant. Meteorologic data, including daily temperature and dew point temperature (a measure of relative humidity), were obtained from the National Weather Service. Daily pollutant levels in each county were then used to compute the mean pollutant concentrations for 6-week periods preceding each day of the study period.

Sagiv et al. conducted a Poisson regression analysis using counts of preterm births for each day and both SO_2 and PM_{10} jointly over time. The final models included co-pollutant concentrations, long-term trends in preterm birth, and weather (temperature and dew point) based on their established relationship with air pollution and likely associations with SO_2 and PM_{10}. Additionally, the authors evaluated and used smoothing techniques and spline functions to improve fit, and parametric functions to achieve more conservative standard errors and to account for long-term trends. The offset was number of gestations at risk.

The authors explored average daily concentrations of pollutants during the six weeks preceding birth, and acute exposures of one day with lags ranging from one to seven days before birth. For the 6-week analyses, to incorporate county-level information, the authors used a multivariable mixed-effects model with a random intercept for each county. For the one-day acute effect analyses, the authors also controlled for day of week.

Sagiv et al. observed a marginally significant increased risk for preterm delivery associated with exposure to SO_2 in the 6 weeks before birth, RR=1.15; (1.00–1.32) per 15 ppb increase, and the data suggested a monotonic dose-response effect. When SO_2 exposure was categorized into quarters, the RRs were statistically non-significant. Table 40 shows the RRs for SO_2 and preterm birth, for both 6-week and acute (1-day) exposures. For acute exposures, the authors evaluated lags of one to seven days before birth and found an acute effect of exposure to SO_2 three days before birth (RR=1.07; CI, 0.99, 1.15), adjusting for covariates, including temperature, dew point temperature, and day of the week.

Table 40. RRs and 95% CIs for preterm birth and exposure to SO_2 in the six weeks preceding birth.

Exposure window	Exposure to SO_2 (ppb)	RR (95% CI)[a]
Final 6 weeks	Per 15 ppb	1.15 (1.00, 1.32)
	First quarter 0.8 – 4.9	1.00
	Second quarter 4.9 – 8.1	1.02 (0.97, 1.06)
	Third quarter 8.1 – 10.6	1.04 (0.98, 1.10)
	Fourth quarter 10.6 – 17.0	1.06 (0.99, 1.14)
Acute (1 day) with 3-day lag	Per 15 ppb	1.07 (0.99, 1.15)

[a] Offset by number of gestations at risk and adjusted for long-term and seasonal preterm birth trend, and PM_{10}, CO, and NO_2. Acute exposure analyses also control for day of week.

Comments

- Sagiv et al. acknowledge that measurements from stationary outdoor monitors may not represent individual exposure. In this study, the number of monitors and size of the areas in which they are dispersed is not reported, though it appears that one of the four counties had only one monitor. However, the likely consequence of using ambient concentrations is to underestimate the effects of pollution.
- The authors also note that they only examined exposure at the end of pregnancy, although one previous study found an association between preterm birth and SO_2 exposure in the beginning of pregnancy. The authors point out that the observed associations in this study are small, corresponding to one or two excess preterm births for every 100 births exposed to a 15 ppb increase in SO_2. However, they also note that many people live in urban centers and are exposed to high levels of air pollution. Therefore, if the observed associations are causal, they could have significant public health implications.

Sakai, 1984: Fetal abnormality in a Japanese industrial zone.

The purpose of this ecologic study was to assess the impact on fetal outcomes of low concentrations of various man-made agents in the environment in the Keihin industrial zone along Tokyo Bay in Japan. The author examined the spontaneous fetal death rate and the number of LBW (<2500 g) births in three districts in the Keihin industrial zone: Tsurumi and Kanagawa districts in Yokohama City, and Kawasaki in Kawasaki City. A fourth, less industrialized neighboring district, Totsuka in Yokohama City, was also included for comparison.

The author obtained data on fetal outcomes from vital statistics data published by the Health and Welfare Ministry for 1973–1977. Spontaneous fetal death is not defined. There were 2,767 (5.73%) LBW births and 1,710 (3.47%) fetal deaths. Air pollution

data for SO_2, NO_2, NO, and dust for 1975–1977 came from the Air Pollution Monitoring System in three districts in Yokohama City. Thus, the air pollution aspect of this paper covers only 1975–1977.

The author calculated the correlations between fetal abnormality rates and the annual mean concentrations of pollutants (Table 41). SO_2 was positively correlated with the rate of underweight births, and was statistically significantly correlated with the fetal death rate.

Table 41. Correlation coefficients for annual mean concentration of pollutants and rates of LBW (<2500 g) and spontaneous fetal death.

Pollutant	LBW rate	Fetal death rate
SO_2	0.627[a]	**0.704**[b]
NO	-0.569	0.075
NO_2	0.669[b]	0.797[c]
Dust	-0.455	-0.351

[a] p≤0.10, [b] p≤0.05, [c] p≤0.01

Selevan et al., 2000: Semen quality and reproductive health of young Czech men exposed to seasonal air pollution.

This cross-sectional study is the precursor to the Rubes study (Rubes et al., 2007). Selevan et al. compared 18-year old men living in Teplice, a highly industrialized district, to those living in Prachatice, a rural, less polluted district, in the Czech Republic. During the 1980s, Teplice had seasonally elevated levels of air pollution, with SO_2 and associated PM levels frequently exceeding U.S. and Czech air pollution standards in winter, when the use of coal for heating homes increases and thermal inversions cause the retention of air pollution.

All young men turning 18 in the districts in the six months before sampling were sent a letter from their district Hygiene Station with an appointment for a physical examination. Subjects were recruited when they presented for their physical examinations. Sixty-one percent (408 of 670) presented for their examination and completed a questionnaire; of these, 67% (273) agreed to provide a semen sample. One specimen container leaked, so 272 samples were available. Data were collected from participants by structured interviews, physical examinations (including urogenital evaluation), and semen sampling. Subjects provided information on health status, lifestyle, other exposures (e.g., metals, pesticides, and solvents), and reproductive history, including date of last semen emission. The authors describe the semen processing procedures and assays in detail. All appointments were scheduled within one week in early fall 1993 or late winter 1993 or 1994.

Air monitors in the districts measured SO_2, PM_{10}, TSP, NO_x, and CO, and confirmed that pollution levels were higher in Teplice than in Prachatice, and higher in winter than

the rest of the year in both districts. PM_{10} levels were significantly correlated with SO_2 (r=0.81), TSP (r=0.96), NO_x (r=0.58), and CO (r=0.49). Because 90 days is generally considered sufficient for detecting effects on spermatogenesis, air pollution in the 90 days before semen sampling was considered relevant. SO_2 levels are shown in Table 42. Although concentrations of specific pollutants are reported, the exposure data were classified simply as high, medium, or low. Mean pollutant levels in the 90 days preceding fall semen sampling periods in both districts and in late winter in Prachatice were considered low. The authors considered winter 1993 in Teplice a high air pollution period, and winter 1994 in Teplice a medium pollution period. The location and number of monitoring stations is not described. As the authors noted, the time between severe episodes of air pollution and semen sampling was different in the winters of 1993 and 1994; consequently, one might not expect a clear exposure-response relationship. Therefore, Selevan et al. conducted multivariable regression with air pollution levels entered as dummy variables to avoid imposing a linear relationship. Factors considered for inclusion in the models were sexual abstinence interval, high fever in the last three months, wearing briefs vs. loose-fitting underwear, alcohol consumption, cigarette smoking, caffeine consumption, and hobby or work with solvents or metals.

Table 42. SO_2 concentrations (ppb) in Teplice and Prachatice Districts for 90 days preceding collection of semen samples.

Location	Characteristic	Winter 1993	Summer 1993	Winter 1994
Teplice	Mean ± SD	62.6 ± 61.4	11.7 ± 5.7	30.4 ± 15.2
	Median	40.8	9.6	31.0
	Range	5.5–266.2	4.0–26.7	4.3–88.0
	No. days > 57*	26 (28.9%)	0	4 (4.4%)
Prachatice	Mean ± SD	15.8 ± 13.5	2.3 ± 1.2	6.6 ± 4.8
	Median	12.8	2.3	5.0
	Range	2.7–73.2	0.4–5.3	0.4–24.0
	No. days > 57*	1 (1.1%)	0	0

* 24 hour average; Czech standard

Medium or high air pollution was not associated with sperm concentration or total sperm count, after controlling for abstinence <2 days. Semen volume, sperm concentration, and total sperm counts were not significantly associated with pollution levels. The authors observed a significant association between medium, but not high, air pollution exposure and decreased percentage of motile sperm, after adjusting for wearing briefs, high fever, working with solvents, and smoking. Similarly, lower total numbers of motile and progressive sperm were associated with medium, but not high, air pollution levels.

Table 43. Semen outcomes by level of exposure late winter surveys vs. all fall surveys[a] (adapted from (Sram et al., 1999)).

	Prachatice		Teplice	
	Very Low	Low	Medium	High
	Late winter '94 (n=48, mean SO_2 = 17.5)	Late winter '93 (n=28, mean SO_2 = 41.5)	Late winter '94 (n=63, mean SO_2 = 79.4)	Late winter '93 (n=47, mean SO_2 = 164.0)
Total sperm count < 40 million/sample	0.6 (0.2–1.7)	1.5 (0.5–4.5)	1.1 (0.5–2.5)	0.7 (0.3–1.9)
Percent motile < 24%	2.0 (0.7–5.5)	2.0 (0.6–6.8)	9.8 (3.6–27.2)	3.5 (1.1–11.5)
Sperm morphology < 13% normal	0.2 (0.1–0.7)	0.5 (0.2–2.0)	4.1 (1.2–13.9)	10.1 (2.8–36.0)
Sperm head shape < 29% normal	0.5 (0.2–1.6)	0.2 (0.0–1.2)	6.1 (2.0–18.4)	1.4 (0.4–5.0)

[a] For all fall: Teplice: Fall 1992, mean SO_2=23.8, n=36; Fall 1993 mean SO_2=29, n=44; Prachatice: Fall 1992, mean SO_2=8.9, n=17; Fall 1993 mean SO_2=6, n=42.
Odds Ratios (95% CI)

The associations between pollution and measures of sperm motion were mixed, though the authors raise the possibility of bias introduced by the exclusion of sperm samples with fewer than 25 motile sperm tracks. Quality of sperm motion was measured using SCSA measurements, and three were selected to describe different aspects of sperm motion. Distance traveled over time was significantly higher with high pollution exposures, but this effect was largely seasonal and the association was not significant after adjustment for season. The authors state that season is associated with some measures of sperm motility and morphology, but season is also causally associated with use of coal for residential heat and temperature inversions; therefore, adjusting for season may not be appropriate. Average point-to-point velocity was significantly lower after medium pollution exposure and significantly higher with high pollution exposure. Linearity, a measure of swimming pattern, was significantly higher after medium pollution exposure and significantly lower with high pollution exposure.

Analysis of the SCSA data focused on the COMPα variable, which is the percent of sperm with abnormal chromatin (Table 44). The proportion of sperm with poor chromatin integrity was significantly higher in samples obtained after high air pollution exposure.

Table 44. Semen COMPα_t outcome by exposure (adapted from (Selevan et al., 2000)).

	Low	Medium	High
COMP α_t	19.2 ± 12.2	16.2 ± 9.3	28.8 ± 20.4 *

* Different from low and medium by Kruskal-Wallis test, $p<0.05$.

Comments

- As the authors note, the specific components of the air pollution that may account for observed adverse outcomes were not identified.
- Because this study used only one semen sample from each participant, the possibility that adverse semen characteristics preceded the exposures cannot be excluded.
- The authors acknowledge that if the important exposures are peak levels, error may have been introduced given the timing of these peaks within the 90-day periods. The numbers of peaks and time between peaks and semen sampling varied between the two winters in Teplice (high and medium exposure periods). Because the ability to detect the effect of an acute exposure on a susceptible germ cell stage depends on the timing of the semen sampling, this study could have missed such effects.
- The smaller number of subjects in the high exposure group compared with the medium and low exposure groups may have limited the ability to detect differences in sperm parameters between these groups.

Smrcka and Leznarova, 1998: Environmental pollution and the occurrence of congenital defects in a 15-year period in a south Moravian district.

Smrčka et al. studied air pollutants and the sites of minimal and maximal "accumulation" of congenital defects in the district of Břeclav in South Moravia, Czech Republic, 1975–1990. The methods used and outcomes observed in this ecologic study are not clear. The authors report that the most widespread harmful substances in the atmosphere of the region are airborne inert dust in long-term concentrations exceeding 15.3 ppb and 38.1 ppb (0.04 μg/m^3 and 0.10 μg/m^3), SO_2 in long-term concentrations exceeding 22.9 ppb and 38.1 ppb (0.06 μg/m^3 and 0.10 μg/m^3) and other gaseous harmful substances, including odors. The authors found three areas with an increased accumulation of congenital defects; one of the three areas also had a significantly higher occurrence of spontaneous abortions and congenital heart defects within the district. The area with higher spontaneous abortions, heart defects, and other congenital defects had a greater occurrence of organic solvents and phosphoric acid from factories, and emission dust from a ceramic factory. Of the other two areas with higher occurrences of defects, one had dust emissions from a lime factory and the other was an agricultural area with no factories. Smrčka et al. report that the district of Břeclav had sulfurous oxide emissions of 4990 tons/year in 1975 and 6844 tons/year in 1982, but do not otherwise report ambient SO_2 concentrations.

Strickland et al., 2009: Ambient Air Pollution and Cardiovascular Malformations in Atlanta, Georgia, 1986–2003.

Strickland et al. conducted a retrospective cohort study with a time-series analysis to explore the associations between ambient air pollution (O_3, CO, NO_2, PM_{10}, and SO_2) levels during weeks 3–7 of pregnancy and risks of cardiovascular malformations. Each live born or stillborn infant included in the cohort had an estimated date of conception during January 1, 1986–March 12, 2003. Records for infants with cardiovascular malformations were provided by the Metropolitan Atlanta Congenital Defects Program, which conducts active, population-based surveillance on pregnancies of mothers residing in the five Atlanta counties at the time of delivery or termination and that reach at least 20 wks' gestation.

Records of infants with cardiovascular malformations were reviewed by a pediatric cardiologist and classified according to Society of Thoracic Surgeons Congenital Heart Surgery Database nomenclature. Isolated transient newborn cardiac conditions such as patent foramen ovale, were classified as physiologically normal. Infants with more than one malformation were placed in multiple outcome groups only when the malformations were thought to be embryologically independent; otherwise, only the major cardiovascular malformation was coded. Infants with normal cardiac physiology, identified trisomies, evidence of heterotaxy syndrome, and abnormal cardiac looping were excluded.

Measurements of ambient daily 8-hour maximum O_3 and 24-hour average CO, NO_2, PM_{10}, and SO_2 were obtained from the U.S. EPA Air Quality System, Georgia Department of Natural Resources, and the Metro Atlanta Index. For each pollutant, Strickland et al. selected one central monitoring station for use in the analyses.

Each pregnancy with a given conception date was assigned the same pollutant metric, which was a weighted average of the 35 daily ambient air pollution measurements during weeks 3–7 of the pregnancy. Weights were 0.7 for measurements in weeks 3 and 7, 0.9 for weeks 4 and 6, and 1.0 for week 5. Temporal associations between ambient air pollution and daily risks of cardiovascular malformations were modeled using Poisson generalized linear models with a log link and scaled variance estimates. The offset was the natural logarithm of total conceptions. Indicator variables for each week of the year were included to control for factors with seasonal variation. The authors also conducted numerous sensitivity analyses.

Table 45. IQR and mean values, by season and year of conception, for the weighted 5-wk air pollution metric[a] assigned to the cohort of pregnancies reaching at least 20 wks gestation in Atlanta, Georgia, with an estimated date of conception during January 1, 1986–March 12, 2003. (Adapted from (Strickland et al., 2009)).

	24-hour SO_2, ppb[b]
Interquartile range	4.0
Mean value, by season of conception	
March–May	5.4
June–August	5.4
September–November	6.9
December–February	7.1
Mean value, by year of conception	
1986–1991	8.7
1992–1997	5.5
1998–2003	4.0

[a] The air pollution metric is a 5-week weighted average of daily ambient air pollution levels measured at a central monitor 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.

[b] Daily central monitoring station measurements available: 94% (5,966 of 6,315 days) for SO_2. When feasible, missing daily measurements were modeled: 5% (311 of 6,315 days) for SO_2.

The authors report risk ratios corresponding to an IQR increase in the pollutant metric. Table 46 shows the results of the primary analysis for SO_2. Of the 60 associations examined in the primary analysis, no significant associations were observed except an increased risk for patent ductus arteriosus associated with PM_{10}.

Table 46. RR and CI for associations between the weighted 5-wk air pollution metric and cardiovascular malformations among the cohort of pregnancies reaching at least 20 wks gestation in Atlanta, Georgia, with an estimated date of conception during January 1, 1986–March 12, 2003. (Adapted from (Strickland et al., 2009))

	24-hour SO_2, ppb	
	RR	**CI**
Atrial septal defect, secundum	1.00	0.72, 1.38
Coarctation of the aorta	1.04	0.75, 1.43
Hypoplastic left heart syndrome	0.77	0.50, 1.18
Patent ductus arteriosus	1.22	0.86, 1.74
Pulmonary stenosis, valvar	0.70	0.49, 1.00
Tetralogy of Fallot	0.85	0.61, 1.17
Transposition of the great arteries	1.13	0.75, 1.71
Ventricular septal defect, muscular	0.95	0.77, 1.17
Ventricular septal defect, perimembranous	0.99	0.76, 1.28
Conotruncal defect	1.06	0.86, 1.31
Left ventricular outflow tract defect	0.97	0.76, 1.22
Right ventricular outflow tract defect	0.74	0.55, 1.00

Comments

- The authors comment that because of their review and classification of birth defects records, their outcome groups differ from those of previous studies, and the results of their study are difficult to compare with others (e.g., 35% of the records they reviewed were reclassified as “structurally normal”).
- As the authors note, the use of ambient air pollution measurements as a proxy for personal exposure was likely the largest component of measurement error. In this study, all pregnancies with the same estimated conception date were assigned SO_2 exposure from a single monitor; thus, this study did not measure spatial variation in exposure
- The authors noted that there was misclassification in the vital records data, as demonstrated by the fact that 30% of pregnancies had a last menstrual period date listed as the 15th of the month.
- The authors do not discuss the relatively low correlations among pollutants, e.g., the correlation between SO_2 and PM_{10} was 0.41. The low correlations might be explained by the use of a different monitor for each pollutant.

- SO_2 decreased over time. The difference in SO_2 levels from 1986–1991 and 1998–2003 was 4.7, which is substantially more than many studies have seen.

Virro et al., 2004: Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles.

In this study, Virro et al. use a retrospective review and prospective study to assess the relationship between SCSA parameters and conventional IVF and ICSI outcomes.

SCSA measures the percentage of sperm with a high susceptibility to low pH-induced DNA denaturation and is expressed as the DNA fragmentation index (DFI). DFI is a measure of DNA quality that is proportionate to the level of DNA strand breaks in sperm. It is also an indicator of environmental pollution, smoking, and exposure to toxicants. SCSA simultaneously identifies the percentage of sperm with immature nuclear development. The nuclear chromatin structure of immature sperm is abnormal with a characteristically high level of high DNA stainability (HDS).

Two hundred and forty-nine couples undergoing their first IVF and/ or ICSI cycles were the subjects of this study. Data from the first 63 couples were looked at retrospectively. Patients were grouped according to their SCSA data into the following groups:

<30% DFI (low levels of DNA fragmentation; n=178)
≥30% DFI (high levels of DNA fragmentation; n=71)
<15% HDS (low levels of immature sperm; n=207)
≥15% HDS (high levels of immature sperm; n=42)

Seventy-five percent of patients had both conventional IVF and ICSI performed; 18% had all of their eggs injected for male factor infertility, and 7% had only conventional IVF performed. Statistical analysis was completed using the mean DFI and HDS of replicate runs. Pearson's χ^2 test was used to measure the relationship between DFI groups and HDS groups, the presence of male factors and IVF/ICSI outcomes, low blastocyst rate, spontaneous abortion, and chemical pregnancy. Analysis of variance and regression analysis were used to determine the relationships between SCSA parameters and conventional semen parameters.

Men with ≥30% DFI were 6.9 times (CI 3.8, 12.62) more likely to have one or more abnormal conventional semen parameter(s) such as sperm count or morphology. In addition, these men were also at risk for low blastocyst rates (<30%) ($p < 0.003$) and no on-going pregnancies.

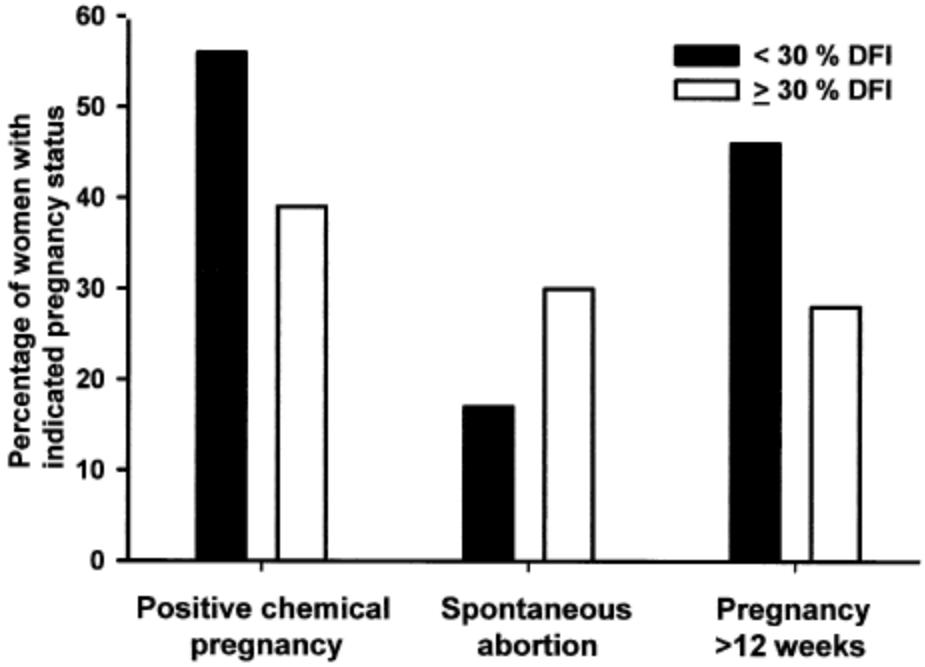

Figure 3. Female partners of men within group 2 (≥30% DFI) had a lower rate of chemical pregnancy (p=0.02) and a higher rate of spontaneous abortion (P=0.11) and a significant decrease in pregnancies ongoing at 12 wks of gestation (P<0.01). HDS groups and the WHO thresholds for normal semen analysis (volume, density, motility, and morphology) were not significantly related to these measures of pregnancy outcome. (Adapted from (Virro et al., 2004))

Figure 3 illustrates that in men with ≥30% DFI there was a cumulative effect of:

1) having a lower chance of initiating a chemical pregnancy (p = 0.02);
2) a trend of increases in spontaneous abortion (p=0.11), to produce a decrease in pregnancy rates compared with men with ≤30% DFI (28% versus 47%, respectively). Fertilization rates were not statistically different between high- and low-DFI groups.

Men with ≥15% HDS were 4.8 times (CI 2.4, 9.3) more likely to have one or more abnormal conventional sperm parameters and had lower IVF fertilization rates. Combined, IVF and ICSI fertilization rates were not statistically related to HDS.

The decrease in pregnancy rates found among couples with men having high levels of DNA fragmentation (≥30% DFI) have been shown to have a significant, negative effect on the number of embryos that develop to the blastocyst stage, which correlates significantly with low blastocyst numbers and poor quality.

Comments

- This study supports the idea that poor embryo quality, decreased implantation rates, and increased spontaneous abortion rates may be associated with male factor infertility.
- Although this study was identified in the literature review by Zini et al. (2008), for their meta-analysis of sperm DNA damage associated with risk of pregnancy loss, it was excluded because a 2x2 table could not be constructed from the data.

Wang et al., 1997: Association between air pollution and low birth weight: a community-based study.

Wang et al. investigated the timing and intensity of maternal exposure to TSP and SO_2 during pregnancy and its relationship to birthweight and LBW (<2,500 g) in four residential areas that cover 45 km^2 in Beijing, China. Dongcheng, Xicheng, Congwen, and Xuanwu are adjacent residential areas in the center of Beijing. There are no major industries, and bicycles are the primary form of transportation. Coal stoves are used for heating or cooking in 97% of households and are the major source of air pollution.

The cohort included 74,671 women who were at least 20 years old at the start of pregnancy and gave birth to live, full-term (37–44 gestation weeks), singleton babies weighing >1,000 g, during 1988–1991. The women were required to register in their local maternal health-care center and were given a manual which contained instructions for pre- and post-natal care, and forms for the obstetrician to record information about the mother, pregnancy, and pregnancy outcomes. The overall mean birthweight was 3,318 g, and there were approximately 2,195 (2.94%) LBW births.

SO_2 concentrations were monitored at the two WHO Global Environmental Monitoring System monitors in Dongcheng and Xicheng. Daily air samples were collected and analyzed for two to three weeks each month, starting in the second week of the month. The mean pollutant concentrations measured at the two monitors were used as the daily air pollution levels. The daily measurements for each pollutant were highly correlated between the two monitors, with Pearson correlation coefficients of 0.92 for SO_2 and 0.93 for TSP. To examine the importance of timing and magnitude of exposure, the authors constructed the following exposure variables for both SO_2 and TSP: 1) mean exposure for the entire pregnancy; 2) Mean exposure during each trimester; and 3) lagged moving averages of exposure, i.e., 1, 2, 3, …*n* weeks before birth. These exposure variables were evaluated individually and jointly. The authors concluded that the best-fitting models included the mean level of exposure during the third trimester. Mean third-trimester SO_2 concentrations ranged from 3.4–117.5 ppb (9–308 $\mu g/m^3$) and were divided into quintiles for evaluating risk.

Birthweight was modeled as a continuous variable and as a binary indicator (LBW). The authors conducted multiple linear regression and logistic regression with

adjustment for gestational age, season, residential area, maternal age (20–24, 25–29, 30+ years), year of birth, and infant gender.

Table 47 shows that the risk of LBW was significantly increased for those infants whose mothers had high levels of SO_2 exposure (4^{th} and 5^{th} quintiles) in the *third* trimester (25 weeks to delivery). The odds of a LBW birth were increased by 16% for exposure in the 4^{th} quintile (Adjusted OR 1.16, CI 1.01, 1.34) and 39% for exposure in the highest quintile (AOR 1.39, CI 1.22, 1.60). The AOR for LBW associated with a 38 ppb increase in 3^{rd} trimester SO_2 exposure was 1.11 (CI 1.06, 1.16). The data suggested an exposure-response relationship between LBW and SO_2; a test for linear trend was highly significant. The AOR of 1.11 for a 38 ppb increase in SO_2 corresponds with an OR of 1.37 for a 114 ppb increase in mean SO_2. Results were similar for TSP (AOR 1.10 for 100 $\mu g/m^3$ increase in TSP). Adjustment for season did not change the point estimates (ORs) but resulted in wider CIs, though they remained statistically significant.

Table 47. Adjusted ORs and 95% CIs of low birthweight (< 2,500g) by quintiles of mean exposure to SO_2 during the third trimester of pregnancy

Exposure (ppb)	AOR	95% CI
3.4 – 6.9 (Referent)		
6.9 – 21.0	1.09	0.94, 1.26
21.0 – 55.7	1.12	0.97, 1.29
55.7 – 91.2	1.16	1.01, 1.34
91.2 – 117.5	1.39	1.22, 1.60
Continuous variable*	1.11	1.06, 1.16

Logistic regression models adjusted for gestational age, residential area, maternal age, year of birth, and infant gender.
* Linear trend test, $p<0.01$, for each 38 ppb increase in SO_2.

The authors also stratified logistic regression analyses by residence, season, maternal age, infant gender, and year of birth to evaluate the consistency of associations. The strength of the association varied for the different strata, but the AORs were almost all >1, the single exception being babies born in 1991, when the AOR for a 38 ppb increase in SO_2 during the third trimester was 0.99 (0.75, 1.28).

Multiple linear regression analyses yielded similar results, showing that higher exposure to SO_2 during the third trimester was associated with reduced birthweight. A test for linear trend was statistically significant. After adjustment for gestational age and other covariates, the estimated reduction in birthweight was 7.3 g for each 38 ppb increase in SO_2 exposure. Similarly, the estimated reduction in birthweight was 6.9 g for each 100 $\mu g/m^3$ increase in TSP.

Table 48. Effects of maternal exposure to SO_2 during the third trimester of pregnancy on birthweight (g)

Exposure (ppb)	β	SE	P-value
3.4 – 6.9 (Referent)			
6.9 – 21.0	-6.4	4.8	0.18
21.0 – 55.7	-4.7	4.7	0.32
55.7 – 91.2	-11.2	4.8	<0.05
91.2 – 117.5	-24.3	4.7	<0.01
Continuous variable[a]	-7.3	1.5	<0.01

Multiple regression models adjust for gestational age, residential areas, maternal age, year of birth, and infant gender.

[a] β represents the difference in birthweight (g) for each 38 ppb increase in SO_2.

Comments

- The authors note a high degree of correlation between SO_2 and TSP (correlation coefficients were not reported), and that it is thus difficult to determine whether one is a more important factor than the other. They did not report multi-pollutant analyses.
- The authors acknowledge they were unable to examine factors know to affect birthweight, such as maternal nutrition and pre-pregnancy weight, weight gain, smoking, history of adverse pregnancy outcomes, and occupational exposures. However, they argue that because such factors are unlikely to be related to daily pollution exposures, they are not likely to have confounded the analyses.
- Wang et al. noticed a small negative association between birthweight and pollution exposures in the first two trimesters. They state this could be explained by seasonality (exposures in the first two trimesters will usually be inversely associated with the third). Another possibility is that exposures in early pregnancy are associated with different outcomes, such as spontaneous abortion. Finally, the authors argue that by limiting this study to term live births, they exclude fetuses that do not survive to term or are born prematurely, but may have been disproportionately affected by early pregnancy exposures to air pollution.
- The authors note that while the effect of maternal smoking in pregnancy is associated with a much larger reduction in birthweight (200g), the attributable risk, i.e., the proportion of LBW in this sample that was attributable to air pollution, was 13%. Wang et al. state this is among the highest attributable risks ever reported for known risk factors of low birthweight.

Williams et al., 2007: Assessing the impact of the local environment on birth outcomes: a case for HLM

Williams et al. conducted a cohort study of the effects of SO_2 on birthweight with the purpose of demonstrating how hierarchical linear models can be used to analyze the relationships among community level environmental data, individual risk factors, and birth outcomes. The authors argue that there is a need to examine how local environmental factors intermingle with individual risk factors to affect birth outcomes, in part because individual level approaches for explaining health outcomes are insufficient. However, using individual risk factors along with variables representing environmental exposures may lead to statistical problems such as bias. Hierarchical linear models, also called multi-level modeling, uses a linear model with variables that account for variations at the individual-level (level 1), and variables that account for larger group or community-level units (level 2) in which individuals are nested. An individual can be nested in only one level 2 unit.

The cohort consisted of 13,559 (95%) of the 14,194 infants born in Tennessee in 2002, whose addresses could be geocoded. Only the firstborn of multiple births was included, and infants were excluded if critical data were missing. The authors do not report whether preterm births were included. Williams et al. selected the air pollutants SO_2, lead, and $PM_{2.5}$ for the study based on the evidence that they can adversely affect birth outcomes.

The level 1 (individual level) variables were:

- infant and birth characteristics (plurality, gender)
- maternal characteristics (previous preterm delivery, previous infant > 4000 g, pregnancy-induced hypertension, chronic hypertension, oligohydramnios, pre-eclampsia, other maternal risk factors, previous pregnancies that did not result in live births, education, smoking, race/ethnicity)
- environmental quality (first trimester average ambient SO_2, lead, and $PM_{2.5}$ concentrations, and number of hazardous waste sites within 5k)

The mean first trimester average SO_2 level was 3.4 ppb, SD 1.1 ppb, and maximum 8.0 ppb. No other information on SO_2 exposure assessment was reported. The average number of hazardous waste sites within 5 km of an infant's residence was 2.26, with a maximum of 13.

The level 2 unit was defined as the residential area within a census tract that shares the same three monitors for lead, SO_2, and $PM_{2.5}$. There were 314 level 2 units in the 132 census tracts. Each level 2 unit had from 1 to 250 infants, with mean 43 and median 31. Level 2 variables were the proportion of residents below the poverty level in the census tract ("poverty") and the interaction between maternal risk factors and poverty. The average proportion of residents of the census tract below the poverty level was 18%, and the range was 0%–79%.

The authors reported on numerous statistical models and results. According to their final model, which included adjustment for level 1 and level 2 variables, a one SD (1.1 ppb) increase in SO_2 in the first trimester was associated with a statistically significant 61 g reduction in birthweight. An increase from zero to the upper 1% of the distribution (5.1 ppb) of SO_2 exposures would be expected to reduce birthweight by 304 g. The highest first trimester average SO_2 level of 8.0 ppb was associated with a 440 g decrease in birthweight. The authors compared the effect of exposure to this level of SO_2 on birthweight to being born to a mother with chronic hypertension or pre-eclampsia. The authors also reported that atmospheric lead exposures during the first trimester were associated with substantial but much smaller decreases in birthweight. Results for $PM_{2.5}$ were not reported. Poverty of the mother's residential census tract also negatively influenced birthweight.

Comments:

- The effect of SO_2 observed in this study is much larger than in any other study.
- Exposure assessment is not reported in detail, e.g., distances between residences and monitors was not addressed.
- If preterm births were included, some of the observed effects on birthweight may be attributable to effects on prematurity.
- Air pollutants such as O_3, NO_2, CO, and PM_{10} were not included in analyses; these unmeasured air pollutants could explain some of the observed effect.

Xu et al., 1995: Acute effects of total suspended particles and sulfur dioxides on preterm delivery: a community-based cohort study.

The acute effects of air pollution on preterm delivery were examined in this prospective cohort study in Beijing, China. The study population included 25,370 resident women who lived in one of four residential areas of Beijing, covering an area of 45 km^2 in 1988; approximately 819 (3.23%) of the deliveries were preterm (for SO_2 analyses, the sample size appears to have been 15,246). The women were required to register in their local maternal health-care center and were given a manual which contained instructions for pre- and post-natal care, and forms for the obstetrician to record information about the mother, pregnancy, and pregnancy outcomes. The analyses included primiparous women at least 20 years of age, whose pregnancy resulted in a singleton live birth, and had complete records.

The authors report that the residential population was stable. Coal stoves were used for heating or cooking in 97% of households and were the dominant source of local air pollution. There were no major industries in the area, and bicycles were the primary mode of transportation. Few homes in the area have air conditioning and windows are

kept open most of the time May to September. Outdoor SO_2 and TSP concentrations were monitored at the WHO Global Environmental Monitoring System sites in two of the four areas in the study. Samples were collected daily and analyzed for two weeks each month, starting in the second week of the month. All study subjects resided within five km of the air monitoring stations. There were seasonal variations in mean temperature, humidity, SO_2 and TSP level, and gestational age, with a crude inverse association between air pollution level and gestational age (figure 4).

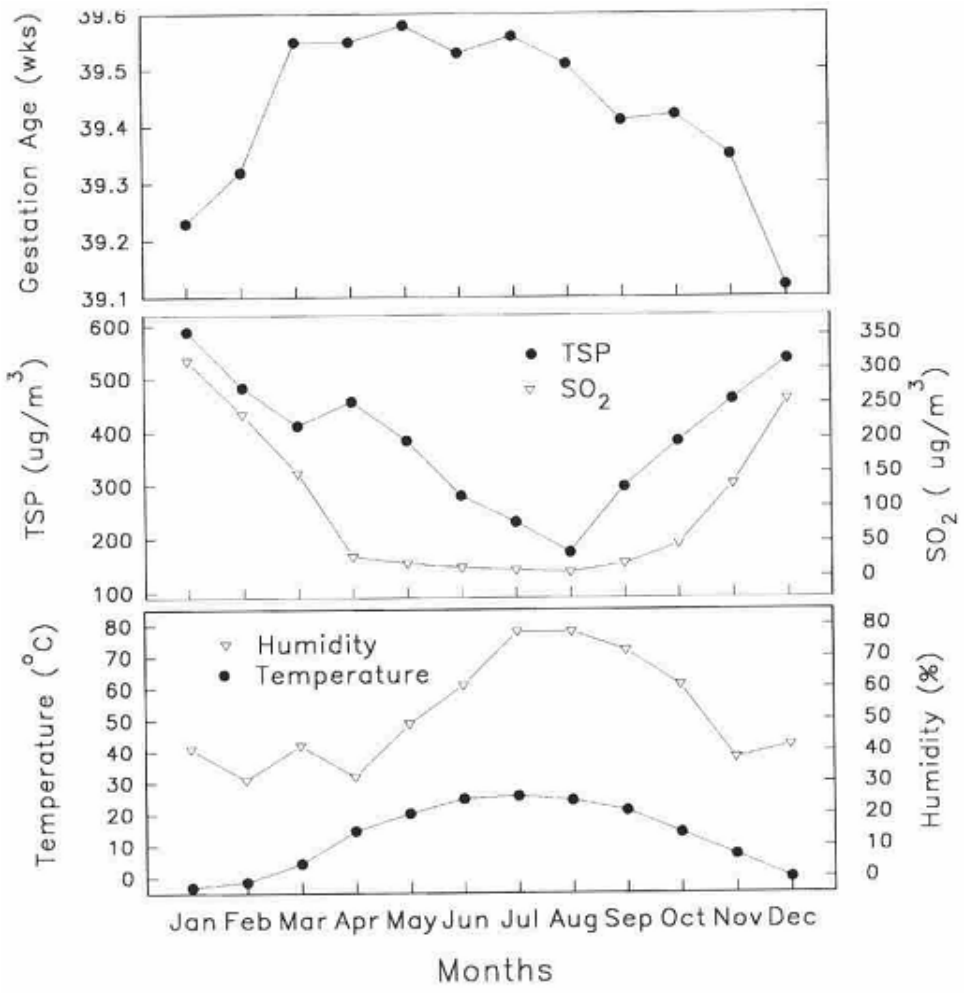

Figure 4. Monthly mean gestational age, TSP and SO_2 concentrations, temperature, and humidity, 1988. (from Xu et al., 1995)

The authors evaluated SO_2 and TSP individually and jointly as predictors of gestational age. Gestational age was evaluated as both continuous and binary variables (i.e., preterm birth, <37 weeks vs. ≥37 weeks gestation) in multiple linear regression and logistic regression models, with adjustment for outdoor temperature, humidity, day of the week, season, maternal age, gender, and residential area. The annual mean concentrations of SO_2 at the two monitoring stations were 41.2 ppb (108 µg/m^3) and 35.5 ppb (93 µg/m^3). Because the daily measurements of SO_2 and TSP from the two

monitors were highly correlated, the authors averaged the data for exposure estimates across the study area.

Xu et al. evaluated concurrent pollution levels, as well as lagged moving averages of air pollution and weather conditions. The effect of SO_2 was greatest for 7- and 8–day lags. For the 7-day lagged moving average, the authors estimated that each 38.1 ppb (100 $\mu g/m^3$) increase in SO_2 reduced the duration of gestation 0.075 week (12.6 hours; $p < 0.01$), adjusted for weather conditions, day of week, season, residential area, gender, and maternal age. However, when SO_2 and TSP were included in the model simultaneously, the estimated effects were reduced by 32% for SO_2 (to 0.051 week, or 8.4 hours) and 36% for TSP, but remained statistically significant. The authors also conducted separate analyses to confirm the nature of the relationships between pollutants and gestational age, and concluded that there is a clear dose-dependent relationship between gestational age and SO_2 (figure 5). They also observed that SO_2 had greater effects on gestational age among younger mothers than older mothers.

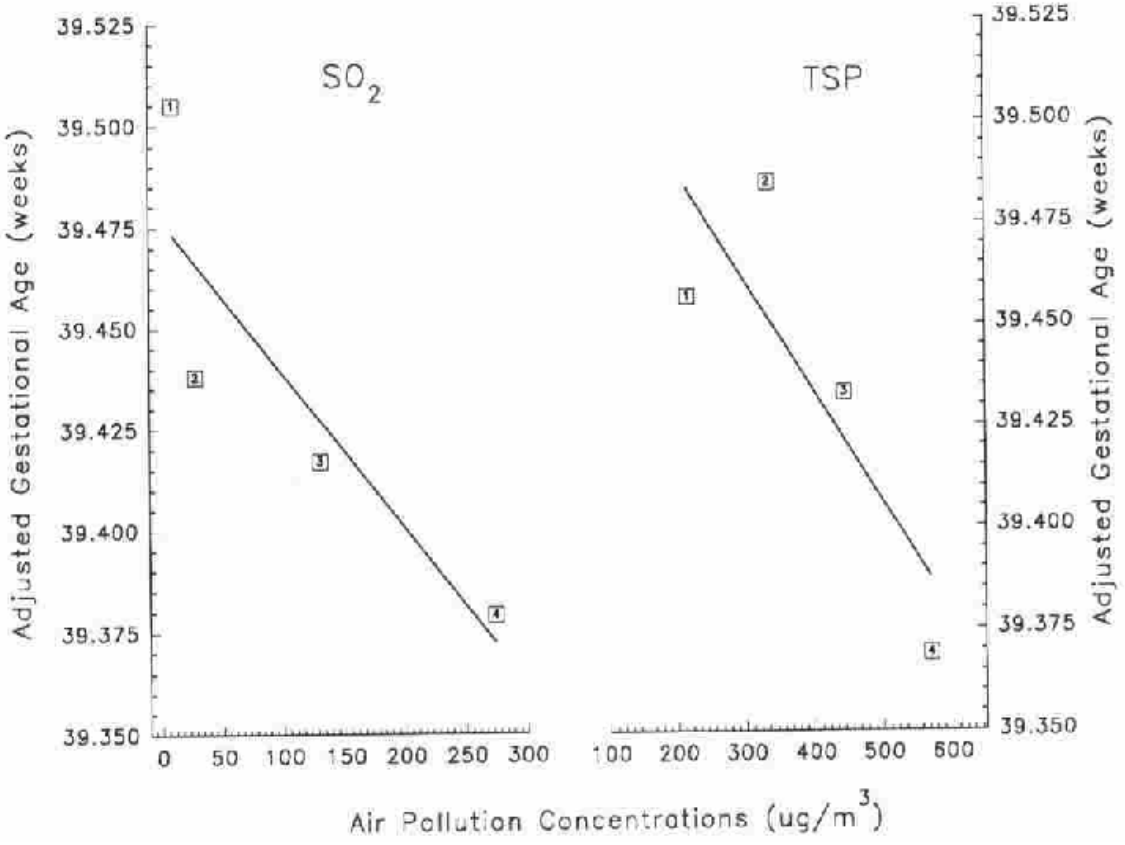

Figure 5. Adjusted gestational age by SO_2 and TSP concentrations (figure from Xu et al., 1996)

The overall rate of preterm births was 3.2%. The adjusted odds ratio for preterm delivery was 1.21 (CI 1.01, 1.46) for each $\log_e(\mu g/m^3)$ increase in SO_2. The authors also reported that the gestational age distribution of high-pollution days was more

skewed toward the left tail (i.e., very preterm and preterm) compared with low-pollution days, suggesting that more babies are born pre-term on high pollution days (figure 6). The results suggest that pregnancies at high risk for preterm delivery may be particularly susceptible to effects of air pollution.

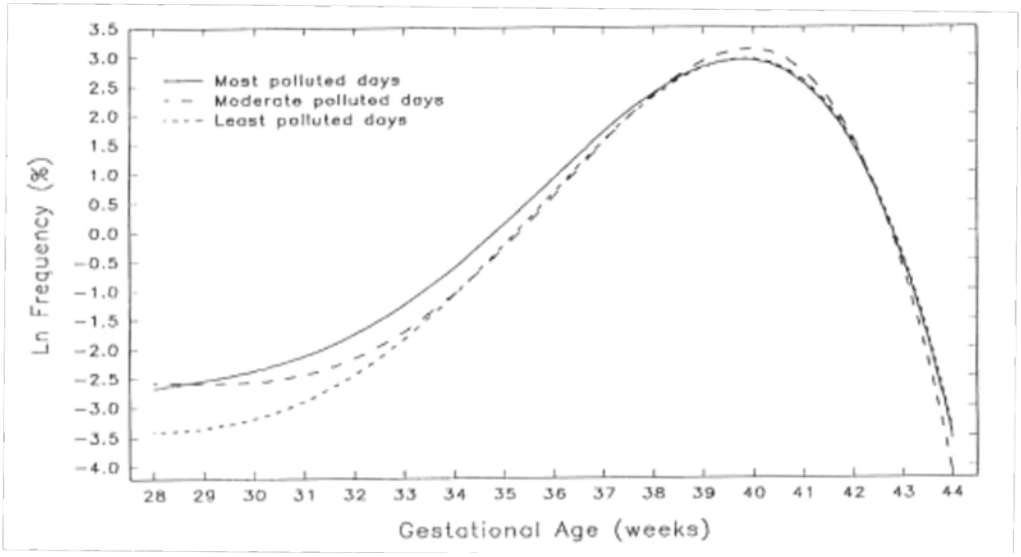

Figure 6. Gestational age distributions, by tertile of SO_2 concentrations (figure from Xu et al., 1995)

Comments

- Xu et al. report a random sample of the households showed that the trend of indoor particulate concentration was similar to that of outdoor, regardless of the presence of coal stoves. They also state that households with coal stoves had higher particulate concentrations.
- The authors note that coal combustion produces CO, CO_2, and volatile organic compounds, in addition to particulates and SO_2; therefore, they cannot rule out the possibility that TSPs and SO_2 are surrogates for a complex mixture of air pollutants.
- The authors appear to have had single daily samples from each monitor and only analyzed the data for 2 weeks each month.
- Exposure window appears to be time of birth (with lagged moving averages for some analyses), but is not clearly reported.
- The authors did not collect data on smoking, ETS, socioeconomic status. However, the authors note the study cohort was homogeneous with respect to sociodemographic characteristics, housing conditions and lifestyle. The prevalence of smoking among Chinese women is extremely low (6.4%), whereas the prevalence of passive smoking is very high (78%). It was also noted that these factors would be expected to be independent of daily air pollution levels and in this study would not affect the association between air pollution and preterm births.
- There was no analysis of effects of early pregnancy exposure to pollutants.

Yadav and Kaushik, 1996: Effect of sulphur dioxide exposure on human chromosomes.

This study by Yadav and Kaushik included 84 individuals. Forty-two were exposed to SO_2 in a fertilizer factory and the other 42 were matched with respect to age, sex, smoking and alcohol consumption. The workers were exposed to an average concentration of 15,906 ppb (41.7 mg/m^3) SO_2. Heparinized venous blood samples were obtained from each subject and short-term lymphocyte cultures were set-up within three hours of sampling. Lymphocytes were harvested after 48 hours for chromosomal aberrations. The Student's t-test was applied to the assessment of chromosomal aberrations (CA) and sister chromatid exchanges (SCE) and the χ^2 test was applied to the assessment of the mitotic index (MI).

Frequencies of both CA and SCE showed a positive correlation with the duration of exposure to SO_2. The frequency of total CA per 100 metaphases was significantly higher in workers (3.262) than the frequency of 0.833 in controls ($p < 0.01$). The mean number of SCE per cell was <4 among controls and 7.27 among workers; and the difference was statistically significant ($p < 0.05$). The mean MI was 4.34 in controls and 7.09 in the exposed workers ($p < 0.05$). MI was the highest (7.83) in the workers for whom the exposure period was up to five years. Thereafter, the MI showed a gradual decline in workers up to 20 years.

Yadav and Kaushik conclude that their results show SO_2 concentrations in the ambient air, even at low levels, causes damage to genetic material at the chromosomal level.

Yang et al., 2003: Effects of air pollution on birth weight among children born between 1995 and 1997 in Kaohsiung, Taiwan.

Yang et al. conducted this retrospective cohort study to explore the association between LBW and maternal exposure to SO_2 and PM_{10} in each trimester of pregnancy. Subjects were 13,396 singleton, full-term (≥37 weeks gestation), live births between January 1, 1995–December 31, 1997 in selected areas around six air quality monitoring stations in Kaohsiung city, Taiwan. Data on pregnancy outcomes were from routine registration of births. The authors excluded babies born at gestation >50 weeks, mothers who resided beyond about 2 km from a monitor, and babies who were not the mother's first child. Approximately 308 (2.3%) of the births were classified as LBW (2,500 g), and the mean birthweight was 3,185 g.

Six AQMS in Kaohsiung maintained by the Taiwan EPA provided measurements of SO_2 and PM_{10}. The authors averaged daily pollutant readings to determine exposure levels during each trimester of each pregnancy based on the birth date and gestational age of each child. Trimester pollutant averages were divided into three tertiles: the 1st tertile (33th percentile) was considered "low exposure" (comparison group), the 2nd tertile (33th to 67th percentile) was considered "moderate exposure", and the 3rd tertile (67th to 100th percentile) was considered "high exposure" (Table 49). Correlations among

trimester SO_2 levels were 0.74 (between first and second trimesters and second and third trimesters) and 0.53 (first and third trimesters). Correlations between SO_2 and PM_{10} were 0.46, 0.45, and 0.45 in the first, second, and third trimesters, respectively.

Table 49. Tertile (percentile) distribution of mean trimester exposures to SO_2 (ppb converted from $\mu g/m^3$).

	1st Trimester		2nd Trimester		3rd Trimester	
Percentile	**33th**	**67th**	**33th**	**67th**	**33th**	**67th**
	9.92	13.76	9.83	13.59	9.68	14.10

The authors analyzed birthweight as a continuous variable in multiple linear regression. Factors considered as potential confounders included: maternal age (≤30, >30 years), season (summer, winter), marital status, maternal education (<12, ≥12 years), gestational age, and gender of baby. The authors also used multiple logistic regression to estimate the risk of LBW associated with maternal exposure to SO_2 and PM_{10}.

High exposures to SO_2 during the first trimester were associated with reduced birthweight. The estimated reduction in birthweight was 18.11 g (1.88, 34.34) when adjusted for all the potential confounders listed above (Table 50). The associations were weaker and non-significant in the second and third trimesters. When SO_2 was a continuous variable, an increase in SO_2 of 0.38 ppb in the first trimester was associated with a statistically significant reduction in the mean birthweight of 0.52 g (0.09, 2.63).

Table 50. Relationship of reduced birthweight to maternal exposure to SO_2 during pregnancy.

Trimester	Exposure	Adjusted RBW (95% CI)[a]
1st Trimester	Low	
	Medium	3.68 (-12.45, 19.21)
	High	18.11 (1.88, 34.34)[c]
	Continuous	0.52 (0.09, 2.63) [c]
2nd Trimester	Low	
	Medium	1.78 (-17.91, 14.35)
	High	13.53 (-2.62, 29.68)
	Continuous	0.19 (-0.78, 1.80)
3rd Trimester	Low	
	Medium	0.43 (-16.56, 15.70)
	High	1.97 (-18.24, 14.30)
	Continuous	0.03 (-1.21,1.37)

[a] Adjusted for gestational age, maternal age, season, marital status, maternal education, and baby gender.
[c] $p<0.05$.

The first trimester results were similar for PM_{10}, with a 0.52 g reduction in birthweight per unit increase in PM_{10}. However, moderate and high exposures to PM_{10} in the third

trimester were also associated with statistically significant reductions in birthweight. The authors conclude that a significant exposure-response relationship between maternal exposures to SO_2 and PM_{10} during the first trimester and birthweight were found.

Comments

- Yang et al. note that they lacked information on maternal nutrition, pre-pregnancy weight and weight gain, cigarette smoking, occupational exposures, and income in the analysis. However, they also note that the prevalence of cigarette smoking among Taiwanese women was 3–4%; so smoking was not likely to be a significant confounder. The authors also state that the factors were unlikely to be associated with pollution levels and therefore probably did not confound the study estimates.
- The authors do not report whether they attempted to account for the correlation among trimester exposures for each pollutant.
- Analyses with both SO_2 and PM_{10} were not reported; this would be particularly interesting for the first trimester exposures, where both pollutants were associated with reductions in birthweight.

Zini et al., 2008: Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis.

In this meta-analysis, Zini et al. systematically review literature on sperm DNA damage and pregnancy loss after IVF and ICSI. The aim of their study was to examine the influence of sperm DNA damage on the risk of spontaneous pregnancy loss after IVF and ICSI.

Zini et al. searched Medline from 1999 to January 2008 and selected studies that evaluated sperm DNA damage in whole or washed semen and spontaneous pregnancy loss in couples undergoing IVF and/or ICSI. Seven eligible reports with 11 studies were identified and assessed. ORs were derived from 11 estimates of pregnancy loss (five IVF and six ICSI studies from seven reports). The 11 studies involved 1,549 cycles of treatment (808 IVF and 741 ICSI cycles) with 640 pregnancies (345 IVF and 295 ICSI) and 122 pregnancy losses. SCSA was used in 6 studies and TUNEL was used in the other 5 studies. In a meta-regression analysis, no significant difference was found in the OR according to treatment type (IVF or ICSI). The combined OR estimates for IVF (five estimates, OR=2.17; CI 1.02, 4.60; P<0.05) and ICSI studies (six estimates, OR=2.73; CI 1.43, 5.20; P< 0.01) were both significant. The summary OR estimates of studies using SCSA (six estimates, OR=1.77; CI 1.01, 3.13; P< 0.05) and TUNEL (five estimates, OR=7.04; CI 2.81, 17.67; P< 0.001) were both significant. However, the meta-regression analysis demonstrated a significant difference in the OR estimates between the TUNEL and the SCSA studies (p<0.012).

The combined OR of 2.48 (CI 1.52, 4.04; p<0.0001) indicates that sperm DNA damage is predictive of pregnancy loss after IVF and ICSI, and is statistically significantly

associated. Meta-regression analysis showed that test accuracy was not affected by treatment type but was related to the type of assay (TUNEL vs. SCSA).

As the authors state, one weakness of this meta-analysis is the highly variable study characteristics: data collection (prospective or retrospective), definition of pregnancy loss (biochemical or clinical), population characteristics (unselected, repeated IVF failures), female inclusion/exclusion criteria, sperm DNA test type and sperm DNA test cutoff. Although the meta-analysis is relatively small, it demonstrates an important relationship between sperm DNA damage and spontaneous pregnancy loss after IVF and IVF/ICSI.

APPENDIX 2: Summaries of Animal Studies on SO_2 and DART Outcomes

Bhattacharjee et al., 1980: Effects on the spermatogonia of mice following treatment with sodium bisulfate.

The effects of sodium bisulfite on differentiating spermatogonia were investigated in 11–13 wk old male Swiss mice (Bhattacharjee et al., 1980). Dosing with sodium bisulfite was performed by i.p. injection as follows:

- controls either untreated or given 0.5 ml distilled water – the same injection volume given to all treated animals
- single i.p. injection
    - 6–8 mice/dose, with 20 mice in the 1000 mg/kg group
    - 500, 600, 700, 800, 900, or 1000 mg/kg
    - animals sacrificed at 3 d post injection (2–4 mice sacrificed/group)
- repeated i.p. injection
    - 6–8 mice/dose
    - 200 or 400 mg/kg
    - dosing repeated 20, 30, or 40 times
    - repeated dosing over 28, 42, or 56 d, respectively
    - over the course of the study, total dose ranged from 4–16 mg/kg
    - animals sacrificed 3 d following final injection (2–4 mice sacrificed/group)

No mortality was observed with either of the repeated doses (200 or 400 mg/kg. No mortality was observed within 24 hours of single doses up to 700 mg/kg. For higher single doses, the frequency of death within 24 hours was as follows:

- 1000 mg/kg – 80%
- 900 mg/kg – 33%
- 800 mg/kg – 17%

Both testes were removed from sacrificed animals, fixed, embedded in paraffin, sectioned at 6µm, and stained. Types of spermatogonia were scored from 99 seminiferous tubules. No effects were found on the numbers of various types of spermatogonia, regardless of the dosing level and repetition.

Dulak et al., 1984: A sulphite oxidase-deficient rat model: reproductive toxicology of sulphite in the female.

Sulfite-induced reproductive toxicity was studied in female Wistar rats. At the start of the experiment, the animals weighed approximately 200–225 g, and were randomly distributed among seven treatment groups. The general design was as follows:

- 16–29 female rats/group
- Four groups designated as controls; normal levels of hepatic sulfite oxidase

- Three groups were made deficient in sulfite oxidase (hepatic enzyme activity levels approximately 1–2% of those in untreated rats)
    - Normal protein, low molybdenum (Mo) diet + high tungsten (W) in drinking water
    - Then, after 21 days of MoW treatment: 0, 25, or 50 mM sulphite (as sodium metabisulfite) in drinking water
- Beginning on experimental day 22, two control groups were pair-watered and pair-fed with one of the sulfite-exposed groups.
    - To control for sulfite-induced decreases in fluid consumption
    - And to control for reduced food consumption resulting from reduced fluid consumption
- All females were mated with untreated males on experimental day 42 (21 days following the start of sulfite treatment)
    - Pairs caged daily until detection of vaginal plug, but for no more than 4 consecutive nights
    - Plug day = day 0 of gestation
    - Treated diets continued during mating and throughout gestation
    - Dams sacrificed on gestation day 21, and uterine contents examined

No effect of sodium sulfite was found on maternal mortality or symptoms of toxicity.

The three sulfite oxidase-deficient groups had similar sulfite oxidase activities, with steady state levels at about 1–2% of controls. Accumulation of endogenously-generated sulfite was consequently measurably higher than in non-deficient controls. Two of the sulfite oxidase-deficient groups also received exogenous sulfite in their drinking water, at concentrations of 25 or 50 mM. Actual sulfite intake for these groups was 2.0 mmol/kg/day or 3.5 mmol/kg/day, respectively.

Sulfite exposure was not found to be associated with any concentration-dependent changes in reproductive performance.

- No effect on mating frequency (hence presumed to be no effect on estrous cycle length)
- No effect on female fertility
- Slight increase in mean number of corpora lutea/dam with 50 mM sulfite (18.1 ± 3.2, compared to 14.9 ± 2.2 for controls), presumed responsible for the significant ($p < 0.05$) increase in pre-implantation loss for this group (20.8%, compared to 6.7% for controls)
- No effect on resorption frequency or live litter size
- No effect on fetal weights or lengths
- No significant treatment-related differences in frequencies of total or specific external, visceral, or skeletal malformations

The authors considered sulfite oxidase-deficient rats to be a better model for humans than normal rats. According to their calculations, normal rats have approximately 10–20x higher sulfite oxidase activity than humans.

When data for the large scale study were combined with data from a pilot study, a small increase in the frequency of anophthalmia seemed to be associated with the high concentration of W (given assodium tungstate) used as part of the treatment to deplete sulfite oxidase. Sulfite did not appear to be a required component for this effect. The authors suggest that under conditions of both high W and sulfite concentration, maternal food and water consumption were severely reduced. Though these data were not provided, compromised maternal nutrition was considered to be a possible factor in development of anophthalmia.

Fiore et al., 1998: Prenatal SO_2 exposure induces changes in the behavior of adult male mice during agonistic encounters.

Adult male and female CD-1 mice were exposed to 0; 5,000; 12,000 or 30,000 ppb SO_2 in air from 9 d before they were caged as breeding pairs (10 pairs per treatment group). Exposure continued throughout the mating period, and up to pregnancy day 14 for females. Exposure is described as "near continuous, covering about 90% of the total exposure time.

Following parturition, litters were culled to eight pups each (four male and four female), and cross-fostered to unexposed, lactating dams. Weaned pups were housed in same litter-same sex pairs, until PND 75–80, after which time each mouse was caged in isolation.

Aggression testing was performed in a neutral cage environment, with a treated male mouse being introduced to an untreated male of the same age and body weight. Encounters were observed and recorded for a single 20-minute session (divided for analysis into four, five-minute segments), and a series of specific behaviors scored for each occurrence and duration. These behaviors consisted of: attacking, freezing, tail "rattling," offensive postures, defensive postures, fleeing, exploration, rearing, wall rearing, body sniffing, digging, and self-grooming.

Results of this analysis found that body sniffing and non-social activities occurred with greater frequency in male mice prenatally exposed to SO_2, while freezing tail rattling, and defensive behaviors decreased in frequency. The frequencies of other behaviors were not affected by treatment.

Table 1. SO_2 concentration and timing of behavioral effects during 20 minute test encounters with untreated, unfamiliar conspecific (n=11–12).

Endpoints	Concentration and time bracket
Self-grooming, duration	Significant decrease ($p < 0.05$) relative to 5,000 ppb group over minutes 15–20 at 0, 12,000; 30,000 ppb
Tail rattling, frequency and duration	Significant decreases ($p < 0.05$) relative to controls over minutes 5–10 at 5,000; 12,000; 30,000 ppb; and over minutes 10–15 at 12,000 ppb
Defensive postures, duration	Significant decrease ($p < 0.05$) relative to controls over minutes 0–5 at 12,000 and 30,000 ppb

When sources of variation were analyzed, some of the changes were found to be stable throughout the testing period, revealing a main effect of SO_2:

- freezing: $F(3, 42) = 2.96^{\#}, 2.89^{\#\#}$, $p < 0.05$ for both values
- body sniffing: $F(3, 42) = 3.07^{\#}$, $p < 0.05$
- rearing, $F(3, 42) = 3.03^{\#}, 2.87^{\#\#}$, $p < 0.05$ for both values

$^{\#}$ = frequency
$^{\#\#}$ = duration

The authors note that changes in responses such as tail rattling, freezing, and defensive postures decreased in a concentration dependent manner. These changes occurred in the absence of significant changes in offensive behaviors. The authors refer to an earlier study from their lab (Petruzzi et al., 1996), in which the same mice showed no alterations in several measures of behavior and learning. Hence, social behavior with other males of the same strain and age appeared to be altered by prenatal SO_2 exposure in the absence of clear effects on general neurobehavioral development.

Hugot and Causeret, 1978: Effect of separated or simultaneous ingestion of tannic acid, potassium metabisulfite and ethanol on reproduction in rats.

In a French language paper with an English abstract, Hugot and Causeret (1978) reported on female reproductive effects in rats fed a diet containing 1% potassium metabisulfite.

Groups of 50, 21-day old female Wistar rats were assigned to either control or treated diets. Diets were available *ad lib*. Thiamine was administered separately to animals on the 1% metabisulfite diet, in order to prevent its destruction by the metabisulfite.

After seven weeks of treatment, females were caged for mating with males of known fertility. Unfortunately, the study is poorly reported and it is not clear if the females were withdrawn from treatment at this point, or whether males were also exposed during the

mating period. Nor is it clear whether or not females were continued on the treated diet throughout gestation and lactation.

The paper indicates that immediately after their live pups were weaned, 30/50 females in each group were sacrificed and their uteri examined for the presence of implantation sites. The experiment was continued with the remaining 20 females/treatment group for another 15 wks, at which point the females were again mated with proven males. It is not clear from the paper at what point(s) potassium metabisulfite exposure had ceased or been resumed.

While the authors note having conducted parametric or nonparametric statistics as appropriate for each endpoint, it is unclear from the text and tables which, if any, endpoints were found to have been significantly altered by treatment. The discussion states that potassium metabisulfite did not affect pup birthweight, but did depress later growth. The linear phase of postnatal growth was said to have been particularly affected, resulting in a mean weight at weaning that was decreased by an average of 7% with treatment relative to controls. Data are presented in Table 2 below.

Table 2. Influence of potassium metabisulfite on reproductive performance

Endpoint	Control	Potassium metabisulfite
n mated	50	50
Maternal weight at mating	217 g	212 g
Maternal weight at end of gestation	332 g	322g
Maternal weight at the end of lactation	237 g	226 g
Fertility index (n pregnant/N mated X 100)	70	62
Gestation index (n live litters/N pregnant X 100)	94	100
Live young at term/litter	11.3	10.7
% male young	49	59
Viability index (N live young on PND 4/n live young at birth X 100)	95	80
Lactation index (N live young at weaning/N live young on PND 4 X 100)	92	97
Mean birthweights	5.5 g	5.5 g
Mean weight gain PND 5–14	20.5 g	18.8 g
Mean weight at weaning	47.2 g	44.2 g
Implants per litter (n=30)	11.4	11.6
Ratio of live births to implants	82	81

Jagiello et al., 1975: SO_2 and its metabolite: effects on mammalian egg chromosomes.

Both *in vivo* and *in vitro* techniques were used to evaluate the capacity of SO_2 to damage meiotic chromosomes in oocytes of the mouse, ewe, and cow. Based on earlier reports in rats and guinea pigs that inhaled SO_2 appeared rapidly in the bloodstream as the potassium or sodium salt, sodium sulfite (Na_2SO_3) was used in these studies.

Harvested mouse oocytes were pooled and placed immediately into the culture system with sodium sulfite added at concentrations ranging from 10 to 10,000 µg/cc, plus an untreated control. Mouse oocytes were then used in three different protocols:

- Five hour harvest for oocytes at first metaphase (M_1)
- 15 hour harvest for oocytes at second metaphase (M_2)
- 14 hours *in vitro* culture with added sodium sulfite and/or pyrimidine bases, to test effect on meiotic progression

Results of these experiments included the following findings for oocytes at the five hour harvest:

- At sodium sulfite concentrations above 10 µg/cc (excepting 100 µg/cc), inhibition of entry into meiosis to the stage of M_1 ($p<0.05$)
- 25, 50, and 100 µg/cc sodium sulfite, initial manifestations of nuclear damage
- 350–500 µg/cc, more severe damage described as “overt atresia”
- 750 µg/cc and above, no oocytes resumed meiosis

At the later harvest of 15 hours:

- A higher threshold of 150 µg/cc sodium sulfite for inhibition of total division rate percent ($p<0.05$)
- Inhibition of entry into M^1 beginning at 100 µg/cc
- At 150 µg/cc, only 50% of cells ultimately reached M_2, as opposed to 83% of control oocytes
- 250–500 µg/cc, atresia of both M_1 and M_2 stages
- 1000 µg/cc, total inhibition of meiosis

The results for oocytes cultured with sodium sulfite and/or pyramidine bases included:

- Addition of pyrimidine bases did not reverse the effects of sodium sulfite on meiotic progression
- Thymine alone, at 1261 µg/cc (10^{-2} M), inhibited division

Ewe and cow ovaries were obtained from a local slaughterhouse, and oocytes harvested under sterile conditions. Sodium sulfite was added to culture media at

concentrations ranging from 50 to 1250 µg/cc. For both species, M_1 and M_2 oocytes were collected after 28 hrs of culture. Results were as follows:

- Ewe
  - No effects of sodium sulfite on meiotic division
  - Atresia and chromosome breaks at 250 µg/cc, more severe effects at higher concentrations
  - Anaphase lagging at 350 and 1250 µg/cc
- Cow
  - Sensitive to meiotic inhibition by sodium sulfite at ≥500 µg/cc ($p<0.05$)
  - Damage to oocyte nuclei progression at ≥250 µg/cc
  - One anaphase lag at 350 µg/cc

Additional experiments involved *in vivo* treatment of female Camm mice with subsequent evaluation of oocytes:

- To detect immediate or delayed effects on oocytes:
  - Groups of six female mice were given sodium sulfite, i.v.
  - Doses of 0, 1.0, 2.5, or 5.0 mg/mouse
  - Oocytes harvested at 96 hrs post-treatment, the duration of one vaginal estrous cycle
- To detect abnormalities presented during one estrous cycle:
  - Groups of six female mice were given sodium sulfite, i.v.
  - 0 or 5.0 mg/mouse
  - Oocytes harvested at 24, 48, 72, or 96 hrs post-treatment
- To detect abnormalities induced during the pre-ovulatory period:
  - Groups of six female mice were given:
  - 10 IU pregnant mare's serum gonadotropin (PMSG) i.p.
  - 5.5 hours later they were given sodium sulfite, i.v., at doses of 0 or 5.0 mg/mouse
  - 24 hrs following sodium sulfite (or 53 hrs following PMSG), animals were given 5 IU human chorionic gonadotropin (hCG), i.p.
  - Oocytes harvested 14 hours following hCG treatment

These experiments did not reveal any effects of i.v. sodium sulfite on subsequently cultured oocytes.

Mamatsashvili, 1970: On the detrimental effect of carbon monoxide and sulfur dioxide on fertility of female rats.

Groups of ten albino rats were exposed for 72 days to one of the following:

- CO, 1 mg/m^3
- CO, 2 mg/m^3
- SO_2, 0.15 mg/m^3 (~57 ppb)

- SO_2, 4 mg/m^3 (~1,500 ppb)
- CO, 2 mg/m^3 and SO_2, 4 mg/m^3

There is no mention of unexposed or sham-exposed controls, but the results section of the paper does mention comparisons to controls. As SO_2 is the subject of this document, only data for that compound will be described and discussed.

Daily vaginal smears were taken to evaluate stages of the estrous cycle. Treated females were mated with unexposed males. It is not clear from the text of the paper whether treatment was completed prior to mating, or continued during mating and/or gestation. The following endpoints were evaluated:

- time to pregnancy
- duration of pregnancy
- number of young per litter
- pup weights and pup weight gain

Estrous cycles of adult F_1 female offspring were also evaluated by daily vaginal smears. F_1 females were also mated with untreated, control males.

Estrous cycles of parental females showed no effect of 0.15 mg/m^3 (~57 ppb) SO_2. The higher concentration of SO_2 (4 mg/m^3 (~1,500 ppb)) and the mixture of CO and SO (again, 4 mg/m^3), however, did result in alterations to the estrous cycle. During the first month of treatment, these changes from controls were manifested as:

- significant decreases in the diestrus and metestrus stages (no statistics provided)
- “some increase in the proestrus stage”
- “marked increase in the estrus stage”

During the second and third months of treatment, estrous cycle length increased in all treated groups, excepting for the group exposed to 0.15 mg/m^3 SO_2 (~57 ppb). No data are provided in the paper, but the authors conclude that, “the severity of disturbances was directly related to the concentration and duration of the poisoning.”

Similar and significant changes in the estrous cycle were also seen in the F_1 female offspring of animals exposed to either 4 mg/m^3 (~1,500 ppb) or the mixture of CO and SO_2. No data or statistics are presented in the paper.

Exposure to 0.15 mg/m^3 (~57 ppb) SO_2 was not shown to affect pregnancy frequency, pregnancy duration, or offspring growth as measured by body weight. Significant changes in these endpoints were observed in the 4 mg/m^3 (~1,500 ppb) SO_2 group, and were even more pronounced in the group receiving a mixture of SO_2 and CO. Litter size was considered to have been increased in all treated groups. No data or statistical analysis are presented in the paper, but the authors suggest that both SO_2 and CO may have had “a stimulating effect on the endocrine system.”

Histopathological changes are described only for the female rats given a combination of CO and SO_2. The paper is not clear as to whether histopathological evaluations were made of animals exposed to SO_2 alone at a concentration of 4 mg/m^3. Normal histopathology is specifically reported for the 0.15 mg/m^3 (~57 ppb) SO_2 group.

Meng, 2003: Oxidative damage of sulfur dioxide on various organs of mice: sulfur dioxide is a systemic oxidative damage agent.

The potential of SO_2 to increase levels of lipid peroxidation and alter intracellular redox status was studied in multiple organs of Kunming albino mice, including testis. Five-wk old mice, weighing 19 $\pm$ 2 g, were divided into treatment groups as follows:

- Sham treated controls, exposed to filtered air 6 h/d for 7 d
    - 10 males
    - 10 females
- 20,000 ppb SO_2, 6 h/d for 7 d
    - 10 males
    - 10 females

At the end of the experimental period, the mice were deprived of food for 18 hrs prior to sacrifice by cervical dislocation. The following organs were removed, weighed and examined for obvious pathology before processing for biochemical assays: brain, lung, heart, liver, stomach, intestine, spleen, kidney, and testis.

While all animals gained weight over the experimental period, mean body weight gain did not differ among groups. As female reproductive organs were not evaluated, only the data for males will be discussed here.

Enzyme levels and activities measured, along with overall results, were:

- Cu, Zn-superoxide dismutase (Cu,Zn-SOD) activity
    - Significantly reduced in all organs tested
- Glutathione peroxidase (GSH-Px) activity
    - Significantly reduced in all organs tested
- Catalase (CAT) activity
    - Significantly reduced only in liver
- Reduced glutathione (GSH) levels
    - Significantly reduced in all organs tested
- (GSH/CSSG) ratio
    - No significant differences between treated and control groups
- Lipid peroxidation (TBARS) levels
    - Significantly increased in all organs tested

Specific data for enzyme levels and activities in testes are presented in Table 3.

Table 3. Effects of SO_2 inhalation on testis in male mice

Parameter	Control	SO_2
Cu,Zn-SOD activity (U/mg protein)	1488.92 ± 133.49	1345.56 ± 137.40*
GSH-Px activity (U/mg protein)	4.23 ± 0.93	3.27 ± 0.62*
CAT activity (U/mg protein)	0.51 ± 0.07	0.60 ± 0.16
GSH levels (nmol/mg protein)	53.86 ± 6.36	40.96 + 6.34**
GSH/CSSG	0.67 ± 0.21	0.57 ± 0.19
TBARS levels (nmol/mg protein	0.214 ± 0.055	0.296 ± 0.075**

*p < 0.05; **p < 0.01

Based on these results, the author concluded that SO_2 causes systemic oxidative damage in all tissues tested. Exposure resulted in significant increases in the lipid peroxidation process in all organs tested of mice of both sexes (though only the male data have been described in this review document). This increase was accompanied by concurrent changes in antioxidant status. Therefore, the author suggests that the oxidative damage produced by inhalation of SO_2 may contribute to toxicological damage to many, if not all organs, and not only to the respiratory system.

Meng and Bai, 2004: Oxidation damage of sulfur dioxide on testicles of mice.

Five wk old albino male mice of the "Kunming" strain were randomly divided into six groups of ten each. Their average body weight at the time the study commenced was 19 ± 2 g. Three of the six groups were exposed to SO_2 at one of the following concentrations:

- 22 ± 2 mg/m^3 (~8,400 ± 760 ppb)
- 56 ± 3 mg/m^3 (~21,00 ± 1,100 ppb)
- 112 ± 8 mg/m^3 (~4,300 ± 3,100 ppb)

Exposure was conducted for 6 hrs/d, over a 7-d treatment period. The other three groups served as controls exposed to filtered air on the same schedule. At the end of the exposure period, the animals were deprived of food for 18 hrs, and then sacrificed for biochemical assays of testicular tissues.

Assays were performed for testicular:

- Levels of thiobarbituric acid reactive substances (TBARS)
- Levels of reduced glutathione (GSH)
- Activities of Cu, Zn-superoxide dismutase (SOD)
- Activities of glutathione peroxidase (GPx)
- Activities of catalase (CAT)

No deaths, morbidity, or clinical symptoms of toxicity were reported for any group. Mean body weight gain was not affected by treatment. Table 4 below shows levels and activities for parameters of testicular biochemistry.

Table 4. Effect of SO_2 on testicular biochemistry in mice

Endpoint	control 22 ± 2 mg/m^3 (~8,400 ± 760 ppb)	control 56 ± 3 mg/m^3 (~21,00 ± 1,100 ppb)	control 112 ± 8 mg/m^3 (~4,300 ± 3,100 ppb)
TBARS	0.170 ± 0.036 ---------- 0.223 ± 0.028*** (+31.18%)	0.214 ± 0.055 ---------- 0.296 ± 0.075*** (+38.32%)	0.192 ± 0.045 ---------- 0.297 ± 0.068*** (+54.69%)
GSH	58.15 ± 10.59 ---------- 56.03 ± 15.73 (-3.65%)	53.86 ± 6.36 ---------- 40.96 ± 16.34* (-23.95%)	54.93 ± 11.78 ---------- 47.36 ± 5.36* (-13.78%)
SOD	979.4 ± 102.3 ---------- 863.8 ± 95.4 (-11.80%)	1515.2 ± 146.9 ---------- 1197.4 ± 220.5 (-20.97%)	2437.2 ± 785.3 ---------- 1703.9 ± 227.2* (-30.09%)
GPx	4.30 ± 0.88 ---------- 4.00 ± 0.91 (-6.98%)	4.23 ± 0.93 ---------- 3.27 ± 0.62* (-22.70%)	4.11 ± 0.52 ---------- 3.29 ± 0.46** (-19.95%)
CAT	0.895 ± 0.217 ---------- 1.140 ± 0.453* (+27.37%)	0.510 ± 0.067 ---------- 0.700 ± 0.164* (+37.25%)	0.726 ± 0.064 ---------- 0.829 ± 0.116 (+14.19%)

Data expressed as mean ± standard deviation (n=10)
Levels of TBARS and GSH are expressed as nM/mg of tissue proteins
Activities of SOD, GPx and CAT are expressed as defined units (U) of activity/mg of tissue proteins
Changed percentages of enzyme activities are expressed in parentheses
Significant differences from controls by t-test at: *p<0.05, **p<0.01, ***p<0.001

Levels of TBARS were significantly increased over controls at all three concentrations of SO_2 ($p<0.001$ in each case). The increases appeared to be concentration-dependent. GSH levels were significantly decreased at the two higher SO_2 concentrations of ($p<0.05$ for both 56 ± 3 mg/m^3 and 112 ± 8 mg/m^3).

A concentration-dependent decrease in SOD activity reached statistical significance ($p<0.05$) at the highest concentration of SO_2 (112 ± 8 mg/m^3). GPx activity showed statistically significant decreases at the two higher test concentrations of SO_2 ($p<0.05$ for 56 ± 3 mg/m^3 and $p<0.01$ for 112 ± 8 mg/m^3, respectively). CAT activity, on the other hand, was significantly increased at the two lower concentrations of SO_2 ($p<0.05$

for both 22 ± 2 mg/m^3 and 56 ± 3 mg/m^3), but not at the highest concentration of 112 ± 8 mg/m^3.

Overall, the study results were taken to demonstrate a SO_2-induced increase in lipid peroxidation in mouse testicles, with accompanying changes in testicular SOD and GPx activities, as well as GSH levels. TBARS, in particular, was found to be significantly increased at all tested concentrations of SO_2, which was interpreted as indicative of endogenous lipid peroxidation. The authors concluded that exposure to SO_2 caused oxidative damage to the testicles of male mice. By extension, they further concluded that SO_2 is toxic to the mammalian male reproductive system.

Meng and Liu, 2007: Cell morphological ultrastructural changes in various organs from mice exposed by inhalation to sulfur dioxide.

Meng and Liu (2007) studied the effects of inhaled SO_2 on the ultrastructure of organs from male mice. Eighteen male, albino mice of the Kunming strain were obtained at a body weight of 18–22 g. The animals were randomly divided into three groups of six animals each, and assigned to one of the following treatments.

- Filtered air control
- 28.00 ± 1.98 mg SO_2/m^3 (reported by authors to be 10 ± 0.71 ppm; calculated by OEHHA to be ~11,000 ± 760 ppb)
- 56.00 ± 3.11 mg SO_2/m^3 (reported by authors to be 20 ± 1.11 ppm; calculated by OEHHA to be ~21,000 ± 1,200 ppb)

Animals were exposed to their respective treatment regimens for 4 hrs/d for 7 d. Food and water were not available during the treatment periods, but were provided *ad lib* at all other times.

Food consumption and body weights were monitored during the study. These data were not presented but, according to the text, body weight gain did not differ among groups. Within each group, body weights significantly increased over the course of the study.

The animals were sacrificed 18 hours following the final exposure session. Brain, lungs, liver, spleen, testes, heart, and kidneys were all removed and prepped for ultrastructure study.

As this Hazard Identification Document (HID) pertains to developmental and reproductive toxicity, the discussion here will concentrate on descriptions of testicular ultrastructure.

Compared to observations made on sections of testis from control animals:

- 28.00 mg/m^3 SO_2 was associated with:

    - altered thickness and density of basement membrane
    - marked folds and fault zones of basement membrane
    - increased breakage of mitochondria in Sertoli cells
    - lysosomes and big vacuolations in cell cytoplasm
    - dilation of cistemae of rough endoplasmic reticulum (RER)
    - occasional lacunae between cells
    - unusual amounts of residual cytoplasm in spermatids, as well as hyperchromatic changes in nuclei
    - in late spermatids:
        - acrosomal granule had disappeared
        - shortened acrosomes
        - vacuolated mitochondria

- 56.00 mg/m^3 SO_2 was associated with:
    - more severe expression of above pathologies
    - parts of basement membranes ruptured
    - increased numbers of damaged spermatids
    - spermatid abnormalities

Overall, the authors concluded that their results demonstrated adverse effects of inhaled sulfur dioxide on ultrastructural morphology of multiple organs in mice. Thus, the toxic effects of inhaled sulfur dioxide were not limited to the respiratory systems.

Murray et al., 1979: Embryotoxicity of inhaled sulfur dioxide and carbon monoxide in mice and rabbits; and Murray et al., 1977: Teratogenic potential of sulfur dioxide and carbon monoxide in mice and rabbits.

Murray et al. report on the potential of SO_2 to cause developmental toxicity in CF-1 mice and New Zealand rabbits. GD 0 was the day a semen plug was observed in mice, or the day of mating in rabbits. Exposure conditions were:

- mice
    - 7 hr/d
    - GD 6–15
    - treatments:
        - filtered room air (control, n=40)
        - 25,000 ppb SO_2 (n=32)
        - 25,000 ppb SO_2 plus 250 ppm CO (n=35)
    - sacrifice on GD 18
- rabbits
    - 7 hr/d
    - GD 6–18
    - treatments:
        - filtered room air (control, n=20)
        - 70,000 ppb SO_2 (n=20)

        - 70,000 ppb SO_2 plus 250 ppm carbon monoxide (CO; n=20)
    - sacrifice on GD 29

The animals were deprived of food and water during the exposure periods. Data collected included:

- maternal variables:
    - daily observations for clinical symptoms of toxicity
    - body weights
        - mice: GD 6, 8, 10, 16, and day of c-section (GD 18)
        - rabbits: GD 6, 9, 12, 15, 19, and day of c-section (GD 29)
    - food and water consumption recorded at 2–3 d intervals
- litter variables:
    - number and position of live, dead, and resorbed fetuses
    - uterine staining for identification of early resorption sites
- fetal variables:
    - weight, sex, crown-rump length (CRL)
    - external malformations and cleft palate
    - internal malformations (1/3 of fetuses from each litter)
    - skeletal malformations and variations

Blood carboxyhemoglobin (COHb) was determined in blood drawn from additional animals given the same treatments. Three rabbits per group and six mice per group were bled immediately following exposure on the first and third days.

Treatment did not result in clinical signs of toxicity, or in changes in body weight or liver weight in either mice or rabbits. Food consumption was reduced over the first few days of exposure to either concentration of SO_2 or SO_2 combined with CO. This effect was described as statistically significant in both species, but no data are presented. Necropsies revealed no effects of any of the treatments in either species on lung tissues or other structures of the respiratory tract.

Treatment affected mean COHb levels in both species as follows:

- 25,000 ppb (mice) or 70,000 ppb (rabbits) SO_2: 1–2% on the 1st and 3rd days of exposure
- 25,000 ppb (mice) or 70,000 ppb (rabbits) SO_2 plus 250 ppm CO: 15–17% on the 1st and 3rd days of exposure

Litter data are presented in Table 5. Implantation and resorption frequencies, mean litter size, and mean fetal CRLs were not affected by SO_2 (without CO) exposure in either species. Mean fetal weights were significantly decreased ($p < 0.05$) in mice prenatally exposed to SO_2, whether or not CO was also present. Fetal weights of rabbits were not affected.

Table 5. Litter variables in mice and rabbits treated with SO_2 or SO_2 plus CO.

	Mice, control	**Mice, SO_2**	**Mice, SO_2 + CO**	**Rabbit, control**	**Rabbit, SO_2**	**Rabbit, SO_2 + CO**
n litters	26	21	22	17	17	16
Implantation sites/litter	11 ± 2	12 ± 3	12 ± 2	8 ± 2	8 ± 3	10 ± 1
Fetuses/litter	10 ± 2	10 ± 4	12 ± 2	7 ± 2	7 ± 2	8 ± 2
Resorptions/litter	1.5 ± 1.6	1.8 ± 1.3	0.8 ±0.8	0.4 ± 0.8	0.7± 1.2	1.4 ± 1.7*
Fetal weights, g	1.05 ± 0.11	1.00 ± 0.08*	0.92 ± 0.10*	38.0 ± 5.0	38.6 ± 5.3	35.7 ± 4.6
Fetal CRL, mm	24.5 ± 0.9	24.2 ± 0.9	23.4 ± 1.0*	96.2 ± 5.2	96.4 ± 4.8	92.7 ± 4.9

*Significant difference ($p < 0.05$) from untreated controls

Data on malformations in mice prenatally exposed to SO_2, or a combination of SO_2 and CO, are presented in Table 6 below. No specific type of malformation was found to be increased by treatment over control levels. Aggregate malformations were slightly, but not significantly ($p = 0.14$), increased with exposure to SO_2 plus CO, but not SO_2 alone.

Table 6. Malformations in Mice Following Prenatal Exposure to SO_2 or SO_2 + CO

	Control	**SO_2**	**SO_2 + CO**
n fetuses (litters)	257 (26)	211 (20)	256 (22)
Cleft palate	1 (1)	4 (1)^	4 (2)
Exencephaly & ablepharia	0 (0)	1 (1)	1 (1)
Omphalocele	0 (0)	0 (0)	2 (2)
Inward rotation of hind limbs	1 (1)	1 (1)^	0 (0)
Multiple defects	0 (0)	2 (1)^	0 (0)
Anterior displacement of ovaries	0 (0)	1 (1)	1 (1)
Fused ribs	1 (1)	0 (0)	0 (0)
Total malformed	**3 (3)**	**6 (3)**	**8 (6)***

^ includes fetuses with multiple malformations
* $p = 0.14$

According to the text of the paper, “delayed ossification of the sternebrae and of the occipital bone, was seen more often among the litters of mice exposed to SO_2 alone or

in combination…." These data, however, were not presented, and therefore do not appear in Table 6.

In rabbit fetuses, no evidence was found for significant effects of prenatal SO_2 exposure on the frequencies of major malformations (Table 7 below). According to the text of the paper, the frequencies of several minor skeletal variations were significantly increased among litters of rabbits exposed to either SO_2 or SO_2 plus CO, but these data are not presented.

Table 7. Malformations in Rabbits Following Prenatal Exposure to SO_2 or SO_2 + CO

	Control	**SO_2**	**SO_2 + CO**
n fetuses (litters)	126 (17)	126 (17)	135 (15)
Dilated cerebral ventricles	2 (1)	0 (0)	0 (0)
Heart: focal thinning of left ventricular wall	1 (1)	0 (0)	0 (0)
Hemivertebrae and asymmetric ribs	0 (0)	1 (1)	0 (0)
Total malformed	**3 (2)**	**1 (1)**	**0 (0)**

Overall, an effect of SO_2 on fetal weights was observed in mice, but not in rabbits. As only one concentration of SO_2 was used, the potential for a concentration-response relationship cannot be evaluated.

Petruzzi et al., 1996: Behavioural disturbances in adult CD-1 mice and absence of effects on their offspring upon SO_2 exposure.

Adult male and female CD-1 mice were exposed to SO_2 in air under the same protocol as described above for another paper from this laboratory (Fiore et al., 1998). Exposure is reported to have been "near-continuous, covering about 80% of the total time indicated." Ten breeding pairs were caged on the morning of exposure day (ED) 9, with the presence of a vaginal plug taken to indicate gestation day (GD) 0. Males were removed on GD 12. Once litters were delivered, they were all culled to four males and four females each, then fostered to untreated dams.

Eight litters from each concentration group were used for assessment of somatic and neurobehavioral development on PND 2–18. Half the pups of each sex were weighed and measured on each of days PND 2–10, and on alternate days from PND 10–16 or 18. Physical and behavioral developmental landmarks were assessed, and the day of acquisition recorded. Specific endpoints evaluated were:

- day of eyelid and ear opening

- day of incisor eruption
- righting reflex
- cliff aversion
- fore- and hind-limb stick grasp reflexes
- fore- and hind-limb placing reflexes
- vertical screen grasping response (in reaction to gentle tail pull)
- weak tactile stimulation response (turning the head in response to a touch on the cheek)

Passive avoidance learning and retention were tested in a footshock apparatus. Male pups only were used for these experiments, which were performed on PND 60. Of two males selected from each litter, one was assigned to the footshock group, the other to unshocked controls. All animals were subjected to a retention trial without footshock.

As evaluated in these experiments, prenatal exposure to SO_2 had no effect on postnatal growth, or physical or behavioral development. The passive avoidance experiments provided evidence for an effect of footshock in accelerating learning of the passive avoidance task. Prenatal exposure to SO_2, however, had no effect on these results.

Because of the lack of evidence for any effect of SO_2 on the physical or behavioral development of prenatally-exposed offspring, the authors chose not to present those data in the paper.

Singh, 1989: Neonatal development altered by maternal sulfur dioxide exposure.

Singh examined the behavior of neonatal mice subsequent to prenatal exposure to SO_2. Timed-pregnant CD-1 mice were exposed to SO_2 as follows:

- GD 7–18 (plug day = day 1)
- SO_2 concentrations of
    - 0 ppb
    - 32,000 ppb
    - 65,000 ppb
- continuous exposure with airflow set at 500 ml/min
- food and water available ad lib

The animals were removed from the exposure chambers on GD 18, and allowed to deliver normally. Litter sizes and pup weights were recorded on the day of birth. Behavioral endpoints were evaluated on 14 litters per group as follows:

- righting reflex, PND 1
- negative geotaxis, PND 10
- aerial righting reflex, PND 12

No maternal toxicity was reported, but the paper does not specify what observations/measurements may have been taken on maternal animals. Mean litter size was not significantly altered at either concentration of SO_2.

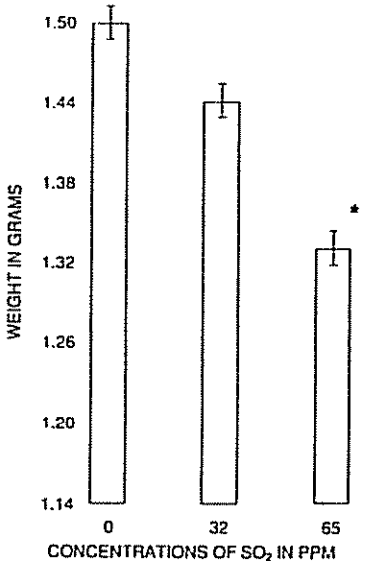

Figure 1. Birthweights, g ± SE
(Singh, 1989)
*p < 0.05

Birthweights were reduced in a concentration-dependent manner, which reached statistical significance (p < 0.05) at the higher SO_2 concentration of 65,000 ppb (Fig. 1).

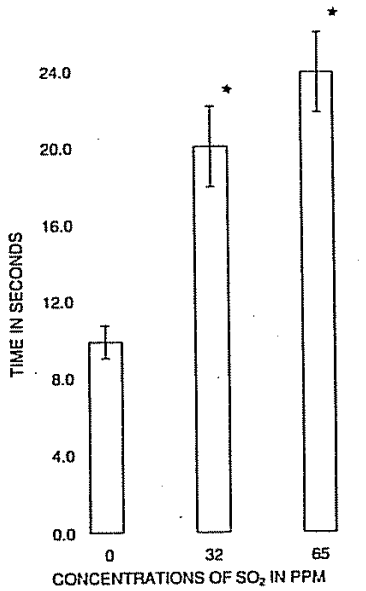

Figure 2. Mean time ± SE for righting reflex on PND 1
(Singh, 1989)
*p < 0.005

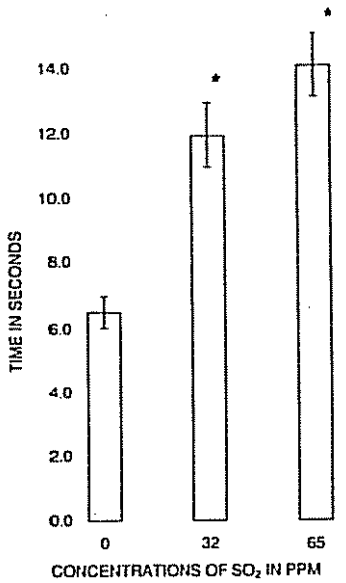

Figure 3. Mean time ± SE for negative geotaxis on PND 10
(Singh, 1989)
*p < 0.005

Statistically significant (p < 0.005) and concentration-dependent increases were found in the times pups required for the righting reflex on PND 1, and for negative geotaxis on PND 10 (Figs. 2 and 3). Aerial righting scores were lower for both groups of SO_2-exposed mouse pups on PND 12, but the apparent effect did not reach statistical significance (Fig. 4).

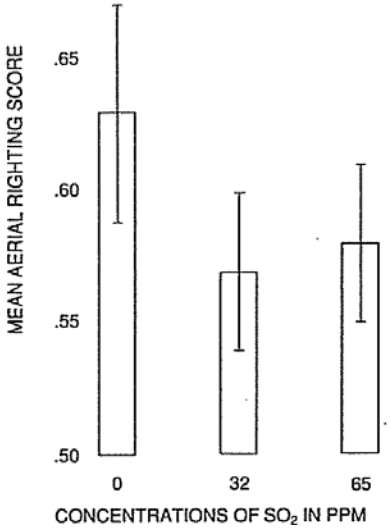

Figure 4. Mean time ± SE for aerial righting on PND 12
(Singh, 1989)

The author concluded that moderate, chronic prenatal exposure to SO_2 had adverse effects on birthweight and reflex development in CD-1 mice. The finding was taken as possible evidence for altered neuromuscular coordination of the developing offspring resulting from a toxic prenatal exposure.

Til et al., 1972: The toxicity of sulphite. I. Long-term feeding and multi-generation studies in rats.

Sodium metabisulfite was added to the diets of Wistar-derived rats for periods of up to two years and over three generations. Concentrations ranged from 0–2%. To avoid confounding by thiamine deficiency due to the known destructive effects of sulfite on thiamine, the diet was enriched with 50 ppm thiamine. The study protocol was as follows:

- Six groups of 20 ♀ and 20 ♂ newly-weaned rats
- Diets contained 0, 0.125, 0.25, 0.5, 1.0, or 2.0 % sodium metabisulfite
- All F_0 rats were mated within their diet group at about wk 21 (to produce a F_{1a} generation)
- Half of the F_0 rats were also mated again within their diet group at wk 34 (to produce a F_{1b} generation)
- 10 ♀s and 10 ♂s from each F_{1a} group were maintained on their diets for 104 wks total, and mated within their groups at wks 12 and 30 (to produce F_{2a} and F_{2b} generations)
- 15 ♀s and 10 ♂s from each F_{2a} group were maintained on their diets for about 30 wks total, and mated at wks 14 and 22 (to produce F_{3a} and F_{3b} generations)

Endpoints evaluated included:

- Changes in body weight
- Food consumption
- Numbers of pups/litter at birth
- Litter weights on PND 1, 8, and 21 (litters culled to no more than 8 pups on PND 1)
- Hematological parameters
- Occult blood in feces
- Serum biochemistry
- Kidney function
- Sacrifice for necropsy: organ weights and pathological observations (including reproductive organs)
    - Interim sacrifice of 5 ♀ and 5 ♂ from each F_0 group at 52 wks
    - Final sacrifice at 104 wks for surviving F_0 and F_1 animals
    - Sacrifice at 30 wks for F_2 animals

Additional experiments were carried out with higher sodium metabisulfite concentrations on fewer rats for a shorter time. As these experiments did not involve reproduction or report on reproductive organs, they will not be discussed further.

The overall condition of rats in all groups, for all generations studied, was considered to be good for the first 72 wks of observations. After that time, symptoms of aging became evident in many rats, and mortality increased. Treated groups generally showed higher survival rates than controls.

Growth rates for the three generations of rats studied are presented in Figures 5, 6, and 7 below. While there appears to have been marginal reductions in weight gain for F_1 and F_2 rats exposed to 2% sodium metabisulfite in the diet, statistical significance was not mentioned.

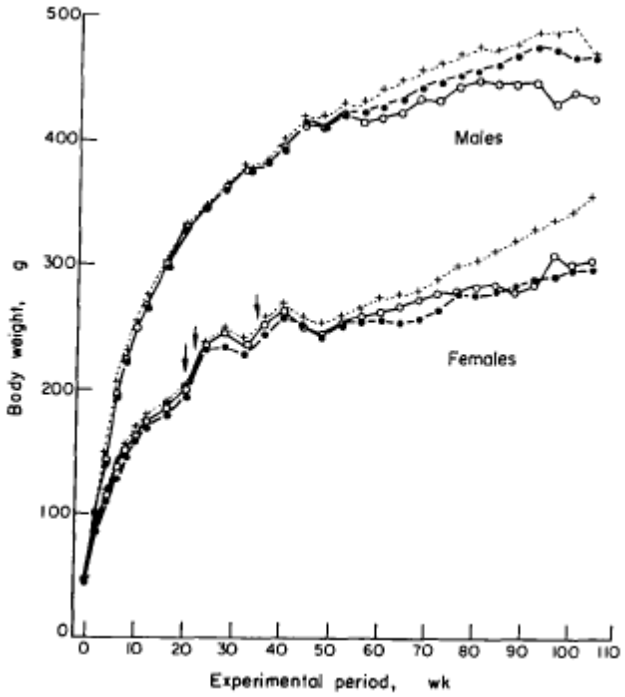

Figure 5. Growth curves of F_0 female and male rats fed sodium metabisulfite in their diets at 0, (○), 1 (+), or 2% (●) for 2 years. Arrows indicate the time of mating. (Til et al., 1972)

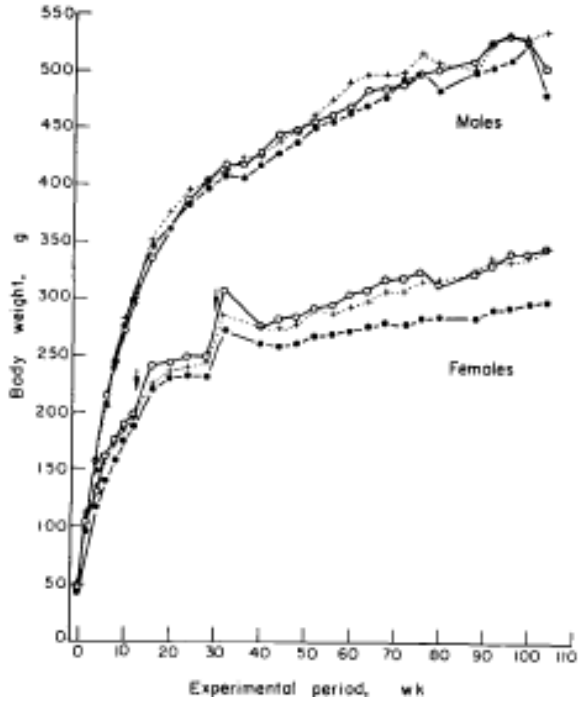

Figure 6. Growth curves of F_1 female and male rats fed sodium metabisulfite in their diets at 0, (○), 1 (+), or 2% (●) for 2 years. Arrows indicate the time of mating. (Til et al., 1972)

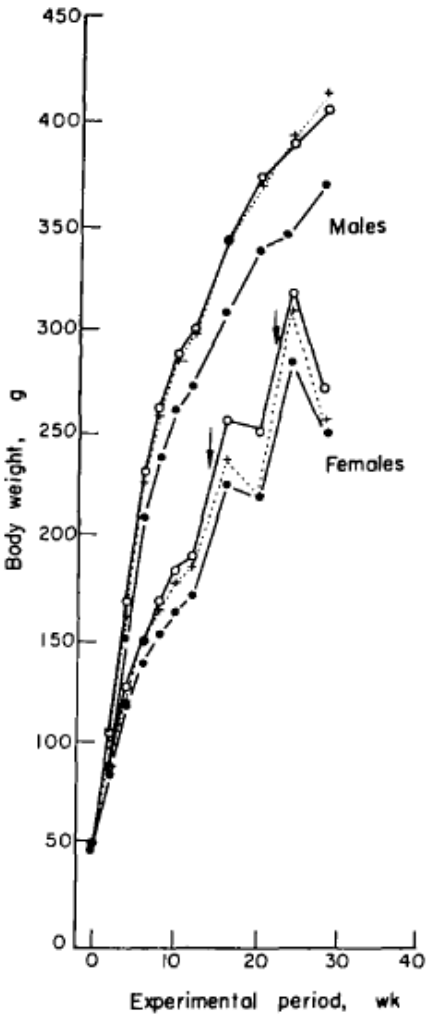

Figure 7. Growth curves of F_2 female and male rats fed sodium metabisulfite in their diets at 0, (○), 1 (+), or 2% (●) for 30 wks. Arrows indicate the time of mating. (Til et al., 1972)

Data on fertility, litter size, offspring weights, and offspring viability for all matings of all three generations are presented in Table 8. Treatment had no effect on fertility at any concentration of sodium metabisulfite, for any generation.

Mean litter size was affected only for the first mating of the F_2 generation; relative to controls, significant ($p < 0.01$ or $p < 0.001$) decreases were seen in litter sizes with exposure to 0.5, 1.0, or 2.0% sodium metabisulfite. Effects of treatment on offspring weights were generally more profound at PND 21, as compared to PND 1 or 8. Weight deficits were also more evident in offspring of F_2 animals, as opposed to offspring of the F_0 or F_1 generations. Significant effects on offspring mortality at birth or weaning appeared to be sporadic across concentrations and generations, with no clear concentration-response effect.

Measures of thiamine content in urine and liver were taken to indicate that the added dietary thiamine had succeeded in preventing thiamine deficiency, even at the highest intake of 2% sodium metabisulfite. Hematology parameters were considered to be generally normal. Findings of occult blood in feces, indicating intestinal bleeding, were common for rats of the highest concentration group in all generations.

No effects of sodium metabisulfite exposure were detected on relative weights of testes or ovaries. No treatment-related neoplastic or non-neoplastic lesions were identified in organs including testes, ovaries, mammary glands, uterus, prostate, seminal vesicles or coagulating glands.

Table 8. Fertility, offspring viability, body weights, and mortality in successive generations of rats fed 0–2% sodium metabisulfite (from Til et al. 1972)

Dietary level of $Na_2S_2O_5$ (%)	No. of females with litters (%)	Mean no. of rats/litter at birth	Mean body weight (g) of young at day			Mortality (%)	
			1	8	21	At birth	At weaning
F_0-generation—first mating							
0	90	8·7	5·9	15·7	44·5	9	13
0·125	90	9·1	5·9	15·0	42·5	1**	23
0·25	75	9·8	5·9	14·3	40·0*	9	25*
0·5	85	9·5	6·1	15·1	42·3	1**	1***
1·0	90	10·6	6·1	15·6	43·2	5	9
2·0	85	9·8	5·9	13·8*	38·1***	8	14
F_0-generation—second mating							
0	80	9·5	6·0	14·6	38·9	8	0
0·125	90	8·6	6·8	14·4	39·4	26***	0
0·25	80	9·3	6·2	17·5*	44·7	8	17***
0·5	100	10·7	6·4	15·7	40·2	0**	5
1·0	90	7·8	6·4	15·8	41·3	0	4
2·0	90	8·9	5·9	14·4	37·6	13	8*
F_1-generation—first mating							
0	90	10·4	6·2	17·9	45·6	0	16
0·125	90	10·7	5·9	14·8*	40·6	1	11
0·25	80	10·0	5·7	16·2*	40·9	0	5*
0·5	90	8·8	6·0	15·6*	43·2	1	15
1·0	70	9·1	6·0	15·0*	38·6*	0	15
2·0	90	9·7	6·2	14·7*	37·7*	2	10
F_1-generation—second mating							
0	100	10·4	6·7	18·3	49·4	0	11
0·125	80	11·0	6·2	16·0*	45·9	1	8
0·25	80	10·9	5·9*	16·9	44·8	3	3
0·5	100	8·7	6·1	19·4	51·9	3	4
1·0	100	9·4	6·5	15·9*	45·1*	5	7
2·0	90	10·8	6·2	15·9*	42·9**	4	3
F_2-generation—first mating							
0	100	11·7	6·2	17·3	47·1	5	2
0·125	100	10·5	5·6*	15·8	42·2*	1	14**
0·25	87	11·2	5·5***	14·6**	40·2***	0**	0
0·5	100	8·9***	6·1	16·8	41·9**	3	10*
1·0	100	8·9**[1]	6·0	15·5*	41·1**	0*	1
2·0	100	8·8***	5·9	15·2**	38·8***	3	2
F_2-generation—second mating							
0	100	10·2	6·4	17·2	44·3	3	8
0·125	100	11·4	6·0	16·8	45·9	2	13
0·25	100	11·3	5·8*	15·3*	40·4*	0	4
0·5	93	9·9	6·2	16·4	44·4	1	71
1·0	100	9·8	6·3	14·3**[1]	44·0	2	21**
2·0	100	10·5	6·2	15·5	41·2	0	7

Values marked with asterisks differ significantly (Student's t test for the first columns and chi-square test for the last two columns) from the control values: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Zhang et al., 2005b: Study of toxicity on male reproductive system of mice induced by SO_2 inhalation. (Chinese language with English abstract and tables).

Four groups of 10, 4-wk old male "Kunming" mice were exposed to SO_2 for 7 d, 4hr/d, at a concentration of 0, 28, 56, or 112 mg/m^3 (0; ~11,000; ~21,000; ~43,000 ppb). The animals weighed 17–19 g at the beginning of exposure.

Treatment had no demonstrable effect on food intake or body weight gain.

Biochemical analyses were performed on homogenates of whole testicular tissues, taken 24 hrs after the final exposure. Statistically significant differences from controls were found in SO_2-exposed animals for:

- Malondialdehyde (MDA) content (nmol/g prot) – at all concentrations, concentration-related
- Glucose-6-phosphate dehydrogenase (G6-PD) activity (nmol/(mg min prot)) – at 56 and 112 mg/m^3 (~21,000 and ~43,000 ppb), concentration-related
- Glutathione S-transferase (GST) activity (µmol/(mg min prot)) – at 112 mg/m^3, concentration-related
- Glutathione (GSH) content (µmol/g prot) – at 56 and 112 mg/m^3, concentration-related

Complete results are summarized in Table 9 below.

Table 9. Effects of SO_2 inhalation on glutathione redox system of mouse testicular homogenates

SO_2 (mg/m^3)	**SO_2 (ppb)**	**GSH (µmol/g prot)**	**GST µmol/(mg min prot)**	**G6-PD nmol/(mg min prot)**	**MDA (nmol/mg prot)**
0	0	58.15 ± 10.59	0.52 ± 0.06	41.50 ± 7.49	0.15 ± 0.02
28	~11,000	56.03 ± 15.73	0.47 ± 0.07	38.97 ± 7.62	0.22 ± 0.03 (1)
56	~21,000	47.36 ± 5.69 (1)	0.42 ± 0.05	35.50 ± 7.54 (1)	0.27 ± 0.04 (1)
112	~43,000	40.96 ± 6.34 (1)	0.39 ± 0.06 (2)	31.75 ± 6.87 (2)	0.50 ± 0.05 (3)

(1) $p < 0.05$ (2) $p < 0.01$ (3) $p < 0.005$

The authors also ran assays to score the length of "comet tails" following electrophoresis of DNA from cells collected from seminiferous tubules. Categories of DNA damage (0, I, II, III) were scored by comparing the length (in µm) of comet tails to that of the comet heads. Results of this assay are presented in Table 10 below.

Table 10. Effect of SO_2 inhalation on comet tail of mice testicular tissue homogenates

SO_2 (mg/m^3)	SO_2 (ppb)	Length of "comet tails" (μm)	DNA damage score				Cells with comet tails (%)	DNA (damage units)
0	0	4.00 ± 2.89	0	I	II	III	51.9	51.9
28	~11,000	5.07 ± 4.37 (1)	48.1	51.9	0	0	78.0	83.4
56	~21,000	10.94 ± 6.54 (3)	19.3	78.0	2.7	0	93.9	117.6
112	~43,000	15.84 ± 8.99 (3)	6.1	74.8	14.5	4.6	100.00	162.4

(1) $p < 0.05$ (3) $p < 0.005$

The authors concluded that SO_2 exposure can influence GSH oxidation/reduction and damage spermatocyte DNA. Based on these mechanistic findings, they further concluded that SO_2 can damage the mouse male reproductive system.