Evidence on the Developmental and Reproductive Toxicity of Sulfur Dioxide

July 12, 2011

Developmental and Reproductive Toxicant Identification Committee (DART) Meeting

Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
Colorless, nonflammable gas, pungent odor

In air pollution – in combination with sulfuric acid, sulfur trioxide, ozone, nitrogen dioxide, and particulates

In ambient air from
- fossil fuel consumption at power generation and other industrial facilities
- wildfires
Sulfur Dioxide (SO$_2$)

- Criteria air pollutant
- US EPA SO$_2$ standards
  - New standard
    1-hour period: 0.075 parts per million (75 ppb)
- Primary route of exposure
  - Inhalation of gaseous SO$_2$
  - Smaller percentage absorbed at low (~40% at 0.001 ppb) vs high air concentrations (>90% at 0.100 ppb)
Male Reproductive Toxicity
Human Studies

Teplice Program (1991-1999)

- Czech government / U.S. EPA
- Very high levels of air pollution
SO$_2$ Levels in Teplice and Prachatice

Benes et al., 2001
Male Reproductive Toxicity Studies in Humans

Retrospective cohort study
- fecundability

- Studies on sperm
  - quality
  - genetic integrity
    - DNA – abnormal chromatin structure
    - aneuploidy
Male Reproductive Toxicity

↑ SO₂ exposure

Humans and animals (in vivo)

Humans (epidemiologic study)

↓ Fecundability or fertility

↑ DNA damage in sperm and germ cells

Humans and animals (in vivo and in vitro)
SO$_2$ levels, Temperature, and Conception

30-day running averages of SO$_2$ levels (in micrograms per cubic meter) and 30-day maximal daily temperatures ($^\circ$C) compared to percent conception in the FUMC by the second month before conception.
SO$_2$ Levels in Teplice

Benes et al., 2001
Adjusted odds ratios (AOR) for conceiving in the first unprotected menstrual cycle by exposure to SO$_2$ prior to conception

<table>
<thead>
<tr>
<th></th>
<th>Medium 15.3 – 30.5 ppb</th>
<th></th>
<th></th>
<th>High &gt; 30.5 ppb</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOR</td>
<td>CI</td>
<td>p-value</td>
<td>AOR</td>
<td>CI</td>
<td>p-value</td>
</tr>
<tr>
<td>1st 2-yr period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 mos</td>
<td>1.58</td>
<td>0.85–2.74</td>
<td>0.16</td>
<td>1.26</td>
<td>0.58–2.71</td>
<td>0.56</td>
</tr>
<tr>
<td>3 mos</td>
<td>0.88</td>
<td>0.49–1.57</td>
<td>0.66</td>
<td>0.86</td>
<td>0.41–1.82</td>
<td>0.70</td>
</tr>
<tr>
<td>2 mos</td>
<td>0.49</td>
<td>0.25–0.96</td>
<td>0.037</td>
<td>0.43</td>
<td>0.20–0.93</td>
<td>0.033</td>
</tr>
<tr>
<td>1 mos</td>
<td>1.14</td>
<td>0.67–1.97</td>
<td>0.62</td>
<td>1.20</td>
<td>0.58–2.48</td>
<td>0.62</td>
</tr>
<tr>
<td>2nd 2-yr period</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4 mos</td>
<td>0.90</td>
<td>0.51–1.61</td>
<td>0.74</td>
<td>0.88</td>
<td>0.41–1.85</td>
<td>0.73</td>
</tr>
<tr>
<td>3 mos</td>
<td>0.85</td>
<td>0.45–1.57</td>
<td>0.59</td>
<td>0.96</td>
<td>0.45–2.03</td>
<td>0.91</td>
</tr>
<tr>
<td>2 mos</td>
<td>0.67</td>
<td>0.36–1.28</td>
<td>0.22</td>
<td>0.59</td>
<td>0.36–1.28</td>
<td>0.20</td>
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<tr>
<td>1 mos</td>
<td>1.16</td>
<td>0.59–2.29</td>
<td>0.66</td>
<td>1.15</td>
<td>0.59–3.59</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Adapted from Dejmek et al., 2000
Influence of distance from the monitor on the adjusted odds ratios of conceiving in the second month (30-60 days) before conception

<table>
<thead>
<tr>
<th>Distance</th>
<th>Medium</th>
<th></th>
<th>High</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOR</td>
<td>CI</td>
<td>AOR</td>
<td>CI</td>
</tr>
<tr>
<td>&lt; 3.5km</td>
<td>0.56</td>
<td>0.31–1.00</td>
<td>0.36</td>
<td>0.17–0.73</td>
</tr>
<tr>
<td>&gt; 3.5km</td>
<td>0.58</td>
<td>0.31–1.08</td>
<td>0.70</td>
<td>0.34–1.45</td>
</tr>
</tbody>
</table>
Evidence of a Causal Association

• Reduced odds of conception with SO$_2$ exposure $>15.3$ ppb in 2$^{nd}$ month before conception

• Timing of the effect coincides with critical period

• Dose-response association with SO$_2$ exposure

• Stronger association when considering distance

• Decreased fecundability only seen with SO$_2$

• Effects on sperm motility and morphology appeared reversible
Human Studies on Sperm

• Air pollution - adverse effects on sperm quality and sperm chromatin

• SO₂ increased DNA damage in sperm

• SO₂ increased risk of aneuploidy in sperm
Animal Studies Male Reproductive Toxicity

*Mice exposed to SO$_2$ by inhalation showed toxic effects in the testis, as well as other organs:*

- Altered testis basement membranes, damaged Sertoli cells and spermatids ($>\sim$11,000 ppb)

- Altered testicular biochemical parameters ($>\sim$8,400 ppb), increased DNA damage

- ↑ levels of lipid peroxidation, altered intracellular redox status in mouse organs, including testes (at $\sim$20,000 ppb)
Summary

Male Reproductive Toxicity

- Decreased fecundability
- Decreased sperm quality
- Toxic effects to the testes
- Increased DNA damage in sperm
Questions?
Developmental Toxicity
Developmental Toxicity

• Preterm birth
• Low birth weight
• Congenital malformations
• Pregnancy loss
• Asthma
• Other developmental effects
Preterm Birth

• Ten studies - eight report significant findings

• Higher SO₂ exposure - increased risk of preterm birth

• Three studies reported dose-response associations

• Studies varied
  • window of exposure
  • adjustment for distance from monitor
  • level of exposure
Reported SO$_2$ Exposures (ppb) in Preterm Birth Studies

- Brauer et al., 2008
- Jalaludin et al., 2007
- Liu et al., 2003
- Sagiv et al., 2005
- Leem et al., 2006
- Darrow et al., 2009
- Bobak, 2000
- Jiang et al., 2007
- Xu et al., 1995
- Xu et al., 1995
- Mohorovic, 2004
Preterm Birth Forest Plot

Risk estimates with 95% confidence intervals from 10 studies

Brauer et al, 2008
Jalaludin et al, 2007

Liu et al, 2003
Sagiv et al, 2005
Leem et al, 2006
Darrow et al, 2009
Bobak, 2000
Jiang et al, 2007
Xu et al, 1996
Mohorovic, 2004
Study by Xu et al.

- High levels of SO$_2$
- A large gradient of SO$_2$
- Monitored and adjusted for seasonal changes
- Controlled for total suspended particles (TSP)
- Close proximity of population to air monitoring stations
- Investigated different number of lag days (exposure windows)
Adjusted Gestational Age, by SO₂ and TSP Concentrations

Derived from locally weighted regressions, adjusting for temperature, humidity, day of the week, season, maternal age, gender of child, and residential area.
Gestational Age Distribution by Tertile of SO$_2$ Concentration
Results from the Study by Xu et al.

- Dose-dependent relationship between gestational age and SO$_2$ and TSP
- Adjusted odds ratio for PTB = 1.21 (95% CI= 1.01, 1.46 for each log$_e$ (ug/m$^3$) increase in SO$_2$
- Pregnancies at high risk for PTB may be particularly susceptible to effects of air pollution
Fetal Growth
Fetal Growth

Outcomes examined in humans

• Low birthweight (<2500 g)
• Birthweight (continuous)
• Intrauterine growth restricted (IUGR), Small for gestational age (SGA; <10th percentile for sex and gestational age)
• Very low birth weight (<1500 g)
• Fetal ultrasound scan measurements, e.g., femur length, head circumference
# Fetal Growth

## 22 epidemiologic studies

<table>
<thead>
<tr>
<th># of Studies</th>
<th>Association with fetal growth</th>
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<tbody>
<tr>
<td>13</td>
<td>↑ risk only</td>
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<td>5</td>
<td>No significant associations</td>
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## Fetal Growth

### Spatial and Temporal Exposure Assessment

<table>
<thead>
<tr>
<th>Study</th>
<th>Association with fetal growth restriction, comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lin et al., 2004</td>
<td>↑ risk LBW at medium and high levels</td>
</tr>
<tr>
<td>Dugandzic et al., 2006</td>
<td>↑ risk LBW, 1\textsuperscript{st} trimester</td>
</tr>
<tr>
<td>Williams et al., 2007</td>
<td>Very high ↑ risk LBW; outlier</td>
</tr>
<tr>
<td>Yang et al., 2003</td>
<td>Slight ↓ in birth weight, 1\textsuperscript{st} trimester</td>
</tr>
<tr>
<td>Hansen, et al., 2008</td>
<td>↓ ultrasound measurements, low SO\textsubscript{2}</td>
</tr>
<tr>
<td>Brauer et al., 2008</td>
<td>No significant associations, low SO\textsubscript{2}</td>
</tr>
</tbody>
</table>
Fetal Growth

Co-pollutant confounding

• Carbon monoxide (CO)
• Particulate matter:
  < 10 μm (PM$_{10}$)
  < 2.5 μm (PM$_{2.5}$)
  Total suspended particulates (TSP)
• Nitrogen dioxide (NO$_2$)
Fetal Growth

22 epidemiologic studies

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## Fetal Growth

### Multi-pollutant analyses

<table>
<thead>
<tr>
<th>Study</th>
<th>Co-pollutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gouveia et al., 2004</td>
<td>CO, PM(_{10}), (NO(_2), O(_3))</td>
</tr>
<tr>
<td>Bobak and Leon, 1999</td>
<td>TSP, NO(_x)</td>
</tr>
<tr>
<td>Hansen et al., 2008</td>
<td>PM(_{10}), NO(_2), O(_3)</td>
</tr>
<tr>
<td>Lin et al., 2004</td>
<td>CO, PM(_{10}), NO(_2), O(_3)</td>
</tr>
<tr>
<td>Liu et al., 2003</td>
<td>CO, NO(_2), O(_3)</td>
</tr>
<tr>
<td>Williams et al., 2007</td>
<td>PM(_{2.5}), lead</td>
</tr>
<tr>
<td>Nascimento and Moreira, 2009</td>
<td>PM(_{10}), O(_3)</td>
</tr>
</tbody>
</table>
Fetal Growth

Lin et al., 2004 - Taipei and Kaohsiung, Taiwan

- Assessed spatial and temporal variation
- Restricted cohort: 3 km from monitors
- Adjusted for CO, PM$_{10}$, NO$_2$, O$_3$
- Adjusted for season
- High SO$_2$ levels
- Exposure gradient
Fetal Growth

Lin et al., 2004

Results for LBW and SO₂ (entire pregnancy)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>SO₂ conc. (ppb)</th>
<th>AOR* (95% C.I.)</th>
</tr>
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<tbody>
<tr>
<td>Low</td>
<td>&lt; 7.1</td>
<td>1.00</td>
</tr>
<tr>
<td>Medium</td>
<td>7.1 – 11.4</td>
<td>1.16 (1.02, 1.33)</td>
</tr>
<tr>
<td>High</td>
<td>&gt;11.4</td>
<td>1.26 (1.04, 1.53)</td>
</tr>
</tbody>
</table>

* Adjusted for gestational week, gender, birth order, season, maternal age & education, and co-pollutants (CO, PM₁₀, NO₂, O₃)
Fetal Growth

Lin et al., 2004

Results for LBW and SO$_2$ (third trimester)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>SO$_2$ conc. (ppb)</th>
<th>AOR* (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>&lt; 6.8</td>
<td>1.00</td>
</tr>
<tr>
<td>Medium</td>
<td>6.8 – 12.4</td>
<td>1.13 (0.99, 1.28)</td>
</tr>
<tr>
<td>High</td>
<td>&gt;12.4</td>
<td>1.20 (1.01, 1.41)</td>
</tr>
</tbody>
</table>

* Adjusted for gestational week, gender, birth order, season, maternal age & education, and co-pollutants (CO, PM$_{10}$, NO$_2$, O$_3$)
Fetal Growth

Lin et al., 2004

Limitations

• Kaohsiung and Taipei
• Maternal characteristics
• CO was associated with ↓ risk of LBW
• Correlations among pollutants not reported
Fetal Growth

Animal studies

• ↓ birthweight in mice at 65,000 ppb; concentration-dependent
• ↓ fetal weight, no change in crown-rump length in mice at 25,000 ppb
• No effect on fetal weight in rabbits at 70,000 ppb
Congenital Malformations
Congenital Malformations

Epidemiologic Studies

Methodological challenges

• Confounding
• Multiple comparisons
• Case identification
• Case groupings, syndromes
Congenital Malformations
Epidemiologic Studies

Case groupings

• Any/all birth defects
• Chromosomal vs. non-chromosomal defects
• Heart defects
• Oral clefts

Inconsistent findings
Congenital Malformations

Animal study

• No association with specific or aggregate malformations in mice at 25,000 ppb or rabbits at 70,000 ppb SO$_2$
Pregnancy Loss
Pregnancy Loss

Spontaneous abortion

• No association in a cross-sectional occupational study
Pregnancy Loss

Stillbirth

• Fetal death after 28 weeks gestation, or >1,000 g

• Ecologic studies

• Correlation with SO$_2$ (r=0.7; p≤0.05)
Pregnancy Loss

Animal studies

Gestational exposure to SO$_2$ did not result in changes in mean litter size or resorption frequencies at:

– 25,000 ppb (mice)
– 70,000 ppb (rabbits)

Exposure to 32,000 ppb or 65,000 ppb SO$_2$ was not associated with changes in litter size
Asthma

Prenatal exposure to SO₂ associated with ↑ risk of childhood asthma...

• High correlations between pre- and postnatal exposure

• High correlations among co-pollutants

• Traffic-related pollutants had stronger associations
Other Developmental Outcomes

Mice

• Effects on male-male social behavior at 12,000 & 30,000 ppb
• Delays in acquisition of certain postnatal reflexes at 32,000 ppb
Developmental Toxicity

Summary

• Preterm birth
• Low birth weight
• Congenital malformations
• Pregnancy loss
• Asthma
• Other developmental effects
Questions?
Female Reproductive Toxicity
Female Reproductive Toxicity

*In vitro* fertilization (IVF)

- SO$_2$ was consistently but not significantly associated with ↓ odds of live birth
- Other pollutants more strongly associated with odds of live birth
Female Reproductive Toxicity

Animal Study

Rats exposed by inhalation to SO$_2$ at $\sim$1,500 ppb showed effects on:

- Estrous cycle length in F$_0$ and F$_1$ offspring
- Pregnancy frequency and duration
- Offspring growth (body weight)
- No changes observed at $\sim$57 ppb
Female Reproductive Toxicity

Related studies: Sodium sulfite

• *In vitro* exposure of sheep or cow oocytes resulted in fragmentation of chromosomes, with or without rearrangement

• No effects were seen in mouse oocytes exposed either *in vitro* or *in vivo*
Female Reproductive Toxicity
Summary

• Humans: IVF

• Animals: Estrous cycles and pregnancy

• Related studies
Questions?