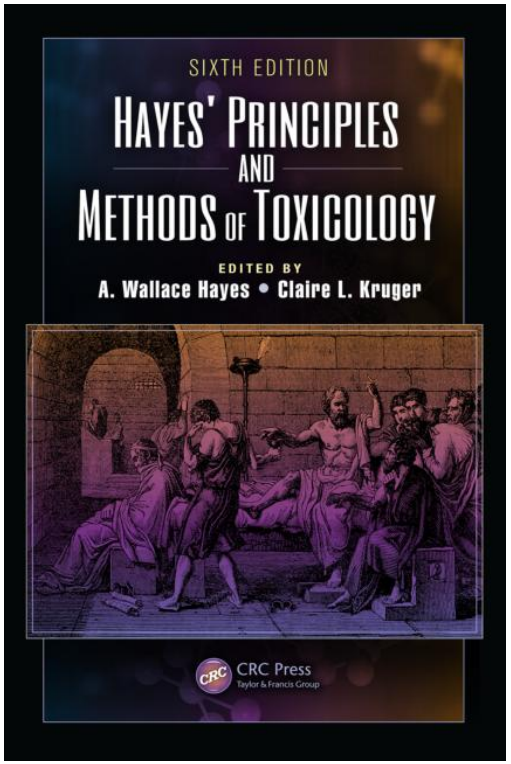
Toxicopanomics: Applications in Predictive Mechanistic Toxicology

Agenda

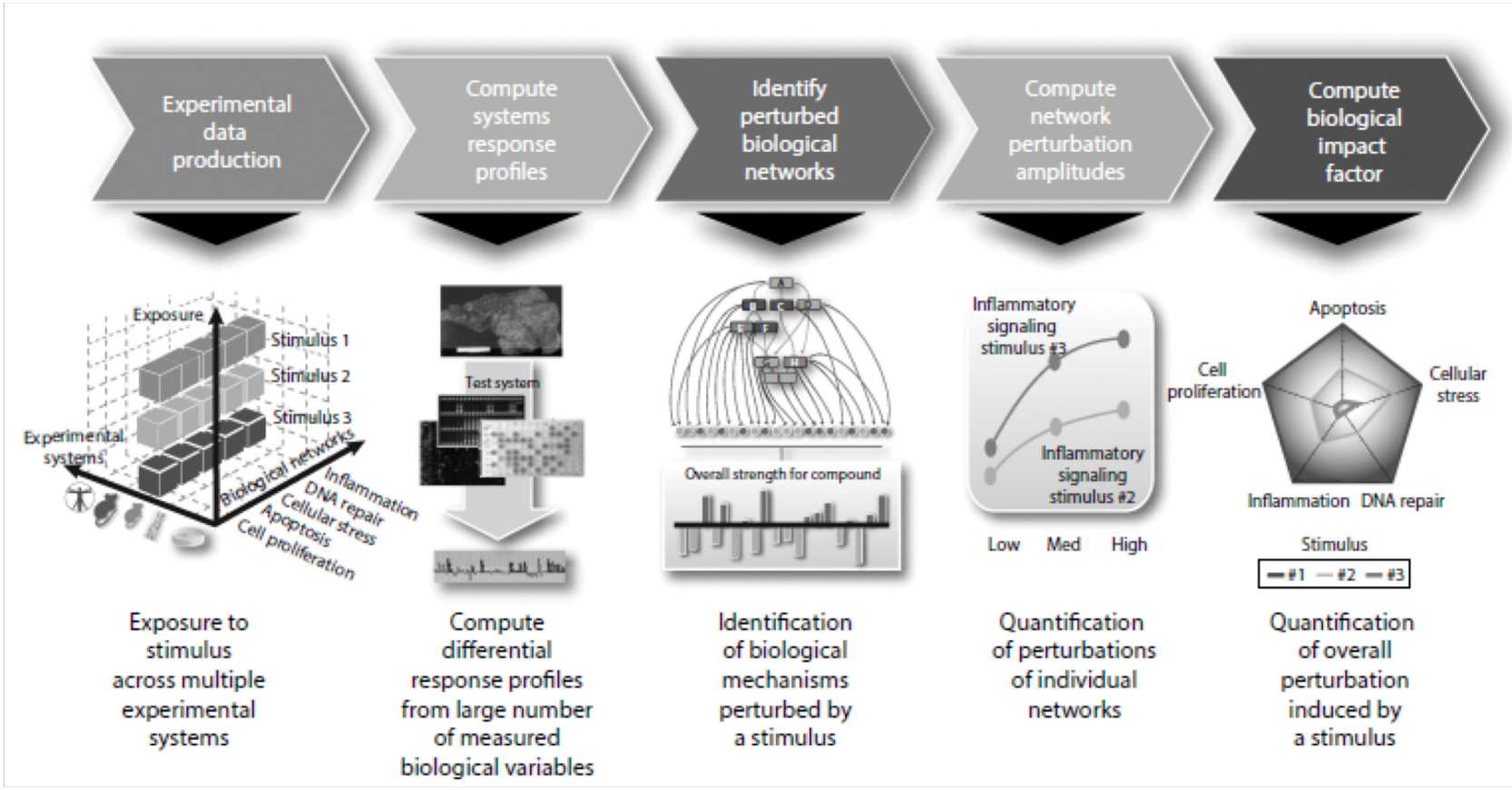
- Definition – What is Toxicopanomics?
- The Network-Based Approach
- Experimental Design
- Important Techniques
- Example Data
- Discussion

What is Toxicopanomics?

- Paradigmen shift in toxicology of the 21th century
- Identify Pathways of Toxicity (PoT)
- Focus towards predictive toxicology
- Evolved by National Research Council (NRC)
- Reduce or replace animal models
- Europe: SEURAT-1 Initiative, OECD, EPAA



The Network-Based Approach



Mode of Action (MOA) - Network Perturbation Amplitudes (NPA) - Biological Impact Factor (BIF)

How to design a systems biology experiment?

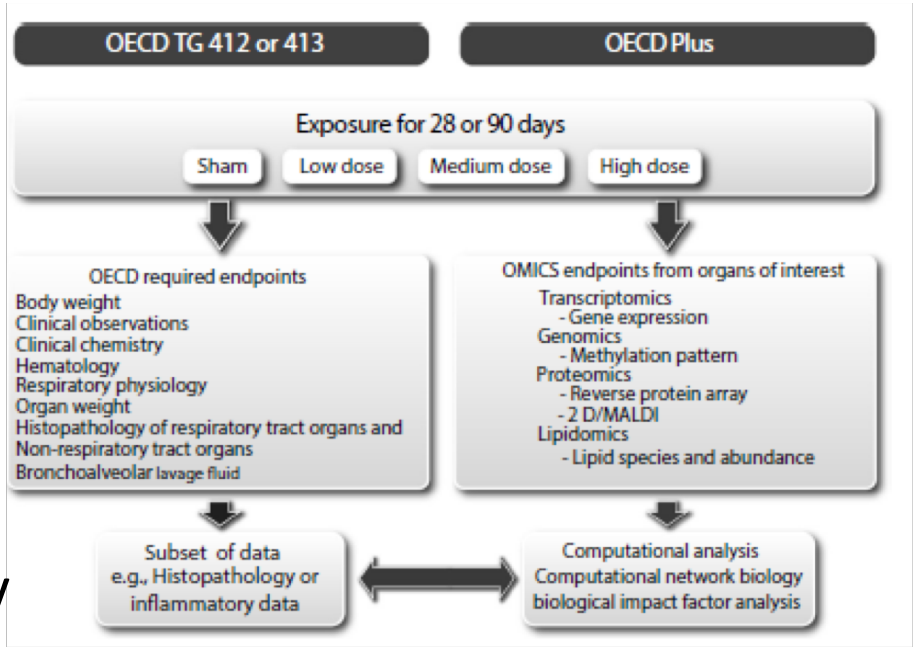
- Preparation:

  - Formulate scientific question
  - Select most adequate method & exposure
  - Statistically empower the data

- Determine reactivity, capture MOA, identifiy/rank stimuli associated with MOA, threshold/dose-response
- Handle variation, eliminate systemic/tech bias, sufficient number of biological replicas

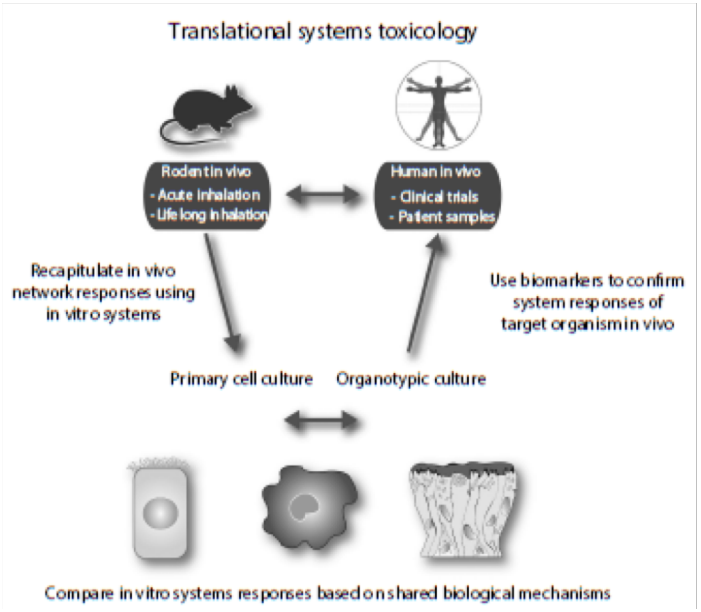
In Vivo Experiments – The OECD Guidelines

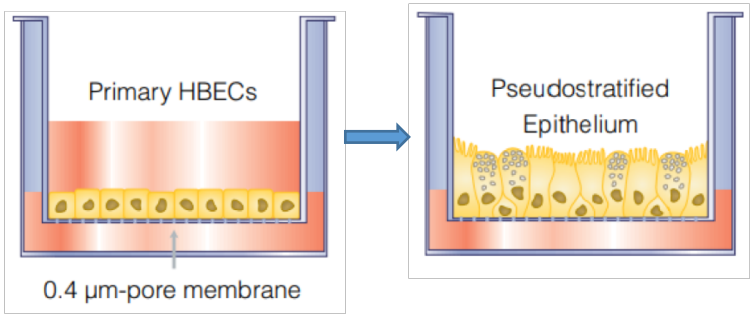
- Collection >100 testing methods in inhalation toxicology
  → TG 412/413
- At least three concentrations, control/vehicle control
- In-life observations, clinical pathology, histopathology
- Include satellite groups, BAL, neurologic/clinic (histo)pathology
- Additional animals for OECD plus



In Vitro Experiments

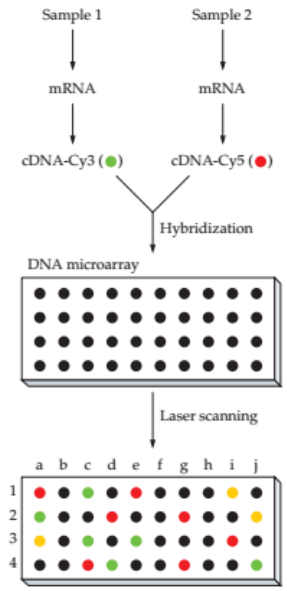
- In Vivo: Expensive, time-consuming, ethically controverse, poor predictability
- Primary cells: Preserved cell-type-specific functions, response in different donors
- 3D cell culture: HBECs
- Cells polarized & differentiated, develop tight junctions, comparable gene expression
- Next step: Perfused systems, microorganoids

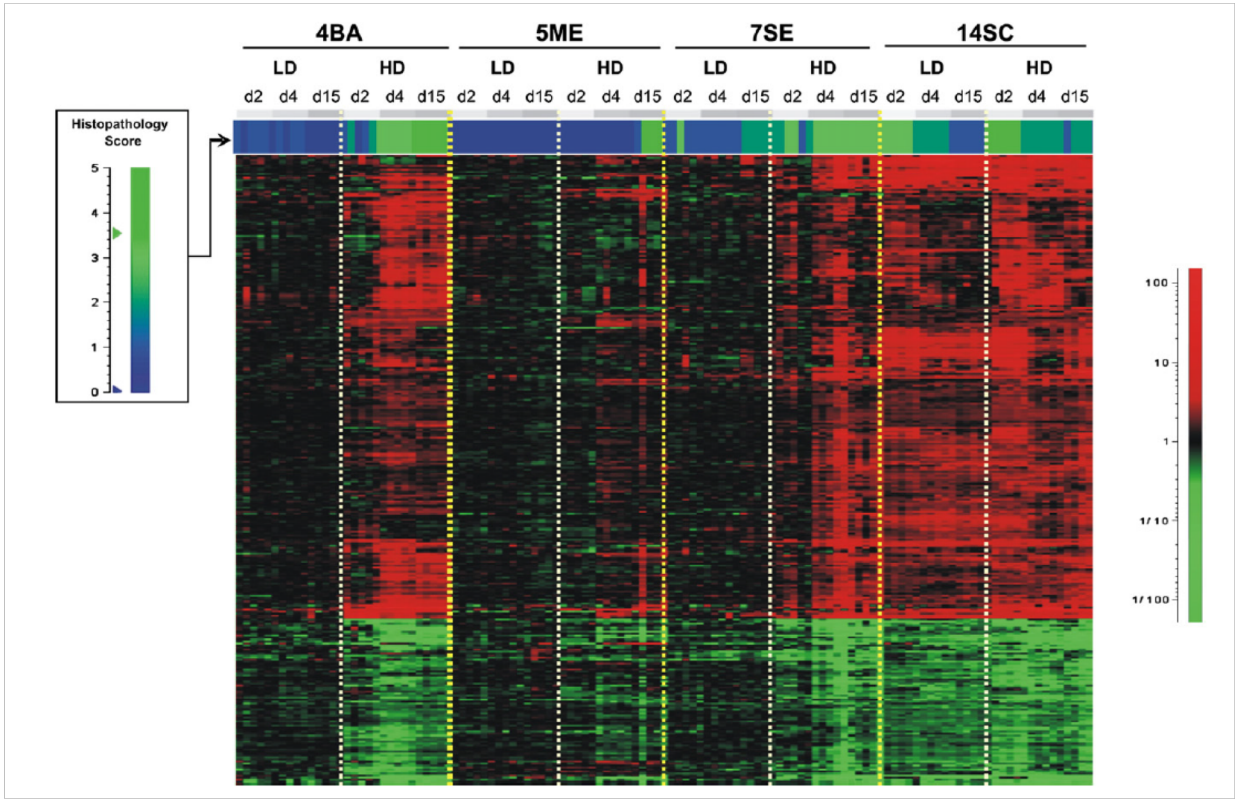




Genomics – Gene Expression Profiling

- cDNA Microarray: RNA isolated, labeled (Cy3, Cy5), hybridized on the same array





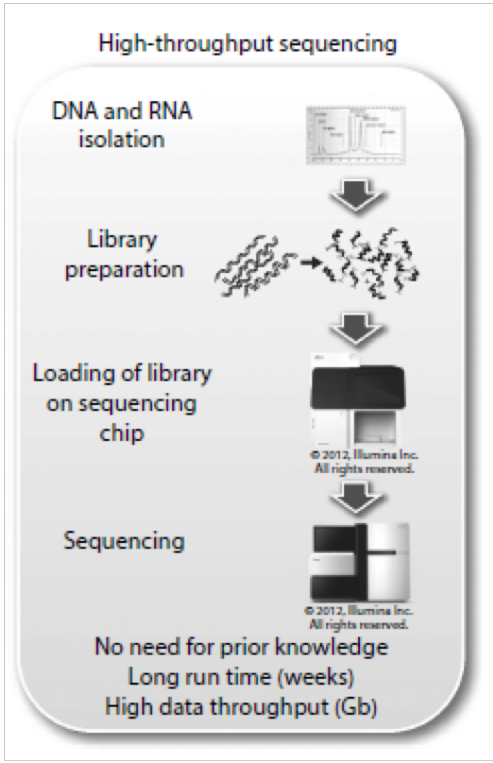
Genomics – New Methods

- Next Generation Sequencing (NGS):

  - Advantage in accuracy, throughput, flexibility
  - No prior knowledge, no designed chips
  - CNV, SNPs, deletions with DNA-seq
  - Whole-genome DNA methylation (MethylC-seq)

- Histone Modification with ChIP:

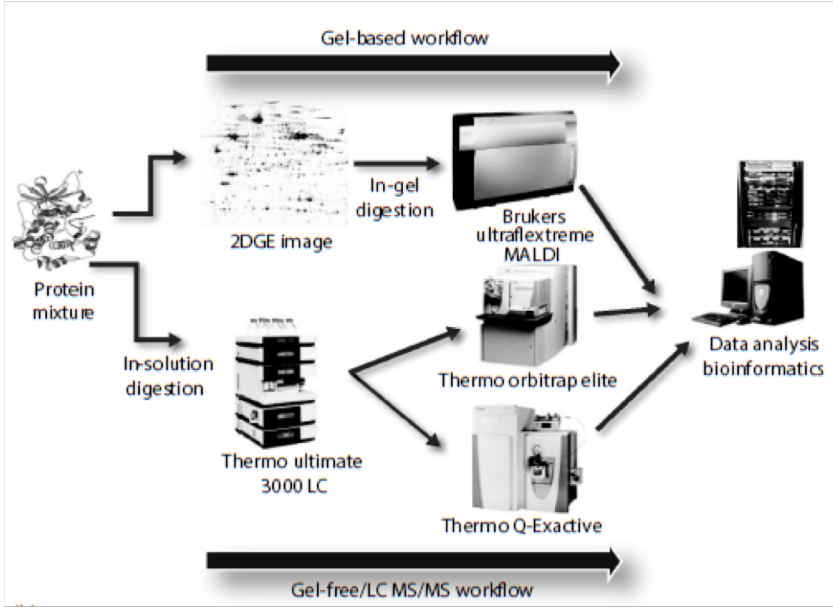
  - Antibodies specific for histone modification
  - Precipitated chromatine hybridized with either ChIP-chip or ChIP-seq



Proteomics – Biomarker Discovery

- Gel-based (DIGE):

  - Difference in gel electrophoresis
  - Label with cyanine dyes (Cy2,3,5)
  - Protein abundance, reproducibility



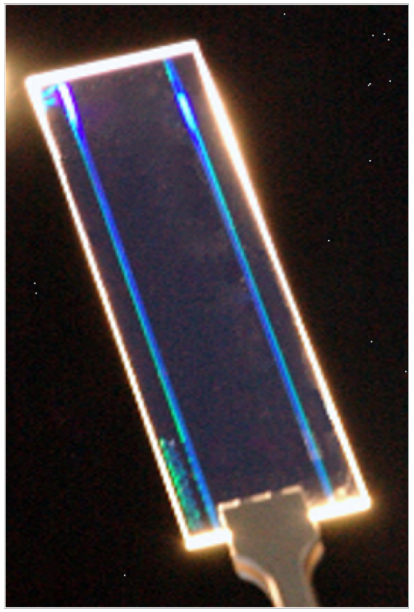
- Gel-free (LC-MS/MS):

  - Label free: Multiple samples, broad dynamic range, no sample treatment but error prone and large data amounts
  - Lable-based (iTRAQ): Eight samples, pooled before MS, low error but similar protein profile & low dynamic range

Proteomics – Biomarker Quantification

- MS-based: Selected reaction monitoring (SRM)

  - Previous selection of protein/peptide/transition
  - Absolute & relative quantification, highly reproducible, molecular specificity but limited number of measurable proteins in one run, sensitivity cannot reach entire proteome

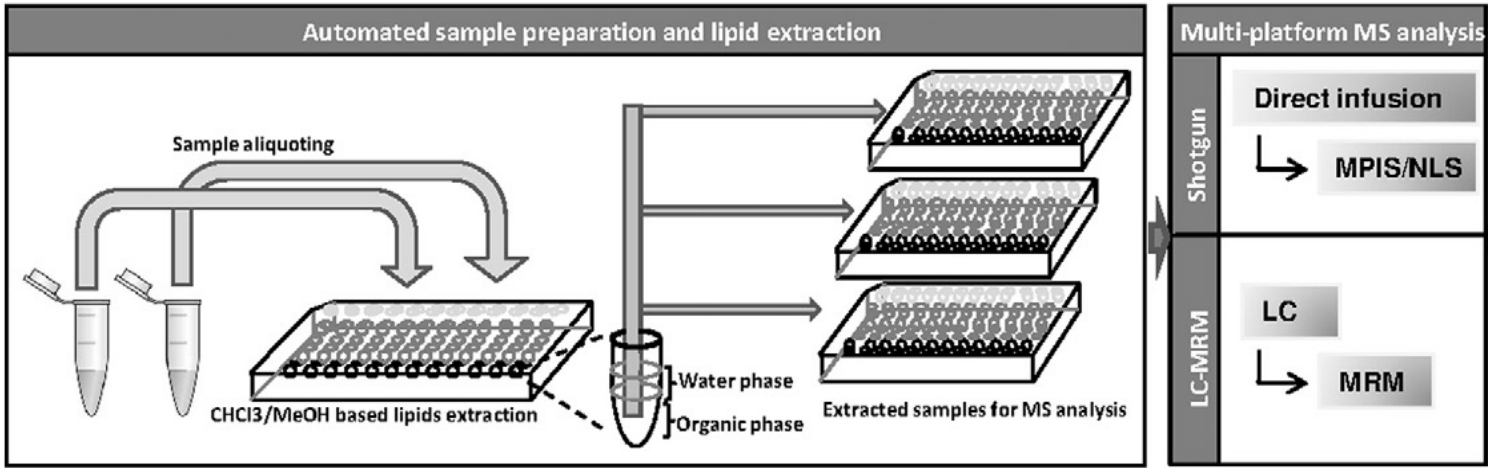


- Antibody-based: Reverse Protein Array (RPA)

  - Analysis of 192 different lysates with 200 antibodies
  - Sensitivity to detect posttranslational modifications
  - Antibodies must be highly specific and thoroughly validates

Lipidomics

- Measuring changes in the cellular/tissue lipid composition
- Overall status of cells, identification of potential biomarkers in early stage toxicity (short exposure and low concentration)
- Reproducible & precise, hundreds of molecular lipids in high & low abundance quickly identified and further quantified



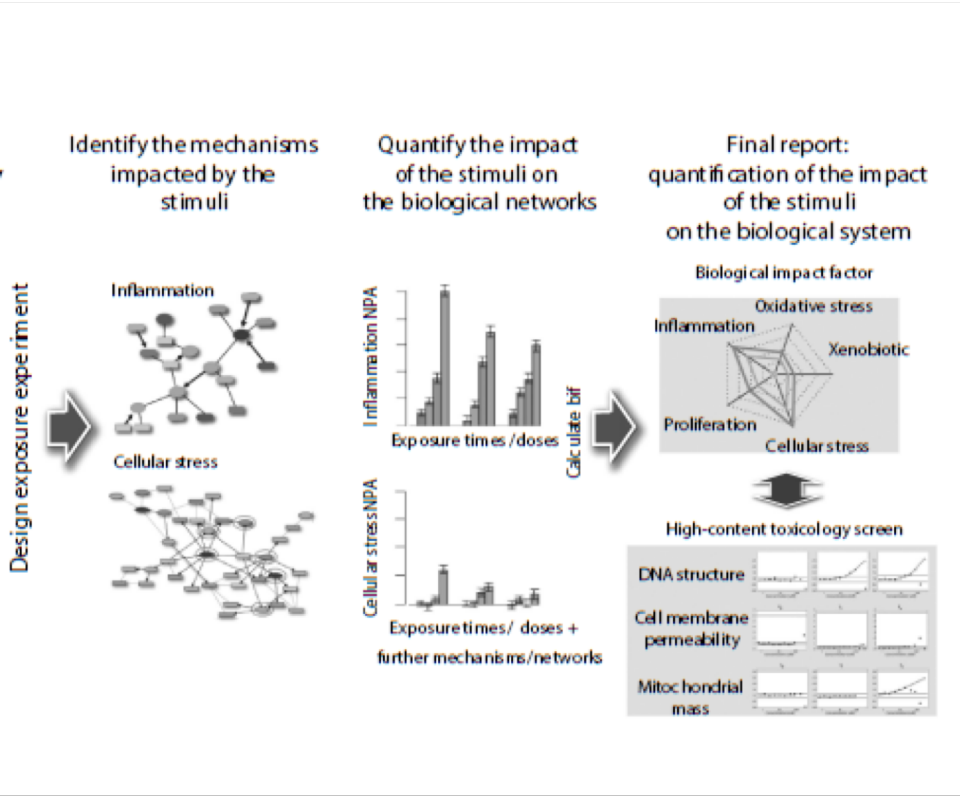
High Content Screening (HCS) & Data Analysis

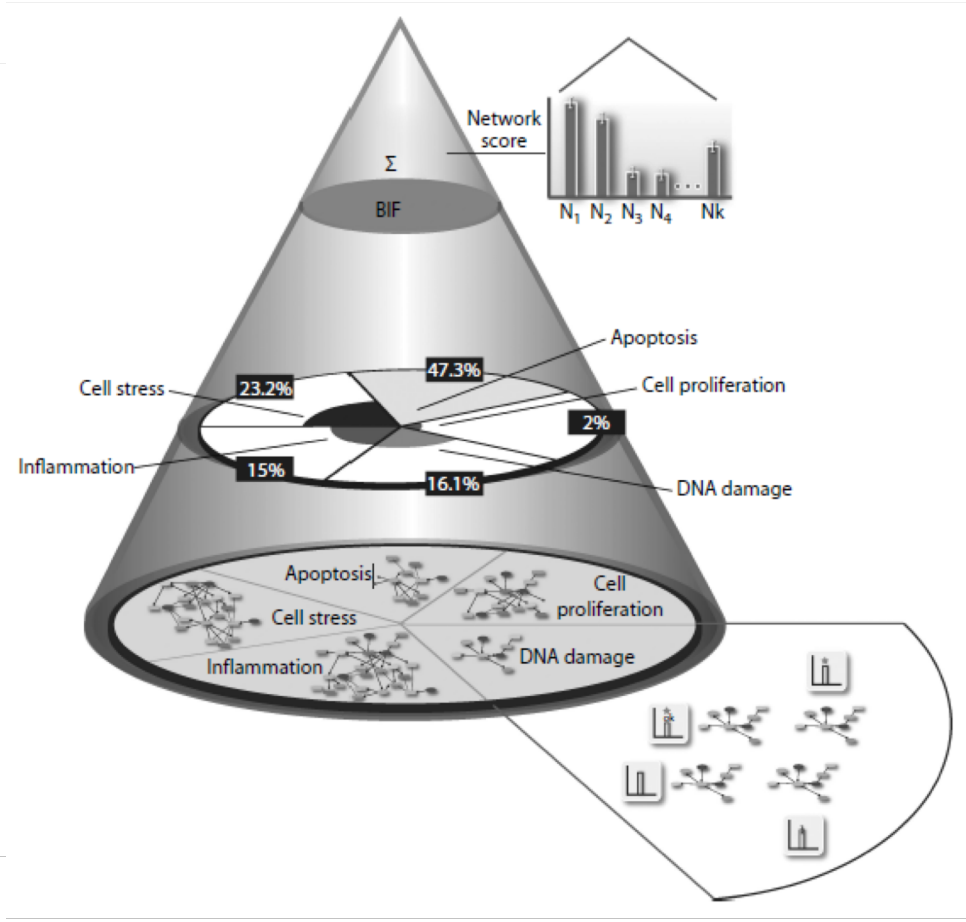
- Phenotypic assessment, visual detection of biomarkers
- Fixed & labeled cells or directly in living cells during exposure
- Changes in gene expression or morphology

→Target apoptosis/autophagy, proliferation & viability, cytotoxicity and oxidative stress,..

- Automated digital microscopy, large data sets
- Platforms to capture detailed information, enable to interpret and reproduce experiments
- Employ a common toxicology ontology, coordinate activities

From data to BIF

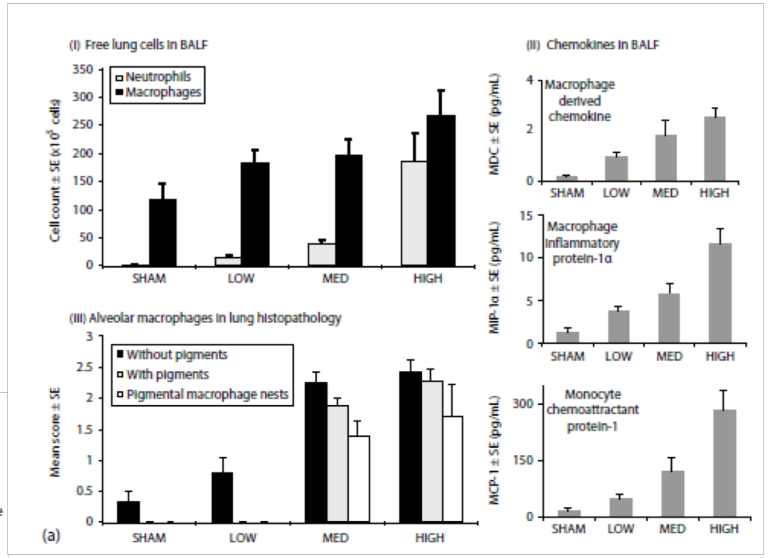


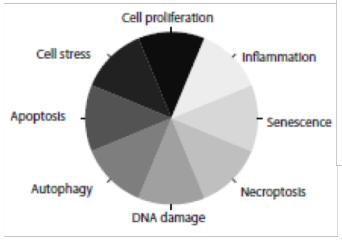


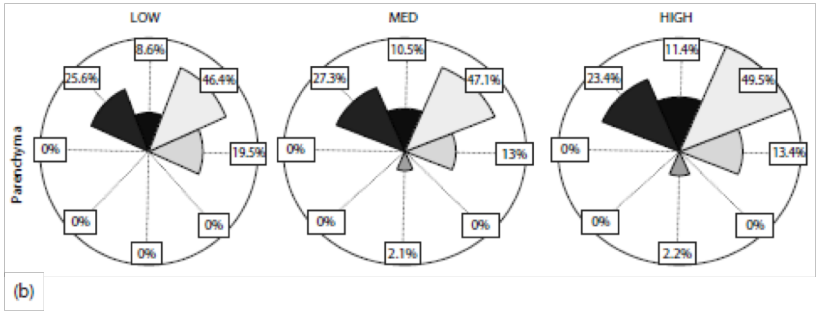
Example Data

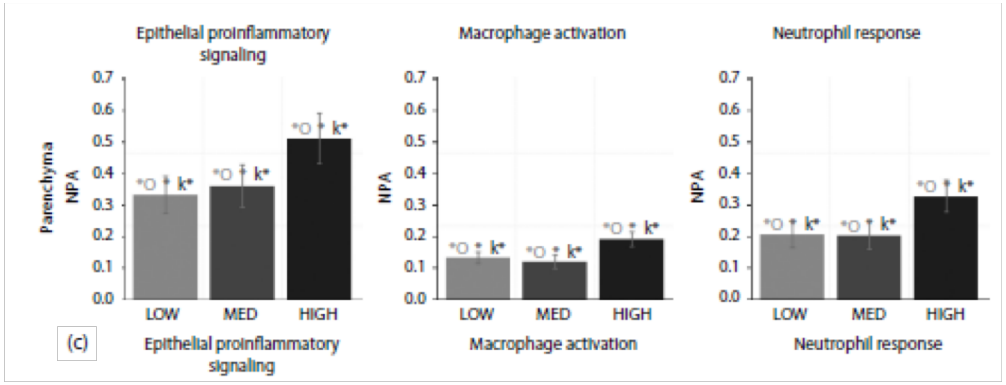
28-day rat inhalation study (CS or filtered air):

- BALF analysis (OECD TG 412 guidelines), gene expression analysis, histopathology (a)
- BIF at network level, contribution to cellular processes (b)
- Perturbation in subnetworks (c)









Thank you for attention!

Feel free to ask questions

Sources

- Heyes‘ Principles and Methods of Toxicology
  Chapter 7: Toxicopanomics: Applications of Genomics, Transcriptomics, Proteomics, and Lipidomics in Predictive Mechanistic Toxicology
- H.Ellinger-Ziegelbauer et al.: The enhanced value of combining conventional and “omics” analysis in early assessment of drug-induced hepatobiliary injury; Toxicology and Applied Pharmacology 252 (2011) 97-111
- H.R.Jung et al.: High throughput quantitative molecular lipidomics; Biochimica et Biophysica Acta 1811 (2011) 925-934
- Barski et al.: High-Resolution Profiling of Histone Methylations in the Human Genome; Cell 129 (2007)
- Doi: 10.1007/978-3-319-24932-2_7
- http://www.biomedcentral.com/1752-0509/5/168
- Doi:10.1093/toxsci/kfn237
- https://www.nmi-tt.de/microdevices/zeptochip/