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Metabolomics applied to Toxicology : The Aristolochic Acids Nephropathy Case

SWEMSA - ERDING - October 21-23 2019

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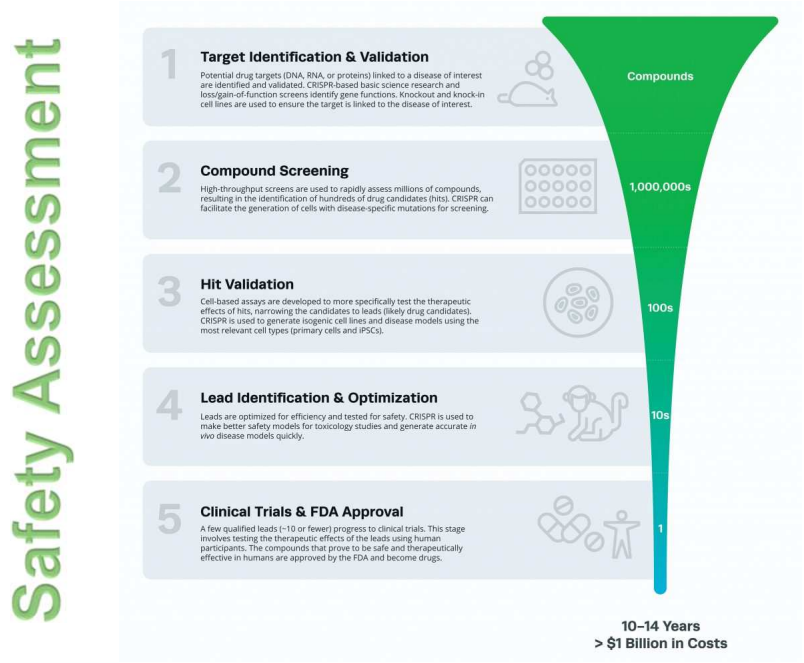
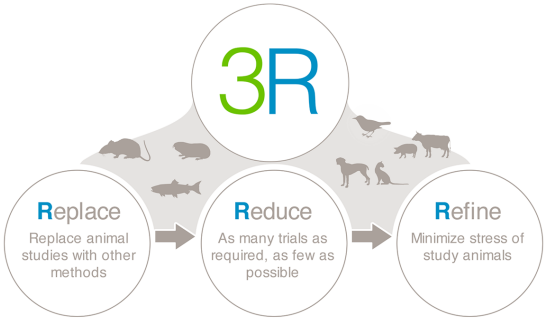
Faculté
de Médecine
et de Pharmacie

Jean-marie.colet@umons.ac.be

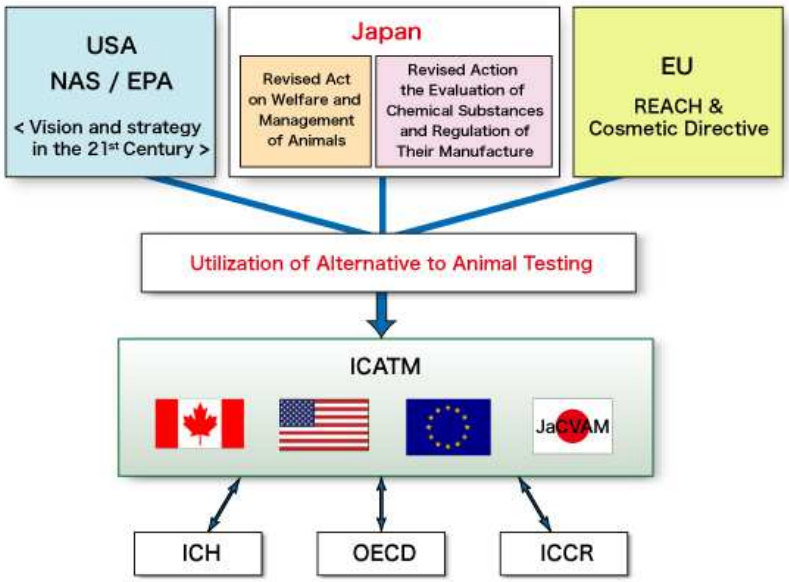


2

ANIMAL
TESTING


3R
Replace
Replace animal studies with other methods
Reduce
As many trials as required, as few as possible
Refine
Minimize stress of study animals

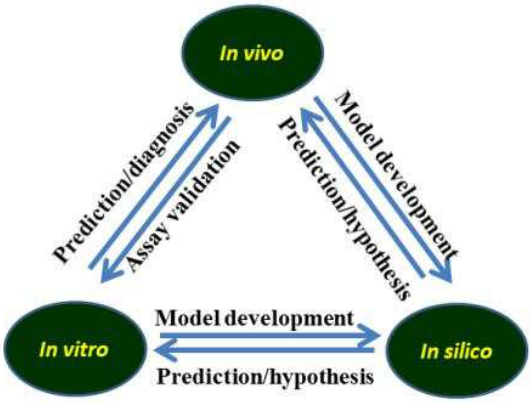
3


USA
NAS / EPA
< Vision and strategy in the 21st Century >
Japan
Revised Act on Welfare and Management of Animals
Revised Action the Evaluation of Chemical Substances and Regulation of Their Manufacture
EU
REACH & Cosmetic Directive
Utilization of Alternative to Animal Testing
ICATM
JaCVAM
ICH
OECD
ICCR

4

5

What is our in-house workflow?



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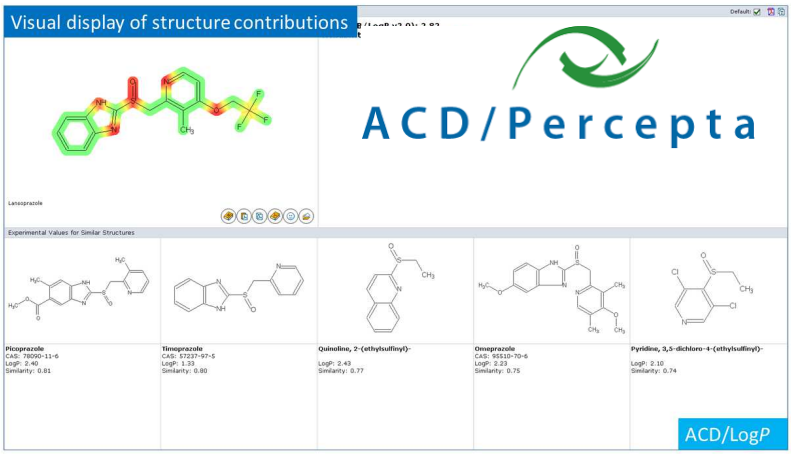
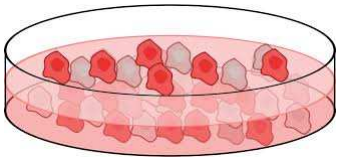
QSAR approaches

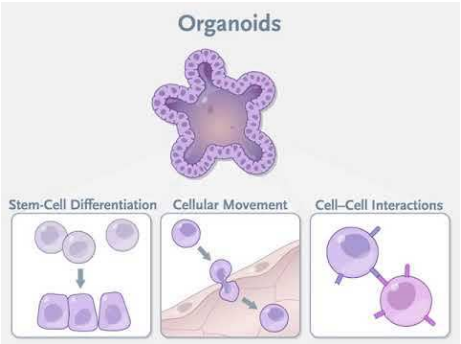
REPLACE



7

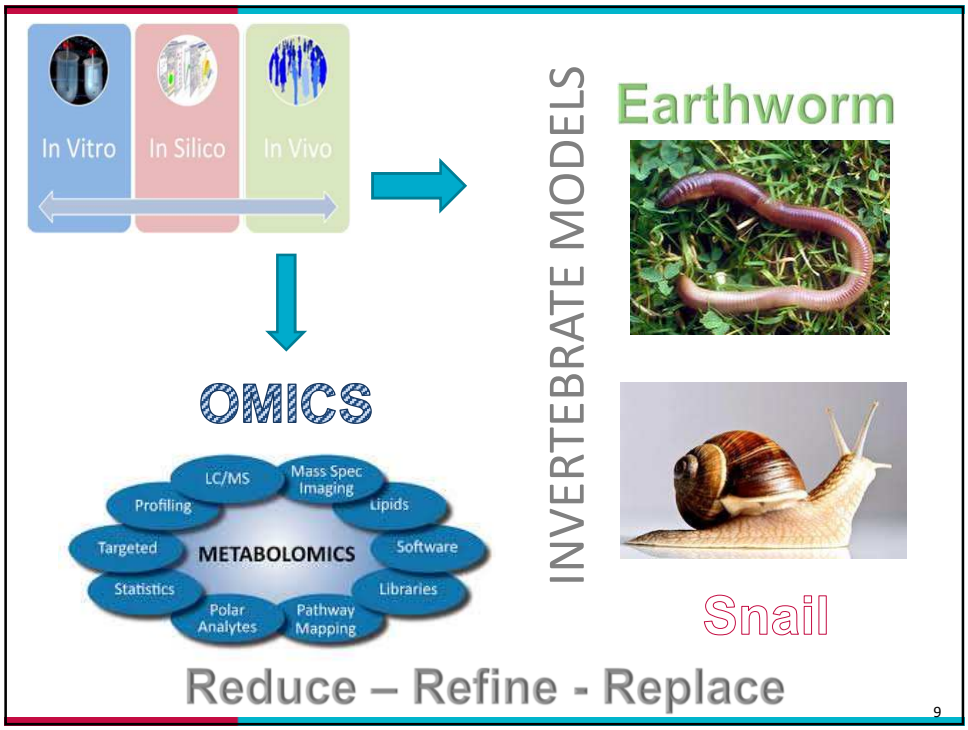
REPLACE





Liver
Kidney
Heart
Neurones
Endothelial cells

8


In Vitro
In Silico
In Vivo
OMICS
LC/MS
Mass Spec Imaging
Profiling
Lipids
Targeted
METABOLOMICS
Software
Statistics
Libraries
Polar Analytes
Pathway Mapping
INVERTEBRATE MODELS
Earthworm
Snail
Reduce – Refine - Replace
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BIO
profiling
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ULB
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BIOPROFILING PLATFORM IN A FEW WORDS …

- Financial support from the European Union and Wallonia under the ERDF-ESF 2014-2020 program
- Interuniversity project (Brussels-Wallonia region)
- 3,4M€ for equipment acquisition
- Located at UMONS (Mons, Belgium)
- Open to all actors in the biotechnology sector

BIO profiling

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Two complementary platforms :

Meta-Vision

- Small molecules
- NMR-based

MS-Quanta

- Macromolecules
- MS-based

WWW.BIOPROFILING.EU

BIO profiling

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Meta-Vision

Equipment

NMR Spectrometer
600 MHz Bruker AVANCE™

BIO profiling

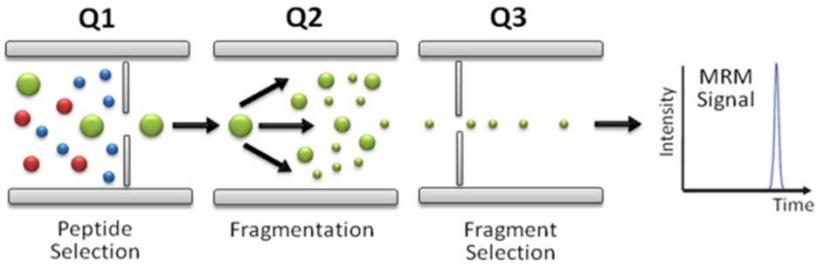
MS-Quanta

1. **High Resolution mass Spectrometers :**
   Exploratory quantitative analysis in DDA and DIA mode
   Relative quantitative profile of more than 2000 targets
   Triple TOF 3600+ (SCIEX) Q Extractive (Thermo Scientific)
   Triple TOF 6600 (Sciex) Q Extractive Plus (Thermo Scientific)
2. **High sensitivity and high speed mass spectrometers :**
   SRM/**MRM**/PRM analysis
   Simultaneous quantification (multiplex mode) > 50 targets
   Qtrap 6500+ (Sciex) Xevo TQ-S (Waters)
3. **High resolution mass spectrometers in mass or space :**
   FT-ICR SolarX XR (Bruker)
   MALDI-TOF/TOF Rapiflex (Bruker)

BIO profiling UMONS ULB LIEGE université

Multiple Reaction Monitoring

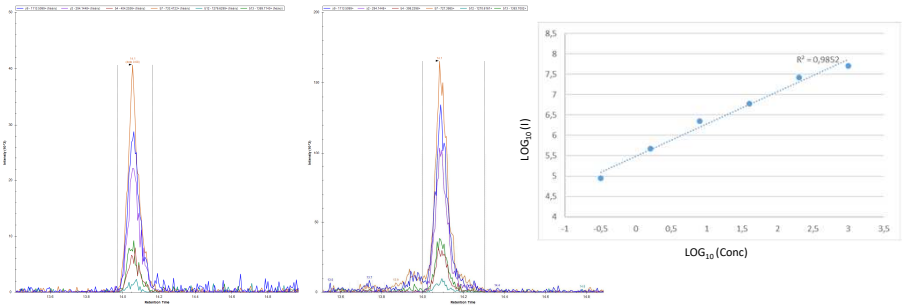
Monitoring of transition (precursor ion + fragment ion) along LC separation of peptides



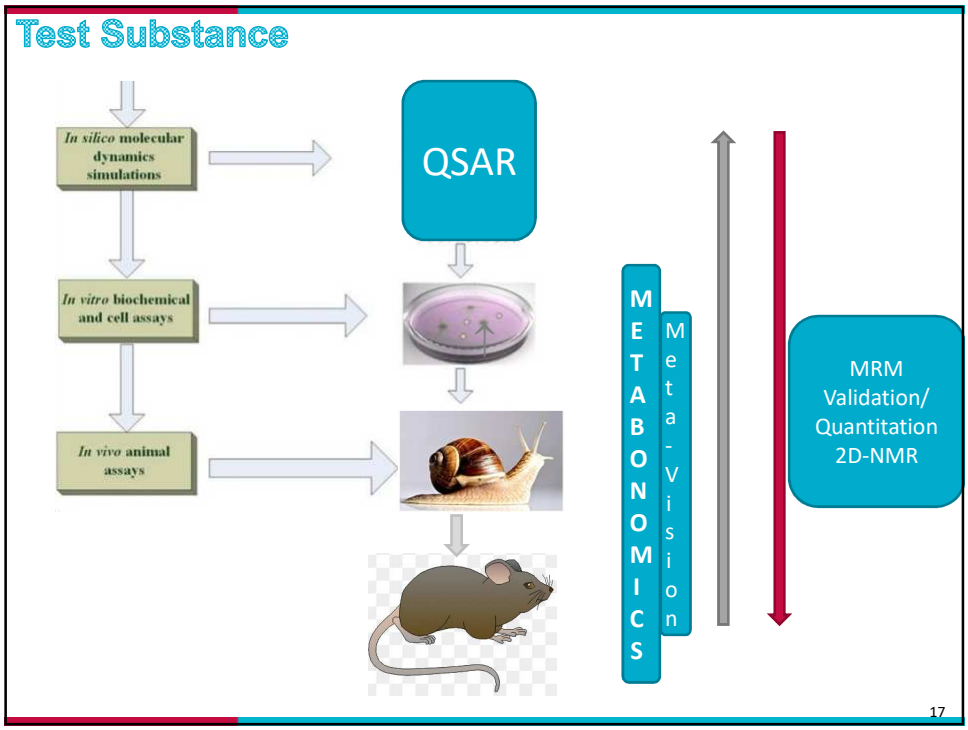
15

MRM case study: absolute quantification

Absolute quantification based on internal (heavy) standard peptide



16


Test Substance
In silico molecular dynamics simulations
In vitro biochemical and cell assays
In vivo animal assays
QSAR
METABONOMICS
Meta-Vision
MRM Validation/ Quantitation 2D-NMR
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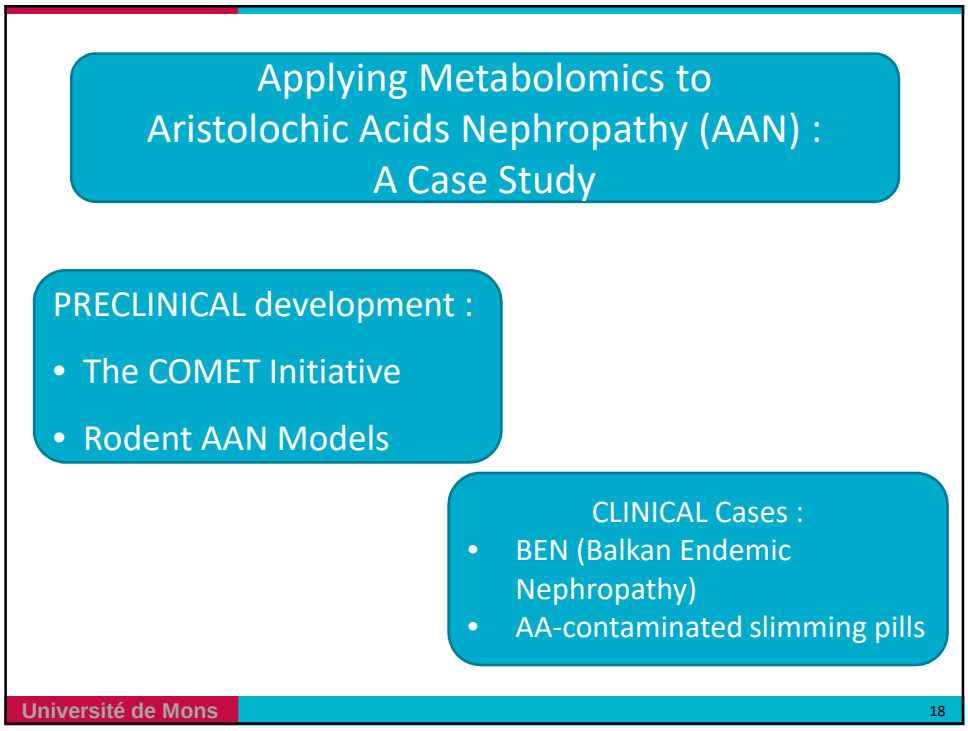
Applying Metabolomics to Aristolochic Acids Nephropathy (AAN) : A Case Study
PRECLINICAL development :
• The COMET Initiative
• Rodent AAN Models
CLINICAL Cases :
• BEN (Balkan Endemic Nephropathy)
• AA-contaminated slimming pills
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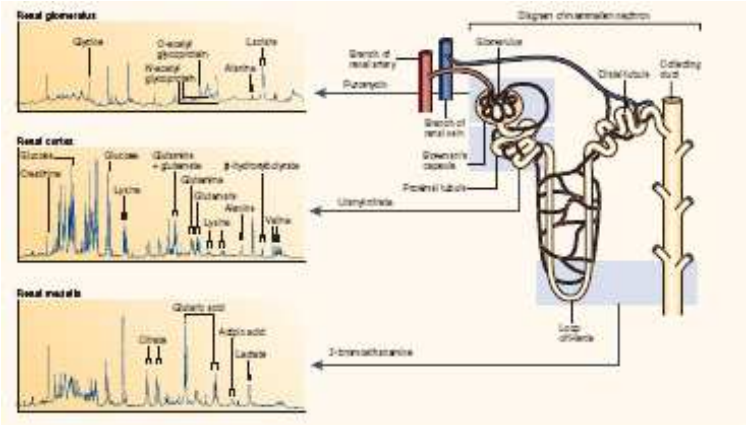
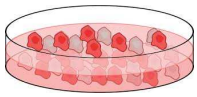


Nicholson JK Nat rev Drug Disc 1(2):153-161 (2002)

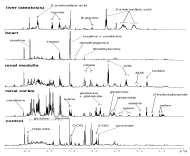
Metabolism cages on refrigerated racks

Urine

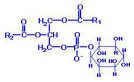
Blood



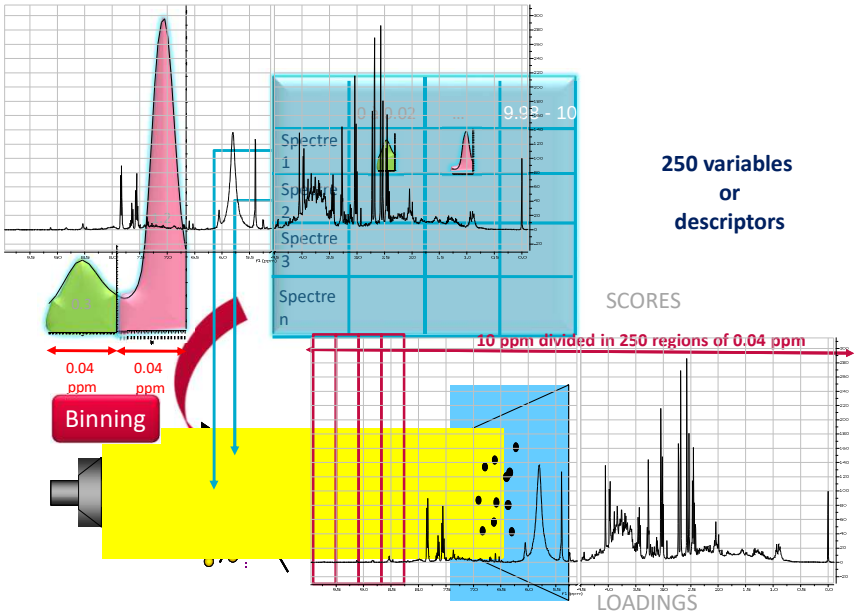
^{1}H NMR



Pattern recognition



Potential biomarkers



Creating an Excel table

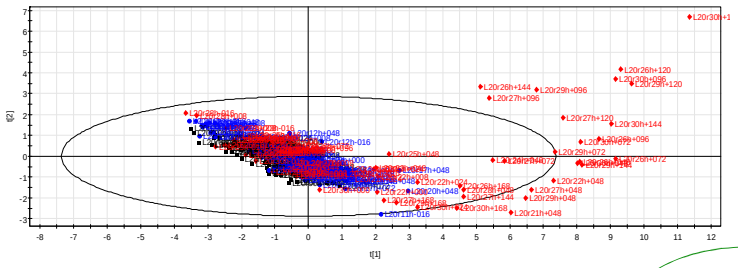
Median Chemical Shift of the descriptor 9.86 to 9.90 ppm

AUC in a descriptor

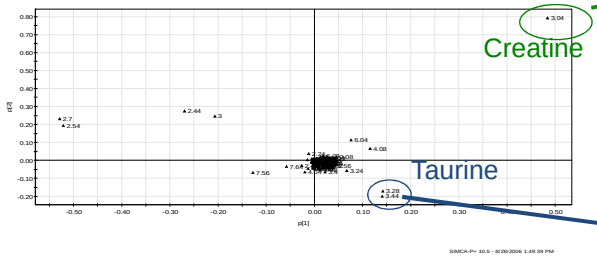
	9.96	9.92	9.88	9.84	9.8	--	0.4	0.36	0.32	0.28	0.24
L20r01h-016	0.000983	0.0012	-0.00207	0.00186	0.00156	--	0.0076	0.00836	0.00545	0.00793	0.00748
L20r02h-016	0.000161	-0.0021	-0.00026	-0.00063	0.000468	--	0.00684	0.00464	0.00512	0.0104	0.00614
L20r03h-016	0.000428	-0.00026	0.0013	0.00138	0.0026	--	0.0125	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



3.04 ppm

Loadings Plot

3.28 & 3.44 ppm

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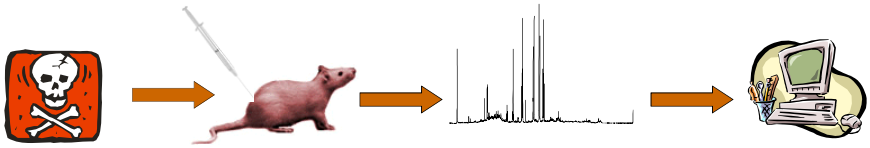
COMET

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

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COMET: Control Model

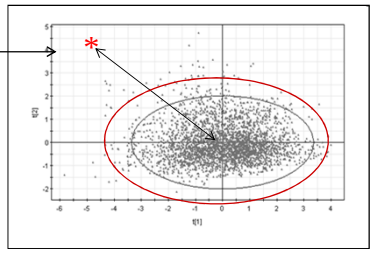
Prediction and Classification of Drug Toxicity Using Probabilistic Modeling of Temporal Metabolic Data: The Consortium on Metabonomic Toxicology Screening Approach
T. Ebbels, H. Keun, O. Beckonert, M. Bollard, J. Lindon, E. Holmes, and Jeremy K. Nicholson
Journal of Proteome Research/2007

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%



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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

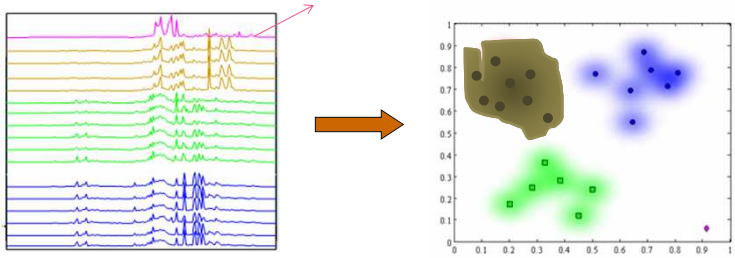
- PNN-based model (Probabilistic Neural Network – Specht 1990)

Test sample

Cardiac toxicity

Renal toxicity

Liver toxicity



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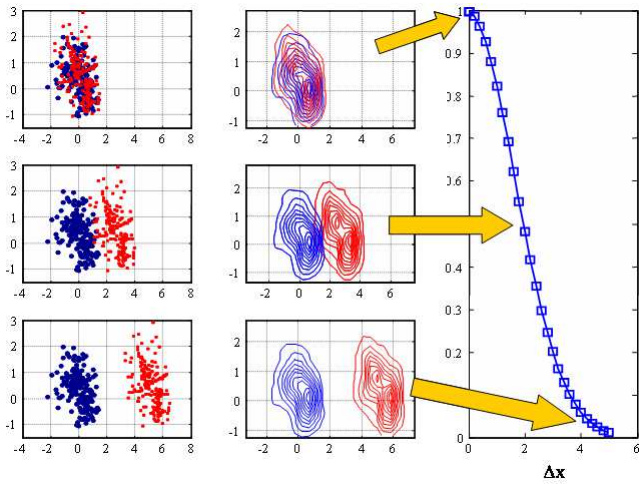
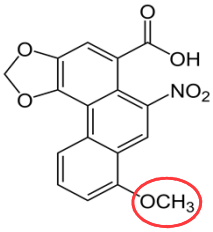
Clouds approach



Classification based on similarity

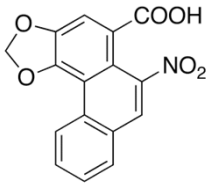
Aristolochic Acids Nephropathy

- **Mixture**



Aristolochic Acid I (AAI)

+



Aristolochic Acid II (AAII)

➢ Active compounds from *Aristolochiaceae*

Aristolochia

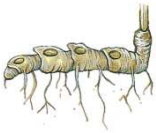
www.atleurdepau.com

Asarum

Braganti

etc.usf.edu

Natural Remedies in traditional medicines



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Rhizomes :
- Gastric stimulant
- Fever
- Malaria

Leaves :
- Skin troubles
- Digestive troubles
- Leishmaniasis

Fruits :
- Respiratory disesas
- Wounds
- Malaria

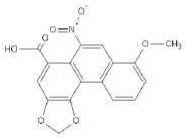
Flowers :
- Diuretic

Racines :
- Type II diabetes
- Snake bite

Rods :
- Anti-inflammatory
- Diuretic
- Poor blood cirulation

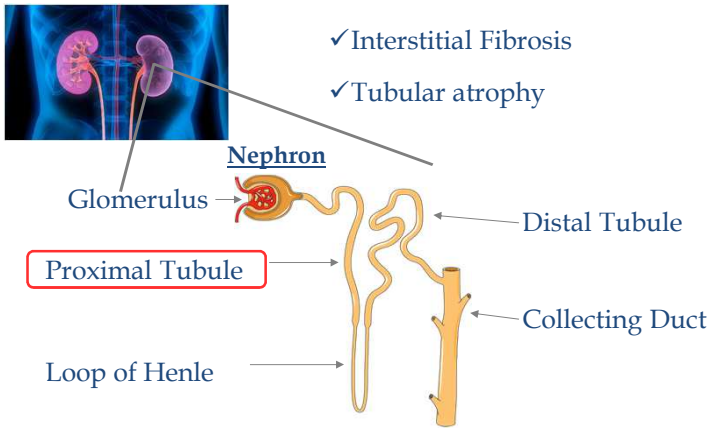
Seeds :
- Liver troubles
- Abdominal pain

QSAR analysis (PERCEPTA®)

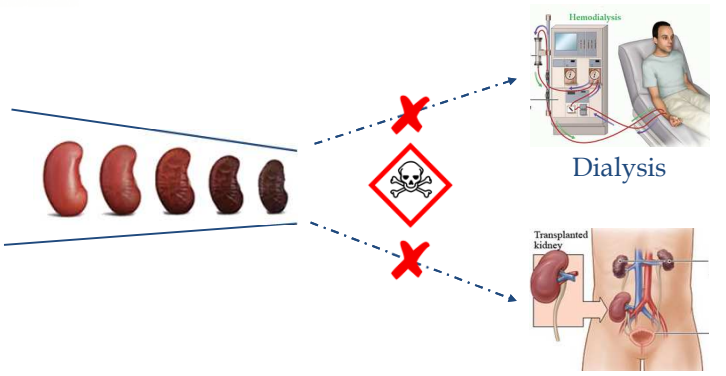
Structure	Compound name	Toxic effect	Route	Species	Studies
	Aristolochic acid	Changes in tubules; Kidney tumors	Intravenous; Oral	Human male; Rat	Acute; Carcinogenicity

Toxicity of Aristolochic Acids

- **Kidneys (Nephrotoxicity)**

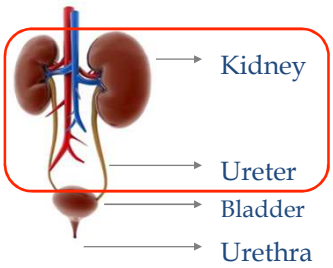


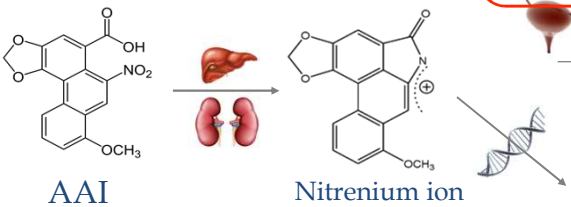
✓ CHRONIC RENAL FAILURE



➢ Carcinogenicity

✓ Urothelial cancers





AAI

Nitrenium ion

✓DNA adducts

✓Genetic mutations

✓Cancer lesions

Balkan Endemic Nephropathy (BEN)

✓**Chronic renal disease**

- ✓ Interstitial Fibrosis
- ✓ Tubular atrophy
- ✓ Proteinuria
- ✓ DNA adducts
- ✓ Urothelial cancers

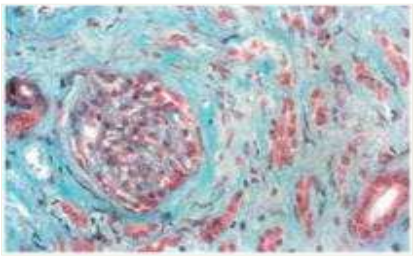
✓**Balkan Endemic Region**

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

- ✓ Environmental intoxication
- ✓ *Aristolochia Clematitis*
  - ❖ AA's contaminated bread
  - ❖ Diseased inhabitants > 100 000

A belgian clinical case of AAN

- ✓ 90's in Belgium
- ✓ > 100 female patients
- ✓ Ingestion of slimming pills
- ✓ Chinese herbs
- ✓ Formulation error



Debelle et al., Kidn.Int. 2008

➢ *Stephania Tetrandra* ➔ *Aristolochia Fanghi*

MODE of ACTION (MOA) of AAs → Biomarkers

Metabonomic

Experimental

Clinical

Unveil molecular & cellular modes of action of AAs

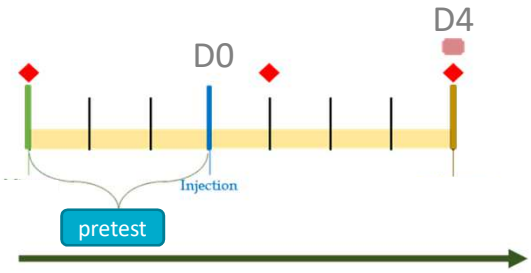
New therapeutic targets

Identification of early and non-invasive biomakers

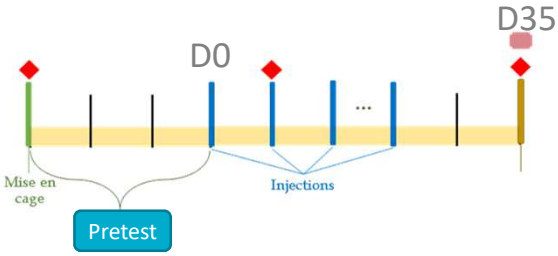
Early screening of AAN

Rodent models of AAN

Acute

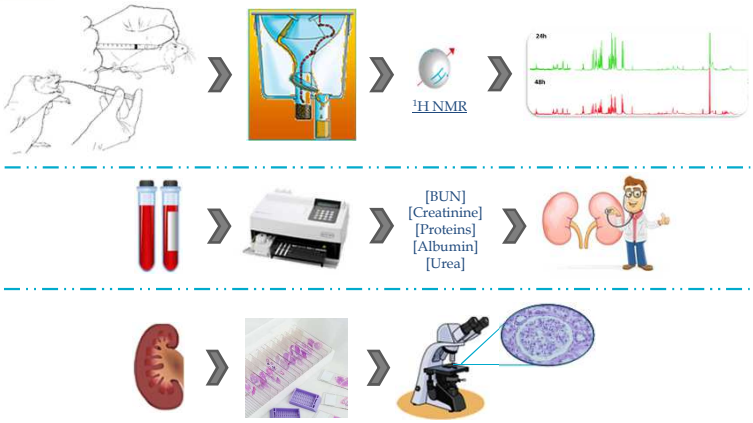


(sub)-Chronic

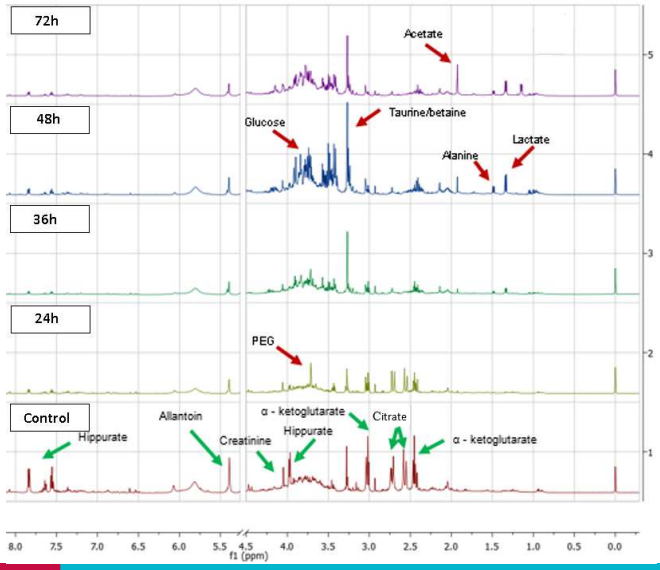


Blood sampling
Euthanasia
Urine collection

Rodent models of AAN



Urine samples from rat exposed to AAI over time



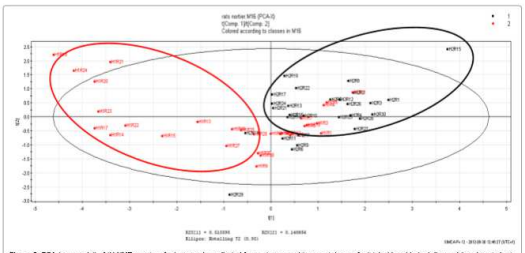


Figure 2: PCA (scores plot) of ^{1}H-NMR spectra of urine samples collected from rats exposed to repeat doses of aristolochic acids (red diamonds) and control rats (black squares).

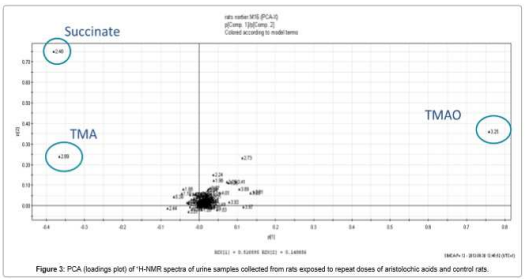
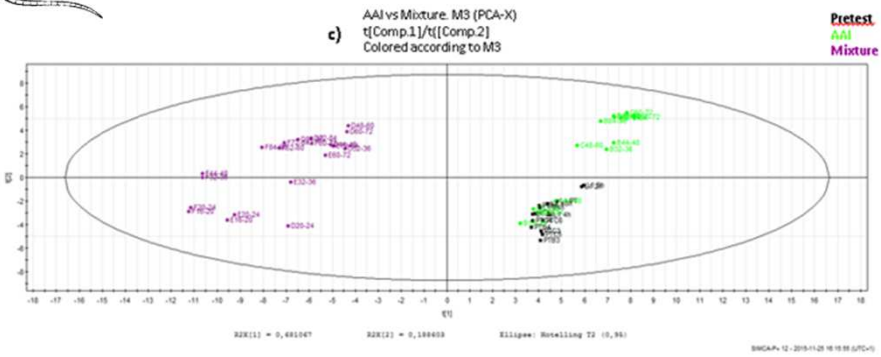


Figure 3: PCA (loadings plot) of ^{1}H-NMR spectra of urine samples collected from rats exposed to repeat doses of aristolochic acids and control rats.

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

AAI versus mixture (AAI + AAII)



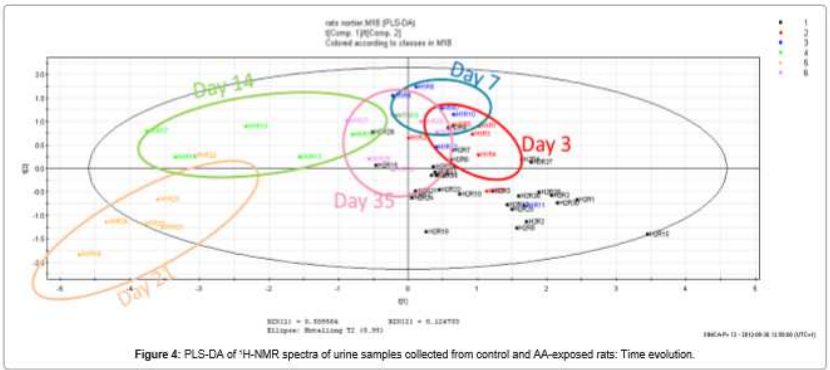
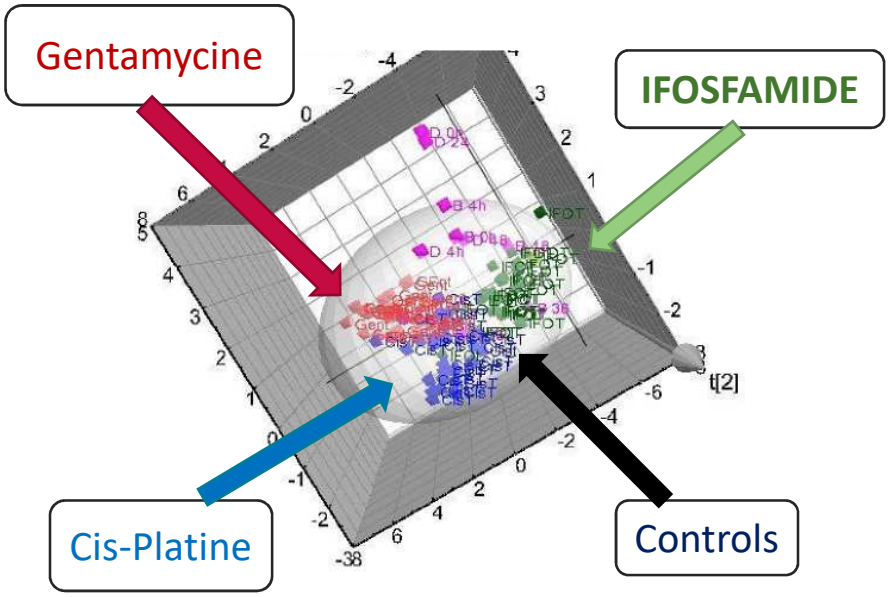


Figure 4: PLS-DA of ^{1}H-NMR spectra of urine samples collected from control and AA-exposed rats: Time evolution.

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



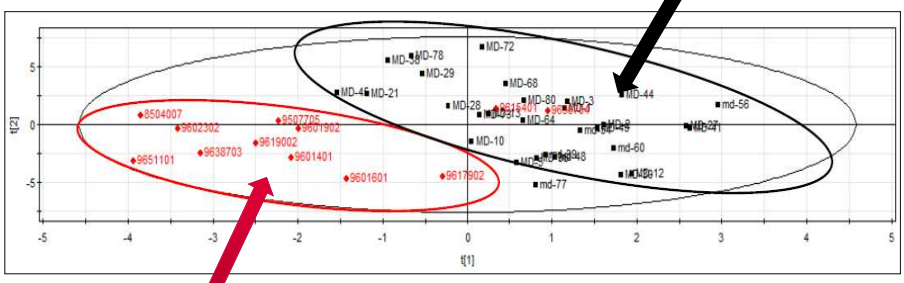
Metabonomic findings in rats :

Ifosfamide-induced Fanconi syndrome

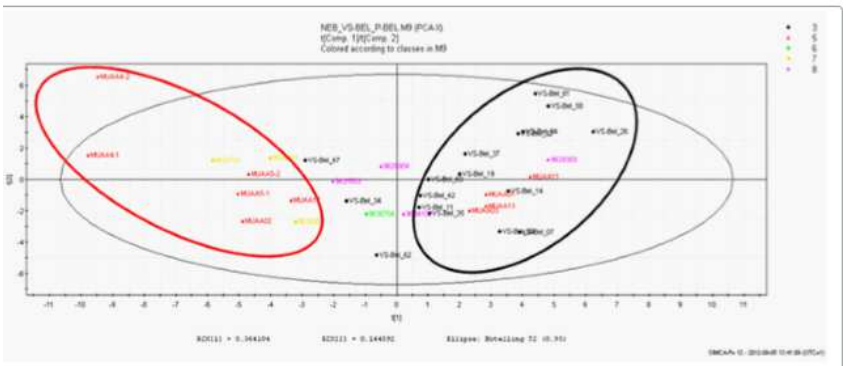
- Proximal tubular necrosis
- Hemorrhagic cystitis
- Oxidative stress
- Role of free radicals
- with depletion of glutathione (GSH)
- Hypoxia episodes

AA's similar to ifosfamide (early phase)

Healthy volunteers



Croatian diseased women



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

Clinical findings

- Renal impairment reminding of Fanconi syndrome
- Oxidative stress (mitochondrial dysfunction)
- Cognitive disorders (♀ : β-hydroxybutyrate & Methylguanidine)

Contribution of METABONOMICS in AA's toxicology

Experimental models

- Better understanding of molecular and cellular MOA
- Dynamic evaluation of the successive adverse effects affecting the kidneys (low vs high doses + acute vs chronic exposure)
- Evaluation of possible antidotes
- Identification of transposable biomakers

Contribution of METABONOMICS in AA's toxicology

Clinical

- Identify metabolic changes which are specific of the type and stage of renal lesions
- Propose a classification of patients that is closer to the progression of the renal disease
- Identify early and non-invasive biomarkers of AKD and CKD with a better understanding of the AKD-to-CKD transition (potential for new therapeutic strategies)

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All my
colleagues

THE END

THANK YOU FOR YOUR ATTENTION!