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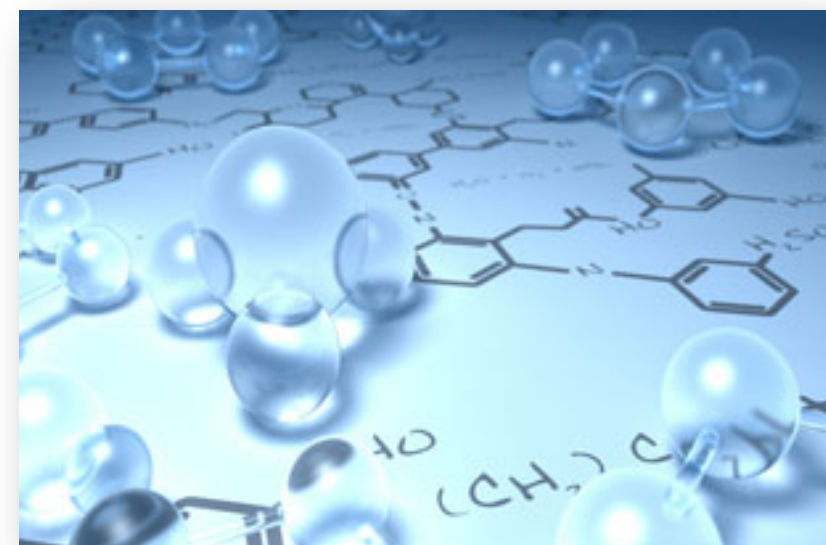
Accelerating the impacts of metabolomics in regulatory toxicology – an academic view

*RSC / IGHRC Joint Awareness Day, London
13 October 2017*

Professor Mark Viant
University of Birmingham, UK

Overview of presentation

- Metabolomics is a mature science in academia, but less so in regulatory toxicology
- Five thoughts on *Why?*
- Case studies in metabolomics
 - Discovering MoA (and an endpoint for a regulated bioassay?)
 - Insights into toxicokinetics
- Progress on Best Practice guidelines for regulatory metabolomics
- Accessibility to metabolomics tools



Metabolomics is a mature science *in academia*

Community: large International Metabolomics Society, with national affiliates, extensive conference programs, scientific working groups...

Data: European Bioinformatics Institute's MetaboLights database, *fastest growing of all 'omics databases at EBI*

Publications: >59,000, several in *Science* and *Nature* (Web of Science, 2017)

Journals: *Metabolomics*, *Metabolites*, *Journal of Metabolomics*...

Investment: US NIH invested \$111 million (2012-17)

Outreach: first Massive Open Online Course (MOOC) >6,000 registrants

Scale: Global market expected to reach \$3.5 billion by 2021

(<https://www.mordorintelligence.com/industry-reports/global-metabolomics-market-industry>)

Metabolomics is a mature science ... particularly *in human disease*



International
Phenome
Centre
Network

International Phenome Centre Network

Press Release

For Release on November 29, 2016, 13:45 GMT

www.PhenomeNetwork.org

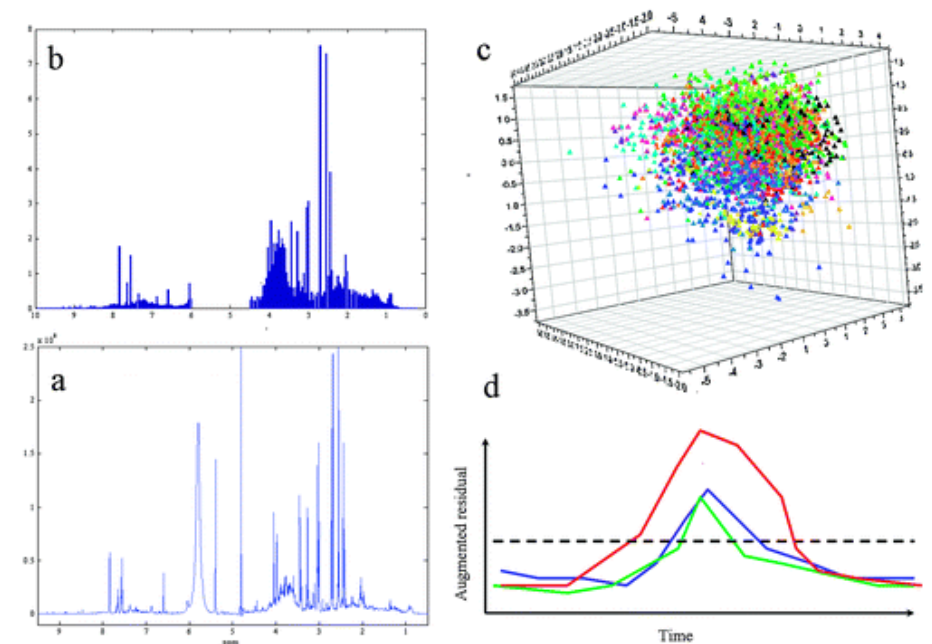
The **International Phenome [Metabolomics] Centre Network** is a research consortium working to transform health care globally and improve disease prevention, detection and treatment **by understanding disease mechanisms**

Includes more than a **dozen international partners** with hubs in: Australia, Canada, China, Japan, Singapore, Taiwan, the United States, and the United Kingdom

www.birmingham.ac.uk/phenome-centre

What *major activity* in metabolomics and toxicology?

- 2002-05, Consortium for Metabonomic Toxicology (COMET)
- Led by Jeremy Nicholson, Imperial College London and five pharmaceutical companies
- Objective: to generate a metabolomics database using ^1H NMR spectroscopy of rodent urine and serum and to build a predictive expert system for target organ toxicity in rat
- >10 publications
- Reported as highly successful



e.g. *J. Proteome Res.*, 2007, 6:4407–22

What *major activity* in metabolomics and toxicology?

- BASF's metabolomics program
- Led by Ben van Ravenzwaay with Metanomics Health
- Constructed a metabolomics database using mass spectrometry of rat serum, discovering numerous metabolome patterns for different toxicological targets
- Example: *Metabolomics: A tool for early detection of toxicological effects and an opportunity for **biology based grouping of chemicals**—From QSAR to QBAR*
Mutation Research 2012, 746:144–50
- >600 chemicals studied, >10 publications
- *Database not open access*

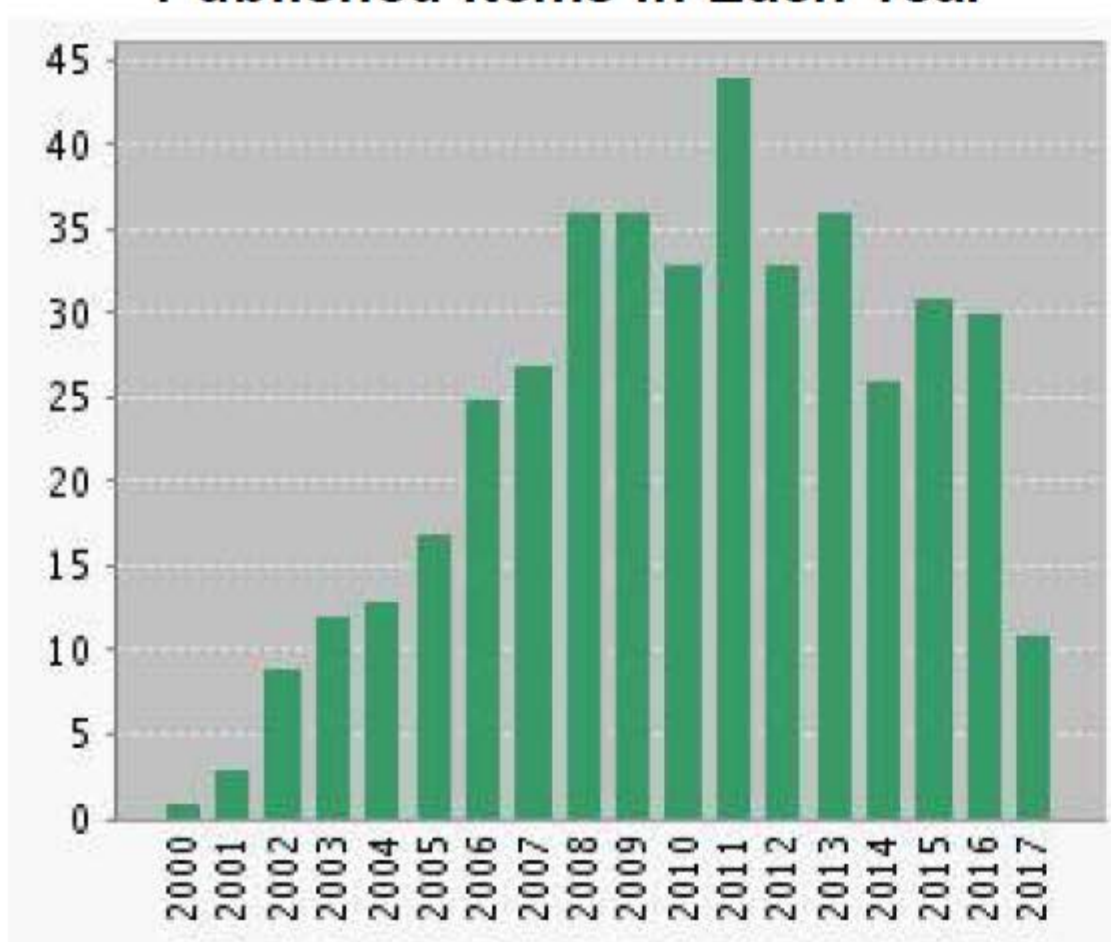
423 publications: topic of metabolomics and toxicology

Citation Report: 423

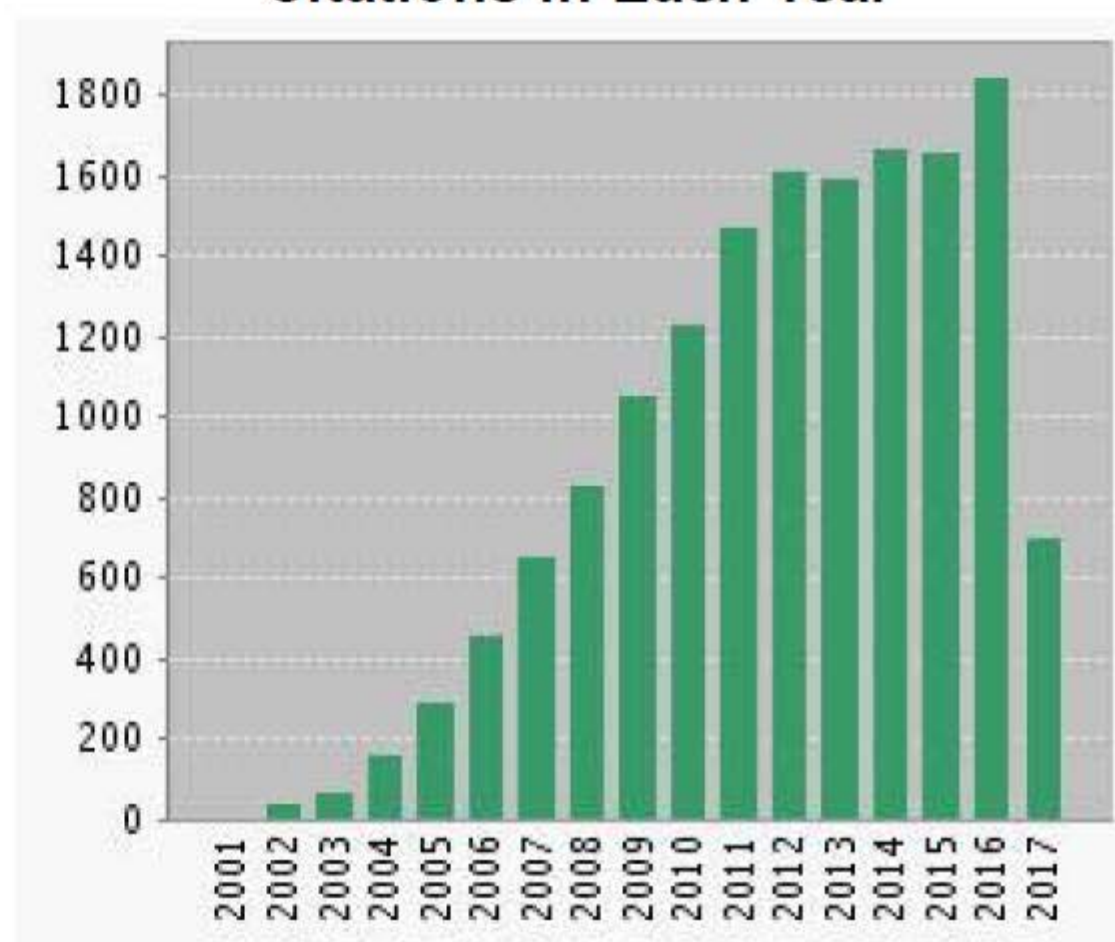
(from Web of Science Core Collection)

You searched for: **TOPIC:** ((metabolomic* or metabonomic*) and toxicology)

Published Items in Each Year



Citations in Each Year



Why isn't metabolomics already widely used in regulatory toxicology?

Key question – do the regulatory agencies have any interest in metabolomics?

Regulators are very interested in metabolomics

ECHA: New Alternative Methods (NAM) workshop report, April 2016

*“Conclusions: NAM data from **metabolomics** have provided a robust and interpretable basis for the mechanism of action which has supported the hypothesis of category membership and facilitates read-across”*

UK Health & Safety Executive: Prof George Loizou, Animal-Free Chemical Safety Assessment (*Frontiers in Pharmacology* 2016; 7:218)

*“**Metabolomics**... has the advantage of being high-throughput and currently provides the best approach to delineating and understanding a biological mechanism preceding an effect”*

EFSA: Jean Lou Dorne, Modern methodologies and tools for human hazard assessment of chemicals (*EFSA Journal* 2014; 12:3638)

*“In the future, publicly available databases combining in vitro and in vivo **OMIC** datasets for large databases of compounds with MoA/AOP knowledge will help considerably to a) identify biomarkers associated with specific AOPs, and b) to bring new tools for predictive toxicology.*

What are roadblocks to using metabolomics in regulatory context? (1)

- 1. Lack of relevant, appropriately designed, sufficiently large, open-access metabolomics case studies that address a regulatory (not academic) purpose**
- 2. Lack of Best Practice guidelines for conducting a regulatory metabolomics study and lack of validation and reporting standards**

ECHA NAM workshop – *“There are a number of R&D needs including a database to support metabolomics, standardisation, validation and reporting formats.”* (2016)

What are roadblocks (**and progress**) to using metabolomics in regulatory context? (2)

3. Accessibility to metabolomics tools (analytical and computational)

- **Commercial service providers, Phenome Centres, etc.**
- **Open-access workflows: W4M, EU PhenoMeNal, etc. (using Galaxy)**

4. Lack of education and training courses in (regulatory) metabolomics, both 'awareness training' and 'hands-on training'

- **Birmingham Metabolomics Training Centre provided 2-days of omics training to ECHA (www.birmingham.ac.uk/bmtc)**

5. Robust metabolite identification

- **Academic pursuit!**
- **Metabolite ID and Model Organism Metabolomes Task Groups of International Metabolomics Society, etc.**

1. Lack of relevant, appropriately designed, sufficiently large, open-access metabolomics case studies that address a regulatory (not academic) purpose

Examples...

- Enhancing chemical grouping and read-across
- Deriving points of departure for hazard assessment
- Discovering MoA(s), e.g. to identify new bioassays for screening

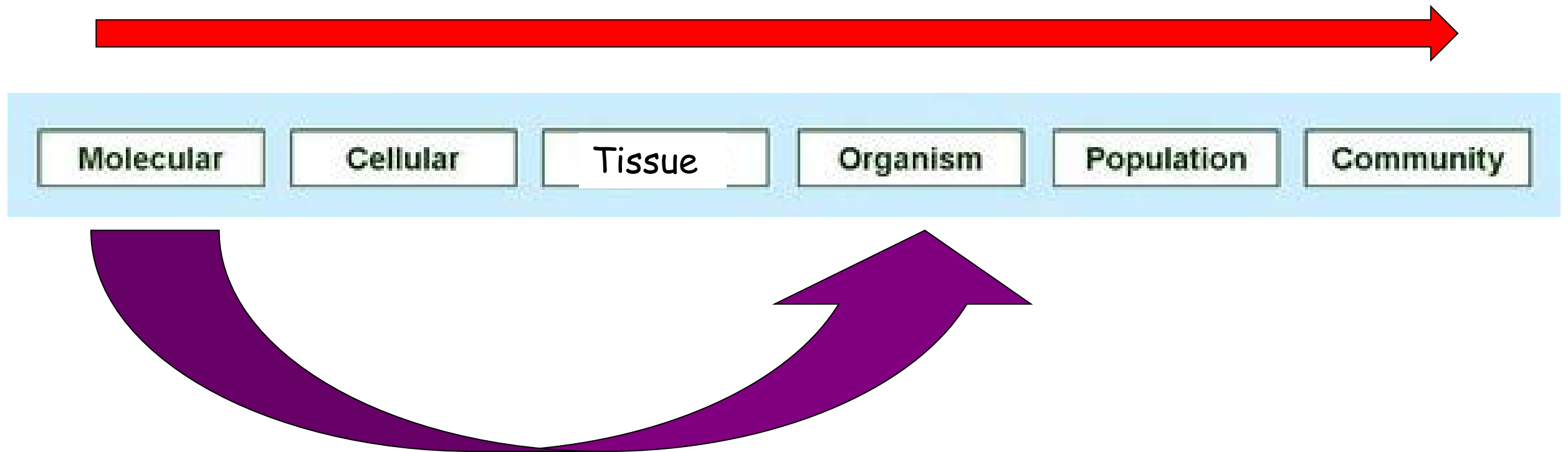
Strengths of metabolomics – highlighted in following examples

1. [Not unique] Shares several strengths with complementary omics techniques: non-targeted, **highly effective at discovery science**, e.g. insights into MoA, guiding selection of bioassays for screening
2. [Unique] Measures **actual cellular function**; metabolites can be causally associated with apical endpoints and adverse outcomes
3. [Unique] Potential to measure simultaneously the exposure chemical and its metabolites (**toxicokinetics**) and the disruption to cell/organism's endogenous metabolism

Discovering MoA
(and an endpoint for a regulated bioassay?)
using metabolomics

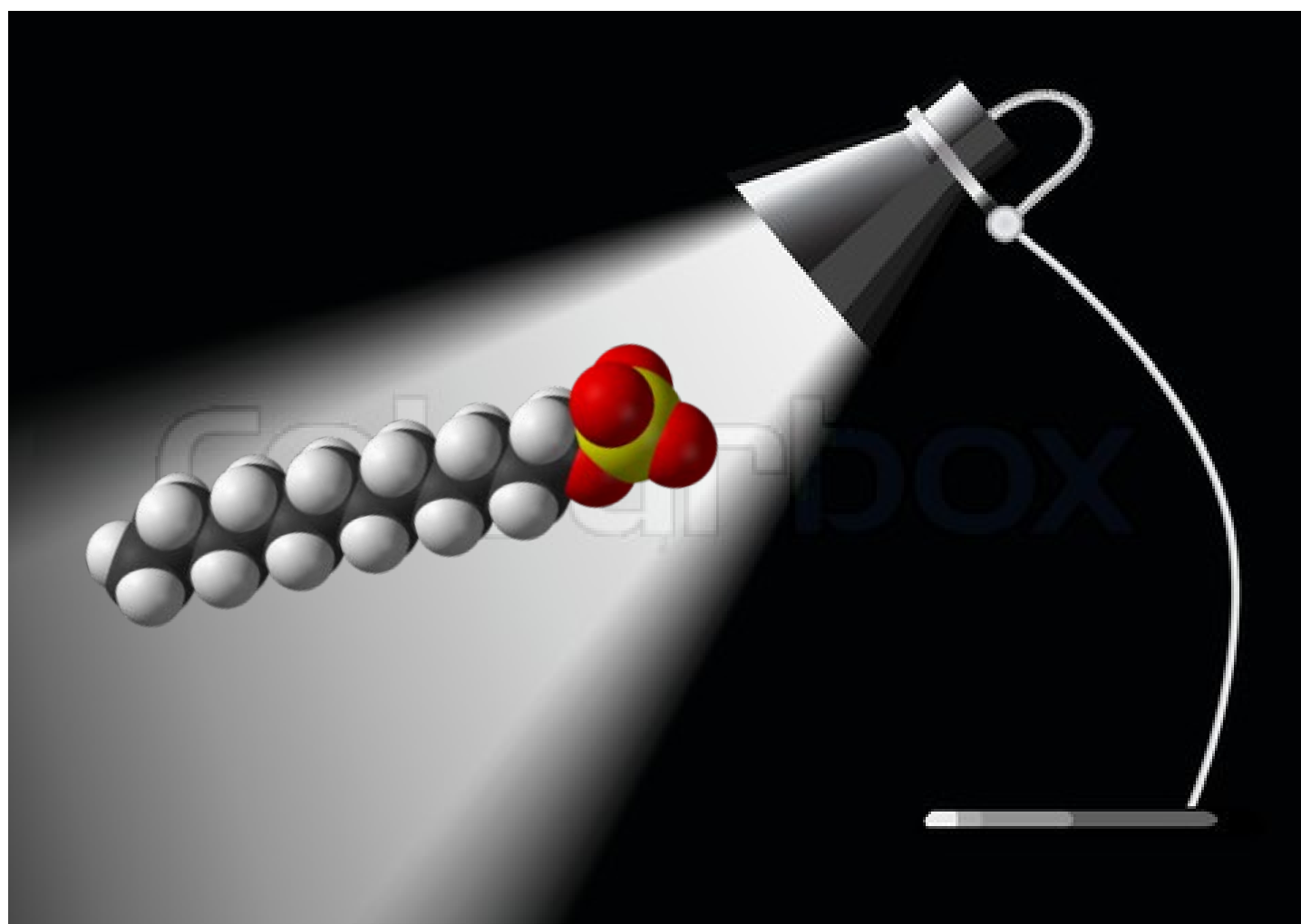
Adverse Outcome Pathway

relevance



Can metabolomics discover molecular markers that are predictive of adverse outcome at an organism (or higher) level?

Power of metabolomics for initial discovery



Translating the discovery to regulatory toxicology

Relevance



Molecular

Cellular

Tissue

Organism

Population

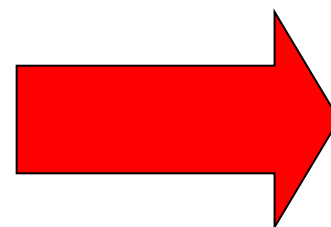
Community



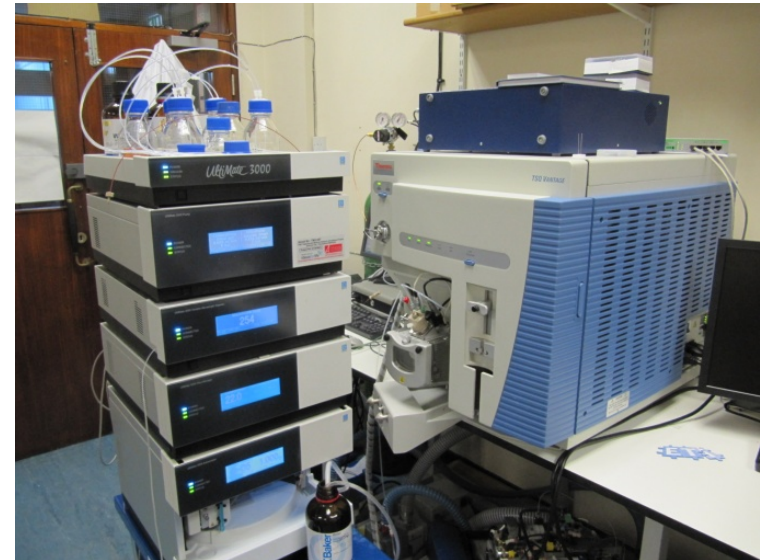
Metabolic markers predict
higher level responses

Specialised metabolomics
facilities & expertise

Validated, high-
throughput, targeted
LC-MS/MS assay



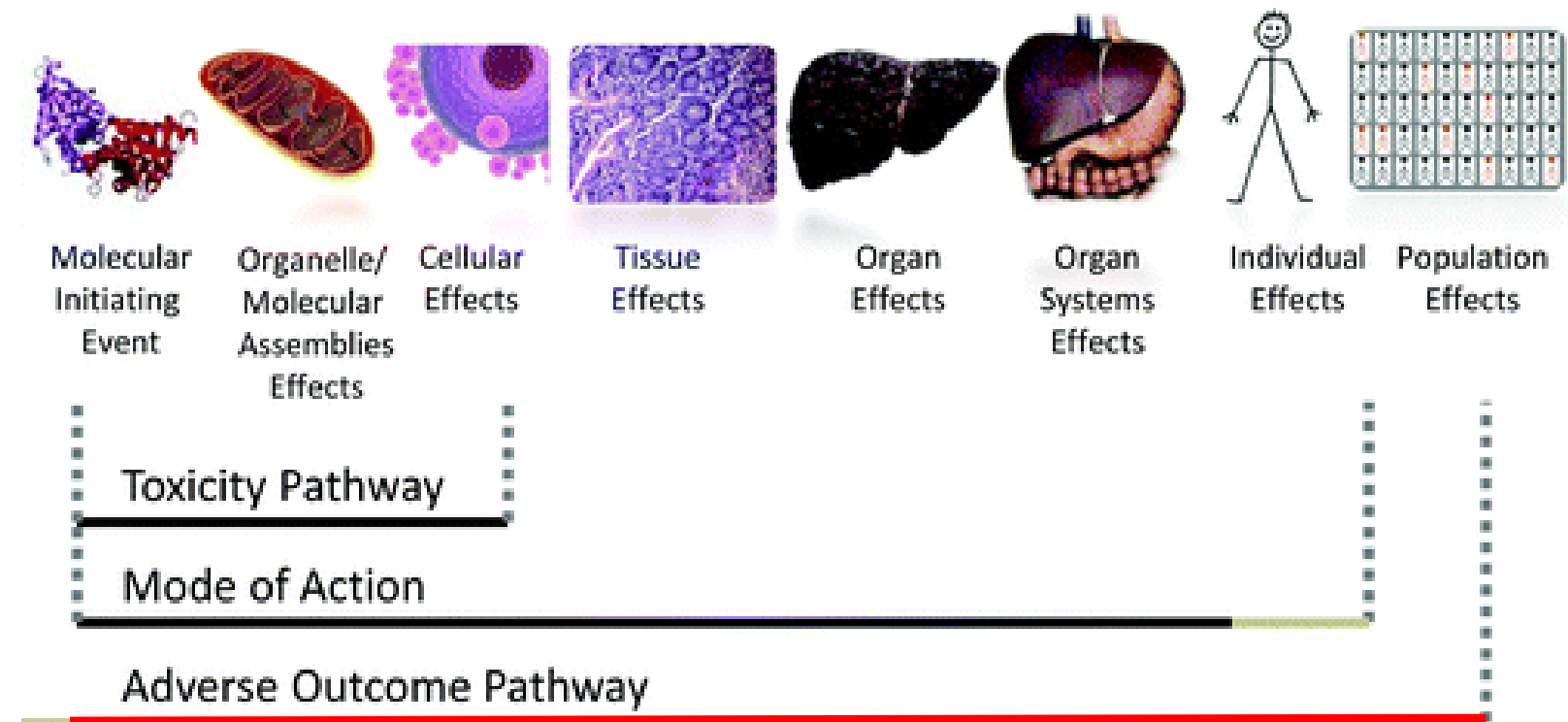
Translate



Take home messages from ZnO study

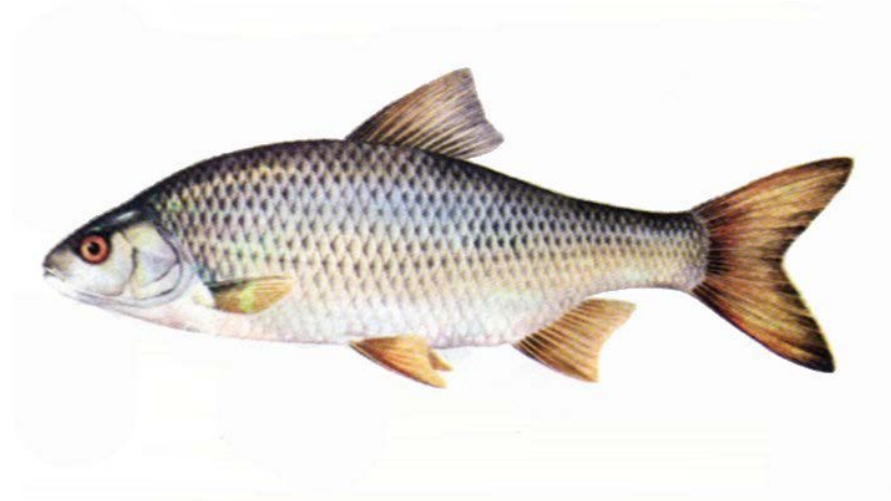
- **Metabolomics discovered novel mechanism** of toxicity of ZnO nanoparticles that disrupts predator-prey signalling; ecological implications still to be determined
- Technologies exist for **translating** this 'metabolic Key Event' to a validated regulatory assay

- **Two species
AOP !?**



Insights into toxicokinetics using metabolomics

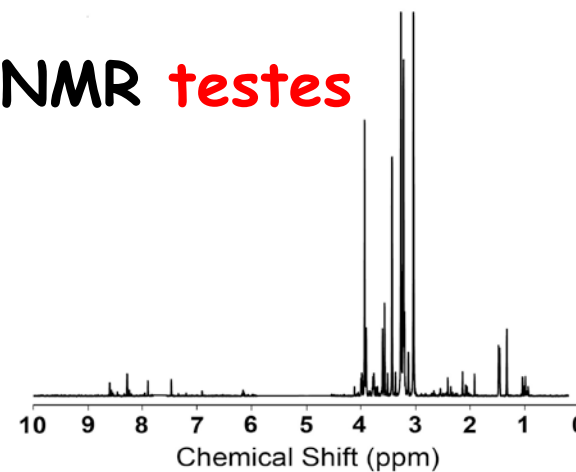
Effect of AChE inhibitor



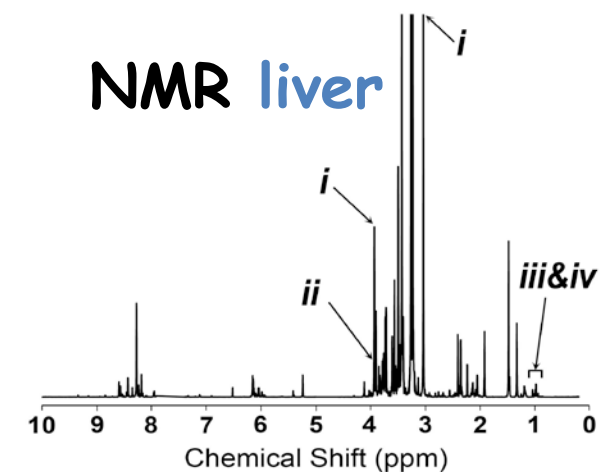
**Non-targeted
metabolomics**

Discovered fenitrothion
significantly disrupts
acetylcholine, disrupts
steroids, affects energy
metabolism and disrupts
phenylalanine metabolism

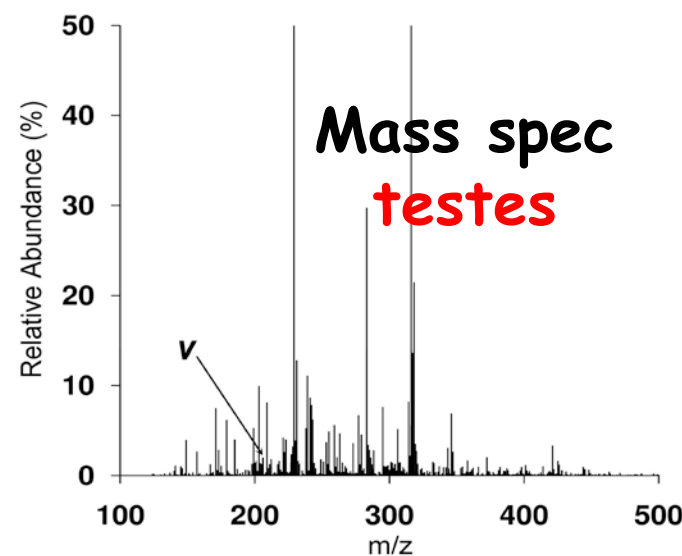
NMR testes



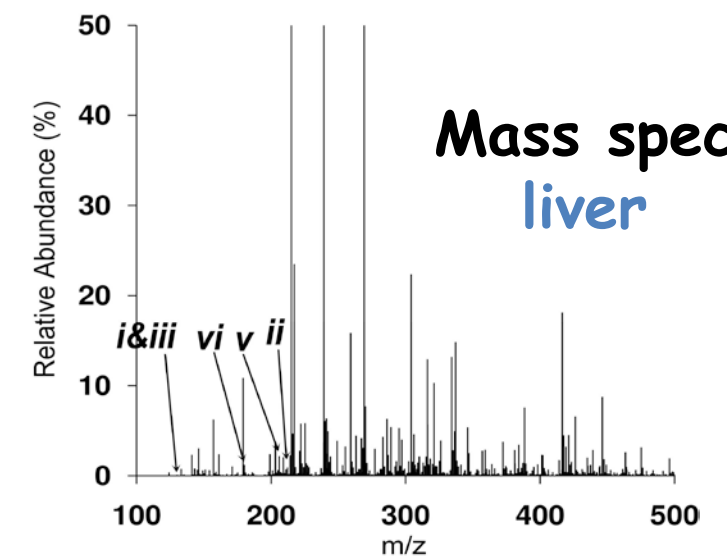
NMR liver



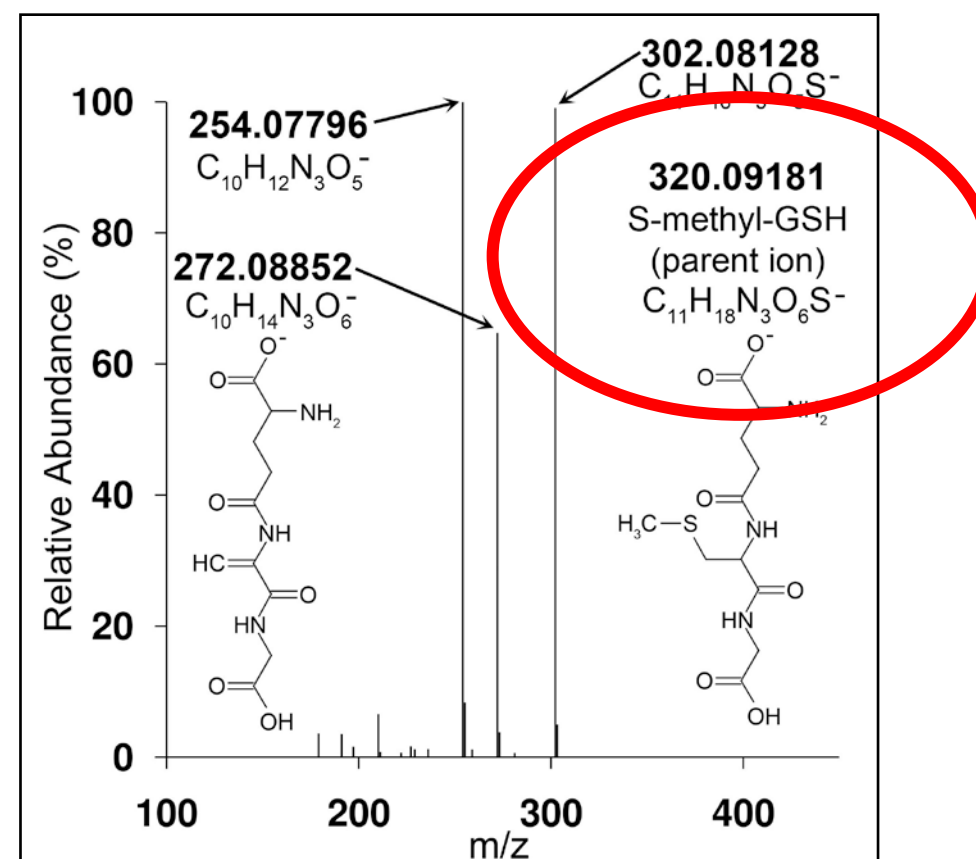
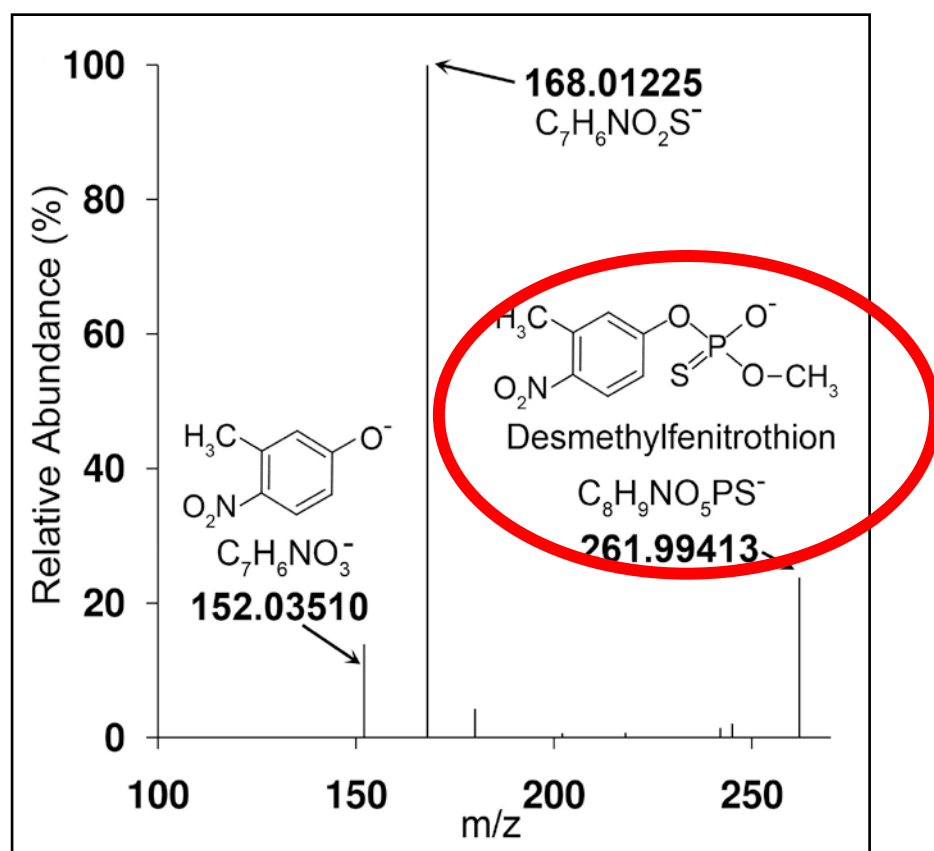
**Mass spec
testes**



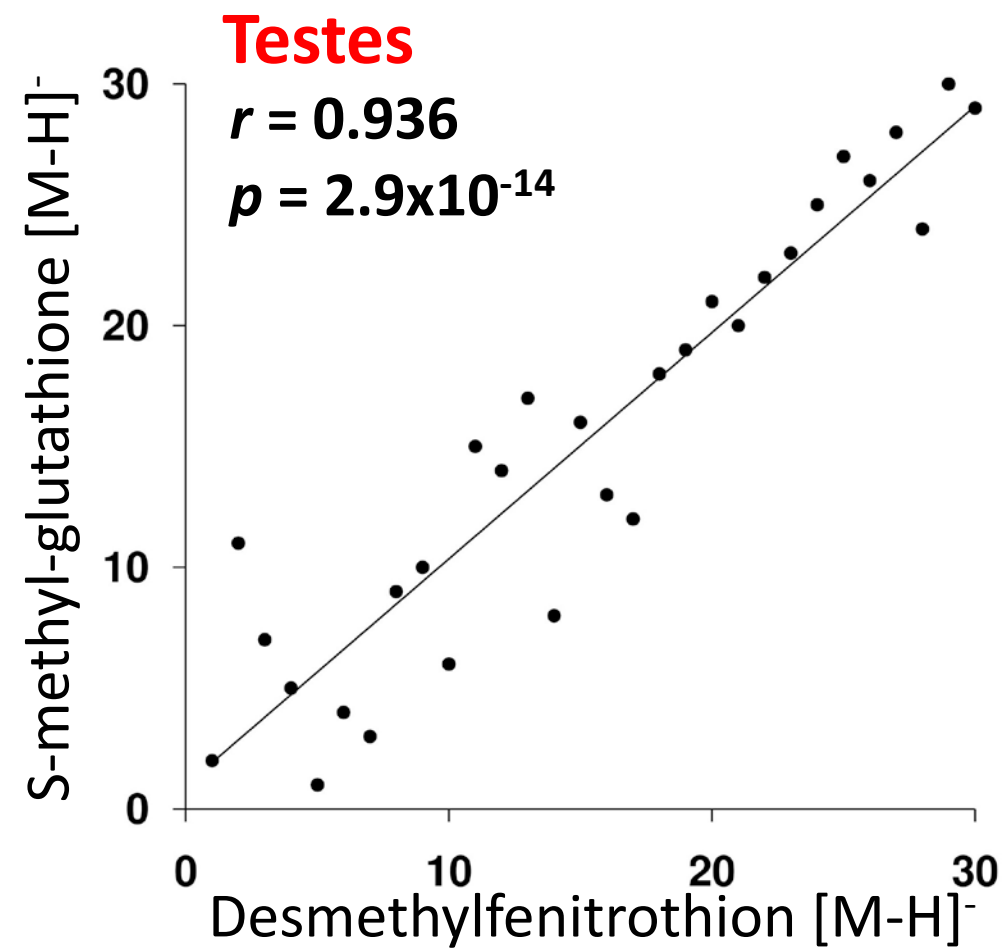
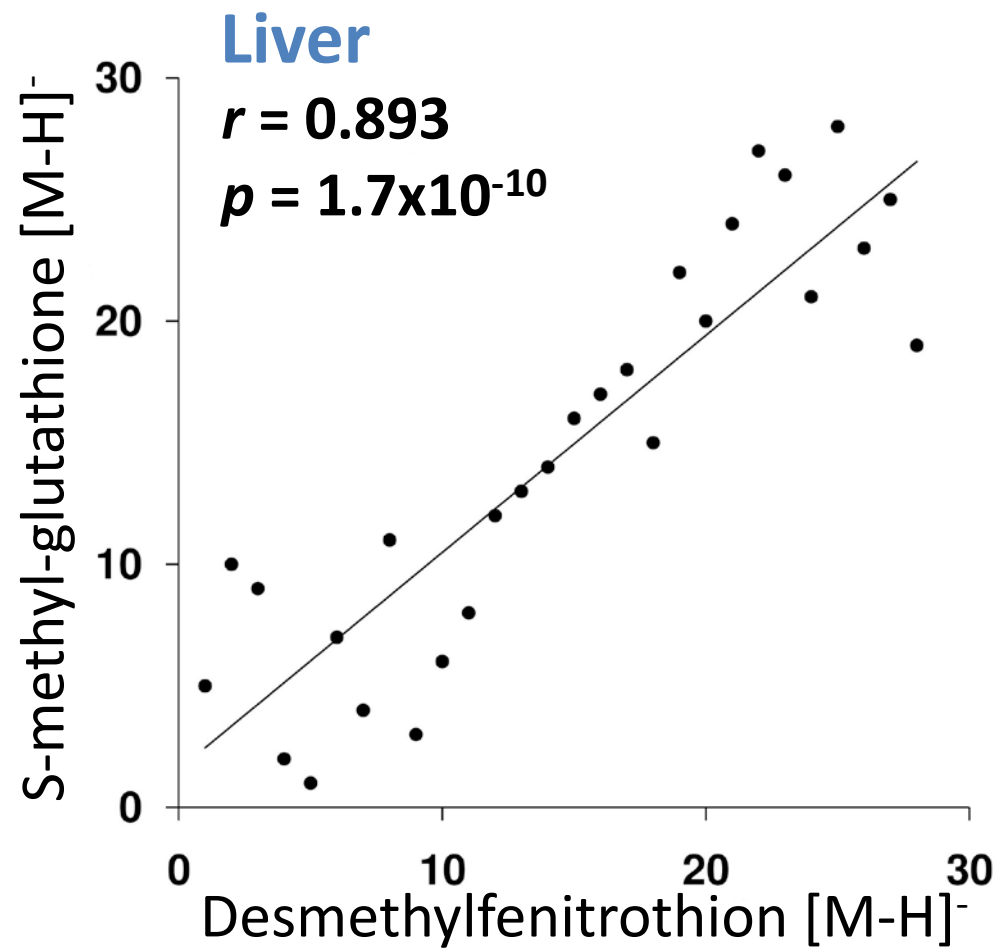
**Mass spec
liver**



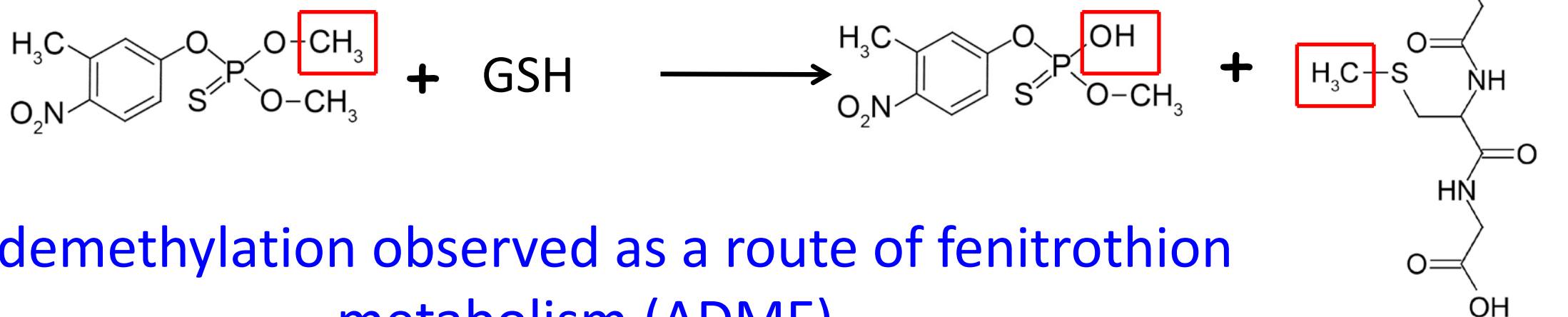
Desmethyl fenitrothion and S-methyl-GSH both detected by mass spectrometry metabolomics



Fenitrothion metabolism



Fenitrothion de-methylation:



O-demethylation observed as a route of fenitrothion metabolism (ADME)

2. Lack of Best Practice guidelines for conducting a regulatory metabolomics study and lack of validation and reporting standards

Progress in best practice, validation, reporting standards

MEtabolomics standaRds Initiative in Toxicology (MERIT)

- Developing best-practice guidelines and minimal reporting standards for the acquisition, processing and analysis of metabolomics data
- Funded by ECETOC, with industry, government and academic scientists

OECD Extended Advisory Group on Molecular Screening & Toxicogenomics

- Developing Transcriptomics Reporting Framework (co-led by Tim Gant), Metabolomics Reporting Framework, ...

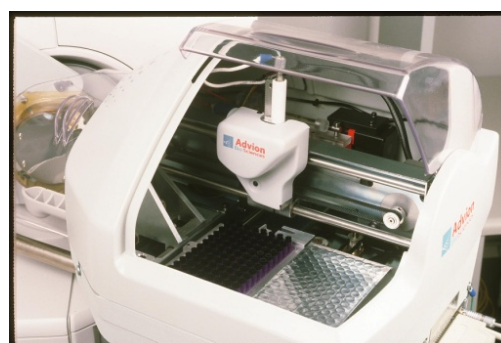
3. Accessibility to metabolomics tools (analytical and computational)

A complete workflow for high-resolution spectral-stitching nanoelectrospray direct-infusion mass-spectrometry-based metabolomics and lipidomics

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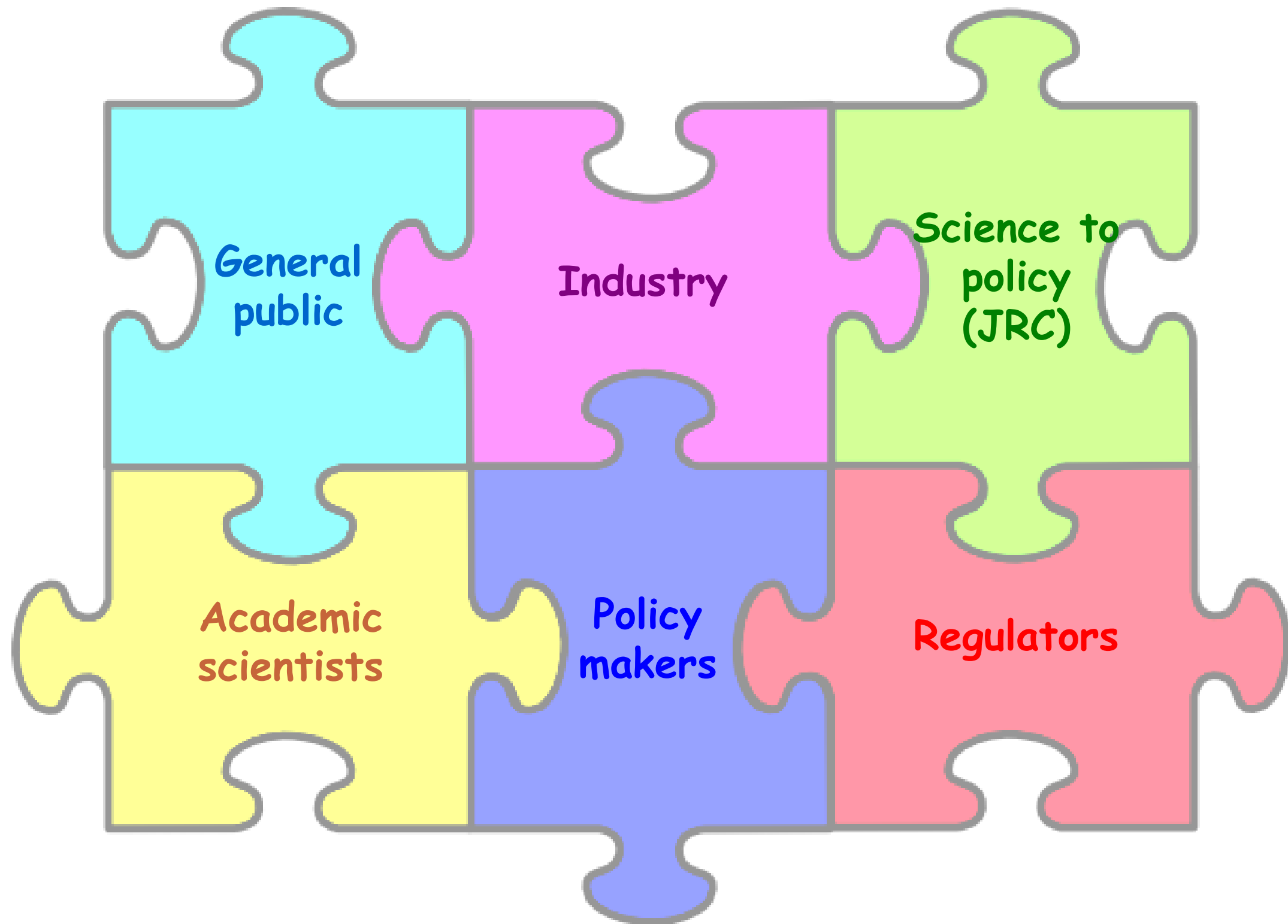


ThermoFisher
SCIENTIFIC

Take home messages

- There are several challenges to applying metabolomics in regulatory toxicology:
 1. Relative lack of robust case studies in regulatory metabolomics
 2. Lack of best practice, reporting frameworks, and 'awareness training'
- Metabolomics is a mature science in academia, the roadblocks are being addressed, and efforts to translate this technology towards regulatory toxicology should now be accelerated

Accelerated while engaging all parties...



Metabolomics @ University of Birmingham

