Final version 20 October 2017
Prepared by Valeria Dulio and Robert Kase

Recommendations for the use of effect-based methods for monitoring of estrogens in surface waters – Outcomes of the Dübendorf Workshop (21 June 2017)

In January 2012 the European Commission proposed *inter alia* that the highly potent estrogens Ethinylestradiol (EE2) and Estradiol (E2) should be prioritised for reduction measures in continental and coastal surface waters. This led to the decision of the Commission to include Ethinylestradiol (EE2), Estradiol (E2) and Estrone (E1) in the first EU Watch List for Union-wide monitoring (Commission Decision 2015/495) [1].

To address the analytical difficulties in monitoring these substances at reasonable cost, at the required threshold values (PNEC), two workshops were organised in February and in March 2013 and a first recommendation paper [2] was published, setting the basis for the actions needed for improved monitoring of estrogens and stressing the need to test the applicability of effect-based methods. The text is included in the annex of the EU technical report on effect-based tools [3].

The recommendation paper triggered the launch of the Science to Policy Interface (SPI) Estrogen monitoring project in 2014, as a voluntary initiative of 12 countries and 24 organisations in Europe under the leadership of Robert Kase and Mario Carere (http://www.ecotoxcentre.ch/projects/aquatic-ecotoxicology/monitoring-of-steroidal-estrogens/). The project demonstrated the feasibility of the use of *in vitro* effect-based methods for the measurement of estrogenic activity in water bodies.

Three years later, thanks to progress made in the estrogen monitoring project and in other research programmes, a new scenario has developed. The EU Water Directors welcomed and supported the proposal by the Commission to consider a more holistic approach for regulation of chemicals in the aquatic environment in view of the WFD review [4]. An Effect-Based Methods (EBM) Activity has been launched as part of the CIS-WFD programme [5]. And finally, the EBM results of the Estrogen monitoring project now provide concrete demonstration data about the performance of the tested effect-based methods (see upcoming publication Könemann et al. 2017 [6] and Kase et al. 2017 [7]).

A further ”Estrogen Monitoring recommendation workshop” was organised by Ecotox Centre at Eawag on 21th June 2017 in Dübendorf (Switzerland) to update, in the light of the new results of the estrogen monitoring project, the list of recommendations to be addressed to DG ENV as an input to the EBM Activity group for operational implementation of EBM in the regulatory framework as tools for assessment of estrogenic activity in surface water bodies. The workshop was moderated by Valeria Dulio and many EBM Activity participants as well as NORMAN and SOLUTIONS experts contributed to this outcome.

As a result of the workshop, the participants agreed on the following conclusions and recommendations.

Regulation of estrogenic activity in the aquatic environment

Estrogenicity is a highly relevant endpoint for aquatic ecosystems and human health, which needs to be monitored and controlled / reduced when the detected activity in the aquatic environment is above the critical levels defined by the legislation.

Estrogen Receptor (ER)-mediated effects should be included as a new endpoint in the regulatory framework of the WFD.

The role of effect-based and chemical analytical methods for regulatory monitoring of estrogens

Chemical analysis of the three substances on the Watch List is now possible thanks to the progress made in recent years.

However, first monitoring results of the EU Watch List substances in 2017 show that this analysis is still challenging for member states. In 21 EU member states the LOQs for EE2 and E2 were above their EQS in > 95% and approximately 50% of the analyses, respectively. This means that in many cases the samples could not be assessed for the population-relevant risks these substances pose.

In the SPI Estrogen monitoring project it was possible to demonstrate that all the effect-based methods tested achieved the required sensitivity criteria and 100% of samples could be assessed. Moreover estrogenicity was quantified with high specificity and sensitivity comparable to chemical analytical methods.

Because of the sufficient low LOQs and acceptable absolute variability in LOQs, effect-based methods are reliable and suitable for risk assessment and prioritisation of polluted sites.

Besides, effect-based methods are the only currently available methods to address unknown mixture risks. Bioassays are able to respond to the recently recognised need to change the paradigm; that is, instead of measuring three individual substances, it makes more sense to measure the effects caused by these substances plus other unknowns that may contribute to the overall effect.

In conclusion, EBM are able to provide more complete information about the estrogenic activity present in the water bodies, which can then be related to specific chemical compounds via chemical analysis.

Next steps for implementation of EBM under the WFD

In the light of these considerations, no participant voted to stick with chemical analytical assessment alone. The majority recommended the use of *in vitro* effect-based methods as screening tools to reduce chemical analytical monitoring burden.

As regards the implementation framework, around 30% of the participants were in favour of a status assessment using *in vitro* effect-based methods in combination with chemical analytical substance identification, only in cases where it is not possible for water managers to identify the pollution source(s). In other words, when the cause / source of the observed estrogenic activity is known, water managers can apply reduction measures without need for chemical analysis.

In terms of practical implementation of EBMs in the regulation, participants agreed that the procedure (i.e. protocol for sample preparation, extraction, etc.) developed in the estrogen monitoring project should be further tested in the Watch List mechanism before inclusion in the WFD review. A new effect-based Watch List project [8] started in September 2017.

The determination of effect-based trigger values (EBT) is another important short-term action which is needed to allow water managers to distinguish between more and less polluted sites. Different approaches to deriving EBTs have been proposed for the ER-mediated activity [9], [10], [11]. They all have advantages and disadvantages, but they all result in generally comparable EBT levels.

All currently proposed EBT values were tested in the SPI Estrogen Monitoring project. A screening EBT of 400 pg/L BEQ (bioanalytical equivalent concentration) was identified as sufficient to identify a high percentage of population-relevant mixture risks (see upcoming publication Kase et al. 2017 [7]). This EBT value will be further tested in the upcoming effect-based Watch List project.

Participants recognised that it is important to take into account that the response of EBM to chemical contaminants may vary depending on the bioassay applied: this is mainly due to the relative sensitivity of different assays to a given compound. This could lead to the need to define a specific EBT for each EBM. However, this is not a practical solution for routine use of EBM in a regulatory context. The great majority of the participants voted for an alternative option, which is to combine a common screening EBT of 400 pg/L BEQ with the application of test-specific sensitivity factors (i.e. (screening EBT) ÷ (test-specific sensitivity factor) = test-specific EBT). The upcoming publication by Escher et al. 2017 [12] will develop an additional approach for the derivation of EBTs which can be compared with the sensitivity factor proposal.

In any case it is recommended that the way to proceed when an EBT is exceeded should be as follows. A common Mode-of-Action-specific EBT should be defined as a guidance value to assess estrogenicity of a surface water, e.g. if the measurement result is < EBT, this will mean low risk probability; ~ EBT = risk possible; > x EBT = high risk probability. The risk quotient (RQ), defined as RQ= BEQ/EBT, will characterise the risk of the sample and allow a prioritisation of samples.

The use of screening methods must aim to avoid false-compliant results. For example, in the dioxin guidelines EC/644/2017 and ISO 19040, for the results from screening methods to be accepted as reliable, it is necessary to show that they fulfil the requirement of a false-compliant rate of less than 5%. It is recommended that the same approach should be used for the selection of relevant effect-based methods (*in vitro* and *in vivo*) which can be used alongside chemical methods for the evaluation of complex mixtures occurring in different types of aquatic environments.

Consideration of the comparability of the results given by the different methods, and as far as possible the definition of quality control criteria for these methods in the context of the WFD, on the lines of the criteria defined by the QA/QC Directive, should be addressed.

Workshop participants list

Name	Institute	Country
Aliaksandra Shuliakevich	RWTH – University of Aachen	Germany
Anja Duffek	Federal Environment Agency Germany (UBA	Germany
Anke Hofacker	Swiss Federal Office for the Environment (FOEN)	Switzerland
Armelle Hebert	VEOLIA and Eureau	France
Beate Escher	UFZ Leipzig and University of Tübingen	Germany
Brigitte von Dannwitz	LANUV Nordrhein-Westfalen	Germany
Carole Chalon	ISSeP	Belgium
Christoph Scheffknecht	Umweltinstitut Vorarlberg	Austria
Cornelia Kienle	Swiss Centre for Applied Ecotoxicology. Eawag-EPFL	Switzerland
Daniel Olbrich	Swiss Centre for Applied Ecotoxicology. Eawag-EPFL	Switzerland
Dorien ten Hulscher	RWS	The Netherlands
Els Smit	RIVM	The Netherlands
Francois Brion	INERIS	France
Henner Hollert	RWTH – University of Aachen	Germany
Inge Werner	Swiss Federal Office for the Environment (FOEN)	Switzerland
Jochen Türk	IUTA	Germany
Klara Hilscherova	RECETOX	Czech Republic
Lut Hoebecke	VMM	Belgium
Manfred Clara	Federal environmental agency	Austria
Manfred Frey	STW	Germany
Marco Scheurer	TZW Technologiezentrum Wasser	Germany
Maria Fürhacker	BOKU	Austria
Mario Carere	Istituto Superiore di Sanità	Italy
Olivier Perceval	Agence française biodiversité	France
Peter Behnisch	BDS Ltd	The Netherlands
Premysl Soldan	VUV	Czech Republic
Robert Kase	Swiss Centre for Applied Ecotoxicology. Eawag-EPFL	Switzerland
Ron van der Oost	Waternet	The Netherlands
Sarah Crawford	RWTH – University of Aachen	Germany
Sarah Könemann	Eawag	Switzerland
Sebastian Buchinger	Federal Institute of Hydrology (BfG)	Germany
Selim-Ait-Aissa	INERIS	France
Valeria Dulio	INERIS	France
Werner Brack	UFZ-Leipzig	Germany
Yael Schindler	Swiss Federal Office for the Environment (FOEN)	Switzerland
Yves Marneffe	ISSeP	Belgium

Acknowledgements

We would like to thank Stéphanie Schaan – European Commission DG ENV – for the useful suggestions she provided for improvement and clarification of the final version of this document.

References

[1] Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council, Off. J. Eur. Union L 78 (2015) 40, n.d.

[2] C. Heiss et al., (2013). Recommendation for a monitoring strategy for estrogens in coastal and continental surface waters, included in the annex of the Technical report on aquatic effect-based monitoring tools. https://static-content.springer.com/esm/art%3A10.1186%2Fs12302-015-0039-4/MediaObjects/12302_2015_39_MOESM2_ESM.pdf page 49.

[3] European Union, (2014). Technical report on aquatic effect-based monitoring tools. https://static-content.springer.com/esm/art%3A10.1186%2Fs12302-015-0039-4/MediaObjects/12302_2015_39_MOESM2_ESM.pdf

[4] EU WG Chemicals, Priority Substances – Outcome of Water Directors meeting, (2016). https://circabc.europa.eu/sd/a/096c7e10-1be3-4ffa-b5d3-e8cab1b86c7f/WG Chem 2016-12 (10) WD2016962 outcome Priority Substances.pdf. Last access 25.08.17.

[5] EU WG Chemicals, Activity: Effect-based tools / methods for WG Chemicals as part of the Water Framework Directive CIS Work Programme (2016-2018) endorsed by the Water Directors, (2016). https://circabc.europa.eu/sd/a/e39a14f1-ae0a-4ec7-9dec-2a8c6c98645a/Draft Terms of References for the Effect-Based Activity - 08-07-2016.docx. Last access 13.04.17.

[6] S. Könemann et al. Effect-based and chemical analytical methods to monitor estrogens under the European Water Framework Directive. In prep. (2017).

[7] R. Kase et al. Screening and risk management solutions for steroidal estrogens in surface and wastewater. In prep. (2017).

[8] R. Kase and A. Duffek, (2017). Applied effect-based monitoring of EU watch list samples. https://circabc.europa.eu/w/browse/209ca179-1ab3-4dc1-81f9-0b3e7fdb8885. Last access 13.04.17.

[9] B. Jarosova, L. Blaha, J.P. Giesy, K. Hilscherova, What level of estrogenic activity determined by in vitro assays in municipal waste waters can be considered as safe?, Environ. Int. 64 (2014) 98-109 doi:10.1016/j.envint.2013.12.009.

[10] R. Kase, P.Y. Kunz, H. Hollert, I. Werner. Contribution on bioanalytical assays for steroidal estrogens, in EU JRC report by R. Loos "Analytical methods relevant to the European Commission's 2012 proposal on Priority Substances under the Water Framework Directive" (2012). doi:10.2788/51497.

[11] R. Van der Oost, G. Sileno, M. Suárez-Muñoz, M.T. Nguyen, H. Besselink, B. Bram, SIMONI (Smart Integrated Monitoring) as a novel bioanalytical strategy for water quality assessment; Part I: model design and effect-based trigger values, Environ. Toxicol. Chem. accepted (2017).

[12] Escher et al. Effect-based trigger values for in vitro bioassay supporting the environmental quality standards (EQS) of the European Water Framework Directive. In prep. (2017).