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Metabolomics and the Kidney : Perspectives of Development

BVN-SBN Vilvorde, December 13th 2018

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UMONS

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

Presentation Layout

METABOLOMICS Methodology

Applying Metabolomics to
Aristolochic Acids Nephropathy (AAN) :
A Case Study

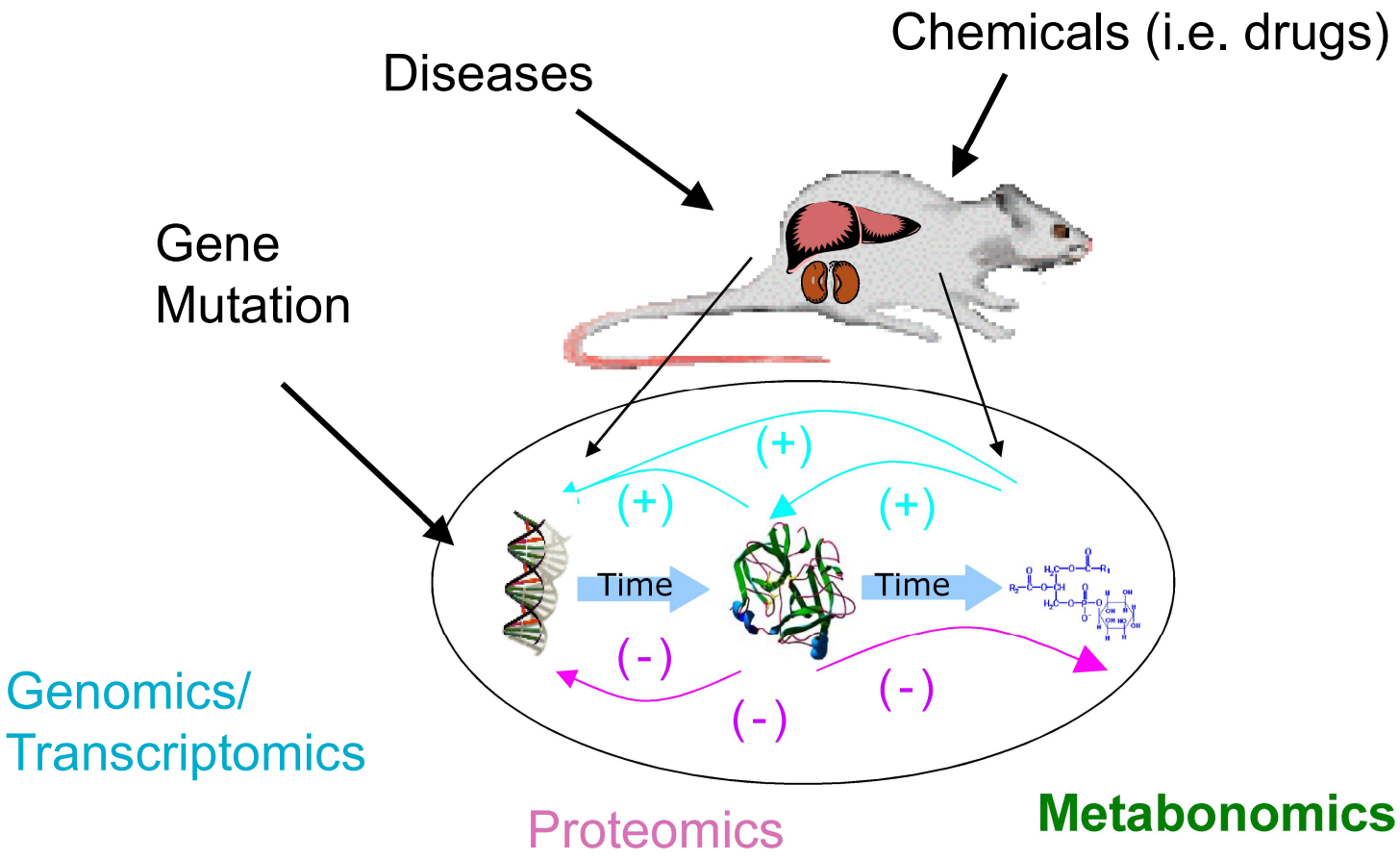
PRECLINICAL Development :

- The COMET Initiative
- Rodent AAN Models

CLINICAL CASES :

- BEN (Balkan Endemic Nephropathy)
- AA-contaminated slimming pills

Systems biology



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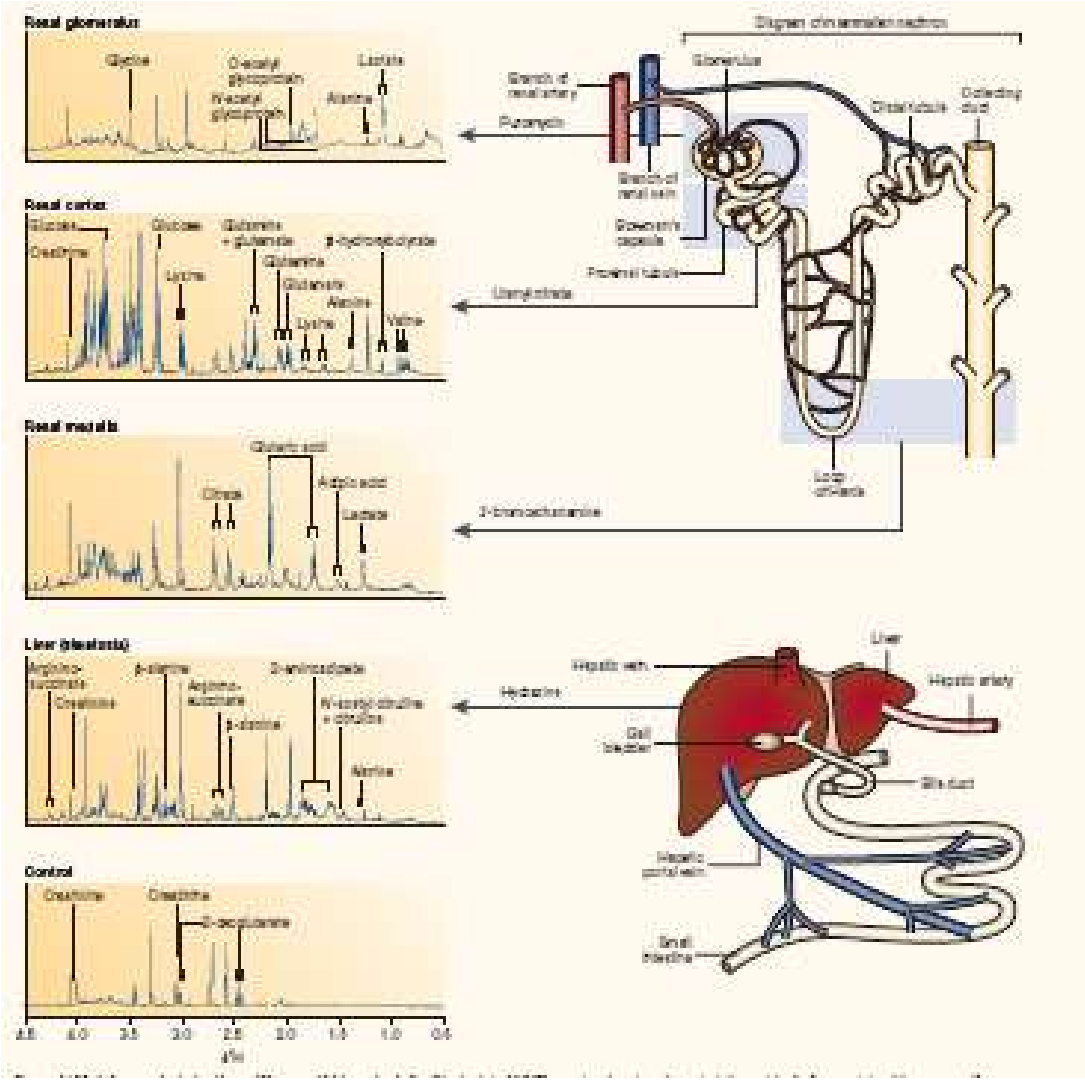
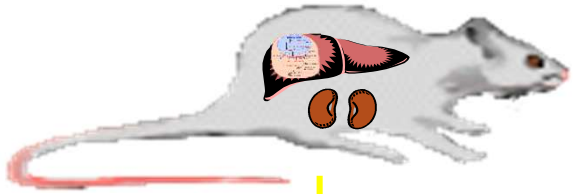


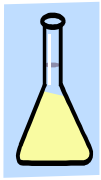
Nicholson JK Nat rev Drug Disc 1(2):153-161 (2002)

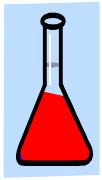
Experimental Protocol



Metabolism cages on refrigerated racks

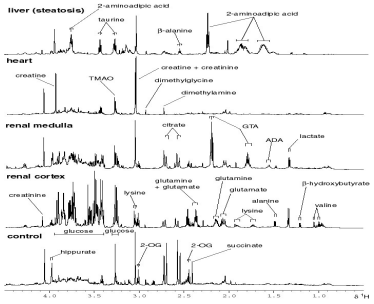
Urine



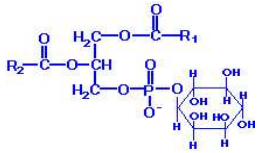


Blood

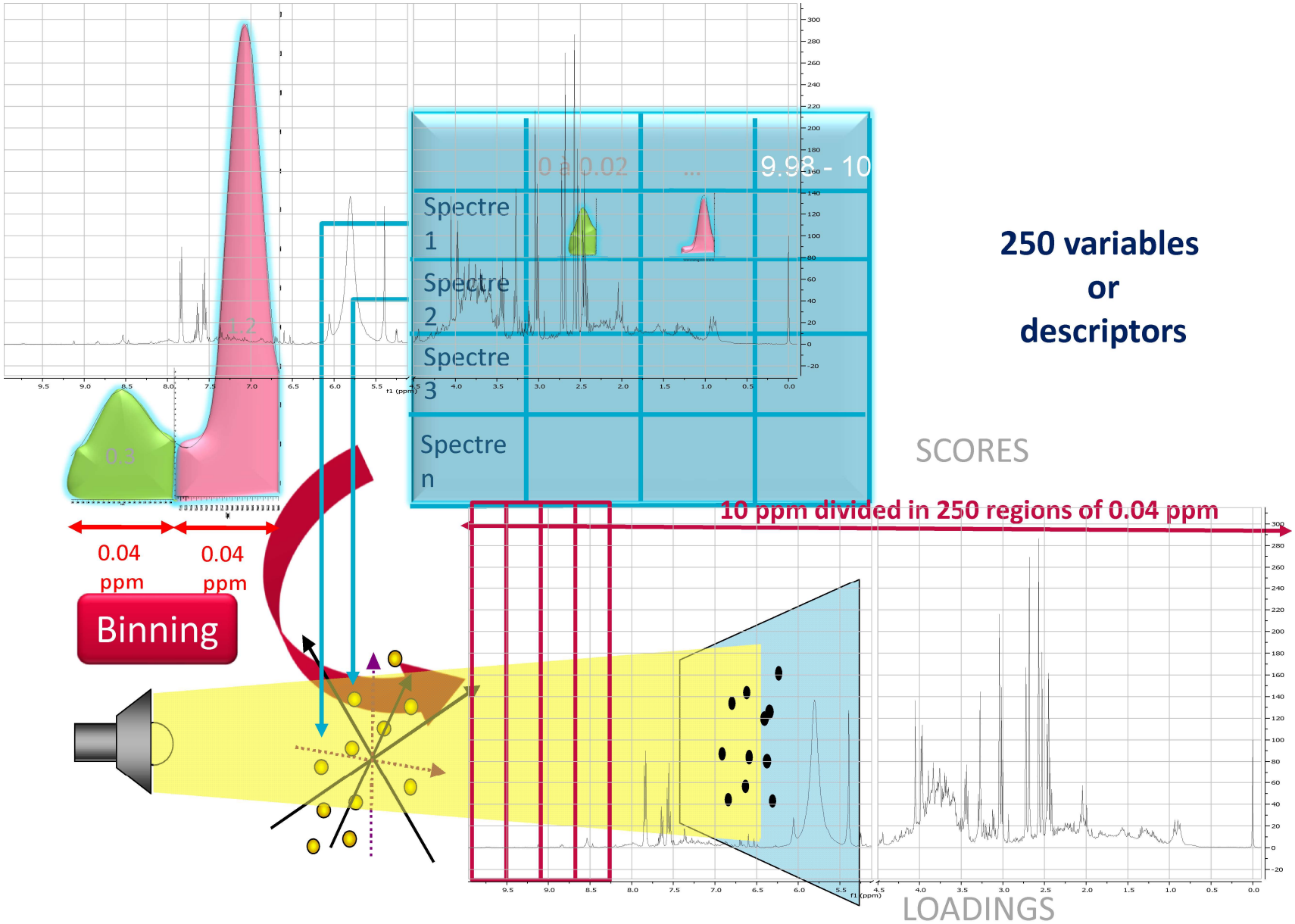
^{1}H NMR



Pattern recognition



Potential biomarkers



250 variables or descriptors

Creating an Excel table

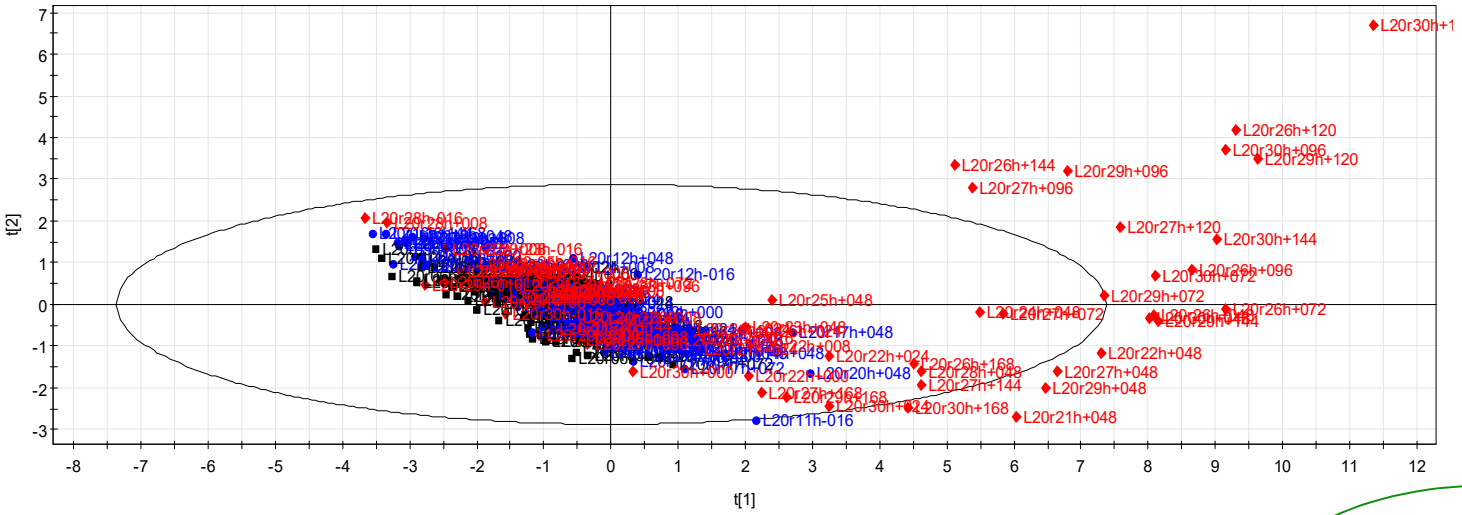
Median Chemical Shift of the descriptor 9.86 to 9.90 ppm

AUC in a descriptor

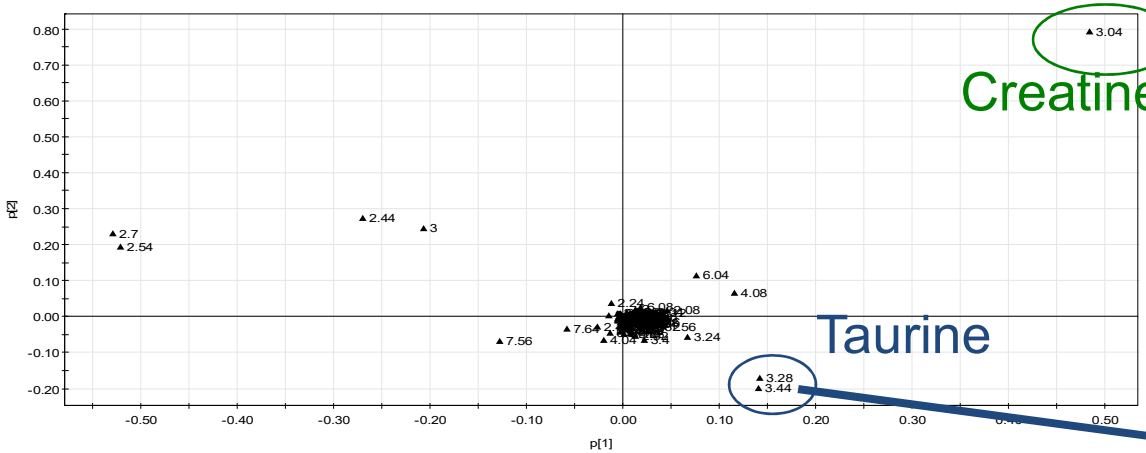
	9.96	9.92	9.88	9.84	9.8	--	0.4	0.36	0.32	0.28	0.24
L20r01h-016	0.000983	0.0012	-0.00207	0.00186	0.00156	--	0.0076	0.00836	0.00545	0.00793	0.00748
L20r02h-016	0.000161	-0.0021	-0.00026	-0.00063	0.000468	--	0.00684	0.00484	0.00512	0.0104	0.00614
L20r03h-016	0.000428	-0.00026	0.0013	0.00138	0.0026	--	0.0125	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

Multivariate MBX data analysis



Scores Plot



Creatine

3.04 ppm

Loadings Plot

Taurine

3.28 & 3.44 ppm

COMET

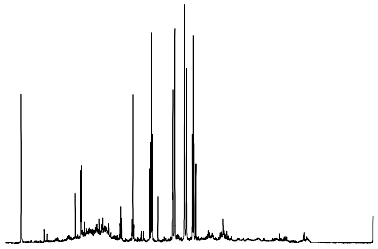
Consortium on Metabonomic in Toxicology

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

COMET: Control Model

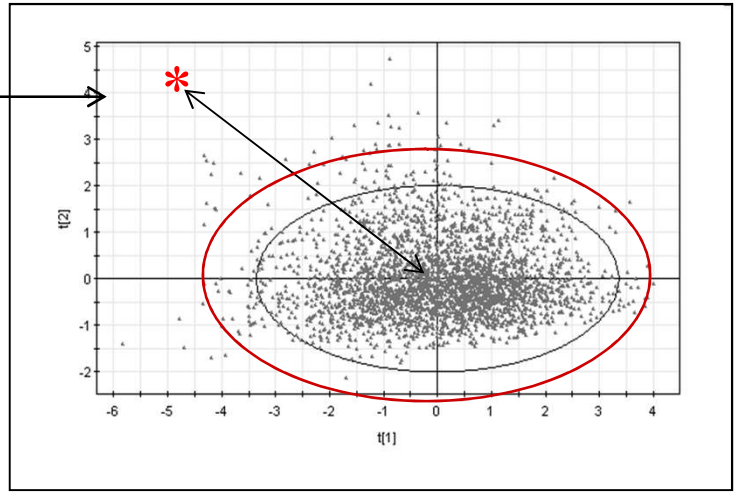
Prediction and Classification of Drug Toxicity Using Probabilistic Modeling of Temporal Metabolic Data: The Consortium on Metabonomic Toxicology Screening Approach
T. Ebbels, H. Keun, O. Beckonert, M. Bollard, J. Lindon, E. Holmes, and Jeremy K. Nicholson
Journal of Proteome Research/2007

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%

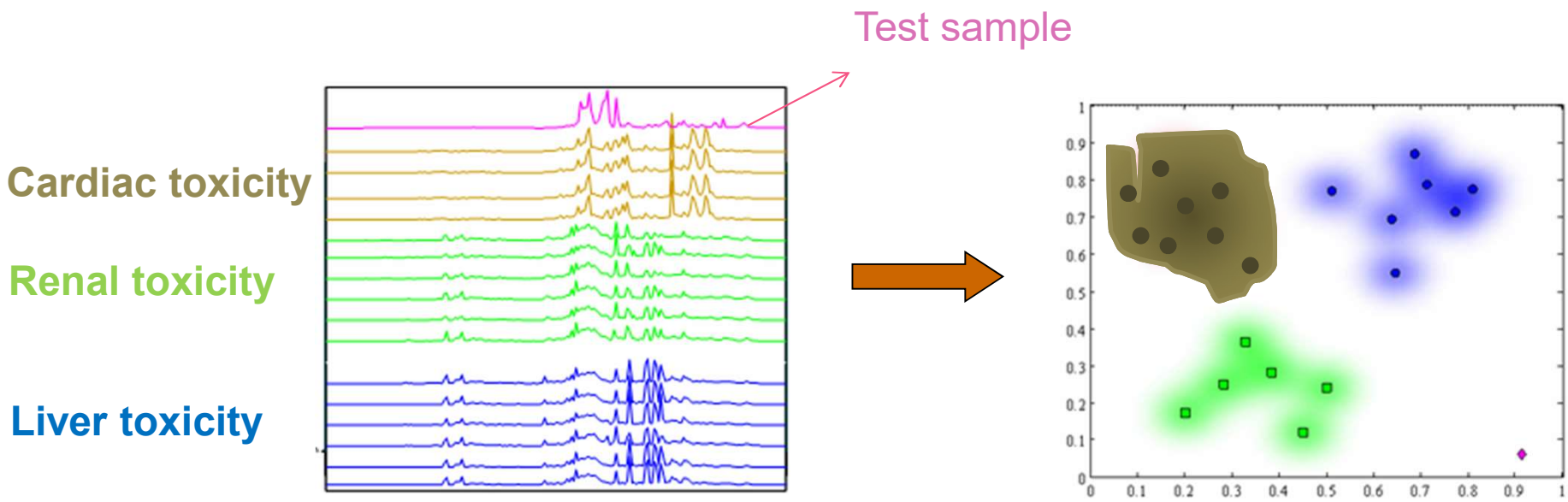


COMET: CLOUDS Model (Classification Of Unknowns by Density Superposition)

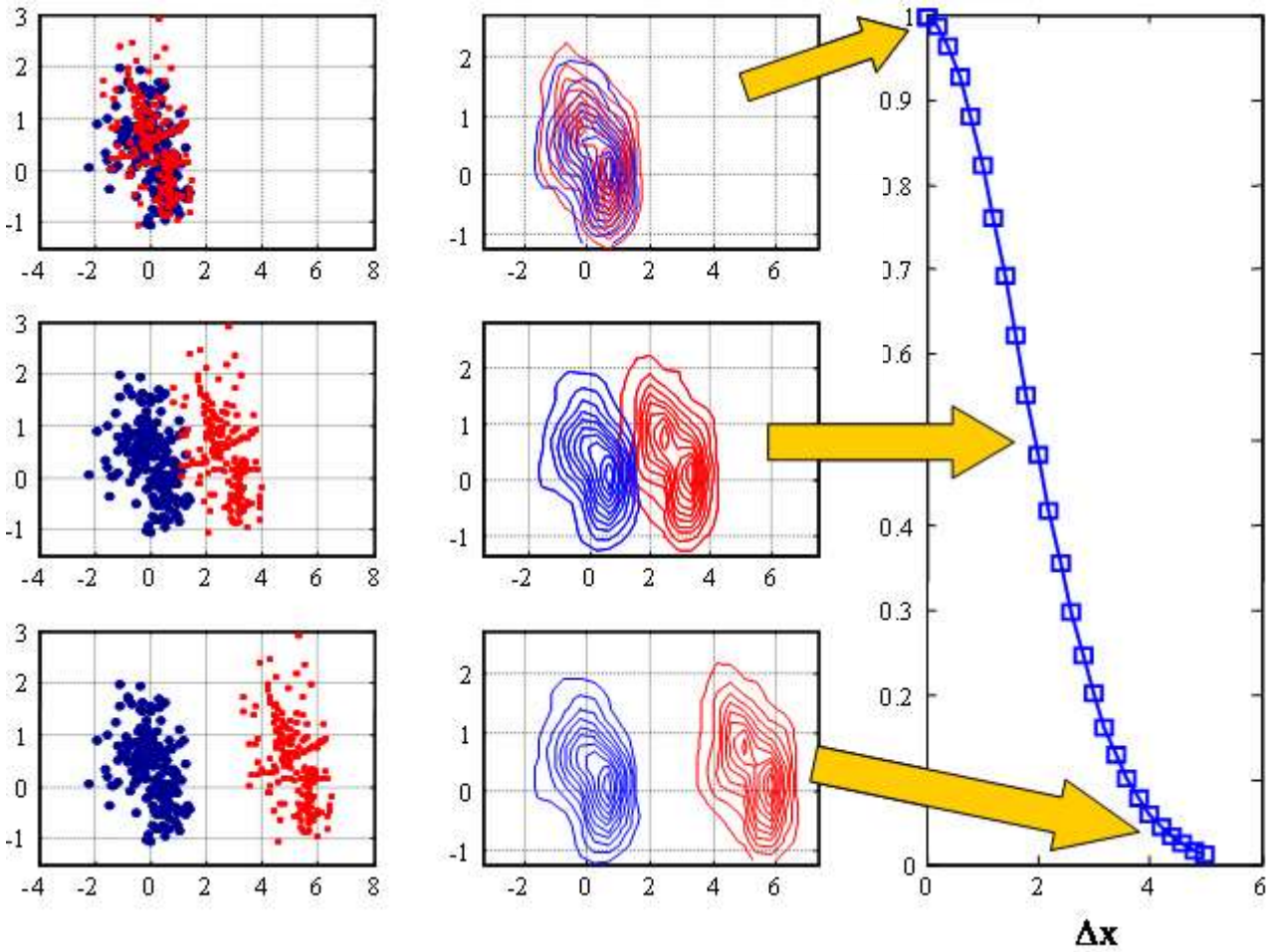
Prediction and Classification of Drug Toxicity Using Probabilistic Modeling of Temporal Metabolic Data: The Consortium on Metabonomic Toxicology Screening Approach
T. Ebbels, H. Keun, O. Beckonert, M. Bollard, J. Lindon, E. Holmes, and Jeremy K. Nicholson
Journal of Proteome Research/2007

Identifying toxicity type

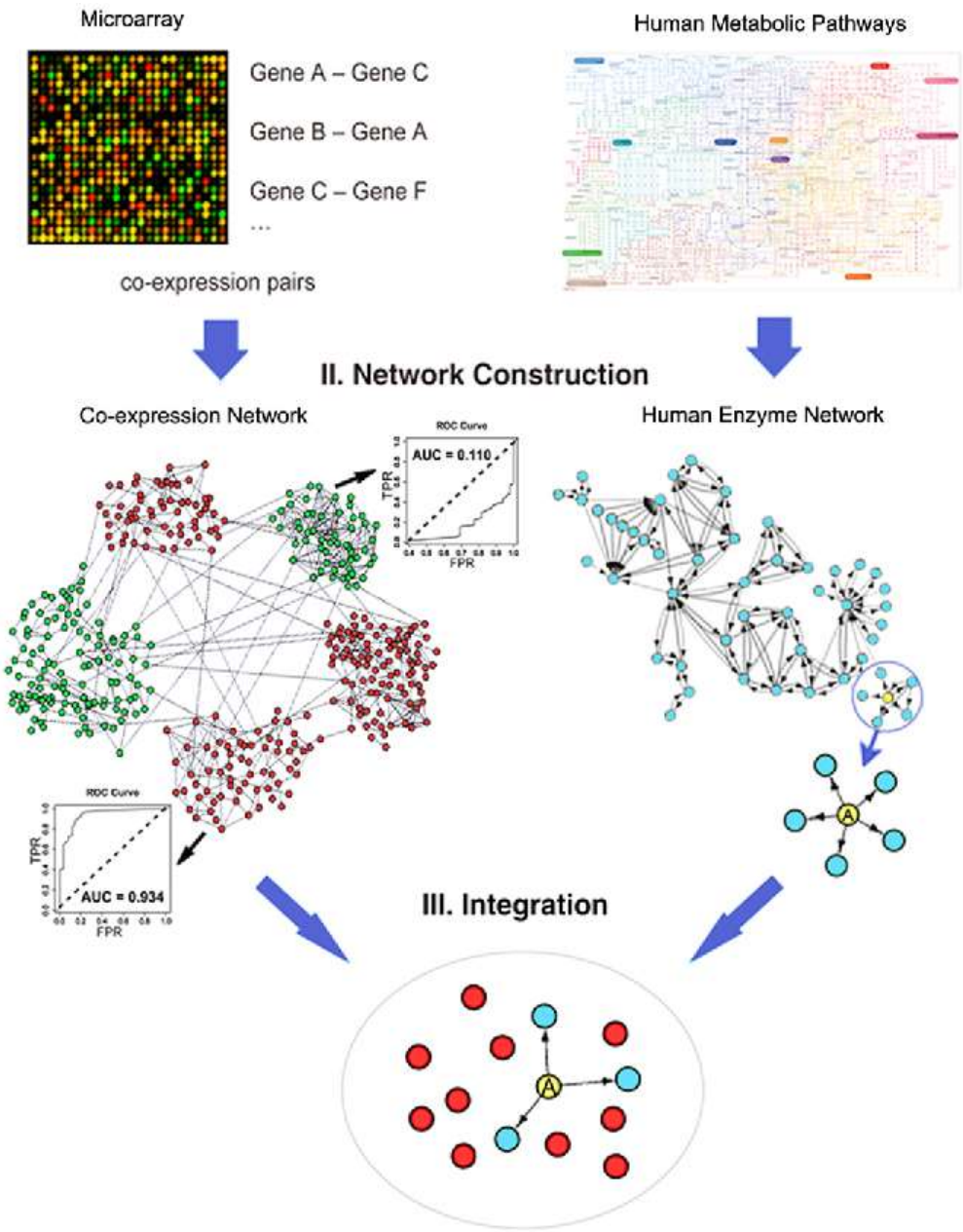
- PNN-based model (Probabilistic Neural Network – Specht 1990)



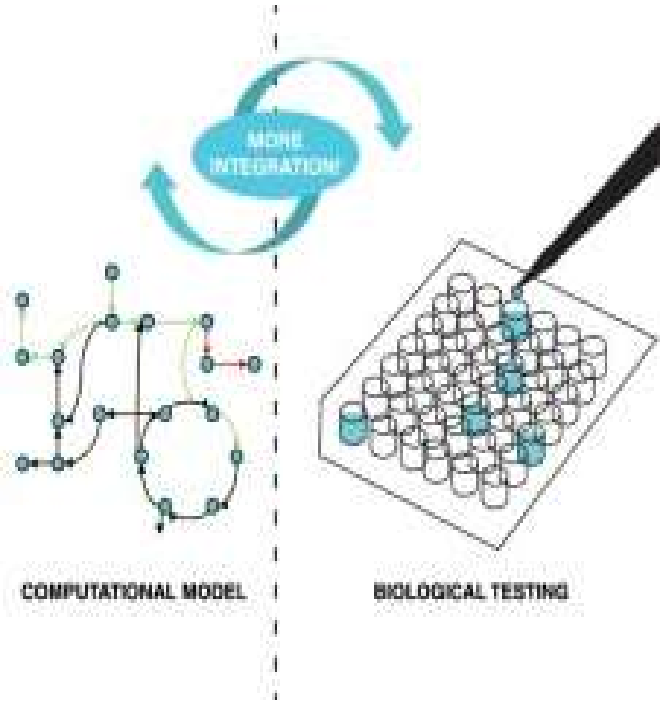
Clouds approach



Classification based on similarity



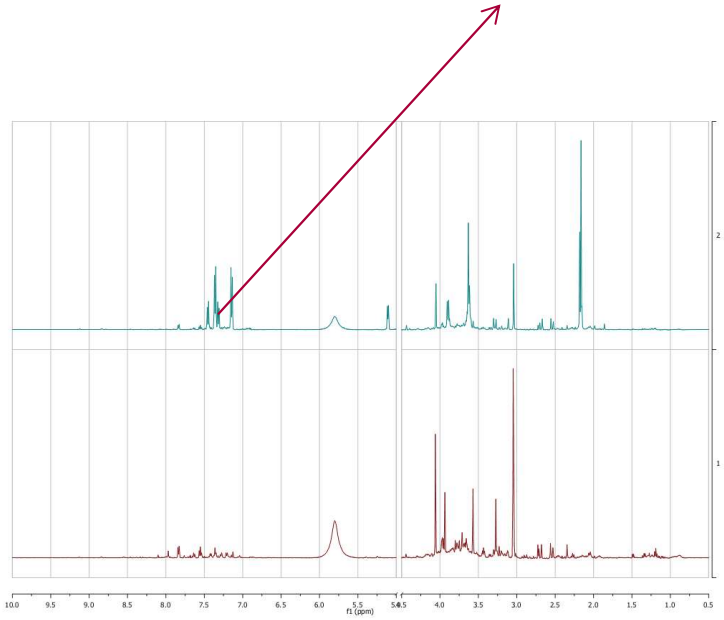
DOI:10.1021/pr400162t

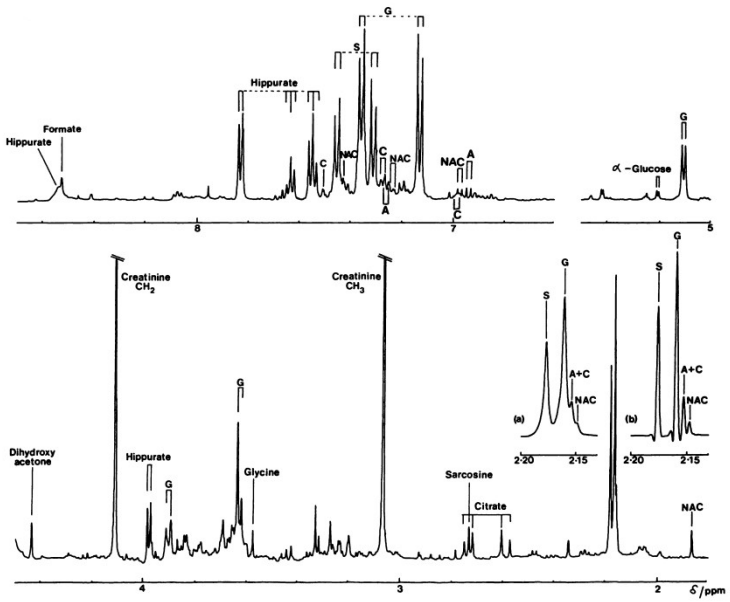


https://doi.org/10.1016/j.copbio.2014.12.010

DRC's (DRUG RELATED COMPOUNDS)

Acetaminophen and/or metabolites





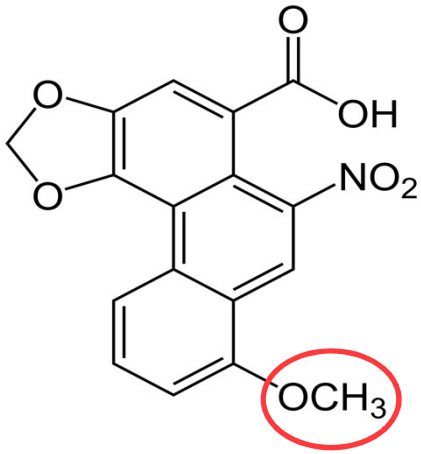
Clin Chem. 1984 Oct;30(10):1631-6.

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

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

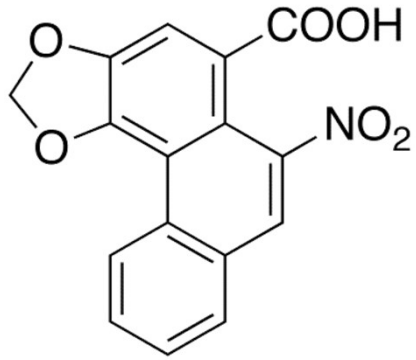
Aristolochic Acids Nephropathy

- **Mixture**



Aristolochic Acid I (AAI)

+



Aristolochic Acid II (AAII)

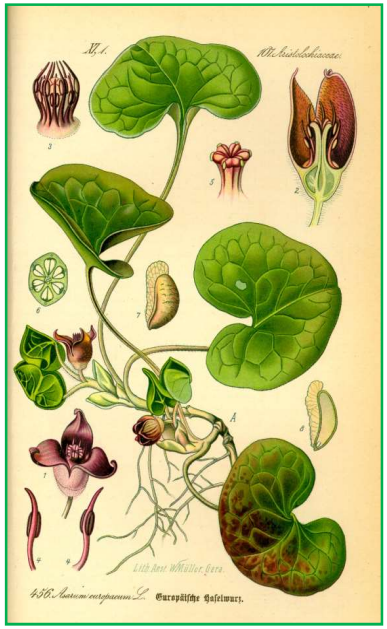
Marilyn Duquesne's Thesis - 2018

➢ Active compounds from *Aristolochiaceae*

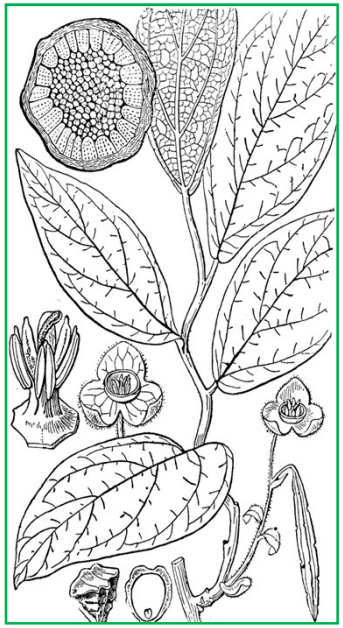
Aristolochia

www.afleurdepau.com

Asarum

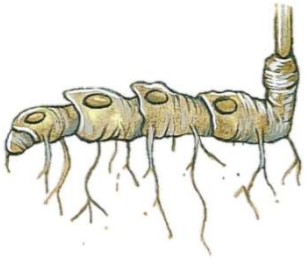


Braganti



etc.usf.edu

Natural Remedies in traditional medicines



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Rhizomes :

- Gastric stimulant
- Fever
- Malaria

Racines :

- Type II diabetes
- Snake bite

Leaves :

- Skin troubles
- Digestive torubles
- Leishmaniasis

Rods :

- Anti-inflammatory
- Diuretic
- Poor blood cirulatio n

Fruits :

- Respiratory dise sas
- Wounds
- Malaria

Seeds :

- Liver troubles
- Abdominal pain

Flowers :

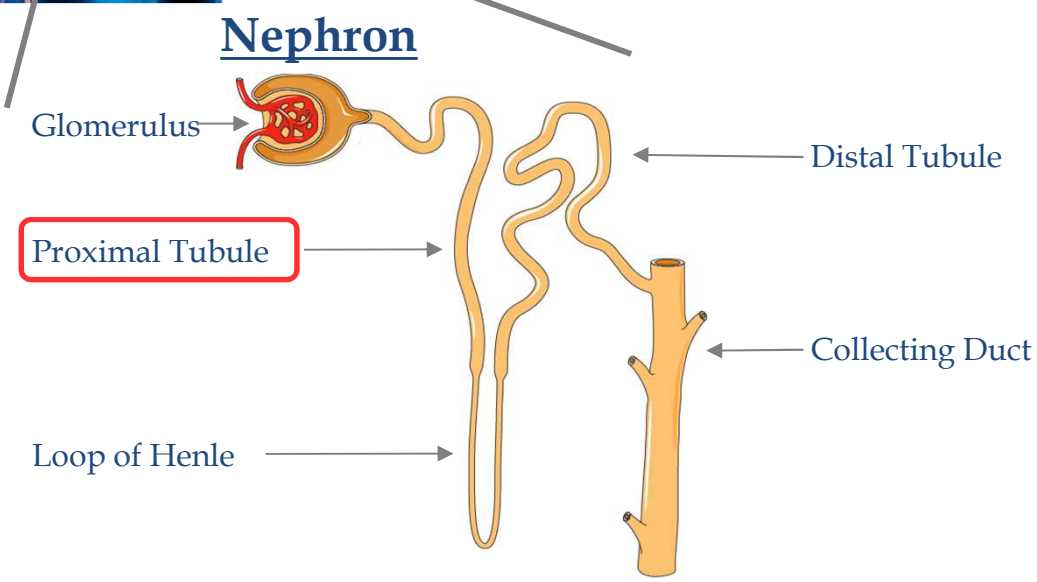
- Diuretic

Toxicity of Aristolochic Acids

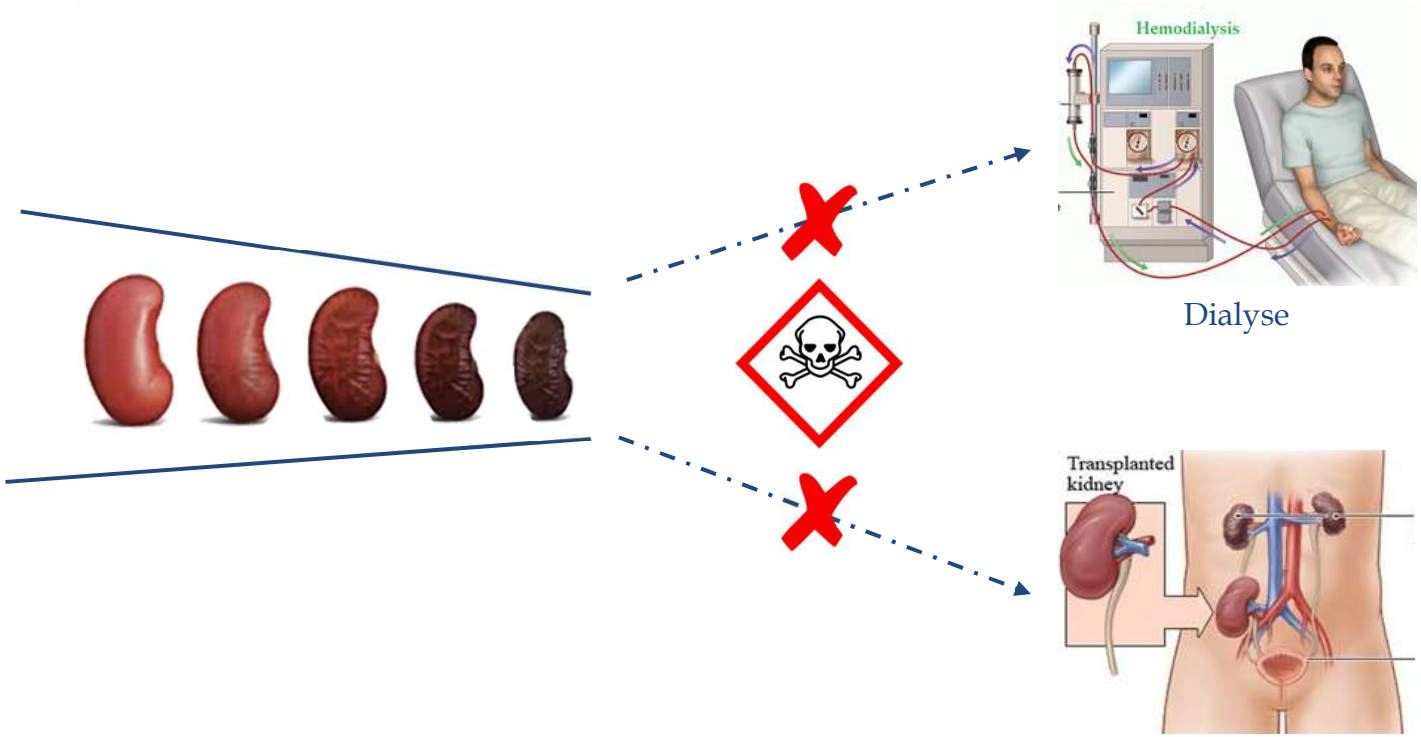
- **Kidneys (Nephrotoxicity)**

✓ Interstitial Fibrosis

✓ Tubular atrophy

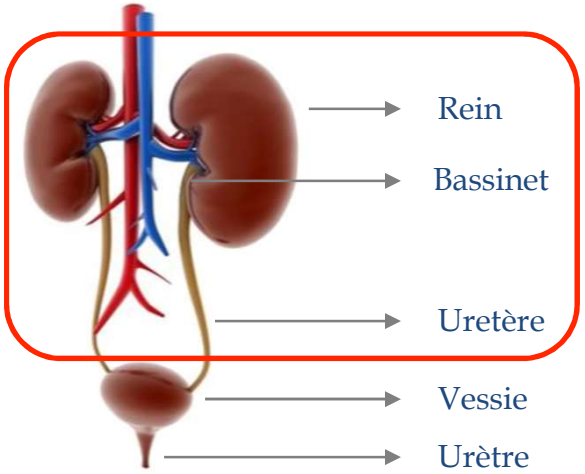


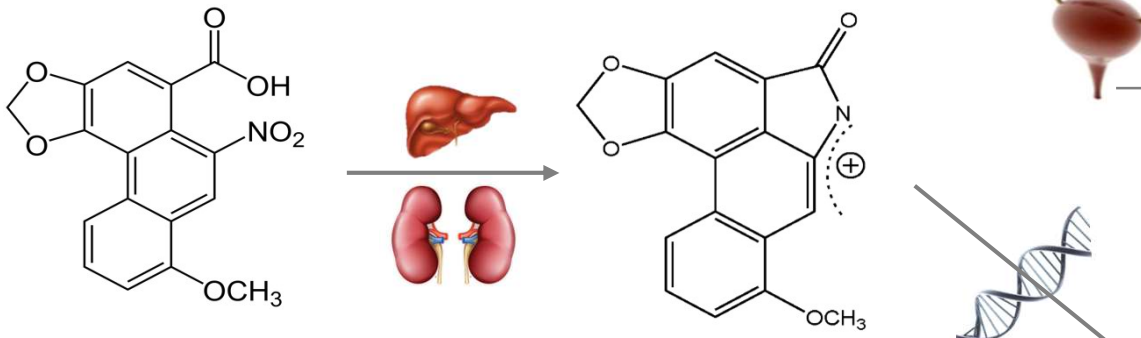
✓ CHRONIC RENAL FAILURE



➢ Carcinogenicity

✓ Urothelial cancers





AAI

Nitrenium ion

✓ DNA adducts

✓ Genetic mutations

✓ Cancer lesions

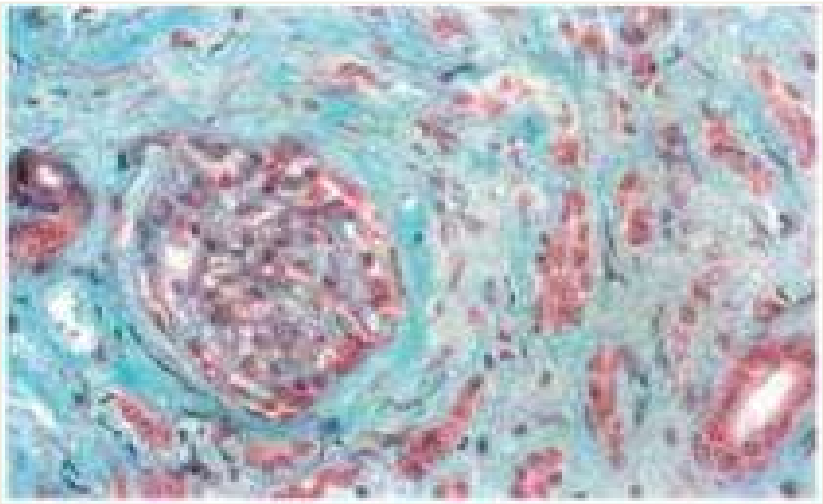
Balkan Endemic Nephropathy (BEN)

- ✓ **Chronic renal disease**
  - ✓ Interstital Fibrosis
  - ✓ Tubular atrophy
  - ✓ Proteinuria
  - ✓ DNA adducts
  - ✓ Urothelial cancers
- ✓ **Balkan Endemic Region**
- ✓ Environmental intoxication
- ✓ *Aristolochia Clematitis*
  - ✓ AA's contamination
  - ✓ Soils, crops, bread
  - ✓ Diseased inhabitants > 100 000 ; Suspected: > 25 000

Grollamn. Proc. Natl. Acad. Sci. 2007 ; Stefanovic and Radovanovic, 2008

A belgian clinical case of AAN

- ✓90's in Belgium
- ✓> 100 female patients
- ✓Ingestion of slimming pills
- ✓Chinese herbs
- ✓Formulation error
  - ✓*Stephania Tetrandra* ➔ *Aristolochia Fanghi*



Debelle et al., Kidn.Int. 2008

Biomarkers of AAN

Metabonomic

Experimental

Unveil molecular & cellular modes of action of AAs

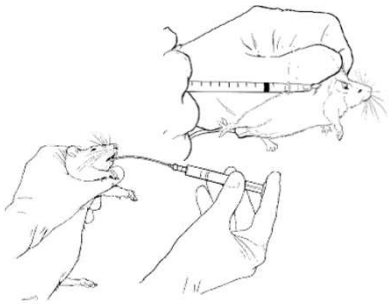
New therapeutic targets

Clinical

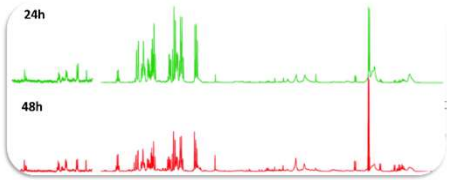
Identification of early and non-invasive biomakers

Early screening of AAN

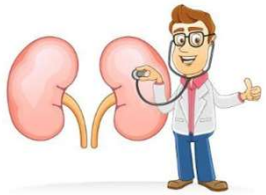
Rodent models of AAN

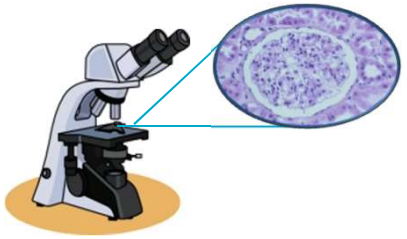


^{1}H RMN



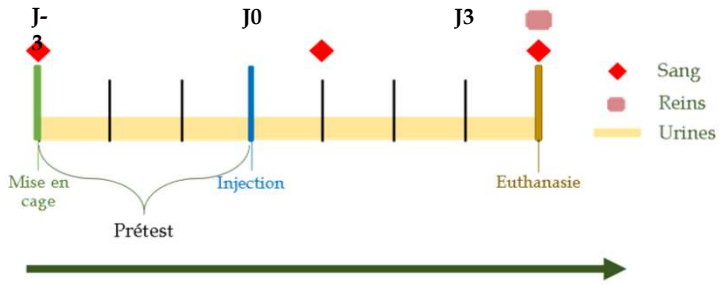
[BUN]
[Créatinine]
[Protéines]
[Albumine]
[Urée]



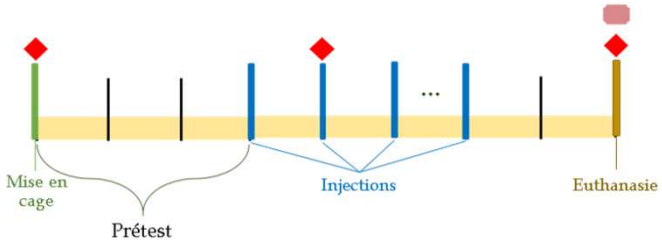


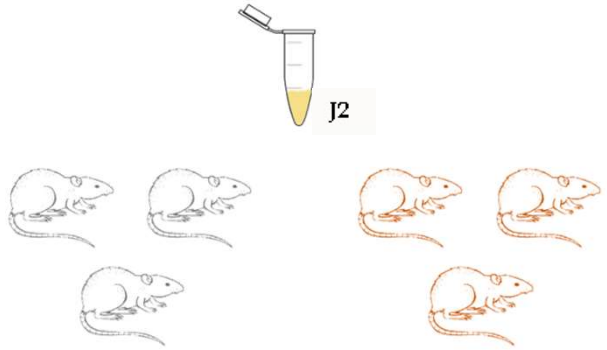
Rodent models of AAN

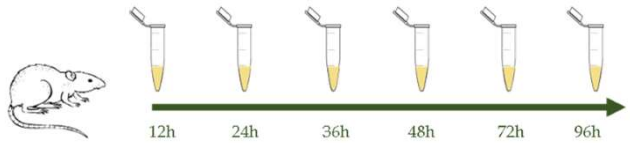
Acute



Chronic







Paramètres physiologiques

- ↘ Poids
- ↘ Prise de nourriture
- ↗ [Créatinine]

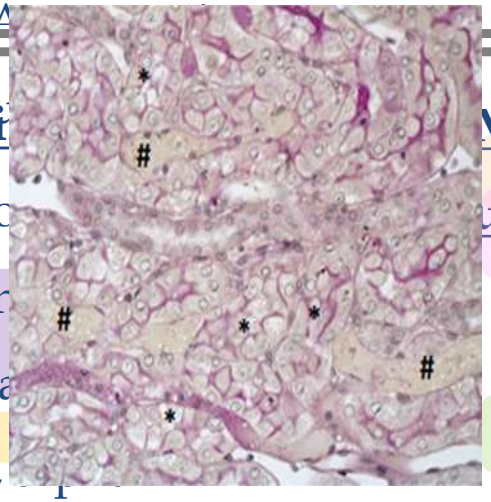
Résultats sanguins

Filtration

- ↗ [A…
sangu…

Résultats histologiques

- Perte de la bordure en brosse (*) — Mort cellulaire
- Capillaires péritubulaires engorgés (#) — Hypoxie, Réabsorption
- Protéinurie



Métabolites urinaires [↗]

Cycle de Krebs

- Allanto…
- Dimeth…
- Succina… citrate, … ketoglutarate
- Hippurate

Sécrétion

Mort cellulaire

Métabolisme anaérobie

- Lactate
- Acétate
- Glycine
- Taurine
- Glucose

Réabsorption

Nephrol Dial Transplant. 2000 Aug;15(8):1175-82.

The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats.

Erdem A[1], Gündoğan NU, Usubütün A, Kilinç K, Erdem SR, Kara A, Bozkurt A.

➢ Rôle néphroprotecteur de la taurine

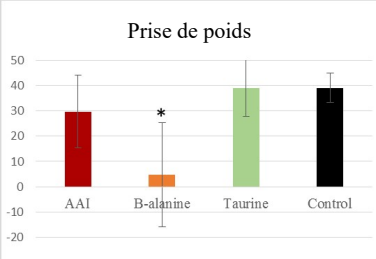
- Régimes:
  - AA
  - AA + Taurine
  - AA + Taurine + β-alanine
  - Control

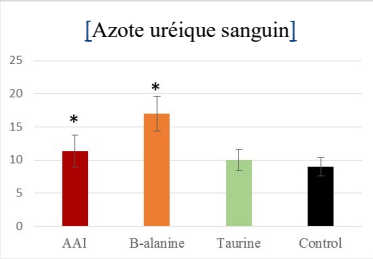
Compétiteur taurine

[Taurine]:

- -
- ↗
- ↘

- Résultats:
  - + Taurine : pas de changements significativement différents
  - + β-alanine: aggravation de la toxicité

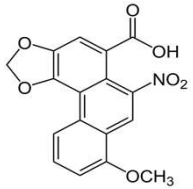




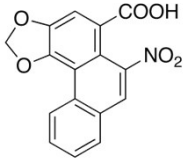
➢ Comparaisons de toxicités

1. AAI, AAII, mixture

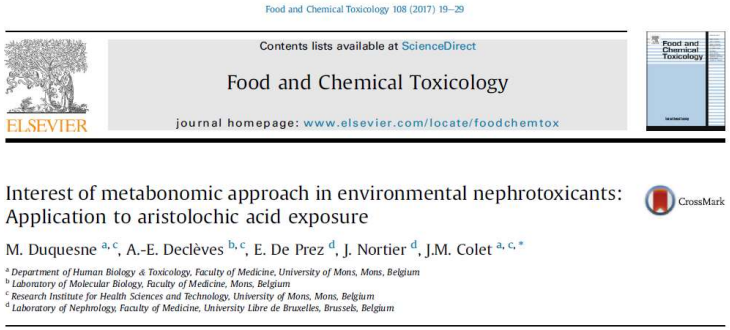
- 4 groupes:
  - AAI
  - AAII
  - AAI + AAII
  - Control



AAI



AAII



Food and Chemical Toxicology 108 (2017) 19–29

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ELSEVIER

Interest of metabonomic approach in environmental nephrotoxicants: Application to aristolochic acid exposure

M. Duquesne [a, c], A.-E. Declèves [b, c], E. De Prez [d], J. Nortier [d], J.M. Colet [a, c, *]

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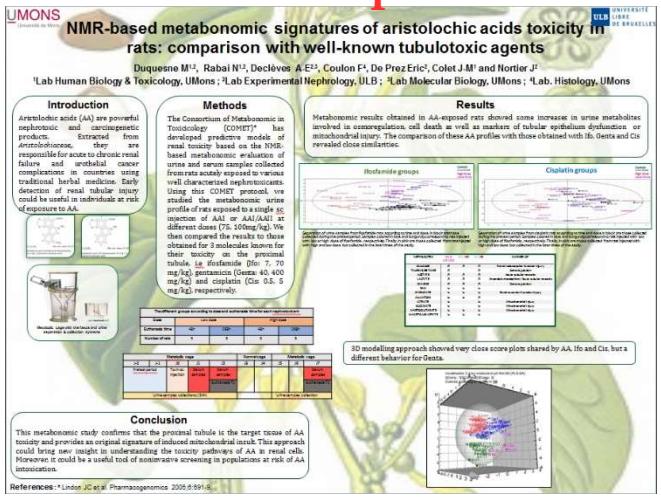
[d] Laboratory of Nephrology, Faculty of Medicine, University Libre de Bruxelles, Brussels, Belgium

➔ AAII > Mixture > AAI

2. Néphrotoxiques connus

- 5 groupes:
  - AA
  - Cisplatine
  - Gentamicine
  - Ifosfamide
  - Control

➔ AA ≈ Cisplatine



UMONS

NMR-based metabonomic signatures of aristolochic acids toxicity in rats: comparison with well-known tubulotoxic agents

Duquesne M[1,2], Rabai N[1,2], Declèves A.-E[2,3], Coulon F[4], De Prez Eric[3], Colet J.-M[1] and Nortier J[2]

[1]Lab Human Biology & Toxicology, UMons ; [2]Lab Experimental Nephrology, ULB ; [3]Lab Molecular Biology, UMons ; [4]Lab. Histology, UMons

Introduction

Aristolochic acids (AA) are powerful nephrotoxic and carcinogenetic products. Extracted from *Aristolochiaceae*, they are responsible for acute to chronic renal failure and urothelial cancer complications in countries using traditional herbal medicine. Early detection of renal tubular injury could be useful in individuals at risk of exposure to AA.

Methods

The Consortium of Metabonomic in Toxicology (COMET)* has developed predictive models of renal toxicity based on the NMR-based metabonomic evaluation of urine and serum samples collected from rats acutely exposed to various well characterized nephrotoxicants. Using this COMET protocol, we studied the metabonomic urine profile of rats exposed to a single ip injection of AAI or AAI/AAII at different doses (75- 100mg/kg). We then compared the results to those obtained for 3 molecules known for their toxicity on the proximal tubule, i.e ifosfamide (Ifo: 7- 70 mg/kg), gentamicin (Genta: 40- 400 mg/kg) and cisplatin (Cis: 0.5- 5 mg/kg), respectively.

Results

Metabonomic results obtained in AA-exposed rats showed some increases in urine metabolites involved in osmoregulation, cell death as well as markers of tubular epithelium dysfunction or mitochondrial injury. The comparison of these AA profiles with those obtained with Ifo, Genta and Cis revealed close similarities.

Ifosfamide groups

Cisplatin groups

3D modelling approach showed very close score plots shared by AA, Ifo and Cis, but a different behavior for Genta.

Conclusion

This metabonomic study confirms that the proximal tubule is the target tissue of AA toxicity and provides an original signature of induced mitochondrial insult. This approach could bring new insight in understanding the toxicity pathways of AA in renal cells. Moreover, it could be a useful tool of noninvasive screening in populations at risk of AA intoxication.

References: * Lindon JC et al. Pharmacogenomics. 2005;6:691-6.

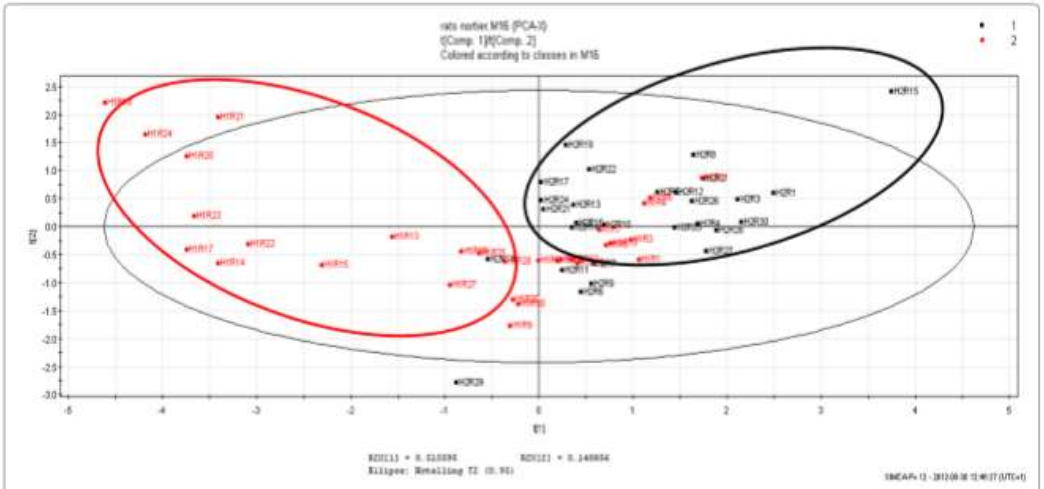


Figure 2: PCA (scores plot) of ¹H-NMR spectra of urine samples collected from rats exposed to repeat doses of aristolochic acids (red diamonds) and control rats (black squares).

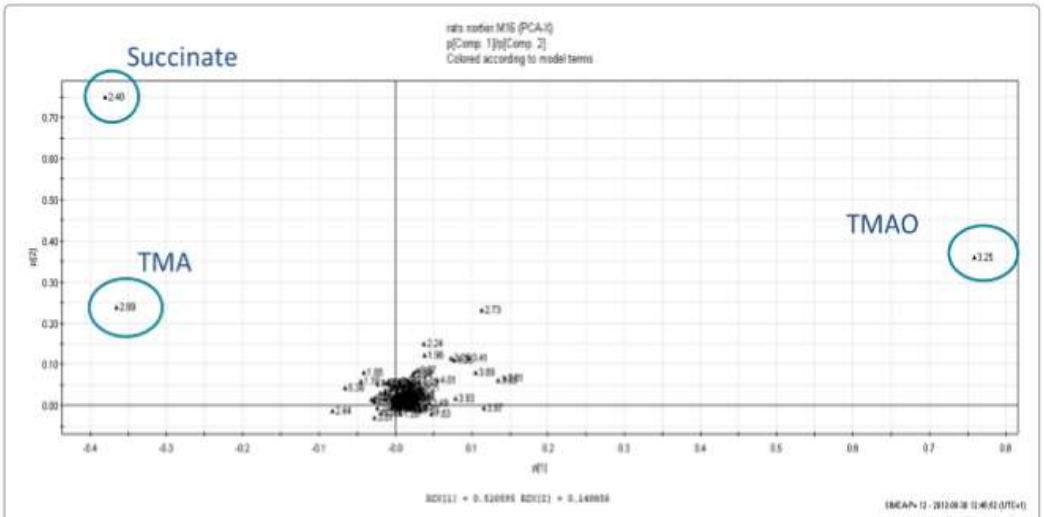


Figure 3: PCA (loadings plot) of ¹H-NMR spectra of urine samples collected from rats exposed to repeat doses of aristolochic acids and control rats.

Duquesne et al., J Cancer Sci Ther 2012, S18
DOI: 10.4172/1948-5956.S18-002

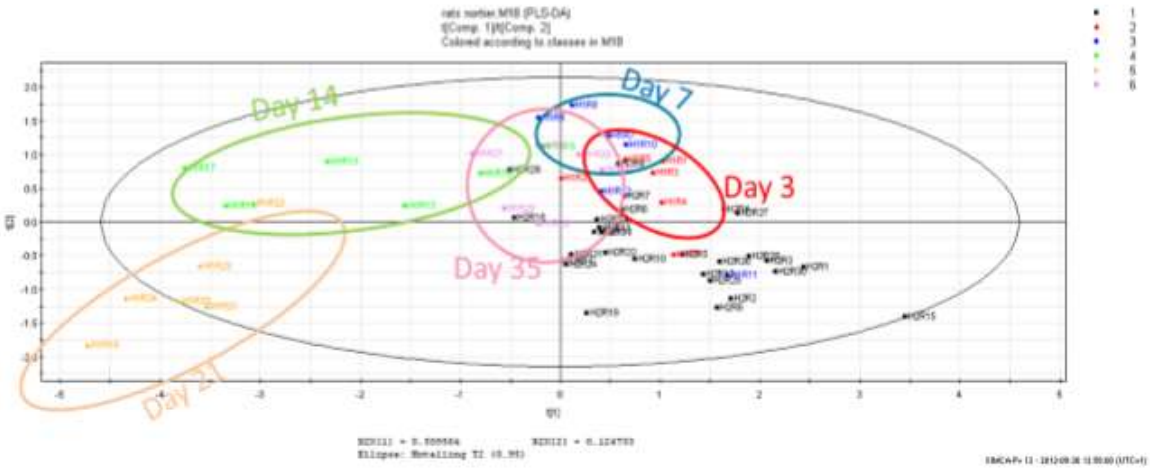


Figure 4: PLS-DA of ¹H-NMR spectra of urine samples collected from control and AA-exposed rats: Time evolution.

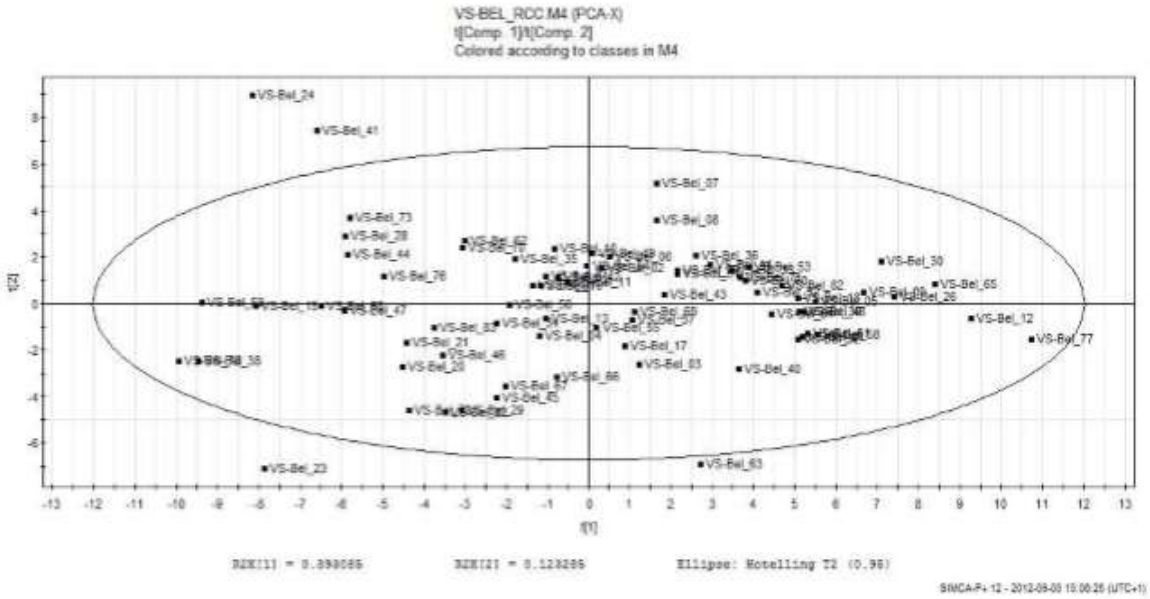
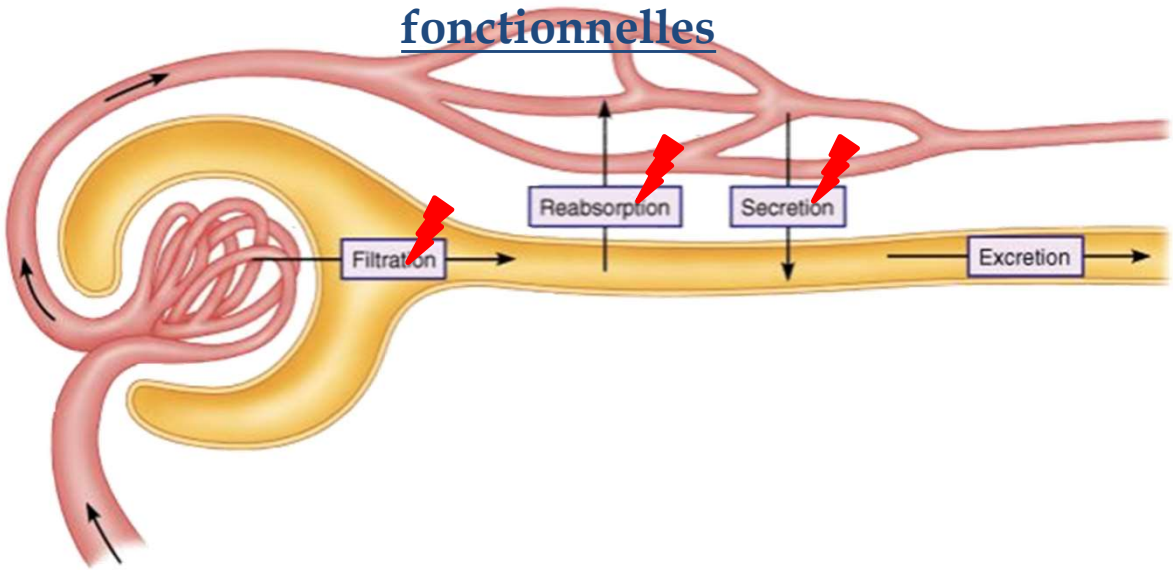


Figure 5: PCA (scores plot) of ¹H-NMR spectra of urtine samples collected from Belgian healthy volunteers.

Duquesne et al., J Cancer Sci Ther 2012, S18
DOI: 10.4172/1948-5956.S18-002

➢ Atteintes fonctionnelles



Filtration glomérulaire

↗ créatinine sérique, azote uréique sanguin

Réabsorption tubulaire

Protéinurie

↗ glucose urinaire

Sécrétion tubulaire

↘ Hippurate urinaire

➔ Entrée cellulaire (OAT)

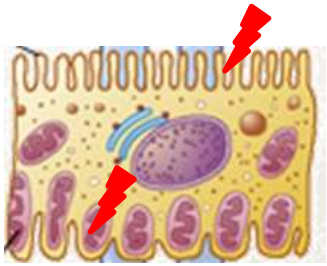
➢ Atteintes structurelles

Perte de la bordure en brosse

↗ lactate ➔ **nécrose**

↘ cycle de Krebs

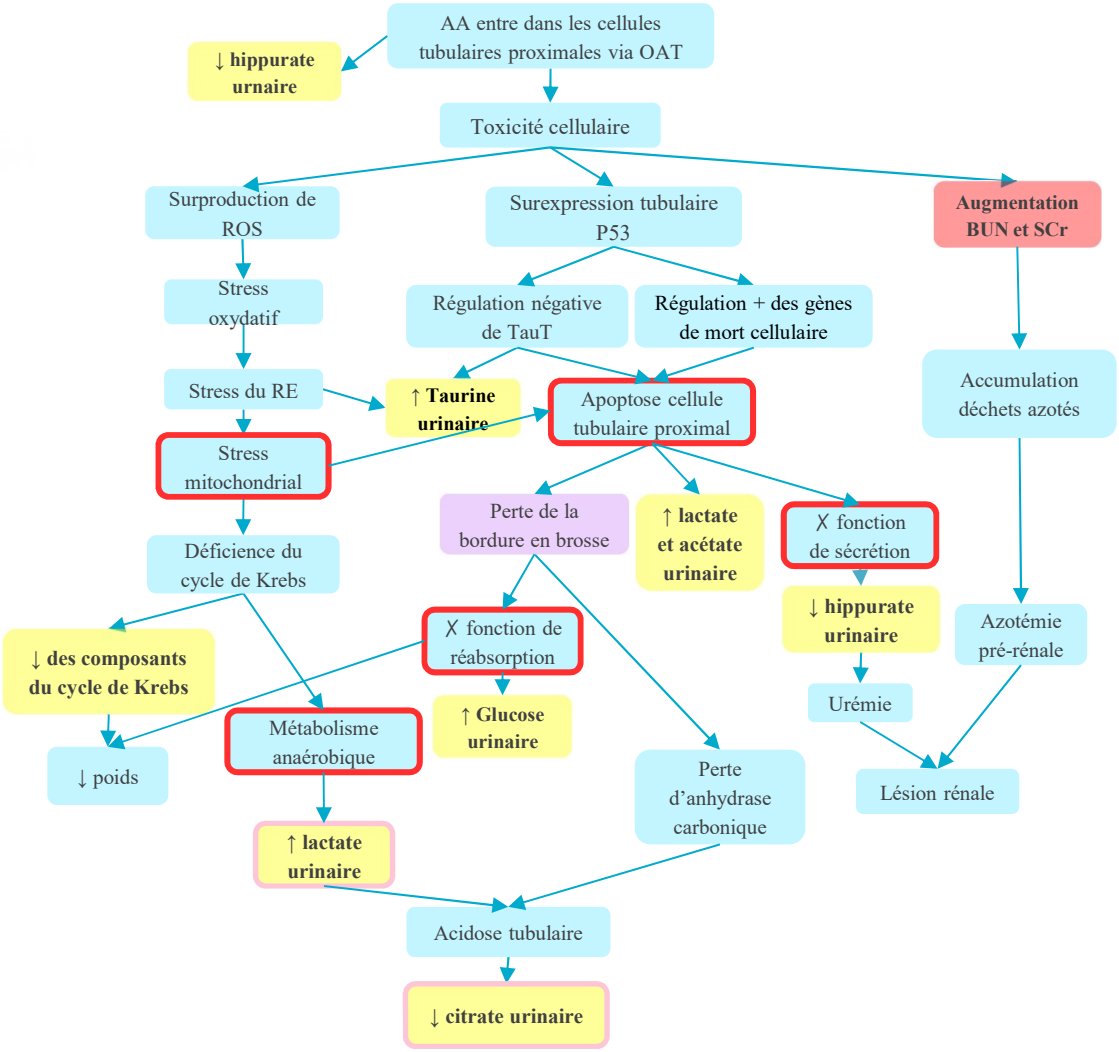
➔ **Mitochondries**



➢ Stress oxydatif

➢ Métabolisme anaérobie

➢ Hypoxie


AA entre dans les cellules tubulaires proximales via OAT
↓ hippurate urnaire
Toxicité cellulaire
Surproduction de ROS
Surexpression tubulaire P53
Augmentation BUN et SCr
Stress oxydatif
Régulation négative de TauT
Régulation + des gènes de mort cellulaire
Stress du RE
↑ Taurine urinaire
Apoptose cellule tubulaire proximal
Accumulation déchets azotés
Stress mitochondrial
Perte de la bordure en brosse
↑ lactate et acétate urinaire
X fonction de sécrétion
Déficience du cycle de Krebs
↓ hippurate urinaire
Azotémie pré-rénale
↓ des composants du cycle de Krebs
X fonction de réabsorption
Urémie
↑ Glucose urinaire
Métabolisme anaérobique
↓ poids
Perte d'anhydrase carbonique
Lésion rénale
↑ lactate urinaire
Acidose tubulaire
↓ citrate urinaire

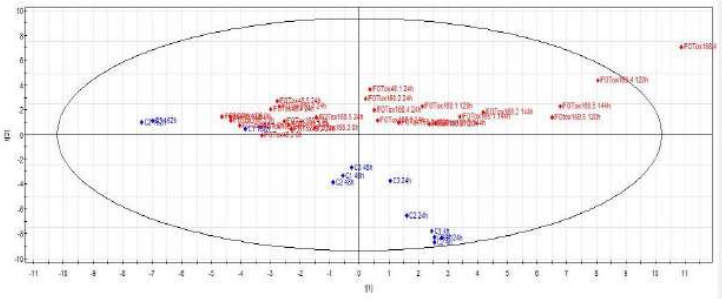


Figure 29 : Scores plot montrant la disposition des échantillons urinaires des rats intoxiqués avec une forte dose d'AAI, en bleu, ou d'ifosfamide, en rouge.

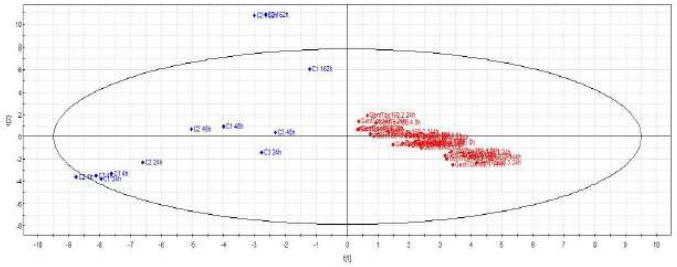


Figure 25 : Scores plot montrant les échantillons urinaires des rats intoxiqués avec une forte dose d'AAI en bleu (100mg/kg) par rapport à ceux exposés à une forte dose de gentamycine, en rouge

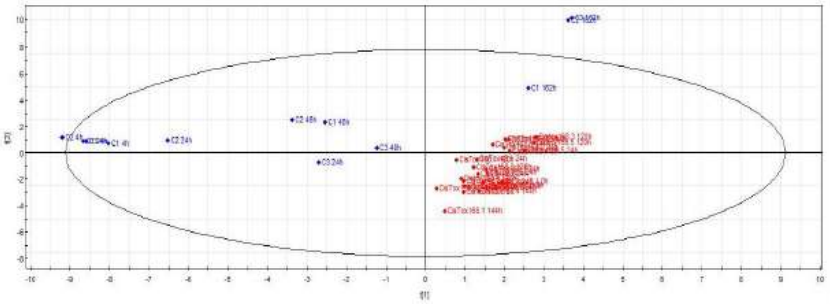


Figure 21: Scores plot exposant la disposition des échantillons urinaires des rats intoxiqués avec une forte dose d'AAI (dose), en bleu ou de cisplatine, en rouge (5mg/kg).

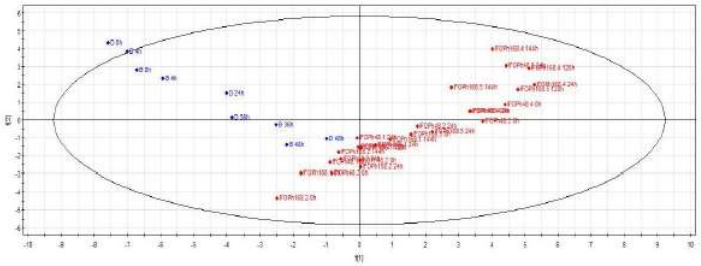


Figure 27 : Scores plot montrant la disposition des échantillons récoltés chez des rats intoxiqués avec une faible dose d'AAI, en bleu, et ceux exposés à une faible dose d'ifosfamide, en rouge.

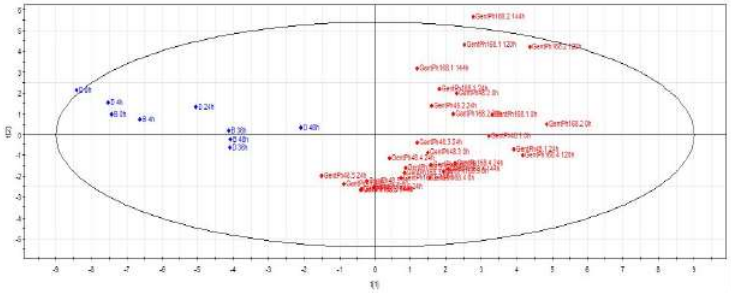


Figure 23 : Scores plot montrant la séparation entre les rats intoxiqués avec une faible dose d'AAI (50mg/kg), en bleu ou de gentamycine, en rouge.

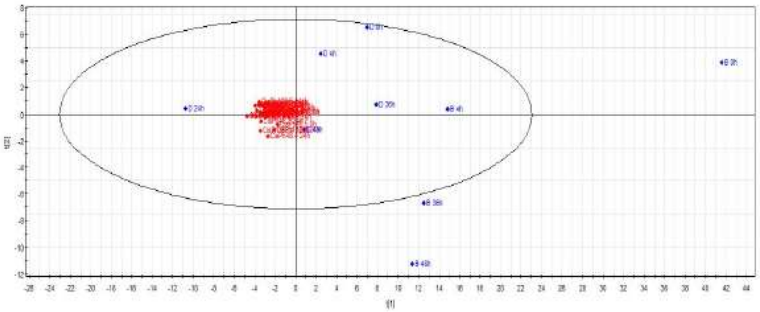


Figure 19 : Scores plot montrant l'évolution de la toxicité entre les rats exposés à une faible dose de cisplatine en rouge, et ceux intoxiqués à une dose non-toxique d'AAI en bleu.

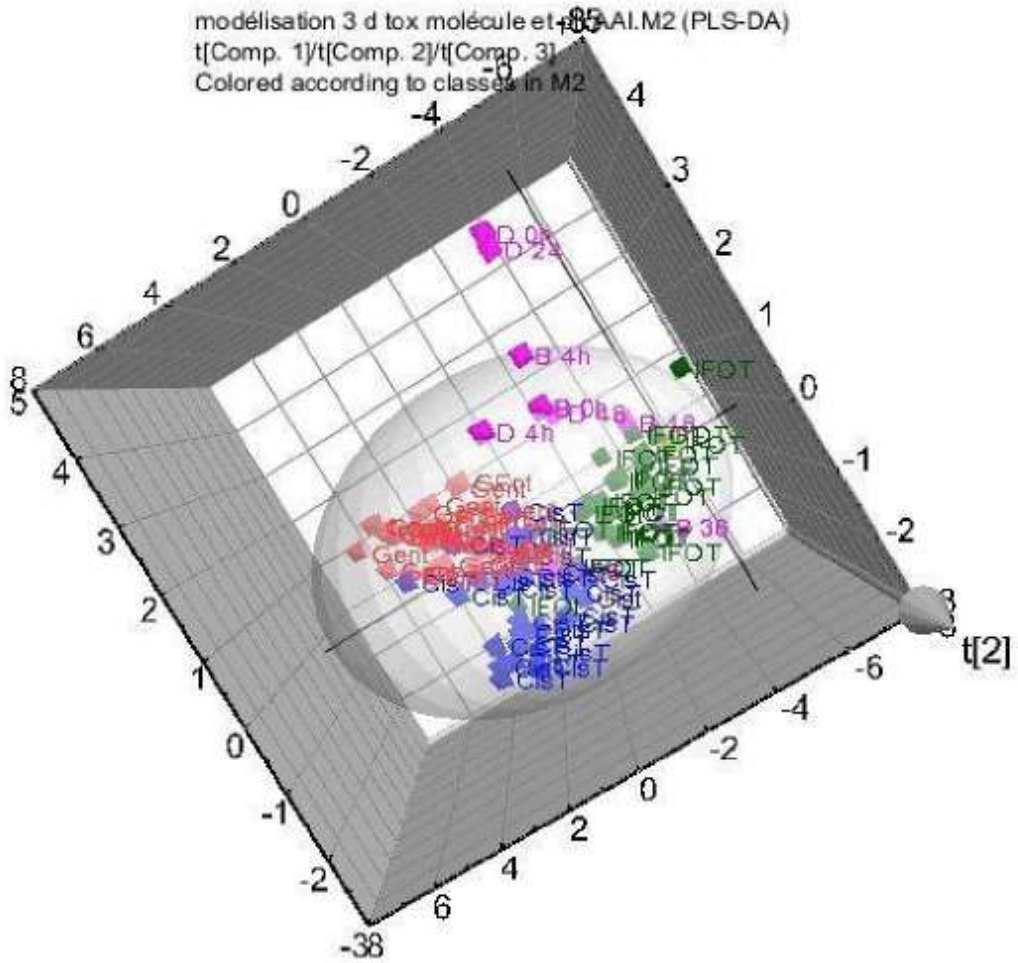
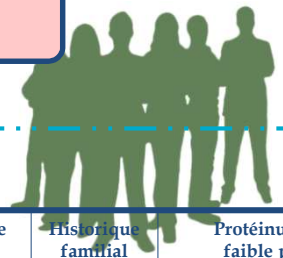


Figure 31 : comparaison 3D des trois molécules néphrotoxiques à l'AAI. En rouge se trouve le groupe exposé à une forte dose de gentamycine, en bleu le groupe exposé à une forte dose de cisplatine et en vert le groupe exposé à une forte dose d'ifosfamide. Le groupe en ros est celui exposé à une faible dose d'AAI (50mg/kg)

Cohorte Belge

- Population contrôle (♂ ♀)
- ♀ NAA



Expé

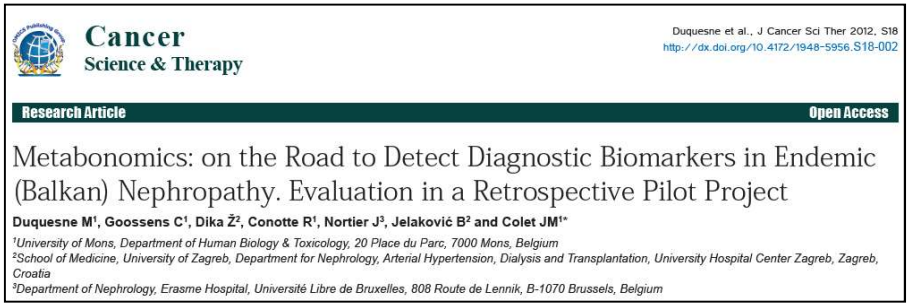
Cohorte Croate

- Malades
- Suspects
- À risques
- Autres
- Non endémiques

Clinique

Groupe	Historique familial	Protéinurie de faible poids moléculaire	Azotémie	Anémie	15 ans dans région endémique
		+	+	+	
Malade	+	+		+	+
		+	+	+	+
Suspect	+	+			
				+	
A risque	+				
Autre	-	-	-	-	

✓ Premiers résultats


Cancer Science & Therapy

Duquesne et al., J Cancer Sci Ther 2012, S18
http://dx.doi.org/10.4172/1948-5956.S18-002

Research Article — Open Access

Metabonomics: on the Road to Detect Diagnostic Biomarkers in Endemic (Balkan) Nephropathy. Evaluation in a Retrospective Pilot Project

Duquesne M[1], Goossens C[1], Dika Ž[2], Conotte R[1], Nortier J[3], Jelaković B[2] and Colet JM[1*]

[1]University of Mons, Department of Human Biology & Toxicology, 20 Place du Parc, 7000 Mons, Belgium
[2]School of Medicine, University of Zagreb, Department for Nephrology, Arterial Hypertension, Dialysis and Transplantation, University Hospital Center Zagreb, Zagreb, Croatia
[3]Department of Nephrology, Erasme Hospital, Université Libre de Bruxelles, 808 Route de Lennik, B-1070 Brussels, Belgium

Métabonomique et pathologie rénale

- Population contrôle
- Paramètres importants:

1. Age
   - Vieillissement rénal:
     - ♀ : 55 ans
     - ♂: 60 ans

3x plus de métabolites affectés

2. Sexe
   - Différence hormonale

Comparaison NAA et NEB

- Similitudes
  - Etiologie commune
- Différences
  - Exposition, doses ≠

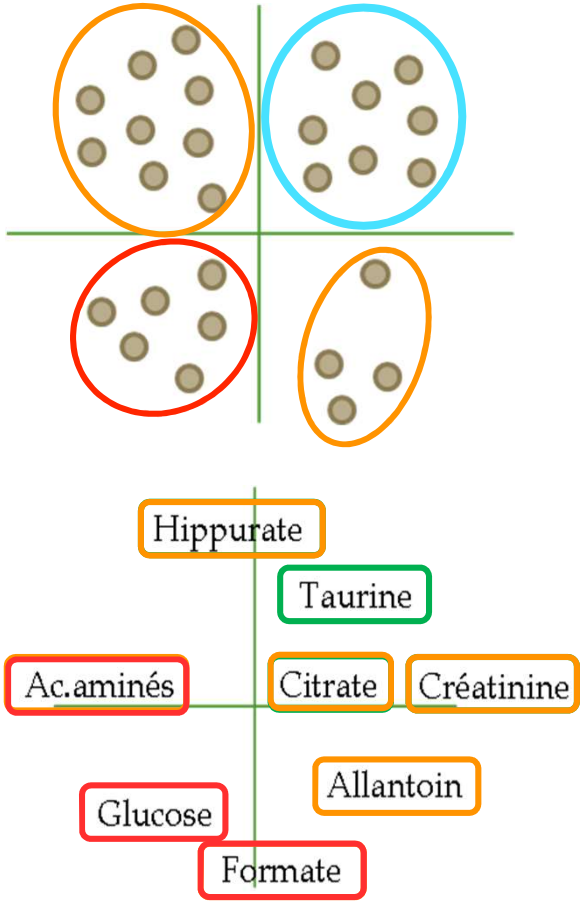
Atteintes AA

- Premier approche
  - ↓ [creatinine], [hippurate]
  - ↑ [glucose], [lactate]

➢ Hommes

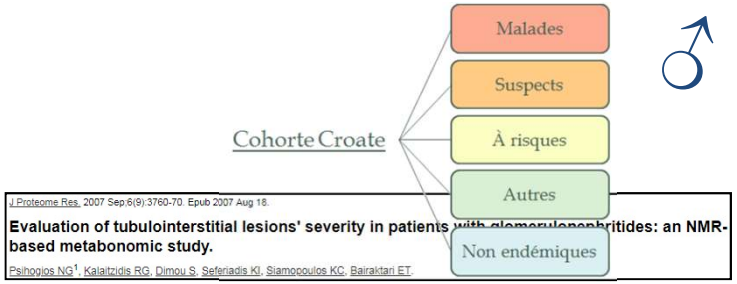
Belge vs Croate

	Métabolites urinaires	
	Présent s/augmentés	Absents/diminués
Groupe 1	Hippurate Citrate Creatinine Taurine	Glucose
Groupe 2	Creatinine Citrate Formate Allantoin	Taurine Hippurate
Groupe 3	Hippurate Acides aminés	Creatinine Citrate Taurine Glucose
Groupe 4	Glucose Formate Acides aminés ++	Hippurate Citrate Creatinine Taurine



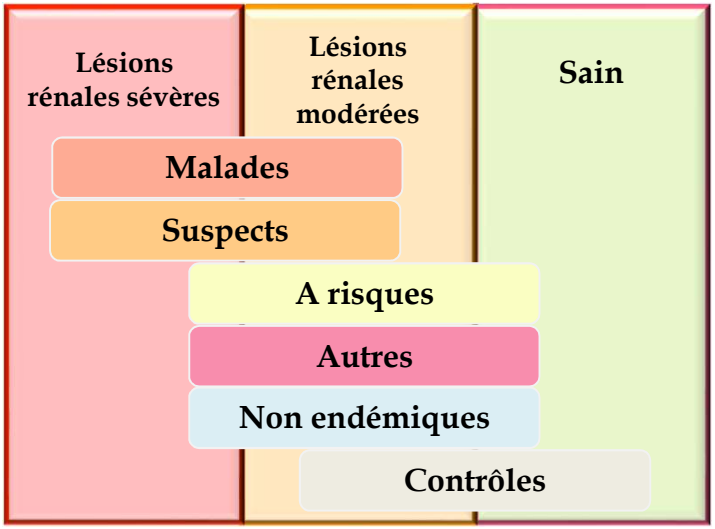
➢ Hommes

Belge vs Croate



	Métabolites urinaires	
	Présent s/augmentés	Absents/diminués
Groupe 1 **Profil métabolique :** **Sain**	Hippurate Citrate Creatinine Taurine	Glucose
Groupe 2 **Profil métabolique :** **Lésions rénales modérées**	Creatinine Citrate Formate Allantoin	Taurine Hippurate
Groupe 3 **Profil métabolique :** **Lésions rénales modérées**	Hippurate Acides aminés	Creatinine Citrate Taurine Glucose
Groupe 4 **Profil métabolique :** **Lésions rénales sévères**	Glucose Formate Acides aminés ++	Hippurate Citrate Creatinine Taurine

↓ Creatinine ➔ **x filtration glomérulaire**
↓ Citrate, hippurate ➔ **x sécrétion tubulaire**
↑ Acides aminés, glucose ➔ **x réabsorption tubulaire**

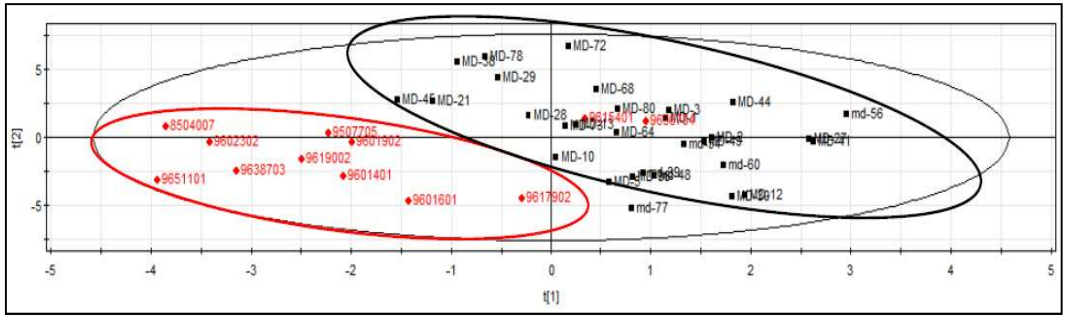


➢ Femmes

♀

Belges « contrôles » vs Croates « malades »

	Métabolites urinaires	
	Présent s/augmentés	Absents/diminués
Croates « malades »	Acides aminés Glucose Lactate Methylguanidine	Citrate Hippurate Créatine N-acetylaspartate β-hydroxybutyrate



↓ Citrate, hippurate ➔ **X sécrétion tubulaire**

↑ Acides aminés, glucose ➔ **X réabsorption tubulaire**

↑ Lactate ➔ **lésions tubulaires proximales**

↓ Créatine, N-acétylaspartate ➔ **X métabolisme/structure neuronale + troubles cognitifs**

↓ β-hydroxybutyrate ➔ **source d'énergie alternative (x glucose, x lactate) + troubles cognitifs**

↑ Methylguanidine ➔ **troubles neurologiques, stress oxydatif.**

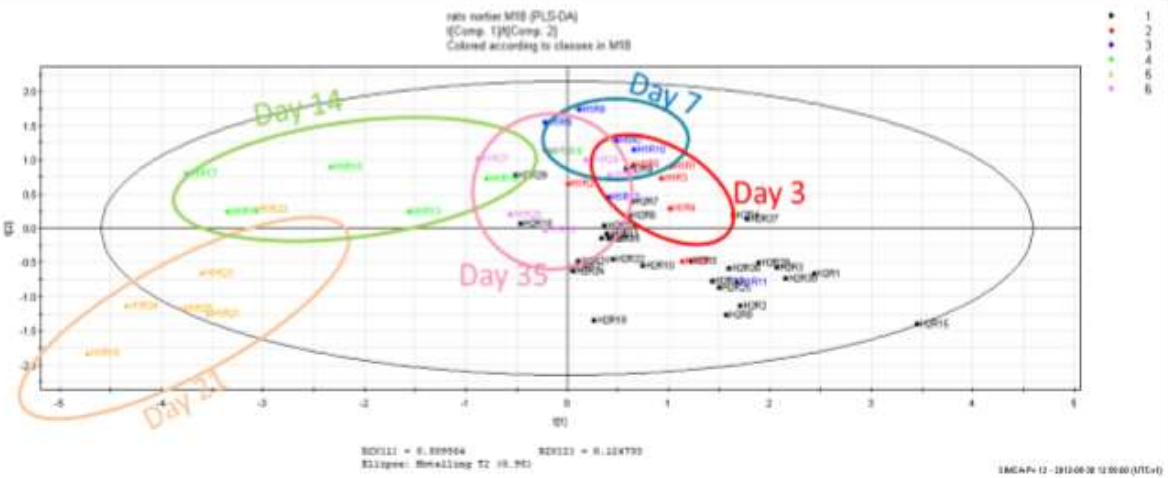


Figure 4: PLS-DA of ¹H-NMR spectra of urine samples collected from control and AA-exposed rats: Time evolution.

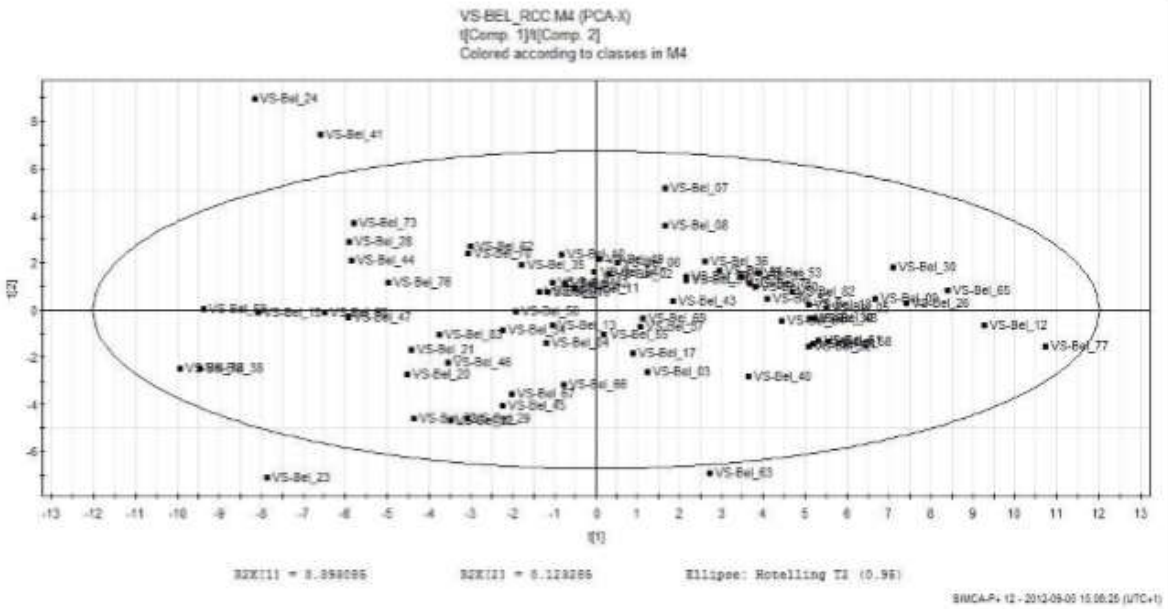


Figure 5: PCA (scores plot) of ¹H-NMR spectra of urtine samples collected from Belgian healthy volunteers.

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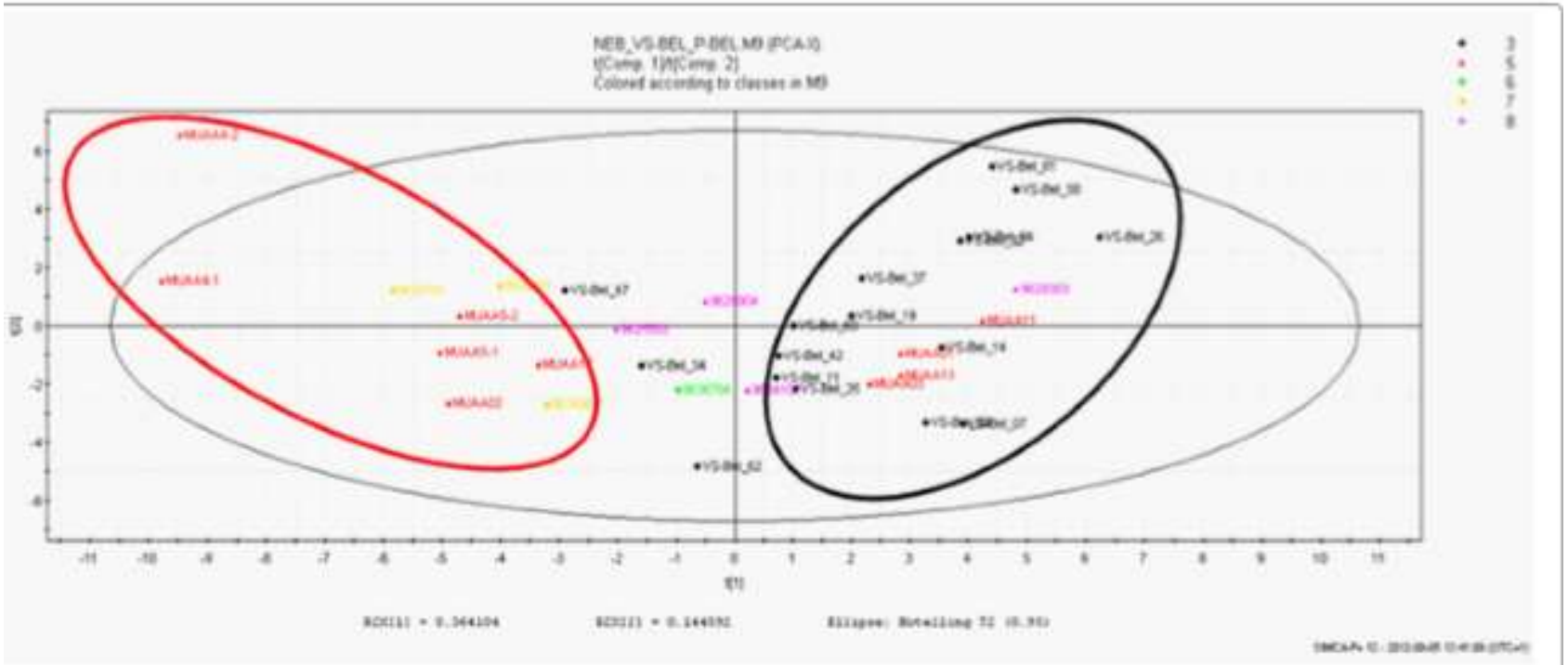
DOI: 10.4172/1948-5956.S18-002



igure 8: PCA (scores plot) of ¹H-NMR spectra of urine samples collected from female subjects: Belgian healthy women (black squares), Belgian AAN patients (red iangles); Croatian EN-patients (green circle); EN-suspected Croatian women (yellow diamonds), EN-at risk Croatian women (magenta diamonds).

Duquesne et al., J Cancer Sci Ther 2012, S18
DOI: 10.4172/1948-5956.S18-002

Clinique

- Troubles fonctions rénales
- Stress oxydatif
- Métabolisme alternatif (♀)
- Troubles cognitifs (♀)
  - Indicateur sévérité

Expérimental

- Meilleure compréhension des mécanismes de toxicité de l'AA
- Cascade d'évènements: l'exposition AA ➔ Lésions rénales
- Identifier biomarqueurs d'exposition à l'AA

Clinique

- Reclasser les patients Croates selon la sévérité de leurs lésions rénales
- Identifier les changements métaboliques caractéristiques du type et stade des lésions rénales
- Identifier des biomarqueurs précoces et non-invasifs de lésions rénales chez l'humain

✓ Analyse métabonomique sur rats est transposable à l'humain.

✓ Altérations rénales induites par l'AA

✓ Pistes intéressantes pour comprendre le mécanisme de néphrotoxicité de l'AA

➔ **Nouvelles possibilités de recherches/stratégies thérapeutiques**

Zambie

Egypte

Colombie

Mali

Grèce

Nigeria

Mexique

Japon

Bolivie

Taiwan

Canada

Maroc

Afrique du Sud

Philippines

Sri Lanka

Paraguay

Chili

Soudan

Vietnam

Thaïlande

Népal

Venezuela

Argentine

Cambodge

Iran

Jordanie

Brésil

Inde

Madagascar

Birmanie

Merci

Expérimental

Développer nouveaux modèles

1. Rats femelles
2. Souris

étudier si différence selon le genre

utilisés souches différentes cibles spécifiques

➔ Confirmation de nos hypothèses

Clinique

- **Confirmer nos premières observations avec plus d'échantillons**
- **Développer un test de dépistage précoce**

Thank you for your attention

- 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 !