Computational Toxicology and Exposure Communities of Practice

Sharing research and promoting collaboration

Thursday, February 22, 11 AM-12 PM ET

Agenda:

- **Introduction: Sammy Hanf**
  Communications Specialist, ORD Center for Computational Toxicology and Exposure
- **Presentation: Jonathan Mosley**
  Research Chemist, ORD Center for Environmental Measurement and Modeling
- **Q&A**
- **Closing remarks: Sammy Hanf**

For more information on the CompTox CoP, visit:
epa.gov/chemical-research/computational-toxicology-communities-practice

Recent Metabolomics Advancements: Standardization, NAMs, and MATCHING for Chemical Safety

Jonathan Mosley
Research Chemist
ORD Center for Environmental Measurement and Modeling

- **New Approach Methodologies (NAMs) in Chemical Safety Testing**
- Introduction and Importance of Standardization in Untargeted Metabolomics
  - What is metabolomics?
  - The Metabolomics Quality Assurance and Quality Control Consortium (mQACC)
- Overview of mQACC's Living Guidance document
- Role of Metabolomics as a NAM and its Growing Significance in Regulatory Contexts
  - Recent advancements
  - Grouping and read across
- Metabolomics for Chemical Grouping (MATCHING) project
  - Key findings
  - Implications for stakeholders (chemical industry, governmental agencies, etc.)

Disclaimer

The findings and conclusions in this presentation have not been formally disseminated by EPA and should not be construed to represent Agency determination or policy.

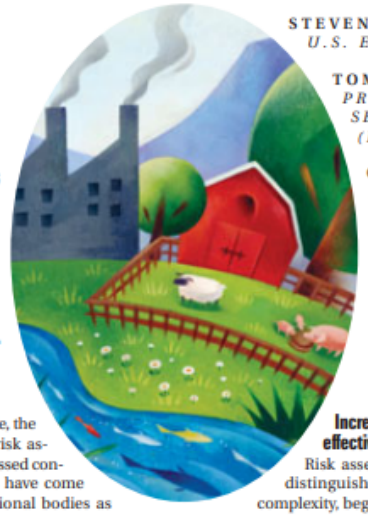
Meeting *the* Scientific Needs *of* Ecological RISK Assessment *in* a Regulatory Context

STEVEN P. BRADBURY
U.S. EPA

TOM C. J. FEIJTEL
PROCTER & GAMBLE SERVICES COMPANY NV/SA (BELGIUM)

CORNELIS J. VAN LEEUWEN
EUROPEAN COMMISSION

Three strategies could move both science and regulation forward.



During the past decade, the field of ecological risk assessment has progressed considerably. Advances have come from such international bodies as the Organisation for Economic Co-operation and Development (OECD), the World Health Organisation (WHO), the European and Mediterranean Plant Protection Organisation (EPPO), and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (*1–8*). Risk assessments have played a critical role in the development of various regulations within the European Commission (EC) as well as in other parts of the world, including the United States, Canada, and Japan (*9–17*). But scientists and regulators are faced with three significant challenges: streamlining the risk-assessment process, quantifying risks in a spatially explicit manner, and acquiring the correct kind of environmental data to enable regulatory programs to effectively focus on future environmental protection activities.

Increasing efficiency, cost-effectiveness, and focus

Risk assessment is a tiered process distinguished by levels of increasing complexity, beginning with the preliminary categorization step, followed by a refined or screening assessment, and progressing to the full, comprehensive risk assessment (*4, 18, 19*). For each tier, a minimum level of information is required. For example, OECD has established an international program—called the Screening Information Data Sets (SIDS)—for surveying high-production-volume chemicals (HPV) for potential effects. SIDS include the basic information needed to perform a preliminary assessment of a chemical's potential risk (*20*).

Applying the current risk-assessment paradigm and meeting the associated data-generation requirements, combined with the increased need to evaluate the potential effects posed by thousands of industrial chemicals, are big challenges for the chemical industry, national and international regulatory

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Traditional testing with defined batteries of in vivo tests

- Too many chemicals
- Too costly
- Too much time to generate and interpret
- Too many animals
- Inefficient
  - Typically, only a subset of the data are used for the assessments

"The challenge is to move .. to [a paradigm] in which a hypothesis- and risk-driven approach can be used to identify the most relevant in vivo information"

New approach methodologies (NAMs)

- **NAMs**: any technology, methodology, approach, that can provide information on chemical hazard and risk assessment without the use of intact animals, including *in silico*, *in chemico*, *in vitro*, and *ex vivo* approaches (ECHA, 2016b; EPA, 2018d).
- Under EU REACH legislation for chemical safety, industry has the option to reduce animal testing using the alternative method of 'grouping and read-across' (REACH, 2003).

ECHA (2016b). *New approach methodologies in regulatory science*. Proceedings of a scientific workshop. Helsinki: European Chemicals Agency. doi:10.2823/543644.

EPA (2018d). Strategic plan to promote the development and implementation of alternative test methods within the TSCA program. U.S. Environmental protection agency. EPA-740-R1-8004. Available at: https://www.epa.gov/sites/default/files/2018-06/documents/epa_alt_strat_plan_6-20-18_clean_final.pdf

REACH (2004). Directorates General Enterprise and Environment. Legislative Proposal Concerning the Registration, Evaluation, Authorisation and Restrictions of Chemicals, Volumes 1–7; DG Enterprise: Brussels, Belgium; Oct 29, 2003; www.europa.eu.int/comm/environment/chemicals/reach.htm.

Overview

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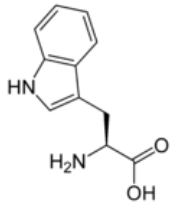
What is metabolomics?

(AT EPA-ATHENS)

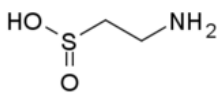
Metabolomics = the study of changes in “many” endogenous metabolites in response to stressors

metabolome = all endogenous metabolites

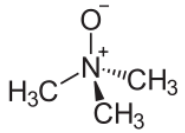
Advanced analytical instruments (NMR, LC-MS and GC-MS) are used to detect and characterize changes in these chemically and functionally diverse biomolecules



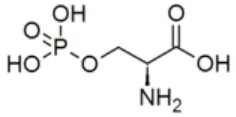
tryptophan



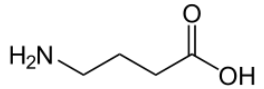
hypotaurine



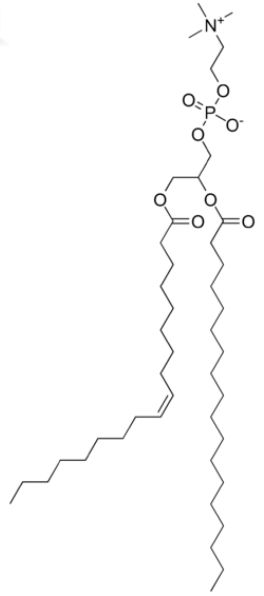
trimethylamine N-oxide



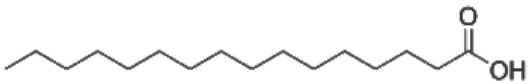
phosphoserine



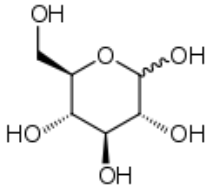
gamma-aminobutyric acid (GABA)



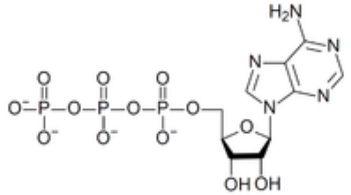
phosphatidylcholine



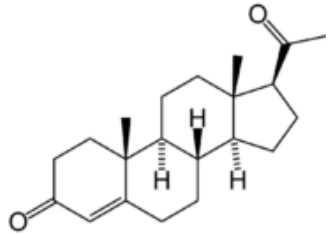
palmitic acid



glucose



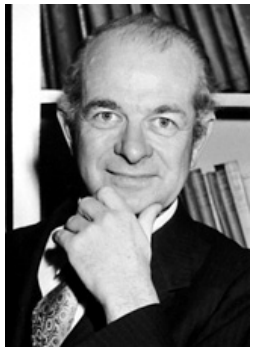
adenosine triphosphate (ATP)



progesterone

Metabolomics:

Although the "field" and the term are relatively new, the concept is not



Linus Pauling – 1971.

"the thorough quantitative analysis of body fluids might permit differential diagnosis of many diseases in a more effective way than is possible at the present time."

Pauling, L.C., Robinson, A.B., Teranishi, R., and Cary, P., Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography, *Proc. Natl. Acad. Sci.* (1971) 68, 2374-2376.

Metabolic Biochemists

Have been assessing the impact of changing levels of endogenous metabolites for many years (e.g. inborn errors of metabolism).

Clinical Chemistry

urinalysis, blood panels, etc.

Metabolomics has proven very useful for:

(a partial list)

- **screening chemicals for adverse effects**
- **classifying chemicals according to adverse outcome pathways**
- **developing biomarkers of chemical exposure**
- **tracking compensation and recovery**
- **informing dose response**
- **conducting cross-species extrapolations**
- **elucidating toxicity pathways**
- ***In vivo* & *in vitro* assessments**

Towards High Quality Data Generation in Untargeted Metabolomics

METABOLOMICS QUALITY ASSURANCE AND QUALITY CONTROL CONSORTIUM (MQACC)

Metabolomics Quality Assurance & Quality Control Consortium (mQACC)

https://mQACC.org/

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Metabolomics Quality Assurance & Quality Control Consortium (mQACC)

History and early developments

- **mQACC** was formed following a Think Tank meeting at the National Cancer Institute in October 2017
- **Mission**: To engage the metabolomics community to communicate and promote the development, dissemination and harmonization of best QA/QC practices in untargeted metabolomics
- **Membership:** 106 scientists across 4 continents from academia, industry and government organizations

Metabolomics (2019) 15:4
https://doi.org/10.1007/s11306-018-1460-7

SHORT COMMUNICATION

CrossMark

Towards quality assurance and quality control in untargeted metabolomics studies

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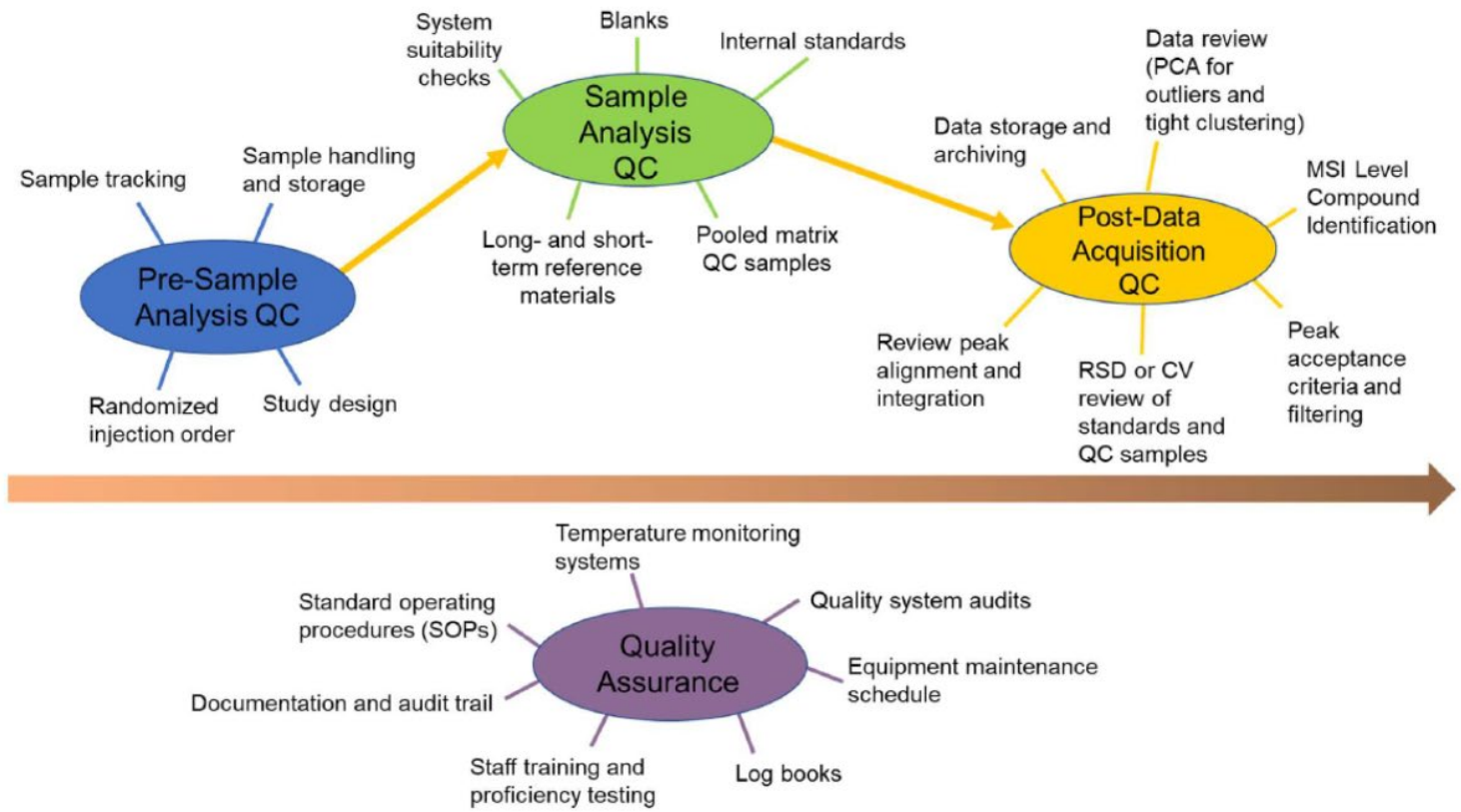
Abstract
We describe here the agreed upon first development steps and priority objectives of a community engagement effort to address current challenges in quality assurance (QA) and quality control (QC) in untargeted metabolomic studies. This has included (1) a QA and QC questionnaire responded to by the metabolomics community in 2015 which recommended education of the metabolomics community, development of appropriate standard reference materials and providing incentives for laboratories to apply QA and QC; (2) a 2-day 'Think Tank on Quality Assurance and Quality Control for Untargeted Metabolomic Studies' held at the National Cancer Institute's Shady Grove Campus and (3) establishment of the Metabolomics Quality Assurance and Quality Control Consortium (mQACC) to drive forward developments in a coordinated manner.

Keywords Quality assurance (QA) · Quality control (QC) · Community engagement · Test materials · Reporting metrics

What is quality control and quality assurance?

- **Quality Control:** Processes related to the procedures applied **during and after data acquisition**



- **Quality Assurance:** Processes related to the procedures applied in **preparation for data acquisition**

Metabolomics Quality Assurance & Quality Control Consortium (mQACC)

mQACC Operations

Coordinating committee

- Jennifer Kirwan (Chair 2024), Jonathan Mosley (will be chair in 2025), Annie Evans (will be chair in 2026)

Monthly videoconferences

- To provide conduit for information dissemination and consortium-wide discussions

Working Groups

Reference & Test Materials
Clay Davis & Raquel Cumeras

Best Practices
Dajana Vuckovic & Georgios Theodoridis

Reporting Standards
Jennifer Kirwan & Nichole Reisdorph

Community Engagement
Claire O'Donovan, Brianna Garcia (V.C. H. Chatelaine and G. Gouveia)

NMR
Leo Cheng & Panteleimon Takis

GC-MS
Oliver Fiehn & Michael Herold

Quality Assurance
Srujana Golla, Rafea Naffa (V.C. A Ochoa and S. An)

Living Guidance Dissemination
Dajana Vuckovic & Georgios Theodoridis

Overview

- New Approach Methodologies (NAMs) in Chemical Safety Testing
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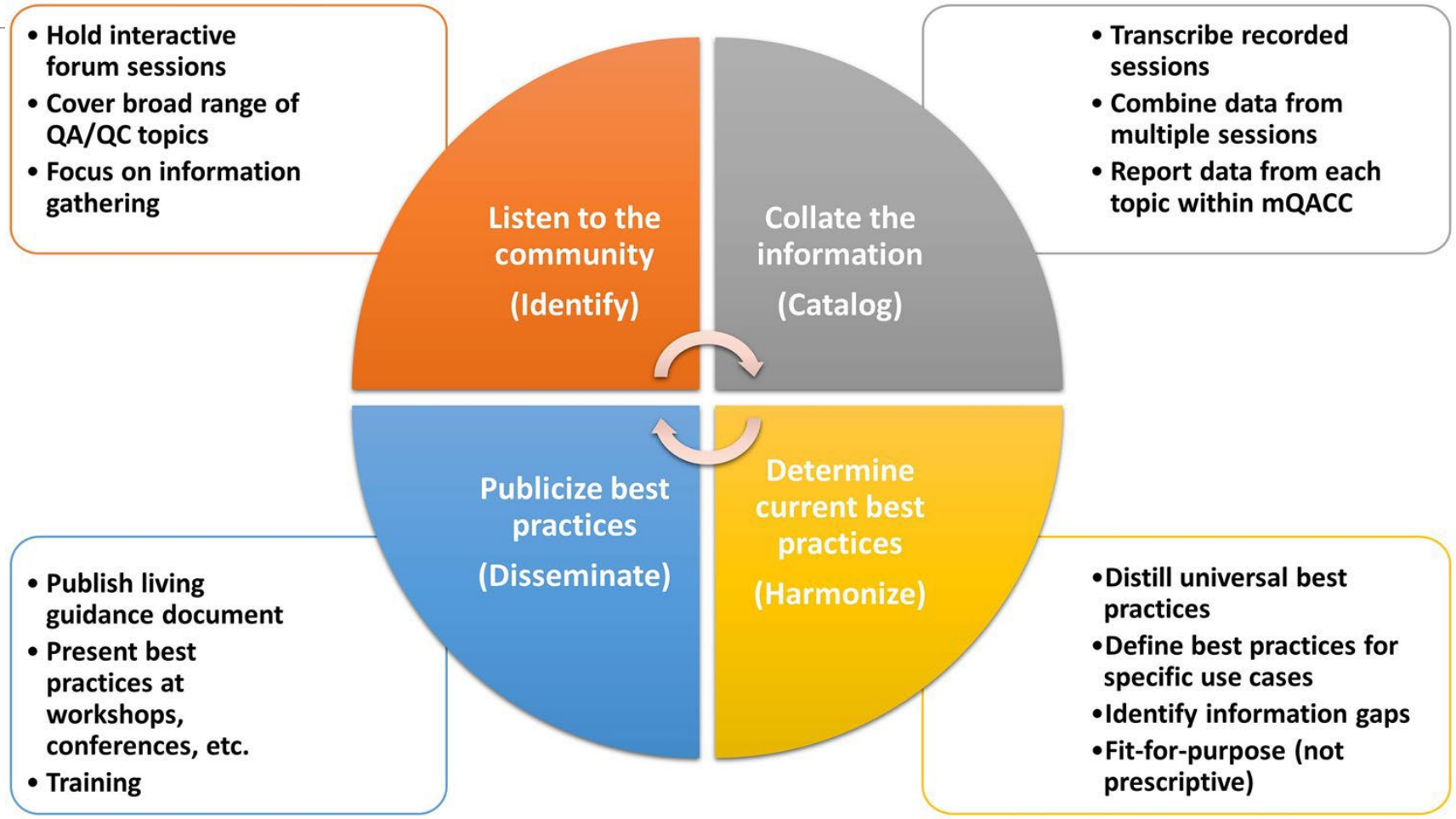
Living guidance document

- One mQACC objective is to construct a living guidance document to support the untargeted metabolomics community in all aspects of QA and QC
- Living document = added to and revised periodically
- Would include
  - minimum requirements and
  - optional good practices
  - examples of use/case studies
- Non-prescriptive
- How to contact mQACC with input regarding guidelines

Metabolomics Quality Assurance & Quality Control Consortium (mQACC)

Community-driven Guidance



Mosley JD, et al. Metabolomics. 2024 12 Feb, 20:20. https://doi.org/10.1007/s11306-023-02080-0

Metabolomics Quality Assurance & Quality Control Consortium (mQACC)

Community-driven Guidance

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Establishing a framework for best practices for quality assurance and quality control in untargeted metabolomics

Review Article | Open access | Published: 12 February 2024

Volume 20, article number 20, (2024) Cite this article

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Jonathan D. Mosley, Tracey B. Schock, Chris W. Beecher, Warwick B. Dunn, Julia Kuligowski, Matthew R. Lewis, Georgios Theodoridis, Candice Z. Ulmer Holland, Dajana Vuckovic, Ian D. Wilson & Krista A. Zanetti

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Table 1 Information-gathering activities conducted by the mQACC Best Practices WG from 2019 to 2023

Event	Date	QA/QC Topic	Approximate No. of Participants
1st annual MANA conference workshop	November 16th, 2019	Use of Pooled QCs in LC-MS-based Untargeted Metabolomics	30
European RFMF Metabomeeting 2020 community survey	January 22nd – 24th, 2020	Use of Pooled QCs in LC-MS-based Untargeted Metabolomics	30
mQACC-HHEAR virtual meeting interactive forum (part 1)	June 19th, 2020	Use of Pooled QCs in LC-MS-based Untargeted Metabolomics	15
mQACC-HHEAR virtual meeting interactive forum (part 2)	July 14th, 2020	Use of Pooled QCs in LC-MS-based Untargeted Metabolomics	15
2nd annual MANA conference virtual workshop	September 14th, 2020	System Suitability Evaluation prior to LC-MS-based Untargeted Metabolomics	25
mQACC virtual interactive forum	February 23rd, 2021	System Suitability Evaluation prior to LC-MS-based Untargeted Metabolomics	45
mQACC virtual interactive forum	April 29th, 2021	Use of Internal Standards in LC-MS-based Untargeted Metabolomics	45
mQACC virtual interactive forum	June 14th, 2021	Design of the Analytical Batch in LC-MS-based Untargeted Metabolomics	30
mQACC virtual interactive forum	November 30th, 2021	Quality of Metabolite Annotation & Identification in LC-MS-based Untargeted Metabolomics	35
mQACC virtual interactive forum	March 10th, 2022	Use of Reference Materials in LC-MS-based Untargeted Metabolomics	25
mQACC virtual interactive forum	May 26th, 2022	Data Quality Review in LC-MS-based Untargeted Metabolomics	20
18th annual conference of the Metabolomics Society workshop	June 19th, 2022	State of QA/QC Best Practices in LC-MS-based Untargeted Metabolomics	190
19th annual conference of the Metabolomics Society workshop	June 19th, 2023	Moving Toward Consensus: mQACC Community Engagement on Best QA/QC Practices in LC-MS-Based Untargeted Metabolomics	100

MANA Metabolomics Association of North America, *RFMF* French-speaking Metabolomics and Fluxomics Network, *HHEAR* Human Health Exposure Analysis Resource

W5: State of QA/QC Best Practices in LC-MS-Based Untargeted Metabolomics, Informed Through mQACC Community Engagement Initiatives

Presenters

Warwick Dunn, University of Liverpool, UK
Tracey Schock, National Institutes of Standards and Technology, USA
Dajana Vuckovic, Concordia University, Canada
Julia Kuligowski, Health Research Institute La Fe, Spain
Jonathan Mosley, U.S. Environmental Protection Agency, USA

W9: Moving Toward Consensus: mQACC Community Engagement on Best QA/QC Practices in LC-MS-Based Untargeted Metabolomics

Presenters

Jonathan Mosley, U.S. Environmental Protection Agency, USA
Warwick Dunn, University of Liverpool, UK
Tracey Schock, National Institute of Standards and Technology, USA
Dajana Vuckovic, Concordia University, Canada
Matthew Lewis, Bruker Life Sciences Mass Spectrometry, UK

Learning Outcomes

1. Understand the community feedback received by the mQACC Best Practices Working Group and recognize how the feedback will be used to support QA/QC best practices for untargeted LC-MS-based metabolomics.
2. Be able to identify how to participate in mQACC, including mechanisms to contribute to the best practices community engagement efforts

Acknowledgements

Design of the analytical batch

- Bryce Geiling
- Jonathan Mosley
- Matthew Lewis
- Claire O'Donovan
- Tracey Schock
- Candice Ulmer
- Dajana Vuckovic
- Krista Zanetti

Pooled QCs

- Jonathan Mosley
- Ioanna Ntai
- Krista Zanetti
- Dajana Vuckovic
- Tracey Schock
- Claire O'Donovan
- Matthew Lewis
- Warwick Dunn
- Jennifer Kirwan

Internal standards

- Bryce Geiling
- Julia Kuligowski
- Matthew Lewis
- Ioanna Ntai
- Claire O'Donovan
- Tracey Schock
- Candice Ulmer
- Dajana Vuckovic
- Krista Zanetti

System suitability

- Jonathan Mosley
- Krista Zanetti
- Ioanna Ntai
- Stephanie Myers
- Bryce Geiling
- Tracey Schock
- Dajana Vuckovic
- Matthew Lewis
- Julia Kuligowski
- Claire O'Donovan

Metabolomics Quality Assurance & Quality Control Consortium (mQACC)

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- Claire O'Donovan
- Candice Ulmer
- Dajana Vuckovic
- Krista Zanetti

Reference Materials

- Julia Kuligowski
- Matthew Lewis
- Jonathan Mosley
- Claire O'Donovan
- Dajana Vuckovic
- Krista Zanetti

Data Quality Review

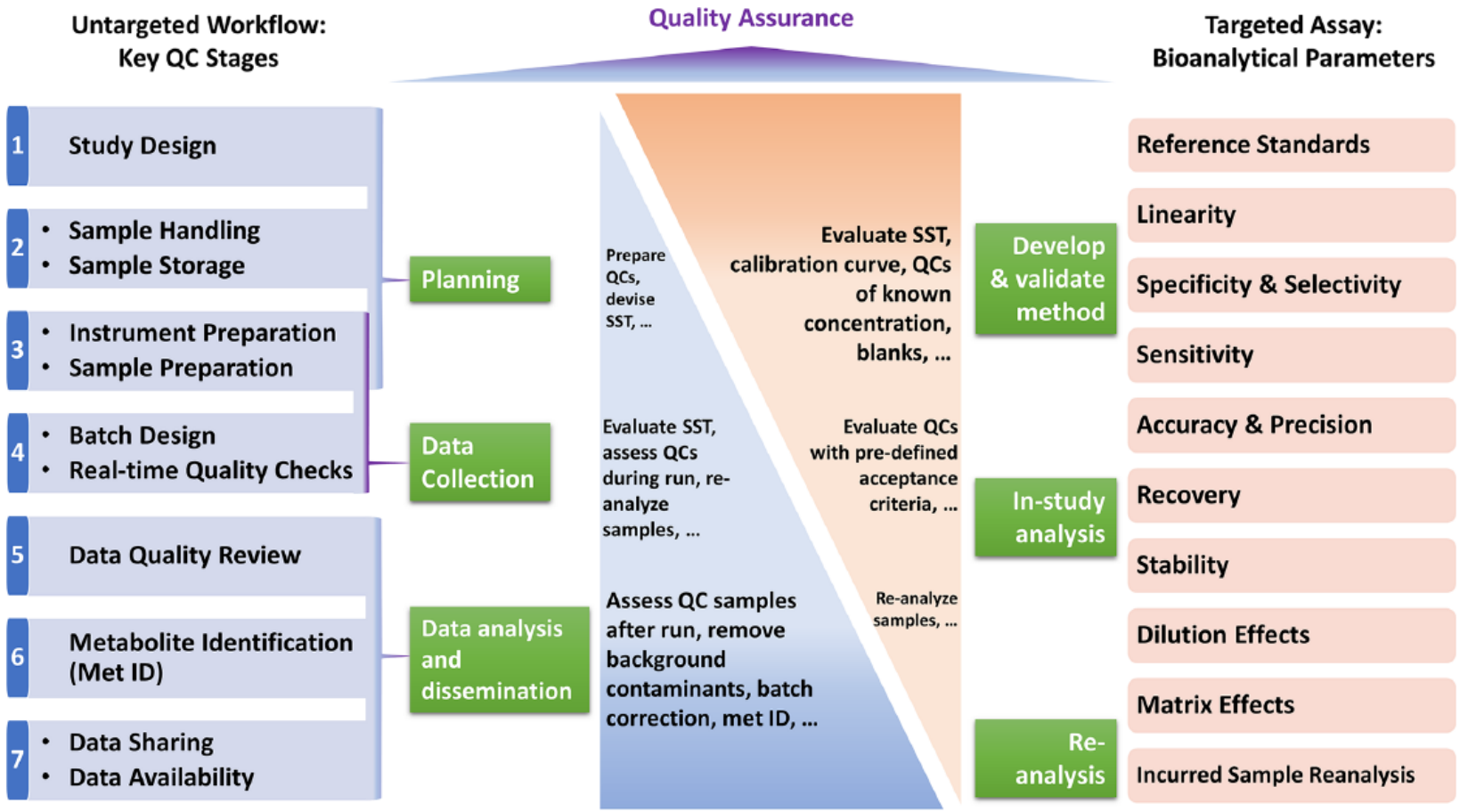
- Helen Gika
- Julia Kuligowski
- Matthew Lewis
- Jonathan Mosley
- Candice Ulmer
- Dajana Vuckovic
- Ian Wilson
- Krista Zanetti

Quality Assurance

- Annie Evans
- Oliver Fiehn
- Michael Herold
- Matthew Lewis
- María Eugenia Monge
- Jonathan Mosley
- Sindhu Nair
- Oliver Schmitz
- Panteleimon Takis

Metabolomics Quality Assurance & Quality Control Consortium (mQACC)

Community-driven guidance



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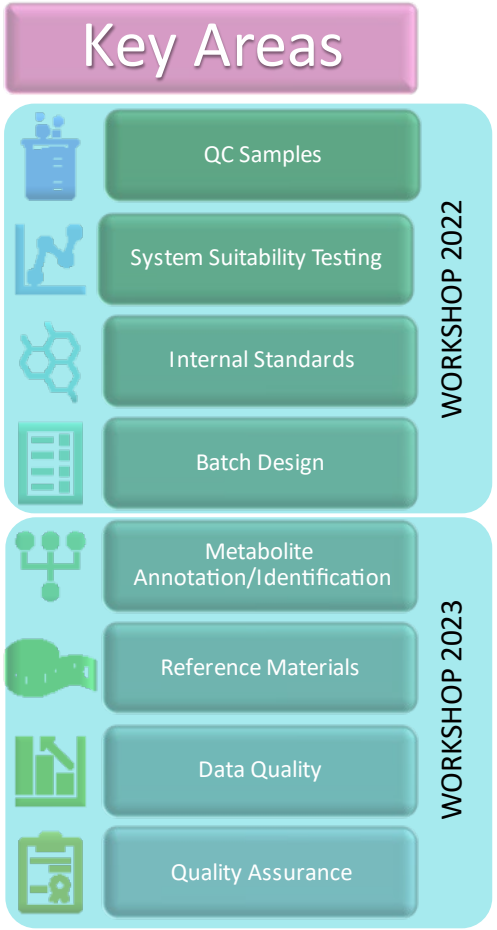
Metabolomics Quality Assurance & Quality Control Consortium (mQACC)

Community-driven guidance



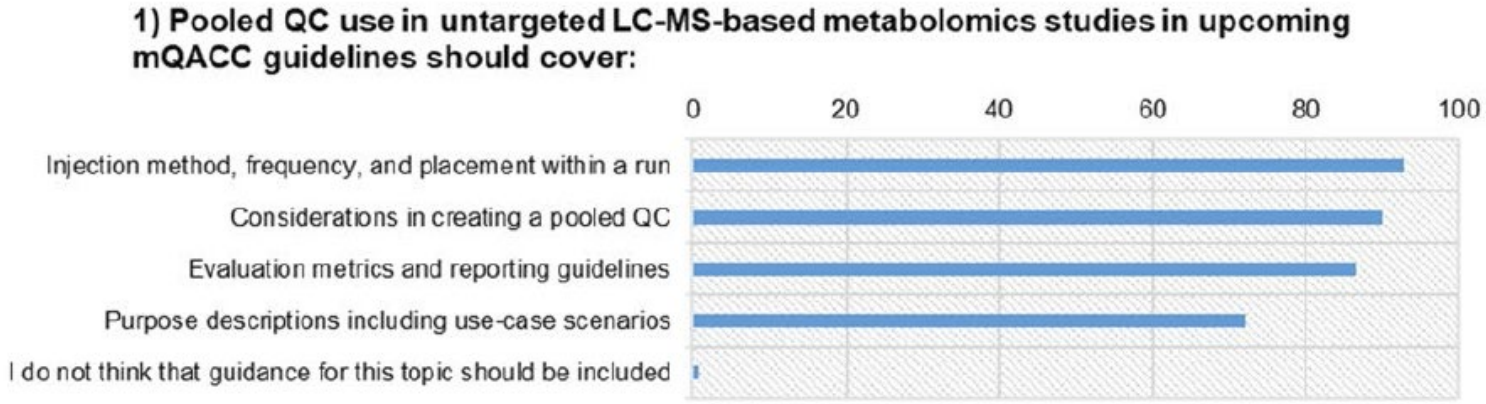


Fig. 2 Polling questions administered to the audience during the workshop by using an on-line tool. Note: All questions were 'choose all that apply' questions. Number of responses N = 140 (Question 1);

Dunn WB, et al. Metabolomics, 2023, 19:93

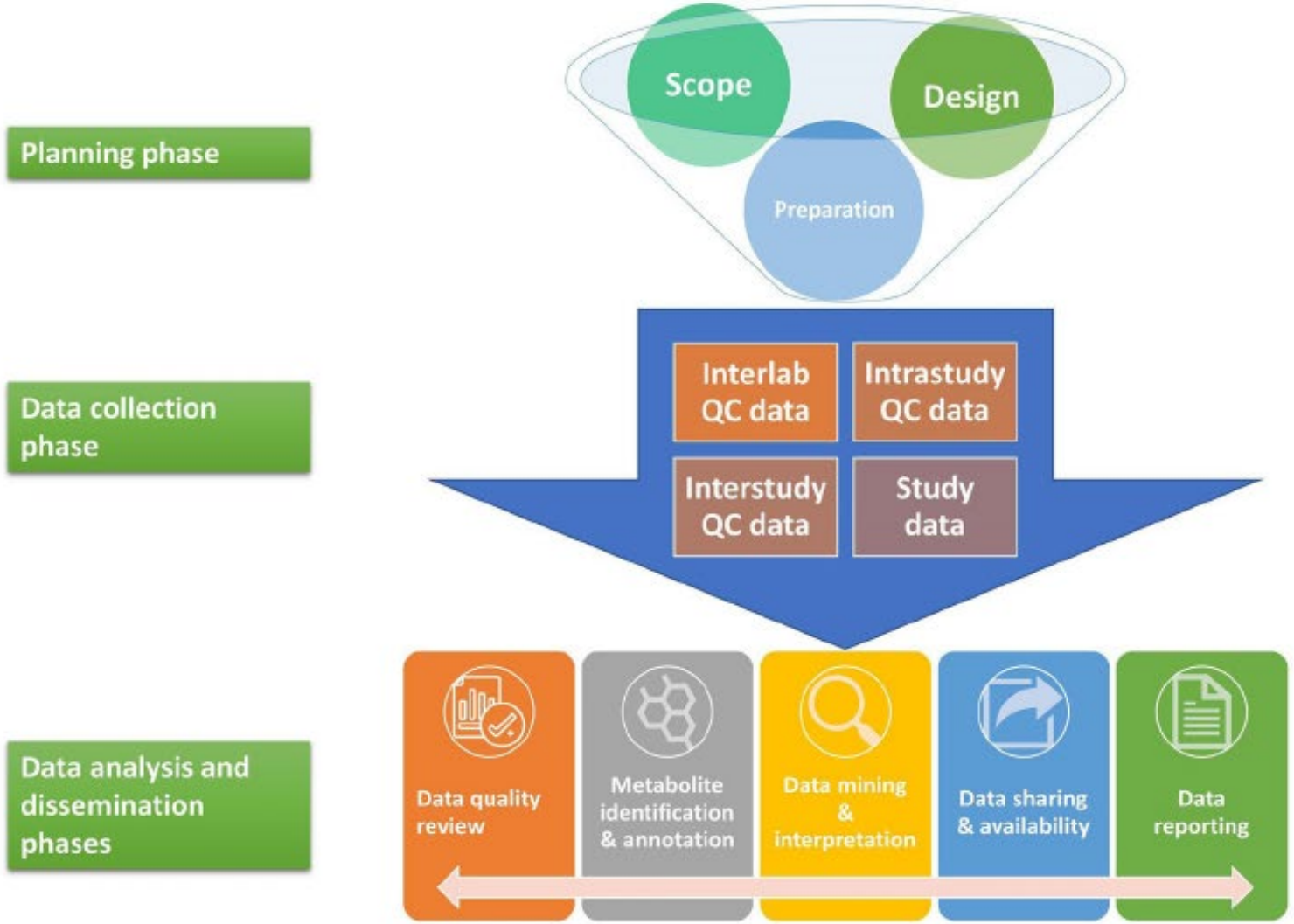


Fig. 3 A proposed framework for the living guidance document that mQACC plans to publish. Initial guidance will be grouped into three main phases dictated by the basic tenets of a metabolomics research study. As additional considerations develop, they can be incorporated into any of these phases where appropriate. Thus, the guidance can grow along with the field

Mosley JD, et al. Metabolomics. 2024 12 Feb, 20:20. https://doi.org/10.1007/s11306-023-02080-0

Metabolomics Quality Assurance & Quality Control Consortium (mQACC)

Possible avenues to contribute

1. Lend your voice during workshops and meetings
2. Report QC practices in manuscripts
3. Require fit-for-purpose quality measures when acting as a reviewer
4. Become a member of mQACC

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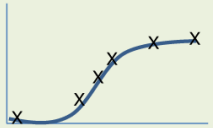
Recent Advancements

- Metabolomics data submission to ECHA
  - European Chemicals Agency (ECHA)
  - Chemical registration dossier built on 20 years of research
  - Dec 2023 – ECHA funded NAMs development
- Best practices publication by MERIT
  - Metabolomics Standards Initiative in Toxicology (MERIT)
  - Published in Nature Comm.
- Development of an OECD Omics Reporting Framework
  - Guides reporting of data to regulators
  - Multi-omic reporting

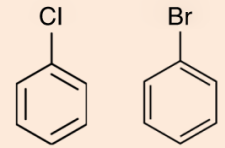
Scenario 1 - Deriving points of departure via benchmark dosing

- Benchmark dosing (BMD) approach has been demonstrated for transcriptomics to determine the level of chemical exposure that activates gene expression.
- Similarly, metabolic points of departure (PODs) will be derived from metabolomics datasets.



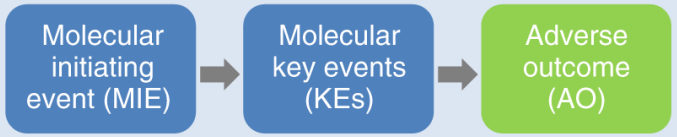
Scenario 3 - Chemical grouping for read-across

- Metabolomics data used to assess the similarities of the biological responses to chemicals, thereby forming chemical groups.
- Read-across of an adverse (apical) outcome from one chemical to the next will be based on similarity of the metabolic responses.



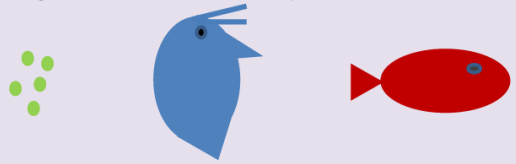
Scenario 2 - Discovery of chemical mode(s) of action and molecular key events

- Discovery approach to help identify molecular key events (KEs) and accelerate construction of adverse outcome pathways (AOPs).
- Time-series metabolomics measurements will provide mechanistic data linked to an adverse (apical) outcome.



Scenario 4 - Cross-species extrapolation of toxicity pathways

- Environmental chemical risk assessment currently focused on only three test species (algae, *Daphnia*, fish).
- Metabolomics and multi-omics data will enable an understanding of cross species toxicity through knowledge of molecular pathways.



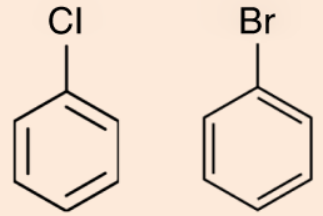
From: [Use cases, best practice and reporting standards for metabolomics in regulatory toxicology](#) Viant et al., Nature (2019)

Grouping & Read Across

- 1st step: present scientific justification
  - 'source' chemical –(grouping)→ 'target' chemical
  - Existing in vivo toxicity data "read across"
  - Avoids further animal testing
- One of most common alternatives to animal testing
  - Poor quality == rejection of chemical dossier
  - "omics" data can strengthen scientific justification

Scenario 3 - Chemical grouping for read-across

- Metabolomics data used to assess the similarities of the biological responses to chemicals, thereby forming chemical groups.
- Read-across of an adverse (apical) outcome from one chemical to the next will be based on similarity of the metabolic responses.



From: [Use cases, best practice and reporting standards for metabolomics in regulatory toxicology](#) Viant et al., Nature (2019)

Overview

- New Approach Methodologies (NAMs) in Chemical Safety Testing
- Introduction and Importance of Standardization in Untargeted Metabolomics
  - What is metabolomics?
  - The Metabolomics Quality Assurance and Quality Control Consortium (mQACC)
- Overview of mQACC's Living Guidance document
- Role of Metabolomics as a NAM and its Growing Significance in Regulatory Contexts
  - Recent advancements
  - Grouping and read across
- **Metabolomics for Chemical Grouping (MATCHING) project**
  - Key findings
  - Implications for stakeholders (chemical industry, governmental agencies, etc.)

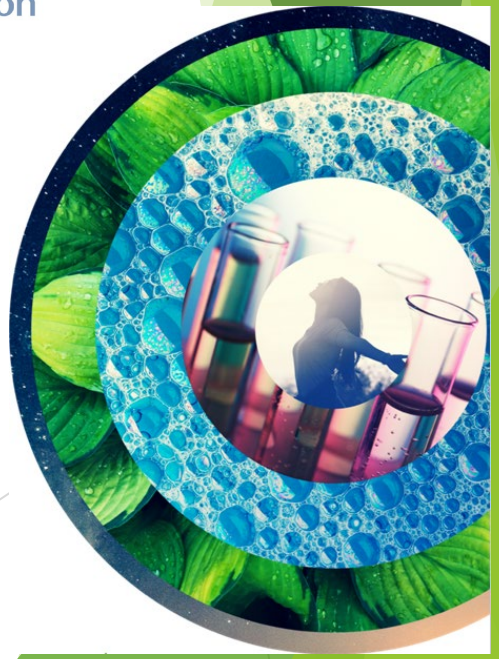
Cefic LRI C8 – MATCHING

*Met**A**bolomics ring-**T**rial for **CH**emical group**ING***

European Chemical Industry Council

Assessing the Reproducibility of Metabolomics Within a Regulatory Context Through a Multi-laboratory Ring-trial

- **Aim**
  - Conduct a blinded ring-trial to demonstrate that six metabolomics labs, each generating, analysing and reporting metabolomics data from a single rodent toxicity study, can arrive at the *same regulatory conclusion*
- **Impact**
  - (a) support changes in regulatory practice by demonstration of high reproducibility of metabolomics assays, or
  - (b) identify technological improvements needed before metabolomics can be more widely adopted into regulatory toxicology



The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

Specific objectives and work packages

Objective 1

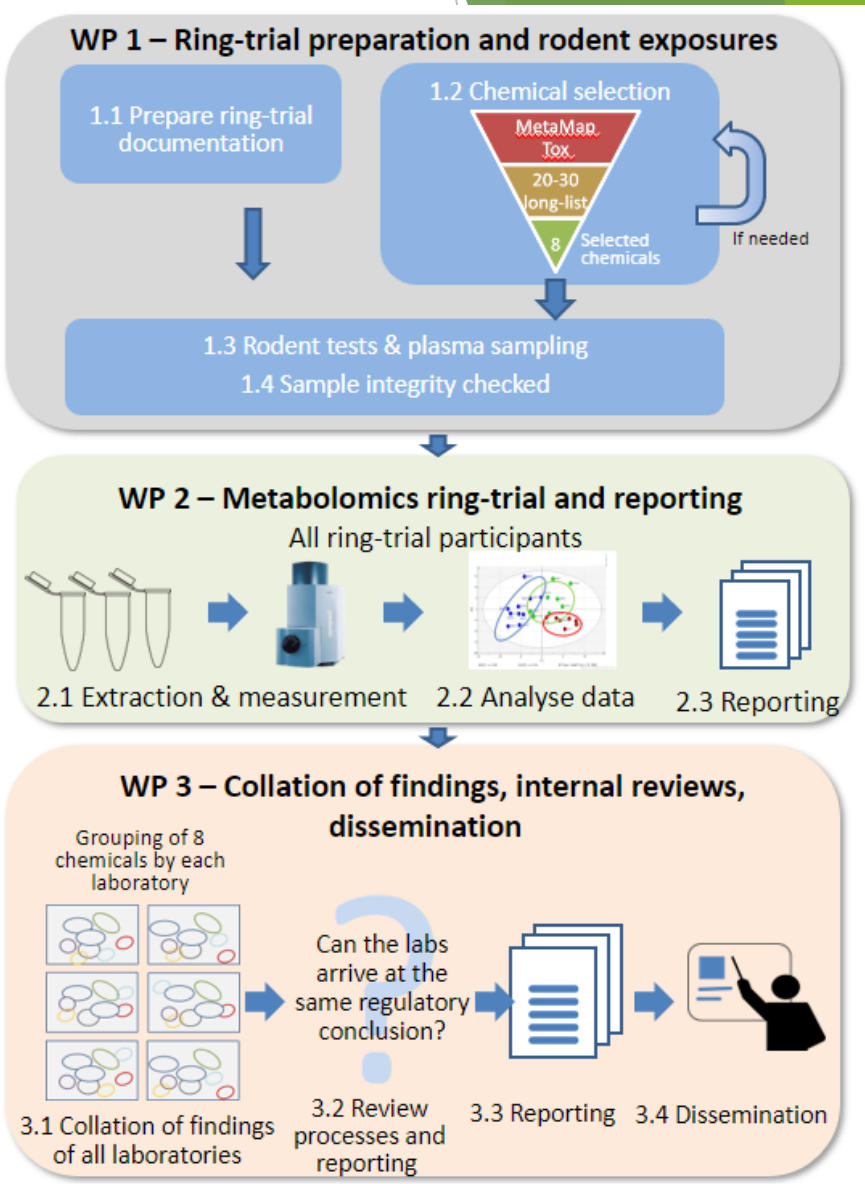
Prepare a set of quality-checked **plasma samples** for the metabolomics ring-trial, derived from **blinded** **28-day rodent tests** using **eight chemicals** (test substances selected by ECHA and BASF from the MetaMap®Tox database)

Objective 2

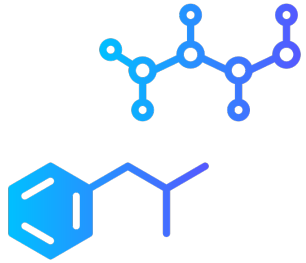
Conduct a blinded ring-trial comprising of six metabolomics labs, and report the chemical grouping results and conclusions of regulatory relevance to ECHA (including using the OECD Omics Reporting Framework)

Objective 3

After ring-trial partners have reported (blinded), ECHA collates and all partners evaluate the ring-trial findings and conclusions, and disseminate project outcomes to regulators and industry



Methods

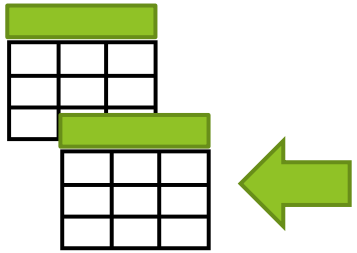


Animal Study

- Rats treated with compounds for 28 days
- Plasma samples QC'd and sent to partners
- Study followed German animal welfare law in AAALAC-certified laboratory (BASF)

Metabolomics

- Partners applied in-house LC-MS-based metabolomics to plasma samples
- QC checks applied
- Individual data assessment strategies to group 8 chemicals
- Data reported to ECHA following OORF guidelines



ECHA EUROPEAN CHEMICALS AGENCY

syngenta

Imperial College London

UNIVERSITY OF BIRMINGHAM

VU VRIJE UNIVERSITEIT AMSTERDAM

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

BASF We create chemistry

- 8 test substances
  - **Route of administration**
  - **Dosing vehicle**
  - **Dose levels**
  - **Known MoA**
- MoA-defined grouping

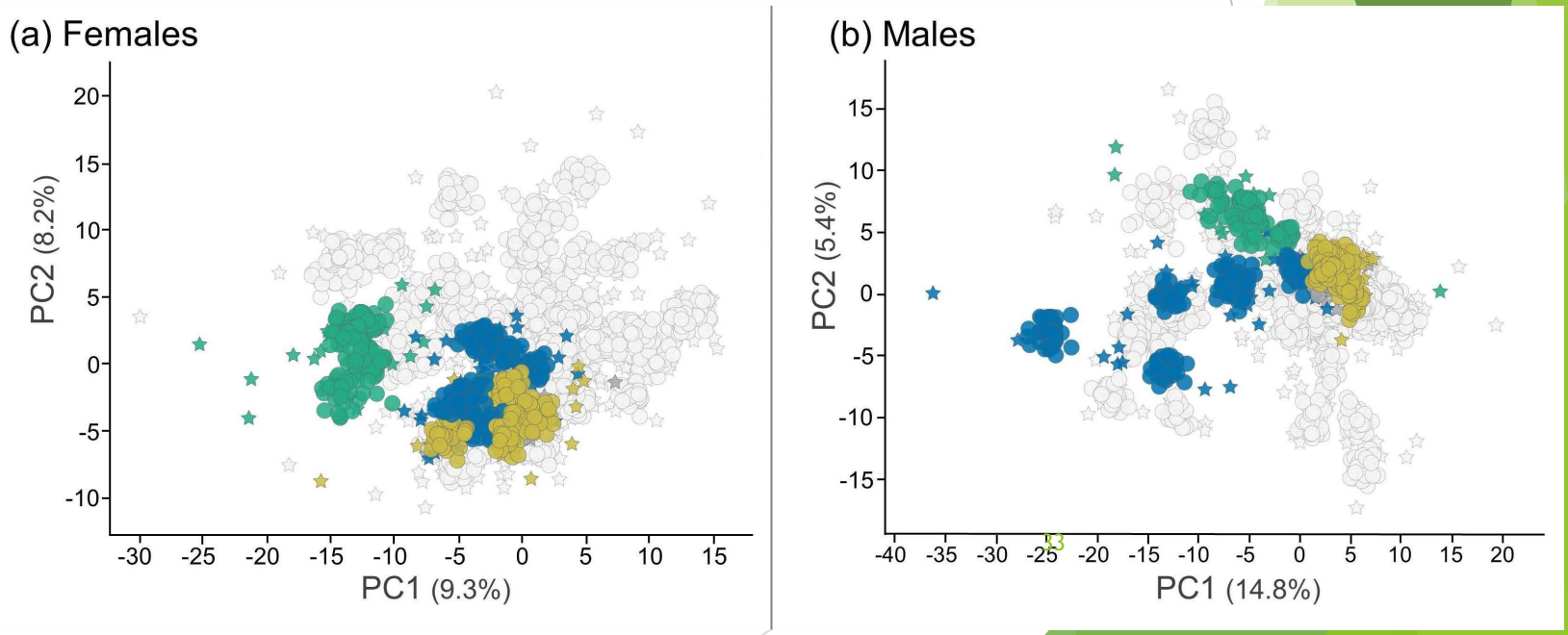
Substances blinded to ring trial partners

Code	Test substance	CAS no.	MoA	High dose	Low dose	Vehicle
TS1	WY-14643	50892-23-4	PP	1200 ppm	400 ppm	In diet
TS2	4-Chloro-3-nitroaniline	635-22-3	Anaemia	90 mg/kg b.w.	30 mg/kg b.w.	In corn oil
TS3	17a-Methyl-testosterone	58-18-4	AR	80 mg/kg b.w.	20 mg/kg b.w.	In corn oil
TS4	Trenbolone	10161-33-8	AR	30 mg/kg b.w.	10 mg/kg b.w.	In corn oil
TS5	Aniline	62-53-3	Anaemia	100 mg/kg b.w.	10 mg/kg b.w.	In aqua bidest
TS7	Dichlorprop-p	15165-67-0	PP	2250 ppm	1000 ppm	In diet
TS8	2-Chloroaniline	95-51-2	Anaemia	160 mg/kg b.w.	40 mg/kg b.w.	In corn oil
TS9	Fenofibrate	49562-28-9	PP	400 mg/kg b.w.	100 mg/kg b.w.	Drinking water containing 0.5% CMC

MetaMapTox
- metabolomics (biological) response database
- Mapping – 8 of 29 substances

3 MoA categories
- PP, Anaemia, AR
- Moderately separated

AR = Androgen Receptor Agonism
PP = Peroxisome Proliferation



Reporting

- Methods
- Quality of data
- Results of grouping the 8 test substances
- Supporting evidence

- Prepare OECD Omics Reporting Framework
- Send directly to ECHA by the agreed deadline
- **All of this was *blinded***

Grouping results - Males

Group	Test substances	Confidenc
A		
B		
C		
…		

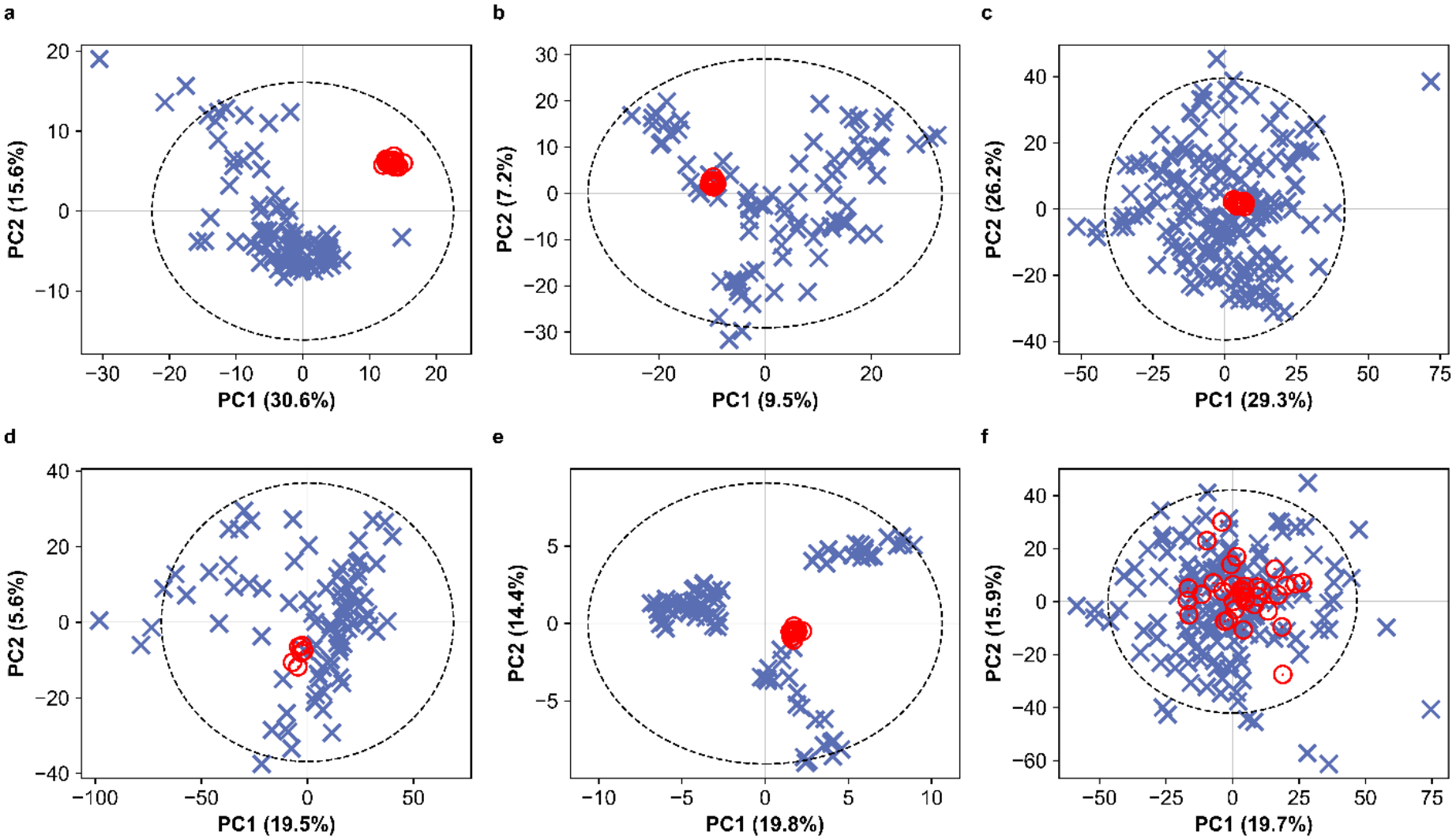
Grouping results - Females

Group	Test substances	Confidence
A		
B		
C		
…		

OORF reporting element	Range of methods reported
OORF Data Acquisition and Processing Reporting Module: QA/QC practices	
Intrastudy QC precision report	Median RSD of intrastudy QCs, Median RSD of intralab QCs, PCA scores plot of QC and biological samples
OORF Data Analysis Reporting Module: Multivariate analysis	
Unsupervised	HCA, Correlation, PCA, Bootstrap PCA, Consensus PCA
Supervised	HCA, PLSDA, OPLSDA, LDA, SUS plots, Correlation, Bootstrapping

QC results

- Unblinding – 5 of 6 labs obtained high quality data
- Partner #6 – analytically noisy data, poor QA/QC results

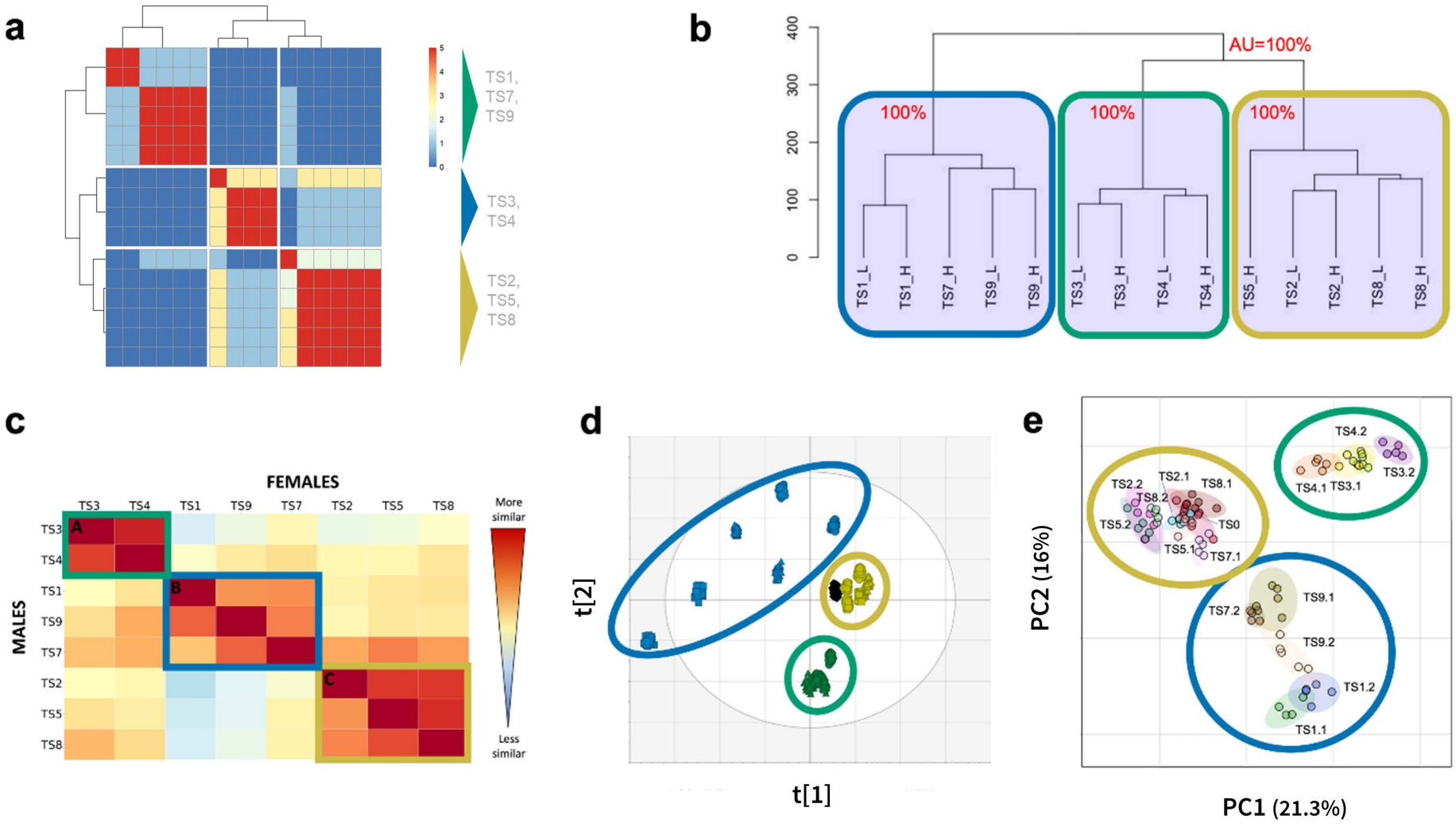


Chemical Grouping Results: Summary

Test substance code	Group	Males					Females				
		RP1	RP4	RP5	RP6	RP7	RP1	RP4	RP5	RP6	RP7
TS1	A										
TS7							HD*	HD*			HD*
TS9											
TS2	B										
TS5								HD*			
TS8											
TS3	C										
TS4											

- Grouping was performed based on **individual statistical strategies**
- Analysis showed **similar grouping** for 5 partners who finished data analysis

Chemical Grouping Results: Visual



Learnings

- The identical grouping results were achieved across the five labs without harmonised
  - instrumentation
  - data types: untargeted, targeted, hybrid
  - sample preparation protocols
  - LC-MS methods
  - data processing workflows and statistical evaluation
- Diversity in approaches was encouraged from the start to be representative of the metabolomics community.
- Partners with identical grouping results observed good data quality – against each lab's own acceptance criteria. The Partner who did not achieve sufficient data quality ultimately did not go on to group the data.
- Each Partner used multiple approaches to answer the grouping question and/or to enhance their confidence in the grouping results.

Statistical grouping approaches	Approaches to derive confidence in grouping
- HCA - Correlation - Multivariate visualisation	- HCA - PLSDA, OPLSDA - LDA - SUS plots - Correlation - Bootstrapping

Conclusions & Next steps

MATCHING study has demonstrated high reproducibility of metabolomics analyses

Initial assessments suggest that ‘good metabolomics practice’ is sufficient to achieve reproducible results across laboratories

The project paper is now published in Archives of Toxicology

As a follow up, the project team will assess the comparability of the metabolomic signatures that delivered the reproducible grouping results.

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Cefic-LRI is proud to fund research such as the MATCHING Project 🔬 !

Great collaboration between industry, regulators and academia!

"This is a massive step forward to improve the existing grouping and read-across approach. The fact that five labs from different countries all got the same, correct results while using different methods and instruments, their own procedures and statistical analysis shows that metabolomics is a reliable method," said Katherine Santizo, Cefic-LRI Programme Manager.

University of Birmingham Mark Viant European Chemicals Agency Imperial College London Syngenta BASF BASF Metabolome Solutions GmbH US Environmental Protection Agency (EPA) Vrije Universiteit Amsterdam (VU Amsterdam)

#metabolomics #research #CeficLRI #chemicalsafety #chemistry #chemicals

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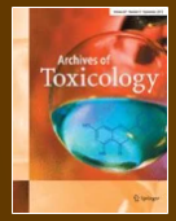

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> by making the grouping and read-across approach more robust by using metabolomics, the number of lab rats being tested could be dramatically cut.
>
> **Professor Mark Viant, School of Biosciences, University of Birmingham**

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March 12: Social Systems and Justice in the Fifth National Climate Assessment

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