The Potential Role, Mechanisms and Relevance of Pollutant-Induced Oxidative Stress in Preterm Birth

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Preterm birth
United States, 1999-2009

Preterm is less than 37 completed weeks gestation.
Preterm Birth Medical Consequences for Infant

• Leading factor in infant death
  – 19.7% of infant deaths

• Medical complications
  – Respiratory distress syndrome
  – Bleeding in the brain (intraventricular hemorrhage)
  – Nearly half of all congenital neurological defects (e.g., cerebral palsy)
  – Chronic lung disease (bronchopulmonary dysplasia)
  – Apnea
  – Retinopathy of prematurity (can lead to blindness)
  – Anemia
  – Infections
Preterm is less than 37 completed weeks gestation.

Cost of Preterm Birth
United States, 2005

Preterm is less than 37 completed weeks gestation.

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Preterm Birth Rates in US & Puerto Rico
1990 - 2005
PROTECT CENTER

- Project 1: Non-targeted Analysis
- Project 2: Mechanistic Toxicology
- Project 3: Epidemiology/Targeted Analysis
- Project 4: Fate and Transport
- Project 5: Green Remediation

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Phthalate Contamination in Puerto Rico
Phthalates

- CDC biomonitoring study of NHANES data detected phthalates in nearly all urine samples
- Because phthalates are rapidly excreted, this suggests that exposure to phthalate is a daily occurrence for most Americans
Mono-2-ethylhexyl phthalate (MEHP) is a biologically active metabolite of the plasticizer di-2-ethylhexyl phthalate (DEHP)
Associations between Human Exposure to Phthalates & Birth Outcomes

- Latini et al. 2003, *EHP* (n = 84)
  - The DEHP metabolite MEHP in cord blood was inversely and significantly associated with decreased gestation length
  - Urinary DEHP metabolites inversely associated with gestation length
  - Women with the highest MEHP quartile delivered 5 days earlier than lowest quartile
  - DEHP and DBP or metabolites in cord blood/meconium was associated with dose-dependent increased odds for low birth weight
- Wolff et al. 2008, *EHP* (n = 404)
  - Urinary MEHP associated with increased gestation length
  - DEHP metabolites associated with increased gestation length

DEHP Metabolite Ratios and Preterm Birth

- MEHP% is the sum of DEHP/MEHP oxidative metabolites divided by MEHP.
- Geometric mean MEHP urine concentrations in women delivering term or preterm stratified by MEHP% median.

Popular Model of Parturition Activation

Challis, JR et al., Reprod Sci 16, 206-215, 2009

Inflammatory stimuli

Toll-like receptors

Proinflammatory cytokines
(IL-1β; TNFα; IL-8; IL-6)

Prostaglandins
Matrix metalloproteinases
Macrophages and neutrophils

Myometrial contractility
Ruptures of membranes
Cervical ripening

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Role of Reactive Oxygen Species (ROS) in Parturition

LPS

Antioxidant (N-acetyl cysteine)

Premature birth

Buhimschi et al, 2002

Pro-labor mediators (prostaglandins, inflammatory cytokines)

Lappas et al, 2003
Toxicant Activation of Parturition

Diagram adapted from A.D.A.M Interactive Anatomy 2009.
In Vitro Models

- HTR-8/Svneo cells
- Primary placental macrophages
- Gestational membrane tissue explants
- Gestational membranes mounted on Transwell inserts
Hypothesis

*tert*-Butylhydroperoxide (TBHP) – Model pro-oxidant
Mono-2-ethylhexyl phthalate (MEHP) – Phthalate metabolite
*S-(1,2)-Dichlorovinyl-L-cysteine (DCVC) – Trichloroethylene metabolite

Placental cell

$O_2^-$ ·$OH$
$H_2O_2$

ROS

Signal Transduction

Prostaglandins
Cytokines
Apoptosis

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A 1-h exposure to MEHP stimulated cellular production of reactive species in HTR-8 cells as measured by dichlorofluorescein (DCF) assay

Manuscript under review
Deferoxamine Inhibits MEHP-Stimulated Generation of ROS in HTR-8 cells as measured by dichlorofluorescein (DCF) fluorescence
Oxidative DNA Adducts

Mass tag profiling allows improved detection of DNA adducts from oxidative stress

Thymine → [O] → Hydroxy thymine

or
A 24-h exposure to 90 or 180 µM MEHP increased oxidative damage to DNA of HTR-8 cells as measured by mass tag profiling for detection of hydroxylated thymine

Manuscript under review
MEHP stimulated PTGS2 mRNA Expression in HTR-8 cells after 4 and 8 h of exposure

Arachidonic Acid → PTGS2 → ROS → PGH₂ → Prostaglandins (PGE₂, PGF₂α) → Parturition

Increased gestational tissue PTGS2 expression is critical for induction of parturition

Manuscript under review

Manuscript under review
Increased PGF2α and PGE2 in Amniotic Fluid with Gestational Age

Lee 2008 The Journal of Maternal-Fetal and Neonatal Medicine,

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Prostaglandin Screening Assay

- Prostaglandin E1
- Prostaglandin E2
- Prostaglandin F1α
- Prostaglandin F2α
- Prostaglandin F3α
- Prostaglandin E2 Ethanolamide
- 6-keto Prostaglandin F1α
- 8-iso Prostaglandin F2α
- 8-iso Prostaglandin E2
- Prostaglandin D2
- 8-iso-2,3-dinor
- Prostaglandin F1α
- Prostaglandin E3
- Thromboxane B2
Primary placental macrophages secrete increased amounts of total prostaglandins after a 24-h exposure to 180 µM MEHP

Manuscript under review
Human Gestational Membrane Explant Model

• Gestational tissue collected from normal, term, not in labor, cesarean deliveries
• 12-mm disks punched from tissue and floated in medium
• Incubated overnight, then treated with MEHP for 24 h
Explants of Gestational Membranes secrete increased amounts of total prostaglandins after a 24- h exposure to 180 µM MEHP

Manuscript under review
Oxidative Stress and Inflammation in US Population

• Used the NHANES databases
  – Publicly available data collected by CDC
  – Exploratory analyses of phthalate metabolite associations with potential response biomarkers

• Putative biomarkers of oxidative stress
  – NHANES 1999-2006 database
  – Bilirubin (a potent antioxidant), inverse association
  – Gamma glutamyltransferase (GGT)
Oxidative Stress and Inflammation

• Putative biomarkers of inflammation (positive associations)
  – NHANES 1999-2006 database
  – C-reactive protein (CRP)
  – Alkaline phosphatase (ALP)
  – Absolute neutrophil count (ANC)
  – Ferritin
  – Fibrinogen
Adjusted Regression coefficients (95% confidence intervals) for change in GGT associated with urinary phthalate metabolite quintiles. Adjusted for age, sex, race and ethnicity, serum cotinine, income, BMI, and urinary creatinine.

Hypothesis Model

Molecular Insult → Reactive Oxygen Species

Pathway Activation:
- DNA Damage
- Membrane Lipid Oxidation
- Gene Transcription

Cellular Response:
- Cell Death
- Prostaglandins
- Inflammatory Response

Pathophysiologic Response:
- PPROM
- Uterine Contraction

Birth Outcome:
- Preterm Birth

PPROM: Premature preterm rupture of membranes

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A More Comprehensive Model: The Prematurity Puzzle


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Summary

- Phthalates may have a role in preterm birth
- Experimental and human observational data support oxidative stress as a potential mechanistic link
  - May have relevance for other environmental contaminants and human health effects
- Multidisciplinarity is needed to address complex environmental health challenges
  - NIEHS Superfund Research Program Centers facilitate these partnerships

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