Russian Children’s Study: Dioxins and Semen Quality

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Russian Children’s Study – primary aim was to prospectively study the impact of environmental exposures on pubertal development and growth

- Study Site description
- Study Design
- Exposure and Outcome Measures
- Results
- Conclusions
Study Site: Chapaevsk, Russia

- population 72,000, in Samara region, central Russia
- 950 km south-east of Moscow
- half of Chapaevsk area was occupied by chemical industries
- wide range of exposure to organochlorine compounds, including organochlorine pesticides
Prospective longitudinal cohort study
Study population (all boys 8-9 yrs old) was identified through health insurance and clinic records

Recruitment and Study Population: Recruitment began in 2003

Recruitment rate: (516/572) ~ 90%
High retention rate over 9 years - 73%

Recruitment flow diagram for boys in Russian Children’s Study

623 boys Identified\(^a\)
51 boys Ineligible\(^b\)
572 boys Eligible
56 boys Refused
516 boys Enrolled
17 boys Excluded\(^c\)
499 boys In cohort

\(^{a}\)used town records; \(^{b}\)dead, moved, not born in Chapaevsk, likely to relocate, or chronic illness; \(^{c}\)identified post-enrollment as orphans
Study Aims

• Assess the association of pre-pubertal serum concentrations of dioxins, PCBs, organochlorine pesticides and lead with:
  – pubertal development
    • onset, tempo and timing
  – somatic growth and body composition
  – reproductive hormones
  – semen quality
Study Design

- Annual physical examinations
  - Height, weight
  - Skin fold thickness
  - Single physician assessed pubertal staging
    - Genitalia (G1-G5): \textit{pubertal onset} G2+
    - Pubic hair (P1-P5, P6): \textit{pubertal onset} P2+
    - Testicular volume (TV: 1-25 cc): pubertal onset > 3 cc

- Questionnaires
  - Birth, demographic, medical and lifestyle
  - Food Frequency (biennial, local & non-local foods)

- Collection of blood (biennial) and urine (annual)
- Bioelectric impedance (body composition)
- Semen collection (since October 2012)
Exposure Assessment

- Baseline analysis at U.S. CDC
- Boys aged 8-9 yrs (n=482) and mothers (n=449)
  - 7 Dioxin congeners
  - 10 Furan congeners
  - 41 PCB congeners
  - 3 Organochlorine pesticides (OCPs)
    - HCB, bHCH, ppDDE
A Longitudinal Study of Peripubertal Serum Organochlorine Concentrations and Semen Parameters in Young Men: The Russian Children’s Study

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Flow diagram of semen quality sub-study nested within the Russian Children’s Study.

- **Collection of blood samples for organochlorine (OC) measurement.**
- **2003 - 2005**
  - **516 boys** recruited
    - (8-9 years old)
  - **124** lost to follow-up
  - **4** missing OC data
- **10 years of follow-up**
  - **383 boys** excluded:
    - **59** too young for semen collection
    - **49** declined participation
    - **144** pending invitation to provide semen sample
    - **3** had chronic disease
- **2015**
  - **133 young men**
    - (18-19 years old)
  - **256 semen samples**

Collection of covariate data: BMI, smoking, and alcohol.

Semen sample collection and measurement of time-varying covariates: season of semen collection, abstinence time, and time elapsed between semen collection and analysis.
Statistical Analysis of serum dioxin concentrations with semen parameters

Linear mixed models with random intercepts to account for multiple semen samples per participant.

Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4

Sperm Concentration (mill/mL) | Total Sperm Count (mill) | Total Motility (%) | Total Motile Sperm Count (mill)

+ Adjustment for covariates
Demographic characteristics and semen parameters of 133 young men (256 samples) in the Russian Children’s Study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (IQR) or n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic characteristics</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>18.3 (18.1–18.7)</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>21.0 (19.2–23.2)</td>
</tr>
<tr>
<td>Smoking status&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68 (51)</td>
</tr>
<tr>
<td>Alcohol consumption&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90 (68)</td>
</tr>
<tr>
<td><strong>Semen parameters</strong>&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>2.4 (1.8–3.5)</td>
</tr>
<tr>
<td>Sperm concentration (million/mL)</td>
<td>51.3 (26.6–78.8)</td>
</tr>
<tr>
<td>Total sperm count (million)</td>
<td>127 (61.0–222.0)</td>
</tr>
<tr>
<td>Sperm motility (A + B + C)&lt;sup&gt;e&lt;/sup&gt; (%)</td>
<td>64.0 (57.0–68.0)</td>
</tr>
<tr>
<td>Total motile sperm count (million)</td>
<td>80.5 (35.8–141.0)</td>
</tr>
<tr>
<td>Abstinence time (days)</td>
<td>2.9 (2.0–6.0)</td>
</tr>
</tbody>
</table>
## Comparative Table of Semen Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Russia (Chapaevsk)</th>
<th>Denmark (Copenhagen)</th>
<th>Spain (Murcia)</th>
<th>Sweden (Malmo)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of subjects</strong></td>
<td>133</td>
<td>4867</td>
<td>215</td>
<td>112</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>18.3</td>
<td>19</td>
<td>20.4</td>
<td>18.3</td>
</tr>
<tr>
<td><strong>Semen volume (ml)</strong></td>
<td>2.4</td>
<td>3.3</td>
<td>3</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Sperm concentration (million/ml)</strong></td>
<td>51.8</td>
<td>48</td>
<td>44</td>
<td>53</td>
</tr>
<tr>
<td><strong>Total sperm count (million)</strong></td>
<td>127</td>
<td>151</td>
<td>121</td>
<td>140</td>
</tr>
<tr>
<td><strong>Motility (%)</strong></td>
<td>64</td>
<td>68</td>
<td>57.2</td>
<td>-</td>
</tr>
</tbody>
</table>

**Median sperm concentration (million/ml)**

- Sweden (Malmo) 53
- Spain (Murcia) 44
- Denmark (Copenhagen) 48
- Russia (Chapaevsk) 51.8

In general, young Russian men have slightly better semen quality than young Spanish men and comparable with Danish and Swedish men.

3. Axelsson et al, 2015
Serum concentrations and TEQs for dioxins, furans, and PCBs measured at study enrollment (age 8–9 years of age) for 133 young men in the Russian Children’s Study.

<table>
<thead>
<tr>
<th>Toxic equivalent/concentration</th>
<th>Min</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEQs (pg TEQ/g lipid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCDD(^a)</td>
<td>0.35</td>
<td>1.77</td>
<td>2.9</td>
<td>4.2</td>
<td>12.1</td>
</tr>
<tr>
<td>PCDD TEQ</td>
<td>0.95</td>
<td>5.69</td>
<td>8.7</td>
<td>13.3</td>
<td>36.0</td>
</tr>
<tr>
<td>PCDF TEQ</td>
<td>0.55</td>
<td>3.20</td>
<td>4.8</td>
<td>7.1</td>
<td>50.6</td>
</tr>
<tr>
<td>Co-PCB TEQ(^b)</td>
<td>0.52</td>
<td>4.66</td>
<td>6.9</td>
<td>10.0</td>
<td>67.2</td>
</tr>
<tr>
<td>Total TEQ(^c)</td>
<td>1.88</td>
<td>16.8</td>
<td>21.9</td>
<td>33.3</td>
<td>107</td>
</tr>
<tr>
<td>Concentration (pg/g lipid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCDD</td>
<td>37.6</td>
<td>115</td>
<td>157</td>
<td>199</td>
<td>1,237</td>
</tr>
<tr>
<td>PCDF</td>
<td>14.4</td>
<td>29.4</td>
<td>44.5</td>
<td>63.3</td>
<td>406</td>
</tr>
<tr>
<td>Co-PCB(^d)</td>
<td>62.5</td>
<td>131</td>
<td>188</td>
<td>273</td>
<td>965</td>
</tr>
<tr>
<td>Concentration (ng/g lipid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΣPCBs(^e)</td>
<td>58.3</td>
<td>152</td>
<td>235</td>
<td>352</td>
<td>1,500</td>
</tr>
</tbody>
</table>
Comparison of dioxin/furan TEQ levels with other populations

PCDD/PCDF TEQ levels (pg/g lipid) in adolescents

- Russia, Chapaevsk (1999, n=30, 14-16 yrs) boys
- Russia, Chapaevsk (2003-05, n=482, 8-9 yrs) boys
- Germany, (1998-99, n=207, 10yrs) boys
- Germany, (2000-01, n=176, 10yrs) boys
- Germany, (2002-03, n=214, 10yrs) boys
- Russia, Chapaevsk (1999, n=30, 14-16 yrs) boys
- Russia, Chapaevsk (2003-05, n=482, 8-9 yrs) boys
- Netherlands, Amsterdam (2005, n=12, 14-19 yrs) mixed gender
- Australia, (2006, n=17, 1.6-16 yrs) mixed gender

RED = MEAN  BLUE = MEDIAN
Percent Contribution of TCDD/Dioxins/Furans/PCBs to Total 2005 TEQs (pg TEQ/g lipid)

- Co-planar PCBs: 31%
- Furans: 21%
- Other Dioxins: 27%
- TCDD: 13%
- Mono-ortho PCBs: 8%
Adjusted mean semen parameters among 133 men (256 samples) from the Russian Children’s Study, by childhood serum TCDD concentrations.

Data are presented as predicted marginal means (95% confidence intervals) by quartiles of TCDD concentrations (represented by the medians) adjusted for BMI, smoking status, alcohol drinker, season of sample collection, and abstinence time at the mean level of continuous covariates and adjusted for frequency of categorical measures. Motile sperm and total motile sperm count models were further adjusted by time elapsed between semen collection and analysis.
Adjusted mean semen parameters among 133 men (256 samples) from the Russian Children’s Study, by childhood serum TCDD concentrations.

Men in the highest quartile of serum TCDD TEQs had:

- 40% lower sperm concentration,
- 29% lower total sperm count,
- 30% lower total motile sperm count, compared to those in the lowest quartile.
Spearman correlations between Serum Dioxin TEQs, Organochlorine Pesticides, and Blood Lead Levels (BLLs).

<table>
<thead>
<tr>
<th></th>
<th>TCDD TEQs (pg/g lipid)</th>
<th>PCDD TEQs (pg/g lipid)</th>
<th>HCB (ng/g lipid)</th>
<th>BHCH (ng/g lipid)</th>
<th>DDE (ng/g lipid)</th>
<th>BLLs (mcg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCDD TEQs (pg/g lipid)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>r=0.77</td>
<td>r=0.57</td>
<td>r=0.41</td>
<td>r=0.49</td>
<td>r=0.49</td>
<td>r=0.04</td>
</tr>
<tr>
<td></td>
<td>p&lt;.0001</td>
<td>p&lt;.0001</td>
<td>p&lt;.0001</td>
<td>p&lt;.0001</td>
<td>p&lt;.0001</td>
<td>p=0.47</td>
</tr>
<tr>
<td>PCDD TEQs (pg/g lipid)</td>
<td>r=0.77</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt;.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCB (ng/g lipid)</td>
<td>r=0.33</td>
<td>r=0.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt;.0001</td>
<td>p&lt;.0001</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BHCH (ng/g lipid)</td>
<td>r=0.44</td>
<td>r=0.49</td>
<td>r=0.41</td>
<td></td>
<td>r=0.49</td>
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</tr>
<tr>
<td></td>
<td>p&lt;.0001</td>
<td>p&lt;.0001</td>
<td>p&lt;.0001</td>
<td></td>
<td>p&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>DDE (ng/g lipid)</td>
<td>r=0.26</td>
<td>r=0.26</td>
<td>r=0.22</td>
<td>r=0.49</td>
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<td>0.06</td>
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<tr>
<td></td>
<td>p=0.0015</td>
<td>p=0.0014</td>
<td>p=0.0081</td>
<td>p&lt;.0001</td>
<td></td>
<td>p=0.41</td>
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<tr>
<td>BLLs (mcg/dl)</td>
<td>r=0.04</td>
<td>r=0.10</td>
<td>r=-0.06</td>
<td>r=0.21</td>
<td>r=0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.47</td>
<td>p=0.10</td>
<td>p=0.45</td>
<td>p=0.01</td>
<td>p=0.41</td>
<td></td>
</tr>
</tbody>
</table>
Do the negative associations between TCDD and semen parameters remain when further adjusting for serum OCPs concentrations (separately) or when further adjusting for blood lead levels?

When models were further adjusted for serum concentrations of hexachlorobenzene (HCB), \( \beta \)-hexachlorocyclohexane (\( \beta \)HCH), \( p,p' \)-dichlorodiphenyldichloroethylene (DDE), and blood lead levels (BLLs), the dose-response relationships between TCDD and semen parameters remained similar.
Data are presented as predicted marginal means (95% confidence intervals) by quartiles of PCDD TEQs concentrations (represented by the medians) adjusted for BMI, smoking status, alcohol drinker, season of sample collection, and abstinence time at the mean level of continuous covariates and adjusted for frequency of categorical measures. Motile sperm and total motile sperm count models were further adjusted by time elapsed between semen collection and analysis.

Adjusted mean semen parameters among 133 men (256 samples) from the Russian Children’s Study, by childhood serum PCDD TEQs concentrations.
Men in the highest quartile of serum PCDD TEQs had

39% lower sperm concentration,

36% lower total sperm count, and

40% lower total motile sperm count, compared to those in the lowest quartile.
Do the negative associations between PCDD TEQs and semen parameters remain when further adjusting for serum OCPs concentrations (separately) or when further adjusting for blood lead levels?

When models were additionally adjusted for serum concentrations of HCB, βHCH and DDE, the associations between PCDD TEQs and semen volume, total sperm count and total motile sperm count became stronger; however, the associations between PCDD TEQs and sperm concentration became somewhat attenuated. Associations remained when models were further adjusted for BLLs.
Conclusions

• Peri-pubertal serum dioxin concentrations (TCDD and PCDD) were associated with reduced semen quality measured ~10 years later among a general population sample of Russian young adult men.

• Associations were robust to adjustment for OCPs and other exposures.

• ? Implications for the sperm epigenome.
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