



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

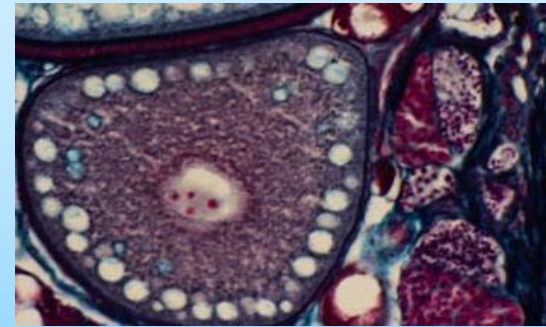
- incompletely removed pollutants
- metabolites formed during treatment processes

(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



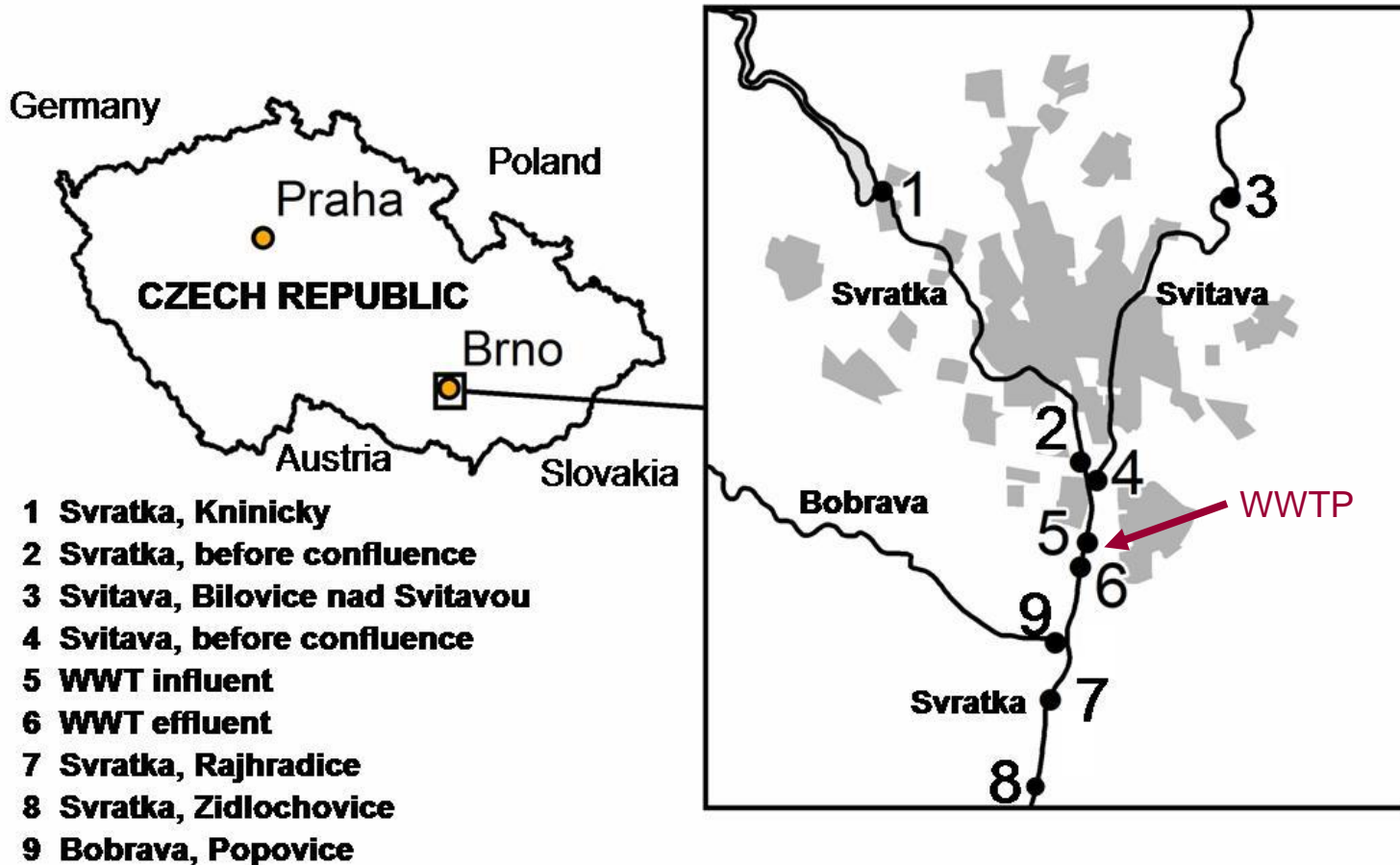
Scope of the study



- 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 - Svatka and Svitava
- 400,000 inhabitants, many industrial activities
- large and modern wastewater treatment plant (WWTP)



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)

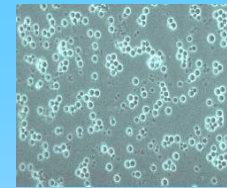
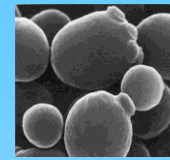


Polar organic chemical integrative samplers (POCIS):

- 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

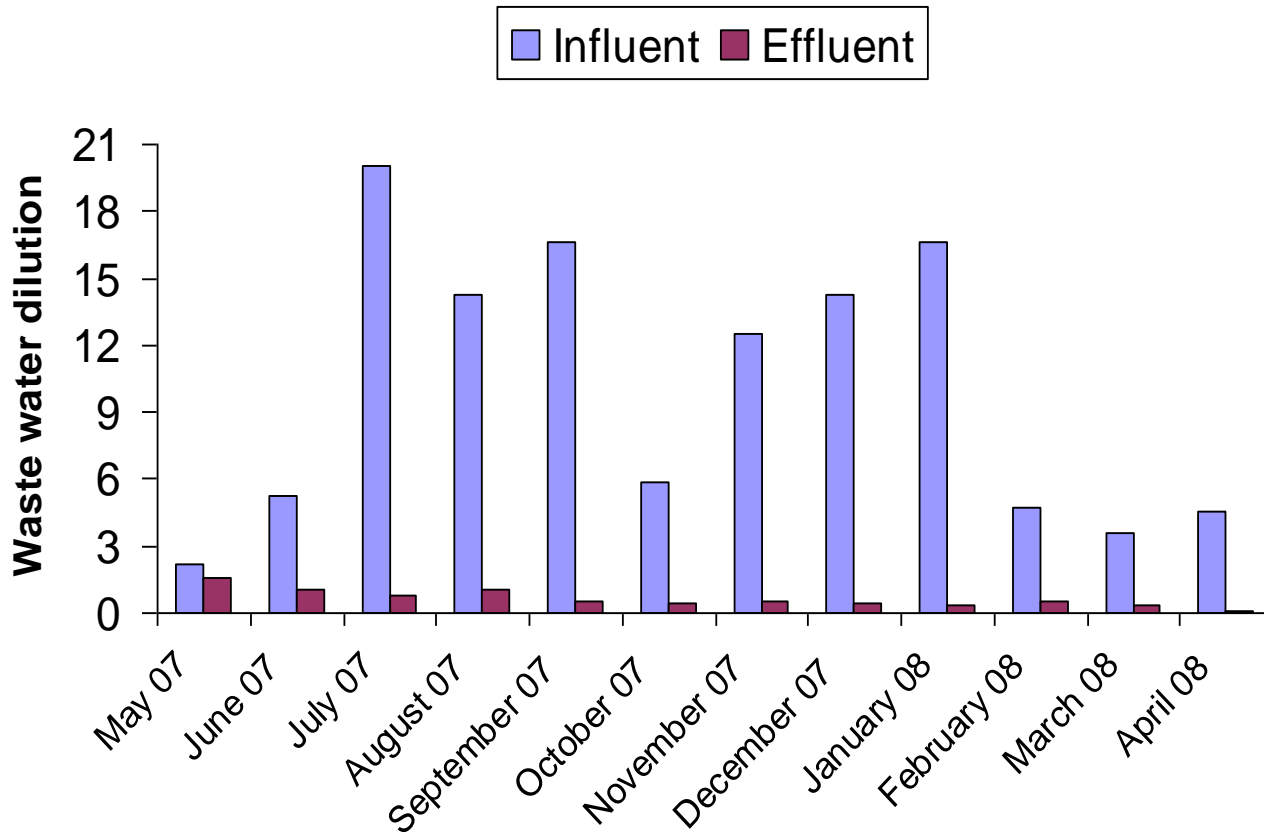


RESULTS 1 - WWTP one year study

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

Cytotoxicity

Waste water dilution causing 25% cytotoxicity



RESULTS 1 - WWTP one year study

➤ POCIS

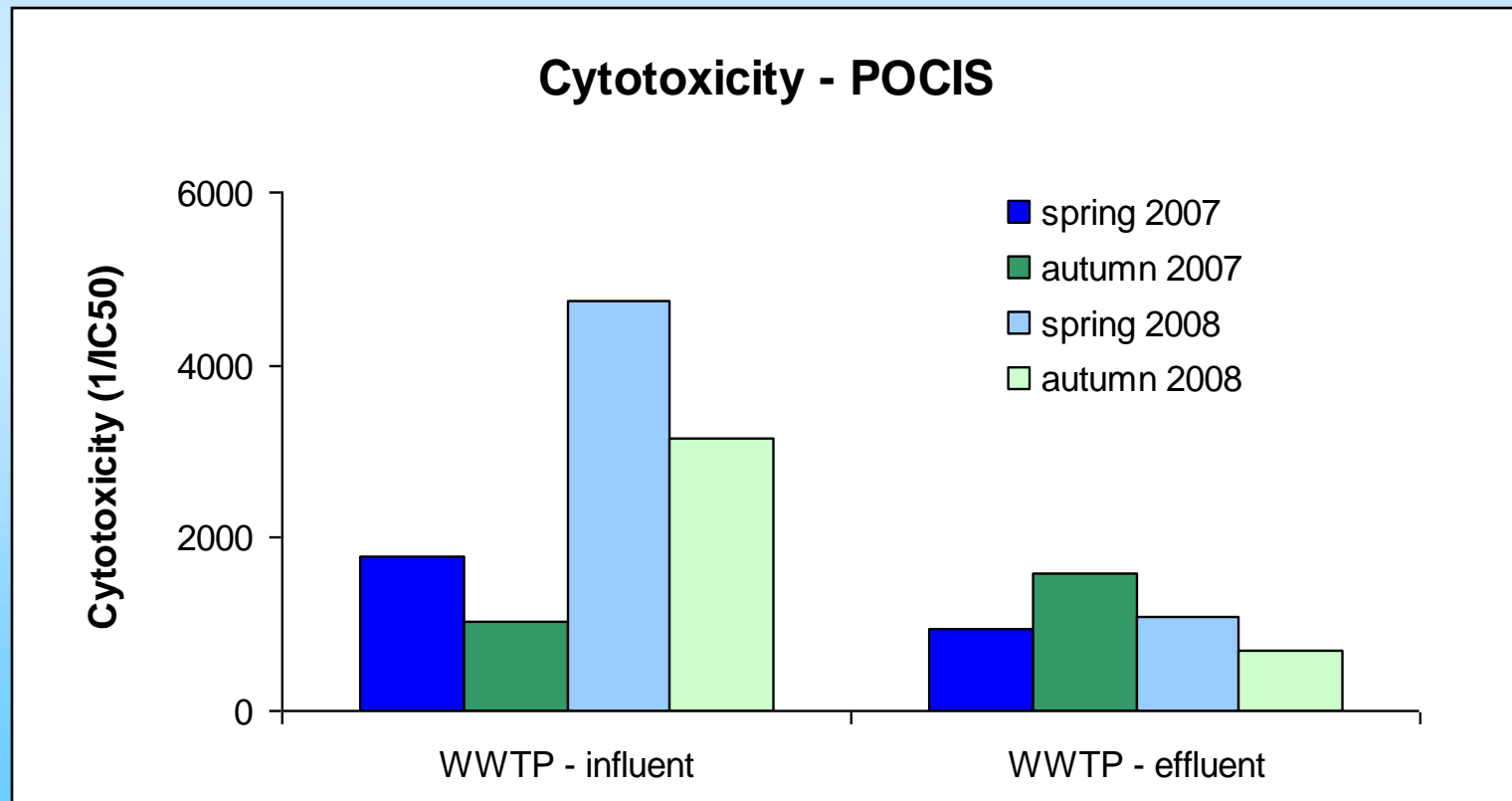
- differences between Influent vs. Effluent less pronounced

Cytotoxicity

? Concentration of the major portion of highly toxic compounds

? Sampling efficiency in dirty waste water

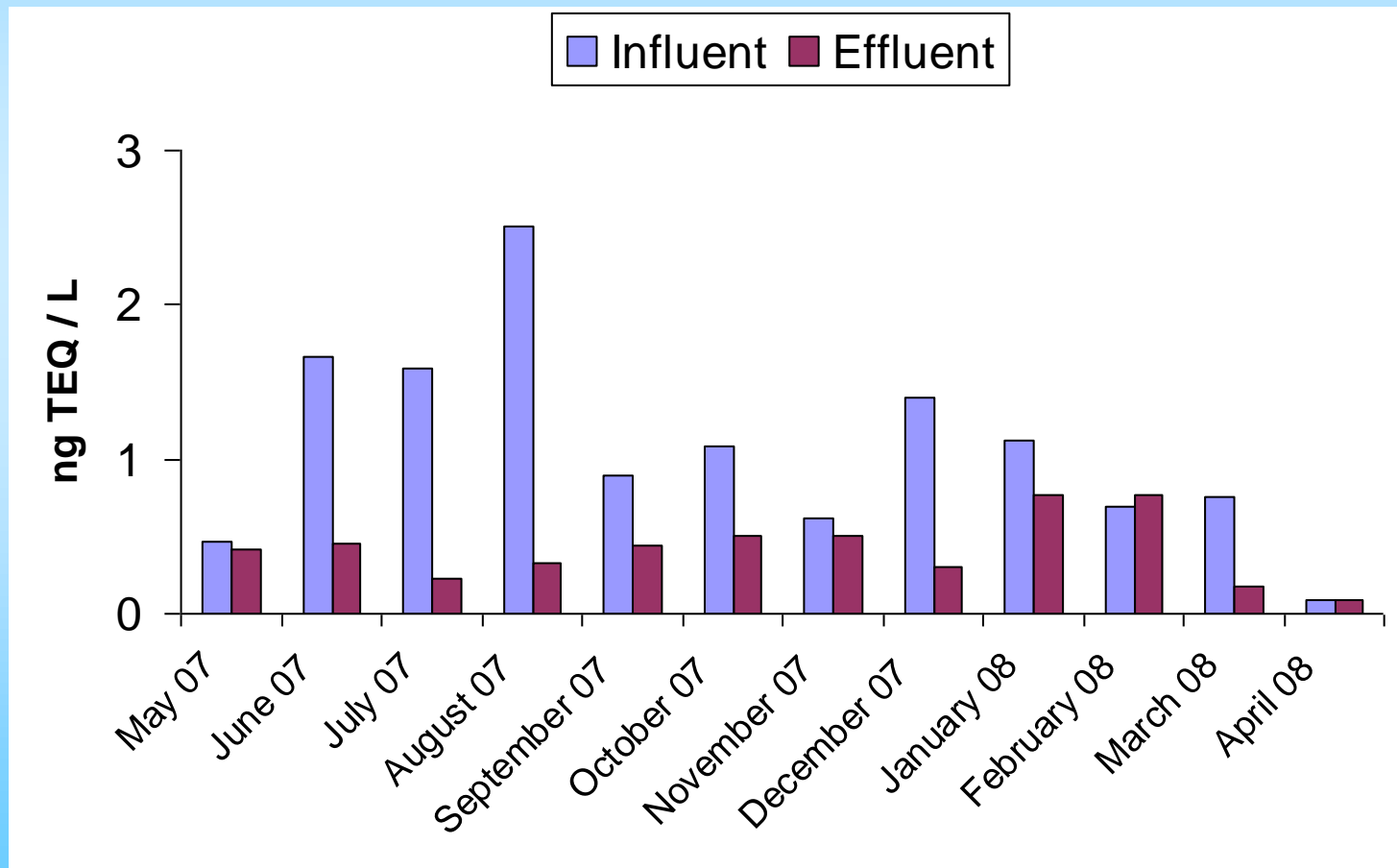
? Biofouling



RESULTS 1 - WWTP one year study

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

AhR-
(dioxin-like)



RESULTS 1 - WWTP one year study

➤ Water PASSIVE SAMPLERS

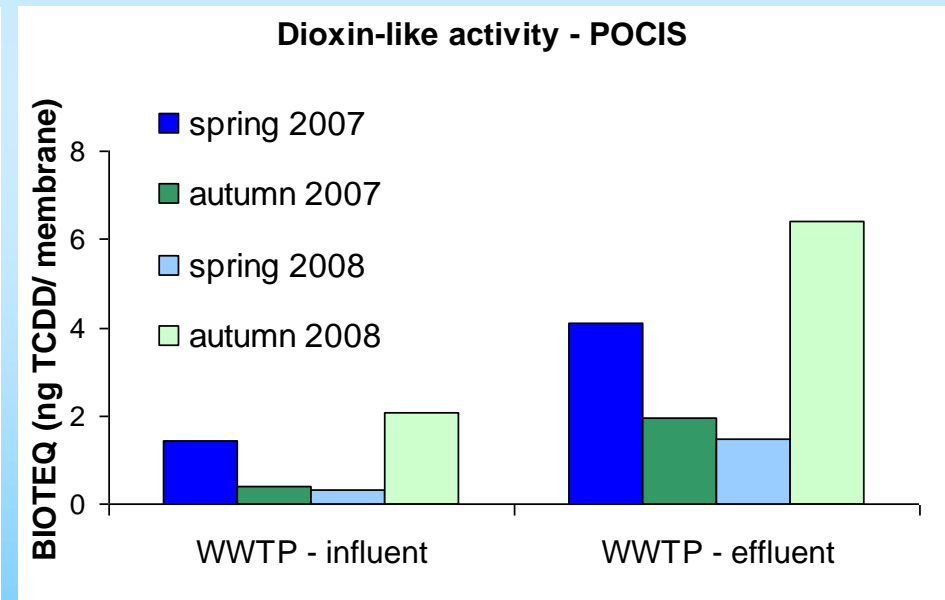
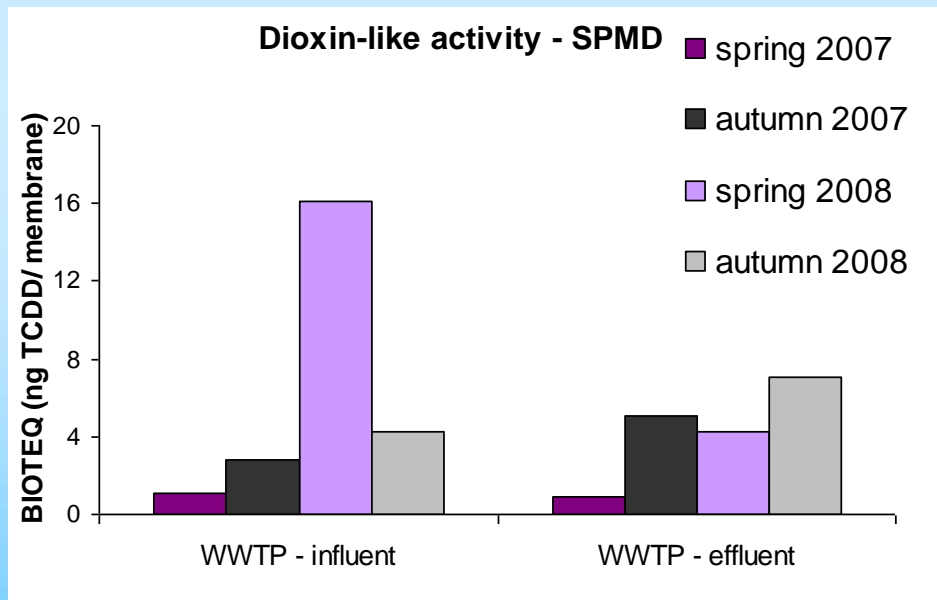
➤ SPMD high effects as expected (hydrophobic cmpnds)

➤ POCIS !?

? *Metabolites / derivatives*

? *Sampling efficiency / biofouling at the influent*

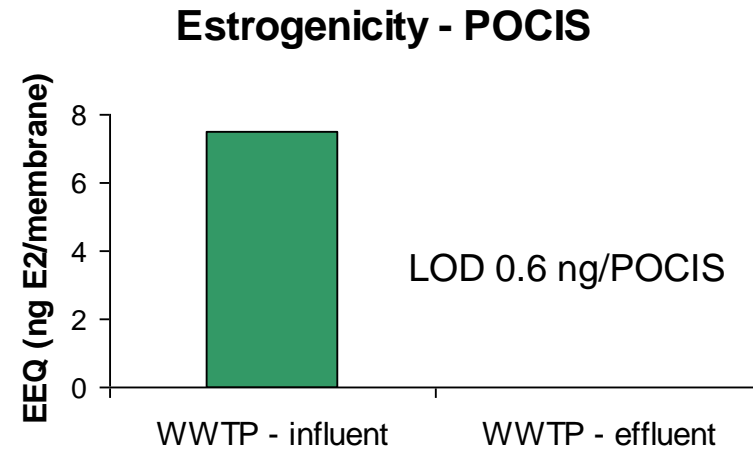
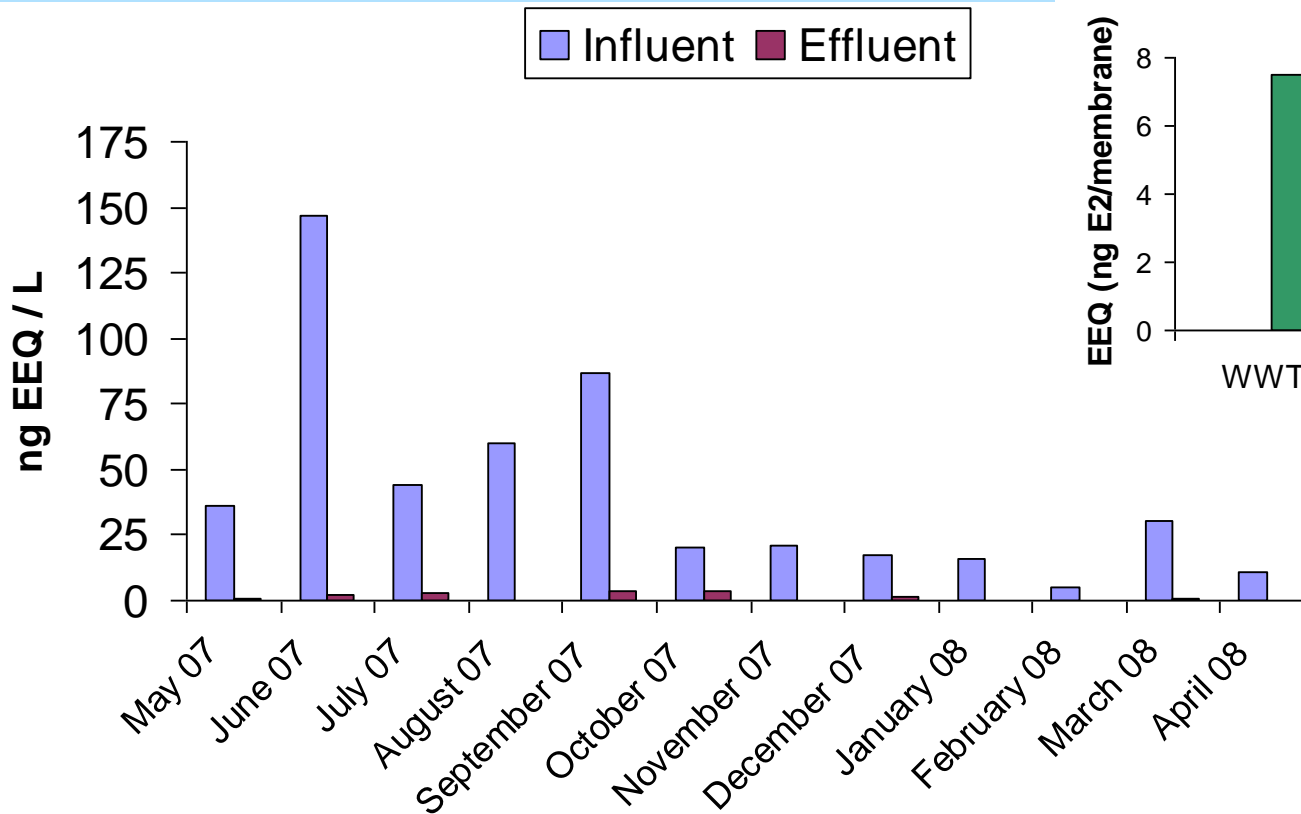
AhR- (dioxin-like)



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



RESULTS 1 - WWTP one year study

➤ GRAB SAMPLES

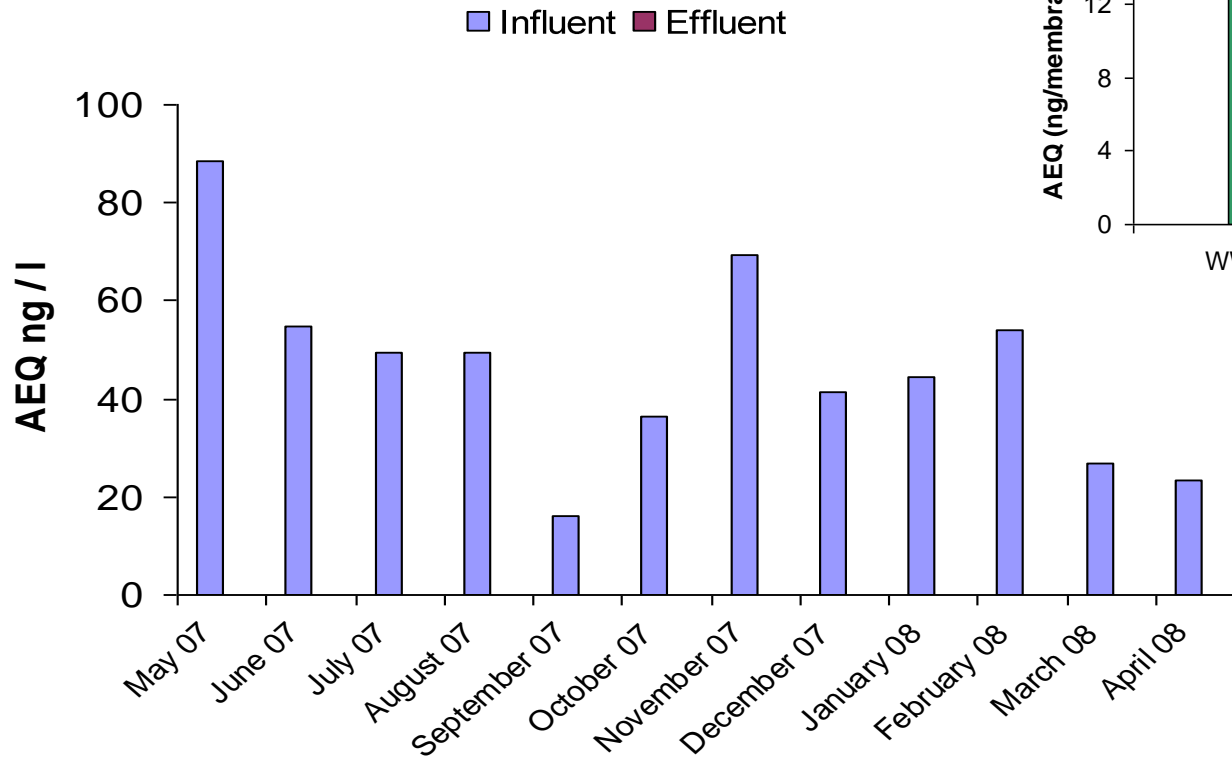
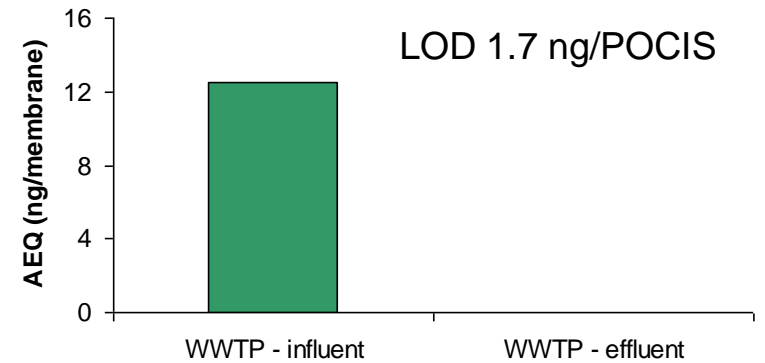
WWTP influent: 18 to 87 ng AEQ/L

WWTP effluent - no androgenicity

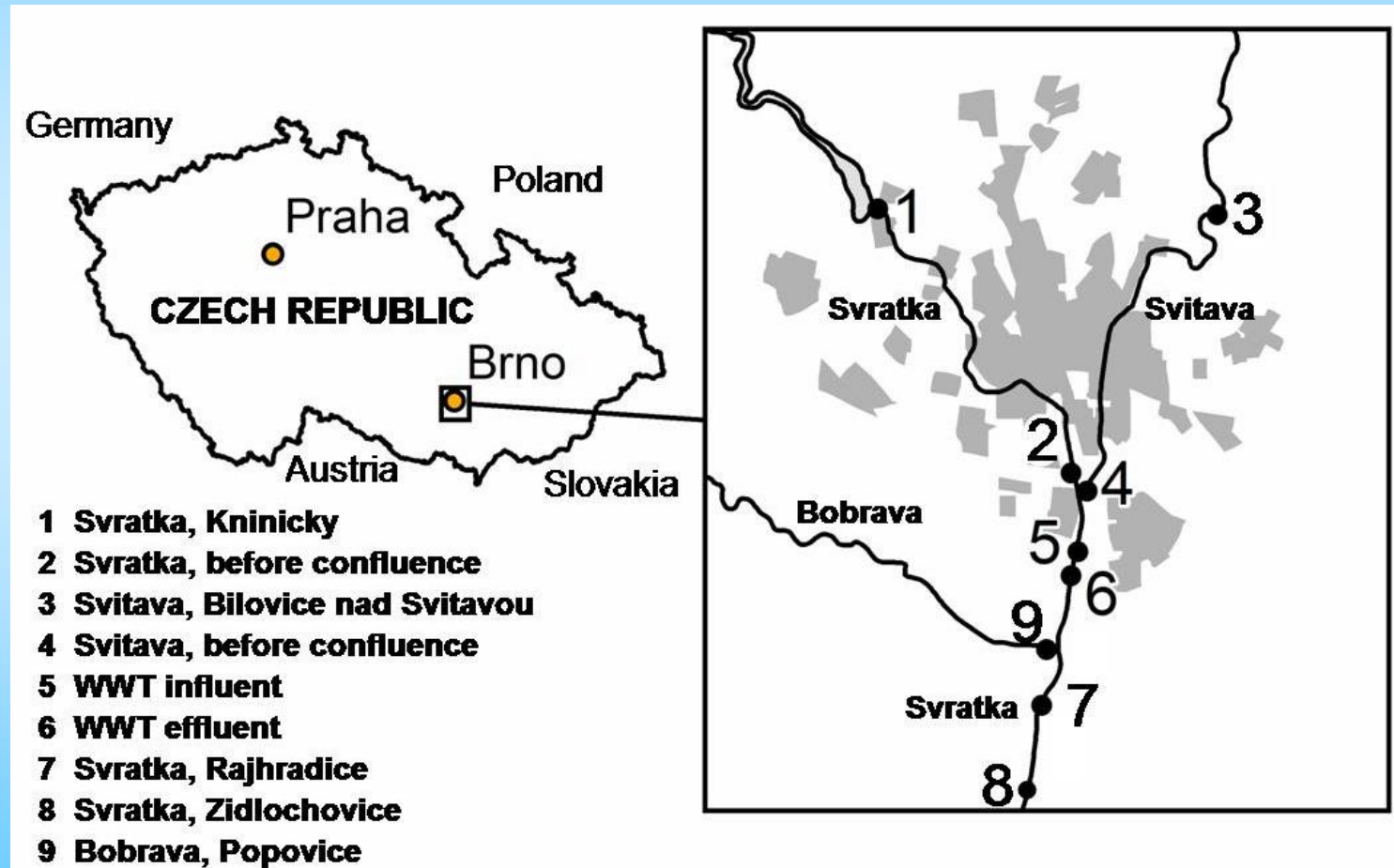
➤ Removal >95% - 99%

Androgenicity

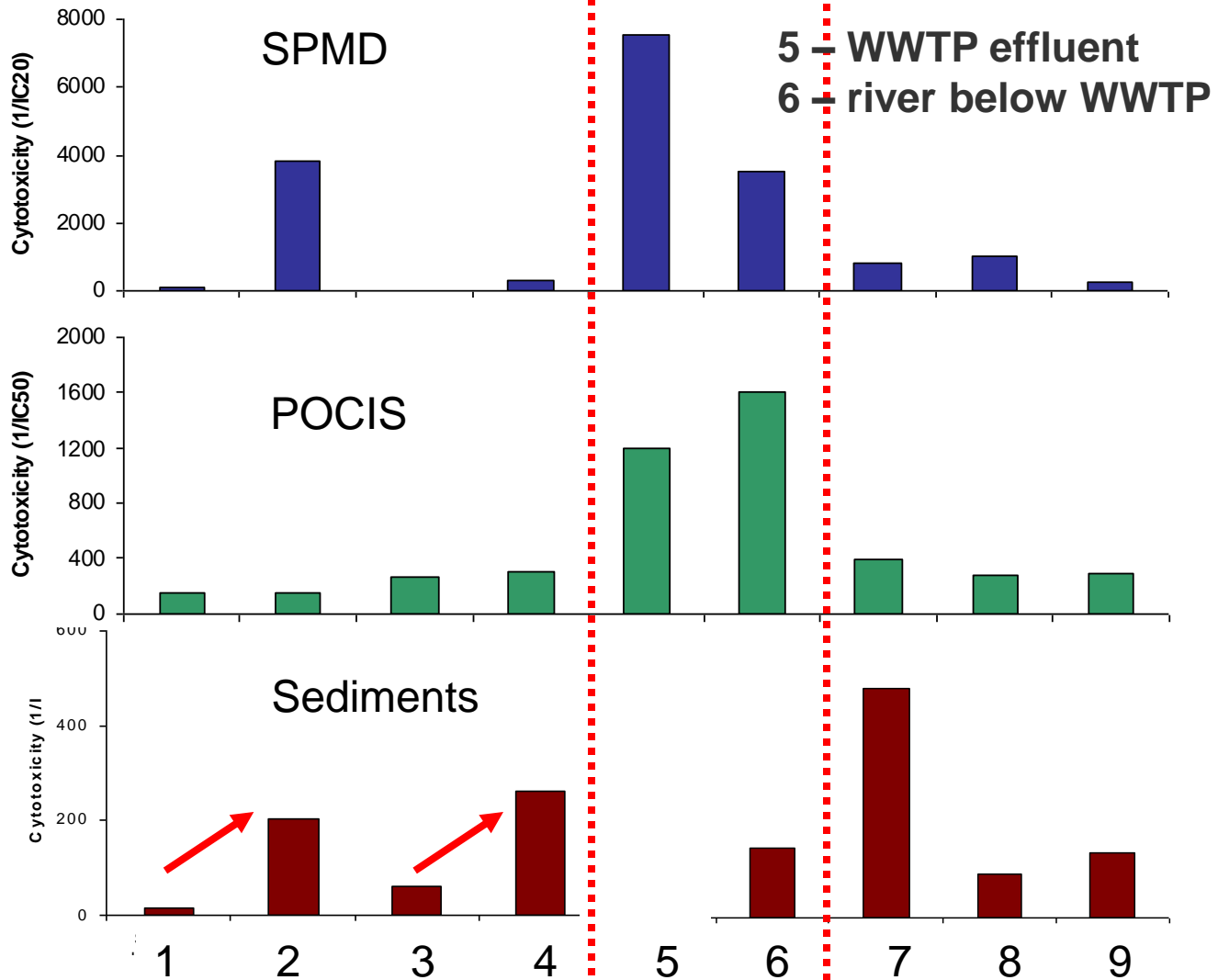
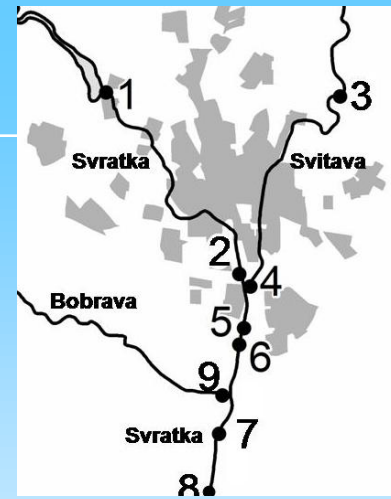
Androgenicity - POCIS



Study 2 - Impact of the Brno city



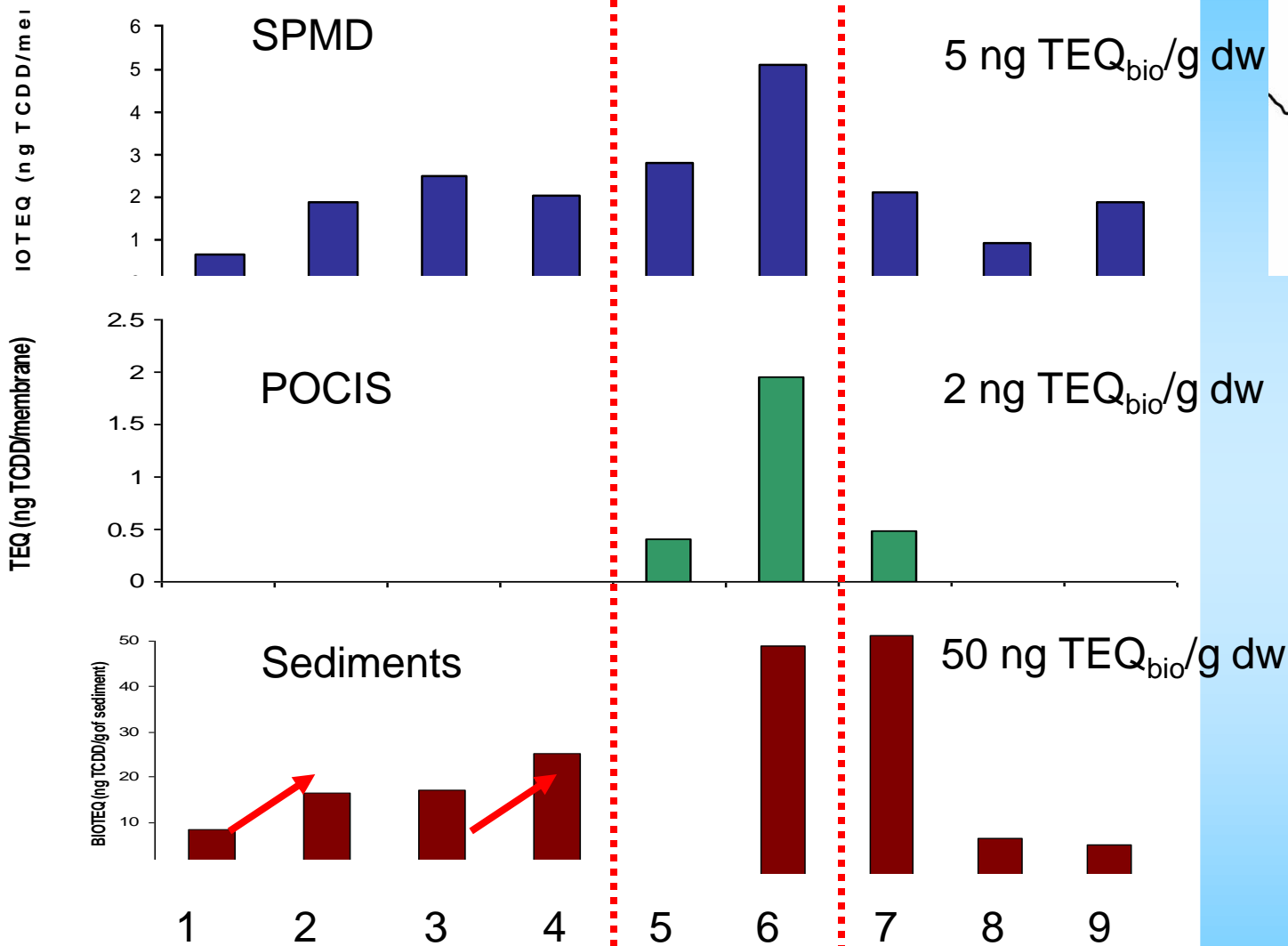
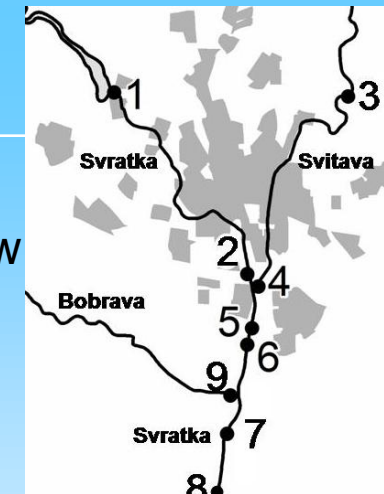
RESULTS 2 - City of Brno



Cytotoxicity

- High cytotoxicity (especially Sediments, POCIS)
- Locality 2 (below Brno), WWTP & sediment 7 (?)

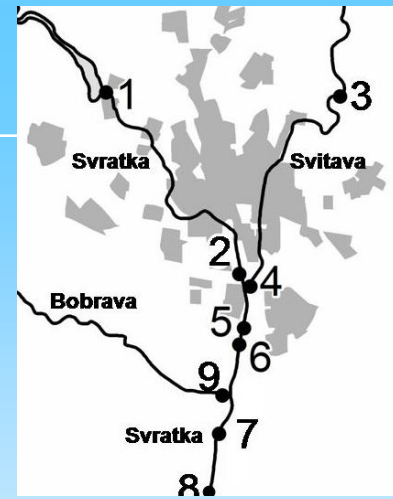
RESULTS 2 - City of Brno



AhR-
(dioxin-like)

- High levels - WWTP effluent & downstream (no. 7)
- Correlation - SPMD vs. Sediments (*hydrophobic cmpnds*)

RESULTS 2 - City of Brno



ESTROGENICITY

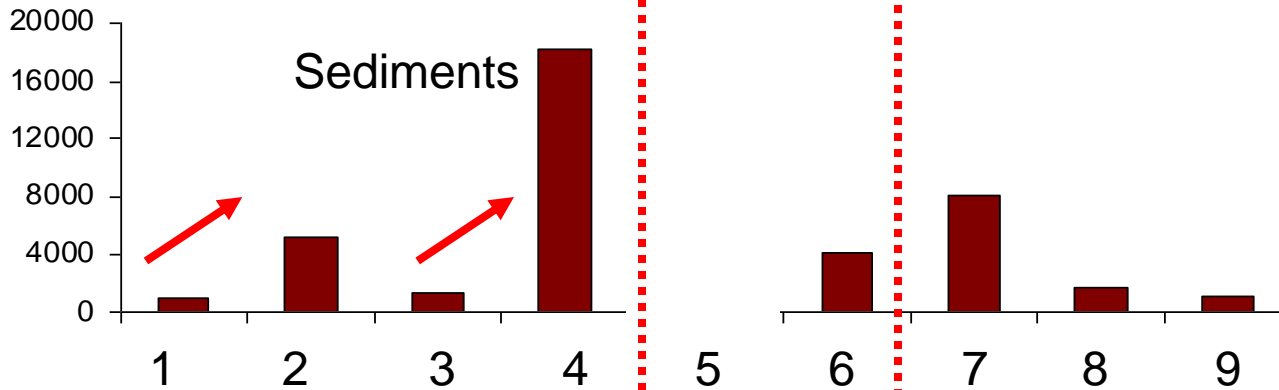
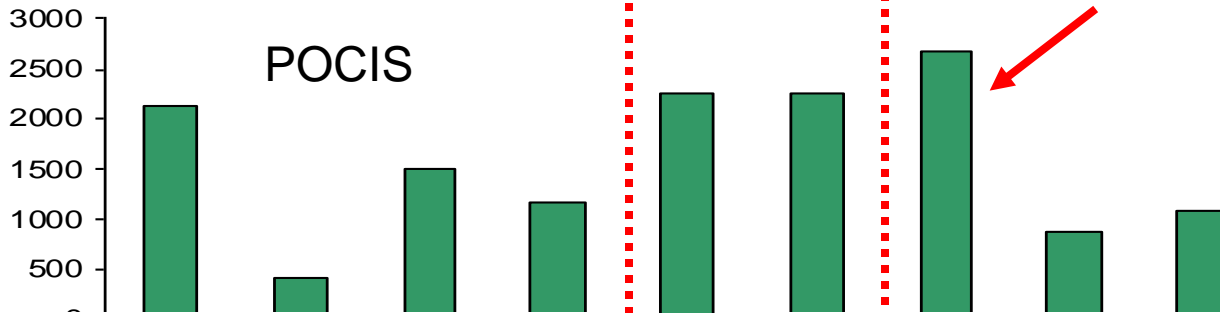
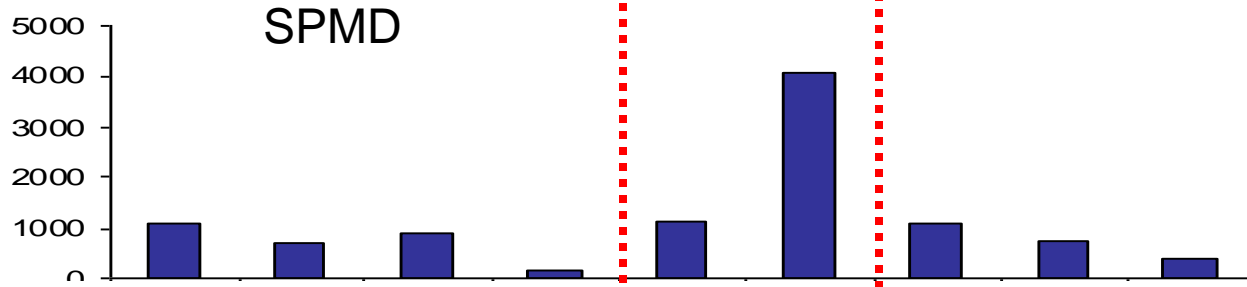
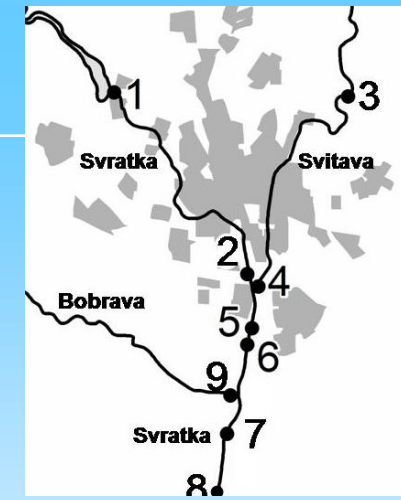
ANDROGENICITY

- no effects in sediments & SPMD
- POCIS - WWTP influent only

Significant „ANTI-“ effects



RESULTS 2 - City of Brno

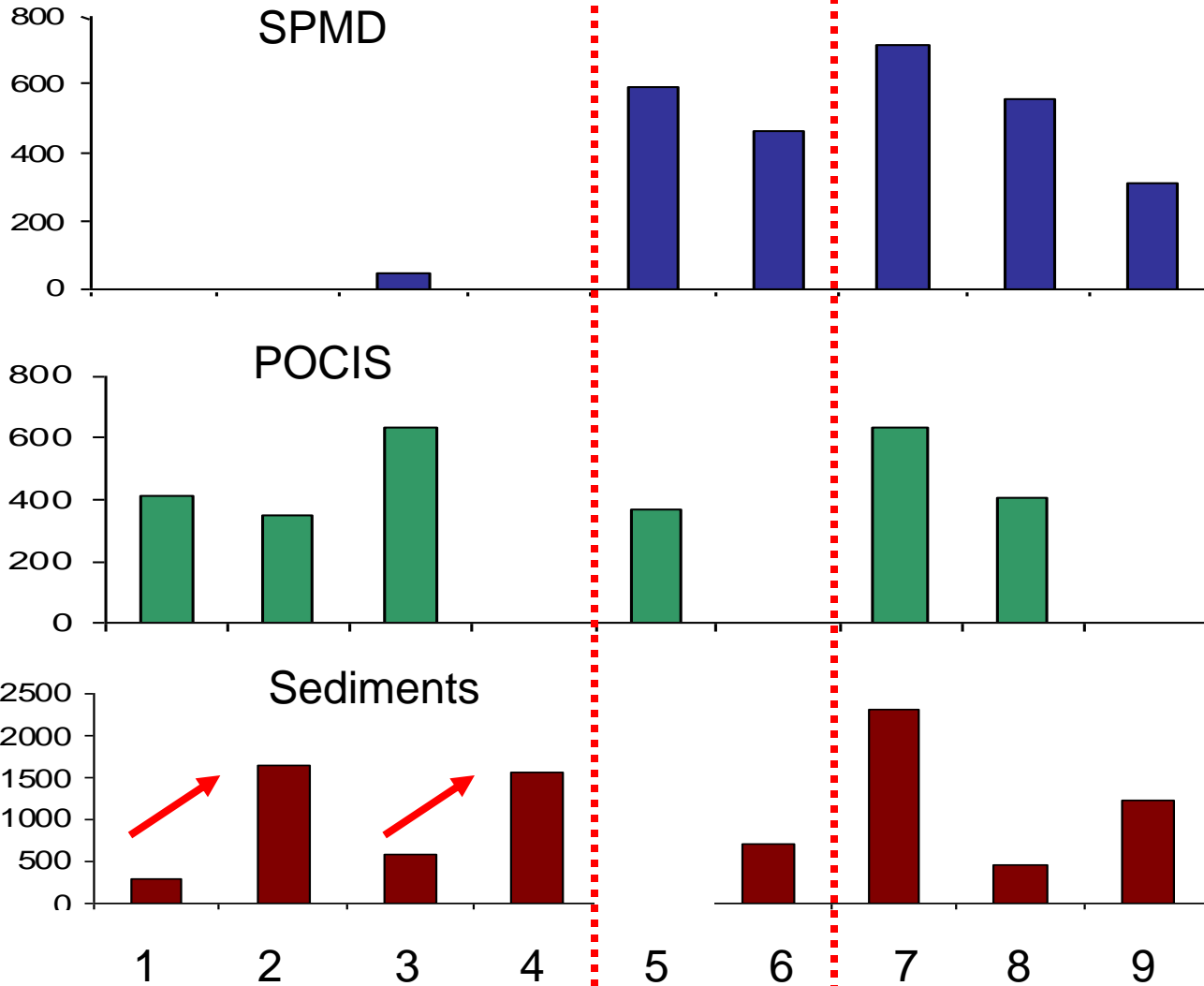
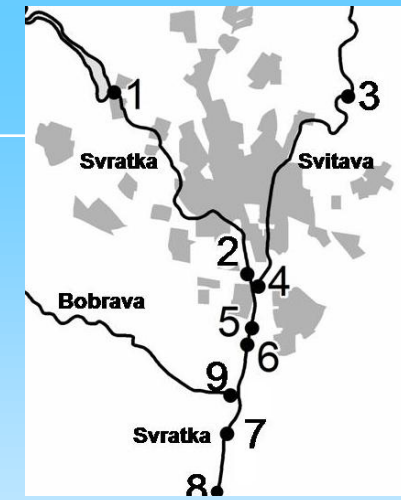


**ANTI-
estrogenicity**

➤ **POCIS !**

➤ **High levels - WWTP effluent & downstream (no. 7)**

RESULTS 2 - City of Brno



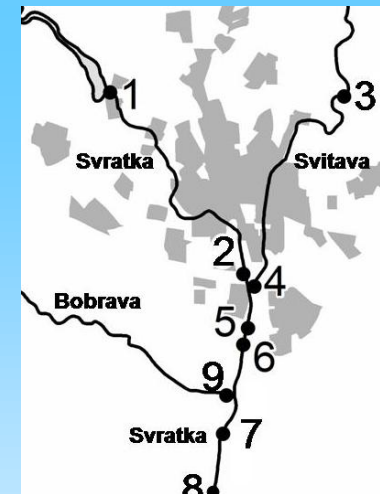
**ANTI-
androgenicity**

➤ **SPMD ! - high levels downstream of Brno**

RESULTS 2 - City of Brno

Chemical analyses

POCIS – polar compounds



POCIS Sampling site	Pesticides	Antibiotics	Other pharmaceuticals		PFOC
			ng/POCIS		
1	376 - 476	12 - 61	389 - 418		5.8 - 8.6
2	285 - 399	2 - 44	356 - 397		3.4 - 5.9
3	382 - 504	28 - 80	1753 - 1774		33.2 - 35.9
4	463 - 616	12 - 61	1548 - 1566		37.9 - 40.7
5	279 - 405	196 - 223	2261 - 2282		12.4 - 15.2
6	2726 - 2857	874 - 892	29297 - 29323		272.4 - 274.1
7	474 - 612	69 - 107	2387 - 2406		29.3 - 31.9
8	342 - 453	56 - 96	2079 - 2100		21.3 - 24.1
9	613 - 735	29 - 86	1951 - 1969		9.9 - 12.4

RESULTS 2 - City of Brno

Chemical analyses – SPMD, Sediments

SPMD						
Sampling site	PAHs	PCBs	OCPs ng/SPMD	Triclosan	MeTriclosan	PBDEs
1	2989	32.6 - 34.8	55.9 - 56.6	32	13	1.0 - 1.6
2	6218	118.8 - 120.4	107.0 - 107.8	25	23	1.1 - 1.8
3	4120	302.8 - 304.6	74.7 - 75.3	42	103	2.2 - 3.0
4	5864	314.8 - 315.5	104.5 - 104.7	42	127	2.5 - 3.0
5	36982	178.8 - 186.3	80.4 - 81.7	5410	16	27.6
6	4380 - 4383	481.1 - 482.6	207.8 - 209.3	1890	6575	36.9 - 37.6
7	4612	223.9 - 225.1	101.7 - 102.2	159	511	3.8 - 4.2
8	2265	140.6 - 141.8	76.7 - 76.9	138	288	2.1 - 2.6
9	3574 - 3577	44.4 - 45.9	44.5 - 45.4	44	72	0.7 - 1.3

Sediment					
Sampling site	PAHs	PCBs	OCPs µg/kg	Triclosan	MeTriclosan
1	2200	4.4 - 4.5	27.3 - 27.9	1.4	0 - 0.1
2	12600	203	97.3 - 97.5	16.7	1.8
3	9700	10.8	21.2 - 21.6	15.9	2.8
4	39600	70.7	57.1 - 57.4	39.7	4.8
6	6700	40	21.9 - 22.5	44.8	12.4
7	13400	96.9	43.2 - 43.7	54.9	9.1
8	15300	20.7	12.6 - 13	3.8	1.2
9	5700	12.9	17.2 - 17.7	14.7	6.1

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

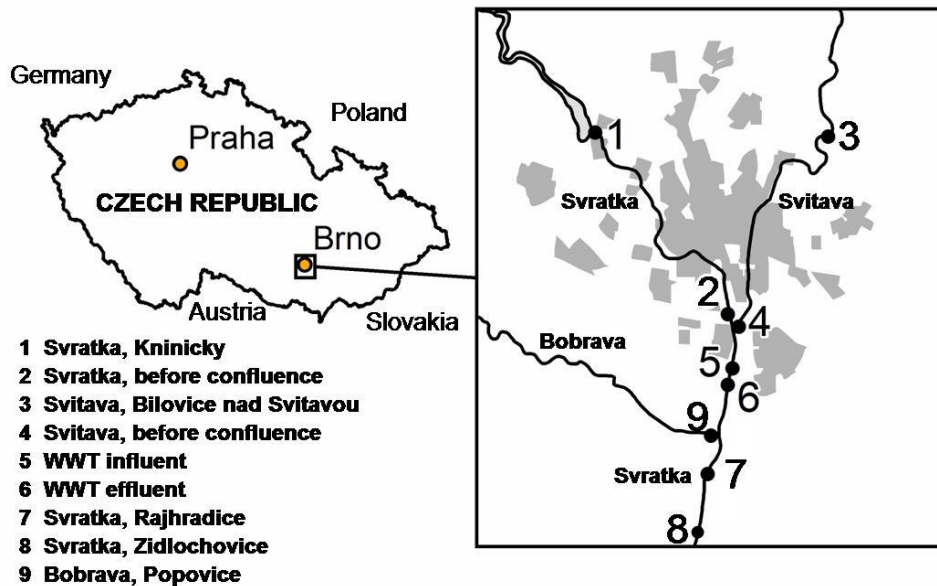
(AhR in SPMD and POCIS, cytotox in POCIS, anti-ER in SPMD)

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

SUMMARY 2

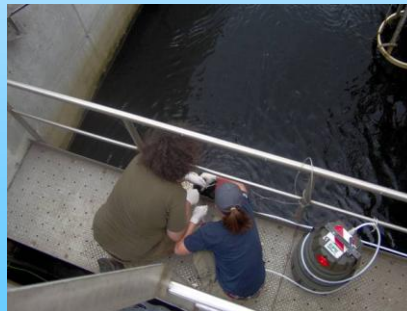
RIVER ECOSYSTEM (POCIS, SPMD, SEDIMENTS)

- Clear pattern in **sediments** - increase be
- 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)



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Thank your for listening!

