

---

## Publishable Summary for 18NRM01 EDC-WFD

### Metrology for monitoring endocrine disrupting compounds under the Water Framework Directive

#### Overview

Natural and pharmaceutical estrogens are key Endocrine Disrupting Chemicals (EDC) which are monitored differently depending on the country, and for which standardised reference methods are currently not available. The overall objective of the project is to develop reliable and harmonised measurement methods for estrogens, to comply with the Water Framework Directive requirements (Directive 2013/39EC, Commission Directive 2009/90/EC and Commission Implementation Decision (EU) 2018/840). The outcomes of this project, in particular the validated mass spectrometry (MS) based reference methods, will be disseminated to CEN/ TC 230 and ISO/ TC 147 to be fed into the documentary standards they develop.

#### Need

It is known that estrogens may end up in surface waters *via* wastewater, and due to their physicochemical properties, they can partition in the different compartments (water and suspended particulate matter (SPM)) of water systems. Despite occurring at ultra-trace levels (below ng/L), it is believed that they are, due to their estrogenic potency, contributing to the large rise in the number of feminised fish and other endocrine disruptive effects. Moreover, they may be a factor in biodiversity loss. Therefore, appropriate measurement methods are necessary which allow estrogen levels below the environmental quality standard (EQS) to be monitored and to show if a water body is at risk.

The objectives of this project were derived from the need expressed by different communities. CEN/ TC 230 "Water analysis" has agreed that there is a lack of standardised analytical methods to monitor three relevant estrogenic substances (17-beta-estradiol, 17-alpha-ethinylestradiol and estrone) in conditions that meet the requirements of the WFD and its derivatives. There are currently no methods available to guarantee the integrity of samples between sampling and analysis, nor quality control tools to ensure reliability. In addition, there are no CEN or ISO standards currently available to address the measurement of EDCs by conventional chemical analysis, and reference materials for validating in-house methods and establishing quality assurance and control measures are not available. Some promising effect-based methods for EDC are under development at ISO but they just complement the classical approach of quantitative methods.

ASLAE, an association representing 50 testing laboratories in the field of water analysis, has highlighted that many of its members face difficulties in developing and validating estrogen measurement capabilities and have failed to achieve the very low limits of quantification that are required by WFD.

For a substance to be added to a regulatory monitoring list, the French Ministry of the environment requires a reliable reference method to be available.

#### Objectives

The overall objective of this project is to develop traceable measurement methods for endocrine disrupting chemicals, with a specific focus on three estrogens of the first watch list (17-beta-estradiol (17 $\beta$ E2), 17-alpha-ethinylestradiol (17 $\alpha$ EE2), and estrone (E1)). Estrogens 17-alpha-estradiol (17 $\alpha$ E2) and estriol (E3) will be included to demonstrate the reliability of the developed methods and to support the requirements of Directive 2013/39/EC, Directive 2009/90/EC and Commission Implementation Decision (EU) 2018/840, hence improving the comparability and compatibility of measurement results within Europe.

The specific objectives of the project are to:

1. Optimise and validate traceable aqueous reference mass spectrometry based methods for the analysis of 5 estrogenic compounds prioritising 3 selected estrogenic compounds 17-beta-

estradiol, 17-alpha-ethinylestradiol, and estrone in whole water samples at environmental quality standard (EQS) levels. Methods will have limit of quantification (LOQ) not exceeding 30 % EQS with a measurement uncertainty of  $\leq 50$  % at EQS.

2. Evaluate the interaction and partitioning of 5 estrogenic compounds prioritising 3 selected estrogenic compounds 17-beta-estradiol, 17-alpha-ethinylestradiol, and estrone between water samples and suspended particulate matter (SPM) and the capability of developed methods to address the different fractions of matrix (whole water and dissolved concentrations of estrogens).
3. Develop production methods for aqueous reference materials (RM), which are as close as possible to real water samples, with proven homogeneity, short- and long-term stability.
4. Improve the comparability of estrogen measurements with selected Effect-Based Methods (EBM) in whole water samples at EQS level. Methods will have been correctly calibrated and information on uncertainty will be provided.
5. Organise and perform an interlaboratory comparison (ILC) to demonstrate the performance of the developed methods using the reference material (RM) for the selected estrogen substances.
6. Contribute to the work of key European and international standardisation organisations e.g. CEN TC 230 and ISO TC 147 ensuring that the outputs of the project are aligned with needs, communicated quickly to those developing the standards and to those who will use them to support the implementation of directives, and in a form that can be incorporated into the standards at the earliest opportunity.

### Progress beyond the state of the art

As a result of the lack of validated measurement methods for estrogens (in terms of limit of quantification (LOQ), accuracy, and uncertainty), the comparability and reliability of measurement results has been proven to be inadequate. The partitioning (distribution) of estrogens between suspended particulate matter (SPM) and dissolved phase of water is not fully understood and the resulting problems in analysis are widely ignored. This knowledge is critical for the comparability of measurements between laboratories and for the evaluation of the chemical status that will be based on whole water measurements. A search in the international reference material database COMAR (International reference material database) revealed that no representative RM (reference material) exists for estrogens. This can be explained by the estrogens' lack of stability in real matrix and missing fit for purpose measurement methods. EBM (Effect-based method) used for identifying estrogen receptor (ER) mediated risk can overcome the LOQ problems encountered with other analytical techniques, e.g. MS-based methods such as LC-MS-MS and GC-MS-MS. These are suitable screening tools for the identification and prioritisation of waterbodies requiring further examination, as well as for measuring the ecotoxicological status in relation to receptor-mediated estrogenicity. Proficiency tests for estrogens are rarely offered and there is low confidence in the quality of estrogen measurements.

#### *Fully validated MS-based reference methods for the detection of estrogen (objective 1):*

This project will optimise and validate MS-based reference methods for the analysis of the 5 estrogenic compounds, prioritising 3 selected ones. This will include complementary sample preparation techniques, stabilisation of samples, extraction, purification and re-concentration to enable reliable measurements of estrogens. To maximise the impact of the project, the performance of the methods for 17-beta-estradiol, 17-alpha-ethinylestradiol, and estrone will be aligned to the requirements of Directive 2009/90/EC in such a way that the methods will have a LOQ not exceeding 30 % EQS, with a measurement uncertainty  $\leq 50$  % at EQS.

#### *Comprehensive study of estrogens partitioning in water (objective 2):*

This project will address this issue through analysis and systematic comparison of the results of filtered and unfiltered water samples, with varying complexity (Dissolved organic carbon (DOC), SPM, etc.). As a result, this project will provide knowledge on the effect of filtration, and comparability of results obtained from filtered and unfiltered samples.

#### *Reference material preparation and characterisation (objective 3):*

This project will produce a representative synthetic (real) matrix RM. At least 1 selected MS-based method will be used to characterise the RM candidates with respect to level of concentration (realistic from

environmental conditions), partitioning of substances between SPM, dissolved phase, homogeneity and stability. The compatibility of the developed materials with effect-based methods (EBM) will also be verified.

*Well characterised bioassays methods (objective 4):*

This project will build on work developed under DG ENV WG Chemicals, and address issues associated with the measurement of estrogens, focusing on metrological aspects and establishing traceability of measurements through appropriate calibration and estimation of uncertainty.

*Standardisation and intercomparison as knowledge transfer to end-users (objective 5):*

This project will establish strong links with CEN/ TC 230, ISO/ TC 147 and national standardisation bodies and provide them documents and reports with the outcomes of this project. The interlaboratory comparison organised by this project will reach end-users, in particular testing (accredited) laboratories, which will facilitate the quick uptake of the developed methods and knowledge by testing laboratories and regulatory bodies.

## Results

*The program of activities and production of results have been impacted by coronavirus (COVID-19) outbreak.*

*Fully validated MS-based reference methods for the detection of estrogen (objective 1):*

BAM and ISPRA with the involvement of all partners have made an overview of the different methods that will be developed by the partners with details of the different methodologies to sustain synergy between partners and keep time. Each partner has started the optimization of their methods to reach the target LOQ (limit of quantification). LNE have made a first estimate of the limit of quantification of its method and demonstrate it can reach the very low targeted LOQ in simple matrix.

On the basis of an overview European database, the selection of 3 representative water samples (synthetic real matrix water) with distinct amounts of SPM (solid particulate matter) and DOC (dissolved organic carbon) has been discussed and agreed. They will be the basis of the development of the matrix-match certified reference material and for the validation of methods.

BAM with all partners discussed and agreed on experimental plan design for the short-term stability study. The optimisation of sample preparation methods

*Comprehensive study of estrogens partitioning in water (objective 2):*

This project will address this issue through analysis and systematic comparison of the results of filtered and unfiltered water samples, with varying complexity (Dissolved organic carbon (DOC), SPM, etc.). This activity has not yet been addressed.

*Reference material preparation and characterisation (objective 3):*

TUBITAK will produce CRMs of five analytes listed on the project (17-beta-estradiol, 17-alpha-ethinylestradiol, estrone, 17-alpha-estradiol and estriol) according to ISO 17034 for further dispatch to project partners after certification. TUBITAK has made the selection of the raw material after been discussed and agreed with all partners. The material has been purchased.

SYKE and BAM organised the production procedures for the candidate RM. The homogeneity and stability study are ready to be launched.

*Well characterised bioassays methods (objective 4):*

IPSRA with all involved partners have organised technical meetings to define the experimental design that will be implemented by each partner to harmonize between partners.

*Standardisation and intercomparison as knowledge transfer to end-users (objective 5):*

Soon the methods and stability design of candidate RM will be achieved, this project will organise and perform an interlaboratory comparison (ILC) to demonstrate the performance of the developed methods using the reference material (RM) for the selected estrogen substances.

## Impact

The website of the project has been created. <http://projects.lne.eu/jrp-Poster>

Two presentations of the project have been realised at Eurachem Workshop - Uncertainty from sampling and analysis for accredited laboratories Berlin, 19-20 November 2019 and ICRAPHE 2nd International conference on risk assessment of pharmaceuticals in the environment (Barcelona, 28/-29 November 2019)

An advisory group composed by : Ulrich BORCHERS (IWW water center CHIEF STAKHOLDER of the project, TC CHAIR CEN/TC230 « Water Quality»), Mario CARERE ( National Institute of Health ISS, researcher, expert for WG Chemical EC), Pierre François STAUB (French Agency for Biodiversity AFB; head project water pollution and metrology), Olivier PERCEVAL (French Agency for Biodiversity AFB; head project ecotoxicology), has been implemented and will be informed on the project regularly. Their recommendations from the first meeting 8th October 2019 have been discussed and amended by the project partners.

### *Impact on industrial and other user communities*

This project will enable harmonised monitoring of endocrine disrupting compounds in water in response to European water policies. Regulatory acceptance of emerging technologies is a slow process, and currently hampers the use of such modern bioassays for compliance testing and regulatory purposes. The outcomes of this project will facilitate the adoption of such technologies. Testing (accredited) analytical laboratories will be targeted to benefit from this project, therefore supporting the provision of services. The impact will be boosted by the implementation of an advisory group with representative of policy-makers, testing laboratories, scientific community. This project will establish specific interactions with JRC-ISPRA that advice the European Commission to allow quick communication of the project's results and outputs.

### *Impact on the metrology and scientific communities*

This project will support the metrology community in handling the long-standing scientific problem of environmental monitoring and risk assessment. This project will have direct impact on different metrology committees, especially the EURAMET Technical Committee of Metrology in Chemistry (TC-MC) and the Organic Analysis Working Group (OAWG) of the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) of the BIPM. CCQM-OAWG is responsible for the CMC (Calibration and Measurement Capabilities) entries related to chemistry. It will contribute to the visibility of EURAMET and its leadership in chemical metrology for environment to the wider metrology and scientific communities. The results of the project will form the basis for developing calibration and measuring capabilities (CMC) entries related to estrogens and comparable substances in water.

The project will communicate the results and scientific knowledge gained in the project to the scientific environmental analytical community via open access publications in peer-reviewed journals, workshops and training. It will increase the awareness of a wider community on quality assurance and quality control (QA/QC) issues as well as metrology concepts that are often misunderstood or misapplied. As measurements are often instrumental for research in diverse scientific fields, the methods developed will provide scientists with guidance to make their measurements metrologically sound.

### *Impact on relevant standards*

The results of this project are expected by CEN/ TC 230/ WG1 "Physical and biochemical methods".

The outputs of this project will have an early impact on current standardisation work by providing the knowledge and scientific reports for future technical report(s), technical specification(s) or standards to CEN/TC 230/ WG 1 and by providing input to ISO/ TC 147 so that they can feed into the revision of ISO 5667-3 and ISO DTS 21231. This project will also complete the set of ISO 19040(1-3) standards providing recommendations.

### *Longer-term economic, social and environmental impacts*

The outcomes of this project will improve the assessment of human and environmental risks related to the occurrence of endocrine disrupting chemicals in the environment through more accurate and reliable measurement data. The project will enable public authorities to provide data with high level of confidence, to ensure an efficient and comparable implementation of the WFD between Member States, and to inform European citizens who have clearly demonstrated their concern about EDC. By providing measurement results with full uncertainty budgets at very low level of concentration, the project will contribute to better decision making by European policy makers and, as a consequence, to a better protection of human health,

aquatic environment and biodiversity. Furthermore, the comparability of data will enable an indirect financial impact by reducing the costs of monitoring and prevention of incorrect decision making.

Project start date and duration:		01 September 2019, 36 Months	
Coordinator: Sophie Lardy-Fontan, LNE		Tel: + 33140433939	E-mail: <a href="mailto:sophie.lardy-fontan@lne.fr">sophie.lardy-fontan@lne.fr</a>
Project website address: <a href="http://projects.lne.eu/jrp-edc-wfd/">http://projects.lne.eu/jrp-edc-wfd/</a>			
Chief Stakeholder Organisation: CEN/ TC 230 "Water Analysis"		Chief Stakeholder Contact: Ulrich Borchers (Chair TC 230)	
Internal Funded Partners:	External Funded Partners:	Unfunded Partners:	
1. LNE, France 2. BAM, Germany 3. NIC, Slovenia 4. SYKE, Finland 5. TUBITAK, Turkey	6. ISPRA, Italy 7. JSI, Slovenia 8. UBX, France		
Linked Third Parties: 9. CNRS, France (Linked to UBX)			
RMG: -			