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

Project highlights

EURION Annual Cluster meeting 20.1.2022
Professor Anna-Liisa Levonen, EDCMET coordinator
Background

• Endocrine disruptor research has focused mainly on reproductive endocrinology and related hormones, which is reflected in the available regulatory test methods.

• The current testing tools do not appropriately identify effects related to less-studied endocrine-mediated pathways and health outcomes, such as disruption of lipid and glucose metabolism, in humans.

• New and improved approaches are needed to increase the quality, efficiency, and effectiveness of the methods to evaluate the effects of these metabolic disruptors and to meet the demanding and evolving regulatory requirements worldwide.
• 11 academic and non-academic partners from several European countries, over 40 experts from various research fields

• During its 5-year journey, EDCMET aims to identify novel ED mechanisms of action, to generate (pre)validated test methods to assess the metabolic effects of EDs, and to predict emergent adverse biological phenotypes by following the adverse outcome pathway (AOP) paradigm.
Key Events (KEs)
- Multi-level omics data from cell lines
- Multi-level omics data from tissues

Molecular Initiating Events (MIEs)
- Omics data for identification with pathways associated with ED AOPs
- Molecular docking (NRs)

Adverse Outcomes (AOs)
- Biomarkers of metabolic phenotype
- Metabolic phenotype in clinical cohorts (biomarkers)

Multi-level omics data from cell lines
- Metabolic phenotype in clinical cohorts (biomarkers)
- Rodent models

ED exposure vs. phenotype
- Rodent models

WP1
- Molecular docking (NRs)

WP2
- NR assays
- Metabolic assays

WP3
- Rodent models

WP4
- ED exposure vs. phenotype

Ref chemicals (EDs, NR ligands)

EDC Met
EURION
AIMS

Identification and prediction of protein-ED interactions and mechanisms behind NR activation

Linking ED exposure to adverse outcomes
Molecular mechanisms and MIEs / In silico

Development of molecular docking and dynamics simulation approaches for protein-ED interaction studies for 14 NRs using NR agonists and ED reference compounds

• Potential ED target proteins?
• What are the molecular triggers for NR structure stabilization and conformational changes upon binding of EDs?
Linking ED exposure to adverse outcomes

- Generation of a novel omics-based classification system and pipeline
  - Key metabolic pathways and genes affected by EDs and set of predictive models utilizing pathway information to prioritize EDs and to predict ED evoked metabolic disease
  - EDTOX R-Shiny application available at https://github.com/vittoriofortino84/EDC_shiny
- Identification of potential biomarkers of exposure and ED effects based on available omics data using machine-learning techniques
- Development of a systems toxicology tool to predict emergent metabolic phenotype from ED exposure

Env Int 2021, 156, 106751; 10.1016/j.envint.2021.106751
AIMS

Development and validation of cell-based and cell-free in vitro MDC profiling assays and the use of omics techniques to understand MDC mechanisms and effects on cellular signaling
In vitro assay development

- NR-cofactor interaction assays for 10 NRs (cell-free)
- NR reporter gene assays for 18 NRs (HepG2)
- ED reference chemical (17) screening

- NCATS and OECD Framework guidance on standardized \textit{in vitro} test systems and scientific assay validation implemented in the development and scientific pre-validation of the assays to ensure robustness and reproducibility of results as well as transferability of the assays
In vitro assay development

- Mitochondrial function assays (HepaRG)
- Lipid accumulation assays (HepaRG)
- ED reference chemical (17) screening
Cellular responses

• Multi-omics approaches for ED effects
  • Hepatic cell lines, mouse and human samples
  • Genomic studies
    • Expression profiling (RNA-Seq)
    • Chromatin accessibility (ATAC-Seq)
    • Chromatin binding (ChIP-Seq)
  • Proteomics
  • Metabolomics

Prediction of mechanistic linkage between MIEs and adverse metabolic phenotype of EDs
AIMS

Development, optimization and testing of in vivo approaches for detection of MDCs

AOPs related to adverse metabolic effects
In vivo rodent models for the assessment of metabolic effects of EDs

Development of standardized *in vivo* protocols to assess and evaluate the adverse metabolic effects of EDs

- *In vivo* experiments are important for understanding the mechanisms of metabolic disruption

The experimental protocol may have very significant effects on results and conclusions

- Applicability of the 28-day toxicity study protocol for the detection of metabolic effects?
- Effects of metabolic challenge (HFD-induced obesity) and in utero exposure
In vivo rodent models for the assessment of metabolic effects of EDs

- Characterization of mode of action and AOPs for MDCs
  - Example: PXR

- PXR activation potentiates HFD-induced liver steatosis
- RNA-seq indicates induction of cholesterol synthesis
- RIF increases plasma LDL cholesterol and cholesterol synthesis in humans

Br J Pharmacol 2021, 178, 2461-2481; 10.1111/bph.15433
AIMS

Assessment of ED levels and ED-related metabolic health outcomes

Detection of possible biomarkers

Estimation of human health risks at population level
ED exposure and metabolic effects

Cohorts from Nordic countries and Spain

Non-occupational exposure to pesticides and health markers in general population in Northern Finland

- NFBC1966 cohort
- Associations with lipid biomarkers (TC, HDL, LDL, TG)
- Baseline study for metabolomics analyses
- Env Int 2021, 156, 106766; 10.1016/j.envint.2021.106766
ED exposure and metabolic effects

Monitoring temporal trends of dioxins, organochlorine pesticides and chlorinated paraffins

- MISA cohorts
- Decreasing temporal trends of legacy POPs
- Particular concern of medium and long chain chlorinated paraffins!
- Env Res 2022, 204, 111980; 10.1016/j.envres.2021.111980

Comparisons of geometric mean serum concentrations (μg/L) of the short and medium chain chlorinated paraffins in the pooled samples of MISA 1 and MISA 2 within period 2007–2009 and 2019.
Impact

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

- Array of new or improved testing tools for risk assessment of MDCs
- Increased human relevance of testing and risk prediction
- Identification of novel mechanisms of action of MDCs
- Linking human exposure and EDC levels with metabolic endpoints and health outcomes
- Increased knowledge on metabolic diseases and novel biomarkers

All data on ED effects as well as test protocols will be made available to the scientific community, stakeholders and regulatory bodies.
EDCMET Management Team

Anna-Liisa Levonen
Project Coordinator
anna-liisa.levonen@uef.fi

Jenni Küblbeck
Scientific Manager
jenni.kublbeck@uef.fi

Maija Hartikainen
Project Manager
maija.hartikainen@uef.fi

Albert Braeuning
Communication and dissemination
albert.braeuning@bfr.bund.de

www.uef.fi/edcmet
@edcmet_eu
https://cordis.europa.eu/project/id/825762
Thank you!

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 825762.