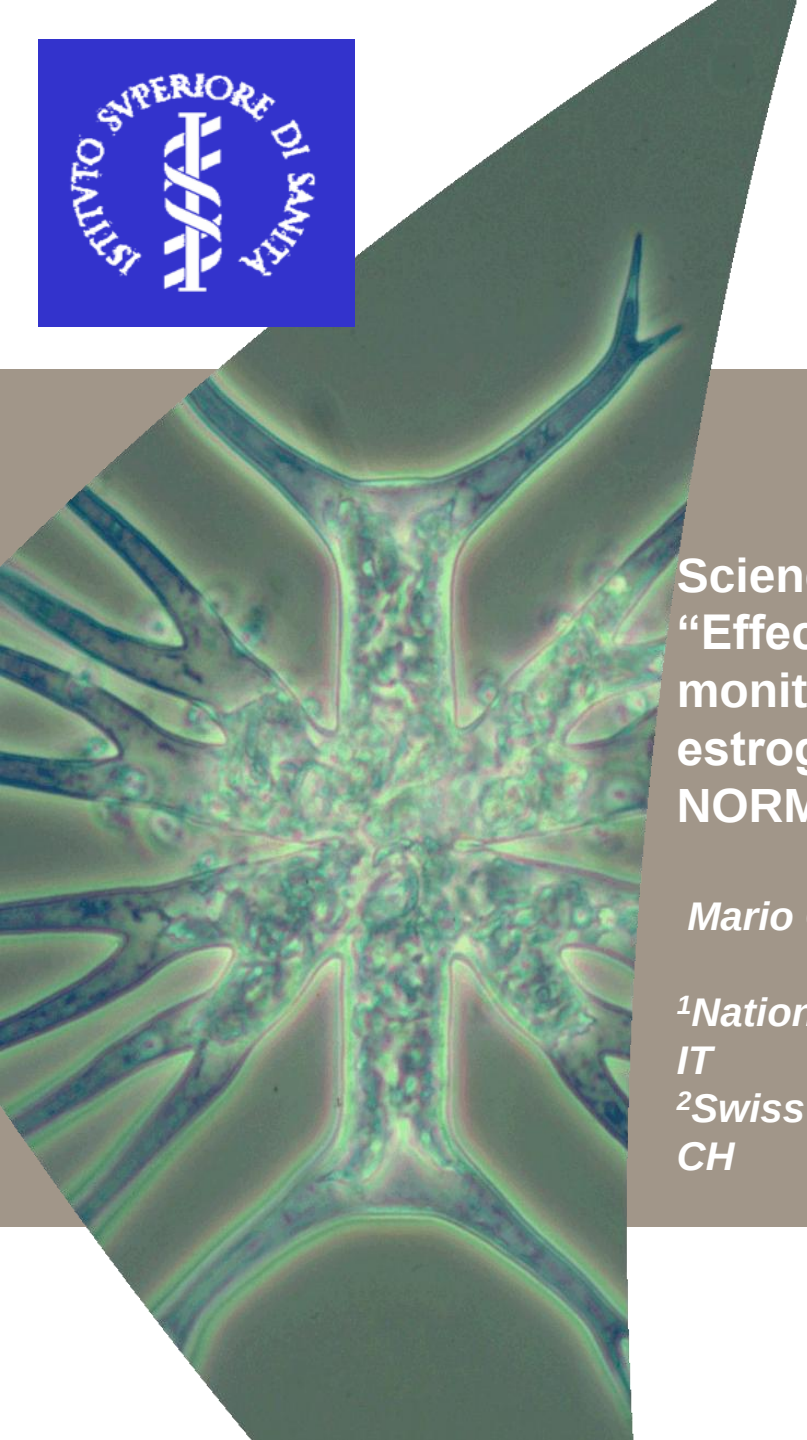

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Schweizerisches Zentrum für angewandte Ökotoxikologie
Centre Suisse d'écotoxigologie appliquée
Eawag-EPFL

Science Policy Interface (SPI) action on "Effect-based and chemical analytical monitoring approaches for steroidal estrogens": project update and plans for NORMAN contribution in 2016

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Norman Meeting Rome 3-4/12/2015

DESCRIPTION OF THE PROJECT

- **Three steroidal estrogens, 17α-ethinylestradiol (EE2), 17β-estradiol (E2) and estrone (E1), are currently included in the so-called “watch list” of the Water Framework Directive (WFD) and are therefore recommended for monitoring.**
- **However, this may be difficult because the detection limits of most existing routine analytical methods are above the biological effect concentrations, and high-end analytical methods are very costly.**
- **Effect-based methods can measure the estrogenic activity of environmental samples in a cost-efficient way at very low concentrations. Therefore, we will compare seven specific effect-based methods with three sensitive chemical analysis methods to measure EE2, E2 and E1.**
- **For this purpose, 39 surface water and wastewater samples are collected and analysed.**

Expected challenges for the watch list substances EE2 and E2

If you want to monitor an exposure related risk for EE2 and E2:

- **A worst case could be a monitoring dataset full of non-detects due to insufficient detection limits (imagine a LOD of 100 pg/L for EE2 risk or no risk?)**
- **The stability of the samples is a critical point in estrogen analysis**
- **Methodical choices and variability will strongly influence the comparability of results**
- **For the relatively low EQS of EE2 and E2 in the sub ng/L range (the best available methods in combination are needed**

Fortunately we have now a promising set of best available chemical analytical and effect-based analytical methods in our project to improve the monitoring and detection

Included methods

Detection methods covered:

- **High end chemical analysis (JRC, BfG, Umea University)**
- **ER-Calux (BDS)**
- **MELN (INERIS)**
- **BG1Luc4E2 + ER-GeneBLAzer (UFZ)**
- **Hela 9903 (RECETOX)**
- **Yeast Estrogen Screen assays (BfG)**
- **T47D-Kbluc assay (RWTH Aachen)**

3 x high end chemical analysis +7 x effect-based analysis, some of them are in OECD validation processses or already in ISO standardisation

All of the screening methods have shown their applicability for single substances, artificial mixtures or environmental samples in different projects.

5 screening methods are already compared in a prevalidation project with single substances and mixtures (Kunz et al. in prep.)

Now we will have in 2016 the chance to compare and characterise all the methods with realistic environmental samples + control samples.

Comparing highly sensitive chemical analytical and effect-based methods

Chemical analytical (BfG)	E1	E2	EE2
LOD	3 pg/L	30 pg/L	10 pg/L
LOQ	10 pg/L	100 pg/L	35 pg/L

Advantage: You can quantify each single analyte

Effect-based ER-Calux (BDS)	E1	E2	EE2
LOD	260 pg/L	5.2 pg/L	4.3 pg/L
LOQ	850 pg/L	17 pg/L	14.2 pg/L

Advantage: You can quantify the receptor activation→ more sensitivity in screening

Preliminary conclusions for effect-based methods

Most prevalidation results of effect-based methods underline the reliability, accuracy, robustness and sensitivity.

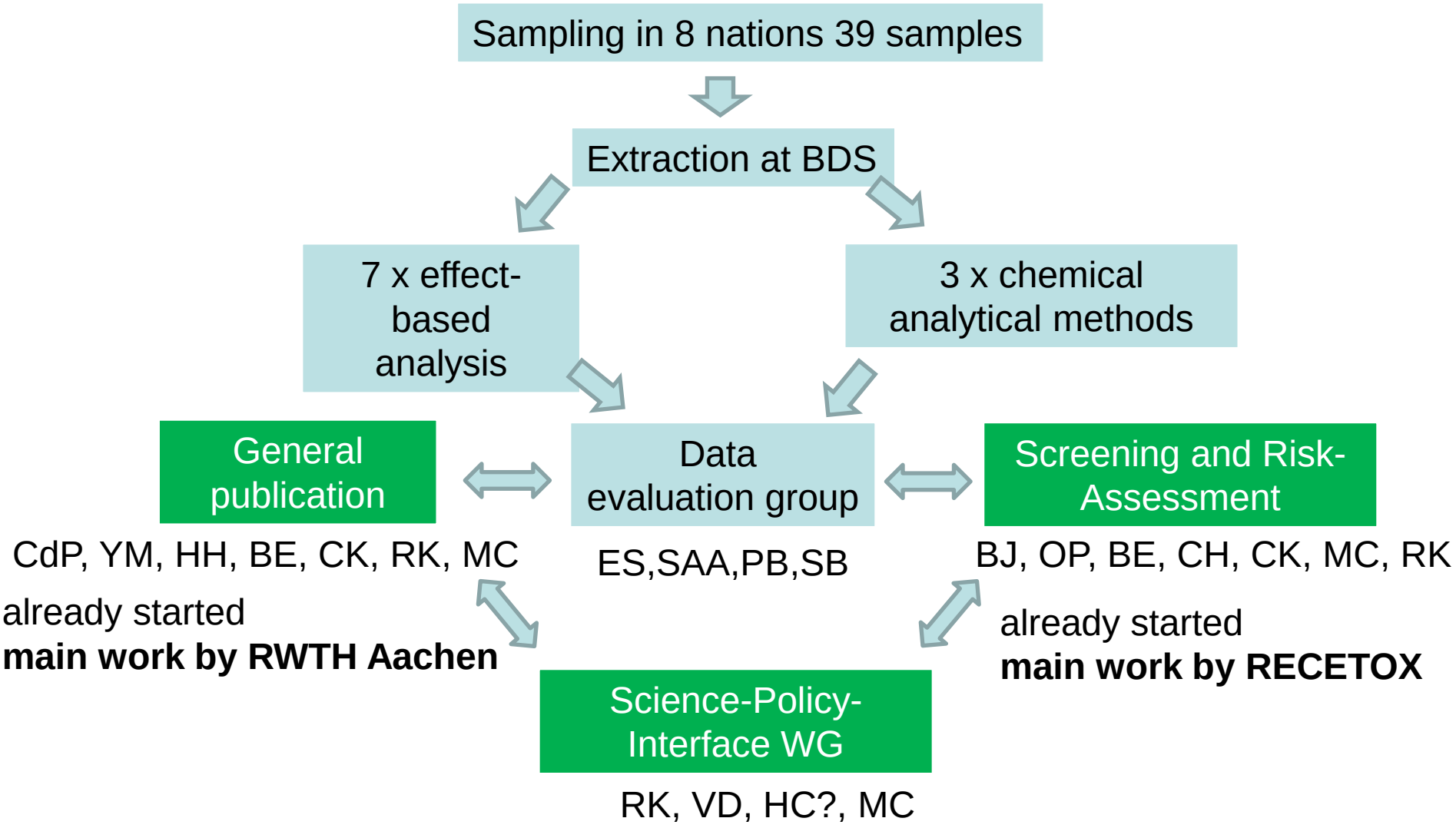
<u>Additionally they are cost-efficient high throughput methods:</u>

Installation cost of high end chemical analytical device > 300k Euro

Laboratory equipment for effect-based methods < 30k Euro

Why not to use the advantages of effect-based and chemical analysis in combination?

Reporting activities



Note: All project partners are invited to be listed, but only a part can do the main publication work.

Initials:

AW = Arne Wick
BE = Beate Escher
BJ = Barbora Jarosova
CdP = Carolina di Paolo
CH =Christiane Heiss
CK = Cornelia Kienle
ES = Eszter Simon
GM = Giulio Mariani
HC = Helen Clayton (tbc)
HH = Henner Hollert
MC = Mario Carere
MS = Michael Schlüsener
OP = Olivier Perceval
RK = Robert Kase
SAA = Selim Ait-Aissa
SB = Sebastian Buchinger
PB = Peter Behnisch
VD = Valeria Dulio
YM = Yvonne Müller

The level of information need by the regulatory bodies will be in 2016 quite high, because the first round of watch list will surely define some space for improvements. We intend to have the right screening results ready to make a clear recommendation how to improve the 2nd round and how to reduce monitoring costs for screening and risk assessment.

To optimise the reporting we ask to support the two main institutes involved in reporting and to support the facilitation of project meetings.

Time plan

April and May 2015: Upload **project homepage** and discussion of the project at **Mulitaleral Meeting** and **SETAC EU**

Most of the project information is now available at:
http://www.ecotoxcentre.ch/projects/aquatic-ecotoxicology/monitoring-of-steroidal-estrogens/

Drafting group results: Sampling, Extraction, Data Evaluation, Screening and Risk Assessment

Q3+Q4 2015: Sampling & extraction
(parallel to the watch list mechanism) → Here we are

Next:15th +16 th February 2016 3rd project meeting at ONEMA, Paris, FR

Q1 2016: Chemical analytical and effect-based measurements of samples extracts

Q2-Q4 2016: Data evaluation and reporting
(2 publications and 1 SPI WG Chemicals report)

Q1 2017: Final project meeting at JRC, IT

Main Tasks in WG Chemicals 2016-2018 WFD

- New Priority Substances review: SG-R re-established in 2014; experts contributing to JRC technical work. Possible de-listing of PS will be considered. Short-list of substances will be needed in 2016.

- **Effect-based tools; and links between chemical and ecological status; mixtures. Possible follow-up of estrogen-screening project. Exchange of information on innovative techniques and approaches; discussion of application in context of WFD.**

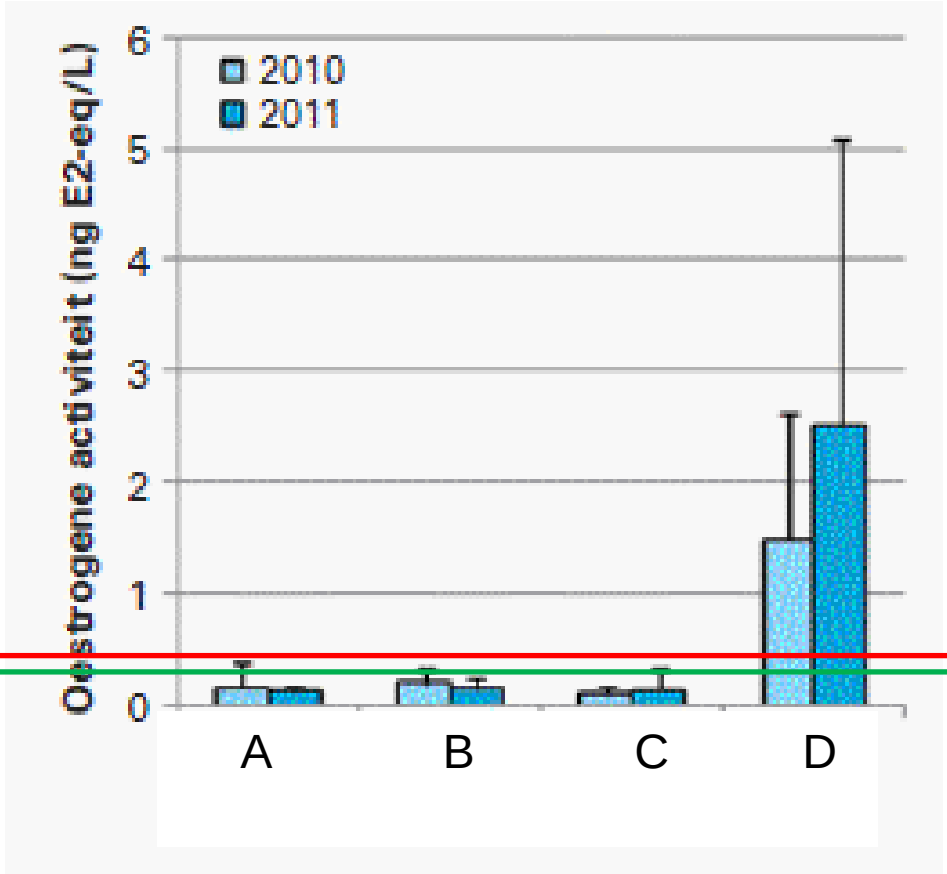
- Passive sampling: exchange of information on latest developments; discussion of application in context of WFD.

- Review of the watch list.

Aims, challenges and options

- Roadmap on how to assess the risks of steroidal estrogens in the future
- Improving monitoring efficiency
- Providing options for more effect-based monitoring in the WFD
- Recommendations of methods for the characterization of municipal waste water quality and surface water quality
- Recommendation of a monitoring strategy reducing monitoring costs
- Offering options for the revision of the WFD

Example: Where do you would like to invest monitoring ressources?



From RIWA 2012: Mean estrogenicity activity in Rhine at Lobith, Lek at Nieuwegein, Amsterdam Rhinechannel and Maas at Keizersveer

AA-EQS for E2 at 0.4 ng/L (Loos et al. 2012)

EEQs-SSE 0.3 ng/L for municipal waste water (Jarosova et al. 2014)

Aim: General effect-based trigger values are proposed, it would be necessary to characterize them in comparison with analytical EE2, E2 and E1 monitoring data for polluted samples !! ➔ test specific trigger values can be elaborated which could allow a more reliable and specific screening

Summary and outlook

With a comparison of screening EEQ values with analytical based risk-quotients for steroidal estrogens, we are able to:

1) Increase the monitoring efficiency for steroidal estrogens

2) To bridge the gap between conventional analytical and an effect-based monitoring

3) Lowering costs for monitoring & providing risk management options for EDCs and pharmaceutical strategies

Please feel free to exchange ideas, observations, suggestions and questions:

Robert Kase (Robert.Kase@oekotoxzentrum.ch)
Mario Carere (Mario.Carere@iss.it)

Thank you for your time and attention !!!

More info at:
http://www.ecotoxcentre.ch/projects/aquatic-ecotoxicology/monitoring-of-steroidal-estrogens/

Included project partners

Joint Research Centre (EC), ONEMA (FR), INERIS (FR), Bio Detection Systems (NL), Swiss Centre for Applied Ecotoxicology (CH), Federal Institute of Hydrology (DE), Federal Environment Agency (DE), Federal Ministry for the Environment (DE), RWTH Aachen (DE), RECETOX (CZ), NORMAN-Network, Helmholtz Centre for Environmental Research-UFZ (DE), IRSA-CNR (IT), Italian Institute of Health (IT), University of Leon (ES), Water Research Institute T.G.Masaryk (CZ), Bavarian State Office for Environment (DE), LANUV (DE), Environment Agency Austria (AT), Umea University of Sweden (SE), ISSeP (Scientific Institute of Public Service) Wallonia (BE), SMAT (IT), Ontario Ministry of the Environment and Climate Change (CAN), McGill University (CAN).

Around 60 colleagues from 24 institutes, agencies and 11 nations are involved.

A very multi-national project including expertise from various agencies and institutes. This participation shows the high level of interest.

We are very grateful that you indicated your collaboration and participation.

And last but not least our special thanks to the NORMAN-Network (www.norman-network.net) for their collaboration and support

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Estrogen Monitoring project in the context of the WFD

Effect-based and chemical analytical monitoring for the steroidal estrogens: An international project to cope with a monitoring challenge

<u>This project is an applied follow up initiative of the:</u>
Science-Policy-Interface (SPI) and
Chemical Monitoring of Emerging Pollutants (CMEP-WFD) activity
with support of numerous project partners!!

<u>Primary aims</u>:
1) A project related to the watch list substances EE2 and E2, E1 with specific effect-based analytical methods can characterise their screening potential in combination with the best available chemical analytical methods.

2) To bridge the gap between conventional analytical and an effect-based monitoring

Technical Report - 2014 - 077

Published in: Wernersson Ann-Sofie; Carere Mario, et al. (2015): The European technical report on aquatic effect-based monitoring tools under the water framework directive. **Environmental Sciences Europe**, 2015; 27 (1) DOI: 10.1186/s12302-015-0039-4. http://www.enveurope.com/content/pdf/s12302-015-0039-4.pdf

TECHNICAL REPORT ON AQUATIC EFFECT-BASED MONITORING TOOLS

In this project:
«We think it is time to demonstrate their application potential in an applied international collaboration project. To bridge the gap between chemical analytical and effect-based analysis for the future.»

Environment

TECHNICAL REPORT ON AQUATIC EFFECT-BASED MONITORING TOOLS

Activity Leaders:

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Chiara Maggi (ISPRA- Institute for Environmental Protection and Research, Italy)

Drafting Group:

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Robert Kase, Cornelia Kienle, Petra Kunz, Etienne Vermeirssen, Inge Werner (Eawag/EPFL, Switzerland)

Craig D. Robinson (Marine Scotland Science, Scotland)
Brett Lyons, Ioanna Katsiadaki (Cefas), Caroline Whalley (Cefas) (UK)

Klaas den Haan (CONCAWE)

Marlies Messiaen (Eurometaux)

Helen Clayton (DG Environment, European Commission)

Teresa Lettieri, Raquel Negrão Carvalho, Bernd Manfred Gawlik (European Commission, DG Joint Research Centre)

Valeria Dulio (INERIS-Norman Network, France)

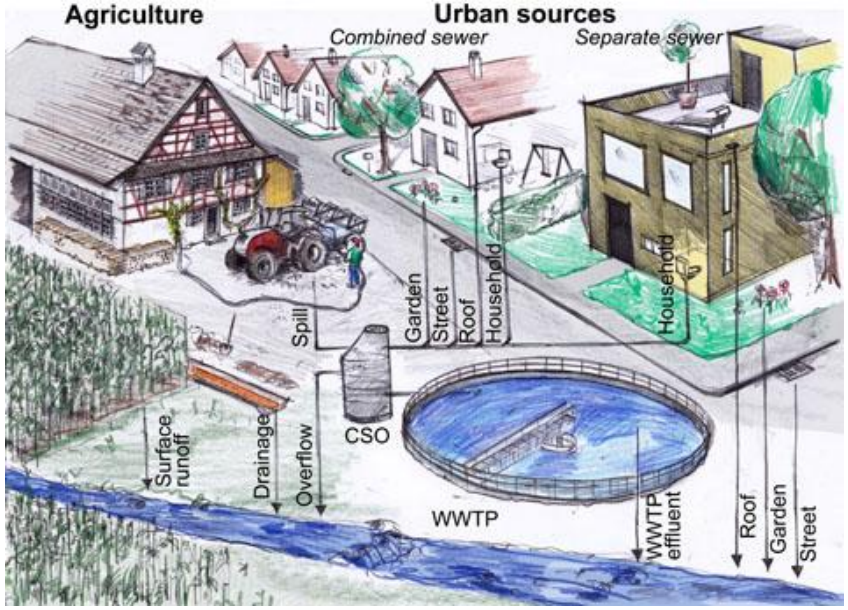
Henner Hollert, Carolina Di Paolo (RWTH Aachen University-Norman Network, Germany)

Werner Brack (UFZ-Norman Network, Germany)

main pathways of E2 and EE2 to water bodies

non-point sources

- Seasonal risks mainly by grasing of livestock
- Application of manure
- Edge of field water bodies
- Source reducing measures available



source: eawag.ch

point sources

- Continuous use and discharge
- Not all estrogens are removed by SWTPs
- For human-pharmaceuticals risk is currently not handled by authorisation
- Lifestyle is hard to influence

E2, E1, EE2 and more

with some analytical challenges and the exposure is mainly modeled

To address the risk posed by EE2, E2 <u>and E1</u> were included in the EU watch list mechanism and <u>should be monitored at their EQS levels 35 pg/L, 400 pg/L, 3600 pg/L</u>

Impressions from waste water sampling in BE and CZ

BE

CZ

Our warmest thanks to Carole, Aurore, Petr, Premysl, Manfred, Christoph, Lomig, Francesca, Sara, Isabel, Julia and many other colleagues !!

Harmonization of methods is needed for a comparability

4 drafting groups provide methodological harmonization for:

1) **Sampling** (EMS): Lead Etienne Vermeirssen, with support of Cornelia Kienle
2) **Extraction** (EMSSEC): Lead Peter Behnisch
3) **Biotest data evaluation** (BEEC): Lead Sebastian Buchinger
4) **Screening and Risk Assessment** (SRAOSE) co-lead Barbora Jarosova & Robert Kase

Initiation Sept 2014 in Koblenz

↓

Formation of drafting groups + 1. drafts Nov 2014

↓

First commenting round Nov-Dez 2014 (3 weeks)

↓

Second commenting round Jan-Feb 2015 (3 weeks)

↓

Final discussion and finalization Feb-Sept

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Providing most of project related information at the project homepage at: https://drive.switch.ch/public.php?service=files&t=930ae34196603db3a70d17c6ac1ea905