



European Cluster to
Improve Identification
of Endocrine Disruptors

EURION Cluster Annual Meeting Report 2023

EURION Cluster Annual Meeting | 30-31 January 2023 | Amsterdam



The 4th Annual Meeting of the EURION Cluster was held on 30–31 January 2023 at the Vrije Universiteit, Amsterdam, to present and discuss the latest advances in research by the EURION Cluster towards improving the identification of endocrine disruptors. The aim of the EURION Annual Meeting was to facilitate cross-project collaboration on topics of common interest and maximize synergies between projects.

Additionally, the aim of the meeting was to inform and engage stakeholders and the EURION International Advisory Panel for Regulatory Affairs (IAP). Both morning sessions on 30 January were open online to the interested stakeholders.

The meeting was attended in Amsterdam by about 140 delegates on site, including Cluster members, IAP members and EURION observers, and 40 more stakeholders online.

Summary of proceedings

The morning sessions on Monday 30 January 2023 were the public facing part of the meeting. The meeting coordinators, Majorie van Duursen (FREIA) and Anna-Liisa Levonen (EDCMET) opened the meeting and gave an **introduction to the day**. This was followed by a presentation by the **DG ENV representative** Mara Ceridono to address EU policy context in relation to EDs and the **DG RTD representative** Alex Mulet Indrayanti, addressing EU policy in relation to EU funded research in environment and health in connection with EDs.

The first session was concluded with an overview of **EURION Cluster highlights in 2022** presented by previous EURION Cluster coordinators Lorenzo Moroni (SCREENED) and Andreas Kortenkamp (ATHENA). The third EURION Cluster meeting, organized as a virtual event in 2022, connected 300 participants to

discuss cross-project collaboration. Main outcomes of the 2022 meeting were a follow-up meeting organized by three EURION thyroid projects and a formation of a new working group on thyroid hormone analysis. This joint meeting between ENDpoiNTs, ERGO and ATHENA aims to build on synergies and explore new areas for collaboration. During this meeting, a need for re-assessment of the current EURION Working Groups to avoid overlaps was established. Another highlight was the joint JRC-EURION Workshop on Test Method Validation, which included presentations and interactive discussion of questions related to the test readiness criteria and technical issues related to the methods. The online EURION Stakeholder Workshop, held on 2 December 2022 with 85 attendees, provided an overview of each cluster project and stakeholder (regulatory authorities, industry, non-governmental organizations) viewpoints e.g., on data gaps, test models, modes of studies and AOP networks. In addition, EURION was presented at the 4th ED forum, several scientific publications have been produced in all EURION projects and cluster news are followed by close to 300 followers on the EURION Twitter account and website.

In Session 2, **EURION project highlights** for each project were presented by the respective coordinators. Presentations were given in Project alphabetical order, by ATHENA (Andreas Kortenkamp), EDCMET (Anna-Liisa Levonen), ENDpoiNTs (Joëlle Rüegg), ERGO (Henrik Holbech), FREIA (Majorie van Duursen), GOLIATH (Juliette Legler), OBERON (Karine Audouze) and SCREENED (Lorenzo Moroni). Prof. Andreas Kortenkamp presented findings on TPO inhibitors, heterotopia, and work on related AOPs in the ATHENA project. Further, the ED criteria, predictivity of thyroid histopathology and usefulness of heterotopia in developmental neurotoxicity was discussed. Prof. Anna-Liisa Levonen presented the highlights of the EDCMET project; several novel or improved *in silico*, *in vitro* and *in vivo* methods and tools have been developed, and some are approaching the pre-validation stage. An overview of recent results from e.g., molecular modeling, omics analyses and *in vivo* mouse studies as well as findings from epidemiological studies, were presented. Prof. Joëlle Rüegg presented the objectives and highlights of the ENDpoiNTs project. They have been focusing on their extensive *in vitro* work to decipher several important hormonal pathways and integrating this information to AOPs. Further, *in vivo* rat studies using six ED compounds and related transcriptomics and steroidomics studies indicating effects on glucocorticoids and thyroid hormones, were presented. Prof. Henrik Holbech gave an overview of the ERGO project and their work on thyroid-related biomarkers and



Opening of the 4th EURION Cluster meeting by coordinators Prof. Majorie van Duursen (FREIA) and Prof. Anna-Liisa Levonen (EDCMET)

endpoints in amphibians and how these can be used in cross-species AOP development to support extrapolation between species. The FREIA project, represented by Prof. Majorie van Duursen, focuses largely on the function of the human ovary and results from perinatal rat studies with the selected EDCs DES and Ketoconazole were presented. Novel biomarkers on EDC-mediated effects on ovarian steroidogenesis were established and will be investigated further. Prof. Juliette Legler presented the battery of methods, including *in silico* approaches, *in vitro* methods on NR activation and functional metabolic endpoints and omics approaches, and IATAs under development in the GOLIATH project. Prof. Karine Audouze also presented the progress in the development of a battery of pre-validated methods in the OBERON project. They have for example developed an open-source workflow for bioinformatics and systems biology models for AOPs as well as an online tool ReadEDTest and webserver AOP-helpFinder 1.0. Prof. Lorenzo Moroni presented the overview of the advances in the SCREENED project, which focuses on the development of 3D *in vitro* models

and EDC effects on thyroid. *In silico* model with experimental data for AOPs and integrated omics platform are to be validated and 3D models to be tested in the near future.

After lunch, the meeting continued on site with EURION members as well as IAP members and EC observers.

During lunch, there was time for poster viewing. There were 40 posters presented. All posters were visited by the poster award jury,



Poster viewing during lunch break on Monday 30 January.

comprised of eight jury members, one from each cluster project, that chose the winning poster of the meeting.

Session 3 consisted of eight short poster spotlight presentations, one from each EURION project. These were:

- ATHENA: Fabian Wagenaars, Inhibition of OATP1C1 and OAT4 mediated thyroxine uptake by endocrine disrupting chemicals
- EDCMET: Thales Kronenberger, Conformational changes in nuclear receptors upon ligand binding
- ENDpoiNTs: Maria Sapounidou, From human biomonitoring to *in vitro* testing - a reverse dosimetry protocol to estimate foetal concentrations for chemicals with suspected neurodevelopmental adversity
- ERGO: Rikke Poulsen, Thyroid hormone quantifications in serum and tissue
- FREIA: Ana Engedal Nissen, QSAR models to predict female fertility disruption MIEs
- GOLIATH: Patrick Balaguer, Nuclear receptor reporter cell lines to characterize EDCs
- OBERON: Nafsika Papaioannou, Multi-omics analysis to investigate the disruption of lipids metabolism induced by exposure to plasticisers and per-and polyfluoroalkyl substances (PFAS)
- SCREENED: Marta Nazzari, Investigation on the effects of phthalates exposure on *in vitro* thyroid models with RNA-Seq and ATAC-Seq.

The later afternoon of Day 1 was dedicated to parallel **Working group sessions**: WG Omics (led by Karine Audouze), WG In vitro (led by Lorenzo Moroni), WG Validation (led by Sharon Munn), WG Epidemiology (led by Carl-Gustaf Bornehag), WG In vivo (led by Sofie Christiansen) and WG In silico (led by Maria Sapounidou).



Poster spotlight presentation by Thales Kronenberger (EDCMET)

Day 2 of the meeting (31 January 2023) opened with three parallel **Cross-cutting EURION cluster meetings**. The aim of these meetings was to allow discussion and sharing of ideas. The topics discussed in these sessions were: Metabolic disorders (chaired by Prof. Anna-Liisa Levonen from EDCMET), Thyroid system disruption and developmental neurotoxicity (chaired by Prof. Andreas Kortenkamp from ATHENA) and Circulating hormones, feedback loops and linear AOPs (chaired by Prof. Majorie van Duursen from FREIA).



Panel discussion with EURION coordinators

Findings and feedback from the cross-cutting meetings were discussed further in a form of a panel discussion chaired by Prof. Joëlle Rüegg (ENDpoiNTs). The main outcomes of the Metabolic disorders session were fruitful discussions on technical aspects of various assays, assay validation approaches and possibilities for collaboration. It was decided to establish a steatosis interest group, as one of the new EURION Working Groups. In the Circulating hormones session, the suitability of linear AOPs to present effects on the endocrine system were discussed, as well as the need for more quantitative knowledge on

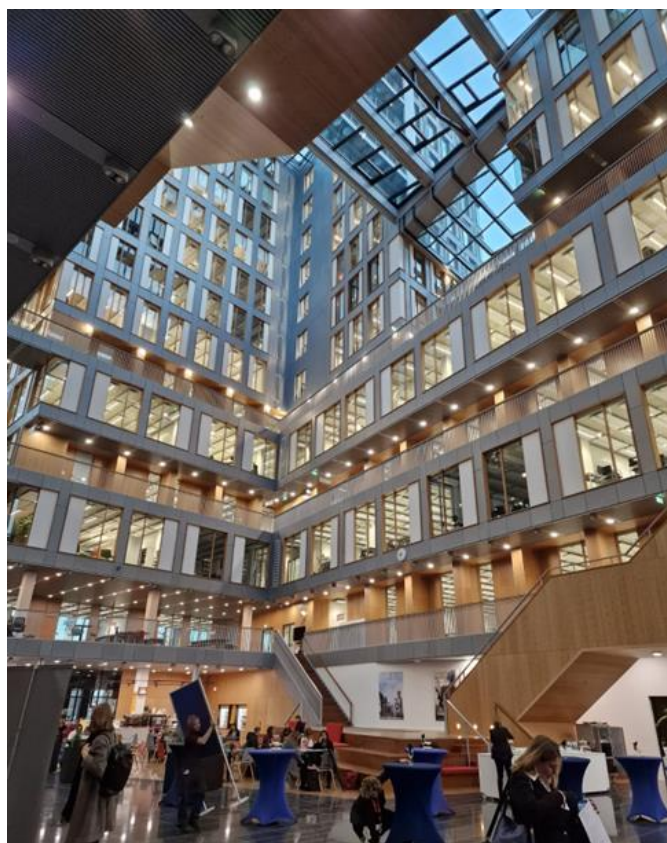
key event relationships and weight of evidence and usability of measuring levels of circulating steroid hormones. The challenges to move away from *in vivo* models and species differences were also discussed. The Thyroid session participants have already been closely collaborating and the group discussed e.g., potential collaborative opinion papers on EDC thyroid effects vs. developmental neurotoxicants. Regulatory and risk assessment aspects related to these were discussed. The overall discussion focused on the definitions of hormones, endocrine disruptors and receptors, as well as opportunities to move more towards mode-of-action based approaches.

The afternoon session of Day 2 was dedicated to the helpful [feedback from and discussion with the International Advisory Panel \(IAP\)](#). A separate report on this session has been created by Sharon Munn, moderator and chair of the IAP.

The cluster concluded with [closing remarks](#) from Majorie van Duursen and Anna-Liisa Levonen and the announcement of the best poster of the meeting. The [winning poster](#) for the EURION Annual Meeting 2023 was entitled: Integrated approach to OMICs markers of DNT in human epidemiology and rodents: Epigenetics, presented by Andrea Cediel-Ulloa from ENDpoiNTs.



Finally, a EURION cluster meeting on site again!



At the Vrije Universiteit Amsterdam VU.NU building.



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