ASSESSMENT OF ENDOCRINE DISRUPTIVE POTENTIAL OF COMPLEX POLLUTANT MIXTURES IN THE RIVER ECOSYSTEM AFFECTED BY A MAJOR CITY: BIOASSAYS AND CHEMICAL ANALYSES

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Wastewater treatment plants (WWTPs)

- **influent mixtures**
  - spectrum of molecules from domestic, agricultural, and/or industrial wastes

- **effluent mixtures**
  - incompletely removed pollutants
  - metabolites formed during treatment processes

*(WWTPs are not a source - environmental problems would be much worse without them)*

Documented effects of WWTPs on reproductive health in wild animals

- intersexuality and reproductive problems
- effects persist far distant from WWTP
Evaluation of ED potency of environmental samples

**Chemical analysis**
- Analysis of wide spectra of potential ED-compounds
- Good quantification of individual chemicals
- Resource- and time-intensive
- Limited information on the biological effects of mixtures
- Do not consider possible interactions among individual chemicals

**Bioassays**
- Reflect overall biological activity of compounds, consider possible interactions
- Indicate toxicologically important compounds (not determined analytically)
- Allow screening of large numbers of samples, fast, cheap
- *Quantification & calibration? Thresholds?*
- *Outputs for risk management?*
Assessment of contamination by ED-compounds in the river ecosystem

Scope of the study

- Impact of major city on the aquatic ecosystem by a complex joint biological / chemical assessment

- Multiple types of samples studied (water, sediments, PAS)

- Series (battery) of *in vitro* bioassays
  - general toxicity (cytotoxicity)
  - compounds with specific modes of action

- Chemical analyses (POPs, polar compounds)
Assessment of contamination by ED-compounds in the river ecosystem

- region of Brno city, Czech Republic, Central Europe
- basins of two rivers - Svratka and Svitava
- 400,000 inhabitants, many industrial activities
- large and modern wastewater treatment plant (WWTP)

1 Svratka, Kninicky
2 Svratka, before confluence
3 Svitava, Bilovice nad Svitavou
4 Svitava, before confluence
5 WWT influent
6 WWT effluent
7 Svratka, Rajhradice
8 Svratka, Zidlochovice
9 Bobrava, Popovice
Studied samples - 1

Study 1 - WWTP - seasonal variability

influent and effluent samples
1 year study *(May 2007 – April 2008)*

Sampled every month (12 periods)
24 h composite / automatic sampler

Solid phase extraction (SPE) concentrated

Focused on effects - bioassays - no detailed chemistry
Studied samples - 2

Impact of the Brno city (2007) - 9 localities

- sediments – grab samples
  - integrative measure of exposure of the aquatic environment
  - sink/source for a number of environmental pollutants

- water - passive samples (PAS)
  - time-weighted concentrations of contaminants
  - include episodic events
  - detection /concentration/ of ultra-trace contaminants

Semipermeable membrane devices (SPMD):

- hydrophobic contaminants (PAHs, PCBs, OCPs, PCDD/Fs, alkylated phenols, organophosphate insecticides, pyrethroid insecticides, neutral organometallic compounds, certain heterocyclic aromatic compounds)

Polar organic chemical integrative samplers (POCIS):

- hydrophilic contaminants - pesticides, natural and synthetic hormones, and other wastewater-related contaminants, drug residues
Bioassays

**Dioxin-like activity:** Aryl hydrocarbon receptor (AhR)-mediated effects
PCDDs/Fs, PAHs, PCBs

- **H4IIE-luc:** transfected rat hepatocarcinoma cells

**Xenoestrogenity / Antiestrogenity:** Estrogen receptor (ER)-mediated effects
PCDDs/Fs, PAHs, PCBs, OH-PCBs, alkylphenols, natural and synthetic hormones

- **MVLN:** transfected human breast carcinoma cells

**Xenoandrogenity / Antiandrogenity:** Androgen receptor (AR)-mediated effects
pesticides, pharmaceuticals

- **MDA-kb2:** transfected human breast carcinoma cell line
- Genetically modified *Saccharomyces cerevisiae*

- **Cytotoxicity** (*specific effects at NON-cytotoxic concentrations*)
WWTP - one year study
RESULTS 1 - WWTP one year study

- GRAB SAMPLES - high cytotoxicity at WWTP influent (20-times dilutions - acute toxicity)
- effluent - mostly no cytotoxic effects

Cytotoxicity

Waste water dilution causing 25% cytotoxicity

<table>
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<tr>
<th>Month</th>
<th>Influent</th>
<th>Effluent</th>
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<tr>
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<td>March 08</td>
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<tr>
<td>April 08</td>
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</tbody>
</table>

Waste water dilution

Viability %
RESULTS 1 - WWTP one year study

- POCIS differences between Influent vs. Effluent less pronounced

- Concentration of the major portion of highly toxic compounds
- Sampling efficiency in dirty waste water
- Biofouling

Cytotoxicity - POCIS

- Spring 2007
- Autumn 2007
- Spring 2008
- Autumn 2008

Cytotoxicity

Cytotoxicity (1/IC50)

WWTP - influent

WWTP - effluent
RESULTS 1 - WWTP one year study

- Water GRAB (24h composite) SAMPLES
- Variable performance of WWTP (removal 0 - 81%)

AhR- (dioxin-like)

![Graph showing AhR levels in influent and effluent over time]

- ng TEQ / L
- Months: May 07 to April 08
RESULTS 1 - WWTP one year study

- Water PASSIVE SAMPLERS
  - SPMD high effects as expected (hydrophobic cmpnds)
  - POCIS ?
    - Metabolites / derivatives
    - Sampling efficiency / biofouling at the influent

Dioxin-like activity - SPMD

Dioxin-like activity - POCIS
RESULTS 1 - WWTP one year study

- Water GRAB (24h composite) SAMPLES
  WWTP influent: 5 to 147 ng EEQ/L
  WWTP effluent: from 0.1 to 4 ng EEQ/L
- Removal 81 to >98%

Estrogenicity - POCIS

- LOD 0.6 ng/POCIS

Graph showing estrogenicity over a year with influent and effluent data for WWTP.

Influent
Effluent

EEQ (ng E2/membrane)

May 07
June 07
July 07
August 07
September 07
October 07
November 07
December 07
January 08
February 08
March 08
April 08

ng EEQ / L
RESULTS 1 - WWTP one year study

- GRAB SAMPLES
  WWTP influent: 18 to 87 ng AEQ/L
  WWTP effluent - no androgenicity
- Removal >95% - 99%

Androgenicity

WWTP influent: 18 to 87 ng AEQ/L
WWTP effluent - no androgenicity

Removal >95% - 99%

Androgenicity - POCIS

LOD 1.7 ng/POCIS
Study 2 - Impact of the Brno city

1. Svatka, Kninicky
2. Svatka, before confluence
3. Svitava, Bilovice nad Svitavou
4. Svitava, before confluence
5. WWT influent
6. WWT effluent
7. Svatka, Rajhradice
8. Svatka, Zidlochovice
9. Bobrava, Popovice
High cytotoxicity (especially Sediments, POCIS)
Locality 2 (below Brno), WWTP & sediment 7 (?)
RESULTS 2 - City of Brno

- **SPMD**
  - 5 ng TEQ\textsubscript{bio}/g dw

- **POCIS**
  - 2 ng TEQ\textsubscript{bio}/g dw

- **Sediments**
  - 50 ng TEQ\textsubscript{bio}/g dw

- **AhR-** (dioxin-like)

- High levels - WWTP effluent & downstream (no. 7)
- Correlation - SPMD vs. Sediments (hydrophobic cmpnds)
RESULTS 2 - City of Brno

ESTROGENICITY

ANDROGENICITY

- no effects in sediments & SPMD

- POCIS - WWTP influent only

Significant „ANTI-“ effects
RESULTS 2 - City of Brno

ANTI-estrogenicity

SPMD

POCIS

Sediments

1 2 3 4 5 6 7 8 9

POCIS !

High levels - WWTP effluent & downstream (no. 7)
RESULTS 2 - City of Brno

- **SPMD**
- **POCIS**
- **Sediments**

- **ANTHI-androgenicity**

- SPMD: high levels downstream of Brno
### RESULTS 2 - City of Brno

**Chemical analyses**

**POCIS – polar compounds**

<table>
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<tr>
<th>POCIS</th>
<th>Sampling site</th>
<th>Pesticides</th>
<th>Antibiotics</th>
<th>Other pharmaceuticals</th>
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### RESULTS 2 - City of Brno

**Chemical analyses – SPMD, Sediments**

<table>
<thead>
<tr>
<th>SPMD Sampling site</th>
<th>PAHs</th>
<th>PCBs</th>
<th>OCPs ng/SPMD</th>
<th>Triclosan</th>
<th>MeTriclosan</th>
<th>PBDEs</th>
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<table>
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<th>Sediment Sampling site</th>
<th>PAHs</th>
<th>PCBs</th>
<th>OCPs μg/kg</th>
<th>Triclosan</th>
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SUMMARY 1

WWTP INFLUENT vs. EFFLUENT

- Seasonal variability in effluents
  - summer > winter/spring - AhR, ER (… with exceptions)

- significant CYTOTOXICITY in influents - correlates with high levels of pollutants

- XENOANDROGENS and XENOESTROGENS in influent – polar compounds

- high elimination efficiency of modern WWTP (>95-98%)

- DIOXIN LIKE COMPOUNDS - lower elimination efficiency (0 – 81%)

??? Effluent values HIGHER than Influent
(AhR in SPMD and POCIS, cytotox in POCIS, anti-ER in SPMD)
  1. lower removal efficiency for these types of compounds
  2. influent (raw waste waters) incomplete sampling compared to effluent
  3. release of particle-bound pollutants during treatment
RIVER ECOSYSTEM (POCIS, SPMD, SEDIMENTS)

- Clear pattern in sediments - increase below the city of Brno
- High levels of DIOXIN-LIKE pollutants in all matrices (sediment, SPMD, POCIS) → presence of both lipophilic and polar compounds / correlation with analyses
- ANTIESTROGENIC and ANTIANDROGENIC activities in most samples (including sites above Brno)
- City (WWTP) – contributes to the contamination with dioxin-like, antiestrogenic and hydrophobic antiandrogenic compounds
CONCLUSIONS

- complex assessment of compounds with specific modes of action in various components of the river ecosystem

- both hydrophobic and hydrophilic compounds contribute

- complementarity of the bioassay results with chemical analysis data (e.g. antiandrogens in SPMDs below the city / no correlation with analyses)

- battery of bioassays
  - applicable for various types of matrices (environmental mixtures)
  - provides a „pattern“ view („toxicity profile“ - NORMAN network approach)
ACKNOWLEDGEMENTS

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Thank your for listening!