Metabolomics
Nature Reviews: Drug Discovery
Nicholson et al. (2002)
Efficacy
Toxicity

Primary Molecules
Secondary Molecules

Filtration
Dilution
Concentration
Resorption

hippurate urea
allantoin creatinine
hippurate creatinine taurine
TMAO
creatinine citrate
2-oxoglutarate succinate

Adapted from D. Robertson, Pfizer Global Research and Development
Metabolomics

Comprehensive Definition:
The quantitative measurement of the time-related multiparametric metabolic response of living systems to pathophysiological exogenous or endogenous stimuli or genetic modification

Operational Definition:
The systematic exploration of biofluid composition using NMR/pattern recognition technology in order to associate target organ toxicity with NMR spectral patterns and identify novel surrogate markers of toxicity.

Adapted from D. Robertson, Pfizer Global Research and Development
Metabolomics:
The study of the total metabolite pool (metabolome), metabolic regulation and fluxes in individual cells or cell types. Can be achieved through a wide spectrum of technologic methods including LC-MS, GC-MS, and nuclear magnetic resonance (NMR)

Metabonomics:
The study of the systemic biochemical profiles and regulation of function in whole organisms by analyzing a metabolite pool (metabolome) in biofluids and tissues. Usually implies that the study is done specifically through nuclear magnetic resonance profiling
**Metabolome:**
The quantitative complement of all the low molecular weight molecules present in cells in a particular physiological or developmental state

**Biofluid:**
A fluid sample obtained from a living system. The donor might typically be a human or an animal. Fluids can be excreted (such as urine, sweat), expressed or secreted (such as milk, bile), obtained by intervention (such as blood plasma, serum or cerebrospinal fluid), develop as a result of a pathological process (such as blister or cyst fluid), or be applied and collected (such as dialysis fluid)
Advantages of Metabolomics

- Identification of target organ, severity, onset, duration and reversal of the effects (time-course)
- Classify sample as “normal” vs. “abnormal”
- Determine mechanisms of action within the organ
- Potential for identifying novel biomarkers of toxic effect
- Non-invasive
- No *a priori* decisions about samples need be made
- No sample processing necessary other than cold collection
- Complete time course data can readily be obtained
- Minimization of compound requirements
- Relatively fast analysis (200-300 samples/day)
- Useful tool for modeling physiological variation and exposure conditions in animals and humans

Adapted from D. Robertson, Pfizer Global Research and Development
NMR spectroscopy

Spectroscopy deals with the interactions between electromagnetic radiation and matter. Spectroscopy is used to derive the properties of matter at the molecular level. Nuclear magnetic resonance (NMR) exploits the magnetic properties of atomic nuclei.

The method functions as follows: A substance is placed in a magnetic field. Some atomic nuclei (e.g. protons, nuclei of hydrogen atoms) then behave like microscopic compass needles, called nuclear spins. Each nuclear spin orientation corresponds to a different energy level. The spins may jump between the levels when the sample is exposed to radio waves whose frequency exactly matches the energy spacing. This is called resonance. One way of measuring the energy is to change the irradiation frequency. At resonance, the spins flip causing an electric signal. The strength of the signal is plotted as a function of frequency in a diagram, the NMR spectrum.

In metabolomics, it is the patterns that occur when many different biochemical entities are detected simultaneously in a mixture using $^1$H NMR that are interpreted.
NMR in Metabolomics:

Pro:
- Non-destructive
- Applicable to intact biomaterials
- More information rich in complex-mixture analyses
- No extraction/derivatization is necessary

Con:
- Less sensitive than MS

History:
- NMR has been used to study metabolites in biofluids for over a decade
- Metabolomics technology as it is known today (600 MHz $^1$H NMR) was pioneered by Jeremy Nicholson, Elaine Holmes and John Lindon of Imperial College in London
- Only recently have advances in flow-through NMR hardware and pattern recognition software made the possibility of “high”-throughput in vivo toxicity assessment a practical possibility
Varian Inova 600
Shielded magnet
120 ul flow probe

Refrigerated Metabolism Cage (0°C)

NMR Acquisition and Gilson 215 Control System

Biomek® Robot

Deuterated Buffer + NaN₃

Frozen Storage

N₂ gas

Gilson 215 autosampler

Data Processing

Adapted from D. Robertson, Pfizer Global Research and Development
Functional NMR Spectrum of Rat Urine

“Biomarker Windows”

Normal Metabolic Profiles

Adapted from D. Robertson, Pfizer Global Research and Development
Techniques and Procedures in Metabolomics

NMR Spectra → Primary Data Processing → Unsupervised mapping of data in 3D space → Supervised classification and calculation of confidence intervals

**Pattern Recognition (PR) Methods:**
PR and related multivariate statistical approaches can be used to discern significant patterns in complex data sets and are particularly appropriate in situations where there are more variables than samples in the data set. The general aim of PR is to classify objects (in this case \(^1\)H NMR spectra) or to predict the origin of objects based on identification of inherent patterns in a set of indirect measurements. PR methods can reduce the dimensionality of complex data sets via 2 or 3D mapping procedures, thereby facilitating the visualization of inherent patterns in the data.

**Principal Components Analysis (PCA):**
This is a data dimension reduction method that involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called *principal components*. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. Use of PCA enables the "best" representation, in terms of biochemical variation in the data set to be displayed in two or three dimensions.
1

ppm

Adapted from D. Robertson, Pfizer Global Research and Development
ALT (IU/L)

Control ALT = 33-94
(Normal Reference Range)

24 hr ALT = 1228 ±1061

48 hr ALT = 994 ±884

96 hr ALT = 161 ±108

Adapted from D. Robertson, Pfizer Global Research and Development
Adapted from D. Robertson, Pfizer Global Research and Development
PCA analysis of vehicle effect on rat urine NMR spectra

Controls, corn oil IP
Controls, untreated
Controls, saline IP

Adapted from D. Robertson, Pfizer Global Research and Development
ANIT 100 mg/kg

day 4

creatinie

day 3

creatinine

day 2

hippurate

TMAO

predose

citrate

2-oxoglutarate

succinate

day 1

Adapted from D. Robertson, Pfizer Global Research and Development
ANIT

Number in parentheses = mean serum total bilirubin (mg/dL)

Adapted from D. Robertson, Pfizer Global Research and Development
ANIT(50 mg/kg) in Mouse

Adapted from D. Robertson, Pfizer Global Research and Development
 Allyl Alcohol (12 mg/kg)

Pretest
24 Hr
48 Hr
72 Hr
96 Hr

Slightly elevated ALP (260-280 IU/L)

Note: Numbers next to symbols are individual ALT levels (IU/L)

Adapted from D. Robertson, Pfizer Global Research and Development
Allyl Alcohol (120 mg/kg)

Concurrent Controls

Elevated 24, 48Hr ALP 359-732 IU/L

Elevated Bilirubin (1.1 mg/dL)

Normal ALP (< 206 IU/L)

Pretest

24 Hr

48 Hr

72 Hr

96 Hr

Necrosis

Elevated ALT

Adapted from D. Robertson, Pfizer Global Research and Development
Allyl Alcohol
Combined Data, 12 (△) & 120 (○) mg/kg

Concurrent Controls

Biliary Toxicity
Elevated ALP & Bilirubin

Necrosis
Elevated ALT

PC 1

PC 2

Pretest
24 Hr
48 Hr
72 Hr
96 Hr
Metabolomic Detection of Liver Toxicity

Metabolomic Detection of Kidney Toxicity

Integrated Metabolomics

Compound libraries

Preliminary activity screening

Pre-development pharm/tox profiling

Conventional/alternative screening procedures

Tissues (MAS-NMR)

Rodent dosing and biofluid NMR

Data reduction and mapping

Expert-system-based decision processes

Normal/abnormal?

Biomarker identification

Toxicity classification

Rate constants for drug transformations and consequent effects on cellular processes in one organ, such as the liver.

Displaced times between effects at different levels of biomolecular organization.

A series of rate constants for distribution, metabolism and effects in the rest of the body.

Limitations of Metabolomics

- Specialized equipment is required
- Extensive expertise is required
- Information is limited to time- and dose-points taken
- High risk of false positive data: a compound causes significant metabolism changes without associated toxicity
- Difficulty in separation of physiological (adaptive) and toxicological (adverse) effects
- Sensitivity of the assay
- Certain pathological states have negligible effects on biofluids: liver fibrosis may go undetected until damage is severe
- Availability of biofluids for certain organ toxicity: CNS vs. urine
- Distinguishing effects of multi-organ toxicants: biomarkers in different biofluids are different and in one biofluid are inter-mixed
Future Directions in Metabolomics

• Develop comprehensive metabonomic database
• Expand metabonomics applications to many species
• Evaluate cryoprobe technology for increased sensitivity or increased throughput
• Expand technology to novel targets:
  – Cardiac toxicity
  – Adrenal toxicity
• “Grand Unification” of Genomic/Proteomic and Metabonomic technologies
**Metabolomics of drugs and drug metabolites**

ANIT trajectory represents the aggregate biochemical effects of the metabolic flux (ANIT to ANIC to ANA). The first metabolic direction of the ANA-dosed animals is in the third principal component (PC3). For ANIC, the second direction is in PC3, as it takes time for ANA to be produced. For ANIT, the PC3 direction change takes longer, as ANA is formed after ANIC. This approach, termed ‘metabolic trajectory deconvolution’, allows effects of drugs and their metabolites to be separated for mechanistic purposes. Endogenous metabolic changes that are caused by the effects of successive drug metabolites must also mirror complex time-related changes in the transcriptomic and proteomic patterns, thus limiting the value of single time-point measurements of genes and proteins.