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Applying Metabonomics to the Development of Metabolic Biomarkers of Xenobiotic-induced Adverse Effects

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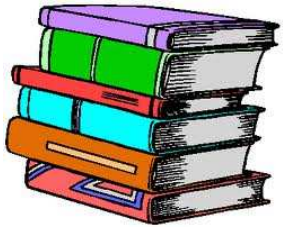
http://portail.umons.ac.be/FR/universite/facultes/fmp/services/toxico/Pages/default.aspx

This presentation will focus on :

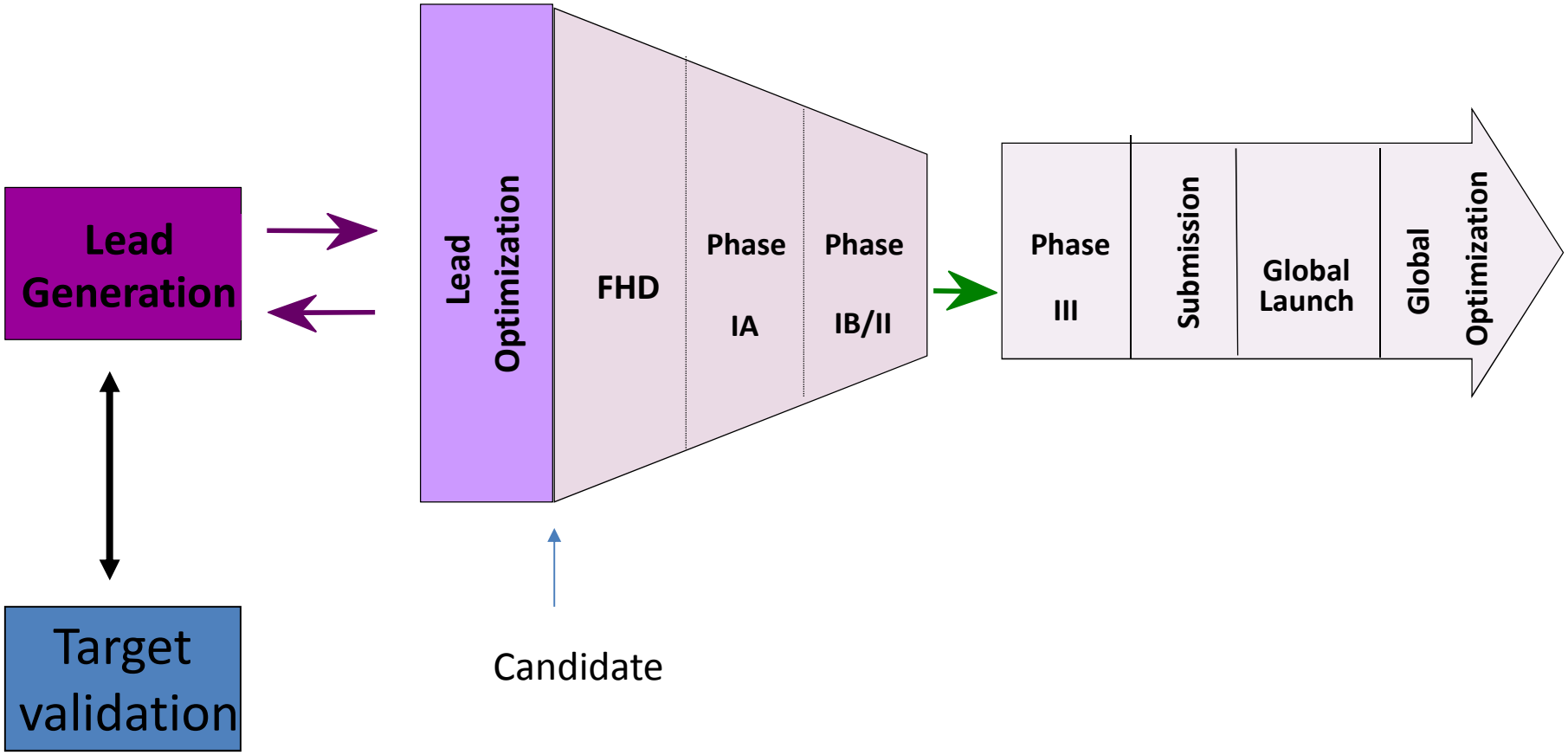
- MBX methodology
- MBX applied to Drug Development
  - ✓ The COMET initiative
  - ✓ Risk Assessment (biomarkers of adverse effects : liver & kidney)
  - ✓ The usual suspects



« Data from literature »

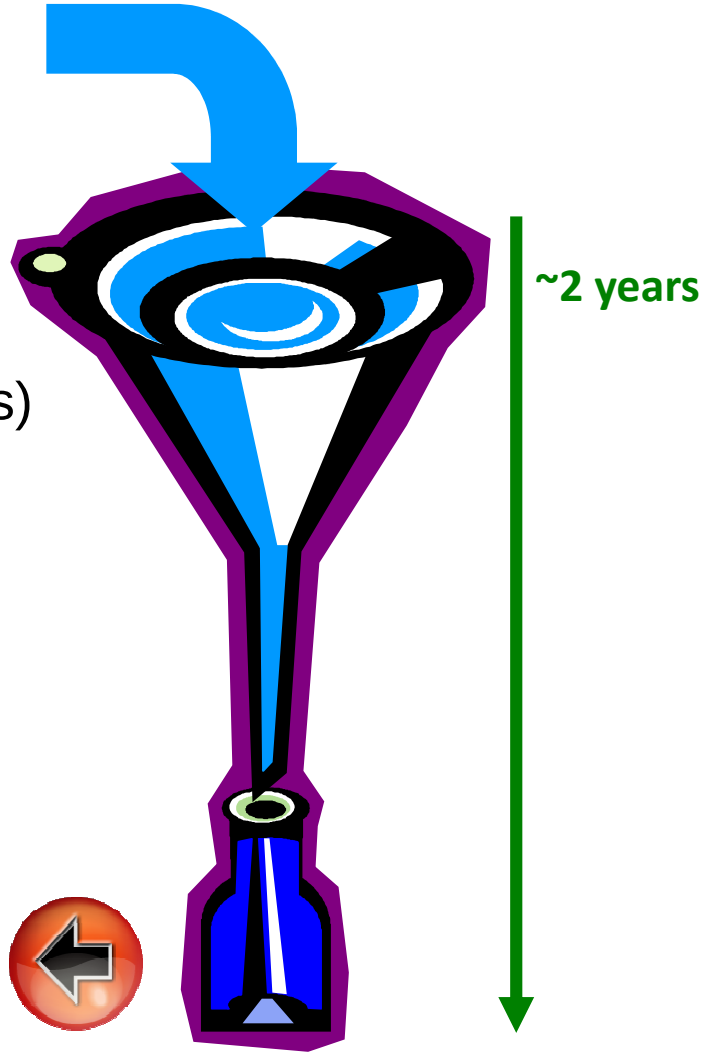
« *In-house* data »

Lead Optimization in the Pharmaceutical Development



Lead optimization acts as a funnel …

Hundreds of molecules derived from one single chemical platform



- Chemistry (initial low amount synthesis)
- Pharmacology (*in vitro* efficacy model)
- ADME (metabolism)
- Pre-clinical Toxicology
- Formulation

Only one candidate molecule to enter clinical stages

Toxicological studies are the bottleneck …

Lack of efficacy

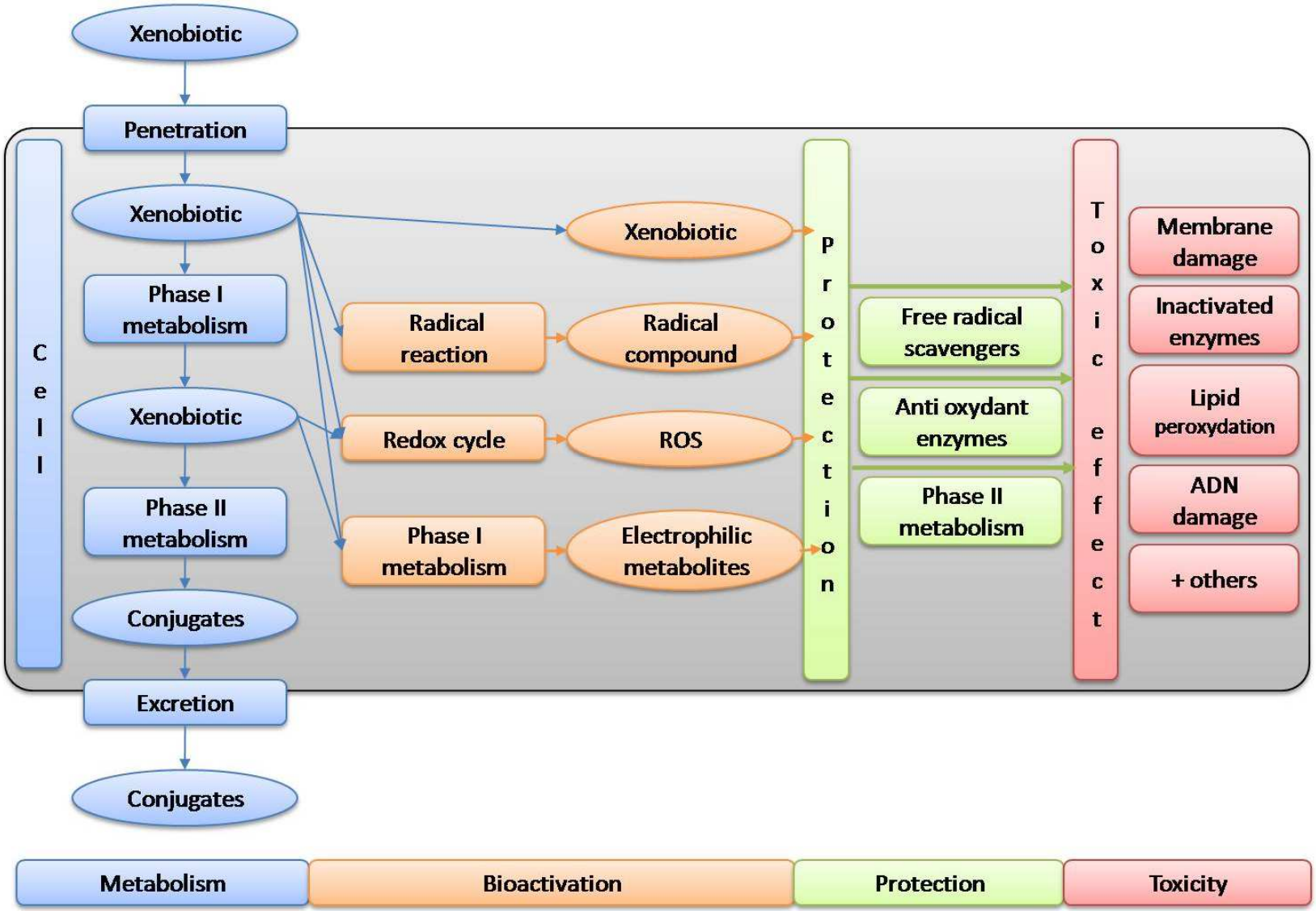
Unexpected/unpredicted adverse events during clinical trials or after launching

→ withdrawal from the market

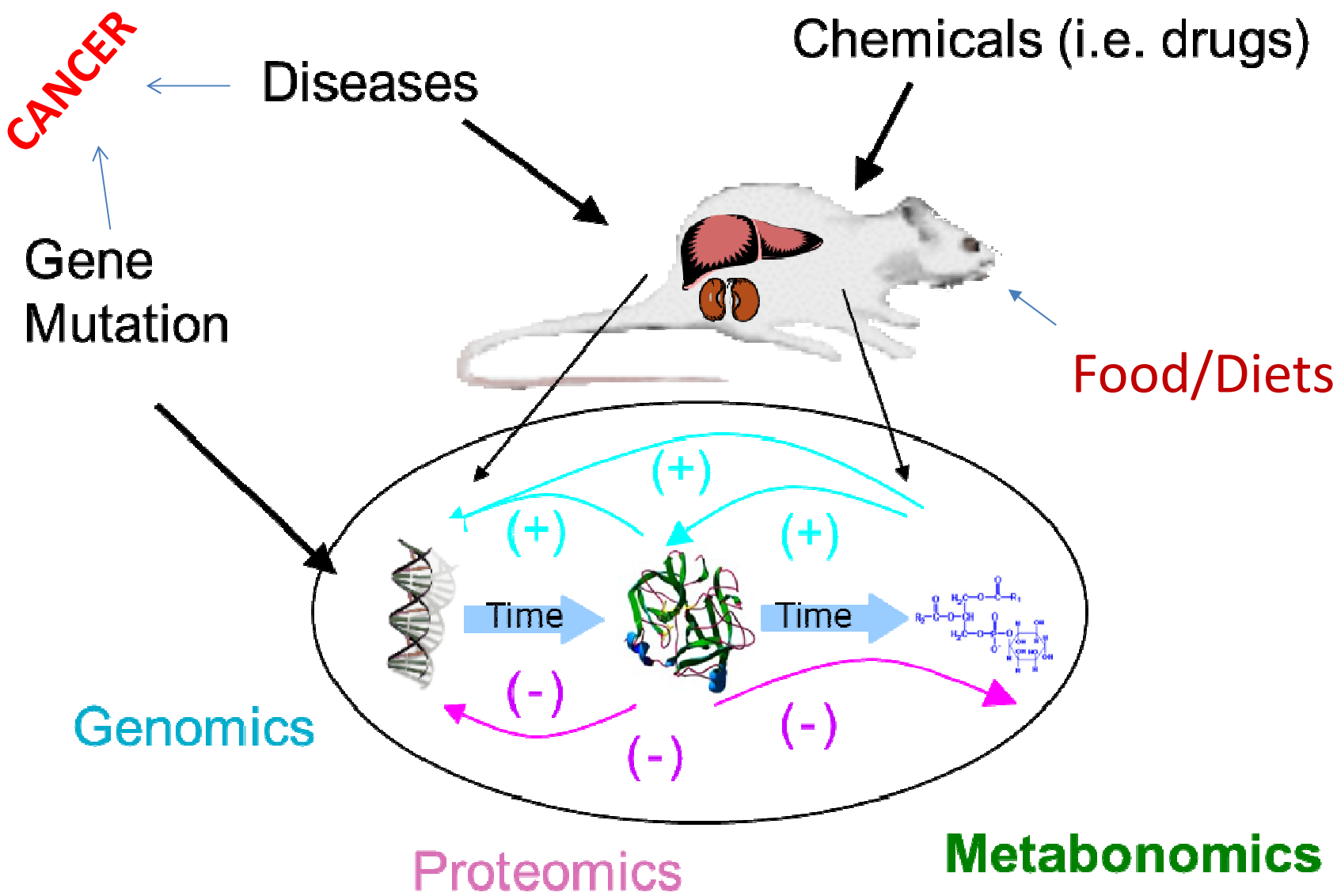
Why are we failing?

- Conventional methods used in Risk Assessment (clinical chemistry, histopathology, …) need the development of moderate or severe lesions to detect changes, …
  - ➢ lack of predictivity

- Preclinical studies usually perfomed in healthy and young animals, while human patients are of all ages and definitely not healthy, …..
  - ➢ Poor transposition from experimental models to clinics


Xenobiotic
Penetration
Cell
Xenobiotic
Phase I metabolism
Xenobiotic
Phase II metabolism
Conjugates
Excretion
Conjugates
Radical reaction
Redox cycle
Phase I metabolism
Xenobiotic
Radical compound
ROS
Electrophilic metabolites
Protection
Free radical scavengers
Anti oxydant enzymes
Phase II metabolism
Toxic effect
Membrane damage
Inactivated enzymes
Lipid peroxydation
ADN damage
+ others
Metabolism
Bioactivation
Protection
Toxicity

What can we do ?



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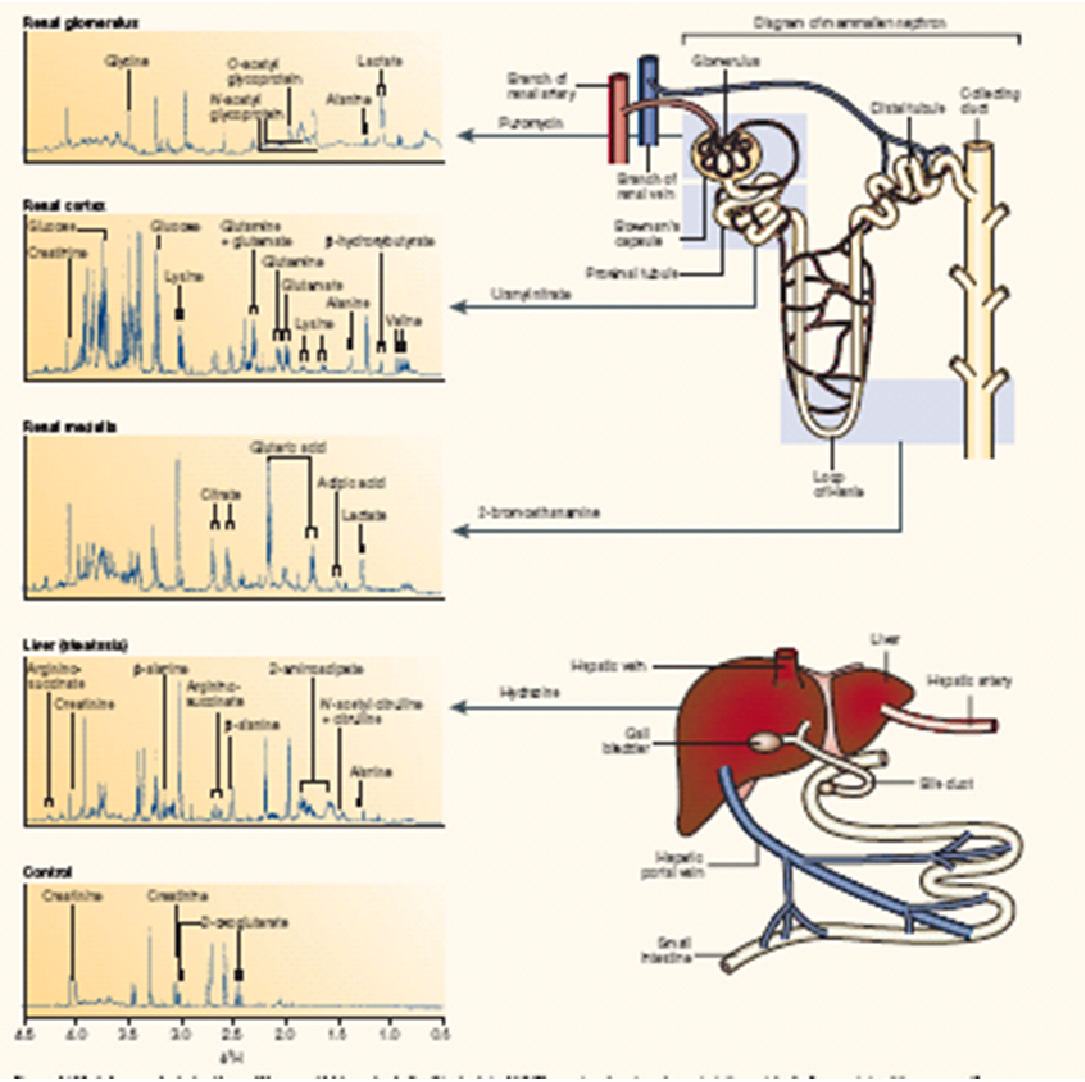
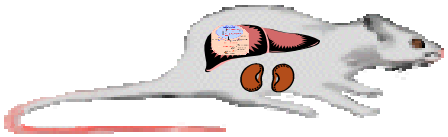
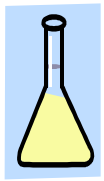


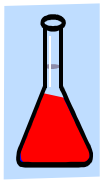
Nicholson JK Nat rev Drug Disc 1(2):153-161 (2002)

Experimental Protocol



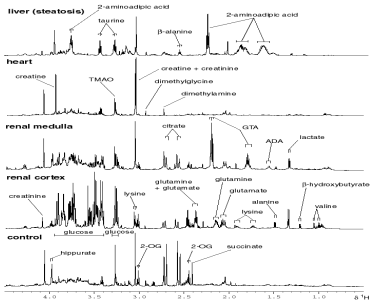


Urine

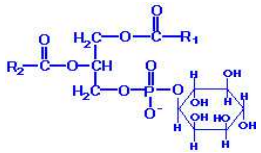


Blood

^{1}H NMR or MS

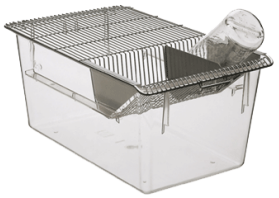


Pattern recognition

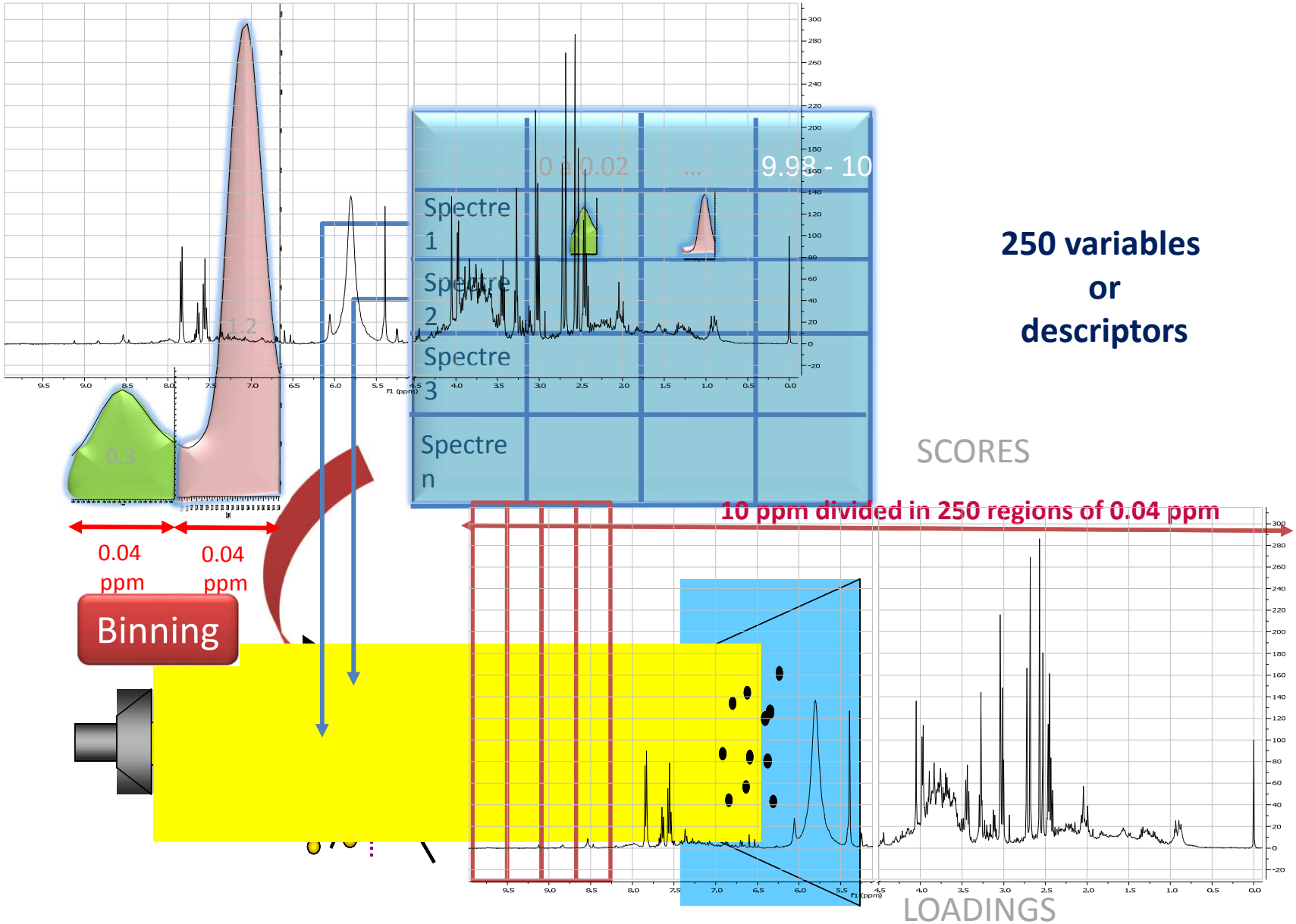


Potential biomarkers

- Animals placed in metabolic cages for urine collection
- Acclimatation (at least 2 days) to minimize stress
- Water and food ad libitum or controlled if necessary

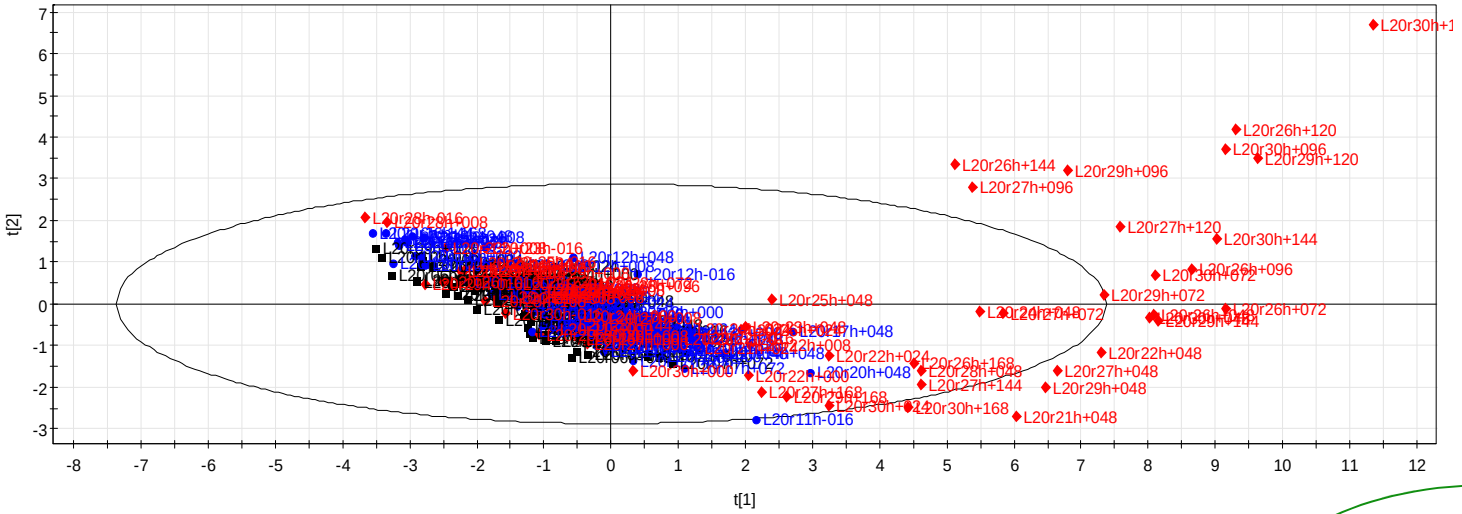


Avoid bacterial contamination !

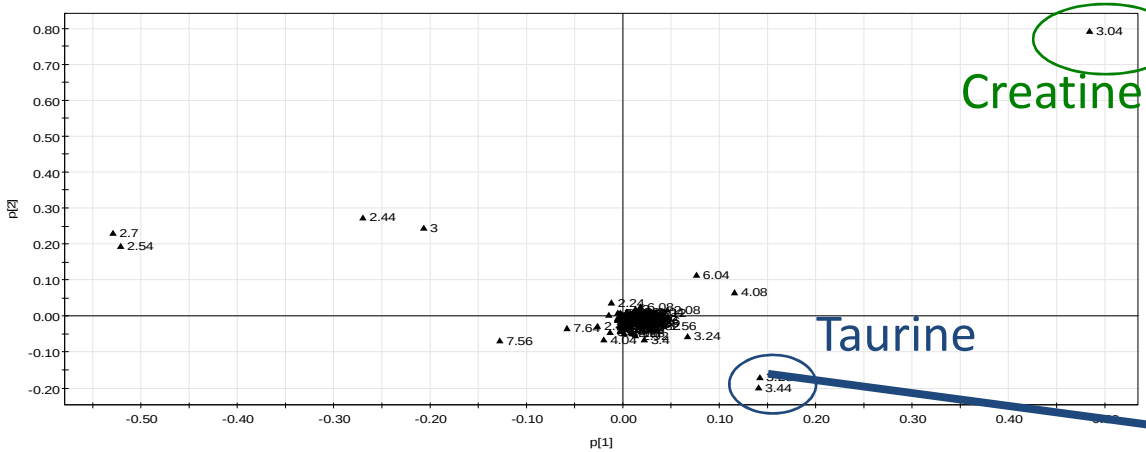


250 variables or descriptors

Multivariate MBX data analysis



Scores Plot



Creatine

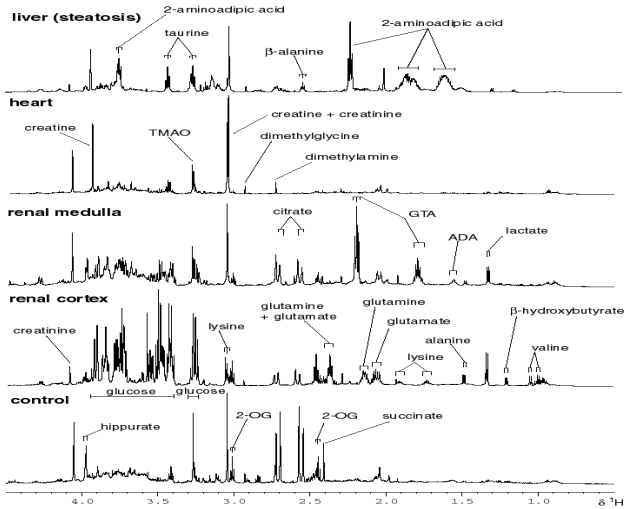
3.04 ppm

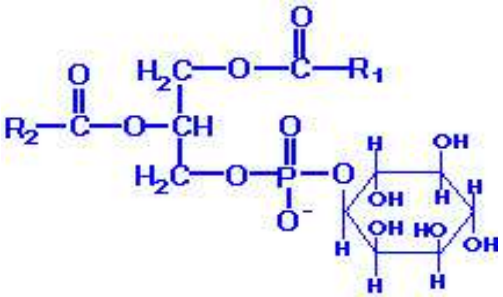
Loadings Plot

Taurine

3.28 & 3.44 ppm

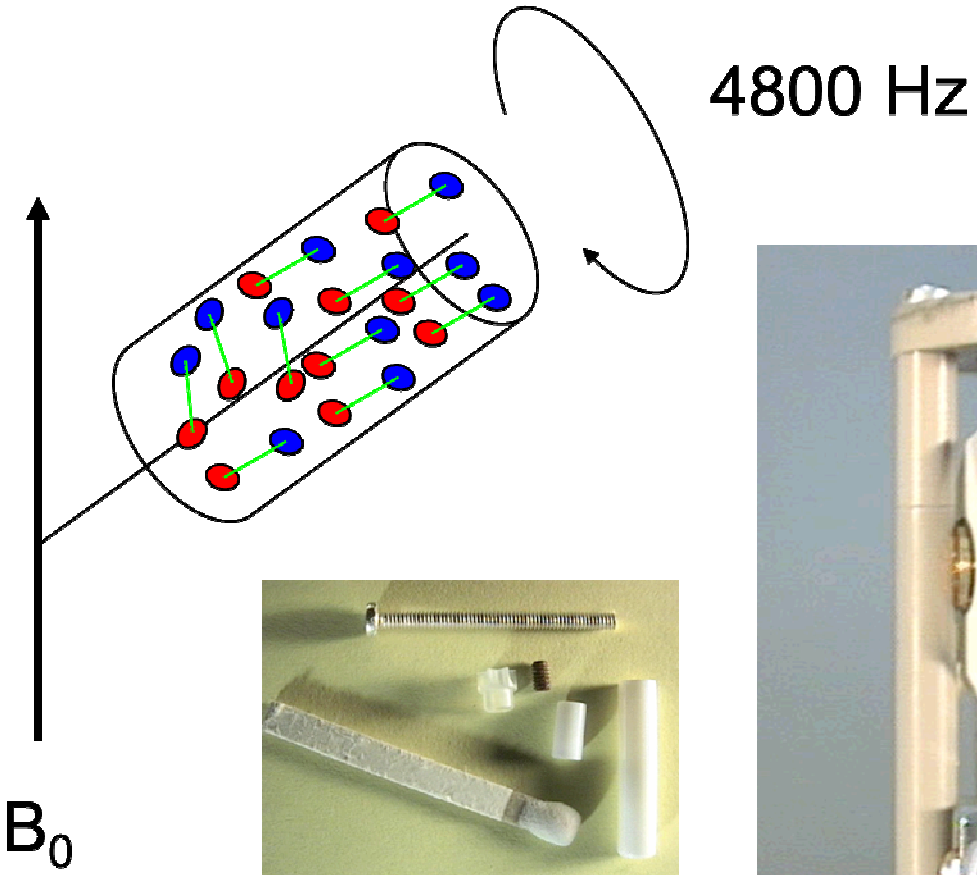
From disease-specific metabolic signatures to biomarkers identification

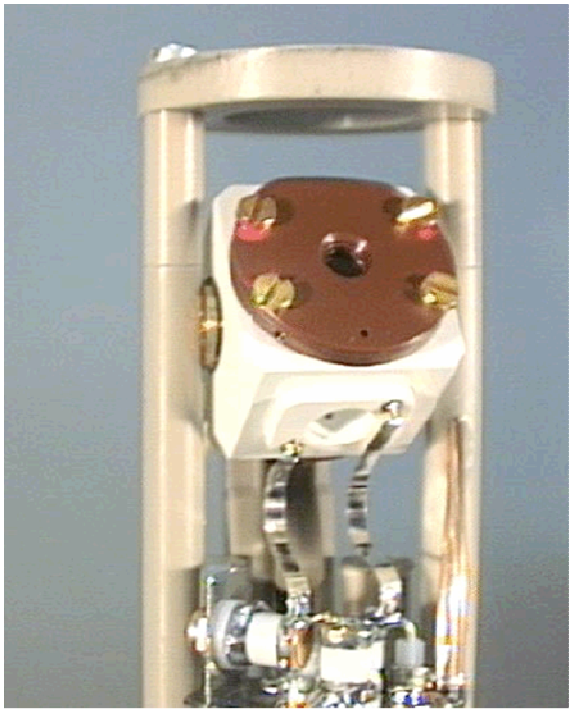




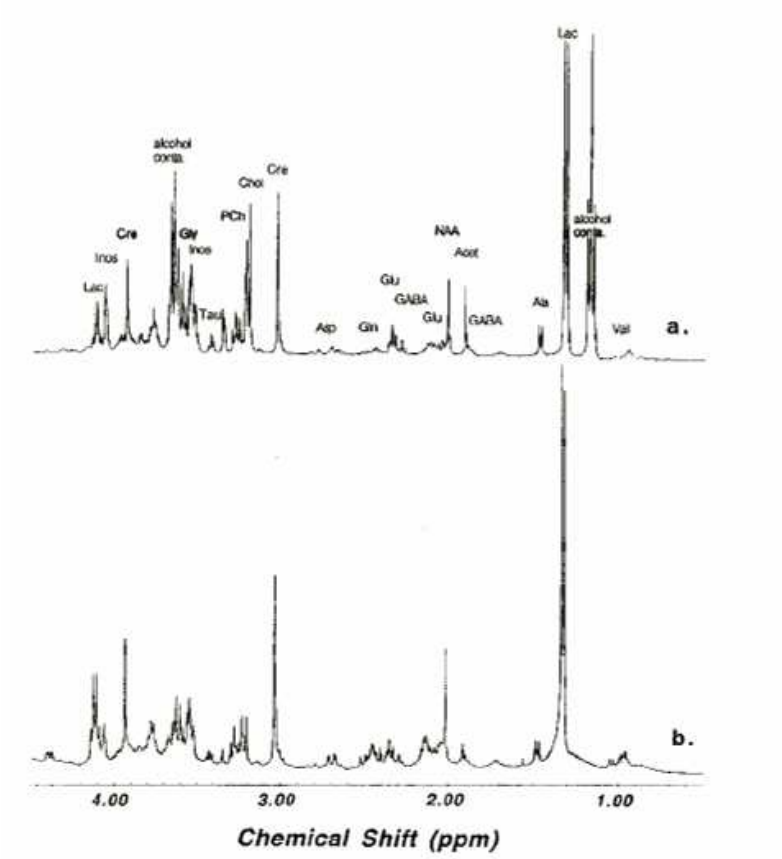
HR-MAS (High Resolution Magic Angle Spinning NMR)

NMR analysis of intact tissues





HR-MAS on brain tissue compared to tissue extracts



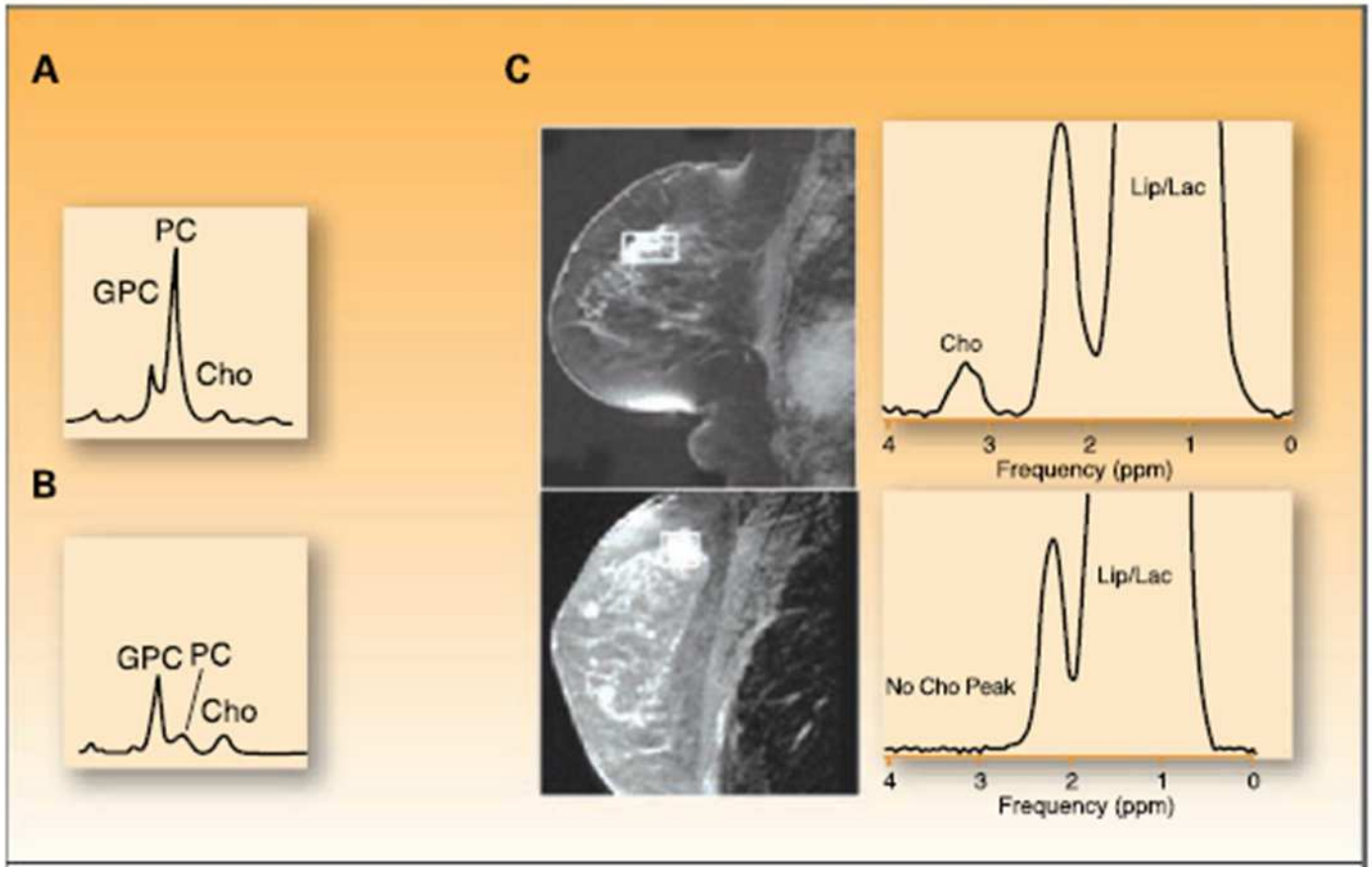
HR-MAS

Liquid extraction

Ref.: L.L. Cheng et.al. *PNAS* **1997**, *94*, 6408-6413.

In VIVO

MRSI : Magnetic Resonance Spectroscopy Imaging



COMET

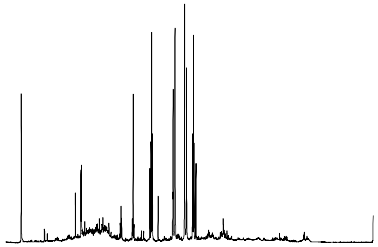
Consortium on Metabonomic in Toxicology

Imperial College-London

BMS Lilly Novo Pfizer Pharmacia Roche

150 prototoxicants

Acute dosing



^{1}H-NMR analysis

Database
+
Chemometrics models for toxicity prediction

COMET: Control Model

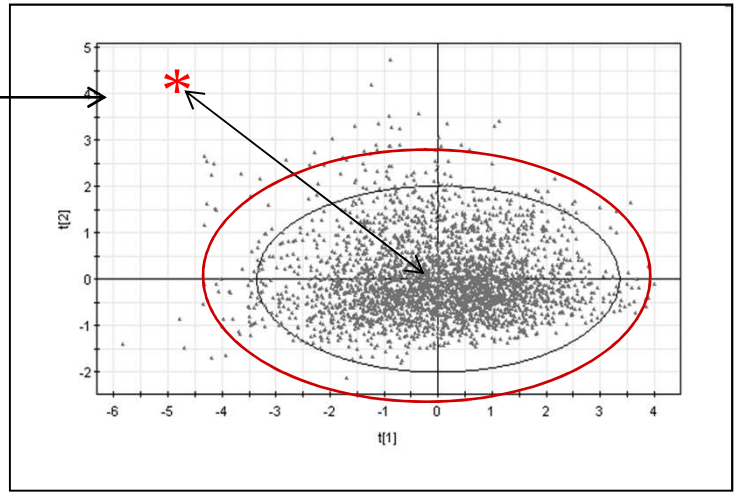
Prediction and Classification of Drug Toxicity Using Probabilistic Modeling of Temporal Metabolic Data: The Consortium on Metabonomic Toxicology Screening Approach
T. Ebbels, H. Keun, O. Beckonert, M. Bollard, J. Lindon, E. Holmes, and Jeremy K. Nicholson
Journal of Proteome Research/2007

Identifying abnormal urine samples

- 4521 “normal” urine samples
- PCA-based approach
- Determine the distance to the model (DmodXPS+) as well as the probability of belonging to the model (PmodXPS+):

Test sample

- Normal
- Marginal: 95%<x< 99%
- Abnormal: <95%

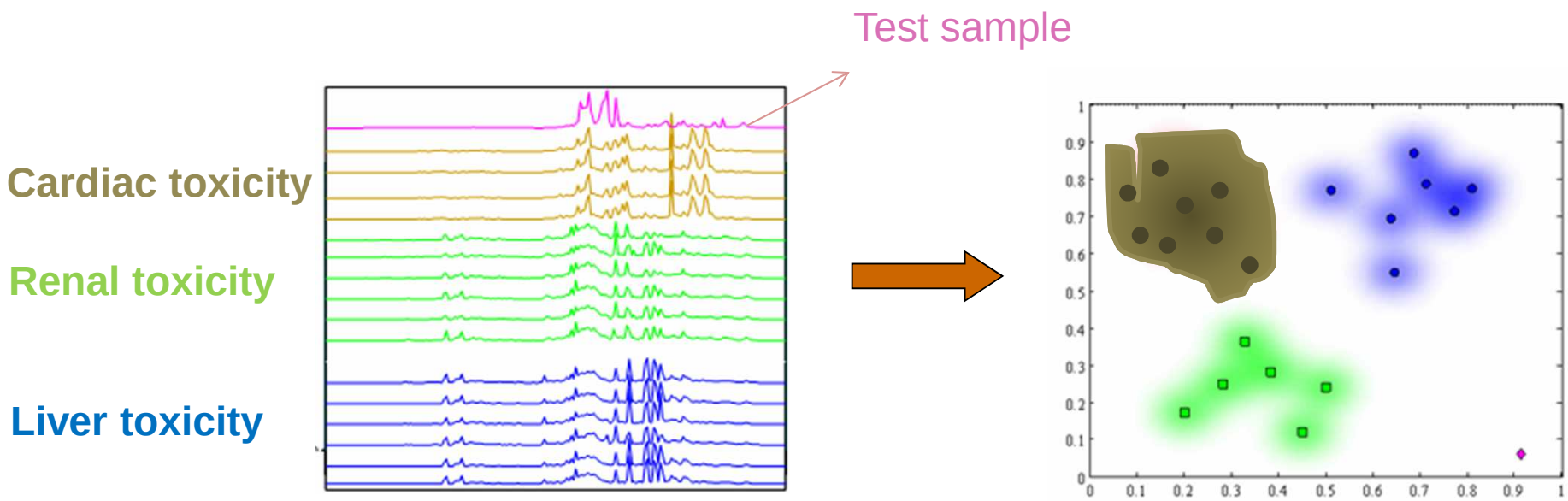


COMET: CLOUDS Model (Classification Of Unknowns by Density Superposition)

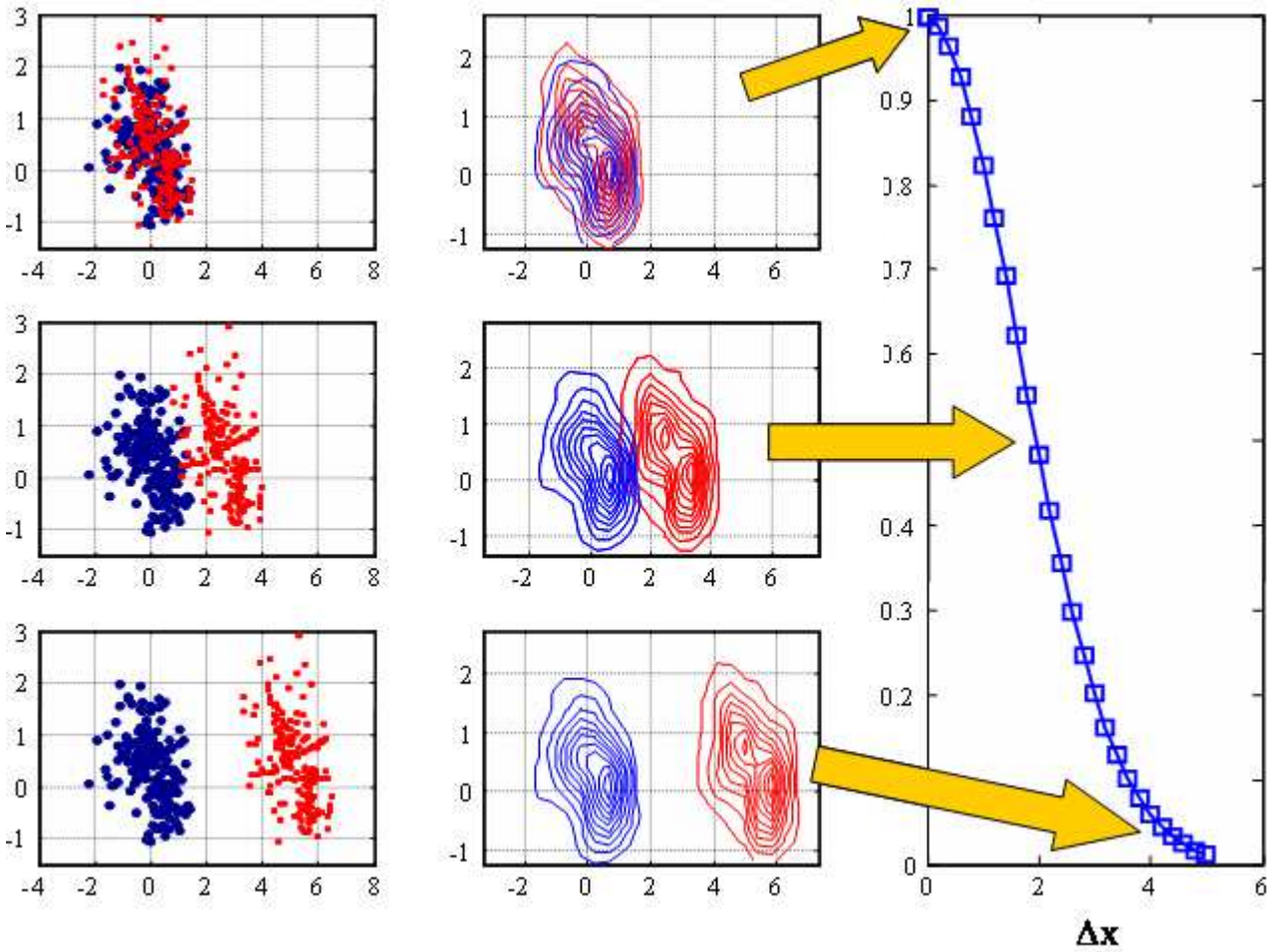
Prediction and Classification of Drug Toxicity Using Probabilistic Modeling of Temporal Metabolic Data: The Consortium on Metabonomic Toxicology Screening Approach
T. Ebbels, H. Keun, O. Beckonert, M. Bollard, J. Lindon, E. Holmes, and Jeremy K. Nicholson
Journal of Proteome Research/2007

Identifying toxicity type

- PNN-based model (Probabilistic Neural Network – Specht 1990)



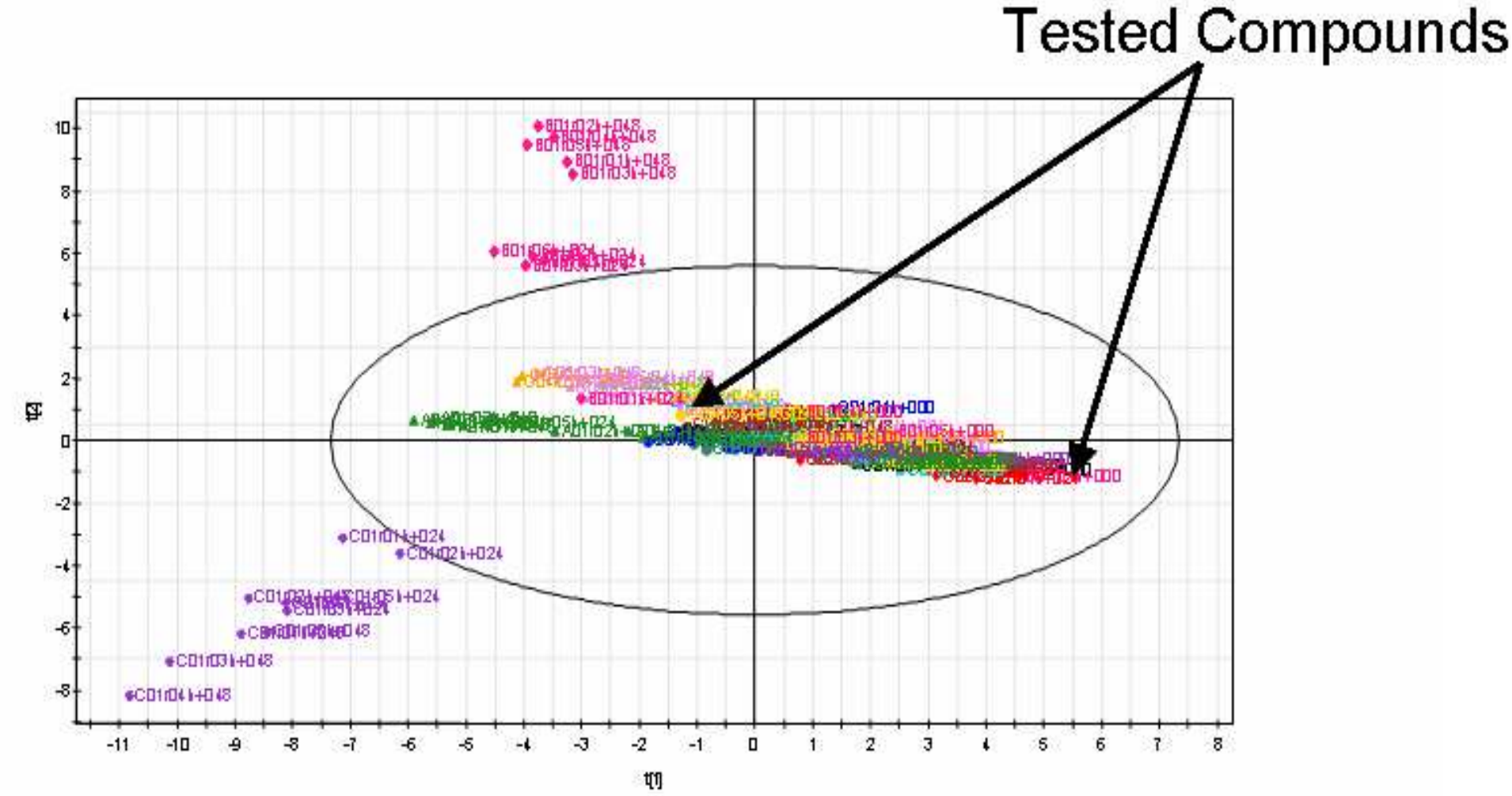
Clouds approach



Classification
based on similarity

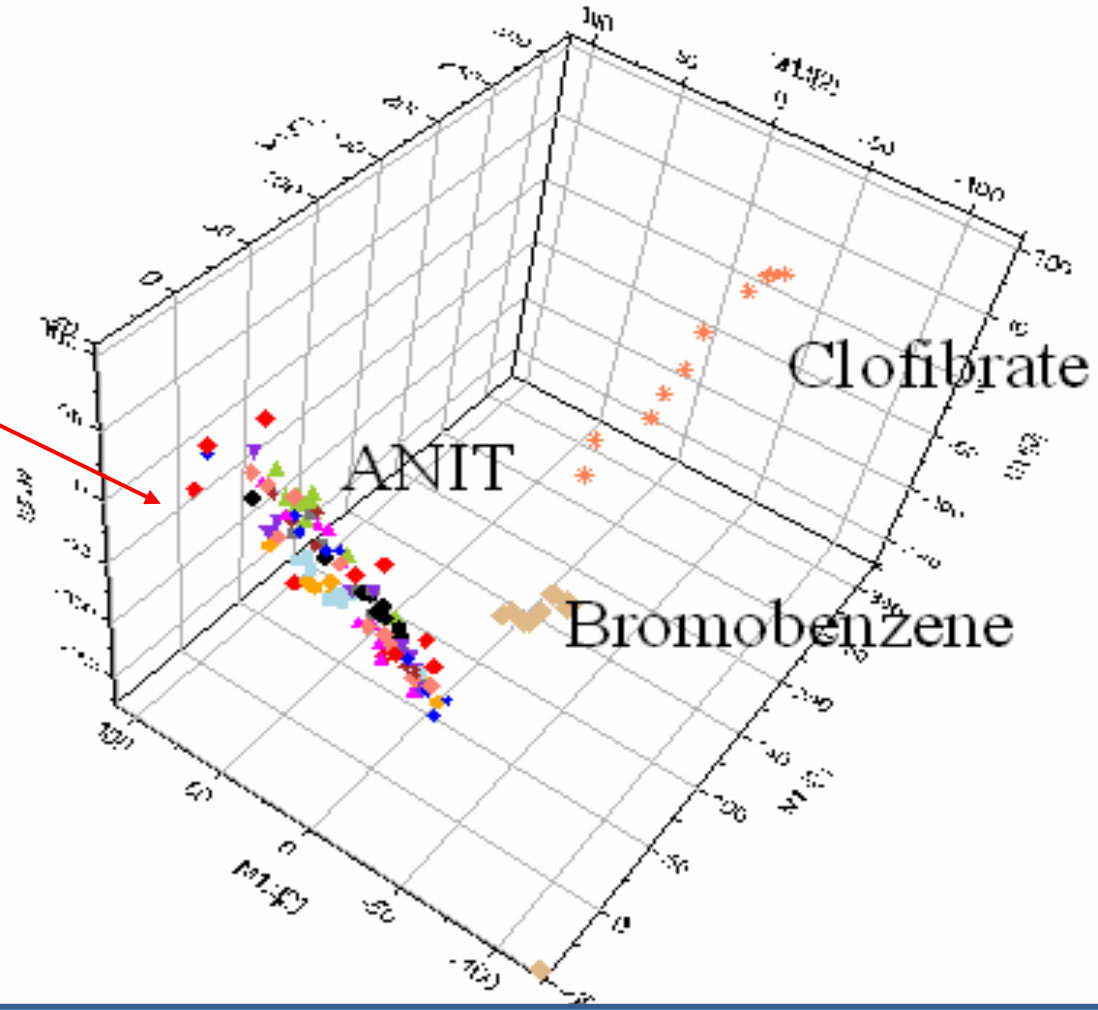
Using CLOUD in Lead Optimization

- 8 compounds with same chemical scaffold selected on the results from conventional 4-Day Tox studies:
  - Vehicules: Corn oil or Acacia
  - 4 hepatotoxicants (A, B, C,D)
  - 4 non hepatotoxicant (E, F, G, H)
  - 3 prototypical liver (ANIT, Bromobenzene, Clofibrate)
- A single dose of 100 mg/kg
- 5 rats / group
- Collection of urine samples (pre-test and at 24h and 48h post-dose)
- Clinical chemistry + liver histopathology (foie) at necropsy (48h post-dose)



Scores plot (PCA): Bromobenzene, clofibrate, ANIT

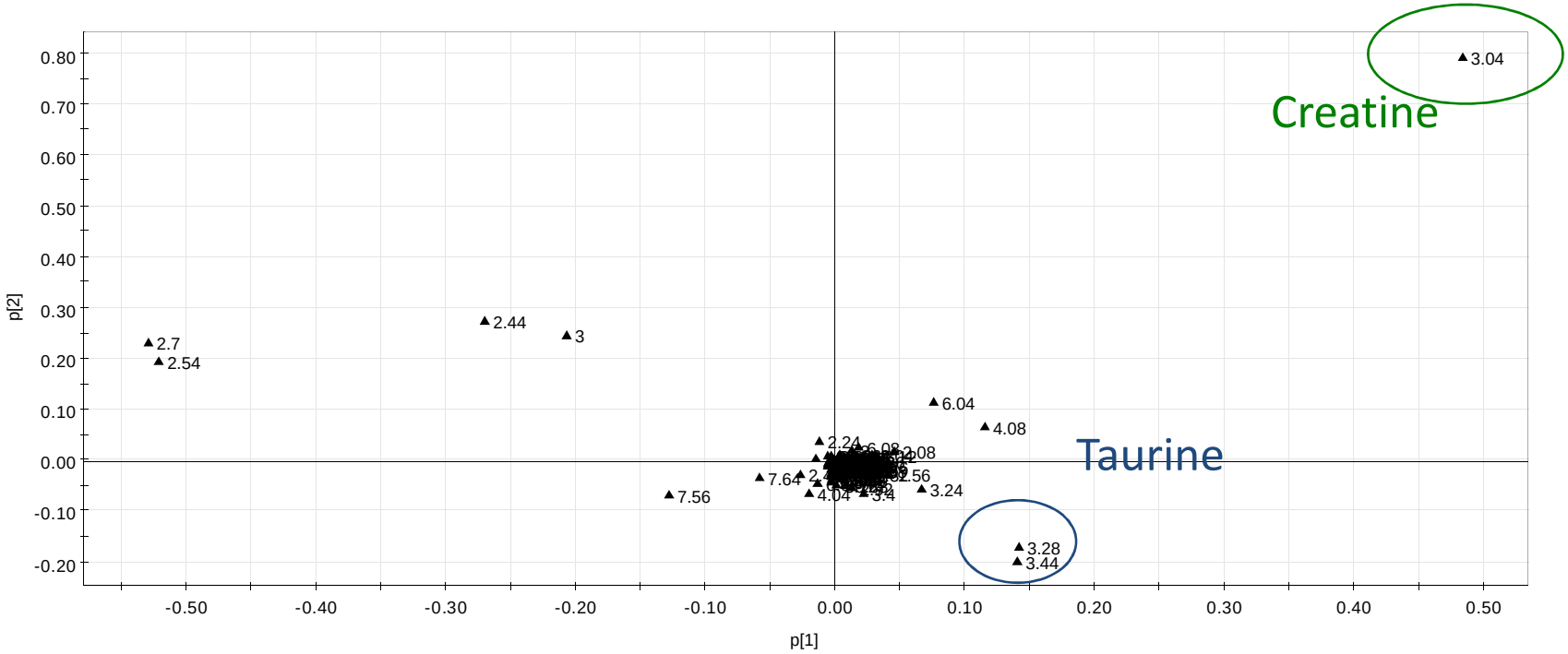
Tested Compounds



Bromobenzene: oxidative stress , lipid peroxydation, Ca^{++} imbalance

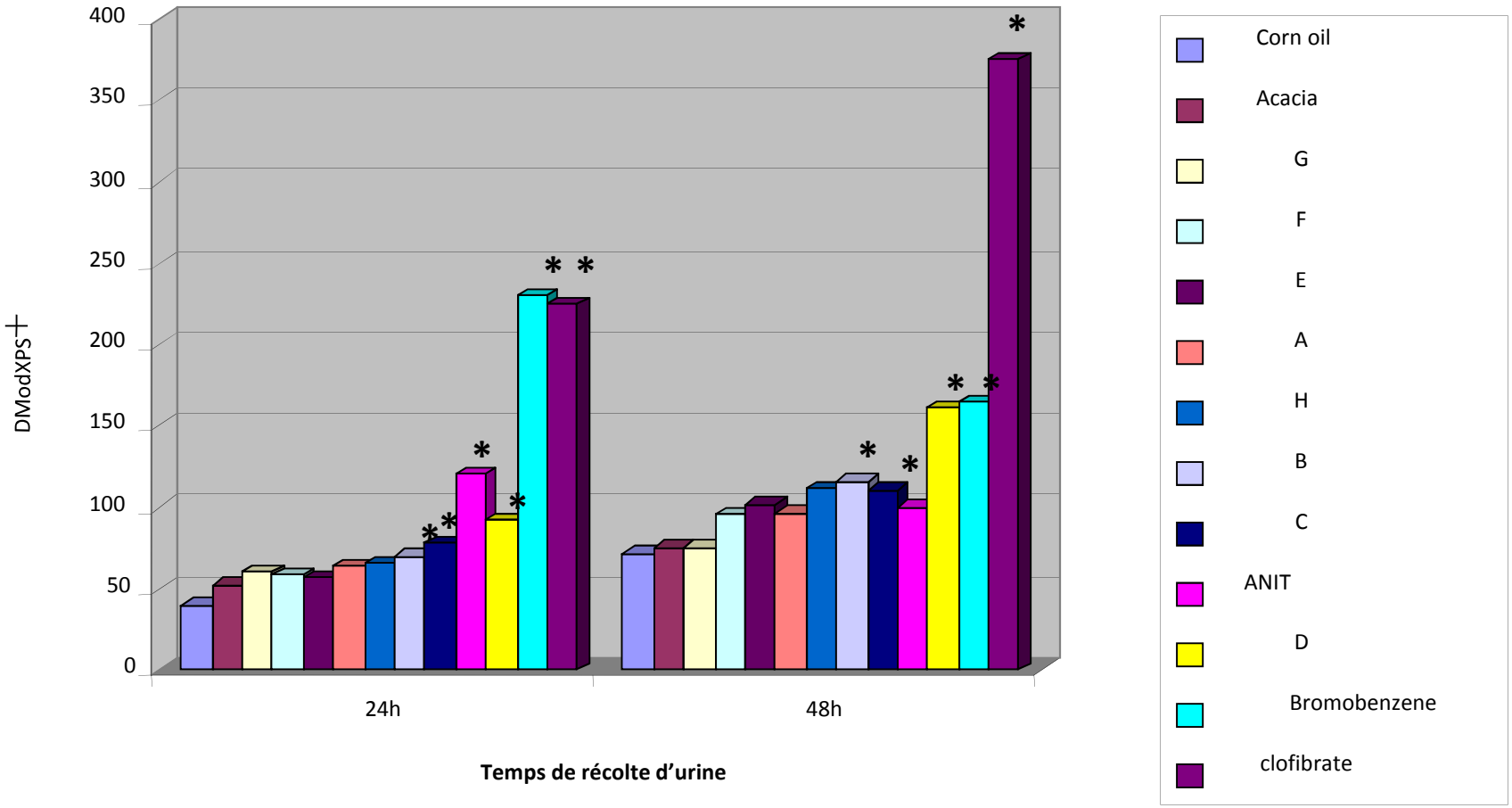
ANIT: Bile ducts damages and disruption of bilirubine metabolism

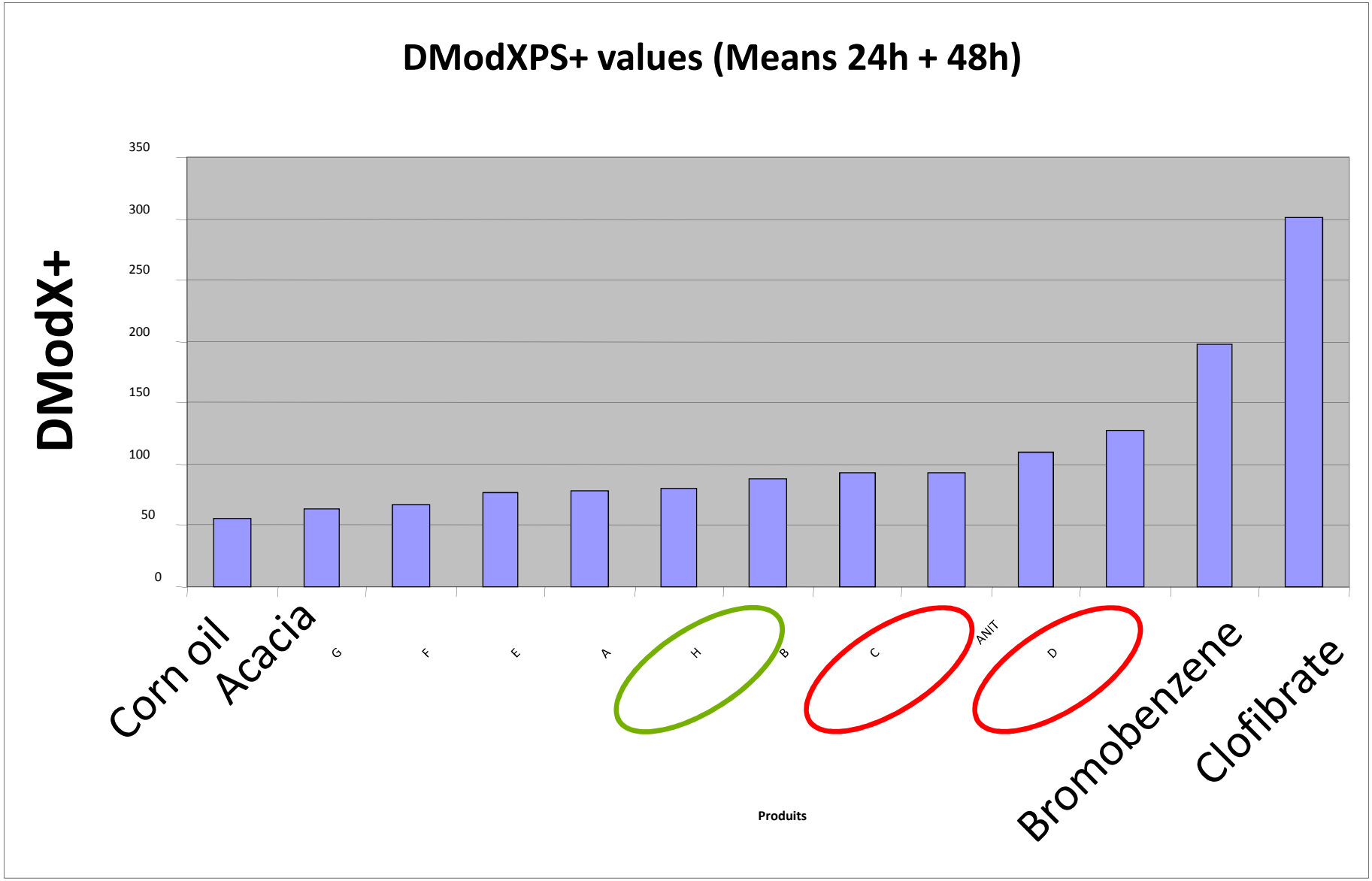
Clofibrate: peroxysomal proliferator



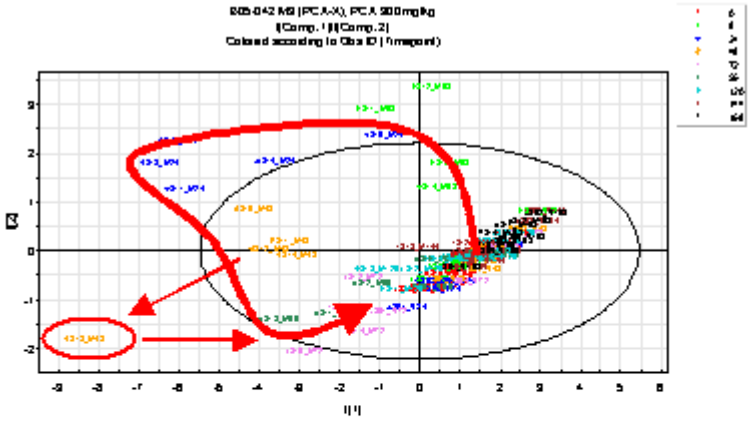
Liver necrosis metabolically characterized by early (before plasma membrane rupture) and simultaneous elevations of urine creatine and taurine

COMPOUNDS RANKING

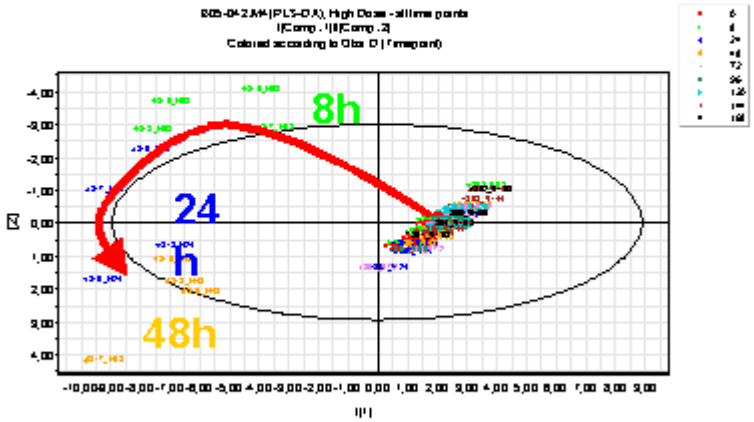



DModXPS+ values (Means 24h + 48h)
DModX+
350
300
250
200
150
100
50
0
Corn oil
Acacia
G
F
E
A
H
B
C
ANIT
D
Bromobenzene
Clofibrate
Produits

Evaluating dose effects



300 mg/kg



600 mg/kg

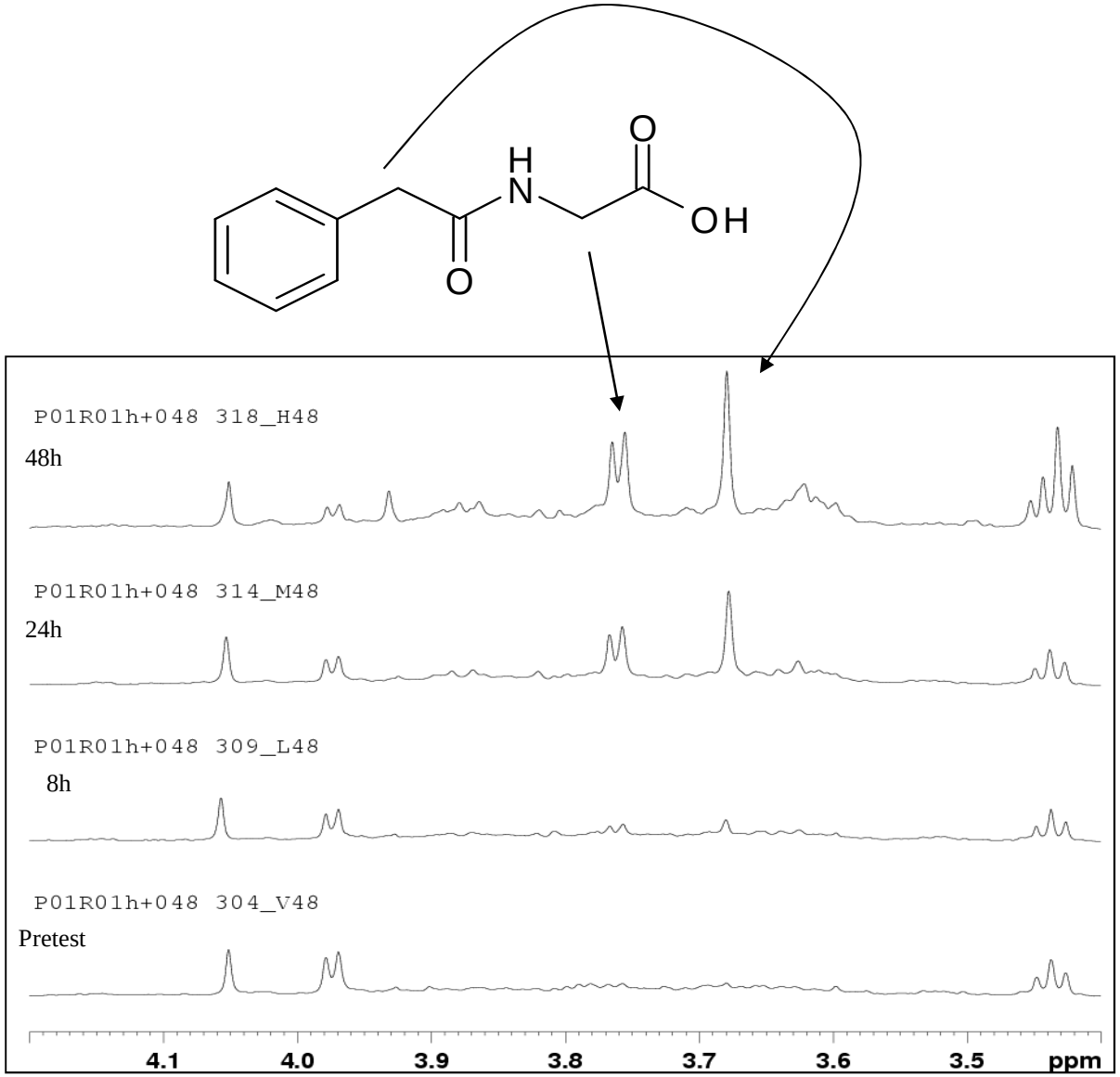
Biomarker of phospholipidosis

From animal models to humans …

- CAD's (Catationic Amphiphilic Drugs)
- sequestring phospholipids in cytoplasmic vacuoles
- reversible effect but …
- often associated with a more severe toxicity (muscle or neuro degeneration)

J. Espina et al Magnetic Resonance in Chemistry (2001); 39(9) : 559 - 565

Phenylacetylglycine



J. Espina et al Magnetic Resonance in Chemistry (2001); 39(9) : 559 - 565

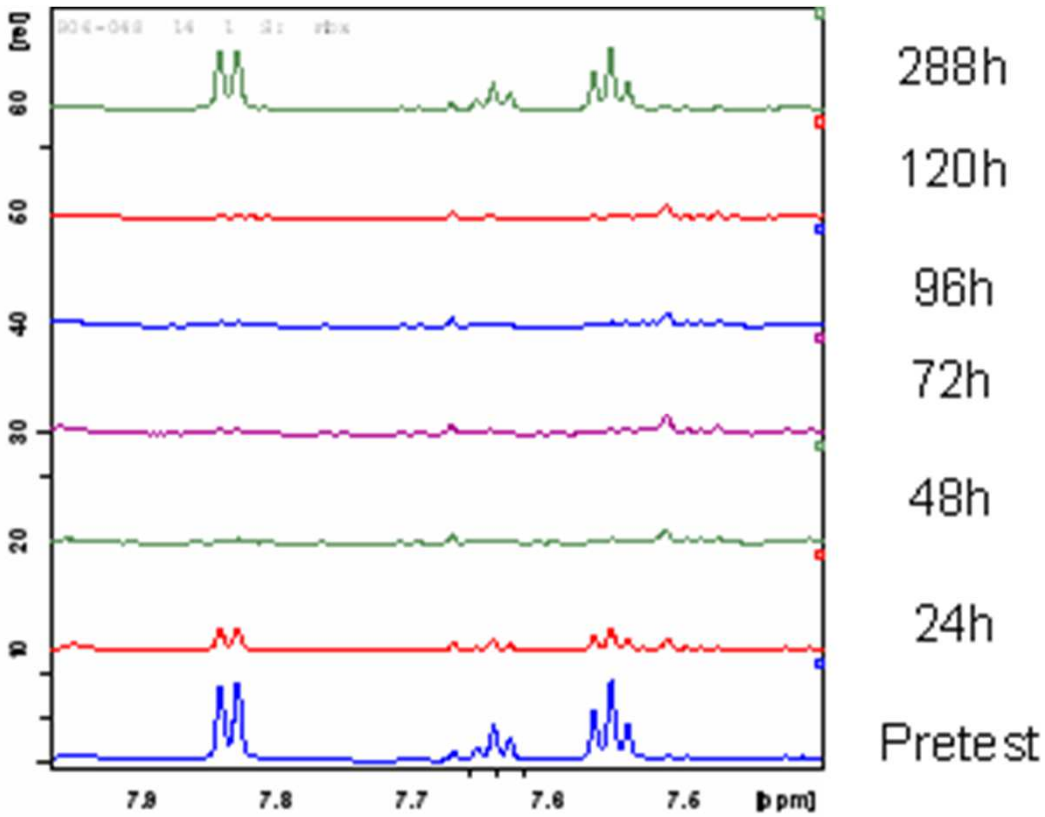
USUAL SUSPECTS ….

List of urine metabolites which frequently change in response to toxicant administration , regardless of the nature of the toxicant, its mechanism(s) of action, of its target(s). Importantly, not all of these molecules change in response to every toxicant, nor do they necessarily follow the same trajectory (temporal response)

The "Usual Suspects"[a]
2-oxoglutarate
acetate
citrate
creatine
creatinine
glucose
hippurate
lactate
succinate
taurine
trimethyl amine/trimethyl amine oxide (TMA/TMAO)

Robertson DG
Toxicological Sciences 5
(2005) ; 85 : 809-822

The case of hippurate



Liver (Σ)

Kidney (OATP1)

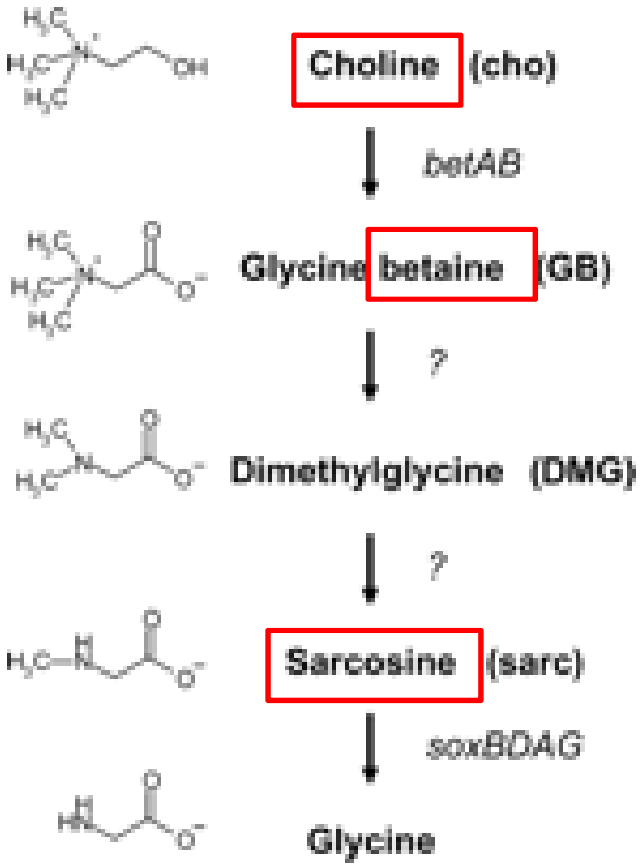
Intestinal flora (Σ)

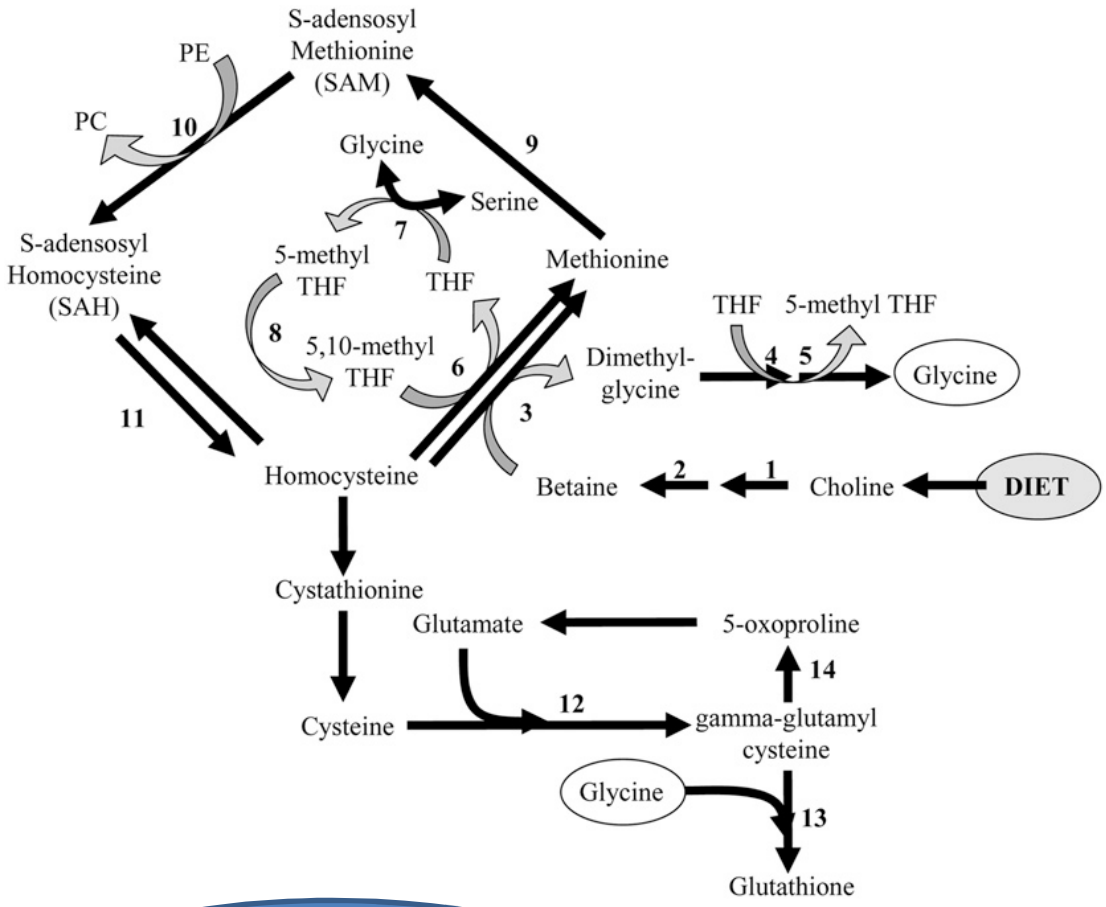
Drug-induced renal proximal tubular injury ⇒ reduction in urine hippurate

BUT

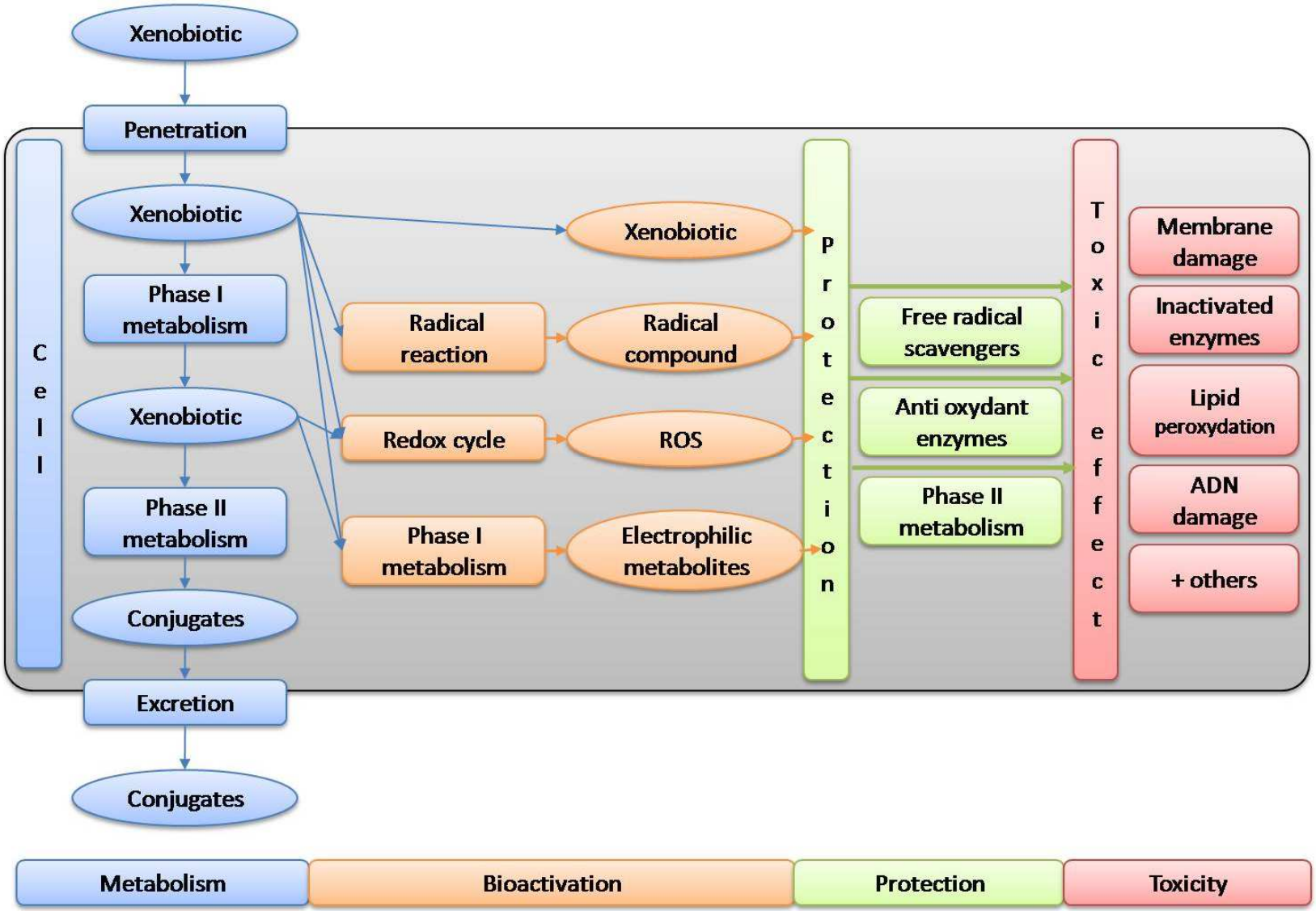
Activation of phase II metabolism ⇒ increase in urine hippurate

Usual suspects … metabolism





Glutathione



↑ Hippurate Choline-sarcosine-betaine allantoin …

↑ Taurine creatine
↓ hippurate
↑ PAG

Conclusions

- Like most technologies, metabonomics will not meet all expectations, but it will certainly add value in many areas of biology and definitely in Risk assessment

- Significant impact in drug efficacy/safety assessment

- Metabonomics will be extremely useful in completing the omics circle from gene (genomics) to protein (proteomics) to metabolite (metabonomics)

Conclusions

What will allow determine the realization of this potential?

- Analytical challenges are nearly met (acquisition of data but more by the volume of data and in the complexity of the profiles generated)

- It is in the experimental design and interpretation of metabonomic data that crucial questions remain

Develop biological models from whole organism to cellular and subcellular levels


THANK
YOU!