EURION

European Cluster to Improve Identification of Endocrine Disruptors

edcmet

GOLIATH

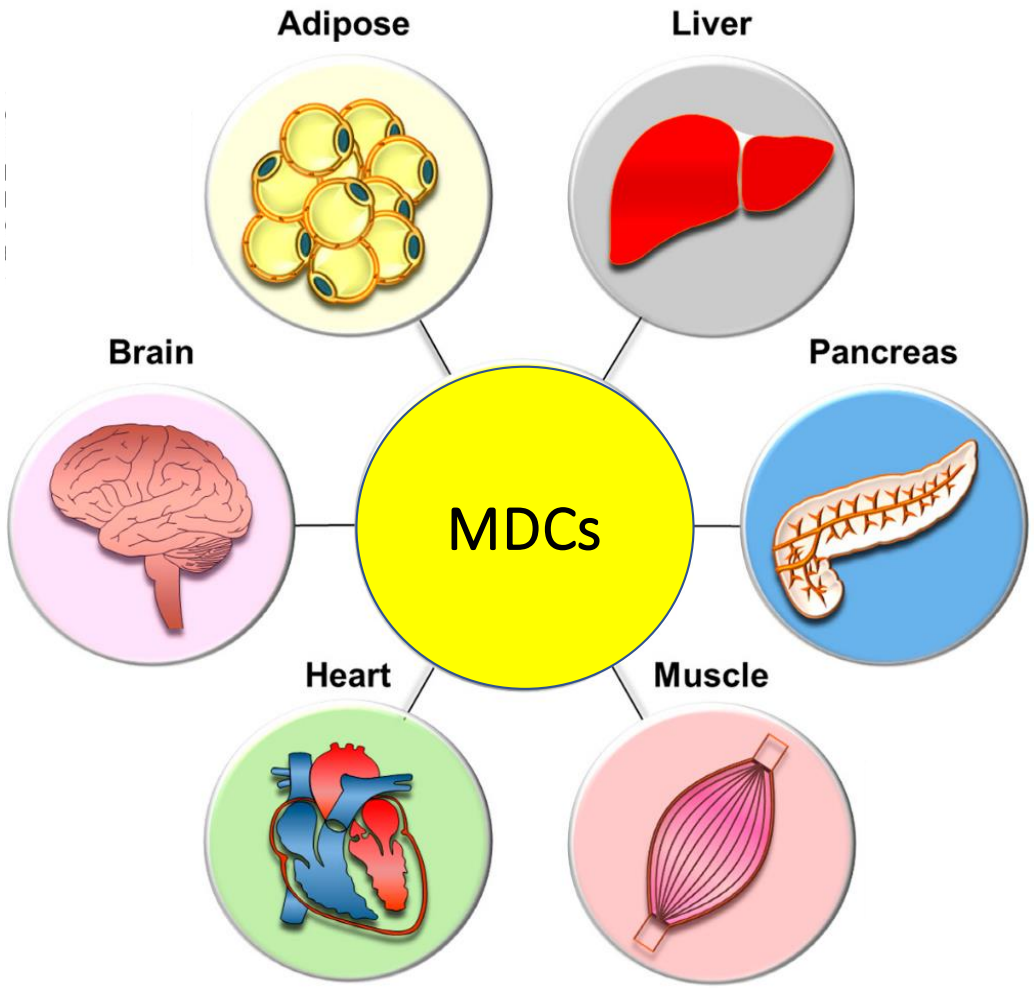
OBERON

EDCs • TESTING • METABOLISM • IATA

Introduction to projects focussing on metabolism disrupting chemicals

Juliette Legler

Dec 17, 2020



Metabolism disrupting chemicals (MDCs)

- class of EDCs that affect energy homeostasis
- affect multiple endocrine mechanisms and cell types implicated in metabolic control
- affect gene expression and biosynthesis of key enzymes, hormones and adipokines essential for controlling energy homeostasis

Definition:
'any EDC that alters susceptibility to metabolic disorders (eg. obesity, diabetes, nonalcoholic fatty liver disease) and includes the terms 'obesogens', 'diabetogens' and 'diabesogens''

Nadal et al, 2017, Nature Rev Endo
Heindel et al, 2017, Repro. Tox.

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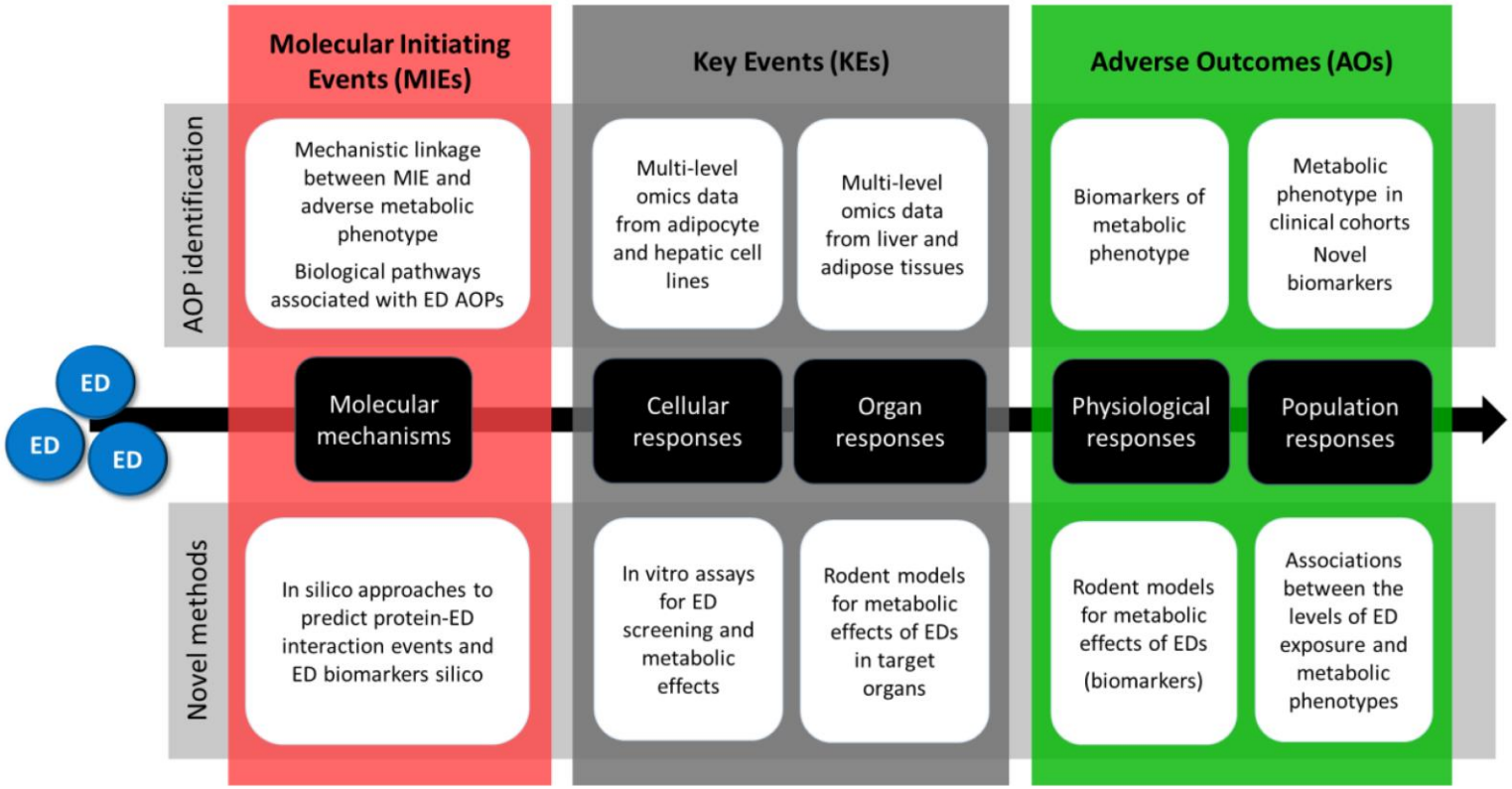
Metabolic effects of Endocrine Disrupting Chemicals: novel testing METhods and adverse outcome pathways

The EDCMET Project: Metabolic Effects of Endocrine Disruptors



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.

Progress thus far
https://cordis.europa.eu/project/id/825762/reporting

Int. J. Mol. Sci. **2020**, *21*(8), 3021; https://doi.org/10.3390/ijms21083021

Example of an *in vitro* assay developed in EDCMET

Reporter gene assays for human nuclear receptor activation

- Based on dual-luciferase assays in hepatic cells on 96-well plates
- AR, CAR, ERα, ERβ, ERRα, ERRγ, FXR, GR, LXRα, LXRβ, MR, PPARα, PPARδ, PPARγ, PR, PXR, RARα, RXRα, TRα (+AhR)
- The assays will go through stability and process studies and scientific pre-validation, including replicate experiment assessment and inter-laboratory comparisons
- Most relevant assays for MDC studies, when compared to data obtained from other developed assays as well as *in vivo* and epidemiological data will be brought forward to the pre-validation process

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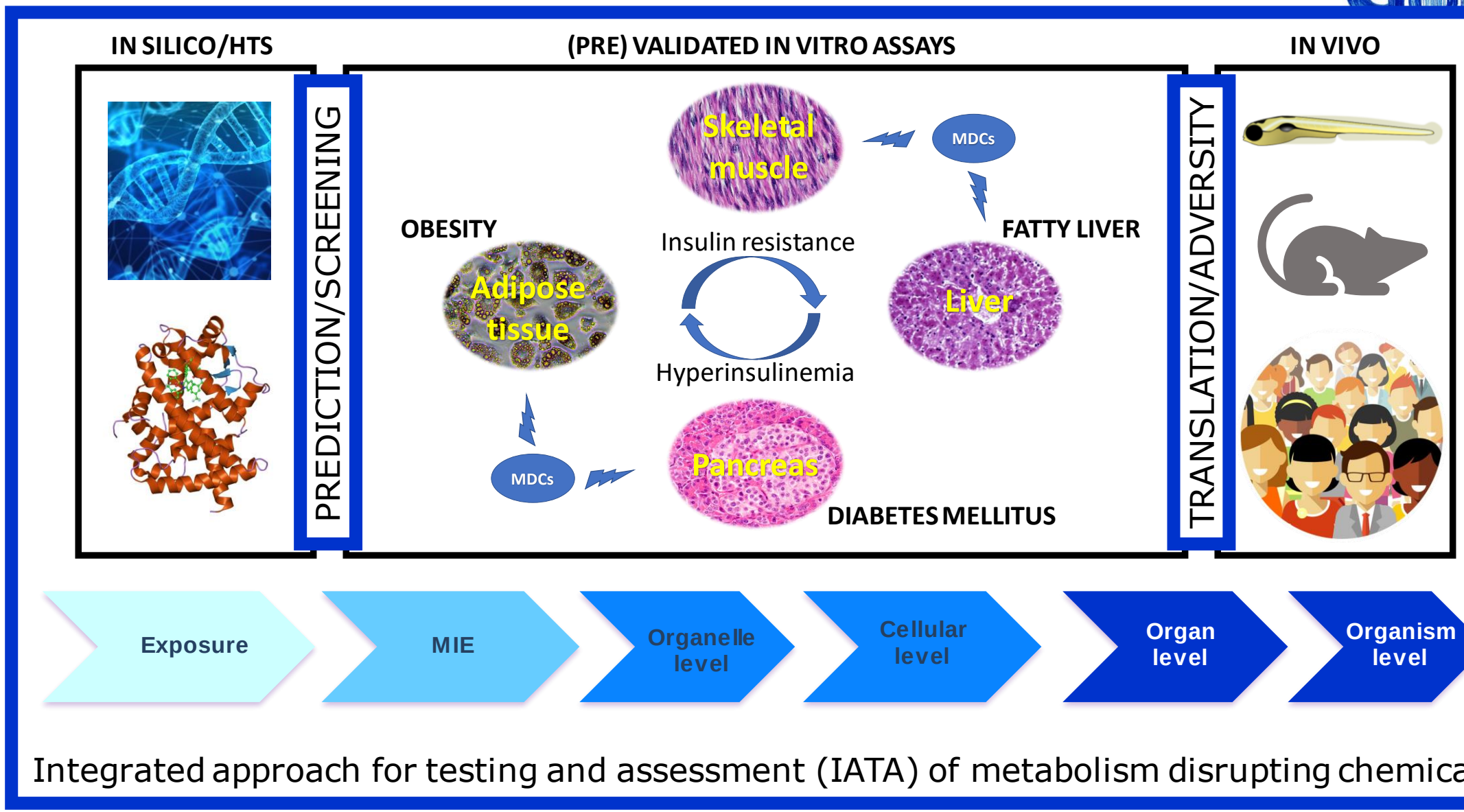
Generation Of NoveL, Integrated and Internationally Harmonised Approaches for Testing Metabolism Disrupting Compounds



Integrated approach for testing and assessment (IATA) of metabolism disrupting chemicals

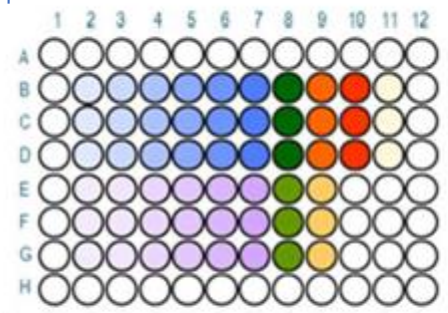
Int. J. Mol. Sci. **2020**, *21*(10), 3480; https://doi.org/10.3390/ijms21103480

CYP induction assay: extension of chemical applicability domain to MDCs

Thawing and Seeding

HPR116 cells
7.2 x 10^4 cells/well
BIOPREDIC

Treatment with chemicals

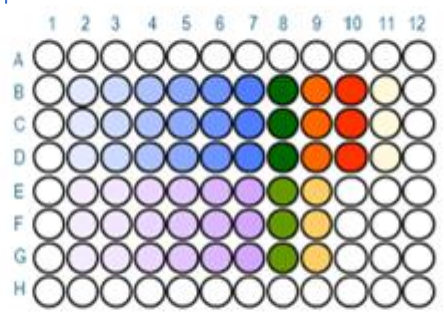


Test items (6 serial dilutions)
Positive inducers
Negative control (DMSO)
Medium

CYP Induction

Test item exposure
48 h

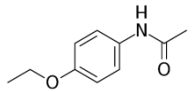
Addition of probe substrate cocktail



Enzymatic probe substrate cocktail

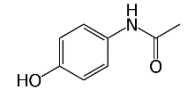
1 h incubation

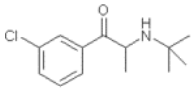
Specific substrates


Phenacetin (26 µM)

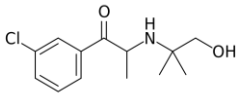
CYP1A2

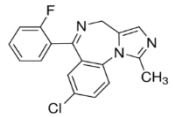
Metabolites


Acetaminophen (ACETA)

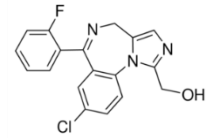

Bupropion (100 µM)

CYP2B6


Hydroxybupropion (OH-BUP)


Midazolam (3 µM)

CYP3A4


1-hydroxymidazolam (OH-MID)

Reference inducers (Positive controls)

CYP1A2	B-naphthoflavone 25 µM
CYP2B6	Phenobarbital 500 µM
CYP3A4	Rifampicin 10 µM

LC-MS/MS
Metabolite quantification

CYP Inducer

https://tsar.jrc.ec.europa.eu/test-method/tm2009-14

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An integrative strategy of testing systems for identification of EDs related to metabolic disorders

10 selected EDs: BPA, BPS, BPF, DEHP, DBP, PFOS, PFAS, Cd, DDE, butyl-paraben

Human exposure children/adolescents & adults cohorts
Measurements, omics,& follow-up studies

Experimental tests
- 2&3D models linked to organs and tissues
- Disease models: zebrafish obesogenic & steatosis tests

Intra- and inter-laboratory reproducibility

Multidisciplinary

Computational studies
PBPK & QSAR models for EDs

Integrative framework
Bioinformatics (omics)
Systems biology

Metabolic disorders prediction, support risk assessment

AOPs
Biomarkers

IATA: battery of tests

Decision tree

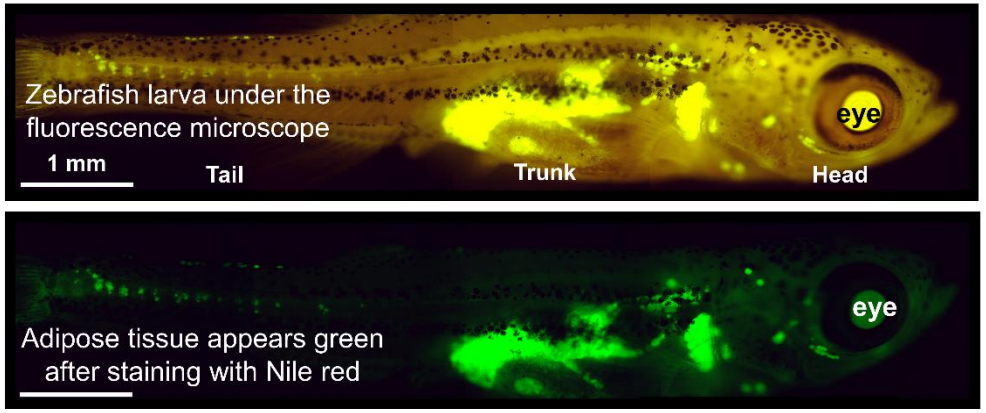
Zebrafish obesogenic test (ZOT)

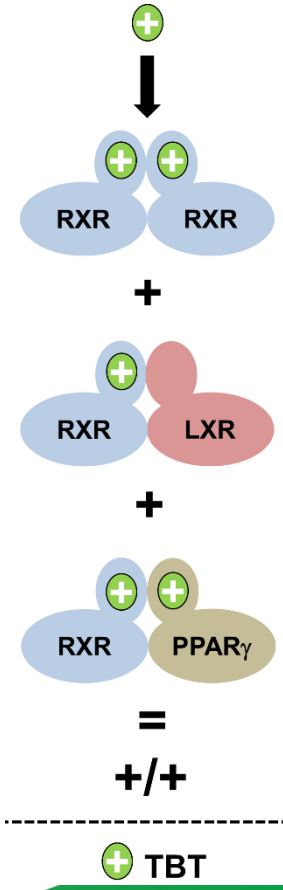
A tool to identify endocrine disruptors chemicals (EDC) involved in obesity

- Whole-organism mechanism-based assay for screening substances acting as potential obesogens.

- *In vivo* assessment of the potential impact and interactions of diet composition, chemical pollutants, and drugs acting as EDCs on white adipocyte lipid droplet size and body adiposity.

- ZOT is selected by PEPPER to boost its pre-validation.





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How EURION MDC projects approach the challenges

- Improve understanding of the endocrine **modes of action** of MDCs
- Develop **assay candidates** for MDCs based on confirmed MoA and key events in target tissues
- Further develop assay candidates into **(pre-)validated test methods**
- Develop an internationally harmonised, integrated approach to testing and assessment (**IATA**) of MDCs, using an Adverse Outcome Pathway (AOP) conceptual framework.

These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825161 (ATHENA), No. 825762 (EDCMET), No. 825759 (ENDpoiNTs), No. 825753 (ERGO), No. 825100 (FREIA), No. 825489 (GOLIATH), No. 825745 (SCREENED), No. 825712 (OBERON). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

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Thank you!

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