3rd HBM4EU Training School

Case study – Combining cohort and in vitro studies for informing AOPs

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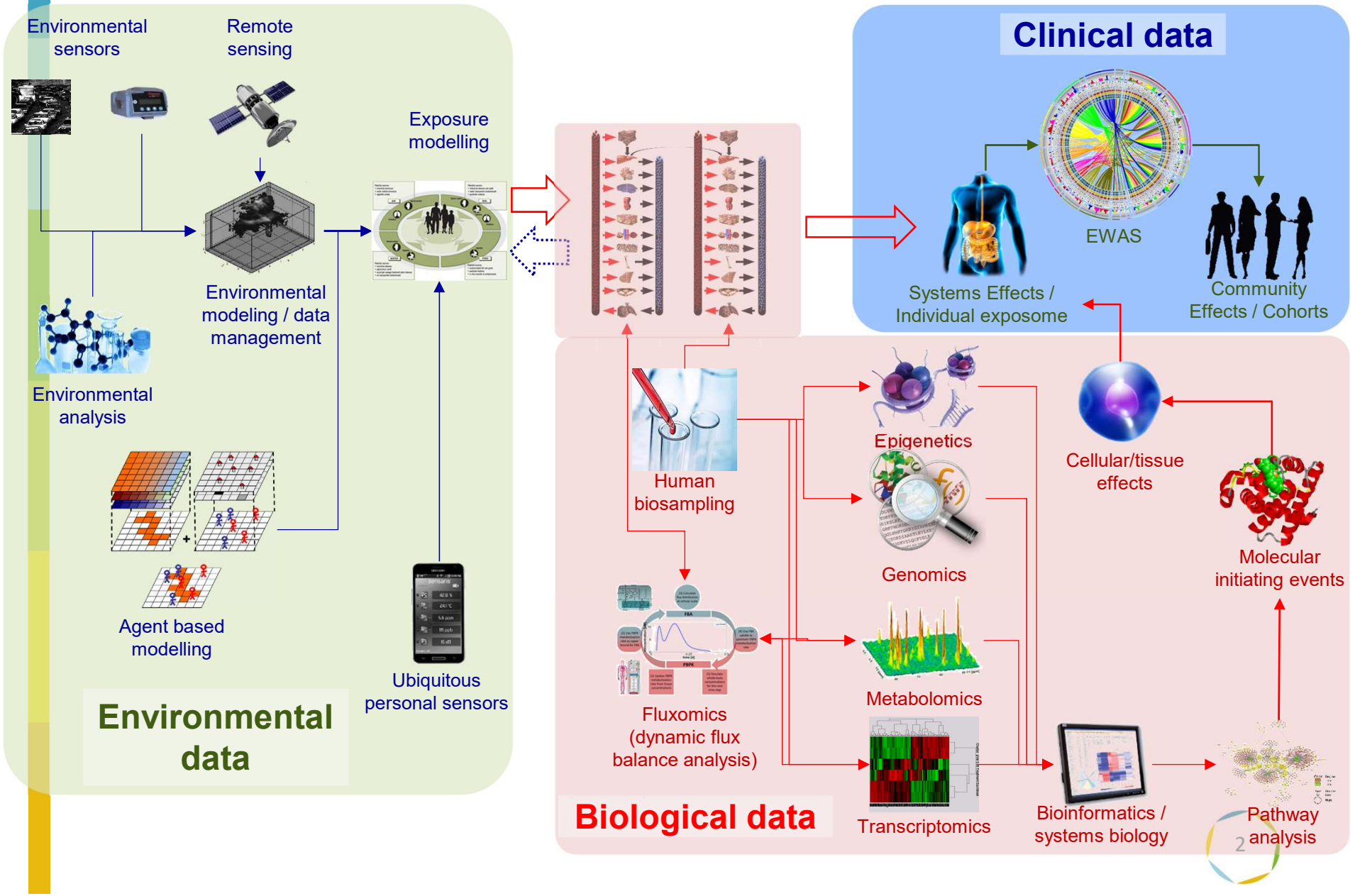
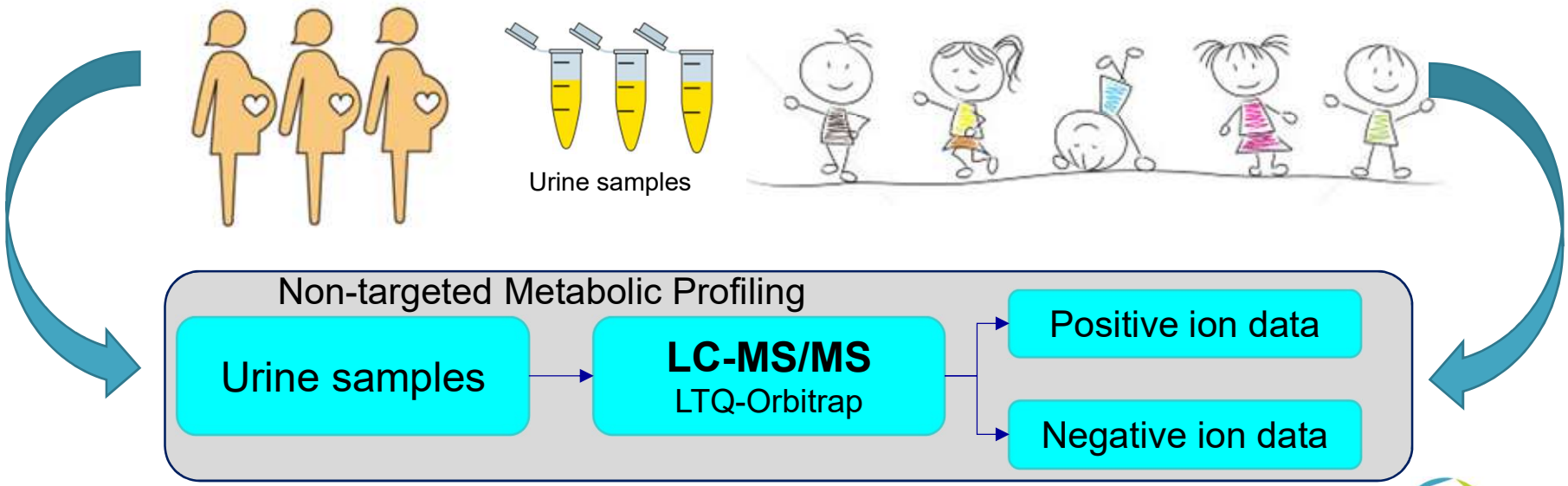
The connectivity approach

Embracing complexity to seek simple solutions to EH problems



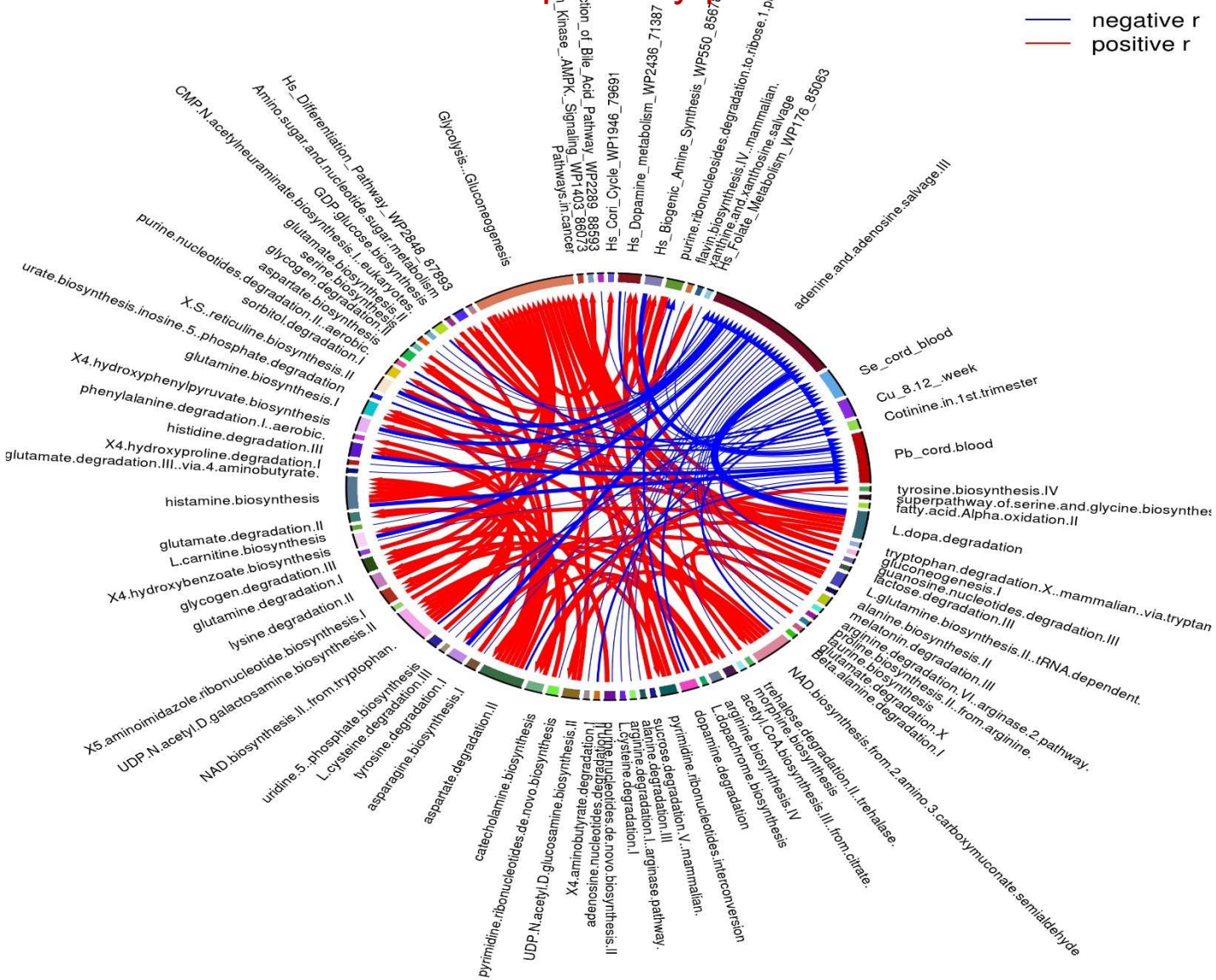
Case Study Repro_PL

- **Urine** and **cord blood** samples of pregnant women exposed to environmental contaminants (phthalates, Pb, Hg)
  - Urinary concentrations of phthalates
  - Cord blood Pb
  - Hair Hg
- EWAS analysis
- LC MS/MS (Thermo Orbitrap) for metabolites identification
- NMR (Agilent 600MHz)
- Agilent Genespring / Mass Profiler Pro for pathway identification



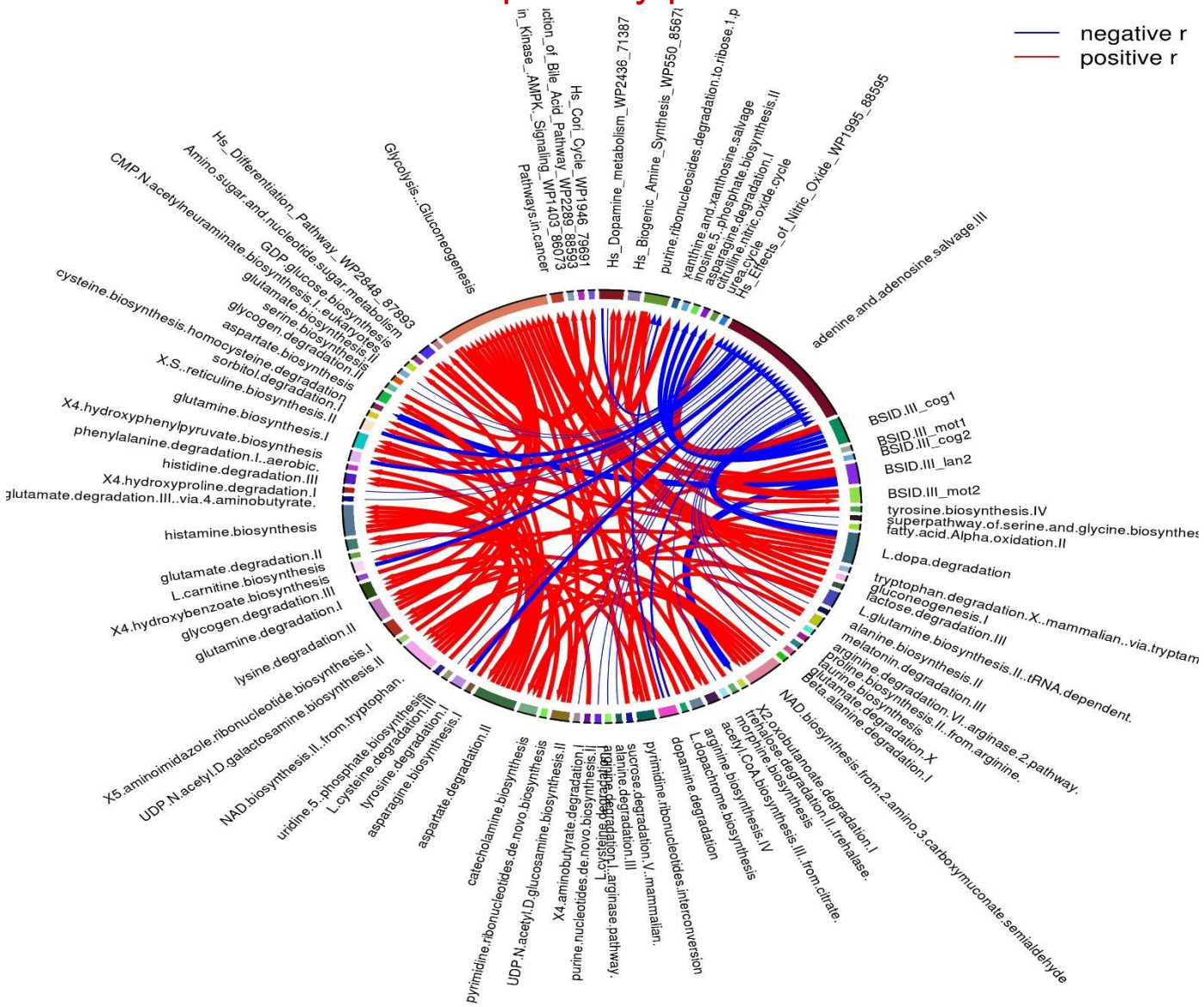
Correlation Globe

External exposure and metabolic pathway perturbation



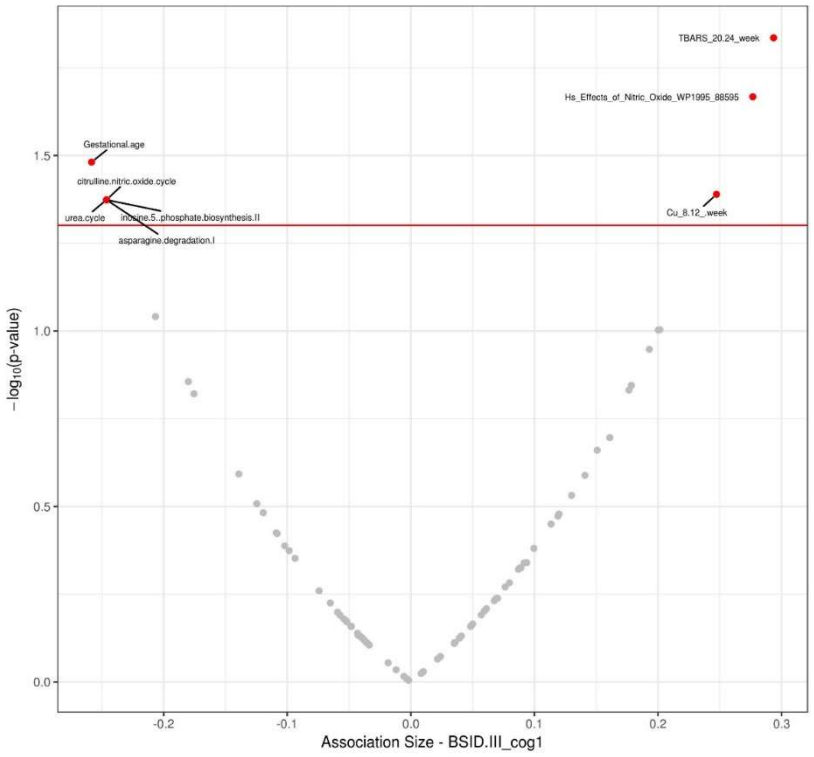
Correlation Globe

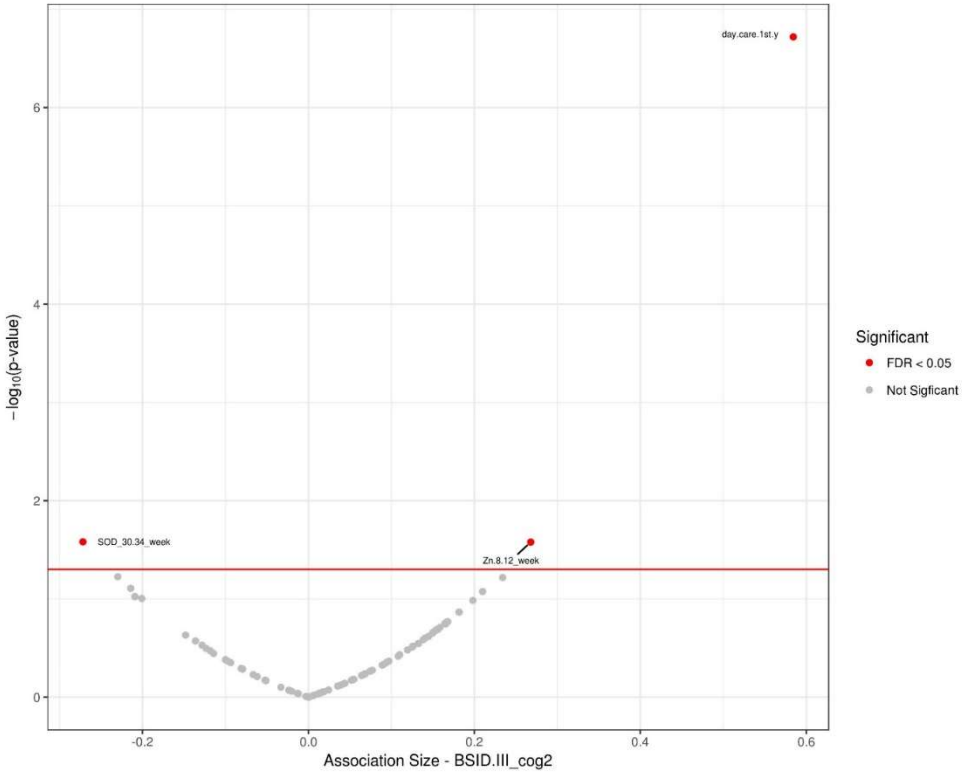
health outcomes
and metabolic pathway perturbations



Volcano Plot

Cognitive development





1st year

2nd year

Volcano Plot

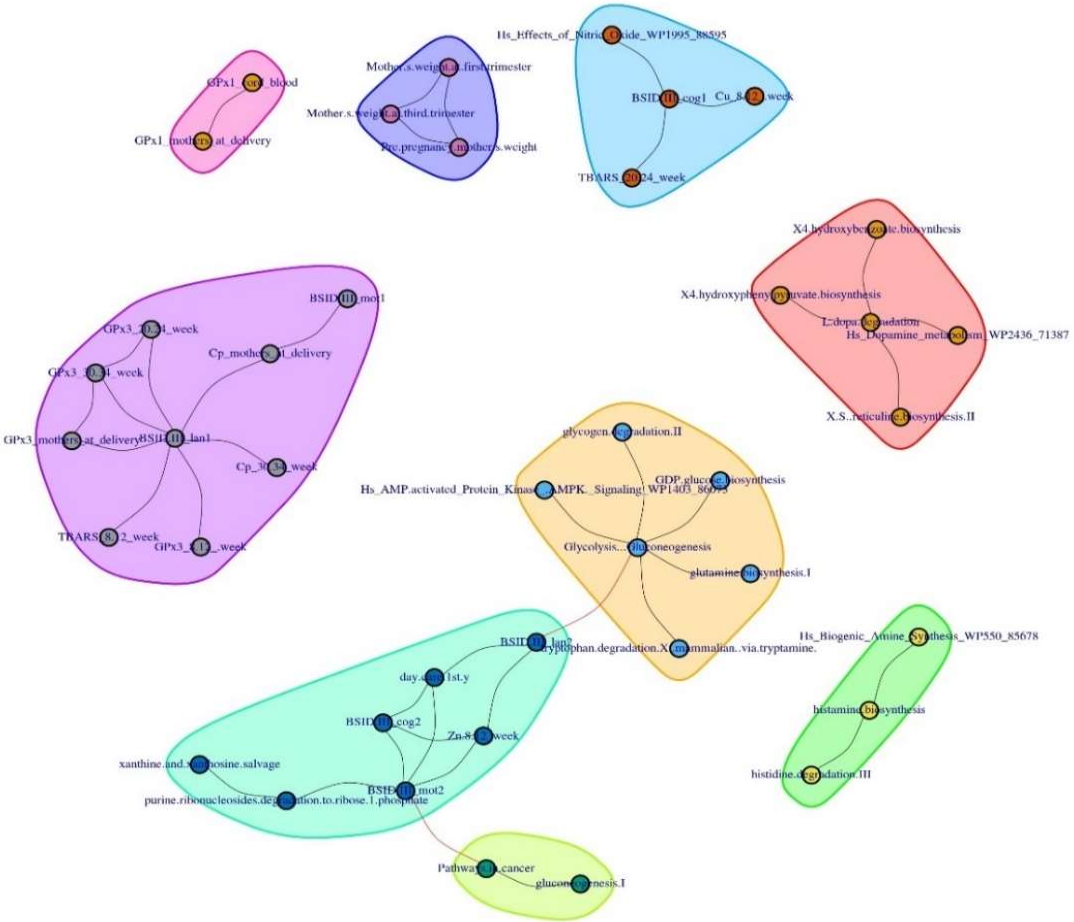
Cognitive development

	BSID.III_cog1
Effects of Nitric Oxide	+
Thiobarbituric acid reactive substances (TBARS_20.24_week)	+
Cu 8-12 week	+
Attendance to day care school during the 2st year after birth	+
Gestational age	-
Inosine 5 phosphate biosynthesis II	-
Asparagine degradation I	-
Citrulline nitric oxide cycle	-
Urea cycle	-

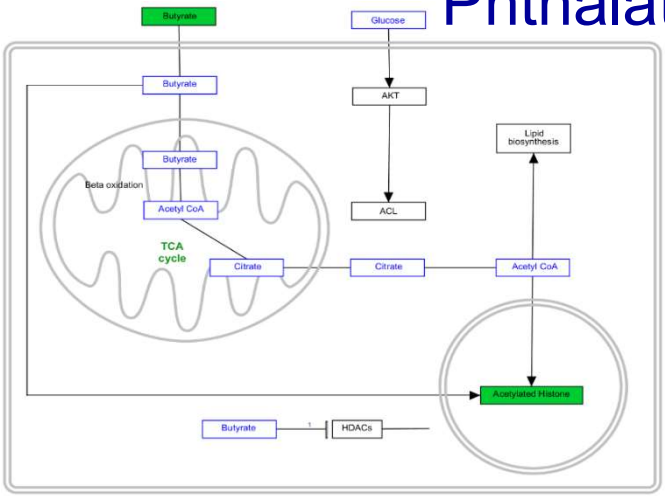
	BSID.III_cog2
Zn 8-12 week	+
Superoxide Dismutase-SOD 30-34 week	-

Repro_PL study

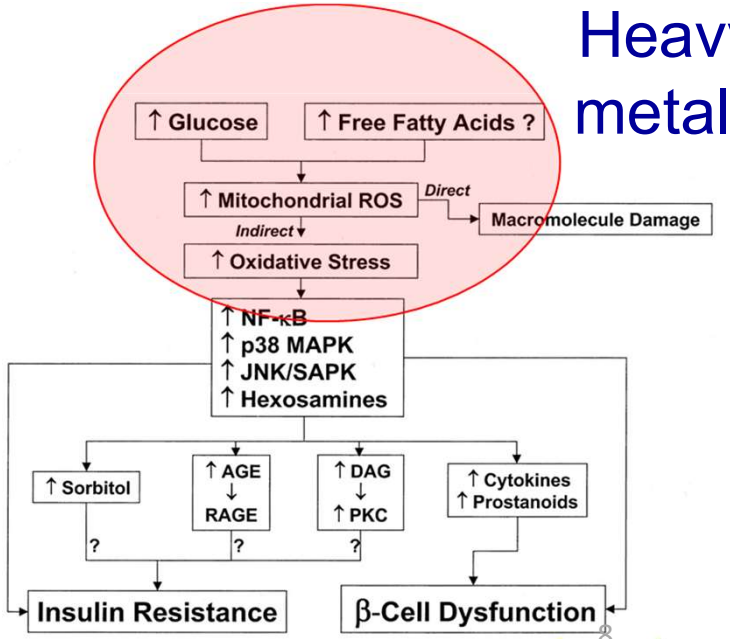
Toxicity Pathway identification



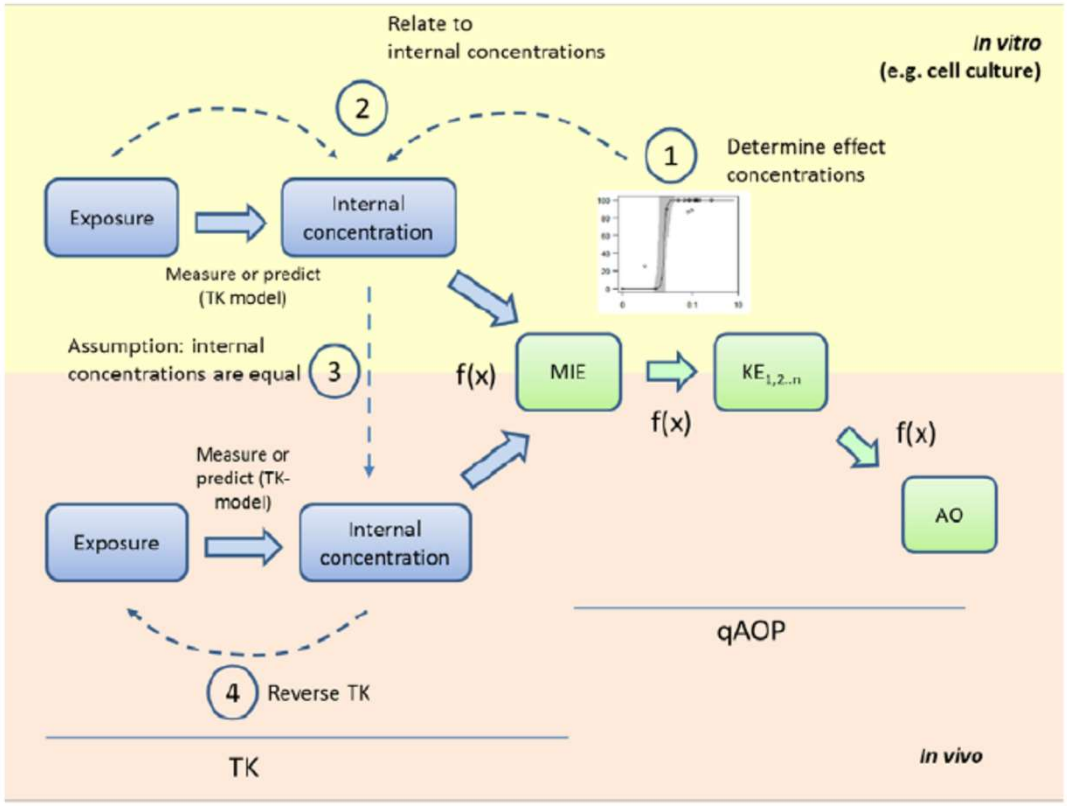
Phthalates



Heavy metals



Internal dose estimated from cohorts → use for in vitro studies



Inference of an external *in vivo* dosing from an *in vitro* effect concentration using reverse toxicokinetics (rTK) and quantitative Adverse Outcome Pathways.

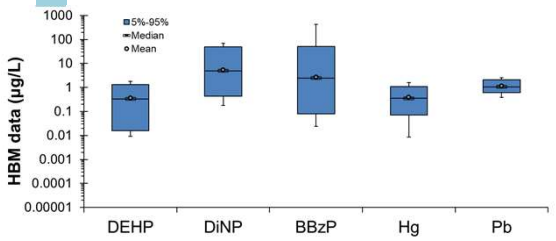
1.Concentrations are determined that perturb activities of an MIE or KE enough to cause significant changes in the final Adverse Outcome, using modeling or experiments.

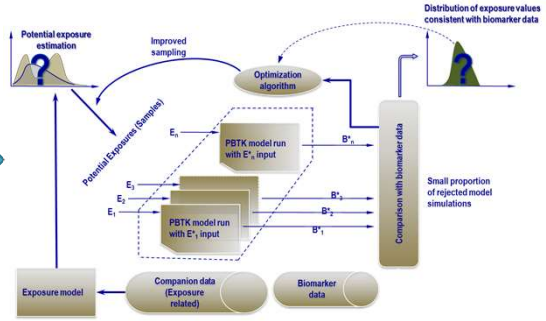
2-3. The concentrations causing effects *in vitro* at the MIE and the AO are assumed to be the same needed at the *in vivo* site of action.

4. The predicted *in vivo* concentration used in combination with reverse toxicokinetics describing metabolism, binding, and clearance functions to determine the external dose required to achieve the internal dose at the MIE.

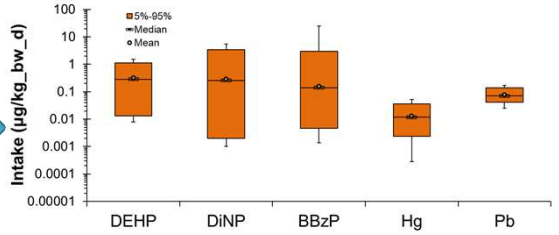
High-D analysis of in vitro data

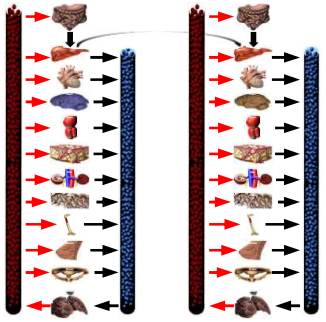
In silico, cross-omics and pathway analysis



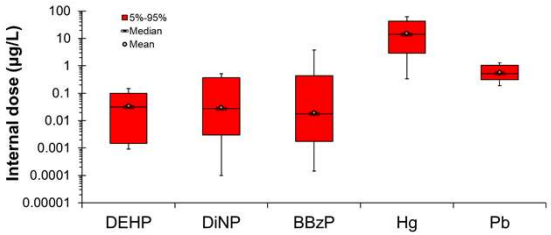


Exposure reconstruction



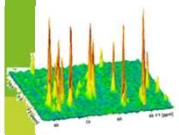


PBBK

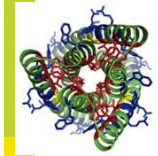


For the treatment of HepaRG, two different mixtures of phthalates and metals were used, M1 and M2. The concentrations of phthalates and metals were 10 times higher in M2

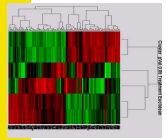
In vitro



Metabolomics



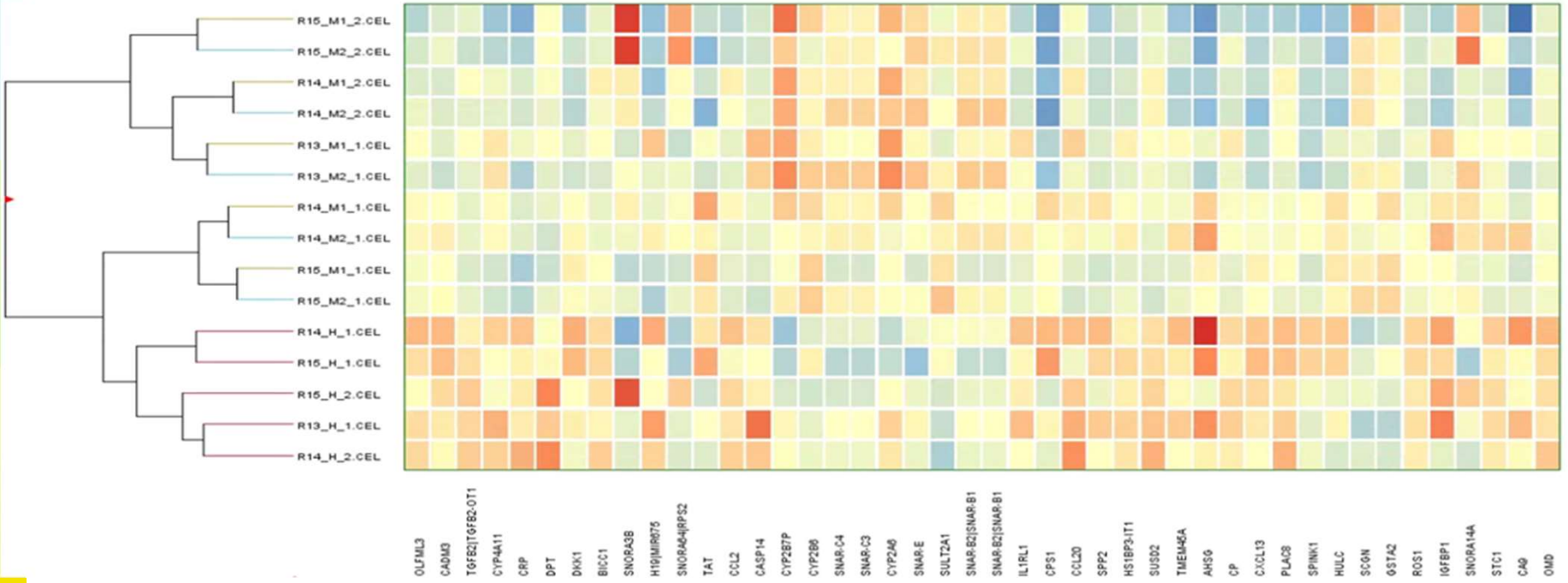
Proteomics



Transcriptomics

Results

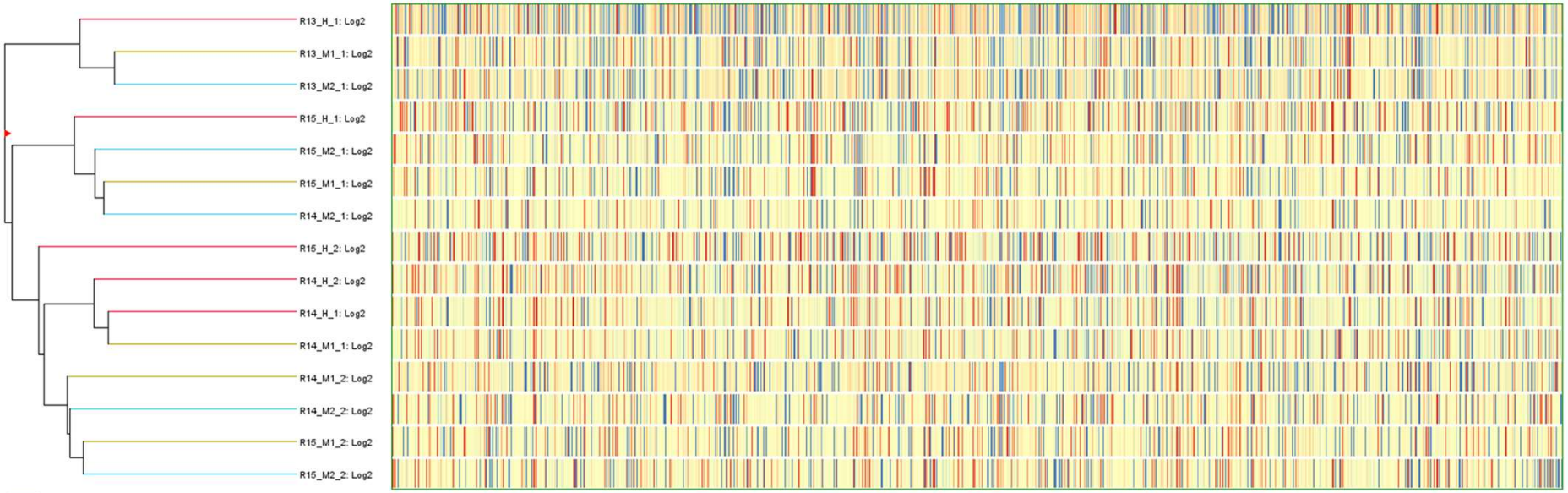
Hierarchical Clustering analysis – Transcriptomics



Euclidean distance was used to determine the distance between intermediate clusters while computing the similarity scores. The similarity between clusters for visualization was calculated base on Ward's method. **Dysregulation of H19, CYP2B6, and AHSG has been linked with neurodevelopmental disorders**

Results

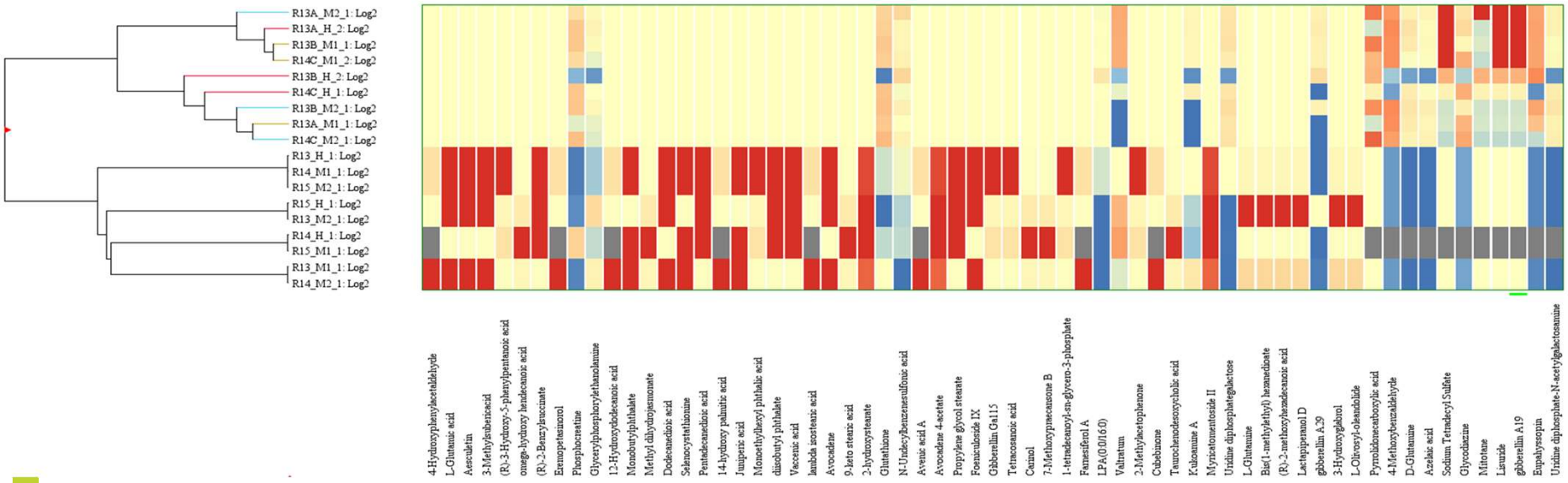
Hierarchical Clustering analysis - Proteomics



Most of these proteins were significantly dysregulated when treated with M2, but not with M1, although tendencies of change were similar in both treatments

Results

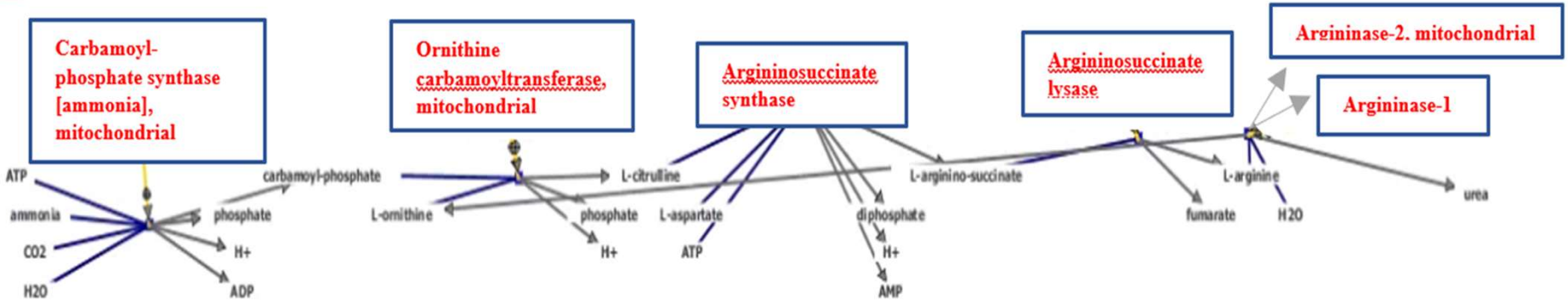
Hierarchical Clustering analysis - Metabolomics



37 and 35 metabolites were down-regulated in case of M1 and M2 respectively, while 37 and 39 metabolites were up-regulated in case of M1 and M2 respectively

Results

Pathway identification – urea cycle

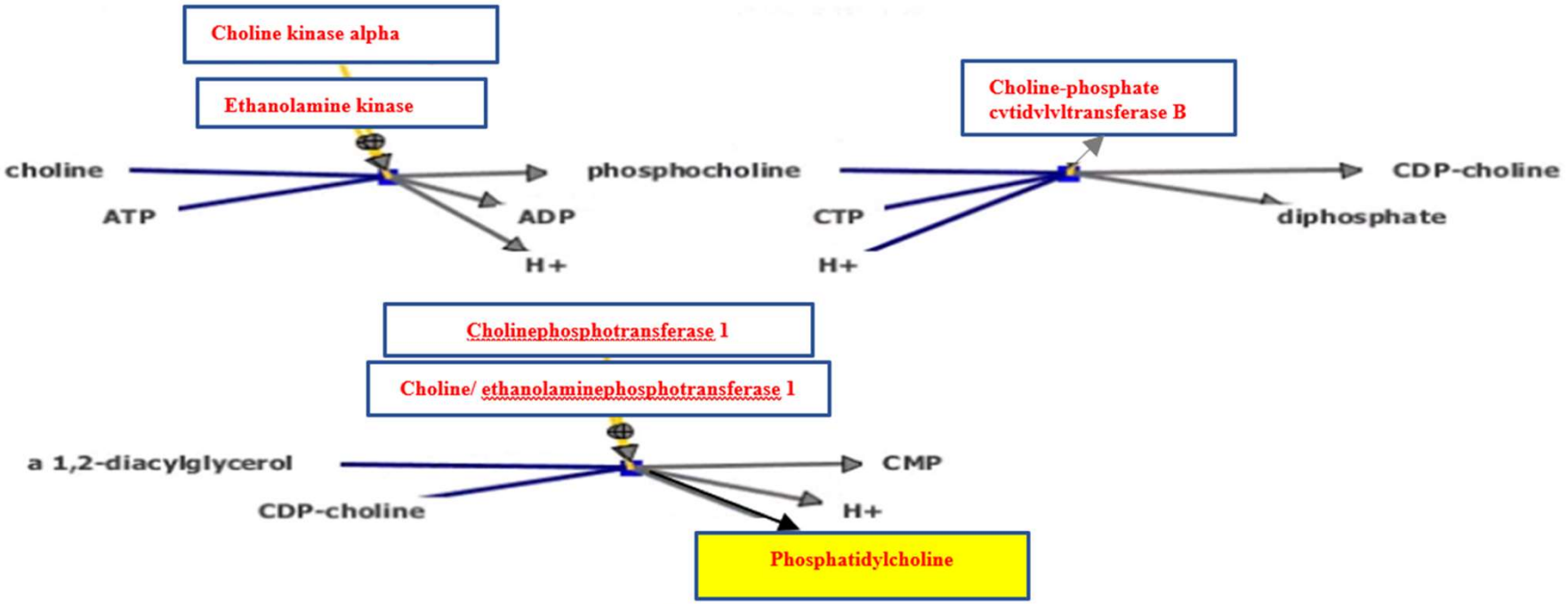


The urea cycle has been co-mapped in case of data integration using transcriptomics and proteomics, due to the statistically significant difference in the expression of arginase-1, arginase-2 (mitochondrial), arginosuccinate synthase, carbonyl-phosphate synthase (mitochondrial), ornithine carbamoyltransferase (mitochondrial) and arginosuccinate lyase.

The identification of the urea pathway is of particular interest since has been also identified in human samples in the **ReproPL cohort** using untargeted metabolomics NMR analysis on plasma samples

Results

Pathway identification – Choline metabolism

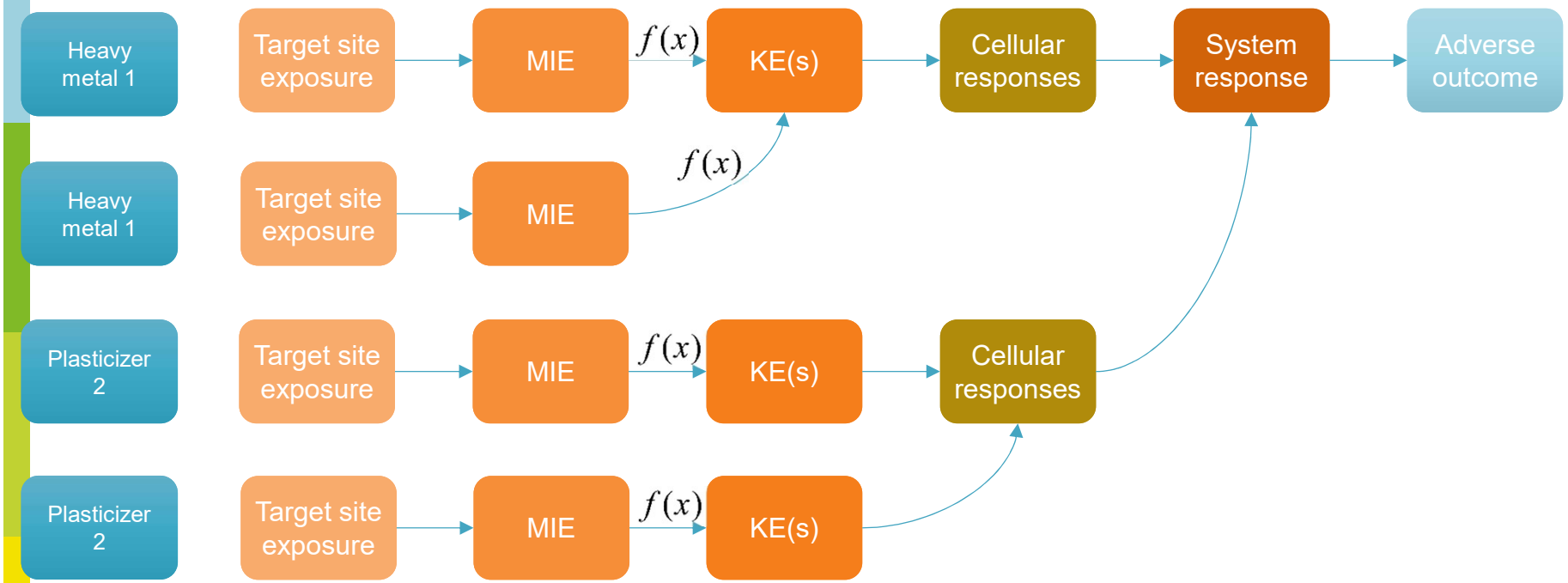


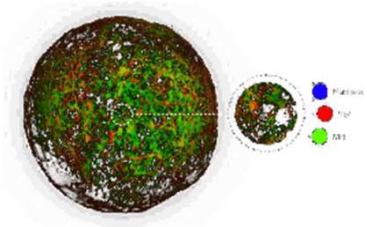
Co-mapping of proteomics and metabolomics data revealed that common drivers are responsible for the allostasis of metabolic pathways related to choline, phosphatidylcholine, phospholipases and triacylglycerol metabolism, due to alterations in the expression levels of phosphatidylcholine, and 1,2-diacyl-sn-glycerol-3-phosphate

Key findings

- Integrated pathway-level analysis of transcriptomics, proteomics and metabolomics data in vitro revealed that co-exposure to phthalates and heavy metals leads to the perturbation of the **urea cycle**, while their common drivers are also responsible for the allostasis of metabolic pathways related to **choline**, **phosphatidylcholine**, **phospholipases** and **triacylglycerol** metabolism.
- The identification of the pathways above is of particular interest since these pathways have been also identified in human samples from the REPRO PL cohort and have been associated with **impaired psychomotor development** in children at the age of three to six.
- Co-exposure to plasticizers and metals disturb biochemical processes related to **mitochondrial respiration** during critical developmental stages that are clinically linked to neurodevelopmental perturbations.
- Pathway analysis supports precise prevention and targeted intervention strategies based on the metabolic profile (e.g. administration of L-carnitine) and exposure related parameters (e.g. change on diet to reduce heavy metals exposure)

Biology based dose-time-response model





In vitro data

Cohort data

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