Biomonitoring and biomarkers to unravel prenatal risk factors for later health outcomes

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VITO and
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Outline

» Prenatal exposure to environmental chemicals and health outcomes later in life
» Biomarkers and biomonitoring
» Biomarkers of exposure
» Biomarkers of effect
» Conclusions
Prenatal exposures and late health outcomes

» The story of DES (diethylstilbestrol) 1938-1950

» DES: a synthetic form of estrogen (female hormone) prescribed to women to prevent pregnancy complications

» DES Daughters:
  » 40 times more likely to develop CCA of the vagina and cervix than women not exposed to DES

» Pregnancy complications and fertility problems

» DES sons: genital abnormalities

Palmer, 2001
Prenatal exposures and late health outcomes : methylmercury

Minamata Disease: methylmercury poisoning in Japan
1950’s
» methylmercury was being dumped into the bay by a plant of the Chisso Corporation
» “…in every case the mother was healthy, and it was not until more than three months after birth that the symptoms were recognized “ Shoji Kitamura (1959)
» 10-12% of villagers developed the disease
» Formal recognition of the Minamata disease causation 1963/68
# Prenatal exposures and late health outcomes: methylmercury

## Fish intake: Faroe island cohort

<table>
<thead>
<tr>
<th>Age/test group</th>
<th>Mercury without adjustment for fish intake</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect</td>
<td>p-Value</td>
<td>Effect</td>
<td>p-Value</td>
<td>Effect</td>
<td>p-Value</td>
</tr>
<tr>
<td>7 Years Motor</td>
<td>−9.74</td>
<td>0.034</td>
<td>25.1</td>
<td>0.010</td>
<td>−12.2</td>
<td>0.0092</td>
</tr>
<tr>
<td>7 Years Verbal</td>
<td>−10.4</td>
<td>0.0018</td>
<td>3.62</td>
<td>0.61</td>
<td>−10.8</td>
<td>0.0017</td>
</tr>
<tr>
<td>14 Years Motor</td>
<td>−7.41</td>
<td>0.033</td>
<td>19.9</td>
<td>0.006</td>
<td>−9.37</td>
<td>0.0082</td>
</tr>
<tr>
<td>14 Years Attention</td>
<td>−8.40</td>
<td>0.029</td>
<td>12.2</td>
<td>0.13</td>
<td>−9.54</td>
<td>0.016</td>
</tr>
<tr>
<td>14 Years Spatial</td>
<td>2.60</td>
<td>0.50</td>
<td>17.3</td>
<td>0.031</td>
<td>1.04</td>
<td>0.79</td>
</tr>
<tr>
<td>14 Years Verbal</td>
<td>−5.97</td>
<td>0.080</td>
<td>9.85</td>
<td>0.16</td>
<td>−6.87</td>
<td>0.049</td>
</tr>
<tr>
<td>14 Years Memory</td>
<td>−2.86</td>
<td>0.39</td>
<td>3.15</td>
<td>0.64</td>
<td>−3.05</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*Effect of true exposure doubling expressed in percent of SD of latent response.*

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*Grandjean, Weihe*
Prenatal exposures and late health outcomes

**Sufficient evidence**, limited evidence

- Developmental milestones: **Methylmercury**, PCBs
- Cognitive function 0-2 yrs: **Methylmercury**, PCBs, lead
- Cognitive function > 2 yrs: **Methylmercury**, PCBs, lead, ETS
- Behavioral problems: PCBs
- Motoric function: **Methylmercury**, PCBs,
- Auditory function: **Methylmercury**, lead, PCBs
- Visual function: **Methylmercury**,
- Childhood lung infections: PCBs
- Childhood middle ear infections: PCBs, DDE (lactational exposure)
- Childhood cancers
  - Lymphoma: ETS
  - Leukemia: herbicides, insecticides, ETS, unspecified solvents
  - Brain: insecticides
- Child growth & Pubertal development: PCBs (post natal growth in height)
- Sudden death infant syndrome: **active smoking**

Prenatal exposure and health outcomes

- Food contaminants (PCBs, methylHg)
- Chemicals in consumer products (bisphenol A, PFOS)
- Air pollution (PM, PAHs)

DOSE ?

- Neurobehaviour,
- Sexual development,
- Immune effects
- Increased cancer incidence
- Changes in growth curves

EFFECT ?
Human biomonitoring: direct measurement in human tissues

**Environmental monitoring** → **biomarkers of exposure** → **biomarkers of effects** → **adverse health effects**

- **air** → Blood lead
- **dust** → Cotinine in urine
- **Consumer products** → MeHg in hair
- **water** → ...
- **soil**

**Birth weight**
**Growth**
**IQ**
**behaviour**
**puberty**
**Asthma**
**Cancer**

**Hormone levels**
**DNA damage**
**Gene expression**
**Epigenetic changes**
Human biomonitoring: direct measurement in human tissues

Preconception
Mothers / fathers
Blood
Urine
Hair

pregnancy
Mothers
Blood
Urine
Hair

Cord blood
Placenta
meconium
Urine

post natal
Breast milk

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Flemish human biomonitoring campaign
Environmental health
Monitoring for action…
2002-2006-2011
Commissioned, financed, steered by the Ministry of the Flemish Community (Dept of Science, Public Health and Environment)
Goals

Develop a surveillance program for environmental health → policy support

» Identify “base line values” or “reference values” for environmental pollutants in the Flemish population

» Has the area of residence an impact on the internal pollutant levels and potential biological effects?

» Early signaling of environmental health risks?
1200 newborns
Sept 2002- Dec 2003
25 maternities + 2 stem cell banks
50 mL cord blood

1600 youngsters
Oct 2003- July 2004
42 schools
18 mL blood, 50 mL urine

1600 adults
Sept 2004- June 2005
43 municipalities
35 mL blood, 50 mL urine
## Biomarker analysis in FLEHS

<table>
<thead>
<tr>
<th></th>
<th>Newborns: 1200</th>
<th>Teenagers: 1600 (14-15y)</th>
<th>Adults: 1600 (50-65y)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Markers of exposure</strong></td>
<td>cadmium, lead&lt;br&gt;marker PCBs, pesticides, dioxine-activity</td>
<td>cadmium, lead&lt;br&gt;marker PCBs, pesticides&lt;br&gt;1-OH pyrene, tt-muconic acid</td>
<td>cadmium marker PCBs, pesticides, dioxine-activity&lt;br&gt;1-OH pyrene, tt-muconic acid</td>
</tr>
<tr>
<td><strong>Markers of effect</strong></td>
<td>Biometry, TSH (heel prick), Apgar score, time to pregnancy&lt;br&gt;&lt;br&gt;<em>Questionnaire:</em> asthma &amp; allergy&lt;br&gt;Follow-up of part of children</td>
<td>comet test&lt;br&gt;hormone balance&lt;br&gt;Biometry, sexual development&lt;br&gt;&lt;br&gt;<em>Questionnaire:</em> asthma &amp; allergy</td>
<td>comet test, micronuclei&lt;br&gt;gene-expression&lt;br&gt;tumour markers&lt;br&gt;8-OH dG&lt;br&gt;&lt;br&gt;<em>Questionnaire:</em> asthma &amp; allergy</td>
</tr>
<tr>
<td><strong>Covariates and confounders</strong></td>
<td><em>Questionnaire:</em> general + food</td>
<td><em>Questionnaire:</em> general + food</td>
<td><em>Questionnaire:</em> general + food</td>
</tr>
<tr>
<td><strong>Markers of susceptibility</strong></td>
<td>Biochemical analyses: cholesterol, iron status cord blood</td>
<td>Biochemical analyses: cholesterol, ferritin, urinary creatinine</td>
<td>Biochemical analyses: cholesterol, ferritin, urinary creatinine</td>
</tr>
</tbody>
</table>
Results:

pollutants in the perinatal period of life

↑

child’s health

A. Neurobehavioral & cognitive development 0-3y (led by Dr Viaene- OPZ)
B. Respiratory health 0-3y (led by Dr. Desager-UA)
C. Biometry (length, weight): at birth, 1-3y
D. Thyroid hormone levels
Neurological behaviour follow-up

- Info on pregnancy
- Postnatal depression

0 1 12 24 36 months

- Cord blood (Pb, Cd, PCB’s, dioxins, TSH, FT3, FT4), nutrition, covariates
- Behaviour child, emotional status mother, life-events
- Cognitive and Behavioural tests child, IQ-test mother, Observation Home Environment, Cortisol

- Monthly questionnaire on food & development
- 3-monthly """"""""
Follow-up: birth weight and growth curves

- Birth weight/length
- Cord blood (Pb, Cd, PCB’s, dioxins, TSH, FT3, FT4), nutrition, covariates
- Weight BMI sds

BMIsds = Child’s BMI - population mean BMI / population standard deviation

--- monthly questionnaire on food & development
--- 3-monthly “ “ “ “

01/07/2010
Biometry:

» 138 mother infant pairs

» Exposures in cord blood: pp’-DDE, PCBs, HCB, dioxins

» Health outcomes:
  » PCBs negative effect on birth weight and birth length
  » DDE had no effect on birth weight or length
  » DDE and PCBs is associated with increased BMI during early childhood
    Verhulst et al., 2009, EHP 117, 122-126

BMI during pre-school age, correlated with adult BMI (Nader et al., 2006; Sachdev et al., 2005).

Higher BMI at that age associated with increased obesity risk in adult life (Whitaker et al. 1998).
**PCBs in cord blood vs. BMI**<sub>SDS</sub> **between 1-3 years of child’s age**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\beta \pm \text{standard error}$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.758 ± 0.588</td>
<td>0.2</td>
</tr>
<tr>
<td>Birth Weight standard deviation score</td>
<td>0.333 ± 0.045</td>
<td>0.001</td>
</tr>
<tr>
<td>Maternal smoking ever</td>
<td>0.368 ± 0.172</td>
<td>0.03</td>
</tr>
<tr>
<td>Maternal BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.046 ± 0.022</td>
<td>0.04</td>
</tr>
<tr>
<td>PCBs (ng/g lipids)</td>
<td>0.003 ± 0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>Age of the child</td>
<td>-0.766 ± 0.069</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**Similar follow-up studies:**
- DDE and/or PCBs ~ height in boys (Gladen et al., 2000)
- DDE ~ weight & BMI in adult females (Karmaus and Eneli, 2004)
- PCB ~ smaller height (Sagiv et al., 2007)
- PCB ~ increased growth in 5-year-old girls (Hertz-Picciotto et al., 2005)
# Effects on birth weight

<table>
<thead>
<tr>
<th>Chemical</th>
<th>N</th>
<th>Biomarker</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOS</td>
<td>428</td>
<td>Maternal serum</td>
<td>Washino et al, 2009</td>
</tr>
<tr>
<td></td>
<td>Japan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFOA</td>
<td>1400</td>
<td>Maternal plasma</td>
<td>Fei et al 2007</td>
</tr>
<tr>
<td></td>
<td>Denmark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCB</td>
<td>100</td>
<td>Maternal plasma</td>
<td>Halldorson et al, 2008</td>
</tr>
<tr>
<td></td>
<td>Denmark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCB</td>
<td>300</td>
<td>Breastmilk</td>
<td>Eggesbo et al, 2009</td>
</tr>
<tr>
<td></td>
<td>Norway</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phtalates</td>
<td>201</td>
<td>Maternal blood</td>
<td>Zhang et al 2009</td>
</tr>
<tr>
<td>(DBP and DEHP)</td>
<td>201</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>China</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Negative relation between POPs and thyroid hormones measured in cord blood

Multiple linear regression model /contaminant/, 200 participants adjusted for plasma total lipids, gestational age, gender, alcohol consumption during pregnancy, and age of the mother. MAERVOET et al., Environmental Health Perspectives (2007)

<table>
<thead>
<tr>
<th></th>
<th>ln fT3 (pmol/L)</th>
<th>ln fT4 (pmol/L)</th>
<th>ln TSH (mIU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>$p$</td>
<td>$n$</td>
</tr>
<tr>
<td>Polychlorinated biphenyls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sum$ 5 PCBs (ng/ml)</td>
<td>-0.198</td>
<td>0.01</td>
<td>195</td>
</tr>
<tr>
<td>Organochlorinated pesticides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCB (ng/ml)</td>
<td>-0.154</td>
<td>0.03</td>
<td>195</td>
</tr>
<tr>
<td>$p,p'$-DDE (ng/ml)</td>
<td>-0.074</td>
<td>0.29</td>
<td>195</td>
</tr>
<tr>
<td>Dioxin-like compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calux-TEQ (pg/ml)</td>
<td>-0.154</td>
<td>0.04</td>
<td>138</td>
</tr>
<tr>
<td>Heavy Metals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium (ng/ml)</td>
<td>-0.084</td>
<td>0.23</td>
<td>186</td>
</tr>
<tr>
<td>Lead (ng/ml)</td>
<td>-0.100</td>
<td>0.15</td>
<td>186</td>
</tr>
</tbody>
</table>
Free thyroxin vs concentration of sum marker PCB’s in cord blood

Clinically irrelevant? (Kimbrough and Krouskas, 2001)

Very subtle changes in $T_4$/TSH homeostasis may affect development of human fetus (Boas et al., 2006; Zöller, 2001)
Asthma/allergy follow-up

Cord blood:
Pb, Cd, PCBs, dioxinlike compounds

Questionnaires
nutrition, infections, Medication, home environment, vaccination, Airway/allergy complaints, pets, hygiene

Gut flora (faeces: 3wk, 6m, 12m)

Exhaled breath:
• condensate proteins
• Gas phase VOC
8-oxodG urine
mAPI index
Exhaled NO
Skin prick tests

Conc. NO$_2$, PM$_{10}$ (4x4 km): 3 trim pregnancy, 3m, 1y
Emissions benzene, NOx (1x1 km): average of 1y

Distance to motorways
voor geboorte

0 1 2 3 4 5 6 …………..12…………………24…………………36 months
Definitions

**Allergy**
positive reaction on 1 allergen in skin prick test (3y)
->egg milk, grass, house dust mite

**Respiratory complaints**

» Coughing during day (1,2 en 3y): “last 6 m regularly coughing during day”
» Wheezing (1, 2 en 3 y): “last 6 m regularly wheezing ”
» Frequent wheezing (3y): mAPI index
Outdoor air pollutants

Symptoms

Pollutants
cord blood
DDE - wheeze

Wheeze 2 y

Family history asthma or allergy  +
Birth weight  +
Birth height  +
Sex  +
Pb cord blood  +
**DDE cord blood**  +
Antibiotics  +

<table>
<thead>
<tr>
<th>Effect</th>
<th>OR</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth height</td>
<td>1,603</td>
<td>1,196 2,149</td>
</tr>
<tr>
<td><strong>DDE (fat, LOG)</strong></td>
<td>1,930</td>
<td>1,078 3,454</td>
</tr>
<tr>
<td>Number of days antibiotics</td>
<td>1,093</td>
<td>1,038 1,150</td>
</tr>
</tbody>
</table>
## DDE - wheeze

**Table 4. Adjusted RR (95% CI) between DDE in cord serum and wheezing at 4 years of age.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Nonatopic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p,p’-DDE</strong></td>
<td>1.32 (1.13–1.54)</td>
<td>1.30 (1.05–1.62)</td>
</tr>
<tr>
<td>Maternal asthma</td>
<td>2.62 (1.46–4.71)</td>
<td>3.45 (1.18–10.10)</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>1.48 (0.89–2.47)</td>
<td>1.03 (0.51–2.10)</td>
</tr>
<tr>
<td>Parity (≥ second child)</td>
<td>1.18 (0.89–2.02)</td>
<td>1.54 (0.74–3.24)</td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>0.62 (0.26–1.46)</td>
<td>0.36 (0.13–1.04)</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.80 (0.32–1.98)</td>
<td>0.37 (0.11–1.19)</td>
</tr>
<tr>
<td>High</td>
<td>0.29 (0.09–1.12)</td>
<td>0.17 (0.03–0.89)</td>
</tr>
<tr>
<td>Male</td>
<td>2.03 (1.15–3.57)</td>
<td>2.84 (1.21–6.68)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>0.87 (0.81–0.95)</td>
<td>0.90 (0.82–1.00)</td>
</tr>
<tr>
<td>Breast-feeding</td>
<td>0.57 (0.33–0.99)</td>
<td>0.34 (0.17–0.69)</td>
</tr>
<tr>
<td><strong>p,p’-DDE in quartile (ng/mL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.57</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.57–1.03</td>
<td>1.00 (0.41–2.43)</td>
<td>1.32 (0.37–4.70)</td>
</tr>
<tr>
<td>1.03–1.90</td>
<td>1.62 (0.70–3.74)</td>
<td>2.63 (0.96–7.20)</td>
</tr>
<tr>
<td>&gt; 1.90</td>
<td>2.36 (1.19–4.69)</td>
<td>2.49 (1.00–6.19)</td>
</tr>
</tbody>
</table>

- **associated with changes in immune system**
  - immune cells (Vine 2001)
  - immunoglobulins (Cooper et al. 2004; Vine 2001)
  - cytokines (Bilrha 2003; Daniel 2002)
- **hormone-like activity of DDE**
  - DDE in TH₂ immune differentiation: interfere with mast cells in airways (express estrogen/progesterone receptors)
  - Direct effect on airway through altering b2-adrenergic responsiveness & increasing production of prostaglandins
Outdoor air pollutants

Symptoms

Pollutants
cord blood
Impact of air quality in perinatal period on airway problems in young children

<table>
<thead>
<tr>
<th>perinatal NO₂, PM₁₀</th>
<th>Wheezing 1y</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM₁₀ first 3 m</td>
<td>Coughing 3y</td>
</tr>
<tr>
<td>perinatal NO₂, benzene</td>
<td>Allergy 3y</td>
</tr>
</tbody>
</table>

» Miller et al. (‘04): PAK’s 3<sup>rd</sup> trim pregnancy -> ↑coughing on 1y
» TRAPCA study: modelled concentrations PM<sub>2.5</sub>, NO₂ or soot in first year of life:
  » coughing on 1y: German cohort (Gehring et al., ’02)
  » dd astma, air way infections, cold on 2y: cohort of the Netherlands (Brauer et al., ’07)
  » Asthma symptoms (nt sign): Swedish cohort (Gehring et al., ‘02)
Overall conclusion...

Flemish birth cohort data support hypotheses exposure to environmental factors early in life, affect young child’s health (0-3y)

<table>
<thead>
<tr>
<th>Biometry</th>
<th>BMI, 3y</th>
<th>PCBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioural &amp; cognitive tests</td>
<td>3y</td>
<td>Pb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCBs</td>
</tr>
<tr>
<td>Respiratory health</td>
<td>wheezing, 2y</td>
<td>DDE</td>
</tr>
<tr>
<td></td>
<td>wheezing 1y, allergy 3y</td>
<td>Outdoor pollutants</td>
</tr>
</tbody>
</table>
Mechanisms of late effects?

» hormone levels,
» oxidative stress
» epigenetic changes?

Biomarkers of effect?

→ mechanistic
→ early warning
Epigenetic changes as biomarkers for environmental exposures: human studies?

<table>
<thead>
<tr>
<th>exposure</th>
<th>N</th>
<th>Biomarker matrix</th>
<th>effect</th>
<th>author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traffic related PAH exposure</td>
<td>56 children</td>
<td>Umbilical Cord white blood cells</td>
<td>Increased DNA Methylation of 59-CpG Island of ACSL3</td>
<td>Perera et al, 2009</td>
</tr>
<tr>
<td>Sum of POPs</td>
<td>70 Greenlandic Inuit</td>
<td>Blood samples</td>
<td>DNA Hypomethylation</td>
<td>Rusiecki et al, 2009</td>
</tr>
<tr>
<td>Peak exposures to traffic particles</td>
<td>718 elderly Boston</td>
<td>Blood leucocyt DNA methylation</td>
<td>DNA hypomethylation</td>
<td>Baccarelli 2009</td>
</tr>
<tr>
<td>benzene</td>
<td>213 adults Milan</td>
<td>Blood DNA samples</td>
<td>DNA hypomethylation</td>
<td>Bollati et al, 2007</td>
</tr>
</tbody>
</table>
OBELIX: OBesogenic Endocrine disrupting chemicals: Linking prenatal exposure to the development of obesity later in life

4 cohorts
Be, Nl, Sv, No

New biomarkers

Food intake

Exposure markers

Gene expression markers

Epigenetic markers

Biochemical markers

Health outcome

www.theobelixproject.org