"From field to lab" and "from lab to field" chemicals, *in vitro, in vivo vs. in situ*

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The in situ problem

Pilnok reservoir

- Ostrava-Karvina region

Pontastacus leptodactylus

INTERSEX:
Females w/ male gonopods?
Males carrying eggs?
Major questions / Objectives

• What is the cause of intersex occurrence?

• Can ED-chemicals be identified?

• Can the mechanism be understood?

• Can we induce ED experimentally?
Integrated assessment

Sediments collected

- Pilnok
- Reference localities
  Karvina pond, Steinlach creek

Organic extraction

Chemical analyses

In vitro effect testing

In vivo effects
Chemical contamination ?

[Images of laboratory equipment]
### Organics (ng/g d.w.)

<table>
<thead>
<tr>
<th></th>
<th>Pilnok</th>
<th>Karvina</th>
<th>Steinlach</th>
<th>Dřevnice</th>
<th>Morava</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>16 PAHs</strong></td>
<td>18420</td>
<td>10075</td>
<td>422</td>
<td>9427</td>
<td>5263</td>
</tr>
<tr>
<td><strong>Σ PCBs</strong></td>
<td>18.7</td>
<td>6.7</td>
<td>0.86</td>
<td>14.8</td>
<td>13.6</td>
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<tr>
<td><strong>Σ DDTs</strong></td>
<td>1.7</td>
<td>2.8</td>
<td>0.33</td>
<td>5.8</td>
<td>22.4</td>
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- High concentrations of PAHs in PILNOK
- Other POPs (PCBs, OCPs) ~ low-average
## Metals (µg/g d.w.)

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<tr>
<td>Pb</td>
<td>47</td>
<td>109</td>
<td>3.5</td>
</tr>
<tr>
<td>Cu</td>
<td>29.1</td>
<td>31</td>
<td>2.9</td>
</tr>
<tr>
<td>Zn</td>
<td>45.7</td>
<td>86</td>
<td>21.5</td>
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Bio-TEQs (H4IIE.luc bioassay)

AhR-mediated activity

- **Steinlach**
- **Pilnok**

**Sediment extracts (mg/well)**

**TCDD calibration**

**ng TCDD/well**

**% TCDD max induction**

**Sediment extracts (mg/well)**

**TCDD**

**Bioioio--TEQs**

**((H4IIE.luc bioassay))**
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</tr>
<tr>
<td>Chem-TEQs*</td>
<td>1.1</td>
<td>1.2</td>
<td>0.002</td>
<td>0.9</td>
</tr>
<tr>
<td>Bio-TEQs&lt;sub&gt;crude&lt;/sub&gt;</td>
<td>70</td>
<td>13</td>
<td>2.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Bio-TEQs&lt;sub&gt;H₂SO₄&lt;/sub&gt;</td>
<td>6.9</td>
<td>0.2</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
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* WHO TEFs for PCBs & IEFs for PAHs (Machala et al. 2001)
Effects *in vitro*?
Assessment of in vitro effects
Assessment of *in vitro* effects

**SYNTHESIS OF STEROID HORMONES**

**ACTION OF STEROID HORMONES**
MVLN bioassay

- PILNOK estrogenic
- Reference sediments - no effects
AR-yeast bioassay

- PILNOK anti-androgenic
- Reference sediments - no effects
Effects on steroidogenesis

- gene modulation (DNA->mRNA)
  Real Time PCR
- protein levels
- enzyme activities
- hormones produced

H295R cell line  bioassay development supported by US EPA grant
Pilnok sediment extracts modulate steroidogenesis in H295R cells

Blaha et al. 2006 Environment International
Effects on steroidogenesis

Cholesterol
- CYP11A↓
  - Pregnenolone
- 3β-HSD↓
  - Progesterone
- CYP21↓
  - 11-Deoxy corticosterone
- CYP11B2↓
  - Corticosterone
  - Aldosterone
  - Zona glomerulosa

CYP17
- 17α-OH-
  - Pregnenolone
- 17α-OH-
  - Progesterone
- CYP21↓
  - 11-Deoxycortisol
- CYP11B1↓
  - Cortisol
  - Zona fasciculata

CYP17
- DHEA
  - 3β-HSD
- CYP17
  - Androstene-dione
  - 17β-HSD
- CYP17
  - Testosterone
  - 17β-Estradiol
  - Zona reticularis
In vivo effects?
Assessment of *in vivo* effects

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*Potamopyrgus antipodarum*
- 2,5,8 weeks: mortality, embryos, hsp70

*Gammarus fossarum*
- 12 weeks: mortality, juveniles, histopathology
Sex-dependent differences in effects – I

! sensitivity of males
Sex-dependent differences in effects – II

variable effects in hepatopankreas

control

male

male

female

female
Pilnok sediments shift reproduction cycle in females (towards maturity)

**OOCYTE STAGE**
(vitellogenesis)

**Relative ratio**

- Pre-
- Early-
- Later-
- Mature

- **Control**
- **Steinlach**
- 50% Pilnok
- 100% Pilnok

1 mm
Pilnok sediments induce production and size of F1 juveniles.
Pilnok sediments (and organic extracts) stimulate production of embryos

No. Embryos / female (% of control)

week

1 2 3 4 5 6 7 8

100 110 120 130 140 150 160

--- O --- Pilnok-Sediment

--- - - Pilnok-Extrakt

75% Pilnok

75% Pilnok

1 mm
SUMMARY 1

1) Routine (PAHs, PCBs, OCPs) analytical data did not clearly indicate excessive EDs
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2) Biological experiments complement chemical analyses and suggest elevated levels of unknown EDs in PILNOK pond ("PAH derivatives").
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3) In vitro assessment indicate „feminization“ - estrogenicity and anti-androgenicity.
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1) Routine (PAHs, PCBs, OCPs) analytical data did not clearly indicate excessive EDs

2) Biological experiments complement chemical analyses and suggest elevated levels of unknown EDs in PILNOK pond (? PAH derivatives)

3) In vitro assessment indicate „feminization“ - estrogenicity and anti-androgenicity

4) New mechanism ? (↑↑CYP11B2 / steroidogenesis)
SUMMARY 2

5) *In vivo* experiments with two invertebrate species revealed EDCs in Pilnok sediments „stimulated reproduction“
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6) Differences

- vertebrate *in vitro*: „feminization“
- invertebrate *in vivo*: „reproduction“
Answers to our questions?

- What is the cause of intersex occurrence?
  - contaminants associated with sediments
- Can ED-chemicals be identified?
  - partially yes: organic (labile) contaminants
- Can the mechanism be understood?
  - (partially) yes: feminization / speeding up reproduction
- Can we induce ED experimentally?
  - Yes!
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