

Recommendation for a monitoring strategy for estrogens in coastal and continental surface waters Part I: Bioassays as screening instruments to detect exposed water bodies

Recommendation for a monitoring strategy for estrogens in coastal and continental surface waters (Part 1):

Bioassays as screening instruments to detect exposed water bodies

In January 2012 the European Commission proposed inter alia the highly potent estrogens Ethinylestradiol (EE2) and Estradiol (E2) to be prioritized for reduction in continental and coastal surface waters. Both substances have a specific mode of action affecting aquatic communities and their toxicity to fish in particular calls for very low environmental quality standards (EQS), particularly for EE2. Some of the very low water concentrations that still cause effects cannot currently be monitored reliably by chemical analysis.

At present no standardized instruments are in place for the detection of EE2 or E2 in routine monitoring of coastal and continental surface waters. An innovative monitoring strategy for both substances needs to be put forward, validated and implemented.

The Federal Institute of Hydrology in Germany (BfG), the Federal Environment Agency (UBA) and the Swiss Centre for Applied Ecotoxicology invited experts from academia and authorities together with Mario Carere (It, joint lead of the Chemical Monitoring and Emerging Pollutants (CMEP) sub-group under the Common Implementation Strategy for the Water Framework Directive (WFD)) and Bernd Gawlik (European Commission – DG Joint Research Centre, joint CMEP lead) to provide an expert opinion on the suitability of bioassays for the monitoring of EE2 and E2 in surface waters.

Two workshops were held, each focusing on a crucial issue of the present methodical discussion on monitoring of estrogens in surface waters:

1. workshop on bioanalytical options for the monitoring of steroidal effects in surface waters am 27.2.2013 at the BfG in Koblenz
2. workshop on the best practice of chemical analyses for estrogens in surface waters and necessary next steps on 12.3.2013 at the UBA in Berlin

Results and conclusions were documented and presentations will be published by the hosts.

In order to bridge the gap between science and regulatory monitoring of EE2 and E2 the participants agreed at the meeting in Koblenz on the 27.2.2013 upon the following conclusions and recommendations:

1. The specific mode of action of EE2 and E2 allows the design of a tailor-made monitoring strategy.
2. Established bioanalytical instruments (*in vitro* tests) can be used to detect estrogen receptor activation in surface water with sufficient sensitivity. They are currently the only methods with sufficient sensitivity to detect effects at the very low concentrations referred to above for which chemical analysis is not yet reliable; results represent the combined effects of all substances with endocrine disrupting properties (unknown mixtures) present in the sample.
3. Continental or coastal waters exposed to waste water or diffuse sources (e.g. agriculture) contain a mixture of unknown substances with endocrine disrupting properties. The environmental quality standards provide protection based on single-substance no effect concentrations (EQS –Directive).
4. A positive bioassay is indicative of the presence of potent estrogens (e.g. E1, E2 or EE2) in the sample, but is not equivalent to the concentration of a single substance. If this screening shows an effect above a certain threshold value, e.g. the EQS under discussion, further investigation is necessary. This

Recommendation for a monitoring strategy for estrogens in coastal and continental surface waters Part I: Bioassays as screening instruments to detect exposed water bodies

should include chemical analysis and information on the potential regional sources of estrogenic inputs.

5. Negative bioassay responses clearly identify water bodies without a risk-potential caused by EE2 and E2 (screening function)
6. For application to surface water samples bioassays still need validation according to commonly agreed principles (e.g. ISO 17025), guidance for sample handling and specific preparation protocols.
7. To avoid redundant work the validation should be undertaken in a coordinated European exercise and should be conducted under the CIS (Common Implementation Strategy). A collaborative mechanism like the JRC EU-wide monitoring concept offers the opportunity to organize a representative exercise in Europe. Within this exercise further crucial questions of monitoring like the financial implications should be evaluated.
8. The workshop participants recommend using freely available human *in vitro* cell cultures (e.g. T47D-Kbluc, MELN or cell lines validated/under validation by international standardization bodies; OECD, ISO) for a pre- validation study using surface water and to compare it with the ER-Calux running in one laboratory.
9. The combination of bioanalytical measurement and validated models of exposure to steroidal estrogens based on consumption data reduces uncertainties and costs of monitoring programs. Validated models on exposure of pharmaceuticals are available for example from the German Laender projects on “Mikroschadstoffe” (micro-pollutants). A consumption based model of waste water driven substances like EE2 is able to focus monitoring efforts on polluted areas with continuous risks. (<http://www.masterplan-wasser.nrw.de/>
www.oekotoxzentrum.ch/projekte/stofffluss/index_EN).
10. A pre-validation and a following European standardization (ISO, CEN) of such a bioanalytical method would be desirable and should be streamlined with on-going work conducted at ISO. To avoid double work, expertise acquired in the DIN working group “Hormonelle Wirkungen” (AK 119-01-03-05-09), the German mirror committee of ISO TC 147 SC5 WG 9 should be integrated and input from other Member States should be sought.

Recommendation for a monitoring strategy for estrogens in coastal and continental surface waters Part I: Bioassays as screening instruments to detect exposed water bodies

List of participants:

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Recommendation for a monitoring strategy for estrogens in coastal and continental surface waters Part II: Expert opinion on the state of chemical analytics in monitoring EE2 and E2

The second expert-meeting focusing on the perspective of the water authorities in the Federal States (Laender) took place at the Federal Environment Agency (UBA) in Berlin at the 12th of March 2013. Conclusions and recommendations can be summarized as follows:

- The technical equipment of laboratories differs significantly between the German 14 Federal States, attending the meeting. Four Federal States already disposing of the analytical equipment are presently developing an adequate analytical method for the detection of EE2 and E2 based on LC-MS/MS. Four Federal States authorized private laboratories to analyze the water samples. Three Federal States are planning to analyze EE2 and E2 in the near future but are still in the process of purchasing new devices. Bavaria conducts an effect based monitoring with fish (analysis of Vitellogenin) in major rivers and surface waters. Three Federal States are already using bioanalytical methods as screening tools for detecting polluted surface waters-sites and some more are generally interested in using these methods in the near future but do not have own operating experience yet.
- Presently the realistic limit of quantification (LOQ) for EE2 and E2 using the best available analytical method for surface waters lies at approximately 0.1ng/l. However at this LOQ an uncertainty of at least 50% up to 100 % cannot be avoided. This range of uncertainty is not unusual for the analyses of organic micro-pollutants, but reduction would be desirable for compliance monitoring.
- Exposure of almost all surface waters analyzed by the Federal States was below LOQ. However Estrone (E1), a metabolite of EE2 and E2 was always detected.
- Experiences of the Federal States confirm the results from literature concerning the state of chemical analysis for EE2 and E2 and the options of optimizing the state of analytical methods which were summarized in an overview presentation.

Local authorities have to cope with three different monitoring tasks in order to implement the Environmental Quality Standards Directive and assessing the chemical quality of surface and coastal waters:

1. Surveillance-Monitoring: As first step water sites that show a need for risk mitigation measures need to be identified.
2. Compliance-Monitoring: The compliance with the EQS for listed substances has to be determined. For this purpose a sufficiently sensitive analytical method should be available, enabling validated and legally reliable results.
3. Trend-Monitoring: Monitoring has to provide reliable data documenting that river-basin management obeys the non-deterioration-requirement and chemical pollution is continuously decreasing.

To solve both challenges the participants recommend an iterative approach for EE2 and E2 which takes into account the costs as well as uncertainties of measurement in the range of concentrations < 1ng/l. The participants advise combining bioanalytical and chemical methods as efficient and promising.

Recommendation for a monitoring strategy for estrogens in coastal and continental surface waters

Part II: Expert opinion on the state of chemical analytics in monitoring EE 2 and E 2

Laboratories should work closely together developing a monitoring strategy and avoiding redundant work. Additionally the combination of bioanalytical and chemical methods requires interdisciplinary information exchange. A targeted cooperation among the Federal States and with the Federal State would be desirable and should combine the following modules:

1. Interdisciplinary exchange between laboratories relating to their experience with bioanalytical and chemical methods
2. Exchange between chemical laboratories in the fields of method development and quality control
3. Comparison of laboratories aiming at the validation of methods and ensuring quality of results between public and private laboratories

The participants agreed that a monitoring campaign of all Federal States would be a supportive next step aiming at an overall picture of the exposure to both substances. This campaign should be stipulated by the German Working Group on surface water issues of the Federal States and the Federal Government (LAWA-AO). The international river basin management commissions will be asked as partners for cooperation. The monitoring campaign should contain the following four work-packages.

1. Sampling of sewage treatment plant effluents relying on the best available technique
2. Supportive information for plausibility check of the analytical results i.e. mass-flux-models
3. Supportive information on the marketing and consumption of EE2 and E2 available at the federal environment agency
4. Development of a monitoring strategy for surface waters

Further options of future cooperation were discussed i.e. the overall evaluation of monitoring data, funding and the organization of a regular exchange of practical experience. To promote the cooperation the participants agreed upon a follow up meeting.

**Recommendation for a monitoring strategy for estrogens in coastal and continental surface waters
Part II: Expert opinion on the state of chemical analytics in monitoring EE 2 and E 2**

List of participants

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