NANOSTRUCTURED ENVIRONMENTAL BIOCHEMICAL SENSOR FOR WATER MONITORING

Nanotechnology and the Environment

Günther Proll, Günter Gauglitz

**Institute of Physical and Theoretical Chemistry
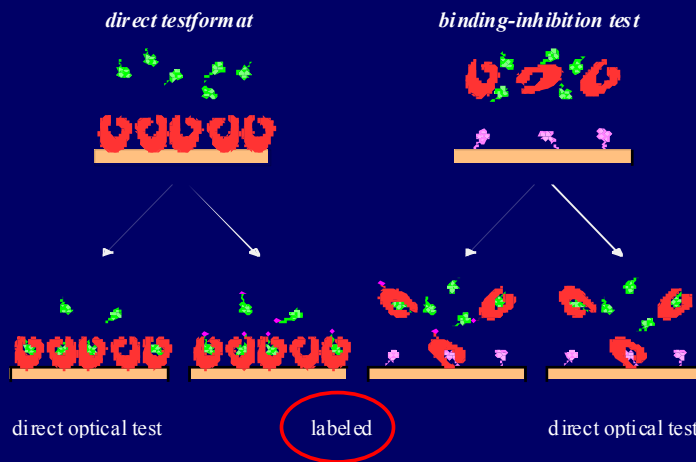Eberhard-Karls-University of Tübingen**

Outline

- **Biosensor Principles and Components**
- **Immunochemistry & Assay Design**
- **AWACSS Project: Overview & Intentions**
- **Instrument & Network**
- **Multi-analyte Measurements**
- **Environmental Assays**
- **Summary & Acknowledgements**

Biosensor principles

direct testformat   *binding-inhibition test*

direct optical test   labeled   direct optical test

Biosensor principles

Labeled Systems

- **ELISA (heterogeneous, competitive)**
- **Direct test format with label:**
  Liegler et al.; Krämer et al.
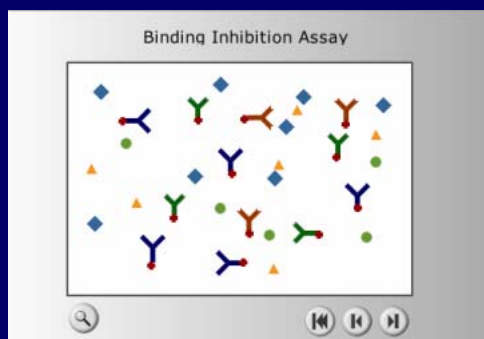- **Binding-inhibition-assay with label**
  RIANA; AWACSS

Immunochemistry

Antigen-Antibody-Interaction:

Binding Inhibition Assay

Specific antibodies which are labelled with a fluorescence dye (Cy5.5^{TM}) recognise organic compounds in aqueous samples.

Components & parameters

Analytical tool	Method	Transduction	Detection	Data Treatment
Antibody	**Assay**	**IO-Chip**	**Optics Electronics**	**AD SW**
•Affinity-chromatography	•Surface chemistry	•Evanescent field	•Filters / PMF	•Drift correction
•Fluorescent dye (Cy5.5, Alexa 680, QDs)	•Regeneration	•Enhancement structure	•Photodiodes	•Calibration
	•Automation		•Amplifier	•Chemometrics

Fully automated biosensor

Nano-scale

Analytical tool	Method	Transduction	Detection	Data Treatment
Antibody ng	**Assay**	**nanostr. IO-Chip**	**Optics Electronics**	**AD SW**

- Affinity-chromatography **pL - nL**
- Fluorescent dye (Cy5.5, Alexa 680, QDs **nanoparticles**)

- Surface chemistry
- Regeneration
- Automation

- Evanescent field **nm**
- Enhancement structure **nm**

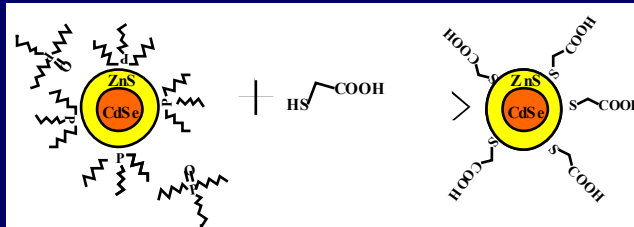- Filters / PMF
- Photodiodes **pW - nW**
- Amplifier

- Drift correction
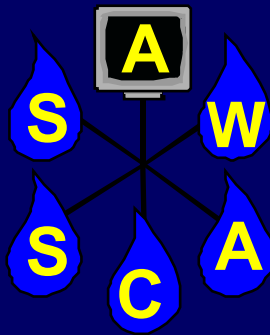- Calibration
- Chemometrics

Fully automated biosensor
LOD: ng L^{-1}
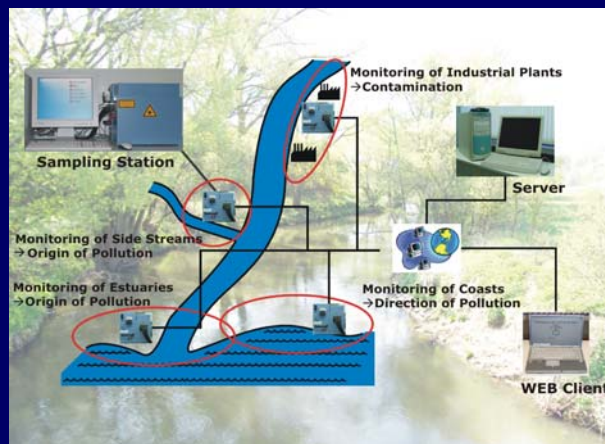

QDs as labels
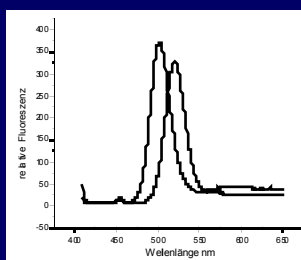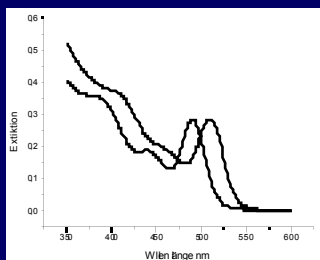
AWACSS-Project

Automated
Water
Analyser
Computer
Supported
System

- EVK1-2000-00045
- Duration: 3 Years, RTD Project
- (March 2001 – March 2004)
- Visit: http://barolo.ipc.uni-tuebingen.de/awacss

Biosensors & Bioelectronics 20 (2005) Part I & II (1499-1508 & 1509-1519)

Goal

Develop a cost-effective, on-line, water monitoring biosensor that will measure a variety of small organic pollutants in short-time with remote control and surveillance.

Monitoring of Industrial Plants
→Contamination

Sampling Station

Server

Monitoring of Side Streams
→Origin of Pollution

Monitoring of Estuaries
→Origin of Pollution

Monitoring of Coasts
→Direction of Pollution

WEB Client

Software & Network

Local stations:
- **Multi-analyte detection**
- **Data acquisition**
- **Data evaluation**
- **Local station control**

Local station

Local station

Control station:
- **Global survey & early warning**
- **Global trend analyses**
- **Local station control**
- **Detection limit control**
  prescribed by EU-Directives

Control station

Target Compounds

Ecotoxicology:

- **Herbicides**

- **Fungicides**

- **Insecticides**

- **Antibiotics**

- **Blue Algae Toxins**

- **Endocrine Disrupting Compounds**

- **Suspected Carcinogens**

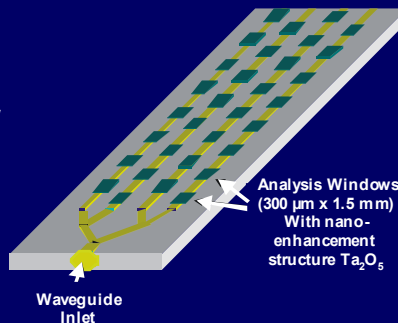- **Industrial Wastes / Chemical Markers**

Integrated Optical Chips

Goal: Develop IO-Chips capable of simultaneously illuminating 32 analysis windows in a miniaturised format.

Advantages:

- **Single mode waveguides allow for efficient and consistant analysis window illumination.**

- **Compact dimensions allow for microfluidic design and reduce sample & reagent volumes.**

- **Spatially defined but resolved analysis windows allow efficient detection and suppress signal crosstalk.**

Analysis Windows
(300 µm x 1.5 mm)
With nano-
enhancement
structure Ta_2O_5

Waveguide
Inlet

Integrated Optical Chips

Goal: Develop IO-Chips capable of simultaneously illuminating 32 analysis windows in a miniaturised format.

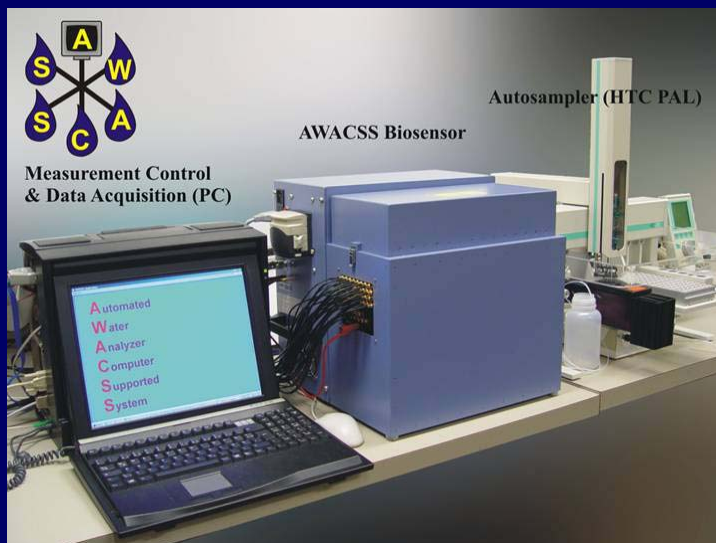### Derivatives:

- **Estrone**
- **Bisphenol A**
- **Atrazine**
- **Sulphonamides**
- **Isoproturon**
- **Propanil**

Analysis Windows
(300 µm x 1.5 mm)
With nano-
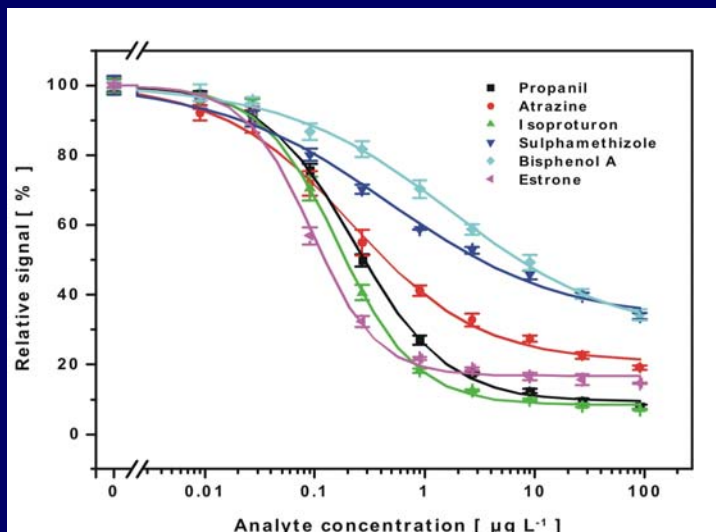enhancement
structure Ta_2O_5

Waveguide
Inlet
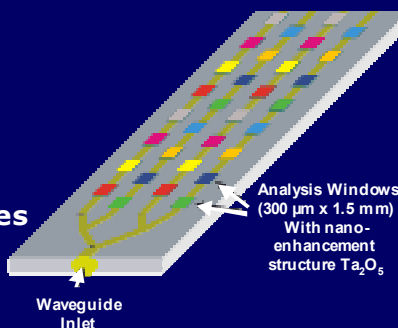
18 minutes per cycle and up to 500 measurements!

Instrument

Multi-analyte Measurements

Validation Parameters

Analyte	A_2	p	IC_{50}	LOD	SD_{zs}
Estrone	16.7	1.46	0.093	0.007	0.6
Bisphenol A	29.5	0.61	1.585	0.008	0.9
Atrazine	20.8	0.76	0.217	0.010	2.7
Sulphamethizole	34.3	0.61	0.419	0.018	2.8
Isoproturon	8.5	1.3	1.263	0.020	2.0
Propanil	9.6	1.08	0.232	0.019	1.9
Dimension	[%]	[ ]	[μg L^{-1}]	[μg L^{-1}]	[%]

Drinking water

Analyt	Estron	BPA	Atrazin	SUL	IP	PRN
RR (0,05 ppb)	106,2 ±3,6	31,6 ±4,2	86,7 ±18,4	110,8 ±5,3	117,4 ±4,4	96,7 ±15,9
RR (0,10 ppb)	119,4 ±6,4	143,8 ±12,8	87,0 ±16,8	67,4 ±4,0	96,9 ±3,8	80,0 ±3,9
RR (0,15 ppb)	113,8 ±11,2	101,8 ±24,8	115,5 ±10,3	109,6 ±9,8	98,0 ±4,4	115,8 ±5,3

- RR recovery rate (70 – 120 % as recommended by the AOAC International)
- RR = c_d / c_t · 100 % (d: detected, t: true)

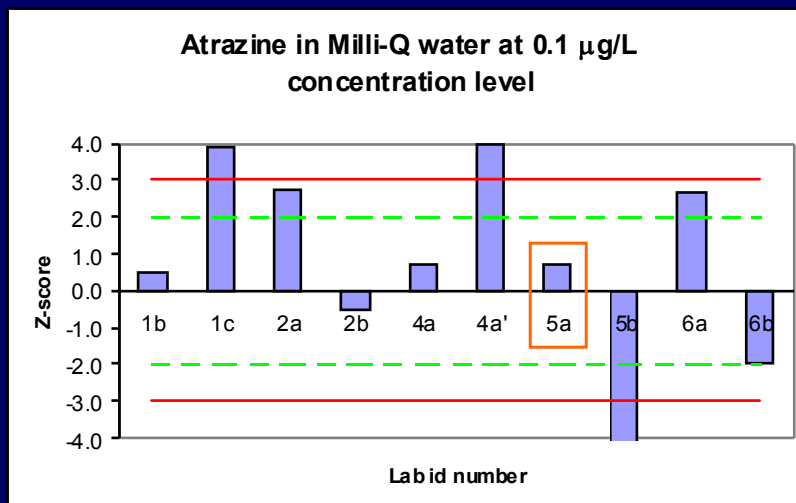# Collaborative Trial

Objectives:

- **Evaluation of the AWACSS performance in comparison to:**

  – **Immunoassay techniques – RIANA and ELISA**

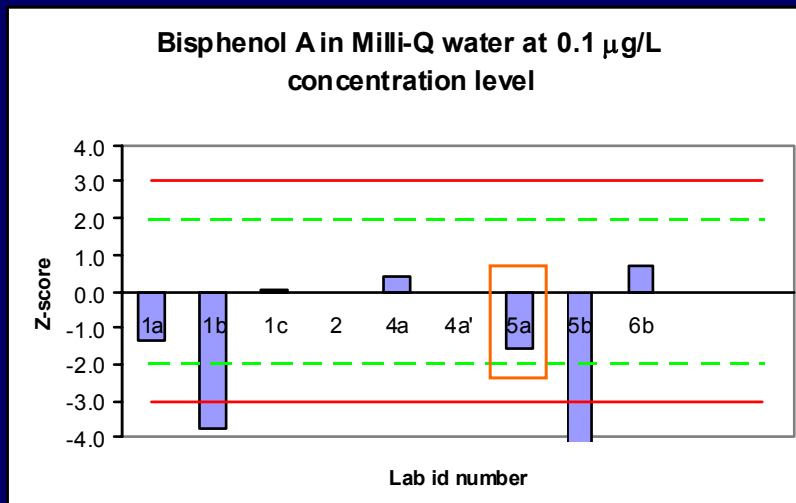 – **Conventional LC- and GC-based analytical techniques**

Collaborative Trial

- **Matrices**
  – Milli-Q water
  – River sediments (homogenized 63 μm fraction, freeze dried)
  – Mixed industrial and municipal wastewater - Novaky Chemical Plant, Slovak Republic

- **Analytes**
  – Atrazine - pesticides, on the list of EU WFD Priority Substances
  – Bisphenol A - endocrine disrupting compound, industrial pollutant
  – Estrone - endocrine disrupting compounds, hormones, municipal WWTP

- **Spiked levels**
  – 0.1 and 1 μg L^{-1} - water samples
  – 50 and 500 ng g^{-1} - sediments

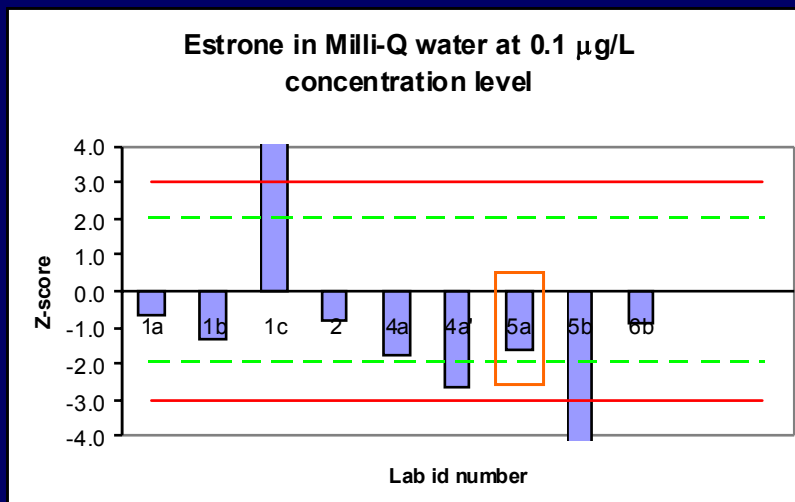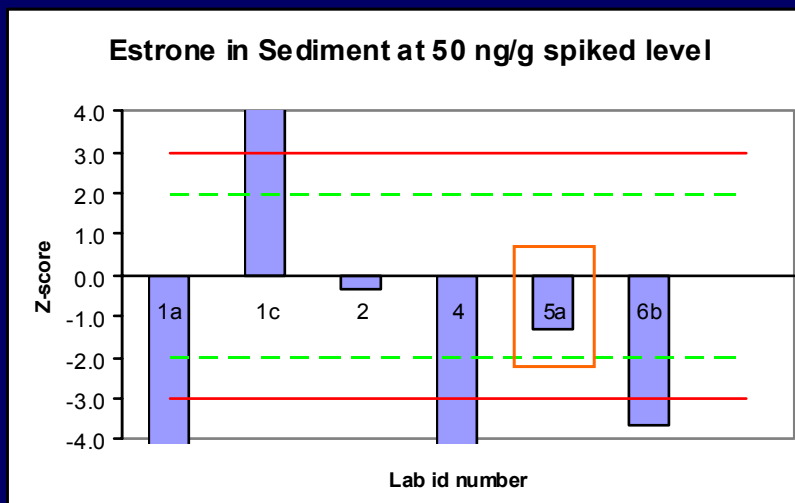Collaborative Trial

Atrazine in Milli-Q water at 0.1 µg/L concentration level

Collaborative Trial

Bisphenol A in Milli-Q water at 0.1 µg/L concentration level

Collaborative Trial

Estrone in Milli-Q water at 0.1 µg/L concentration level

Collaborative Trial

Estrone in Sediment at 50 ng/g spiked level

Collaborative Trial

Compound	Assigned value	Matrix		Assigned value		
		Milli-Q water			Sediment	
	[µg L^{-1}]	Mean [µg L^{-1}]	RR	[µg L^{-1}]	Mean [µg L^{-1}]	RR
Atrazine						
Level 1	0.1	0.11	111	0.1	0.1	100
Level 2	1	1.15	115	1	0.77	77
Bisphenol A						
Level 1	0.1	0.08	80	0.1	0.133	133
Level 2	1	1.25	125	1	0.85	85
Estrone						
Level 1	0.1	0.08	80	0.1	0.071	71
Level 2	1	1.04	104	1	0.71	71

RR: Recovery Rate

Field Test - Sampling System

valve

filter

pump

sampling vessel for AWACSS system
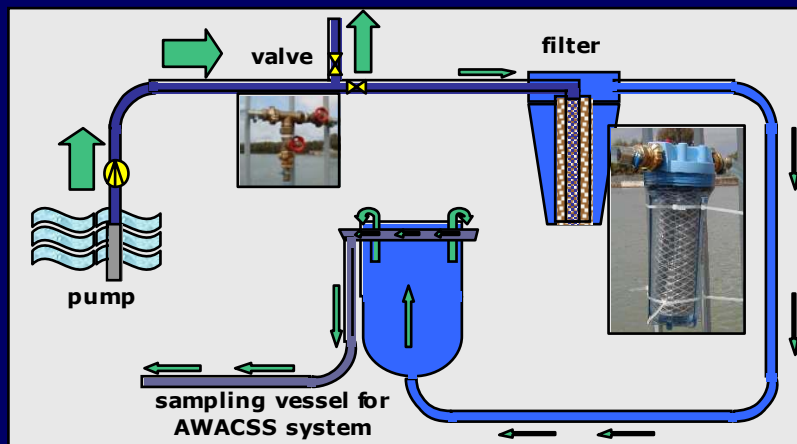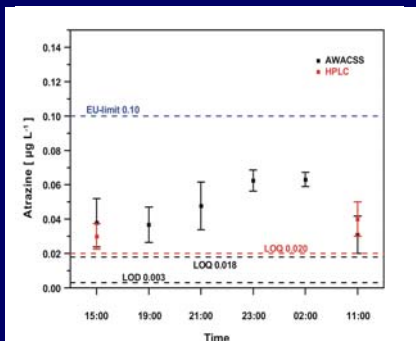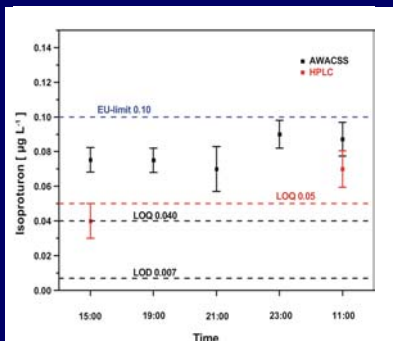
AWACSS Biosensor (Field Test)

Calibration: atrazine, isoproturon, estrone, bisphenol A
Fully automated sampling and detection!

- **Estrone below LOQ (AWACSS and HPLC-DAD)**
- **Bisphenol A contamination from tubes**

Assays: How far it can go ...

Jens Tschmelak · Guenther Proll · Guenter Gauglitz

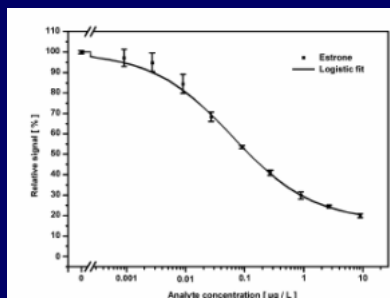## Immunosensor for estrone with an equal limit of detection as common analytical methods


Fig 1 Calibration for estrone from 0 to 9 μg L^{-1} in Milli-Q water with ovalbumin from chicken eggs, phosphate buffered saline and 3 ng anti-total-estrogen in each sample, with total sample volume of 1 mL

Table 1 Values for the logistic fit function and the calculated results for SD$_{blanks}$, LOD and LOQ

Parameters	Values	Calculated results	Values
A_2	17.16±1.54	SD$_{blanks}$	0.67%
x_0	0.062±0.004	LOD	0.19 ng L^{-1}
p	0.639±0.052	LOQ	1.39 ng L^{-1}

LOD: 190 ppq

Progesterone in raw milk (1:10)
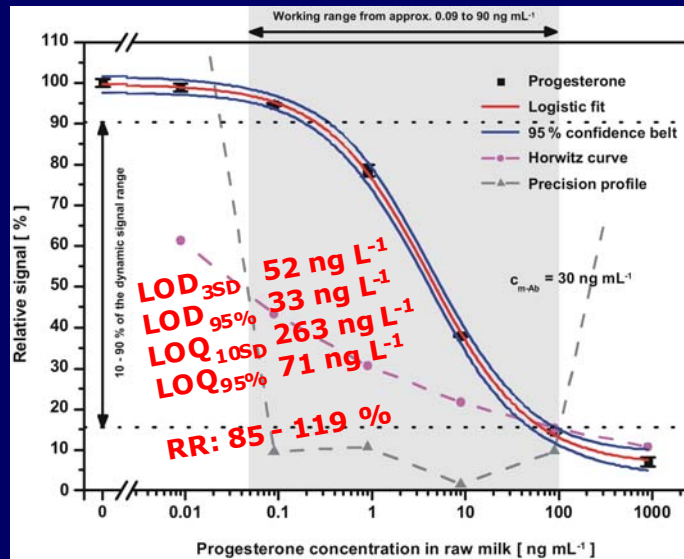
Summary

The AWACSS biosensor is a fully automated water monitoring system and capable for remote control:
- **Quick!**
- **No pre-treatment nor pre-concentration!**
- **Multi-analyte measurements!**
- **Real samples: tap, ground and surface!**
- **Successful field test**
- **LODs below EU limits!**
- **LOD in ppq-range for several compounds:**
  Progesterone, Testosterone, Propanil, Estrone, Isoproturone, Atrazine, ...

Acknowledgements

- **EU for funding AWACSS**
- **All partners of the AWACSS project for the excellent cooperation!**

Thank you for your attention!