EURION

European Cluster to
Improve Identification
of Endocrine Disruptors

EURION Progress Report

Public summary, June 2022

Table of Contents

1 Introduction 3

2 Project summaries 5

2.1 ATHENA project summary 5

2.2 EDCmet project summary 8

2.3 ENDpoiNTs project summary 10

2.4 ERGO project summary 13

2.5 FREIA project summary 17

2.6 GOLIATH project summary 20

2.7 OBERON project summary 23

2.8 SCREENED project summary 26

3 Overview of test methods to be developed 28

4 Cluster highlights 29

5 Working groups 30

5.1 ADVERSE OUTCOME PATHWAYS (AOPs) WG 31

5.2 CHEMICALS WG 32

5.3 ANIMAL STUDIES: RODENTS WG 32

5.4 ANIMAL STUDIES: AQUATIC ORGANISMS WG 33

5.5 IN VITRO MODEL STUDIES WG 34

5.6 REGULATORY AFFAIRS AND POLICY (RAP) WG 34

5.7 INTEGRATED APPROACHES TO TESTING AND ASSESSMENT (IATA) WG 35

5.8 EVIDENCE-BASED TOOLS WG 35

5.9 IN SILICO WG 36

5.10 EPIDEMIOLOGY WG 37

5.11 DATA MANAGEMENT WG 37

5.12 DISSEMINATION AND COMMUNICATION WG 38

5.13 OMICS 39

5.14 VALIDATION WG 39

1 Introduction

The *European Cluster to Improve Identification of Endocrine Disruptors*, EURION, is a cluster of eight European research projects. The projects are focusing on the development of new endocrine disruptor (ED) test methods targeting relevant endpoints that are not sufficiently covered by current regulatory tests. As the current testing tools, including regulatory *in vivo* tests and novel *in vitro* assays, do not appropriately identify ED-induced effects related to certain less studied endocrine-mediated pathways or health outcomes, the EURION projects aim to address these gaps. *EURION,* was launched by DG-RTD in Brussels, Belgium, 31 January 2019 with representatives from the eight projects as well as the International Advisory Panel (IAP) and observers from various EU agencies. The aim was to optimise synergies and avoid overlaps between the projects selected for funding from the call SC1BHC-27-2018 'New testing and screening methods to identify endocrine disrupting chemicals'.

The projects are:

- ATHENA - Assays for the identification of thyroid hormone axis-disrupting chemicals: elaborating novel assessment strategies.
- EDCMET - Metabolic effects of endocrine disrupting chemicals: novel testing methods and adverse outcome pathways
- ENDpoiNTs - Novel testing strategies for endocrine disruptors in the context of developmental neurotoxicity
- ERGO - Breaking down the wall between human health and environmental testing of endocrine disrupters: EndocRine Guideline Optimisation
- FREIA - Female reproductive toxicity of EDCs: a human evidence-based screening and identification approach
- GOLIATH - Beating Goliath: Generation of novel, integrated and internationally harmonised approaches for testing metabolism disrupting compounds
- SCREENED - A multistage model of thyroid gland function for screening endocrine-disrupting chemicals in a biologically sex-specific manner
- OBERON - An integrative strategy of testing systems for identification of EDs related to metabolic disorders

The cluster projects will:

- Deliver novel/improved ED assay candidates for regulatory use (pre-validated);
- Provide support for the OECD work on testing and assessing chemicals for ED identification, especially feeding into the OECD Endocrine Disrupter Screening and Testing Programme.
- Contribute to enhanced international cooperation.
- Contribute to the development of an international strategy and guidelines for testing EDs and assessing associated hazard and risk

As new and improved approaches are needed to increase the quality, efficiency and effectiveness of existing methods to meet demanding and evolving regulatory requirements, EURION enables effective collaboration between the projects to reach and contribute towards the main goal. Being part of the Cluster facilitates

sharing of knowledge, data and expertise as well as allowing the joining of forces for common dissemination and communication activities in order to maximize impact, both at the project and cluster-level.

The EURION Cluster is coordinated by rotating teams consisting of two projects at a time to cover the 5-year life span of the projects. For the first 15 months, the EURION Cluster was coordinated by GOLIATH and OBERON. The next 15 months were coordinated by ENDpoiNTS and ERGO. Since July 2021, ATHENA and SCREENED are the organisers, they will coordinate EURION and organise the annual meeting in January 2022. They hold the position as organiser until October 2022 after which EDCMET and FREIA will act as coordinators during the last quarter of the funding period.

This report summarises the progress and activities of the EURION Cluster and the eight projects during the 18-month reporting period, from 1st July 2020 to 31st December 2021. The first part of the report is focused on providing a progress overview of the individual projects in the form of public summaries. This is followed by an overview of the test methods to be developed, cluster highlights and working group summaries.

2 Project summaries

2.1 ATHENA project summary

Assays for the identification of Thyroid Hormone axis - disrupting chemicals: Elaborating Novel Assessment strategies - ATHENA

Summary of the context and overall objectives of the project

The thyroid hormone system is a complex interplay of physiological components that work together to maintain biological functions such as growth and homeostasis in humans and animals. Levels of thyroid hormone are controlled in the body by a tight feedback loop. Thyroid hormone system disrupting chemicals (THSDCs) can alter levels of thyroid hormone; if chronically, this can lead to pathological conditions like hypothyroidism or cancers in the long term. In pregnant women, even acute exposure can affect the foetus and be particularly damaging in the first trimester when the foetus cannot produce its own thyroid hormone and relies on the mother for this essential supply. It is well-established that maternal hypothyroidism (thyroid under-function) can lead to impaired psychomotor development and lowered IQ in offspring. It has been shown that THSDCs in the maternal system can decrease circulating thyroid hormone, which leads to lower levels reaching the foetus.

The ATHENA project is interested in how brain development *in utero* is affected by THSDCs which disrupt the maternal thyroid hormone system.

There are several points within the thyroid hormone system where THSDCs can act to disrupt the system. Partners within the ATHENA consortium focus on different areas within the thyroid hormone system to develop test methods to identify THSDCs, and also delineate mechanism of action to construct an AOP network to facilitate a comprehensive testing strategy. Current test methods only provide a minimal and inadequate assessment of thyroid hormone system disruption, leaving several gaps in the testing strategy for identification of THSDCs, hence resulting in potential endocrine disrupting chemicals not being identified as chemicals of concern.

Overview of progress and main results achieved so far

Associations with thyroid function in pregnant women: We are interested in studying the association of exposure to various EDCs with thyroid function in pregnant women. We use human epidemiological data from the longitudinal SELMA study and the Generation R study to evaluate associations between maternal urinary and/or serum concentrations of EDCs and thyroid hormone levels. We have performed studies of bisphenol A, phthalates, PCBs and PFAS in relation to thyroid function during pregnancy from the SELMA study. These findings have now been published in several papers in peer-reviewed journals. Our work on phthalates has been extended to investigate associations during pregnancy with human chorionic gonadotrophin (hCG) which may act as a mediator between EDCs and maternal thyroid function.

Brain development and neural stem cell division: We are working towards understanding how THSDCs interfere with brain development and neural stem cell decisions during brain development, by developing innovative 3D *in vitro* test methods that capture key steps of vertebrate neural cell commitment. We then investigate how THSDCs disturb these processes. Two models are under development: human cerebral organoids and mouse neurospheres. We have established TH basal levels in the two systems and identified key endpoints. Both models now give us the opportunity to study disruption of TH action and its consequences for key stages of brain development.

High through-put screening (HTS) assays for screening interactions at non-receptor targets and development of Quantitative Structure-Activity Relationships (QSAR): At present there are no high through-put assays to screen potential THSDCs acting on non-receptor targets like enzymes and transporters, a gap which ATHENA will bridge by developing High Through-put Screens (HTS) and Quantitative Structure-Activity Relationships (QSAR).

We have generated stable cell lines and implemented HTS screens based on the non-radioactive Sandell-Kolthoff reaction platform and tested a library of compounds – the curated results are in QSAR development for several explorative models.
The necessary programming work to make QSAR models freely available via the Danish (Q)SAR Models website has been achieved.

Focus on thyroid hormone transport between mother and foetus: As this transport is essential for brain development, we aim to identify new transporters and to evaluate their suitability for HTS assays. The work focuses on two physiological barriers: the placenta for maternal to foetal TH transport and the blood-brain-barrier (BBB) and blood cerebrospinal fluid barrier (BCSFB) for TH supply from the circulation to the developing foetal brain.

We identified novel candidate transporters in the placenta and followed through with a detailed investigation of their characteristics and are now pursuing a similar approach to find the relevant transporters in the BBB and the BCSFB. We utilised an optimised placenta perfusion model to evaluate the importance of two transporters for transplacental T4 transfer and are producing stable cell lines to progress to HTS Sandell-Kolthoff based assay.

Capturing downstream effects on the developing brain: We are developing i*n vivo* assays for downstream effects of THSDC on brain development. Activities have so far focused on establishing a study protocol for capturing the characteristics of disrupted brain development from thyroid hormone insufficiency, with particular focus on defects in neuronal migration producing misplaced neurons in the white matter (heterotopia). We have conducted a large developmental toxicity study in the rat and assessed a multitude of endpoints, including circulating TH levels and gene expression patterns in the brain. We have newly identified the pesticide amitrole, an inhibitor of TH synthesis, as producing neuronal migration defects. The results of several of these studies have been published.

Developing a testing strategy for THSDCs based on Adverse Outcome Pathway (AOP) networks: The ATHENA project aims to develop a testing strategy for THSDC that is founded on adverse outcome pathway networks relevant to the thyroid hormone system in which we are able to identify the molecular initiating events (MIE) that have the strongest impact on perturbing the system. To gain an overview of the effect patterns seen in mammalians in response to THSDC, in terms of changes in TH and TSH, we completed a systematic evidence-mapping exercise for rodent models. Our systematic approach shows that the canonical view of the HPT axis (increase T4 and decrease TSH) is not supported by the evidence and requires further study. We have also produced a qualitative AOP network of pathways relevant to disrupted brain development in which we identified several important nodes not currently covered by test methods (T4 and T3 in neuronal tissues). This qualitative network will serve as the basis for quantitative modelling to support a testing strategy.

International regulation and harmonisation: The ATHENA project work will ultimately translate into policy decisions regarding THDCs and we are building strategies for thyroid disruptors for international regulation and harmonisation with non-EU trading blocs. A summary and synopsis of current international activities and the characteristics of the relevant global regulatory EDC frameworks has been prepared.

Interaction with the EURION Cluster: ATHENA together with SCREENED are the organisers of the EURION Cluster 2021 - 2022 and will organise the EURION Annual meeting 2022, a training workshop in collaboration

with JRC and a stakeholders' workshop as well as coordinate meetings and facilitate collaborations within the cluster.

Progress beyond the state of the art and expected potential impact

The ATHENA project aims to improve understanding of the thyroid hormone system with regards to targets of THSDCs and predicted outcomes. The test methods established in the ATHENA project will undergo the pre-validation process for incorporation into the OECD test guidelines, together with considerations for international harmonisation. We hope to comprehensively cover gaps in the testing strategy for THSDCs, so that test chemicals that have disrupting properties are properly identified and ultimately prevent negative consequences for human health.

Further information about ATHENA:
Website: https://athenaedctestmethods.net
Cordis: https://cordis.europa.eu/project/id/825161
Twitter: @ATHENAprojectEU

2.2 EDCmet project summary

Metabolic effects of Endocrine Disrupting Chemicals: novel testing METhods and adverse outcome pathways – EDCMET

Summary of the context and overall objectives of the project

Thus far, endocrine disruptor (ED) research has mainly focused on reproductive endocrinology and related hormones, which is reflected in the regulatory test methods assessing only endocrine effects of xenobiotics. Accumulating evidence links ED exposure to increased incidence of metabolic syndrome and further, incidence of fatty liver and type 2 diabetes. EDs may exert their adverse metabolic effects via several mechanisms, such as modulation of nuclear receptors, impairment of mitochondrial respiration or excessive cellular accumulation of lipids. New and improved approaches are needed to increase the quality, efficiency, and effectiveness of existing methods to evaluate the effects of EDs and to meet the demanding and evolving regulatory requirements worldwide. EDCMET project focuses on developing novel or improved computational and *in vitro* methods, such as non-cellular assays and cell culture systems, as well as standardized *in vivo* animal models to assess the metabolic effects of EDs. Unbiased omics techniques will be used to investigate tissue and plasma markers *in vivo*. Epidemiological and field monitoring data is used to gain information regarding the human exposure to EDs and related metabolic effects. EDCMET will also apply the adverse outcome pathway (AOP) paradigm to identify molecular initiating events (MIEs) and predict the emerging adverse biological phenotype. The interdisciplinary approach and complementary expertise of project participants will aid in the identification of novel mechanisms of action and the development of novel or improved validated test methods for regulatory purposes.

Overview of progress and main results achieved so far

Interactions of EDs with NRs as well as the potential mechanisms and molecular triggers behind NR structure stabilization and activation have been explored using optimized *in silico* approaches. A database of omics level data for the action of EDs in hepatic cell models and *in vivo* (rodents) has been established. New transcriptomics data from human hepatic cell models and mice, as well as plasma and liver metabolomics data from mice, have been produced. Metabolomics data is already available from selected cohort samples and possibilities for further analyses are explored. To analyze omics data, a predictive classification pipeline has been developed and published. This pipeline is further expanded and validated by interrogation of *de novo* omics data generated in the project. A systems toxicology tool to predict emergent metabolic phenotype from ED exposure is under development.

NR-coregulator interaction assays are available for ten NRs. Reporter gene assays for predicting the activation of NRs involved in the regulation of metabolic pathways have been developed and evaluated by following the NCATS and OECD Framework to ensure robustness, reproducibility, and transferability of the assays. Mitochondrial respiration assays have been developed to enable assessment of functional effects of EDs on cellular level in hepatic cell models. High-throughput, fluorescence-based AdipoRed assay is available to evaluate the steatotic effects of EDs. A set of ED chemicals has been screened across the assay panel and further compound testing is ongoing. Steps towards regulatory implementation of the developed assays have been initiated in collaboration with EURION and OECD expert group.

Standard Operating Procedures (SOPs) have been developed for insulin tolerance and glucose tolerance tests as well as for inducing obesity, insulin resistance, and non-alcoholic fatty liver disease using high-fat diet in mice. Studies on ED effects on vulnerable individuals (obesity, in utero) are ongoing. Liver transcriptomics and biochemical analyses following pregnane X receptor activation have revealed widespread effects and mechanistic details on effects of xenobiotics on cholesterol synthesis. The biological knowledge gained from *in vitro* and *in vivo* studies, alongside further interrogation of legacy data, will also support AOP development later in the project.

Levels of selected ED compounds have been analysed from cohort samples and analyses on potential associations of exposure levels and systemic metabolism as well as health outcomes are underway. Several scientific papers on the epidemiological findings have been published and a workshop is planned for 2022 to discuss results and further plans.

Information on developed assays, ED test compounds, experimental protocols and cohorts have been shared and discussed in EURION meetings and working groups.

Progress beyond the state of the art and expected potential impact

EDCMET will provide an array of new or improved testing tools for risk assessment of metabolism disrupting EDs. These tools are expected to identify novel disrupted pathways in human liver and to increase human relevancy in testing and risk prediction and further reduce the use of laboratory animals. The developed methods undergo preliminary experimental validation and can contribute to the current OECD test systems. The methods and models will be coupled with human exposure data, linking the levels of EDs with metabolic endpoints and health outcomes. EDCMET will lay the groundwork for future testing and ED toxicity assessment and contribute to a better understanding of human exposure to chemicals and the associated burden of metabolic diseases.

Endocrine-related diseases represent a high cost for healthcare systems in the EU countries and metabolic effects of EDs are poorly understood. EDCMET will increase the knowledge on metabolic diseases by characterization of the affected critical pathways in liver using the AOP approach. EDCMET will also aid in the development of novel biomarkers for metabolic diseases and provide a relevant base for assessing adverse human health effects of environmental chemicals. The availability of fast and cheaper test systems can stimulate research around environmental chemicals and contribute to safer marketed products and thereby have a positive impact on the environment.

Further information about EDCMET:
Website: www.uef.fi/edcmet
Cordis: https://cordis.europa.eu/project/id/825762
Twitter: @edcmet_eu

2.3 ENDpoiNTs project summary

Novel Testing Strategies for Endocrine Disruptors in the Context of Developmental NeuroToxicity – ENDpoiNTs

Summary of the context and overall objectives of the project

ENDpoiNTs is developing a new testing strategy to meet the regulatory, scientific and societal needs for improved hazard and risk assessment of endocrine disrupting chemicals (EDCs).

A significant knowledge gap is how EDCs affect neurodevelopment, and endocrine disruption (ED)-induced developmental neurotoxicity (DNT) is hardly covered by the testing tools in regulatory use. The brain is among the most vulnerable organs with respect to toxic insults, particularly during development, and EDCs have indeed been shown to target the developing brain.

The main objective of ENDpoiNTs is to generate new scientific knowledge on how ED is linked to DNT at the molecular, cellular, tissue, and organism level. Based on this new knowledge, the project will:

- Develop predictive computational tools for chemical screening
- Develop and validate cellular testing and screening tools
- Develop novel molecular endpoints for existing animal-based test guidelines
- Ensure human relevance by linking experimental and epidemiological evidence
- Develop an integrated approach to identify endocrine disrupting chemicals inducing DNT
- Engage with key stakeholders and develop novel strategies for EDC testing and assessment into European and international chemical regulatory frameworks

Overview of progress and main results achieved so far

A number of cellular and animal-based models exist to study DNT endpoints, addressing cellular key events and neurodevelopmental outcomes. However, the predictive value of these models to detect ED-induced DNT is largely unknown. In ENDpoiNTs we are assessing the responsiveness of these DNT endpoints to endocrine interference.

ED pathways potentially relevant for DNT cellular key events were selected using in silico modelling to predict targets of EDCs that are known to affect human neurodevelopment. Using receptor agonists and antagonists, their link to cellular key events was established in a number of *in vitro* models. This screening effort showed that almost all key events investigated are dependent on one or several of the selected hormonal receptors. Thereby, novel roles for hitherto unstudied hormonal receptors in the context of EDCs in neurodevelopmental key events were uncovered. On the other hand, the estrogen and androgen systems, that are beside thyroid hormone the most studied in the context of EDCs, were not prominently involved in DNT-related cellular key events. Some results were in accordance between human and rodent models while others diverged, underscoring species differences. Moreover, when comparing genetic sex in the cell models, some pathways were only affected in one but not the other sex. First results with model chemicals show that some of the cellular endpoints are also affected by low doses of EDCs. For most of the hormone-depended endpoints, standard operating procedures (SOPs) were established, and one of the newly developed cellular assays is currently undergoing pre-validation, including assessment of inter-laboratory transferability.

To link the molecular and cellular key events addressed in the in vitro models to adverse outcomes in whole organisms, several in vivo models are employed. The rat model entails a developmental exposure scheme

to six model compounds (Bisphenol F (BPF), Permethrin (PMT), PFOS, Butylbenzyl phthalate, Triphenyl phosphate, and 1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH) and subsequent molecular as well as behavioural and cognitive measurements. All six studies are finalized, behavioural data is being analysed, and molecular studies are in progress. The molecular analyses include transcriptomics, epigenomics, metabolomics, lipidomics, and steroidomics. For the latter, a new sensitive platform has been developed for plasma and tissue samples to be able to perform targeted investigations using low amounts of samples. Similar analyses with more model EDCs are conducted in zebrafish embryos and tadpoles of Xenopus laevis.

For the management and handling of the experimental data, a standardised database platform was developed. It includes data capture, curation procedures and data treatment and statistical analysis approaches relevant for ENDpoiNTs. Currently, all data of the in vitro models are fed into, and analysed by, this platform in order to compare the sensitivity and specificity between the models and, based on this, a final selection of assays for pre-validation will be made. Furthermore, 12 first tier screening high confidence Quantitative Structure Activity Relationships (QSARs) were developed for predicting the agonistic and antagonistic modes of action of the selected receptors. This is the basis for in silico models identifying chemicals that can induce DNT via these interactions. Using data from the public domain and produced in ENDpoiNTs, the molecular interactions, cellular effects and adverse outcomes are now being integrated using the Adverse Outcome Pathway (AOP) framework. To enable this, the relevant literature has been reviewed to identify brain regions, neurotransmitter systems, and endocrine systems of interest. For establishing human relevance of the test methods, doses producing an adverse effect in test systems will be compared with human exposure data from the ENDpoiNTs cohorts and other international data sets for both single reference EDCs and for their mixture. For comparison, the metrics have been evaluated using established regulatory values translated to biomonitoring equivalent concentrations. Furthermore, a mixture study of EDCs has been initiated, with the aim to compare effects of a real-life mixture, established based on human data, to single compounds in the developed in vitro models.

To ensure the uptake of the developed assays and strategies into the regulatory context, ENDpoiNTs is actively engaging with key stakeholders. Within the EURION cluster, a stakeholder workshop was organised, discussing the views, needs and expectations related to EDC test method development and international strategies and guidelines. Furthermore, communication channels with relevant working groups of the OECD have been established or strengthened to enable continuous discussion of the readiness of the developed assays and endpoints for validation and regulatory implementation. Finally, to disseminate the project's results to the scientific community, key stakeholders, and the public at large, ENDpoiNTs has produced project flyers and animated short films about the aims and first results of the project. It is actively maintaining its webpage (https://endpoints.eu/), as well as continuously publishing its results in scientific journals with open access.

Progress beyond the state of the art and expected potential impact

ENDpoiNTs will advance the current foundations of regulatory science in this area clearly beyond the state of the art by i) enhancing human-relevance of screening methods, and ii) developing an integrated platform of testing strategies and methods to test and assess ED-induced DNT. The development of a strategy for integrating the battery of testing tools in the Integrated Approach to Testing and Assessment (IATA) framework and integrating epidemiological research and data in risk assessment of EDCs will also advance the scientific basis for risk management. Ultimately, this will have impact on policymaking and regulations and enable better protection of vulnerable populations and particularly, the developing brain.

Further information about ENDpoiNTs:
Website: https://endpoints.eu/
Cordis: https://cordis.europa.eu/project/id/825759
Twitter: @ENDpoiNTs_EU

2.4 ERGO project summary

Breaking down the wall between human health and environmental testing of endocrine disrupters: EndocRine Guideline Optimisation - ERGO

Summary of the context and overall objectives of the project

ERGO project will break down the wall between mammalian and non-mammalian vertebrate regulatory testing of endocrine disrupting chemicals (EDCs) by identifying, developing and aligning thyroid-related biomarkers and endpoints (B/E) for linkage of effects between different vertebrate classes. To achieve this, an adverse outcome pathway (AOP) network covering various modes of thyroid disruption (TD) in multiple vertebrate classes will be developed. An AOP starts from a molecular initiating event (MIE) and outlines the sequence of key events (KE) leading to a relevant adverse outcome at the organism or population level. The AOP network will provide the scientifically plausible and evidence-based foundation for the selection of B/E and assays in lower vertebrates predictive of human health outcomes. These assays will be prioritized for validation in ERGO.

ERGO will re-think ED testing strategies from in silico methods to in vivo testing and develop, optimize and validate existing in vivo OECD guidelines and (if required) new in vitro protocols with novel TD endpoints and consequently reduce requirements for vertebrate animal testing by preventing duplication of testing in mammals and non-mammalian vertebrates. The ERGO Integrated Approaches to Testing and Assessment (IATA) strategy will increase the screening capacity to enable more chemicals to be tested for ED properties.

To achieve its goals of providing stakeholders and businesses with better tools and strategies needed to ensure improved management of EDCs, ERGO has four overarching objectives:

- Investigate, develop and validate thyroid B/E predicting effects across vertebrate classes for inclusion in new in vitro and existing in vivo OECD test guidelines (TGs) for improved identification and safer assessment of thyroid disrupting chemicals.
- Develop an AOP network across vertebrate classes for identification of thyroid B/E applicable for assessment of cross-class thyroid disrupting KE.
- Transform new data, tools and understanding into a harmonized IATA testing strategy for regulation of EDCs by inclusion of stakeholders at the global level in the incorporation of the cross-vertebrate class testing approach.
- Publish a guidance document on extrapolation of TD effects across mammalian, fish and amphibian OECD TGs.

Overview of progress and main results achieved so far

WP1 - Project Coordination and Management:

WP1 is organised into a scientific coordination team (CO) and a project office (PMO). It manages and steers the scientific project to ensure that it reaches its objectives and deliverables in time and budget, with the highest scientific level, and in compliance with expectations from all stakeholders, including regulatory agencies and industry. WP1 coordinates ERGO clustering activities with the sister projects under BHC-27-2018 and has taken over EURION chairing in April 2020 with the ENDpoiNTs project.

WP2 - Knowledge management and data infrastructure:

The “Chemical Properties Estimation Software System” named ChemProp has been adapted to be used as the ERGO database system. Furthermore, the chemical selection was made for the in vitro test battery as well as for the in vivo assays. The experts forming the User Reference Group (URG) members have been agreed upon and will be convened in autumn 2020.

WP3 - Adverse outcome pathway (AOP) network development:

The scope for AOP development within ERGO has been established. The AOP development progress has been outlined. Knowledge gaps has been identified based on the current status of the AOPs and relevant to achieving the goals of ERGO.

WP4 - Biotransformation and Modelling:

Data mining has been started, and decisions on amount and type of data to be looked up have been made. For in silico bioavailability triggers, model candidates are under evaluation. Concerning In-vitro-to-in-silico biotransformation, the experimental setup for S9 enzyme mix assays including reference systems has been started. Based on the S9 assay according to literature, respective variants employing fish and rat derived S9 fractions have been established and validated. Finally, 1107 potential EDCs were extracted from the EDC DataBank

WP5 - Case studies for thyroid-related endpoints and biomarkers in ED test systems:

Preparations involve recruitment of personnel (SDU: 3-year postdoc, CNRS&LOR: 2 PhDs, UHEI: 1 PhD) and technical preparations for the experiments (SDU: installation of a zebrafish facility, CNRS: data analysis for choice of compounds and test systems). Multiple partners have performed intensive literature review work to contribute to existing data. First experiments have been performed at UHEI, UA, YCU and MU. Part of the results of these experiments have been critically evaluated by MATT.

The WP5 experimental work that has been started mid/end of 2019, was interrupted due to the COVID-19 lockdown in spring 2020. All partners continued working mostly on desktop-based tasks.

From January-April 2020, SDU coordinated the preparation of an open-access peer reviewed publication in a special issue of Int. J. Mol. Sci. (IJMS) with all WP5 partners contributing. The publication was published as a “project report” outlining the main objectives of ERGO and can be downloaded from: https://www.mdpi.com/1422-0067/21/8/2954

WP6 - Mammalian endpoints and epidemiology:

In the context of selection of in vivo and in vitro reference compounds for long-term fish studies, several in vitro assays, literature and available mammalian studies have been evaluated to give an overview of mechanistic apical data of screened substances.

For evaluation of data from mechanistic studies in mammals with reference compounds, a list of compounds to be tested has been provided. In order to perform genomic analyses, a cohort of mice exposed to control and test substances have been produced and a large collection of samples collected. For the establishment of an AOP based strategy for epidemiological and human exposure studies, MU is building a database on the interactions of pollutants commonly detected in epidemiological studies with priority MIEs for TD identified within ERGO.

WP7 - Pre-validation/validation of thyroid biomarkers and endpoints in ED test systems:

Proposals have been prepared for the development and validation of fish-based thyroid biomarkers, however WP7 starts in month 19 and first deliverable is due month 36.

WP8 - Knowledge Transfer, Communication, Dissemination and Exploitation:

- A Dissemination and Exploitation Plan has been elaborated and submitted.
- A portfolio of communication and dissemination resources and tools was developed in the first year of the project to facilitate promotion and widespread awareness of the project. This included generic PowerPoint and Poster Presentation templates, pull-up banner, project factsheet and annual newsletter. Dissemination activities and external strategic communication is carried out on an ongoing basis by all partners across the full project duration.
- An ERGO Introductory Video has been created, showing the impact of endocrine disruption on everyday life, and is being promoted at events and on social media.
- The ERGO Project website (https://ergo-project.eu/) has been launched, carrying out general dissemination of ERGO results, news, events and progress and with a dedicated section for 'Outreach'.
- ERGO social media has been introduced, the project Twitter page (https://twitter.com/ERGO_EU; @ERGO_EU), disseminating through LinkedIn, promoting ERGO activities and results as well as connecting with sister cluster group projects (EURION)
- Four ERGO publications have been uploaded to the EC Funding and Tender Opportunities Portal, project website and repositories.
- Partners have attended high profile conferences and events representing the ERGO project and promoting its results; in Reporting Period 1, a total of 103 dissemination and communication activities have been recorded by ERGO partners, with an estimated reach of over 3.3 million stakeholders.
- A number of news items and promotional articles has been at regular intervals and distributed via a range of dissemination channels such as the project website, social media channels and e-newsletters.
- The ERGO Data Management Plan has also been developed, including a data inventory table, with contributions from task leaders.
- The ERGO Innovation Task Force (ITF) was activated.

WP9 - Ethics requirements:

For this WP, where the objective is to ensure compliance with the 'ethics requirements' set out in this work package, all deliverables have been submitted by the end of the first year of the project.

The European cluster EURION:

SDU, as coordinator of ERGO, took over the chairing of EURION on 1 April 2020 in collaboration with the ENDpoiNTs project. The EURION chairing runs for 15 months until 30 June 2021. The chairing include contact to the EC and the IAP, organisation of bi-monthly teleconferences with EURION project coordination teams, organisation of annual EURION meeting, organisation of EURION outreach activities including participation in the EC ED Forum in October 2020, preparation of an EURION Newsletter and to act as a general contact point for EURION communication and dissemination activities.

ERGO partners participate in all 13 EURION WGs and has been chairing the AOP WG from the project start and has also taken over the Animal Studies Aquatic Organisms WG in the spring of 2020.

Progress beyond the state of the art and expected potential impact

ERGO is a coordinated attempt to contribute filling the gaps in the field of TD. It will allow to identify both disturbance of the thyroid axis and its potential adverse effects in different vertebrate classes. ERGO is expected to improve methodologies for using cell tests and fish and amphibian assays for early screening of substances and to develop new in silico models for predicting internal dose of TDCs to design physiologically based toxicokinetic modelling (PBTK) models and to link MIEs with AE within an AOP network.

ERGO is expected to increase basic knowledge on the detailed role of TH disturbances. The ERGO approach would be of significant interest for the safety assessment of existing chemicals lacking endocrine and developmental toxicity data and new chemicals at an early stage of their industrial development. This methodology should allow:

- To simultaneously screen chemicals for their potential human TD effects, as well as their environmental impact with similar negative effects on fish and amphibians.
- Significantly reduce the requirement of vertebrate animal testing, with respect to animal welfare.
- A relevant assessment at the vitro scale opens the door to automation and higher throughput screening of chemicals, which would further reduce the cost of their assessment.

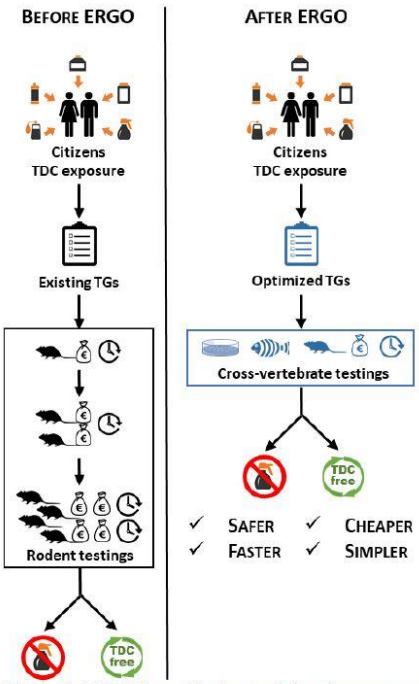


Figure 1.1 ERGO investigates and develops a cross-vertebrate class AOP/testing approach that will deliver optimized and novel ED TGs

Further information about ERGO:
Website: ergo-project.eu
Cordis: www.cordis.europa.eu/project/id/825753
Twitter: @ERGO_EU

2.5 FREIA project summary

FREIA: Female Reproductive toxicity of Endocrine disrupting chemicals (EDCs): a human evidence-based screening and Identification Approach

Summary of the context and overall objectives of the project

The FREIA project aims to provide better test methods to identify human-made chemicals that disturb hormones and their actions on development and function of the reproductive system in women. Currently available test methods are not fit for purpose, which is partly the reason why the effects of such endocrine disrupting chemicals (EDCs) on female reproductive health are often overlooked in regulatory chemical safety assessments. This means that women's reproductive health is at risk globally. Our EU-funded project FREIA will increase our understanding of how EDCs can harm female reproductive health. We will use this information to provide better test methods that enable fit-for-purpose chemical regulation. We will also share our knowledge widely to improve the reproductive health of women globally.

Overview of progress and main results achieved so far

In the FREIA project, we first looked for biological characteristics (biomarkers) for female reproductive toxicity using two well understood EDCs, diethylstilbestrol (DES, a potent estrogen receptor activator) and ketoconazole (KTZ, a blocker of steroid hormone production). Next, we will assess how well our test methods and potential novel endpoints can identify EDCs that cause female reproductive toxicity. Here, we briefly describe the results from the first 36 months of the FREIA project, from January 2019 until December 2021.

Identification of EDCs in a regulatory context still relies heavily on rat studies. We showed that the endpoints that are currently being assessed in regulatory toxicity studies are not sufficiently sensitive to detect an endocrine disrupting effect (https://doi.org/10.1016/j.reprotox.2021.01.003). We found that a delay in activation of the brain to produce Gonadotropin Releasing Hormone (GnRH) is more sensitive to mark a delay in pubertal onset than the standard examination of vaginal opening (VO) in female rats that were exposed in the womb to DES and KTZ. Strikingly, the effect on GnRH was not detected when rat brain cells were exposed to DES or KTZ in a culture dish. This underlines the importance to focus on endocrine axes in the whole animal. We are investigating several additional hormone-sensitive endpoints, such as mammary gland development. A proposal to investigate this was submitted to the Organisation for Economic Co-operation and Development (OECD), a platform for international standard-setting. In addition, we found that the pups had increased blood levels of hormones like progesterone, pregnenolone, androsterone and estradiol after exposure to KTZ in the womb, and to a lesser extent DES.

Studies with cell cultures of fetal and adult human ovaries, bovine oocytes and immortalized ovarian cells all indicate an effect on steroid hormone formation and oocyte ripening by DES and KTZ, albeit in different directions. Ongoing gene expression analyses are designed to elucidate whether similar genes and pathways are affected in cell cultures and how this relates to effects in rats and humans. Differences in susceptibility towards effects of KTZ and DES may partly be explained by the presence of different cell types in the ovary at different ages (https://eovary.ki.se), or the presence of different cell types in our experimental models. Nonetheless, both the rat study and the human ovary cultures show that exposure to KTZ had a stronger

effect than DES, suggesting that chemicals targeting steroid hormone formation (steroidogenesis) may have worse effects on oocyte maturation and quality than those targeting the estrogen receptor (ER).

In current regulatory toxicity testing, the gold standard to study interaction with steroidogenesis is the H295R steroidogenesis assay (OECD test guideline 345). The last step in sex hormone formation, the conversion of testosterone to estradiol, is mediated by the aromatase enzyme. We have developed a computational model that can predict inhibition of aromatase with high precision and accuracy (https://qsar.food.dtu.dk), complementary to the existing H295R assay. Nevertheless, our studies showed that effects of EDCs can also occur earlier in the steroidogenic pathway, or via alternative routes. We are now performing a study with other labs to investigate whether the H295R assay can be improved by measuring more steroid hormones. A project plan for this was submitted to the OECD. Moreover, we showed that steroid hormone profiles from human adult ovary cultures are clearly different from H295R profiles. The implication of this will be investigated further in the next phase of the FREIA project.

Considering the limited effects of known ER activator, DES, on ovarian function during early life, the ER does not seem to be a high impact target for (developmental) female reproductive effects. On the other hand, ER-beta is known to play an important role in differentiation of estradiol-producing cells surrounding the maturing oocyte. We showed that a wide variety of potential EDCs had an ER-beta activating or deactivating effect in our ER-beta assay. Notably, the chemicals that were studied for ER-beta interaction were also detected in the biological fluids surrounding oocytes, the follicular fluids, in Swedish and Estonian women undergoing fertility treatment. The total exposure to these chemicals and some chemicals specifically, decreased a woman's response to ovarian stimulation by hormones. Moreover, some chemicals in the follicular fluids of these women were associated with a reduced chance of the treatment resulting in live birth. Further studies are undertaken to investigate molecular pathways that are affected by the exposures.

Identification of molecular targets and pathways will guide the development of test methods and test strategy, provided that the steps from molecular interaction to female reproductive toxicity are defined. For example, we have developed a computational model to predict peroxisome proliferator-activated receptor (PPAR)-gamma activation, which is linked to female infertility. We have described 16 additional possible pathways (doi: 10.1007/s00204-020-02834-y and 10.1159/000515478). We will evaluate with additional studies and different compounds which of these pathways and targets are suitable additional endocrine-sensitive endpoints to test for female reproductive toxicity by EDCs.

FREIA is one of the eight projects on test method development for EDC identification within the EURION cluster (www.eurion-cluster.eu). On the FREIA website (www.freiaproject.eu), general background information on EDCs and female reproductive health can be found as well as project specific information, including webinar recordings, peer-reviewed scientific publications and databases, and the FREIA factsheet (5 languages) and infographic (14 languages).

Progress beyond the state of the art and potential impacts

FREIA uniquely provides the opportunity to investigate hormonal processes in human ovaries from fetal to adult age in order to improve scientific knowledge on the causes of female reproductive toxicity. Our committed collaborators for policy, advocacy and communicating actions to promote women's health and a healthy society allow FREIA to have a huge societal impact. The FREIA approach will strongly support the work of European regulatory agencies, or even globally through the EURION cluster activities. The tools we are developing perfectly fit the needs of modern-day toxicity testing with a clear regulatory application in mind. Together, the FREIA outcomes will support testing, identification and assessment of EDCs that are toxic for female reproduction.

Further information about FREIA:
Website: www.freiaproject.eu
Contact: info@freiaproject.eu
Twitter: @freiaprojectEU

2.6 GOLIATH project summary

Beating Goliath: Generation Of NoveL, Integrated and Internationally Harmonised Approaches for Testing Metabolism Disrupting Compounds

Summary of the context and overall objectives of the project

The incidence metabolic disorders such as obesity, diabetes and metabolic associated fatty liver disease has reached 'Goliathan' proportions. The worldwide increase in metabolic disorders cannot be explained by lifestyle and genetic factors alone; the role of environmental factors in these disorders has been increasingly acknowledged. Exposure to endocrine disrupting chemicals (EDCs) that disrupt metabolism – chemicals collectively referred to as 'metabolism disrupting chemicals' (MDCs) – is an environmental risk factor that urgently requires more attention. MDCs are natural and anthropogenic chemicals that have the ability to promote metabolic changes that can ultimately contribute to the development of obesity, diabetes and/or fatty liver in humans. Considering the important role these metabolic alterations can play in the global epidemics of metabolic disorders, it is essential that international chemicals regulations require the identification of MDCs and the assessment of the risk associated with exposure.

Within European chemicals regulations, criteria to identify EDCs have been proposed that require information on a chemical's endocrine mode of action (MoA) and related adverse effects relevant for human health. However, currently no regulatory *in vivo* or *in vitro* tests exist to identify the potential metabolism disrupting effects of chemicals. The need for these tests has been internationally recognized, as without them, comprehensive hazard and risk assessment of chemicals for potential metabolism disrupting activity is virtually impossible.

The overall aim of the GOLIATH project (www.beatinggoliath.eu) is to improve hazard and risk assessment of EDCs by generating novel, optimised, integrated and internationally harmonised approaches for testing metabolic disruption. The GOLIATH project spans the entire spectrum of testing, from *in silico* predictive modelling and high-throughput screening, to the development of robust ready-to-use *in vitro* assays and optimisation of current *in vivo* testing guidelines. By incorporating novel omics technologies to translate *in vitro* and *in vivo* assay outcomes into human health effects, GOLIATH will generate new testing strategies for MDCs, and provide novel insights in the mechanisms by which MDCs disrupt metabolic pathways and induce adverse effects on human health. With a consortium comprised of world-leading experts in endocrinology, physiology, molecular biology, endocrine disruption, toxicology, epidemiology, bioinformatics, test method development, validation and chemical regulation, GOLIATH will be pivotal in the development of an internationally harmonised strategy for testing MDCs.

The overall objectives of the project are:

- To improve the understanding of the endocrine modes of action of MDCs.
- To develop assay candidates for metabolic disrupting chemicals based on confirmed MoA and key biological effects in target tissues.
- To select and develop assay candidates into (pre-)validated test methods, in collaboration with OECD, ensuring test method definition, transferability, inter-laboratory testing and assessment of predictivity, which are prerequisites for their regulatory use.
- To develop an internationally harmonised, integrated approach to testing and assessment (IATA) of MDCs, using an Adverse Outcome Pathway (AOP) conceptual framework.

Overview of progress and main results achieved so far

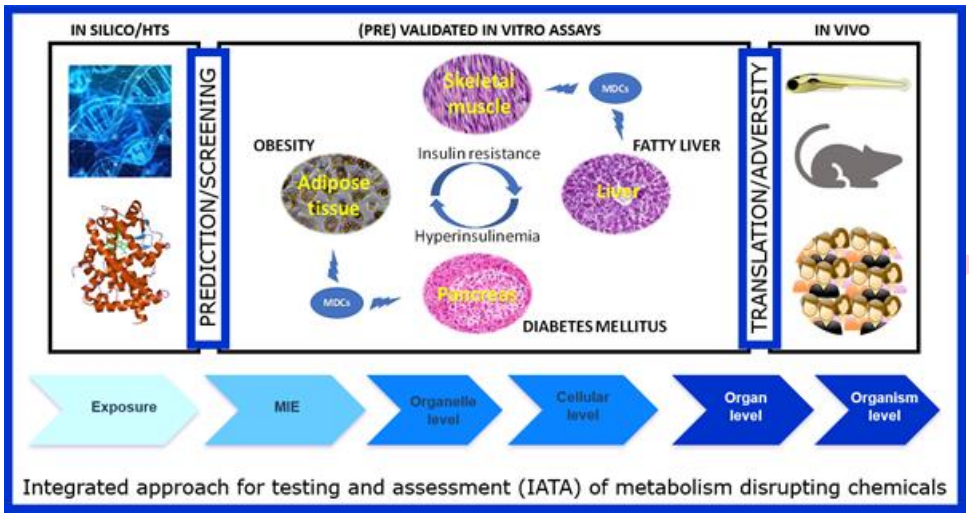
Overview of the main tasks and results of the first 36 months:

1. A review of current state of the science and overview of human exposure to MDCs (published in the International Journal of Molecular Sciences (https://www.mdpi.com/1422-0067/21/10/3480)), to be further elaborated in collaboration with OECD
2. Identification of suitable model chemicals for the selected assays within GOLIATH, and subsequent chemical selection for pre-validation studies has been performed for multiple assays that are developed in GOLIATH (Jacobs et al, submitted)
3. The Edmon platform is now enhanced with machine learning and experimental structural information on PXR interaction with EDCs (https://doi.org/10.1073/pnas.2020551118, http://atome.cbs.cnrs.fr/ATOME_V3/SERVER/EDMon_v3.html)
4. Development of in silico models for prediction of the interaction of organic chemicals with selected molecular initiating events (MIEs) (Sapounidou et al, under revision)
5. Research on interspecies differences of PPAR gamma with model PPAR gamma activators revealed novel insights in species specificity of PPARγ action (doi: 10.1021/acs.est.1c04318)
6. Initial high-throughput screening of chemicals for activation of key nuclear receptors
7. An algorithm named 'DEXOM', has been developed for MoA identification via metabolic networks (doi: 10.1021/acs.est.1c04318)
8. Implementation of a proteome-based thermal shift assay for the identification of soluble proteins interacting with chemicals and EDCs in zebrafish embryo proteome (doi: 10.1016/j.jprot.2021.104382)
9. The CYP induction assay has been optimized and transferred to naïve laboratories for ring trials as part of (pre)validation activities
10. (Pre)validation activities have been commenced for three other assays, namely PPAR gamma and alpha reporter transactivation assays and the human mesenchymal stem cell to adipocyte differentiation assay, including the transferability to naïve labs
11. Further development of *in vitro* assays to assess metabolic disruption in liver, pancreas and adipose tissues, including creation of standard operating procedures (SOPs) and test definitions for pre-validation purposes, including insulin regulation and secretion in selected models.
12. We have further optimized a transgenic zebrafish model for metabolism disruption following early life exposure to MDCs
13. Progress has been made on epidemiological analysis of changes to anthropogenic measures and markers in human cohorts relative to prenatal exposure to MDCs
14. Establishment of standardized protocols to be used by all GOLIATH partners for multi-omics (transcriptomics, metabolomics, lipidomics) analyses.
15. For specific GOLIATH assays, we have developed AOP reporting templates that will add to the conceptualization of IATAs for MDCs
16. An expert elicitation process is underway within the consortium as part of the weight of evidence assessment for MDCs
17. Progress has been made to augment existing test guidelines of animal studies to include measures of metabolic disruption.
18. We are working together with partners from the EURION project (EURION) (https://eurion-cluster.eu/) within various working groups
19. International outreach has been promoted via EURION's International Advisory Board and various dissemination activities

Progress beyond the state of the art and expected potential impact

In order to progress beyond the state of the art, the expected results of the GOLIATH project are to:

- Generate a set of robust, well-characterised and ready-to-use *in vitro* test methods for the effects of MDCs on key target cells in metabolic disorders that will be (pre-)validated and further implemented beyond the duration of the project
- Realize an integrated approach for testing and assessment of MDCs, that will exceed the duration of GOLIATH, by bringing together new and existing test methods, in a framework that will be internationally harmonised and relevant for regulatory purposes
- Describe systematically in an AOP framework the mechanisms by which MDCs disrupt metabolism and contribute to metabolic disorders in humans, thereby providing information on the endocrine MoA which is essential for defining endocrine disruption criteria
- Generate a significant knowledge base on the exposure to and effects of MDCs relevant to the European population, providing a substantial contribution to the weight of evidence for the role of chemicals in metabolic disorders



Further information about GOLIATH:

Website: https://beatinggoliath.eu

Cordis: https://cordis.europa.eu/project/id/825489

Twitter: @beating_goliath

2.7 OBERON project summary

An integrative strategy of testing systems for identification of EDs related to metabolic Disorders - OBERON

Summary of the context and overall objectives of the project

The main objective of the OBERON project is to develop a new battery of tests to detect endocrine disruptors having an impact on metabolic disorders, without the use of animal experimentation. Based on the concept of integrated approach for testing and assessment (IATA), OBERON will combine 1) experimental methods, (2) high throughput omics technologies, 3) epidemiology and human biomonitoring and 4) advanced computational models on functional endpoints related to metabolism.

- WP1 aims at integrating epidemiology and human biomonitoring (HBM) studies with the ED test systems for metabolic disorders, in order to increase the relevance of the ED test system. Various time windows will be considered, including developmental periods of high susceptibility (pregnancy-childhood-adolescence) and adulthood. Sex specificity will be studied. This WP draws on large-scale, population-based study populations including ongoing human biomonitoring (HBM) studies and some of Europe's most informative prospective birth cohorts.
- WP2's goal is to develop whole organism test systems to identify EDCs implicated in metabolic disorders. The main objectives are: 1) to perform the pre-validation of the zebrafish obesogenic test (ZOT) to screen EDCs and mixtures acting as obesogens, 2) to set up and pre-validate zebrafish-based bioassays to screen EDCs involved in NAFLD progression. The endpoints used for these whole-organism alternative testing assays are adiposity, liver steatosis and markers of steatohepatitis.
- The global aim of WP3 is to improve/develop innovative in vitro cellular models which best represents the three main organs and tissues (liver, adipose tissue and pancreas) involved in the etiology of metabolic disorders. These models will serve as a tool for the identification of endocrine disruptors which may increase the risk of developing metabolic diseases like obesity, diabetes or hepatic steatosis. Identification will be based on toxicity mechanisms, omics data and metabolic networks.
- The main objective of WP4 is to develop or adapt existing in silico methods for endocrine disruptor compounds. WP4 will develop QSAR and PK/PBPK models in support of the evaluation and integration of the data streams of the project.
- WP5 aims at integrating information from WP2 (in vivo models), WP3 (in vitro models) and WP4 (in silico models) in order to interpret the WP1 data. Overall objectives of WP5 include the (a) data infrastructure development, (b) development and application of a bioinformatics workflow of cross-omics and the respective biomarkers towards ED exposure/disease pathways, (c) development of new AOPs relevant EDs outcomes, and (e) development of an integrative testing strategy.
- The WP6 will ensure the scientific project management, and that the project reaches the defined objectives. Specifically, WP6 sets up an effective management framework for the consortium, acts as the interface between the consortium and the European Commission, performs strategic decision making.
- WP7 organizes a well targeted dissemination effort to ensure a maximum impact of the project and release the full exploitation potential of the results.

Overview of progress and main results achieved so far

During the first period, we started establishing and analysing several experimental tests across the different EU laboratories. During this period, expected deliverables including ethics, and publications were achieved.

In the second period, biomonitoring studies and birth cohorts of exposure levels of EDS in Europe were finished and respective deliverable were submitted. A couple articles were published. Other cohorts on children, adolescent and pregnant women are either ongoing or samples are under analysis. Respective publications from those analyses are under preparation.

An optimized Zebrafish model has been studied and used to measure the effects of OBERON EDs compounds on adiposity and steatosis. Steatosis analyses are still ongoing with a now optimized bioassay on liver staeatosis. Transferability of the zebrafish experiments is being tested. Publications are expected early 2022.

Liver, adipose tissue and pancreatic cell cultures were and are exposed to the compounds under study in OBERON and effects are measured at different levels in the metabolism of the cell: effects on cell viability, carbohydrate and lipid metabolism, lipid droplet accumulation, mitochondrial activity, hormone secretion and gene expression profile among others. For each tissues, the most promising model was chosen to go onwards.

In silico studies are ongoing with the analyses of existing databases, QSAR models and toxicokinetic models. Some new optimized QSAR models are being developed and included on the VEGA platform set up by partners at IRFMN: https://www.vegahub.eu/. A PBPK model for bisphenols in the Zebrafish eleuthero embryo was finalized and showed good predictive capabilities. The human PBPK model has been parameterized for all the compounds and was applied to translate exposure doses into doses in liver, pancreas and adipose tissue. In addition, biokinetic interactions of co-exposure to 4 phthalates has been elaborated.

The OBERON Integrated Data Management (IDM) system has been developed as well and is currently being implemented in support of collection and provision of access to all datasets collected/developed in OBERON. Metabolomics and transcriptomics have been launched and a pilot study for all the different cell lines and for zebrafish is finished. Samples from the zebrafish experiments as well as for the different cell lines are being sent to the laboratories in Greece for these multi-omics studies. A tool based on artificial intelligence and text mining, was created and called AOP-helpFinder.

Personal, medical issues of some of the scientists and the COVID-19 crisis have shut down some of the tests, experiments and analysis for a long period of time. Work is now picking back up with further collaboration within and between work packages as well as some starting collaborations outside the project within the EURION cluster.

The COVID-19 crisis slowed down the whole project, however some experimental results have already been produced and a science-based model was established. Furthermore, the one-year extension provided by the inclusion in the EURION cluster helps meeting the project deadline.

All these results will be used for computational modelling to create the final tiered tests, and to develop new adverse outcome pathway (AOPs) for metabolism. An AOP working group has been created in order to conjugate the WP efforts and start designing AOPs in accordance with the early results.

Progress beyond the state of the art and expected potential impact

In epidemiological studies on the metabolic effects of EDs, we will not only use traditional phenotypic markers but also a set of relevant molecular markers to improve the links between exposure and effects. A variety of non-animal experimental approaches with a large set of biological outcomes will be integrated using computational approaches to further improve the value and predictive capacity of these sets of tests. Improving the quality of the testing strategy will prevent the dissemination of toxic compounds and will have health, social and economic benefits.

- WP1: The analyses of huge cohorts' data and biomonitoring all along human lifespan provide major information for research in pollutants and allows stronger collaboration between scientists across Europe
- WP2: The new and convenient testing and screening methods for EDCs developed in the context of this research program will be candidates for regulatory use in providing relevant information for environmental and human risk assessments.
- WP3: We are working on setting up human-relevant in vitro test systems to identify putative endocrine disruptors involved in metabolic disorders. Feedback from in vivo and epidemiological studies will reinforce this task. This will definitively have a positive impact as it will be help to provide new tools to support risk assessment. Expanding mechanistic knowledge underlying the potential association between exposure to environmental chemicals and progress to metabolic pathologies is also expected.
- WP4: In silico models will feed databases with new and important parameters and make these tools more precise and relevant. As they feed into in vivo and in vitro experiments they will also help guide those experiments and save them time contributing to the 3R.
- The work done in WP5 supports the mechanistic understanding of the linkage between exposure to EDCs and metabolic disorders through a system biology approach, aiming at identifying perturbations at different levels of biological organisation, as well as inter-species pathway preservation, so us to deliver a comprehensive and usable IATA. This will result in more efficient public health protection covering a broad chemical space, reducing both the cost for testing new chemicals and the socioeconomic impacts related to EDC exposure.

Further information about OBERON:
Website: https://oberon-4eu.com/
Cordis: https://cordis.europa.eu/project/id/825712/fr
Twitter: @OBERON_4EU

2.8 SCREENED project summary

A multistage model of thyroid gland function for screening endocrine-disrupting chemicals in a biologically sex-specific manner - SCREENED

Summary of the context and overall objectives of the project

Endocrine disrupting Chemicals (EDCs) are commonly found in our everyday life, but there is growing evidence that EDCs interfere with the functioning of the thyroid and cause changes in thyroid hormone concentrations, the peripheral metabolism of these hormones and the signalling of their receptors. The mechanism by which they act on the thyroid axis is, however, still far from being elucidated, partially due to the limitations of existing tests.

SCREENED aims to develop 3D in vitro tests to characterise the effects of EDCs on thyroid gland function.

SCREENED will deliver highly innovative "Organ-on-a-chip" models, where thyrocyte cells organised in a 3D structure, will be hosted in a microfluidic cell culture device (hereafter called microfluidic bioreactor). This device will mimic the microenvironment of the thyroid gland, by emulating tissue- and organ-level physiology. The first 3D models will consist of thyroid organoids able to recapitulate the thyroid hormone production functionality of the native thyroid (mouse and human organoid models). In addition, we will work on ECM-scaffold-based and on a bioprinted based 3D models, which will represent an even more complex version of the 3D thyroid models, where the "Organ-on-a-chip" will also be supported by a vascularized network.

Overview of progress and main results achieved so far

In period 2, we have improved the protocol to derive thyroid follicles from mouse embryonic stem cells (mESCs). Besides, we have been working in generating a human-derived thyroid tissue using embryonic stem cells (hESCs). Excitingly, our results demonstrate that we can generate, by genetic and chemical manipulation, structures that are capable to organize three-dimensionally in follicles, produce thyroid hormone in vitro, and rescue the levels of TH when transplanted in thyroid-ablated mice. The establishment of a human thyroid follicles from hESCs constitutes a major breakthrough in the field of thyroid research and will be extremely useful to study thyroid gland illnesses beyond EDCs screening.

In parallel, we have successfully developed a microfluidic bioreactor system that is capable of hosting up to eight 3D thyroid tissue constructs in parallel, under flow conditions and that meets the requirements for high throughput screening. We have proved that, in flow conditions, mESC-derived thyroid follicles were able to recapitulate in vivo-like 3D follicular structures, featuring TG expression and a luminal space outlined by ZO-1 expression. Interestingly, follicles cultured in the bioreactor chips revealed an increase in T4 production and storage in the luminal space, as compared to follicles cultured in static conditions. In the same bioreactor platform, we successfully integrated sensors for continuous in situ oxygen measurement. Finally, our bioreactor and battery prototypes for EDCs screening were fabricated and initial experiments revealed that they are capable of providing leak-tight conditions.

We developed few variations of a rat thyroid 3D in vitro model based on 3D collagen scaffolds, 3D Matrigel environments, or decellularized thyroid ECM scaffolds. The rat thyroid progenitor/stem cells that were seeded or encapsulated in these scaffolds showed to be able to repopulate completely the constructs and to self-organize into thyroid follicle morphology able to secrete thyroid hormone. We further developed a 3D

bioprinted thyroid construct using a microfluidic bioprinting system. We evaluated the possibility to bioprint not only single cells, but also more complex and physiologically relevant structures, such as thyrocyte spheroids and mESCs-derived thyroid follicles. We verified that the bioprinting procedure does not impair the viability and structure of the bioprinted spheroids and follicles. In addition, we demonstrated that follicles maintain their functionality and express thyroid markers at levels comparable to non-bioprinted conditions. Finally, we developed a procedure of seeding of endothelial cells on the bioprinted constructs that seems functional for the creation of a preliminary vascularization of the constructs. To facilitate the position of either thyroid progenitor/stem cells in the decellularized-ECM scaffolds or endothelial cells in bioprinted constructs, these 2 cell populations were magnetized with biocompatible magnetic nanoparticles, which showed to maintain cell viability and steer cell positioning in space without altering their functionality.

We also worked towards an increased understanding of the mechanism by which EDCs interfere with the thyroid gland. As our work began, it soon became apparent that there is little if no existing data on the impact of EDCs on human thyroid cells on gene and protein expression. We have now completed planned investigation of the effect of several EDCs at different concentrations on a human thyroid cell line. The novel data is being used to support the identification of transcript and proteomic signatures of the impact of EDCs and to model cellular responses to EDCs. In more recent experiments the impact of EDC's on mouse thyroid follicles is being examined. We are now in the process of using the data to establish targeted measurement of key EDC responsive proteins. In silico models have been parametrized to correlate in vitro data with in vivo data, whenever available in the literature. Preparatory work has been carried out to identify how a molecular initiating event (MIEs) can eventually lead to an adverse effect in the human organisms, in the context of the Adverse Outcome Pathway (AOPs) conceptual framework. Putative MIEs to which SCREENED will eventually work have been identified

Progress beyond the state of the art and expected potential impact
The establishment of a human thyroid in vitro model constitutes a major breakthrough in the field of thyroid research and will be extremely useful to study thyroid gland illnesses beyond EDCs screening. These models bring a new fast, cheaper and animal-free alternative to screen a list of EDCs toxic effect, using a system that resembles what happens in humans.

SCREENED is advancing the field of "Organ-on-a-chip" devices and foster its adoption by industries. Indeed, the development of reversibly sealed bioreactor chips are compatible with high-throughput screening platforms, which are two major requirements for industrial applicability. Moreover, our system is expected to enable integration of sensing technology for continuous monitoring of physical and biochemical parameters during perfusion culture. The progress made in WP3 has led to the successful generation and in vitro characterisation of human thyroid follicles. This gives us the opportunity to progress beyond the current state of the art and undertake the analysis of the effects of EDCs on these in vitro models of the human thyroid at a gene (transcript) and protein level. The data will also support comparative modelling of the effects of EDs on (i) a human 2D model vs. 3D model, and ii) human vs. mouse 3D models. The transcript data is very comprehensive in its coverage (tens of thousands of genes/transcripts) compared to the proteomic data (a few thousand proteins). We intend to develop targeted protein assays to transcripts that have been shown to change and attempt to circumvent the limitation of protein-based discovery experiments.

Further information about SCREENED:
Website: https://www.screened-project.eu/
Twitter: https://twitter.com/ScreenedH
LinkedIn: https://www.linkedin.com/in/screened-project-8625611b4/
Cordis: https://cordis.europa.eu/project/id/825745

3 Overview of test methods to be developed

The EURION projects are developing and (pre-)validating methods and novel tools for better hazard identification of chemicals, focusing on thyroid system, developmental neurotoxicity, metabolic effects and female reproduction.

Table 1: Overview of test methods to be developed

	ATHENA	EDCmet	ENDpoiNTS	ERGO	FREIA	GOLIATH	OBERON	SCREENED
Definitive tests								
Addition of:								
Molecular readouts to existing test guidelines			X	X		X		
Endpoints to existing test guidelines	X			X	X	X		
More sensitive endpoints to existing test guidelines	X		X	X	X	X		
Biomarkers to existing test guidelines	X		X	X	X	X		
Screening tests								
Cell-based assays	X	X	X	X	X	X	X	X
Cell membrane transporters assays	X							
3D cell models							X	X
Stem cell assays	X		X			X		X
Reporter gene assays	X	X	X	X	X	X		
Non-cellular assays		X						
In vitro biotransformation			X					
Whole organism embryo		X	X	X	X	X	X	
Computational tools for screening (QSAR or other)	X	X	X	X	X	X	X	
Blood cerebrospinal fluid barrier (BCSFB) assay	X							
Blood brain barrier (BBB) assay	X							
High throughput assays	X	X	X	X	X	X	X	X
Molecular mechanism based assays (omics readouts)		X	X	X		X	X	X

4 Cluster highlights

GOLIATH and OBERON chaired the cluster for the first 15 months, followed by ERGO and ENDPoiNTs for the next 15 months.
ATHENA and SCREENED have taken over the organisation as of July 2021 for the next 15 month period and have continued Cluster activities according to the mandate.
Activities for the Cluster coordinators include: organising bi-monthly virtual meetings with the project Coordinators also including representatives from DG-RTD and JRC, organising the Cluster annual meeting, writing a periodic report for the Cluster and interaction with the European Commission's activities. Chairmanship activities have included the formation of fourteen different working groups (WG) with representatives from each of the projects in each WG. These WGs have met multiple times to establish collaboration between projects. In period 2, one new working group has been established (the evidenced-based tools WG).

During the second period, ERGO and ENDPoiNTs organised the second Annual Meeting of the Cluster (28th-29th January 2021). An open session for stakeholders took place in the morning of 28 January, providing highlights of the joint cluster activities and the eight projects. The meeting continued with several EURION-members-only sessions, including a scientific poster session and spotlight presentations as well as discussions on collaboration and cross-project activities. The discussion topics included adverse outcome pathways, validation activities, in silico work, stakeholder interaction, text mining tools and potential update on the state of the science report.

Further EURION activities have included liaising with the IAP, with the goal of (i) providing a bridge to other European and international initiatives and regulatory bodies; (ii) bringing understanding of international stakeholder needs, issues, concerns, drivers, and opportunities; (iii) providing independent assessment and feedback on the regulatory relevance of the test methods (under the different regulatory frameworks); (iv) promoting communication with main stakeholders in the field.
Some of the activities of the EURION Cluster over the past 18 months include contributions as a stakeholder to several EU initiatives: EU Green Deal, EU Chemicals Strategy for Sustainability and SRIP (Research and Innovation Strategic Plan), input into the update of REACH annexes and consultation for EDCs.

Some key achievements of the Cluster include so far:

- Set-up one new working group: Evidence-based Tools WG
- Active collaborations with the PEPPER platform which helps to progress assays towards a successful regulatory process
- Presentation of a first list of candidate assays and endpoints from EURION projects with potential to be added to new/existing guidelines at several EDTA OECD meetings (26th-27th May 2021, 26th-27th October 2021).
- Produced a policy document presenting the EURION Cluster and its contribution to advancing a safer chemicals world. The policy paper includes a series of recommendations to policymakers and is available from the EURION website
- Presentation at the 3rd Annual Forum on Endocrine Disruptors organised by DG ENV, providing an overview of work by the cluster and a vision for future research needs (21th September 2021).

5 Working groups

In total, 14 Working Groups (WGs) have been established, each with a special thematic focus in accordance with the Cluster mandate to optimise synergies and avoid overlaps between the projects. The WGs have leaders from the eight projects as well as from the Joint Research Centre, JRC.

The EURION Cluster WGs are:

- ADVERSE OUTCOME PATHWAYS (AOPS) WG
- CHEMICALS WG
- ANIMAL STUDIES: RODENTS WG
- ANIMAL STUDIES: AQUATIC ORGANISMS WG
- IN VITRO MODEL STUDIES WG
- REGULATORY AFFAIRS AND POLICY (RAP) WG
- INTEGRATED APPROACHES TO TESTING AND ASSESSMENT (IATA) WG
- EVIDENCE-BASED TOOLS WG
- IN SILICO WG
- EPIDEMIOLOGY WG
- DATA MANAGEMENT WG
- DISSEMINATION AND COMMUNICATION WG
- OMICS WG
- VALIDATION WG

In addition, a new WG has been set-up following the EURION 2022 Annual event (20th-21th January 2022) to tackle the difficulties faced regarded analytic measurements of thyroid hormone levels and understanding how these relate to the adverse effects observed. This new WG is labelled as the Thyroid Hormone Analytics Working Group and is coordinated by Rikke Poulsen.

5.1 ADVERSE OUTCOME PATHWAYS (AOPs) WG

AOPs (WG chair: Dries Knapen, ERGO)

Purpose and objectives of the WG:

- To support and facilitate AOP-related activities in the EURION projects by
  - Providing general AOP training
  - Organising discussion groups and meetings focused on specialised topics
- To bring together AOP-structured information and data across projects by
  - Providing generic templates for data entry and sharing
  - Organising AOP development workshops

Progress to date:

- A series of four AOP training webinars was organized. The webinars were held in April, May, June and September 2020.
- An AOP development workshop was organized on January 27 2021, aligned with the 2021 EURION Annual Meeting. More than 100 participants, representing all of the EURION cluster projects, attended the workshop. In the morning session, experimental design considerations for AOP development were discussed to further support the different projects' AOP development initiatives. In the afternoon session, opportunities and topics to collaborate across projects were discussed in three breakout groups, focusing on androgen signaling, thyroid hormone signaling and RXR/LXR/ROR signaling. During the pleneary closing session, the following suggestions were formulated to maximize the collaboration potential across projects:
  - Support AOP development based on general biology, physiology, pathology, …
  - Uncouple development of (downstream) KER descriptions from MIE / specific toxicological context / prototypical stressors
  - Use KE and KER descriptions as the unit of collaborative AOP development
- A number of project-specific and AOP-specific ad hoc follow-up meetings were held during 2021 with more detailed discussions.

Activities in the near future:

- An AOP WG meeting was organized on January 20 2022, aligned with the 2022 EURION annual meeting. All projects provided a status update on their ongoing AOP development activities.
- An AOP WG meeting was organized on April 26 2022. During this meeting, the following action items for the AOP WG to focus on in the near future were identified:
  - Mapping of AOPs under development in all EURION projects: create an overview (e.g. Excel file) containing all AOPs, KEs and KERs being worked on, and identify cross-overs between projects.
  - Establish a pragmatic technical solution for online collaborative AOP development, taking into account existing data confidentiality concerns. E.g., a sandboxed version of the AOP-wiki, AOP-wiki templates as Word documents on a Sharepoint server, using Google Docs, etc.
  - Organize workshops/seminars with invited speakers external to the EURION cluster, allowing more advanced, in-depth topics to be discussed. E.g., Kevin Crofton, Mary Gilbert, Jonathan Haselman, Josef Köhrle, etc.
  - Promote publication of developed EURION AOPs in the scientific literature. Evaluate the possibility of setting up a Special Issue in an open access journal for publishing EURION AOPs.

5.2 CHEMICALS WG

Chemicals (WG chair: Pim Leonards, ENDpoiNTs)

The purpose and of objectives of the WG Chemicals are:

- to provide an actual overview of EDCs studied by the projects,
- to provide an overview of EDCs used by multiple projects, and
- to exchange information on the analytical quality and protocols of the studied EDCs.

Progress to date: The main activity of the Chemicals WG was in the first period of the EURION cluster (e.g. overview of studied EDCs, purity of used EDCs, etc). The current activity was related to an update on the list of EDCs studied in the EURION projects.

Activities in the near future: Many EURION projects use reference compounds to study specific EDC endpoints. Therefore, an overview of all reference compounds used in the *in vitro* and *in vivo* studies will be prepared.

5.3 ANIMAL STUDIES: RODENTS WG

Animal studies: rodents (WG chair: Julie Boberg and Majorie van Duursen, FREIA)

The WG objectives are to:

- Share experience and expertise on *in vivo* rodent studies,
- Share tissues among the EURION projects and beyond, whenever possible.

Progress to date: FREIA is chair of the Working Group *in vivo* rodent studies (Table 2). All partners are represented in the WG, even though some partners do not perform rodent studies (OBERON, GOLIATH).

Table 2. Participants of the EURION WG in vivo rodent studies

Name	Project
Julie Boberg (until July 2021) Sofie Christiansen (per July 2021), Majorie van Duursen	FREIA (chair)
Marta Axelstad	ATHENA
Jukka Hakkola	EDCMET
Walter Lichtensteiger	ENDpoiNTs
Frederic Flamant	ERGO
Claire Beausoleil	GOLIATH
Jan Vondracek	OBERON
Roberto Toni	SCREENED
Sharon Munn, Elise Grignard	JRC (observers)

The WG has held two teleconferences (06/2021 and 11/2021). The meetings were dedicated to update each other on current and upcoming in vivo rodent studies. A table with objectives of the WGs were discussed (see below) and people gave updates on their projects. At the EURION cluster meeting of January 2022, a parallel session was dedicated to this WG and all projects gave a brief update and feedback was given from WG members. We plan to schedule meetings for 2022, every three months, in which 2 projects can present their findings.

A table was drafted in which all participants have added details on the planning of the experiments and design. The table is placed on the MS Teams WG folder and is regularly updated by the participants of the WG. The table also denotes tissues that are not primarily of use for the project who performs the study, and thus can potentially be used by other EURION projects and beyond. Tissues have been shared among projects, e.g. ovaries and uteri from ENDpoiNTs were harvested for FREIA. Livers from FREIA (KTZ) have been sent to partners in EDC-MET project, but analyses have been performed on those yet (late 2021).

5.4 ANIMAL STUDIES: AQUATIC ORGANISMS WG

Animal studies: aquatic organisms (WG chairs: Jessica Legardi, ENDpoiNTS / Lisa Baumann, ERGO)

Purpose and objectives of the WG:

- Insight in models and outcomes used across projects
- Share and compare protocols and chemicals
- Explore cross-project collaboration in ring studies

Progress to date: The WG meets regularly (monthly) in order to exchange information about different topics related to experiments with aquatic species as well as to discuss new scientific results. In the period from July 2020 to December 2021, multiple online meetings with presentations of different scientists took place:

- ***25.11.21: Helmut Segner,*** *Immunotoxic effects of EDCs*
- ***27.10.21: Jonathan Hamm,*** *SEAZIT project, ontologies of zebrafish phenotypes*
- ***30.09.21: Wibke Busch,*** *transcriptomics and mixture effects*
- ***07.07.21: Henrik Holbech,*** *update OECD EDTA meeting*
- ***27.05.21: Sina Volz,*** *olfactory-mediated behavior in zebrafish*
- ***01.04.21: Nicolas Buisine,*** *transcriptomics and RNAseq*
- ***24.02.21: Arif Doenmez,*** *data management platform*
- ***25.11.20: Lisa Baumann & David Du Pasquier,*** *fish and amphibian models*
- ***29.10.20: Jessica Legradi, zebrafish behavior tests***
- ***28.09.20: General discussion of the entire WG,*** *planning of future collaborations and meetings*

Activities in the near future: The WG has decided to extend/open the monthly meetings to external experts and audience who work in the field of endocrine disruption with aquatic species. This will broaden the impact of our meetings, as well as foster further collaborations with relevant working groups.
At the annual EURION meeting, it was discussed that an in-person meeting focused on the needs of PhD students and early postdocs of the EURION projects would be beneficial. This workshop should focus on different methods that are useful to work with aquatic species. Planning of that activity is ongoing and will also depend on further development of the COVID19 pandemic.

5.5 IN VITRO MODEL STUDIES WG

In vitro models (WG chair: Lorenzo Moroni, SCREENED)

Purpose and objectives: The working group has gathered online and in person in the annual meeting of Paris 2020. Initial activities aim at generating a database of cells used in the different projects of EURION to individuate possible synergies and additional collaborations in the context of *in vitro* models for ED screening.

Progress to date: The members of the working group have shared a number of assays and protocols that were developed so far in the 8 projects of EURION. We have a shared space in Teams where this information is collected.
A workshop was held in December 2021 that was open to all members of the 8 projects that are interested in in vitro assays. The workshop was a great success with a lot of participants interested to learn from each other and further explore possible collaborations. As a follow-up, we held a dedicated in vitro WG session during the 2022 edition of the EURION Annual Meeting (20th-21st January 2022). This moment served to triger further collaborations.

Activities in the near future: We will further explore possible collaborations across projects. So far GOLIATH and OBERON are actively collaborating, and this collaboration will possiblity be extended to EDCmet. ATHENA and SCREENED could be also 2 other logical candidates.
We have plan to further share experience on "*How are negative controls or "non-EDCs" chosen and tested in in vitro assays*?". This has already been a topic of discussion, during the 2022 edition of the EURION Annual Meeting, with very good input across the working group members.

5.6 REGULATORY AFFAIRS AND POLICY (RAP) WG

Regulatory Affairs and Policy (WG chair: Andreas Kortenkamp, ATHENA)

The purpose and objectives of the WG: To elaborate proposals for improving the implementation of ED testing in relevant EU regulations and directives. This overall aim is broken down into several short-term and medium-term work steps, such as (but not limited to):

- How are triggers for further ED testing currently interpreted across regulations?
- Which OECD-validated *in vitro* and *in vivo* tests can / should be implemented in the short term to improve ED testing?
- Which methods should be implemented to capture ED in wildlife and non-target organisms (e.g. invertebrates)

In the long run, the group will also examine:

- How ED test data are used further, e.g. for establishing ADIs or TDIs
- How improvements in the EU can impact on ED testing beyond the EU

Progress to date: The group met during the EURION cluster meeting 2022 to discuss the outcome of a workshop organised by the ATHENA project with members of the IAP and other stakeholders in August 2021. The workshop focused on summarising the status quo of international regulations regarding thyroid hormone system-dusrupting chemicals. A second aim was to develop perspectives for the immediate improvement of testing strategies for thyroid hormone system-disrupting chemicals.
In discussions of the WG, the lack of validation of *in vitro* assays was seen as a significant bottleneck. The utility of *in vitro* assays for the prioritisation of chemicals and the need for incorporation of *in vitro* bioassays in a future testing strategy was noted. The absence of assays (*in vitro* and *in vivo*) capable of capturing effects of thyroid hormone system disruption on the developing brain was also seen as a major

shortcoming. As immediate possible improvements, the measurement of thyroid hormone levedls in various OECD test guidelines should be harmonised. The ATHENA workshop report has been circulated for further detailed discussions.

Activities in the near future: Ideas for further activities include using the ATHENA report as a template to compelte similar analyses relevant to other hormone systems of interest to the EURION cluster. We will also consider publishing the report as a scientific paper.

5.7 INTEGRATED APPROACHES TO TESTING AND ASSESSMENT (IATA) WG

IATA (WG chair: Miriam Jacobs, GOLIATH)

Purpose and objectives of the WG:
To build understanding of IATA development, and develop collaborative approaches amongst the EURION cluster members, IATA's are a main deliverable for all EURION projects.

- To review and understand existing IATA guidance
- To review and learn from i) case studies of relatively simple IATAs: e.g eye and skin irritation ii) case studies of more complex IATAs: Non-Genotoxic carcinogenicity
- To agree common understanding of terminology and applications
- To build harmonised bridges across the EURION projects

Progress to date: Three meetings were held, up to the end of 2020. Summary slides of these meetings were provided to consortia members on request, in 2021, but after chair consultation with the group, a further meeting in late 2021 did not go ahead, as the contribution of the other consortia was insufficient to organize a new meeting or workshop of IATA development. Active contribution of consortia is needed to facilitate a multi-sided interaction between the different consortia and ED modalities.

Activities in the near future: The chair will provide a case study presentation on the NGTxC IATA work at a later date in 2022, at a time when higher priority work and deliverable needs have been met. At this meeting brief updates of related IATA work will be first expected from each project.

5.8 EVIDENCE-BASED TOOLS WG

Evidence-based WG (WG chair: Martin Olwenn, Elias Zgheib, ATHENA)

Purpose and objectives of the WG: The evidence-based tool working group was created following discussions during the EURION Annual Cluster meeting in 2021. Its main objectives are:

- To share knowledge and review the use and utility of evidence-based tools and methods, particularly for the development of Endocrine AOPs
- To disseminate knowledge about such tools and methods

Progress to date: This working group was only created about 15 months ago. Originally, 14 people joined the working group and while there has been some flux in terms of participants joining and leaving the group, this overall number has remained relatively constant. In addition to the *ad hoc* breakout group session during the EURION Cluster meeting in January 2021, the WG has met six times since its creation. Earlier sessions were used to collate knowledge of WG group participants about evidence-based tools. Following further discussions on how best to apply these tools, it was decided to submit a perspective article to a Special Issue of Frontiers in Toxicology entitled "Towards Integrated Approaches to Testing and Assessment (IATA) for Endocrine Disruptors - Maximizing Mechanistic Knowledge for Regulatory Purposes". The drafting of this

manuscript was used to focus efforts on the use of evidence-based tools for the development of AOPs. The peer-reviewed manuscript was accepted for publication in December 2021.

Audouze K, Zgheib E, Abass K et al (2021) Evidence-Based Approaches to Support the Development of Endocrine-Mediated Adverse Outcome Pathways: Challenges and Opportunities. *Frontiers in Toxicology* 3:787017. doi: 10.3389/ftox.2021.7870173

Activities in the near future: Both chairs of this WG have moved institutions around March 2022 and this resulted in a period of flux during which activities were suspended. Olwenn Martin will resume chairing this WG. As highlighted in the manuscript , there is some interest in automating some of the time-consuming steps required by systematic evidence-based methods. While some examples of automated methods exist, what is lacking in their evaluation in comparision with manual methods. While such 'validation' of automated or semi-automated methods would be a worthwhile exercise, without dedicated funding, it is unprobable that it will be feasible to conduct such an exercise within the scope of EURION. Future activities, to be discussed with other members of this WG, are therefore more likely entail a series of webinars and/or training sessions to increase familiarity and understanding of various tools and methods.

5.9 IN SILICO WG

In Silico (WG chair: Maria Sapounidou, GOLIATH;ENDpoiNTs)

Purpose and objectives of the WG:

- To share analysis approaches, intelligence around test chemicals etc. and to provide a platform for scientific discussion and inter-laboratory validation
- To generate joint publications exploring the utility of in silico methodologies for risk assessment within the ED space

Progress to date: During the EURION Annual Cluster meeting in 2021, a meeting across interested partners was organised. In a subsequent meeting, it was concluded that participants were not ready to participate in any collaborative efforts due to lack of progress alignment across projects.
During the EURION Annual Cluster meeting in 2022, a meeting across interested partners was organised to revisit progress of participants and rekindle collaborative interaction across EURION in silico groups. There is currently 13 participants within the WG and all EURION projects are represented. Three meetings have been organised to communicate projects in an attempt to find common avenues and overlapping methodologies.

Activities in the near future Currently, an effort is organised for a publication focusing on providing a review on the state-of-the art in silico methods for identification and kinetic profilling of endocrine disrupting chemicals.

5.10 EPIDEMIOLOGY WG

Epidemiology (WG chair: Carl-Gustaf Bornehag, ENDpoiNTs)

The purpose and objectives of the Epidemiology WG, EWG: The overall aim is to compile information on epidemiological studies in the cluster, and more specifically:

- to coordinate the involved epidemiological studies within the cluster
- to establish a data management plan for how to share information/results from different epidemiological studies.

Progress to date: During the EURION Annual Cluster meeting in 2022 a parallel session on metabolic disorders was organized. A total of 13 participants from EDCMET, GOLIATH and OBERON participated.
The main issues pointed out were: 1) try to increase sample size to study some research questions such as epigenetic mechanisms of EDs; 2) work together on two papers: a descriptive paper discussing the challenges about omic data and cardiometabolic outcomes in cohort studies; and a second on the prenatal exposure to EDs and epigenetic markers in cord blood/placenta (including cohorts in the PACE consortium).
The WG is further working with finalizing the excel file on epidemiologic studies. Also, we will start compiling established results from the different epidemiology studies based in different health domains (neurodevelopment, metabolism and growth, sexual development, etc.). Finally, we will carry out an inventory on experimental results from the eight EURION projects supporting the findings in the epidemiological studies.

Activities in the near future: New studies have been set up to assess the obesogenic effects of EDs in cohorts participating in ENDpoiNTs (e.g., SELMA) and OBERON (e.g., INMA, Pélagie). The objective is to increase scientific evidence on the early-life metabolic effects of EDs by incerasing the sample size, using cohorts across Europe with potentially different exposure spectrum and confounding patterns, and having an harmonized outcome definitions.

5.11 DATA MANAGEMENT WG

Data Management (WG chair: Anna-Liisa Levonen, EDCMET)

Purpose and objectives of the WG:

- To discuss and evaluate data management solutions and approaches, including data documentation and databases
- To provide templates and/or instructions how to make data findable and interoperable
- To provide support in data management for projects and aid in the development of project specific data management plans
- Integration and harmonization of data infrastructure of EURION – EURION data management roadmap for environmental exposure /ED health effects

Progress to date: Status of data management and DMPs of projects, sharing of data and use of common databases as well as the functions of the working group were discussed in the working group meetings during the previous reporting period. Individual project DMPs were collected in EURION Teams during the current reporting period. EDCMET coordinator and scientific manager participated in the HBM4EU workshop on data management in Dec 2020, to discuss specifically the management of sensitive personal data and related FAIR principles. The activities of the working group have been on hold due to the Covid-19 pandemic. Data management issues have been discussed also in meetings of other working groups.

Activities in the near future Next teleconference for further discussions on the working group functions and plans will be organized to facilitate further discussions on the functions of the working group. Participants from other working groups (especially *in silico, omics and epidemiology*) will be invited to discuss their data sharing and management approaches and how to avoid overlaps between these working groups.

5.12 DISSEMINATION AND COMMUNICATION WG

Dissemination and communication (WG chair: Cluster chairmanship team)

Purpose and objectives of the WG:

- Promote the activities and results beyond EURION to regulators, scientific community, policy makers and the general public, employing a range of communication and dissemination tools;
- Ensure timely and efficient knowledge management and sharing, while safeguarding that suitable IP management strategies and processes are applied to EURION;
- Capture key messages and outcomes, based on knowledge generated through EURION to ensure effective sharing of knowledge outputs to end users;
- Maximise post-project uptake by developing thorough and forward-thinking plans that clearly outline the potential end-users of the project. Optimise post-project uptake by knowledge transfer activity required to ensure objective and measurable short and long-term impacts of the cluster.

Progress to date: the following activities have been carried out during the period from July 2020 to December 2022:

- Organisation of the 2nd EURION Annual Meeting (28th-29th January 2021) to present the latest advances in research by the EURION Cluster towards improving the identification of ED. More than 300 participants, including reprentatives from regulatory agencies, industries and policy-makers attended the event. The Annual event included a poster session, with more than 62 posters presented.
- Release of one common policy paper about future research needs on Endocrine Disruptors (September 2021) and of one policy brief "towards safer chemicals – reliable test methods to identify endocrine disruptors (January 2022). The policy paper was presented at the 3rd Annual Forum on Endocrine Disruptors (21th September 2021) by Prof. Lorenzo Moroni
- Maximization of EURION outreach through an active presence on the Social Media (48 re-Tweets in 2021 and 244 followers are following the EURION Twitter account at the end of period 2).
- In 2021, the 8 projects of the Cluster released over 40 scientific publications related to the identification of new endpoints for ED, the improvement of development of new test methods, the development of new testing strategies based on AOPs network and read-across between vertebrate classes.

Activities in the near future: the following activities are planned in the near future:

- organisation of the 3rd Annual Meeting (20th-21th January 2022) and 4th EURION Annual Meeting (possibly during the Winter 2023)
- Common dissemination activities at key Conferences in the field of toxicology (i.e. World Congress)
- Publications
- Active dissemination of EURION results through the SoMe.

5.13 OMICS

OMICS (WG chair: Karine Audouze, OBERON)

Purpose and objectives: The main objective is to harmonize the data in order to be able to use them across the different projects, and to establish an 'EURIONOMICS protocol' to have comparable data.
The OMICS working group led by K. Audouze (OBERON), includes 32 participants from six of the seven other ED projects (the project ATHENA does not plan any omics activity), and Jukka Sund, JRC will be part of the working group as EU representative.

Progress to date: Since the start of activities in this WG, an EURION omics leaflet was created, a couple of meetings have been held and 4 webinars have been organized. These webinars introduced tools used by different partners in their own specialties:

- Presentation of GitHub by Thomas Darde from FREIA
- Presentations of TOXsIgN by Indusha Kugathas who is PhD working with Thomas Darde
- Presentation of MetExplore by Fabien Jourdan from GOLIATH
- Cross-omics discovery of adverse outcome pathways linked to exposure to endocrine disrupting compounds was presented by Nafsika Papaioannou from OBERON

Activities in the near future: A meeting will be held in June 2022 in order to make plans for the future of this WG.

5.14 VALIDATION WG

Validation (WG chair: Sharon Munn, JRC)

Purpose and objectives of the WG:

- To provide a platform for cross-cluster discussion on issues relevant to validation through identification of issues and sharing of best practices
- To develop and provide relevant training

Progress to date: In addition to the intial training provided to the EURION cluster at the beginning of 2020, discussions within the validation workign group and EURION project coordinator's steering committee indicated a need for further training with case studies highlighting project-specific validation or pre-validation activities. JRC distributed a template describing seven criteria, with a number of sub-criteria, relvant to the evaluation of an in vitro method's readiness for validation based on apublication by Bal-Price et al, 2018[1], with a scoring scheme, allowing project partners to evaluate their own methods against the criteria. The collective experiences of evaluating methods aginst the criteria was the basis for a further validation workshop held on 1 June 2022. Speakers from JRC provided a recap of Good In Vitro Methods Practice (GIVIMP) and appraoches to validation, followed by an interactive discussion on the questions received to the Test Readiness Criteria and technical issues related to the methods. JRC followed up with pointers to what further work could be considered within the timeframe of the EURION projects to address the identified gaps.

Activities in the near future:

- Comments received on the Technical Readiness Template will be taken into account and used to update the template.
- OBERON have developed an automated Test Readiness Template to faciltate a rapid test readiness self-evaluation which can be shared with the cluster once finalised.
- Questions related to specific methods can be addressed through bilaterals with the JRC.

[1] Bal-Price et al (2018) *Altex* 35 (3), 306-352