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Metabonomics in Drug risk Assessment

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

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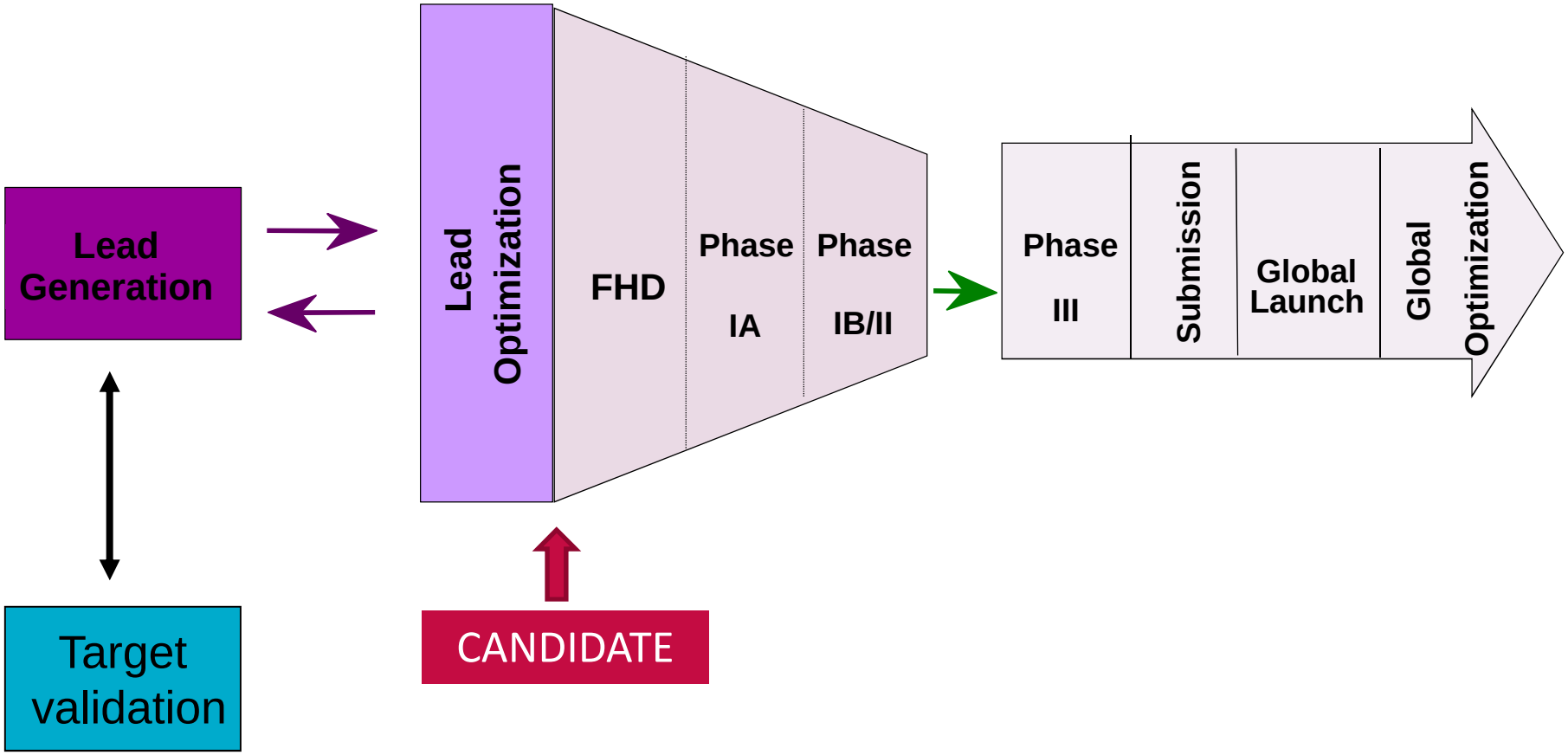
- Implementation of the metabonomics strategy in LEAD Optimization : The COMET project I (2001-2004)

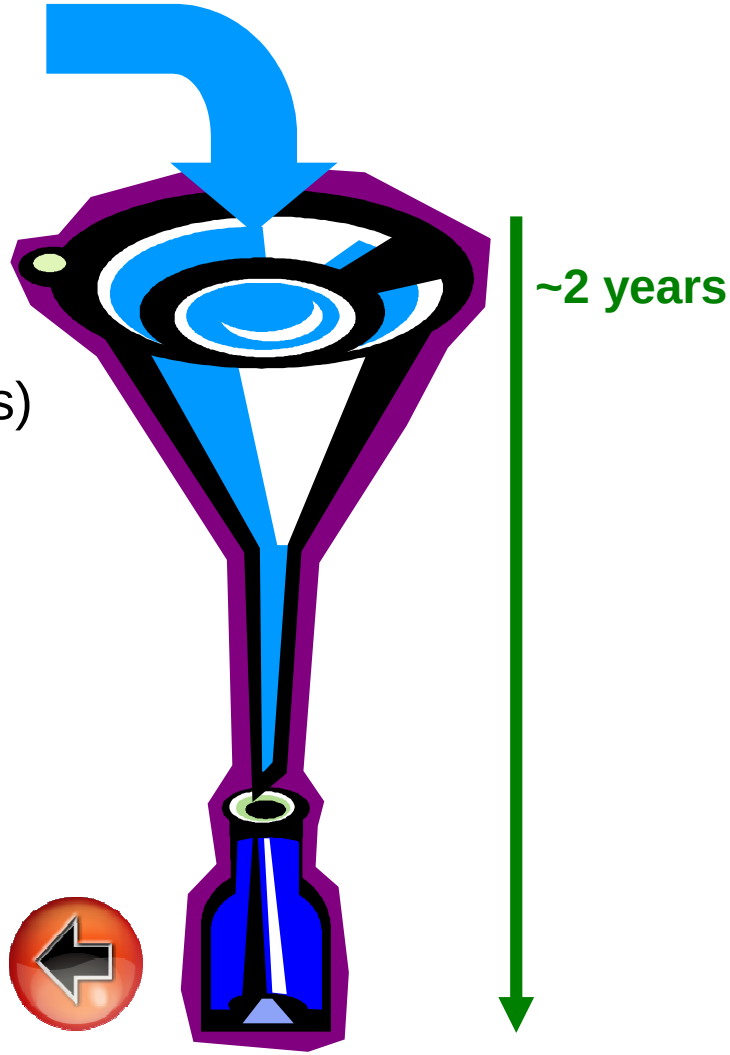
- From « metabonomic signatures » to biomarker(s) of drugs safety/efficacy

Lead Optimization in Drug 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 …

- Time and people-consuming
- Request large amounts of compounds
- Need numerous animals (rodents and non-rodents) ~~3R's~~
- Most important target organs to be addressed:
  - Liver
  - Kidney
  - Heart
  - CNS

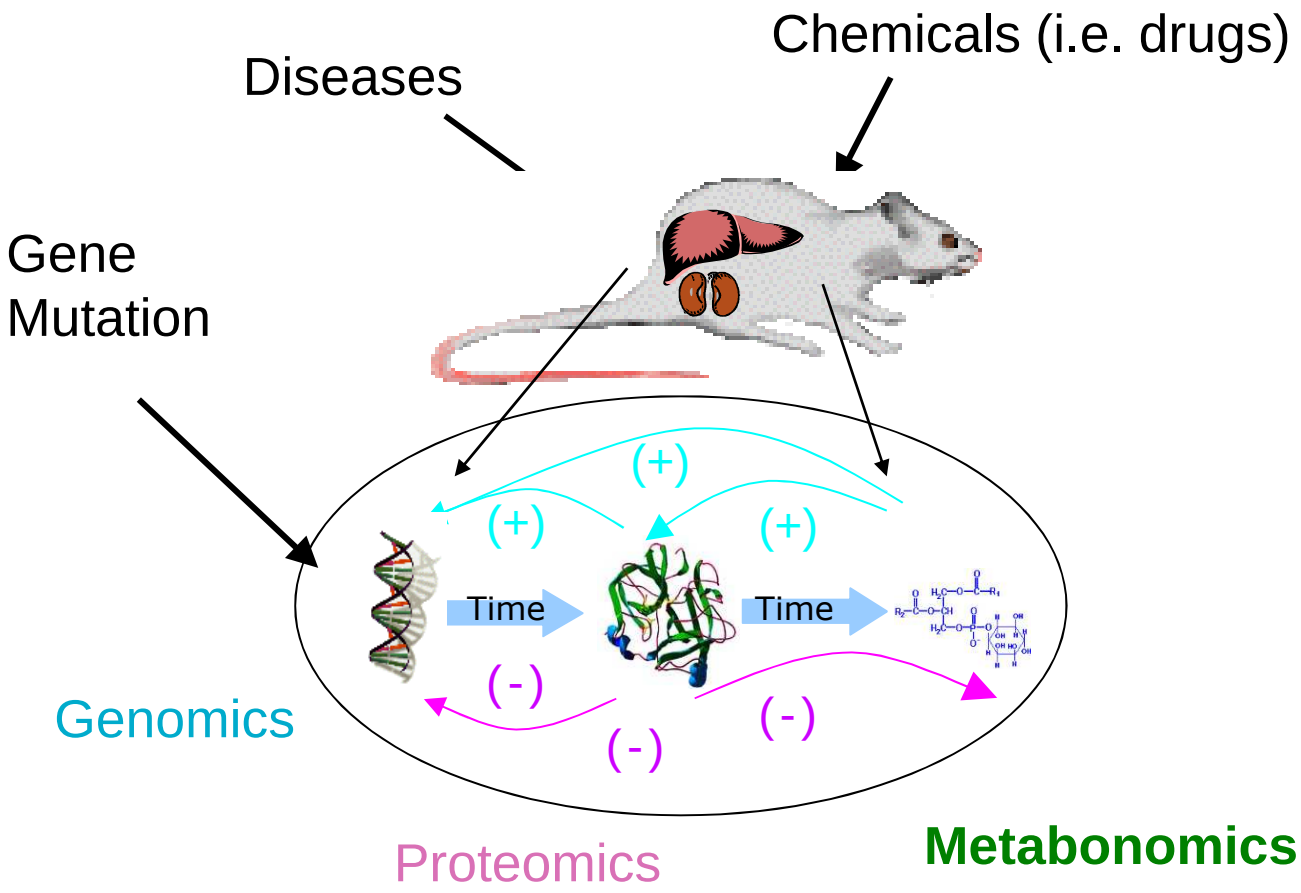
Toxicological studies are the bottleneck …

- Not always predictive of effects seen in humans

Unexpected adverse effects during clinical trials

Idiosyncrasy
⇒ withdrawal from the market

Systems biology to speed up L.O. process



M
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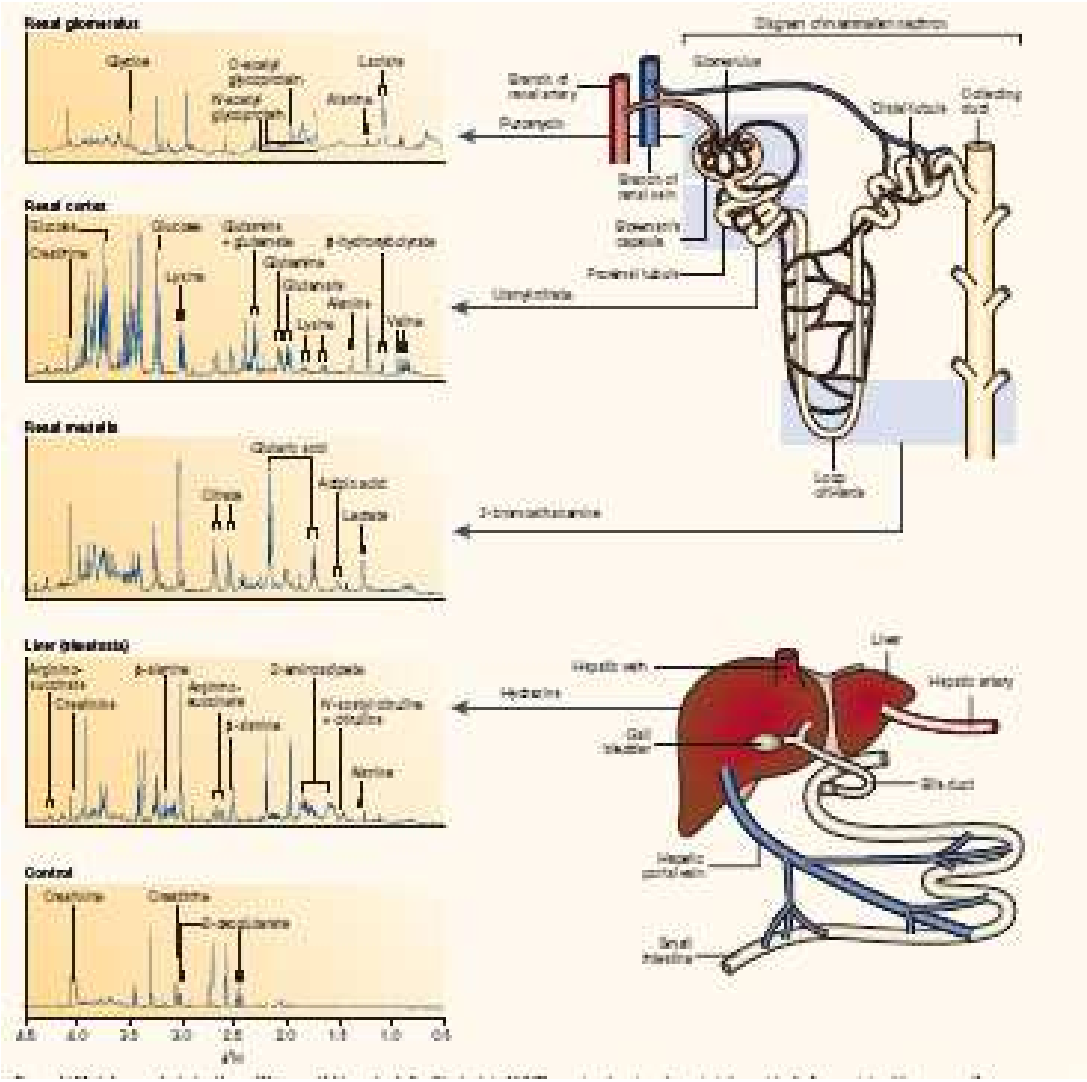


Nicholson JK Nat rev Drug Disc 1(2):153-161 (2002)

COMET

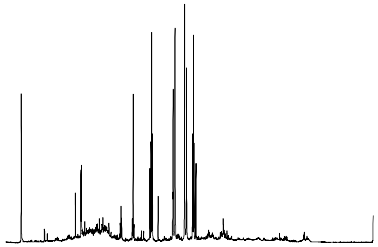
Consortium on Metabonomic in Toxicology

Imperial College-London
BMS Lilly Novo Pfizer Pharmacia Roche

2001-2004

147 prototoxicants

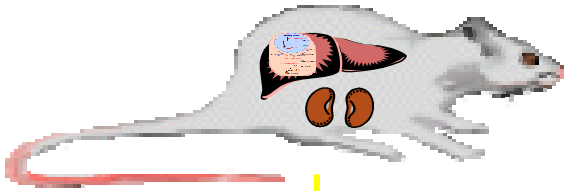
Acute dosing



^{1}H-NMR analysis

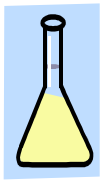
Database
+
Chemometrics models
for toxicity prediction

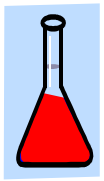
Experimental Protocol



Metabolism cages on refrigerated racks

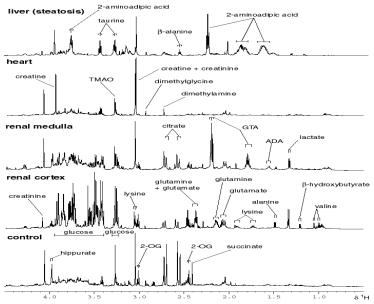
Urine



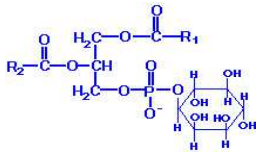


Blood

^{1}H NMR

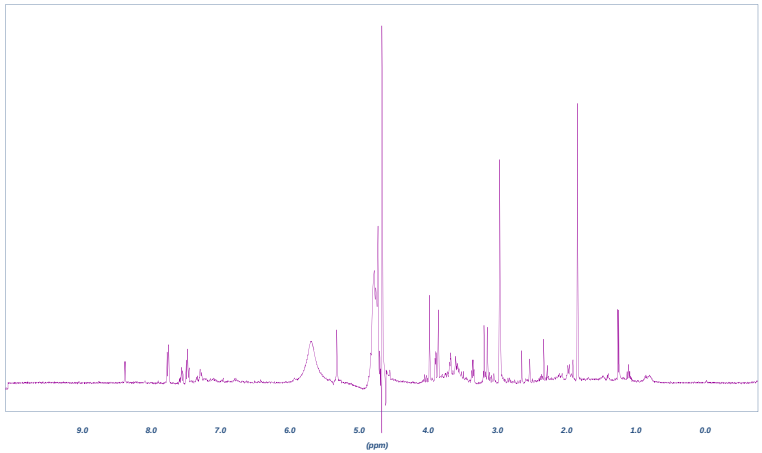


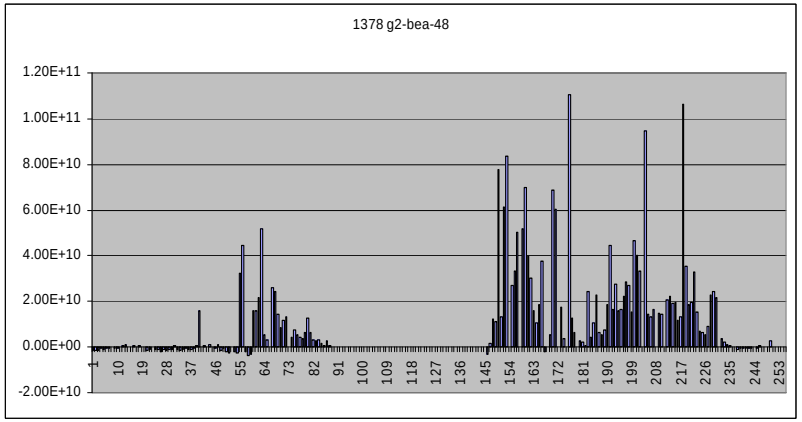
Pattern recognition



Potential biomarkers

Multivariate MBX data analysis





~ 250 descriptors

Binning of the NMR spectra into 250 regions
with same spectral width (0.04 ppm)

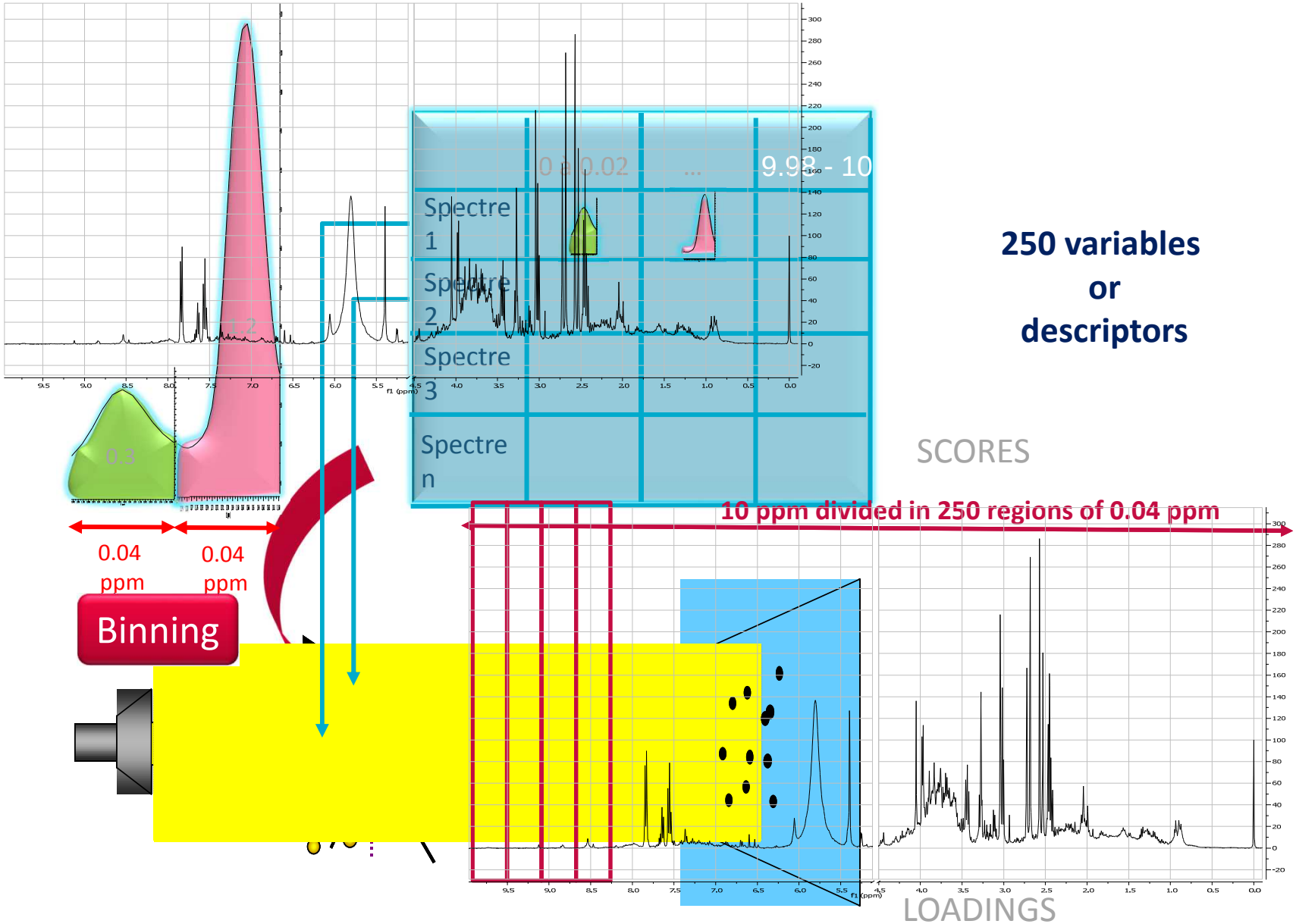
Creating an Excel table

Median Chemical Shift of the descriptor 9.86 to 9.90 ppm

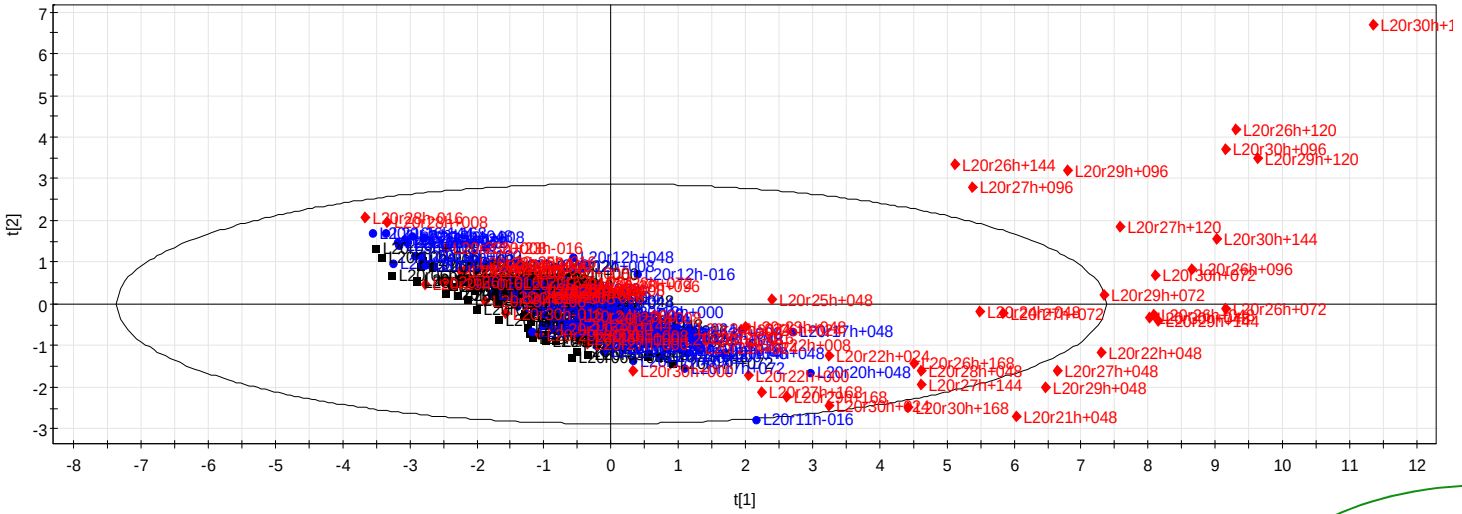
AUC in a descriptor

	9.96	9.92	9.88	9.84	9.8	--	0.4	0.36	0.32	0.28	0.24
L20r01h-016	0.000983	0.0012	-0.00207	0.00186	0.00156	--	0.0076	0.00836	0.00545	0.00793	0.00748
L20r02h-016	0.000161	-0.0021	-0.00026	-0.00063	0.000468	--	0.00684	0.00484	0.00512	0.0104	0.00614
L20r03h-016	0.000428	-0.00026	0.0013	0.00138	0.0026	--	0.0125	0.0102	0.0072	0.0127	0.0111
L20r04h-016	-0.00148	-0.00016	0.000369	0.0016	0.00225	--	0.0117	0.0128	0.00979	0.0169	0.0131
L20r05h-016	-0.00036	-0.00026	0.00152	0.00103	0.00234	--	0.00642	0.00601	0.00581	0.00943	0.00666
L20r06h-016	-0.00061	-0.0006	-0.00058	0.00159	0.00479	--	0.00393	0.00468	0.00291	0.00667	0.00423
L20r07h-016	0.00214	0.00113	-0.00035	0.00188	0.00013	--	0.0104	0.00843	0.0065	0.0141	0.0114
L20r08h-016	0.000229	0.00145	-0.00075	0.000965	0.00115	--	0.00845	0.00668	0.00684	0.00786	0.00444
L20r09h-016	0.000514	0.000777	0.002	0.00194	0.00191	--	0.00659	0.00587	0.00633	0.0101	0.00842
L20r10h-016	0.000283	0.000981	0.00263	0.00134	0.00127	--	0.00415	0.00775	0.00331	0.00958	0.00826
--	--	--	--	--	--	--	--	--	--	--	--
L20r07h+168	-0.00319	-0.00076	0.000934	0.000282	0.00143	--	0.0136	0.0112	0.0105	0.0158	0.0128
L20r08h+168	-0.0013	-0.00024	-0.00041	0.00209	0.00142	--	0.00984	0.0074	0.00839	0.0108	0.00743
L20r09h+168	-0.00049	-0.00045	0.00201	0.000371	0.00199	--	0.0156	0.013	0.0138	0.0168	0.0168
L20r10h+168	-0.00025	0.000264	0.00029	0.00156	0.00154	--	0.0108	0.00908	0.00903	0.0113	0.0116
L20r16h+168	0.00016	0.000599	0.00181	0.00136	0.00153	--	0.00733	0.00573	0.00517	0.00865	0.00589
L20r17h+168	0.00121	0.00016	0.00136	-0.0009	0.000824	--	0.00889	0.00751	0.00737	0.0104	0.00948
L20r18h+168	0.000594	0.00148	0.000818	0.00105	0.000883	--	0.0121	0.0127	0.00947	0.0131	0.0141
L20r19h+168	0.0028	0.000391	-0.00018	0.000905	0.00325	--	0.0104	0.00985	0.00952	0.013	0.0117
L20r20h+168	-0.00058	-0.00076	0.00176	0.00163	0.00126	--	0.00643	0.00641	0.00398	0.0093	0.00709

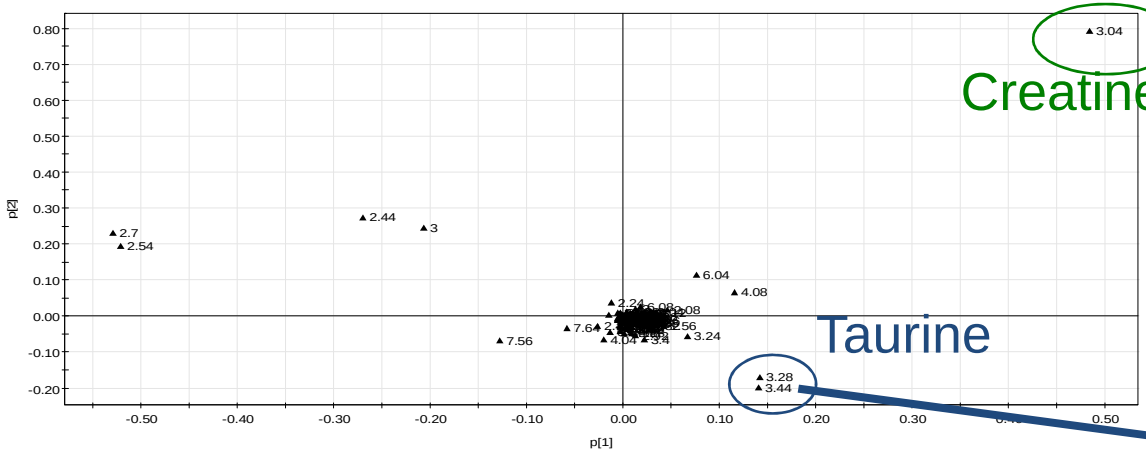
Each line = ^{1}H-NMR spectrum


0 à 0.02
...
9.98 - 10
Spectre 1
Spectre 2
Spectre 3
Spectre n
250 variables
or
descriptors
SCORES
10 ppm divided in 250 regions of 0.04 ppm
0.04 ppm
0.04 ppm
Binning
LOADINGS

Multivariate MBX data analysis



Scores Plot



Creatine

3.04 ppm

Loadings Plot

Taurine

3.28 & 3.44 ppm

COMET: Control Model

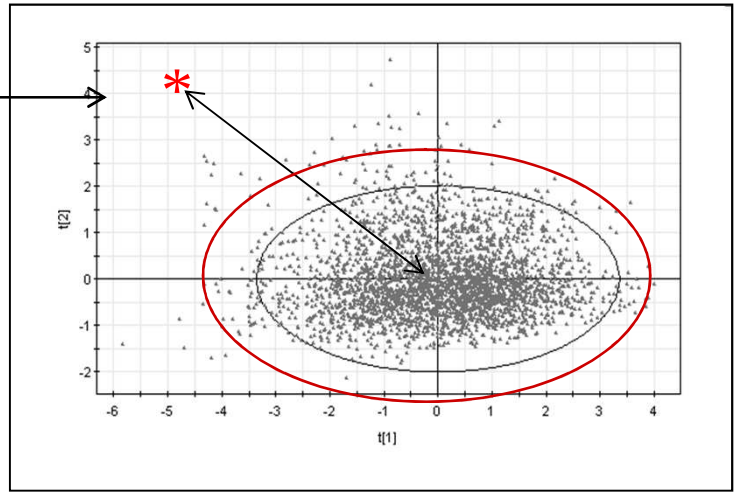
Prediction and Classification of Drug Toxicity Using Probabilistic Modeling of Temporal Metabolic Data: The Consortium on Metabonomic Toxicology Screening Approach
T. Ebbels, H. Keun, O. Beckonert, M. Bollard, J. Lindon, E. Holmes, and Jeremy K. Nicholson
Journal of Proteome Research/2007

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%

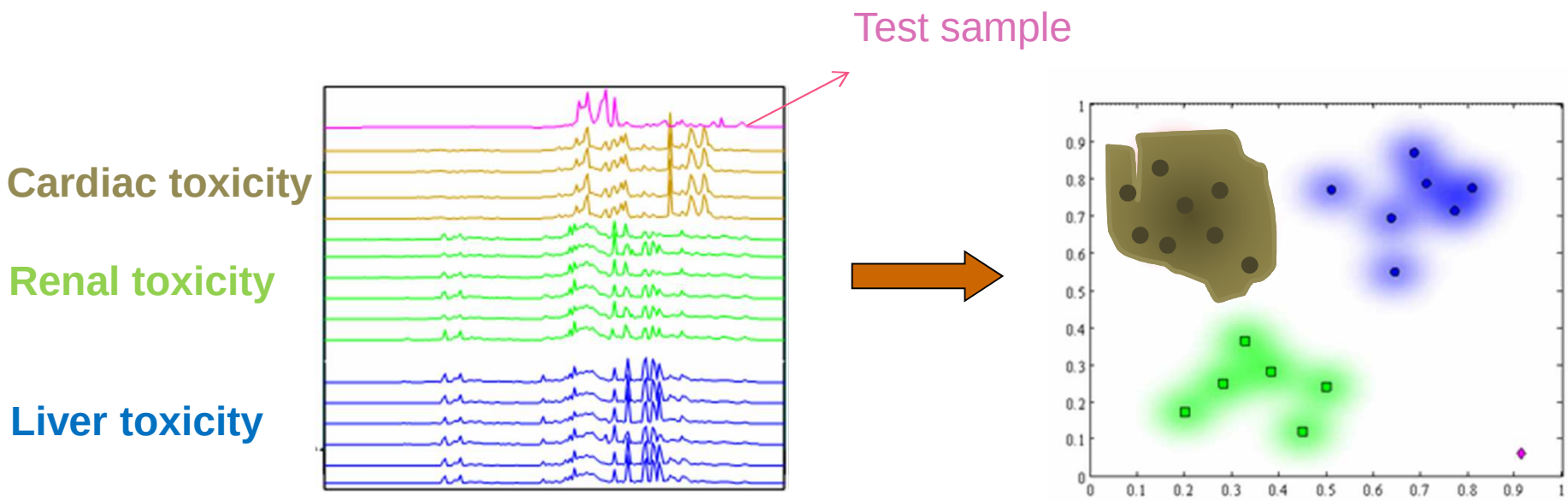


COMET: CLOUDS Model (Classification Of Unknowns by Density Superposition)

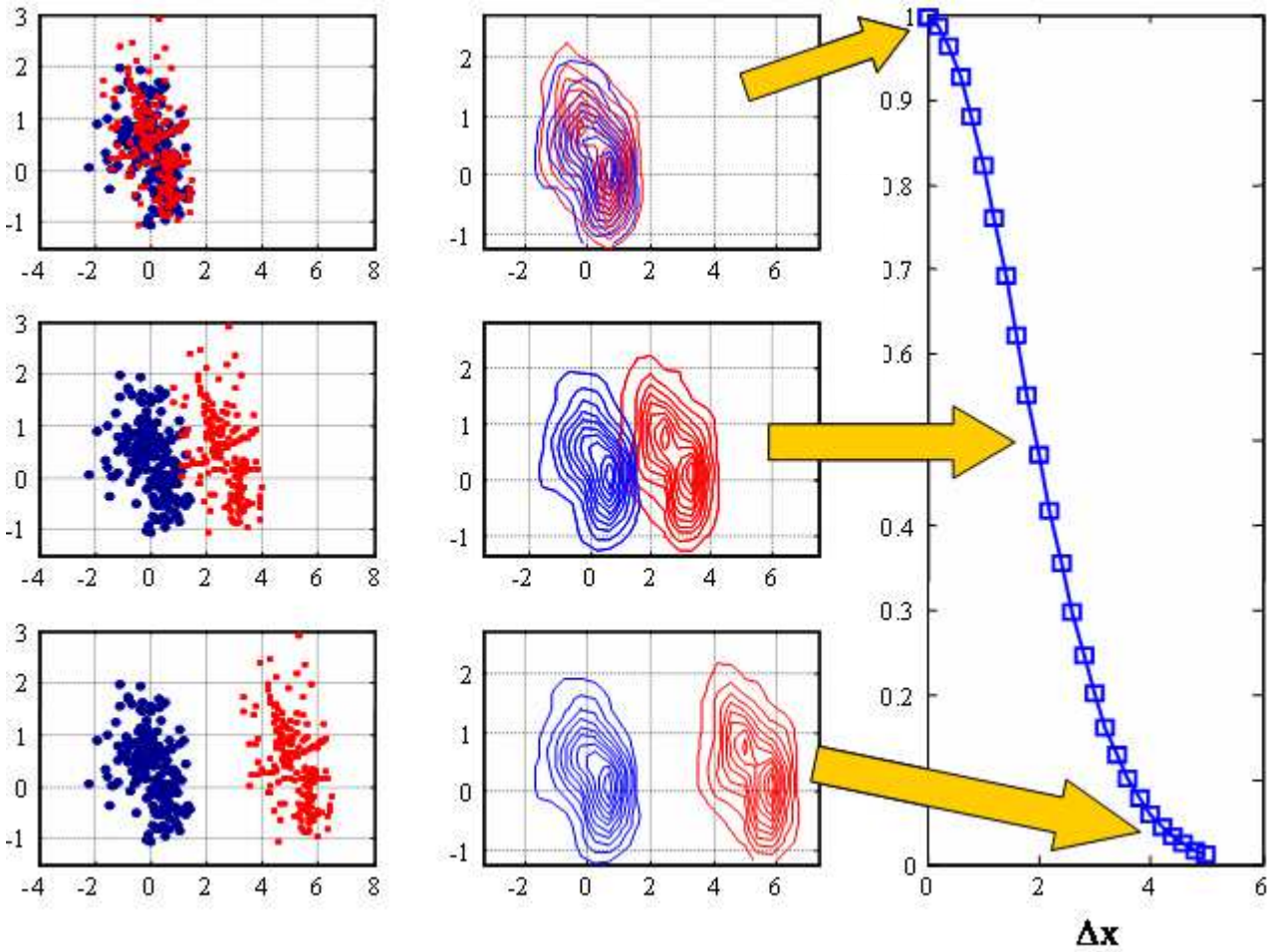
Prediction and Classification of Drug Toxicity Using Probabilistic Modeling of Temporal Metabolic Data: The Consortium on Metabonomic Toxicology Screening Approach
T. Ebbels, H. Keun, O. Beckonert, M. Bollard, J. Lindon, E. Holmes, and Jeremy K. Nicholson
Journal of Proteome Research/2007

Identifying toxicity type

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

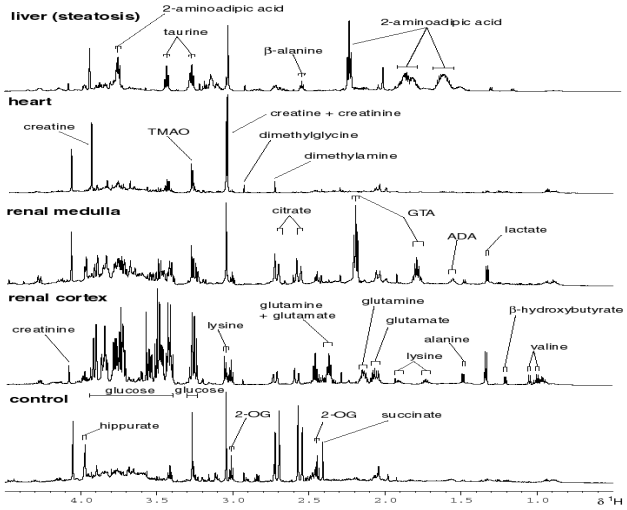


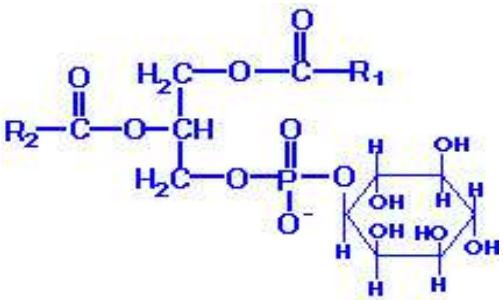
Clouds approach



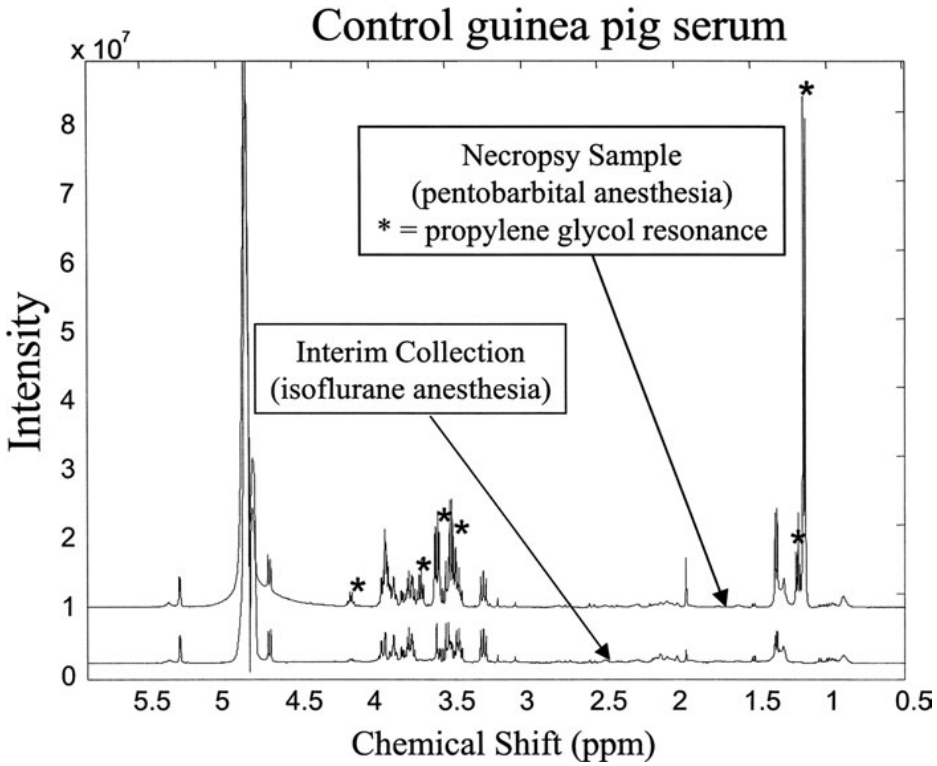
Classification based on similarity

From organ-specific toxic signatures to biomarkers identification





DRC's (DRUG RELATED COMPOUNDS)

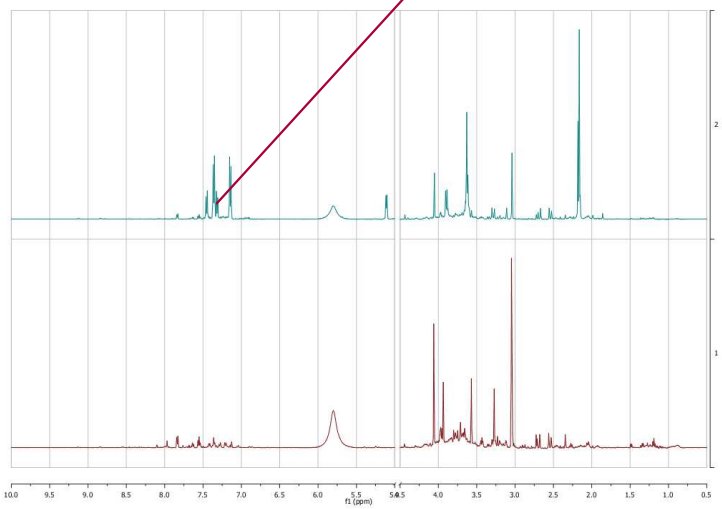


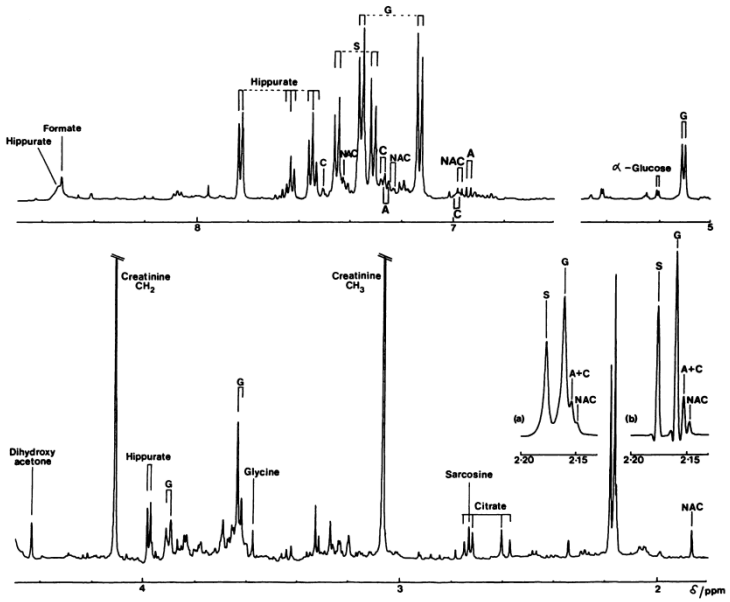
Robertson DG et al. Toxicological Sciences 85, 809–822 (2005)

Comparative 600 MHz ^{1}H-NMR spectra from serum samples from guinea pigs anesthetized with isoflurane (bottom trace) or parenteral pentobarbital containing 40% propylene glycol by volume (top trace). Note the numerous resonances attributable to the propylene glycol in the injected anesthetic.

DRC's (DRUG RELATED COMPOUNDS)

Acetaminophen and/or metabolites



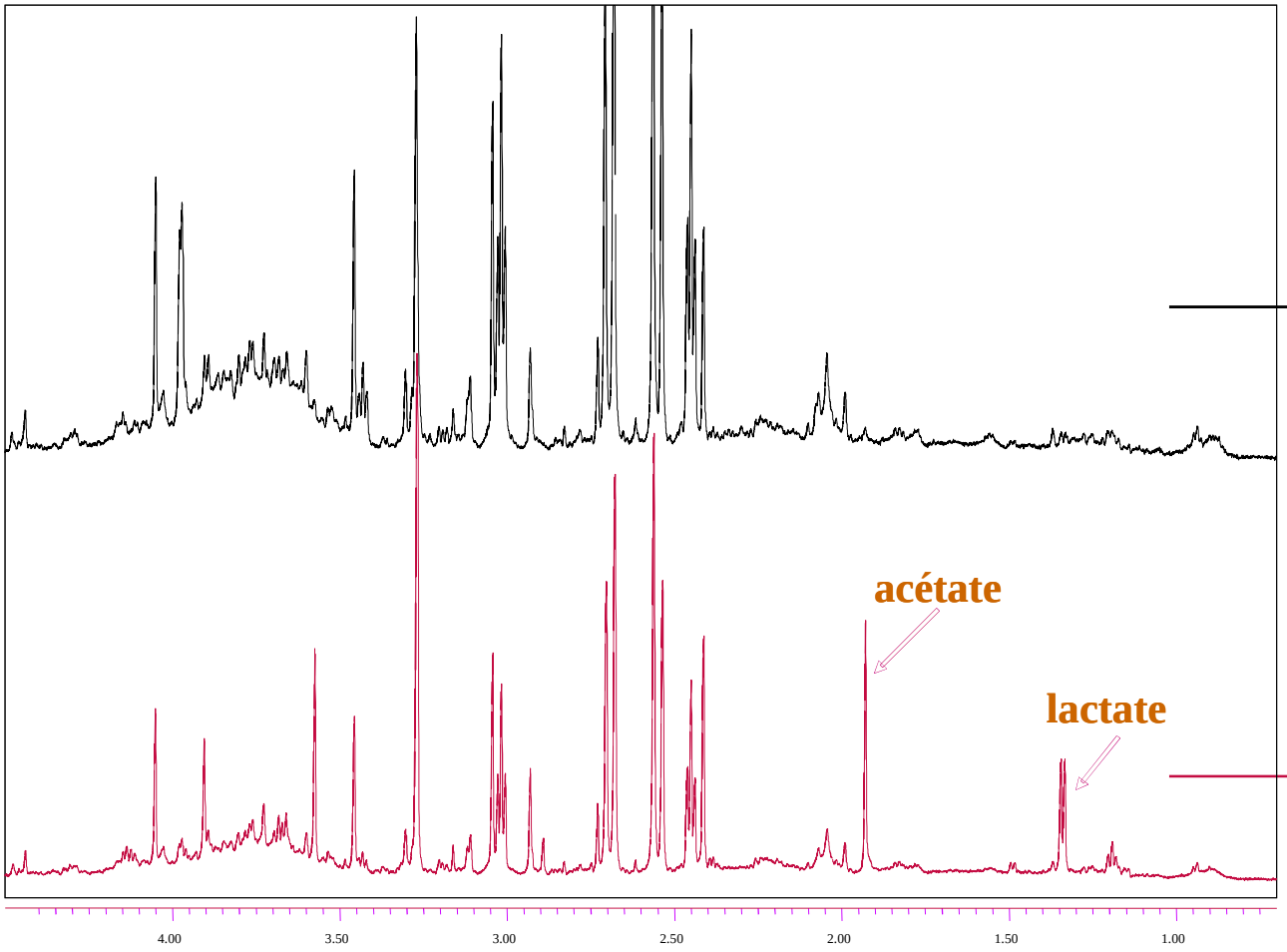


Clin Chem. 1984 Oct;30(10):1631-6.

Urinary excretion of acetaminophen and its metabolites as studied by proton NMR spectroscopy.

Bales JR, Sadler PJ, Nicholson JK, Timbrell JA.

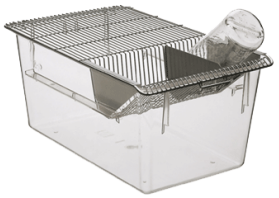
Wrong data interpretation due to bacterial contamination…



Urine collected at 4°C + NaN_3

Bacterial contamination

- 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



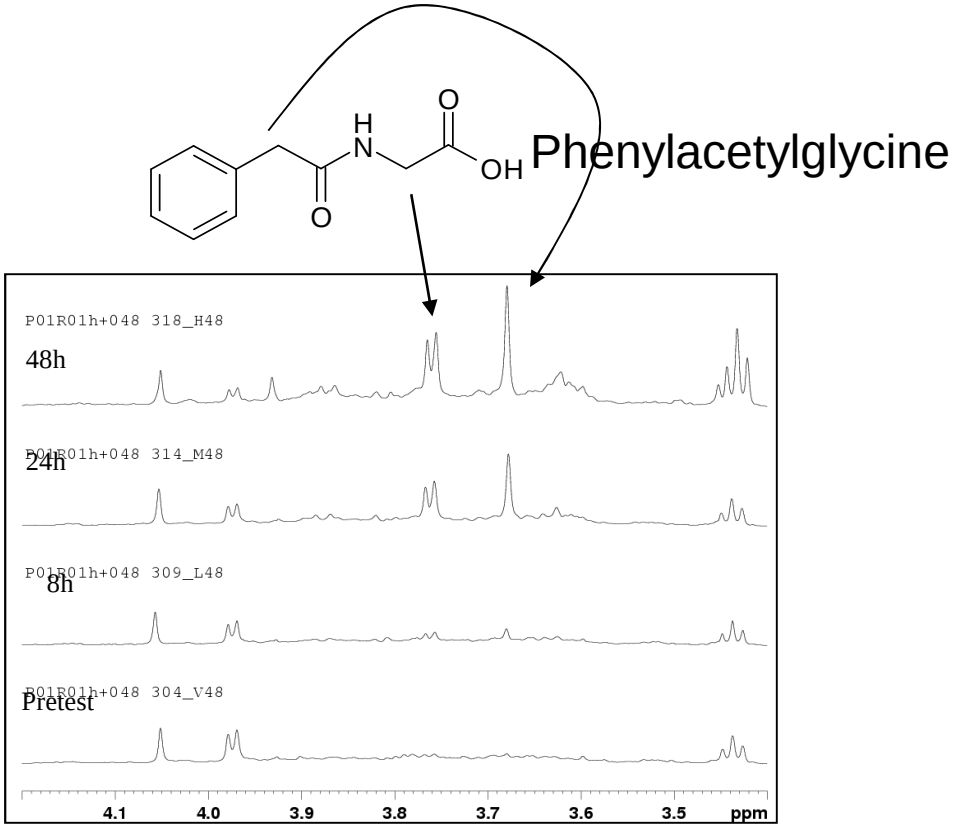
Avoid bacterial contamination !

Biomarker of phospholipidosis

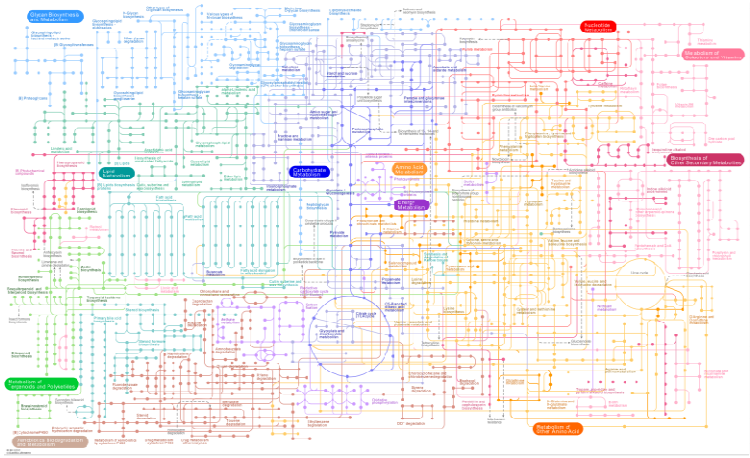
From animals to humans …

CAD's (Catationic Amphiphilic Drugs) sequestring phospholipids in vacuoles

reversible effect but often associated with a more severe toxicity



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



?

Excretion of glycine conjugates in urine is species-dependent :

- Benzoic acid converted in hippuric acid in primates, rodents, and rabbits

- Phenylacetic acid is converted in phenylacetylglycine in rats but in phenylacetylglutamine in humans

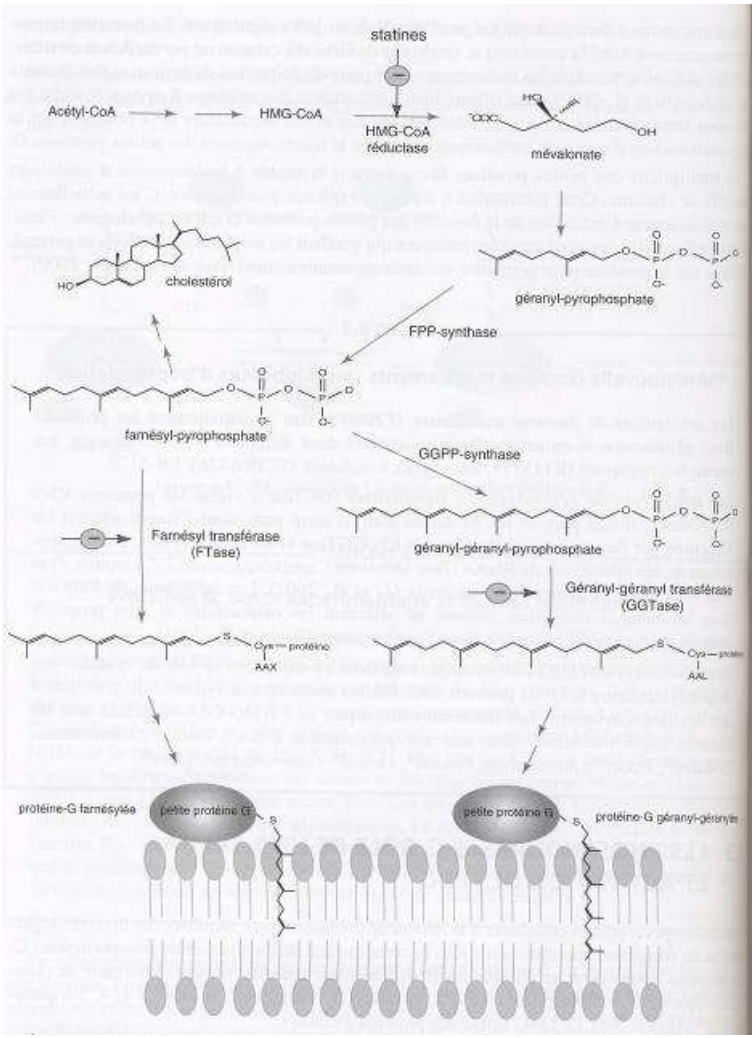
JK Nicholson et al
Nat rev Drug Discov ; 2002; 1(2) : 153 - 61

Biomarkers of inhibitors of isoprenylation (cancer drugs)

Anti-cancer drug strategy

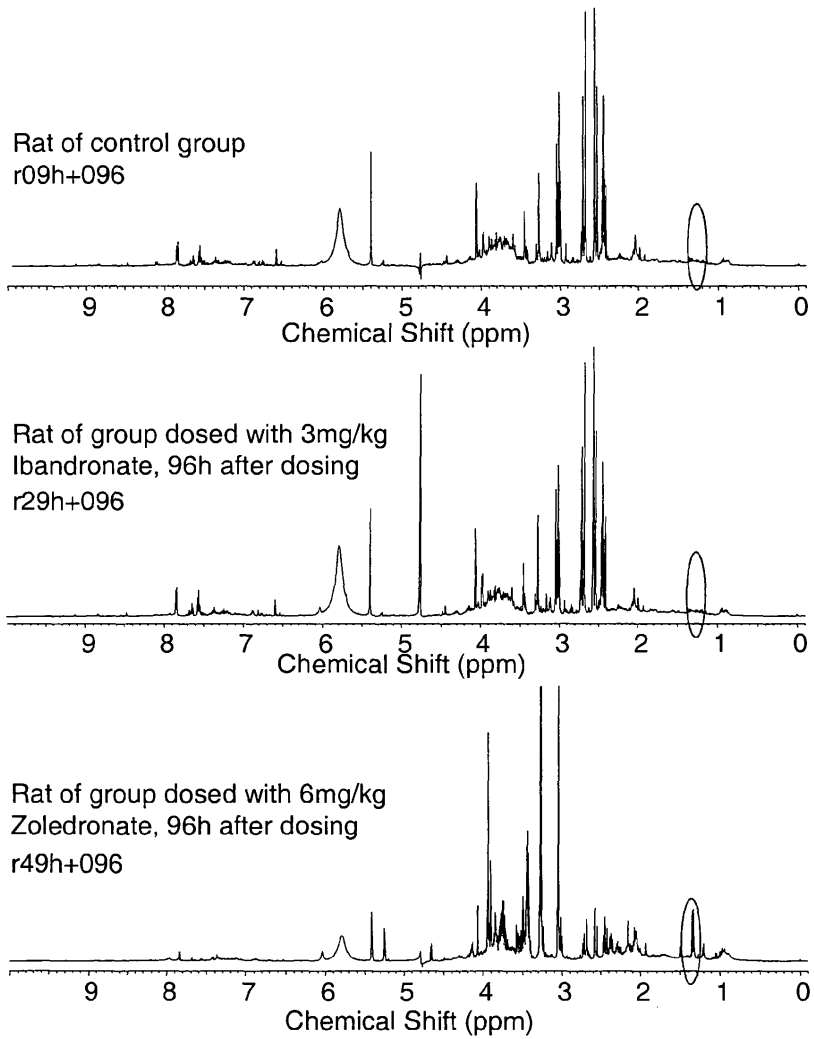
↓

Inhibition of isoprenylation
to prevent binding of
small G proteins
to cell plasma membranes



Biomarkers of inhibitors of isoprenylation (cancer drugs)

Biomarker of drug efficacy



Dieterle F. et al
Chem Res Toxicol. (2007) ;
20(9) : 1291-9

Urine N-acetylfelinine excretion as a biomarker for inhibition of the farnesyl pathway

(Patent of Hoffman-La Roche)

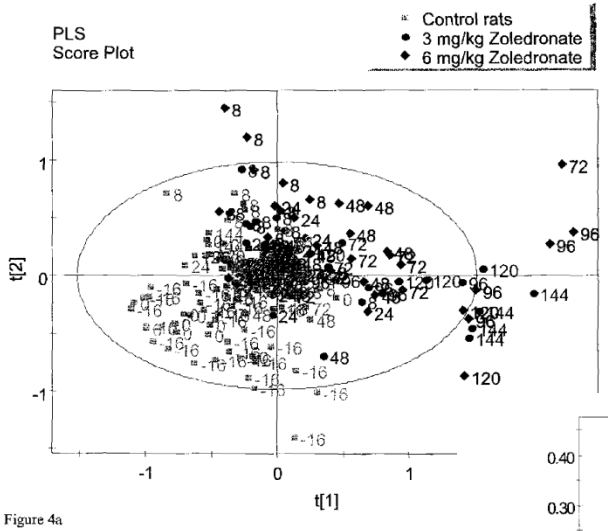
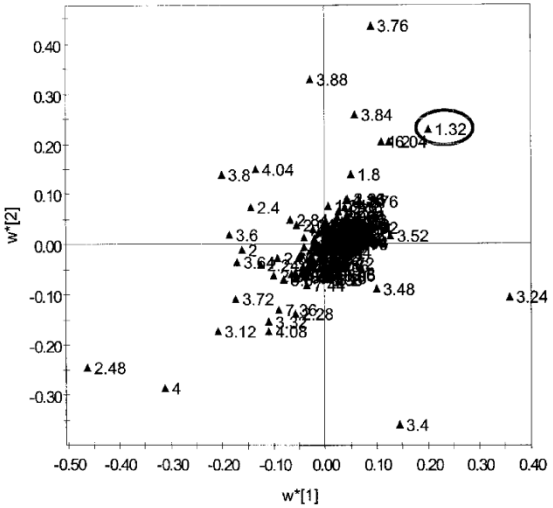
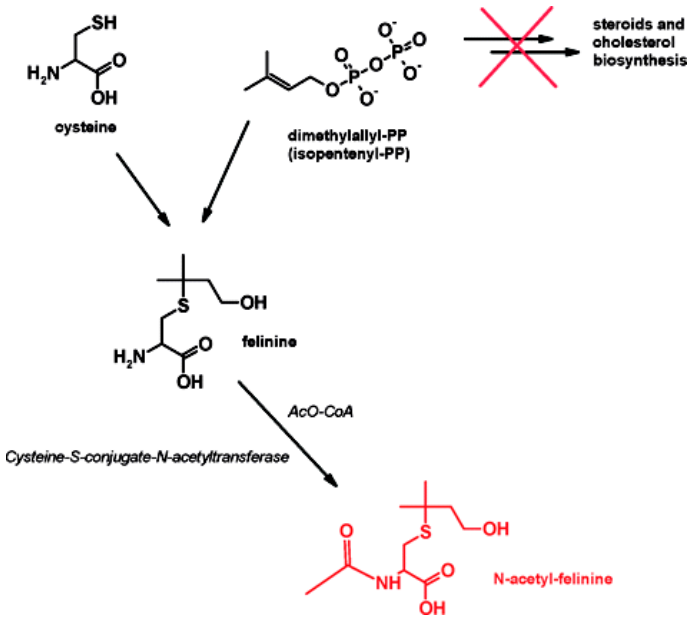


Figure 4a





Confounding intrinsic factors

Drug efficacy ⇔ drug safety

For instance, the large reduction in urinary excretion of the TCA cycles intermediates succinate, citrate, and 2-oxoglutarate observed as a result of metabolic acidosis induced by a non toxic renal carbonic anhydrase inhibiotor, such as acetazolamide, could possibly be mistaken for a toxic response

Metabonomics: a platform for studying drug toxicity and gene function
Jeremy K. Nicholson, John Connelly, John C. Lindon & Elaine Holmes
Nat Rev Drug Discov. 2002 Feb;1(2):153-61

The systemic biochemical effects of oral hydrazine acute dosing have been investigated in male Han Wistar rats using metabonomic analysis of ^{1}H NMR spectra of urine

Hydrazine liver toxicity

Decreased urine excretion	Increased urine excretion
hippurate	taurine
Citrate	Creatine
succinate	threonine
2-oxoglutarate	N-methylnicotinic acid
TMAO	tyrosine
fumarate	arginosuccinate
creatinine	N-acetylcitrulline

APPEARANCE OF RESONANCES CORRESPONDING TO 2-AMINOADIPATE!!

Hydrazine CNS toxicity

Nicholls, A. W., et al..
Chem. Res. Toxicol. (2001); 14, 975–987.

«Mechanistically linked the neurotoxic effects of hydrazine to markedly increased levels of 2-aminoadipate (2AA), which is known to affect kynurenic acid levels in the brain, thus providing a plausible hypothesis for the heretofore unexplained neurotoxic effects of the compound"

Nicholls, A. W., et al
Chem. Res. Toxicol. (2001); 14, 975–987.

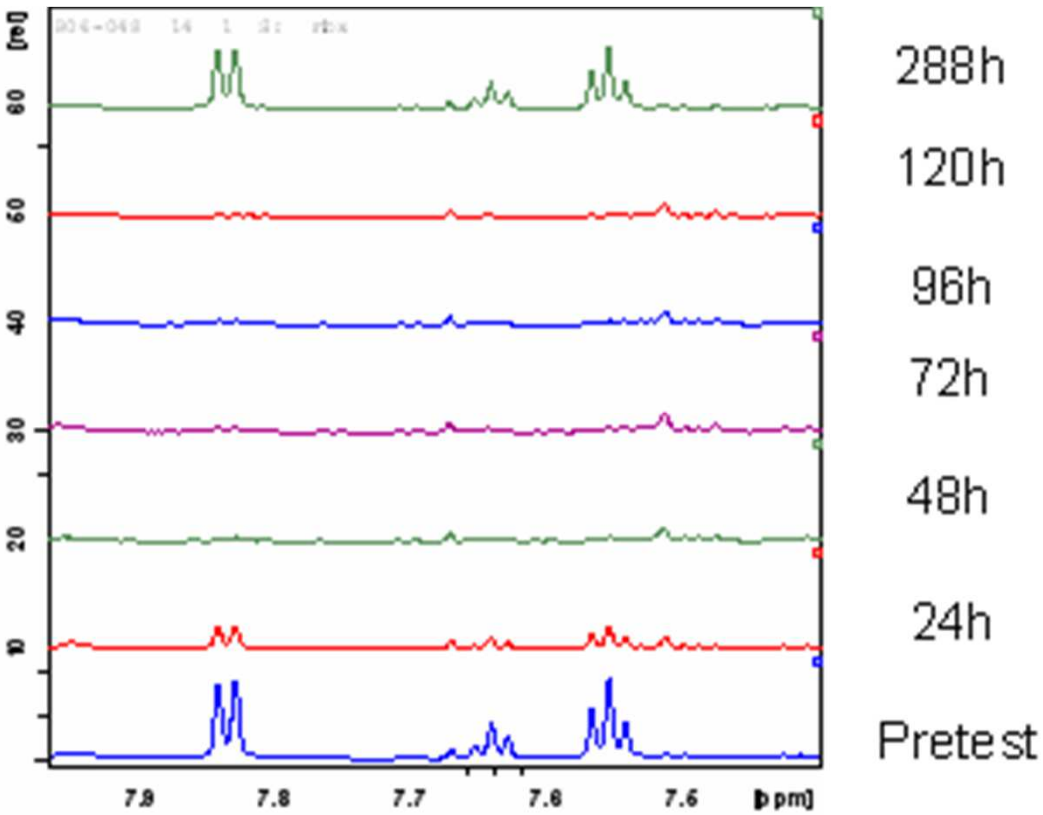
USUAL SUSPECTS ….

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



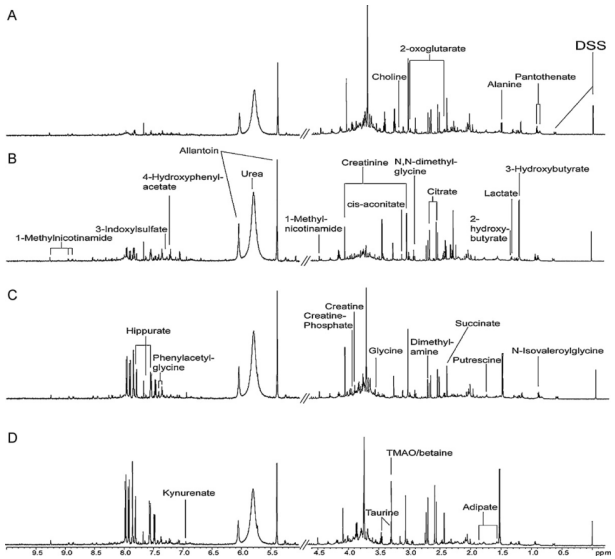
Liver (Σ)

Kidney (OATP1)

Intestinal flora (Σ)

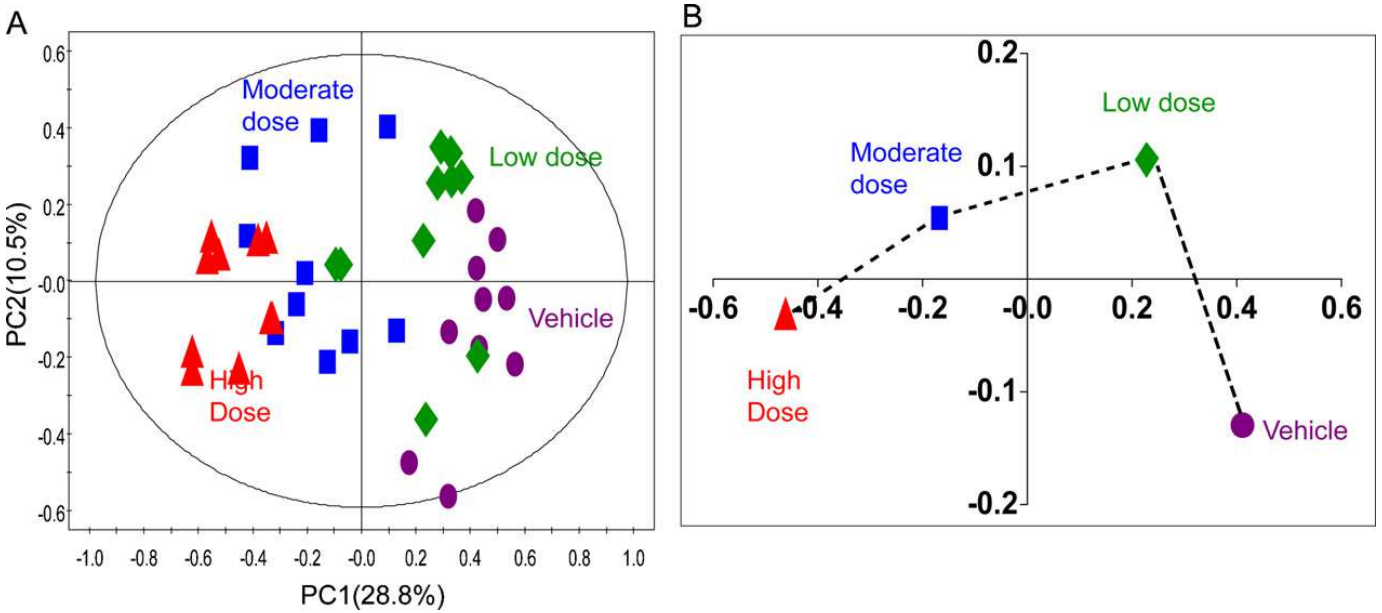
Drug-induced renal proximal tubular injury

Usual suspects … metabolism

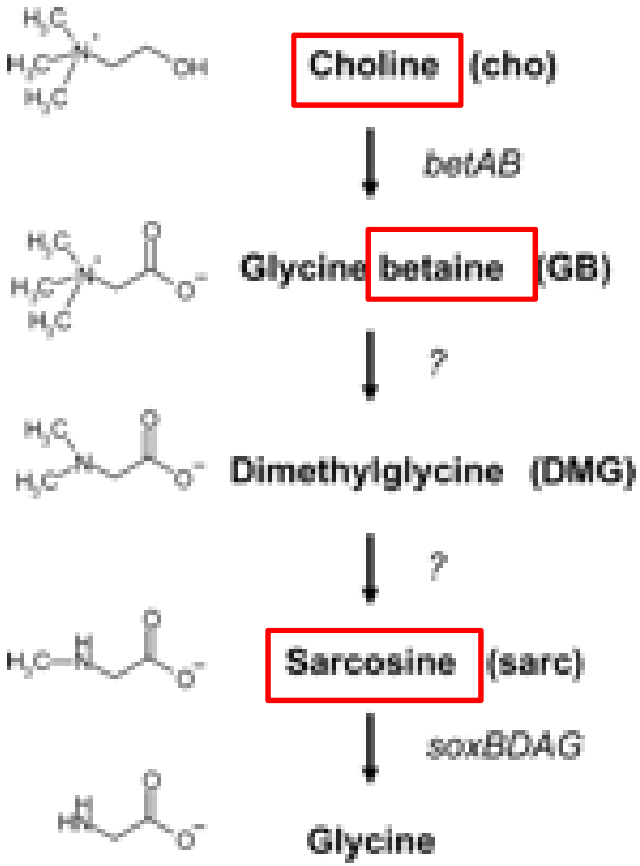


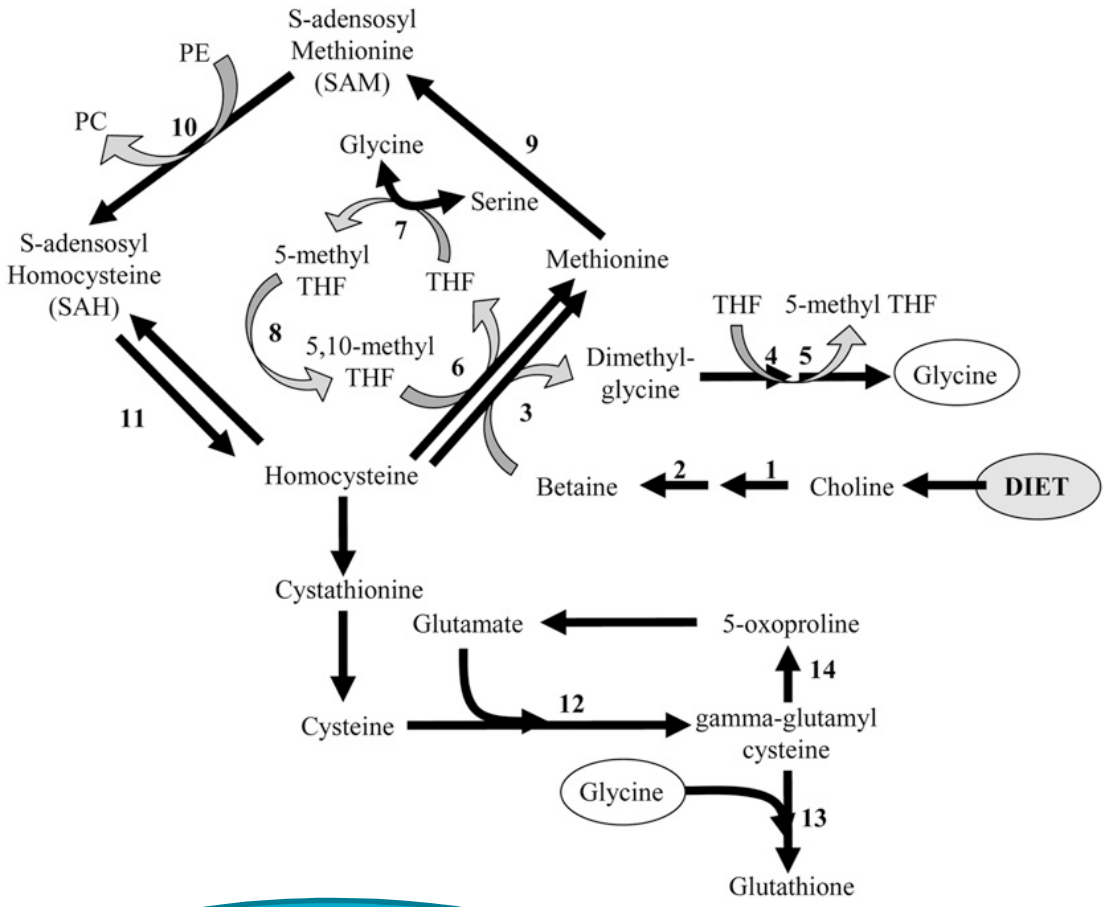
Jeeyoun Jung et al,
Toxicology Letters 200 (2011) 1–7

Fig. 2. Representative 600-MHz 1H NMR spectra of urine from rats treated with vehicle (A) or naproxen at a low (B), moderate (C), or high (D) dose.

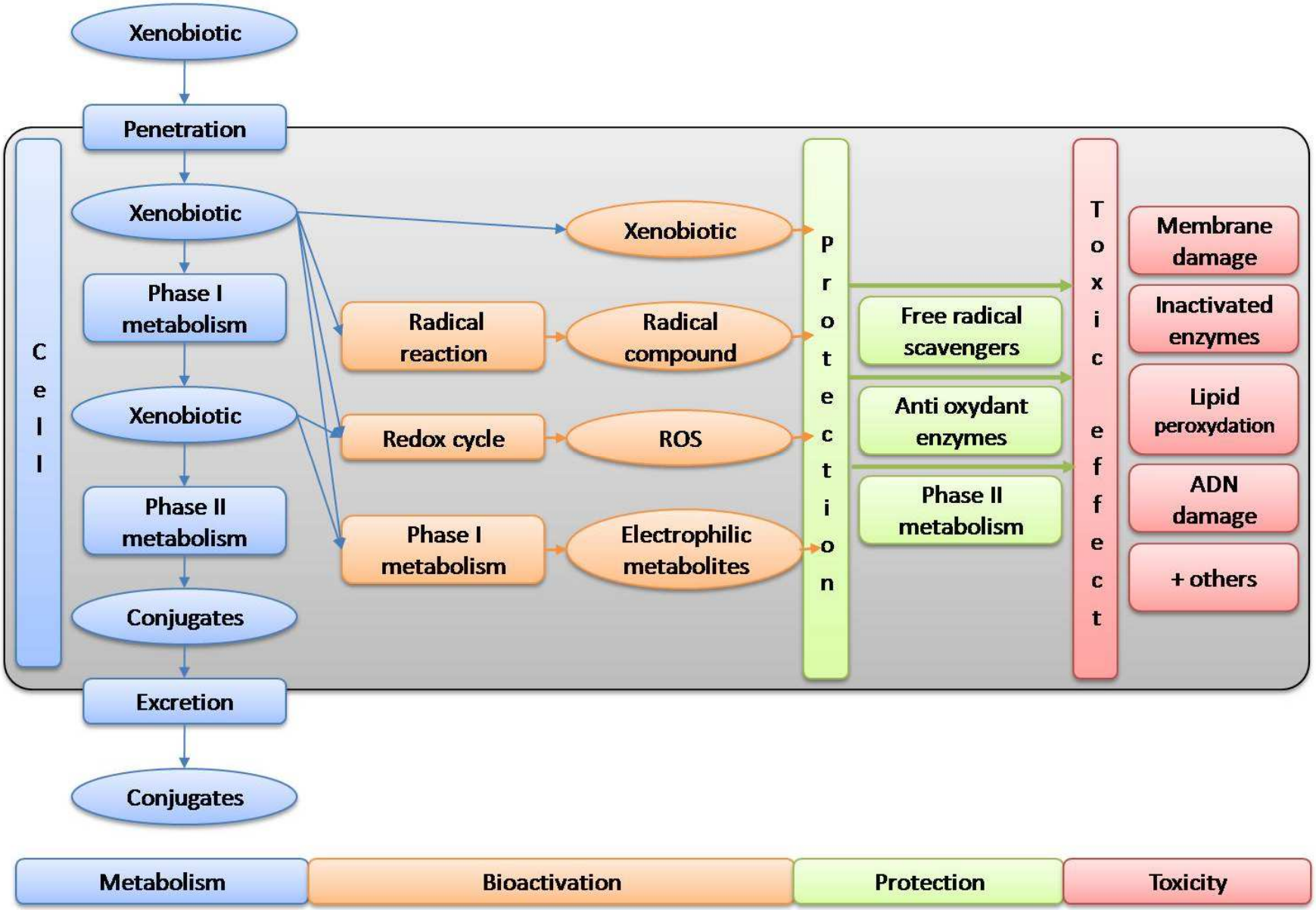


Usual suspects … metabolism





Glutathione


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

Conclusions

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

- Significant impact in drug 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

The team

Thank you for your attention