UPPSALA UNIVERSITET

Environmental Metabolomics; use of non-targeted metabolomics profiling to study possible metabolic effects of HCB and *p,p*'-DDE in a population-based sample

Samira Salihovic, Andrea Ganna, Tove Fall, Stefan Gustafsson, Corey Broeckling, Jessica Prenni, Bert van Bavel, Monica Lind, Erik Ingelsson, Lars Lind.

Department of Medical Sciences, Uppsala University, Sweden.

Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.

Proteomics and Metabolomics Facility, Colorado State University, USA.

MTM Research Centre, Örebro University, Örebro, Sweden.

Occupational and Environmental Medicine, Uppsala University Hospital, Sweden.

2016-07-12

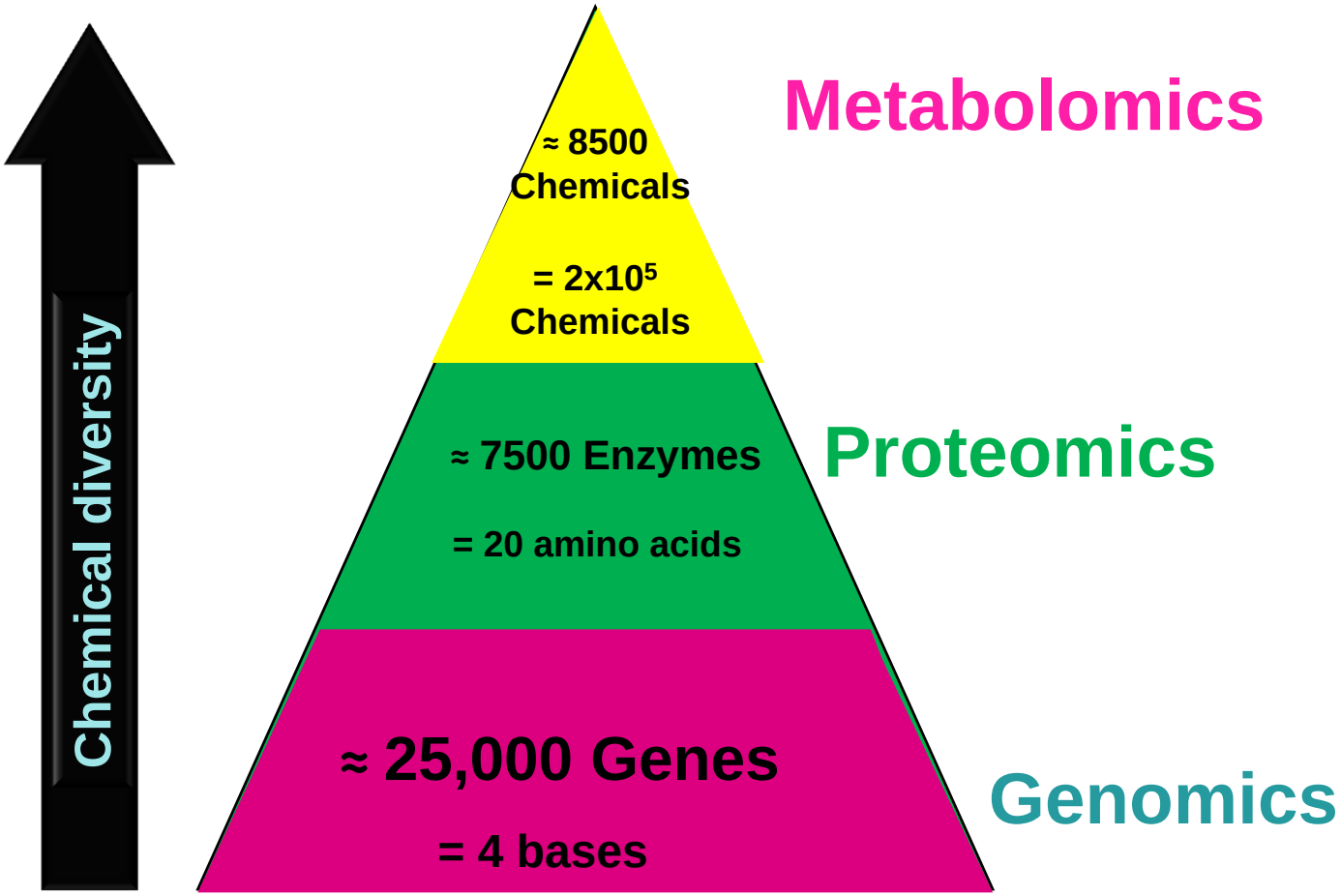
UPPSALA UNIVERSITET

Introduction and definition

- Metabolomics
  - → "Newly emerging" field of 'omics' research
  - → Comprehensive and simultaneous systematic determination of metabolite levels in the metabolome (circulation, urine, cells so forth)
- Metabolome
  - → Refers to the complete set of small-molecule metabolites
  - → Dynamic
- Metabolites
  - → Intermediates and products of metabolism
    - Primary and secondary metabolites
    - Examples include antibiotics, pigments, carbohydrates, fatty acids and amino acids
  - **Organic molecule detectable in the body with a MW < 1, 500 Da**

UPPSALA UNIVERSITET

The pyramid of life



Adopted from Wishart et al. 2012

UPPSALA
UNIVERSITET

Metabolomics in literature

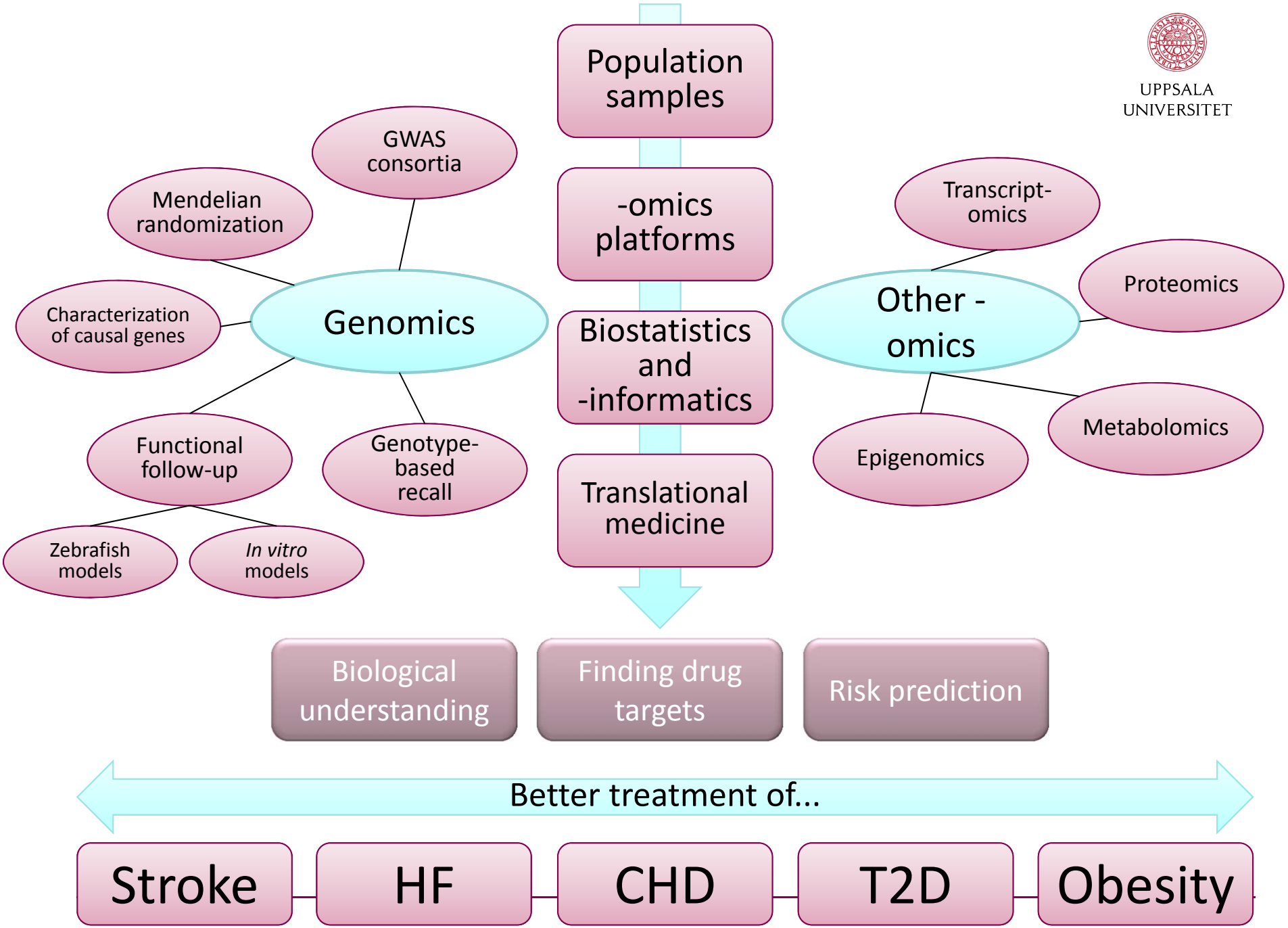
- Metabolomics has most commonly been used to study the association between metabolites and diseases; this is normally done by following healthy people over time and observing the diseases incidence

- Non-targeted metabolomic profiling in the context of large-scale epidemiological studies is mostly performed using state-of-the-art high-resolution mass spectrometry technologies

- Metabolomics is increasingly used in the field of toxicology, with the aim of characterizing the global and dynamic response in living systems after exposure to endocrine disrupting chemicals

UPPSALA
UNIVERSITET

Omics and metabolomics in Mol Epi group



Courtesy of Prof. Ingelsson

Study populations

UPPSALA UNIVERSITET

TwinGene - Serum – DONE!
Case-cohort design
3 replicates *
N subjects=2,397 2,014
N subjects subcohort = 1,519
N features=11,056 or 27,741
Size=1.9 TB

PIVUS - Serum – DONE!
All samples
2 replicates
N subjects=971 970
N features=8,185 or 24,015
Size=523 GB

ULSAM – EDTA-plasma – DONE!
3 replicates *
N subjects=1,150
N samples=2,436 **
Size=~1.2 TB

* we will use only injection 2 and 3
** more samples because glucose tolerance test @ 0,30,120 min

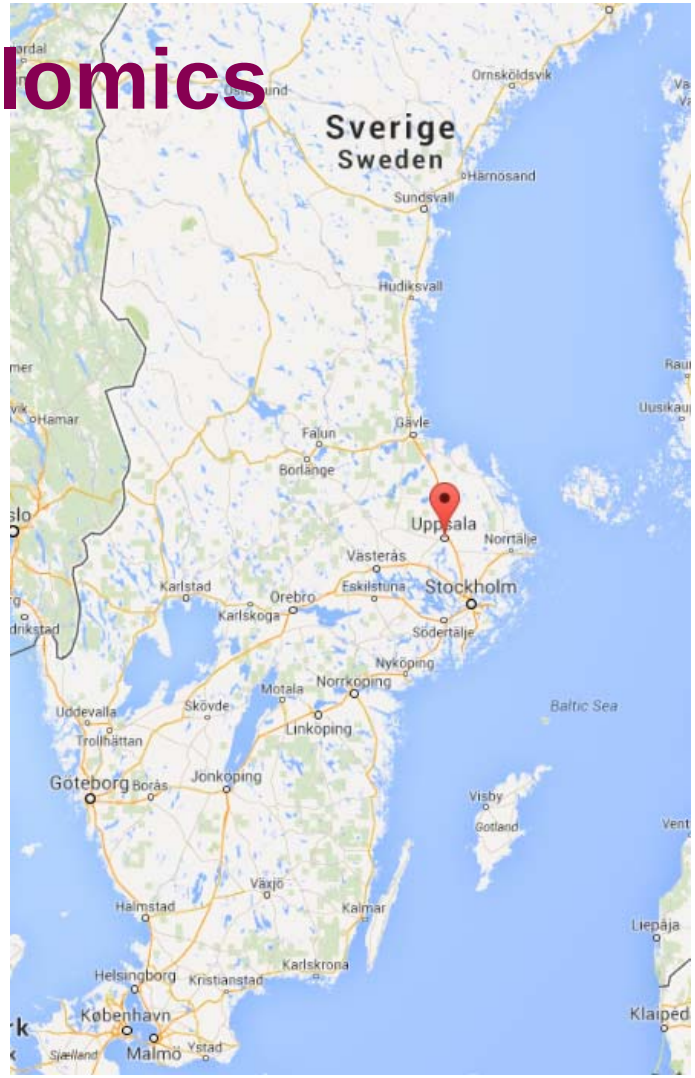
ULSAM – urines – Almost there!
All samples
3 replicates
N subjects=635
N features=18,450
Size=317 GB

Studies are separately processed

UPPSALA
UNIVERSITET

Study population for Environmental metabolomics

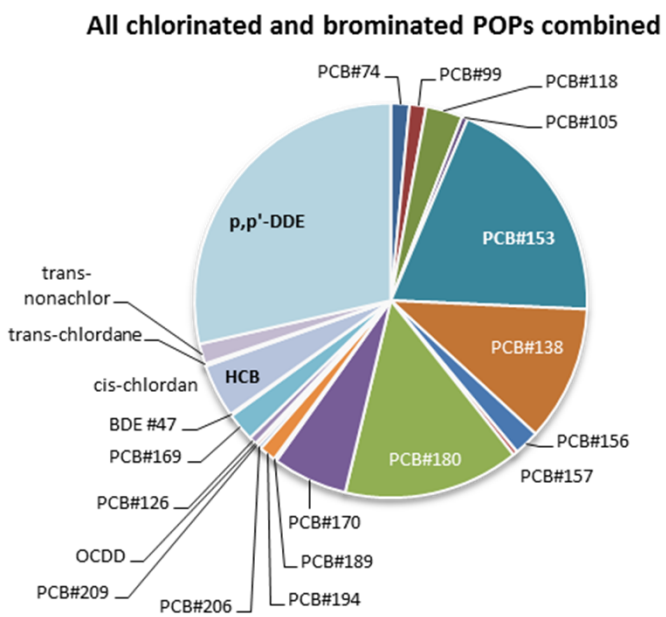
- Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS)
- Inclusion between 2001–2004
- N subjects= 1, 016 (50% women)
- Serum

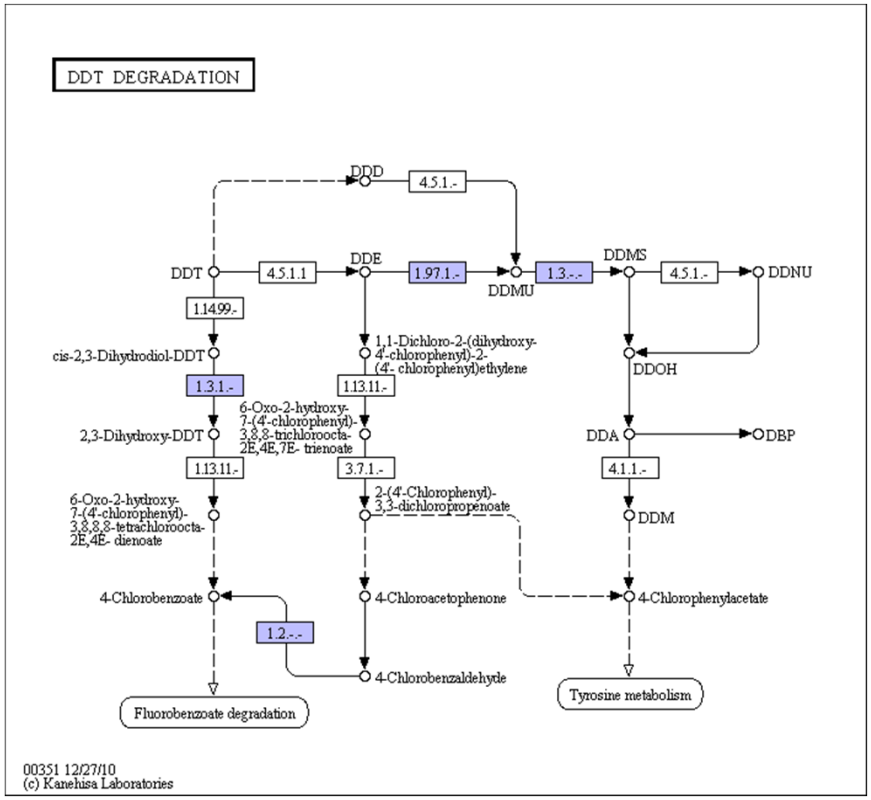


UPPSALA UNIVERSITET

HCB and p,p'-DDE (DDT)

- General population is primarily exposed via diet
- Highest exposure among POPs
- Long biologic half-life
- DDT degradation in humans





Salihovic et al 2012 Env. Int.

UPPSALA UNIVERSITET

How can HCB and p,p-DDE affect human health?

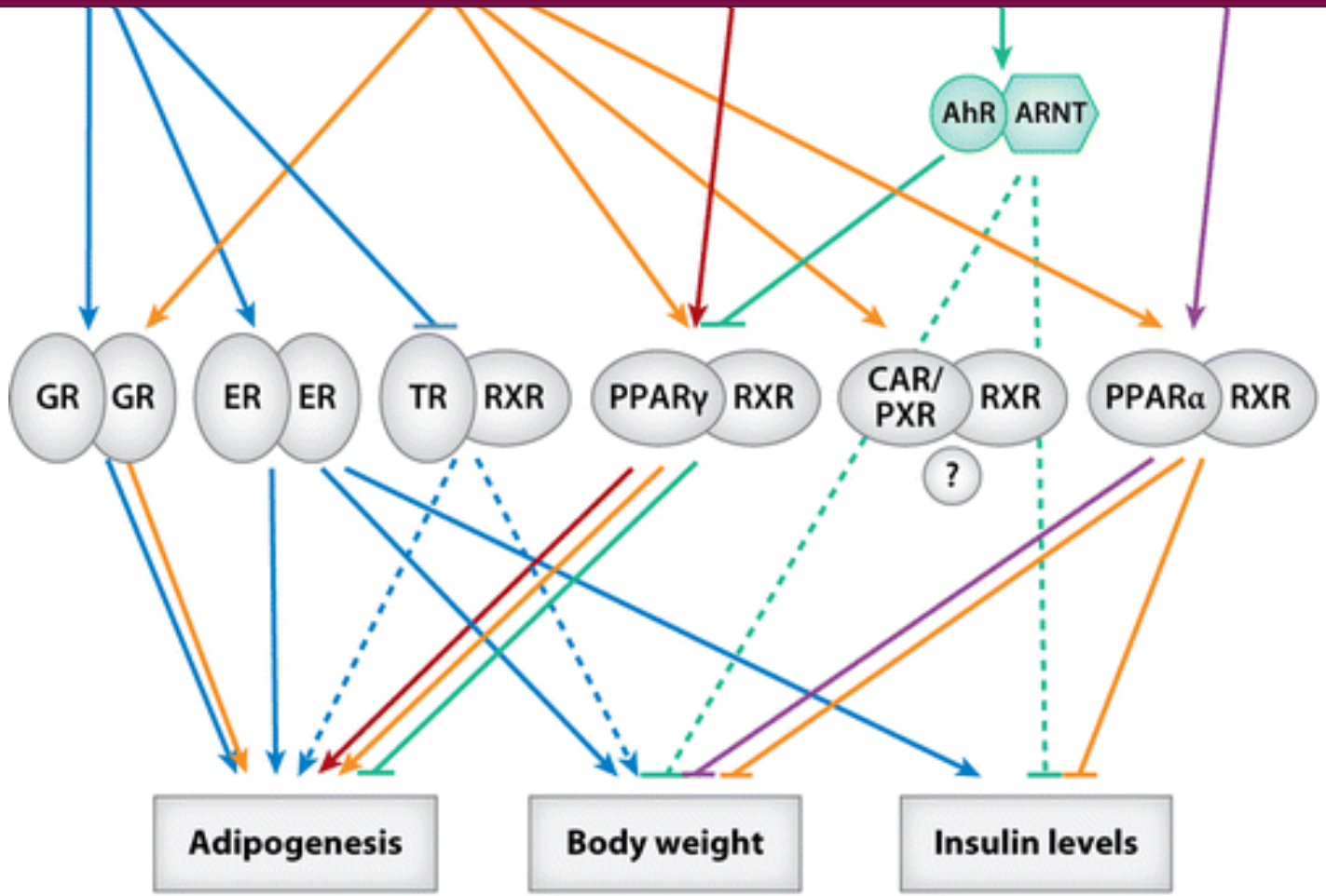
- Both have been found related to neurological, hepatic, reproductive and development effects and are considered to be possibly carcinogenic to humans (WHO IARC, CDC, U.S. EPA)

- Mechanisms of action in humans still largely unknown

Biological dynamics and cell signaling

UPPSALA UNIVERSITET

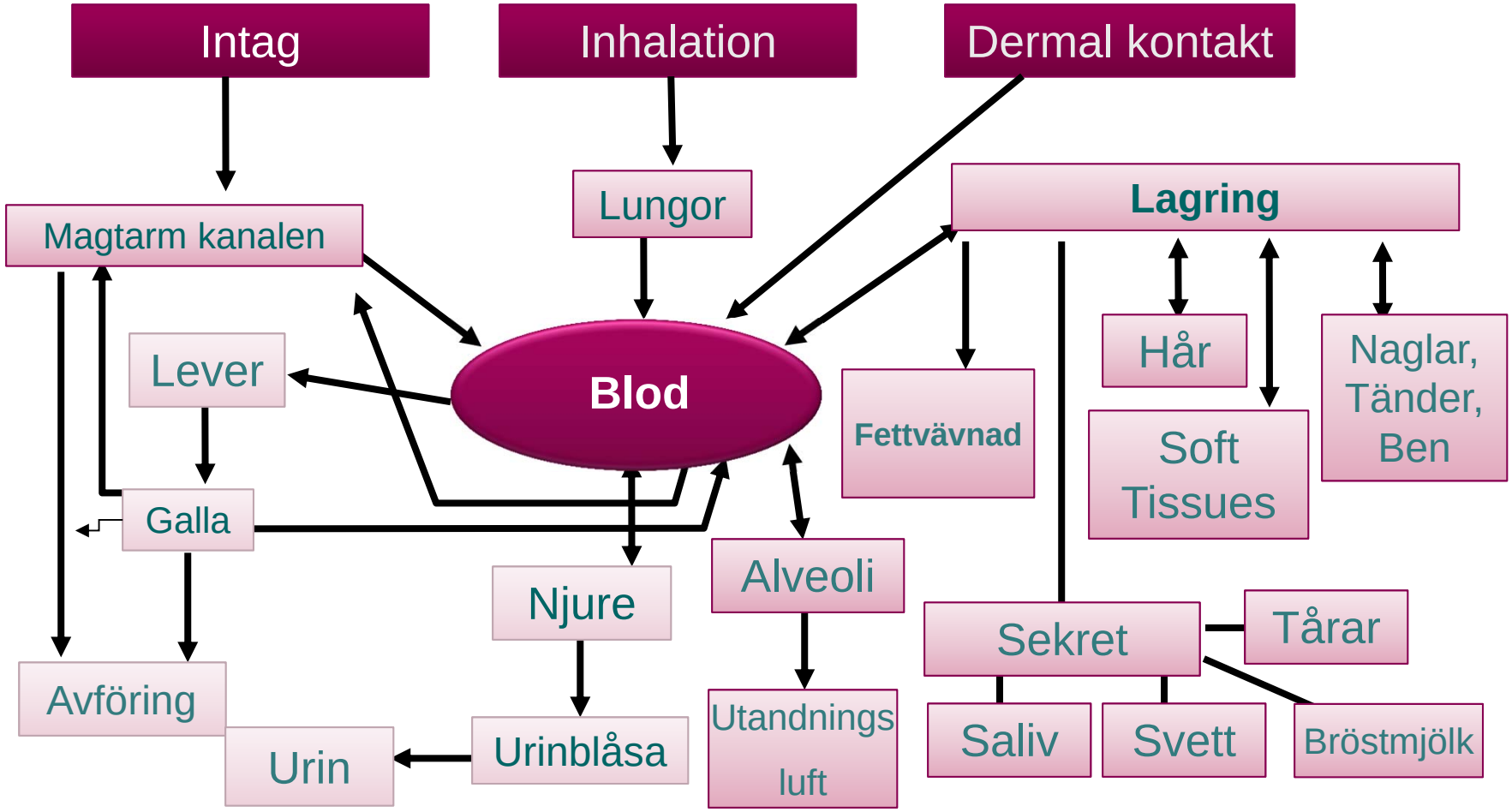
17beta- estradiol, cortisol, retinoic acid, fatty acids, pharmaceuticals,…so many metabolites



Adopted and modified from Casals-Casas et al. 2011

UPPSALA UNIVERSITET

Human exposure, distribution och elimination

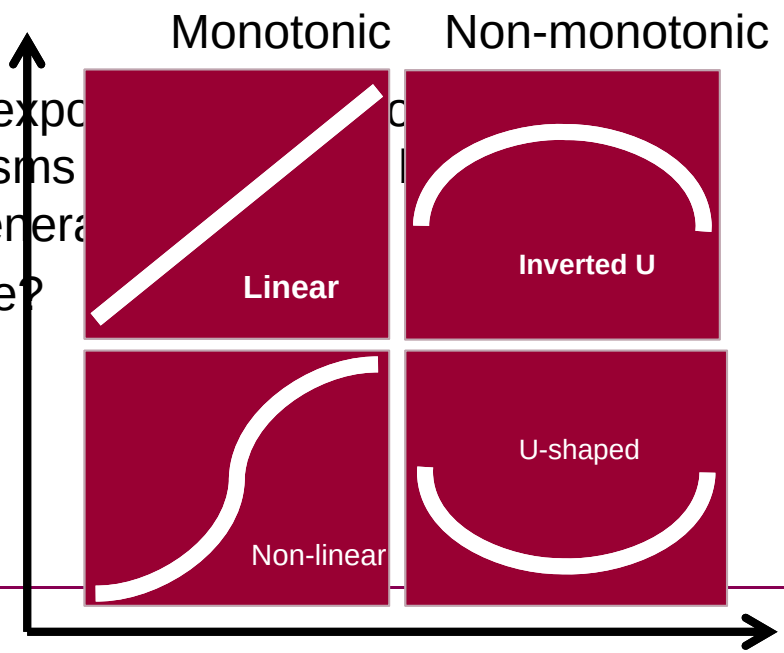


Courtesy of M. Petreas.

UPPSALA UNIVERSITET

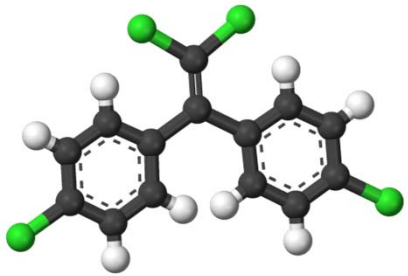
Non-targeted metabolomic profiling offers new possibilities

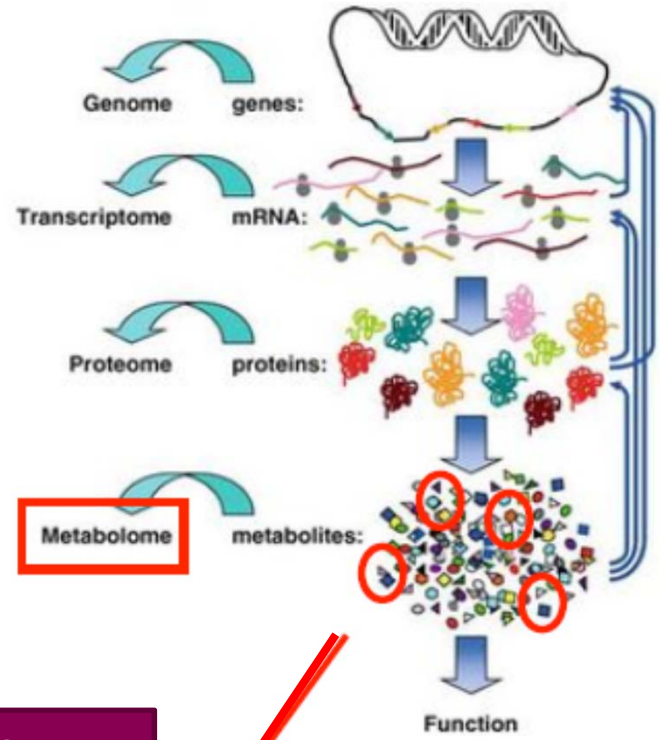
- Explore subtle metabolic changes in humans exposed to endocrine disrupting chemicals
  - → Animals models (cases vs controls)
  - → DDE exposure in humans (occupational or accidental, low vs high)
  - → How can we try to connect POP expo c
    to learn more about the mechanisms l
    and DDE induce toxicity in the genera
  - → Metabolic biomarkers of exposure?



UPPSALA UNIVERSITET

Metabolomic profiling





Does low-dose p,p-DDE exposure induce negative metabolomic effects?

Are the effects measureable in serum from the general population?

Adopted and modified from Zalko et al. 2013

UPPSALA UNIVERSITET

Objective

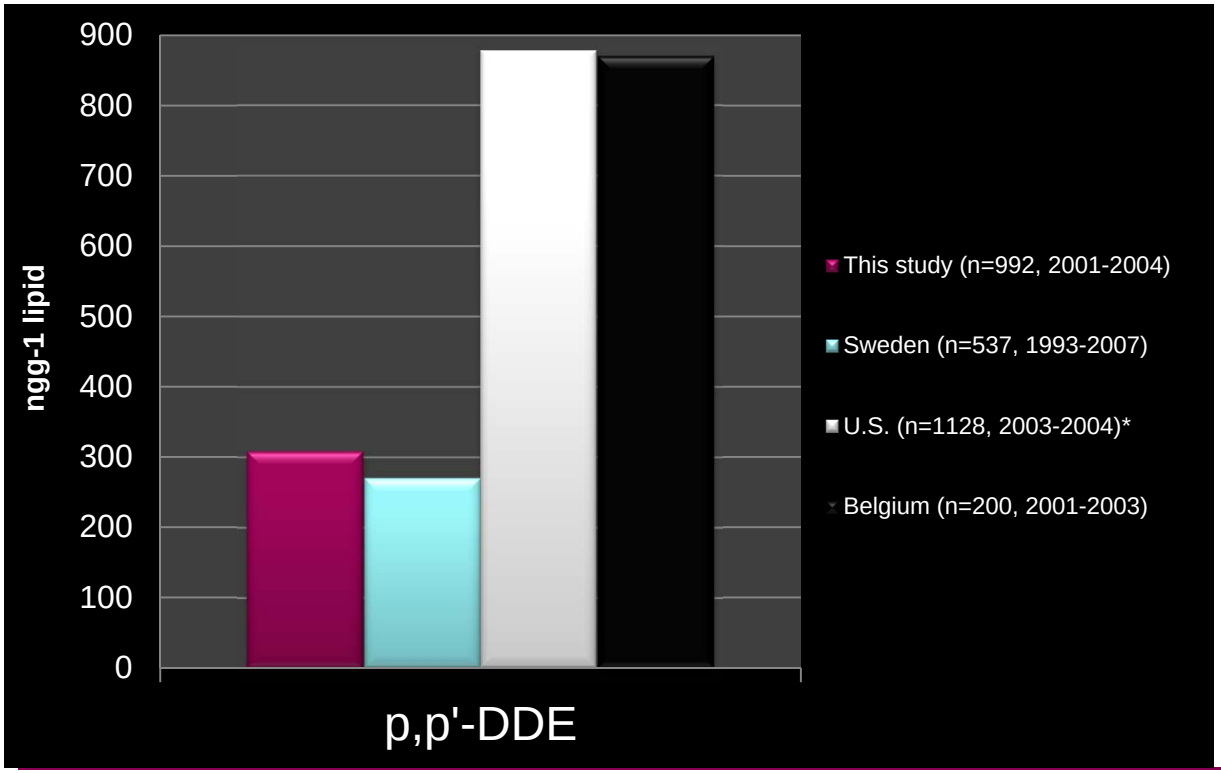
- Study the association between circulating levels of HCB and DDE and metabolites in the general population by using a non-targeted metabolomics approach

- Explore subtle metabolic changes and try to connect HCB and DDE exposure to metabolomic profiles to learn more about the mechanisms and pathways by which DDE induces its toxicity

UPPSALA UNIVERSITET

Targeted analysis of chlorinated and brominated POPs

0.5 mL plasma sample + SPE (Oasis HLB) + Multilayer column → GC-HRMS



- **Marker OCPs**
  - **HCB**
  - Cis-chlordane
  - Trans-chlordane
  - Trans-nonachlordane
  - ***p,p'*-DDE**
- **Marker PBDE**
  - BDE #47

Salihovic et al 2012 Chemosphere

UPPSALA UNIVERSITET

Non-targeted metabolomics analysis - full scan 50-1200 m/z

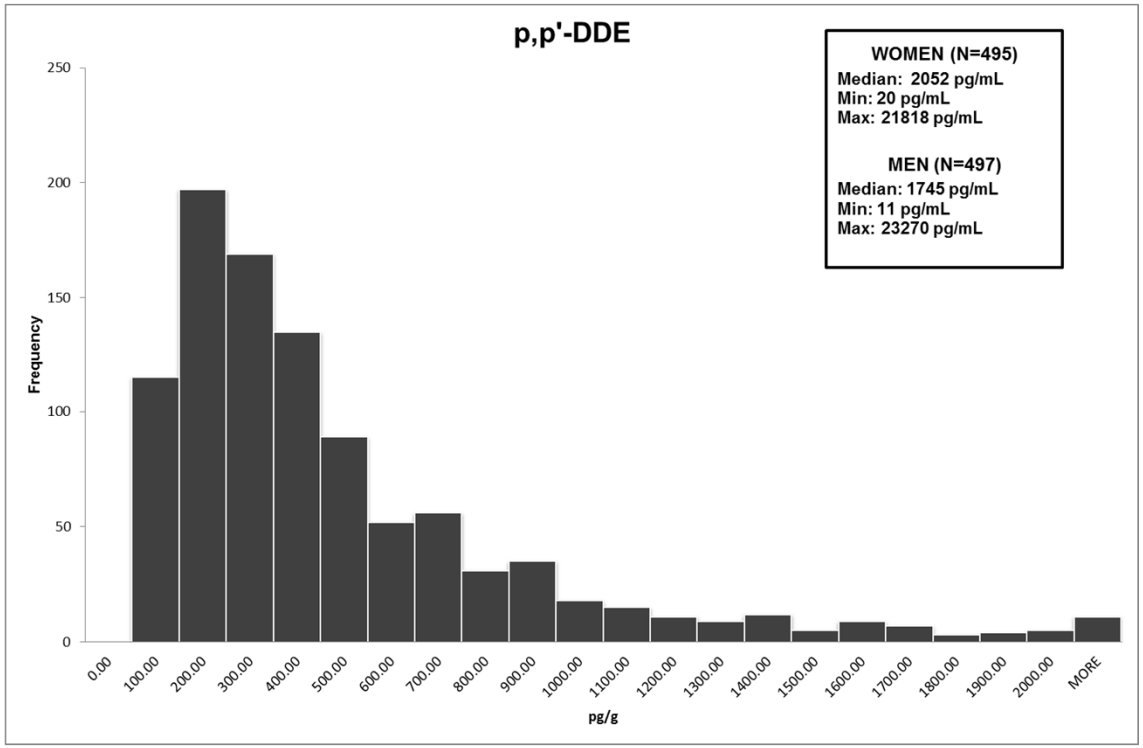
100uL serum + MeOH (protein precipitation)→UPLC-QTOF/MS

- All samples were run in duplicates according to Brockeling et al. (2011)
- Data processing was performed according to the work-flow described by Ganna et al. (2013)

UPPSALA
UNIVERSITET

HCB and p,p'-DDE detection

- Both detected in all 992 PIVUS subjects
- Broad distribution
- Long-term bioaccumulation and toxicological potency



Salihovic et al 2012 Env. Int.

Annotation workflow from raw data to metabolites

UPPSALA UNIVERSITET

Raw Data

MODULE 1
(feature detection)

- Peaks detection
- Peaks alignment
- Peaks grouping
- Filling missing features

MODULE 2
(quality control)

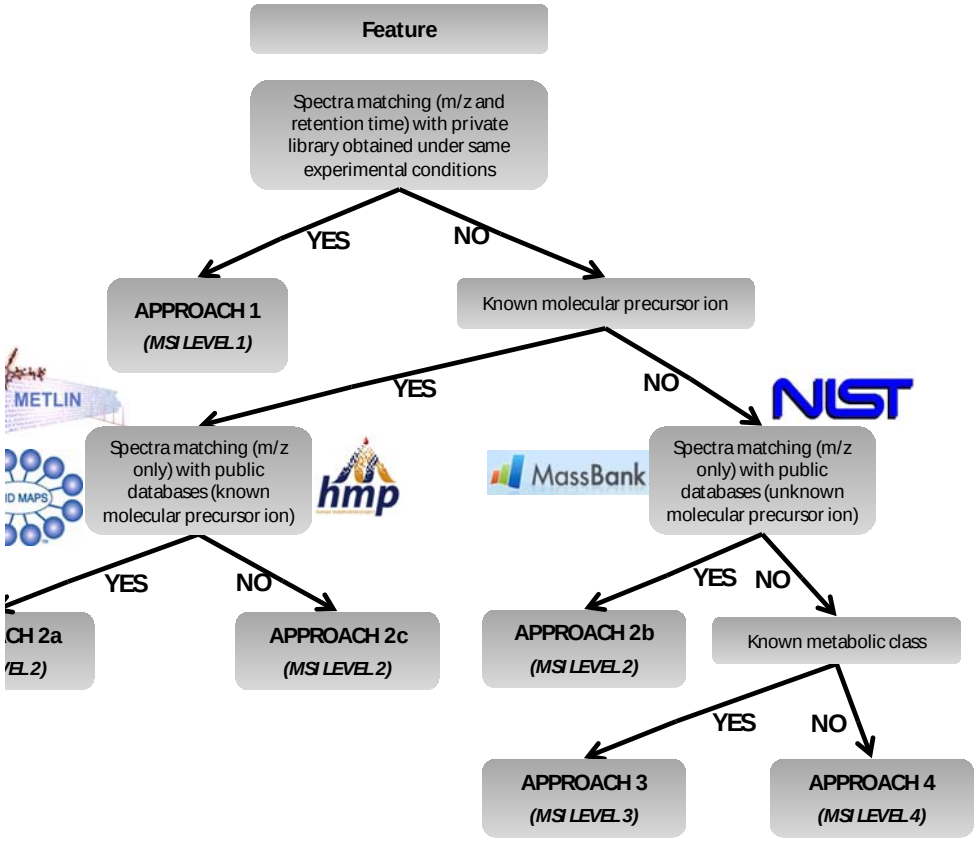
- Log-2 transformation
- Outliers exclusion
- Normalization
- Average between replicates

MODULE 3
(analysis)

- Univariate data analysis (UMWAS)
- Replication

MODULE 4
(annotation)

- Spectra generation
- Annotation



Ganna et al 2015 Metabolomics