LOA REACH CONSORTIUM

Webinar:
Utility of Metabolomics to Support Read-Across for UVCB substances under REACH

30th November 2021 13:00-16:00 CET

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Utility of Metabolomics to Support Read-Across for UVCB substances under REACH
Webinar
30th November 2021

Introduction

Mike Penman

LOA REACH CONSORTIUM

Webinar Programme

Introduction	**Mike Penman (LOA)**	**13:00 (CET)**
Read-Across in REACH; application to UVCBs	**Dr. David Bell (ECHA, Helsinki)**	13:10
Introduction to Metabolomics	**Prof. Mark Viant (University of Birmingham, UK)**	13:25
Use of Metabolomics for Read-Across	**Prof. Bennard Van Ravenzwaay (BASF)**	13:45
Break		14:05
Use of Metabolomics to Assess Biological Coherence in UVCB Read-Across and Category Justification		
A. Introduction	**Mike Penman (LOA)**	14:10
B. Experimental Design and Data Review	**Prof. Hennicke Kamp (BASF)**	
C. Conclusion	**Dr. Martijn Rooseboom (Shell/LOA)**	
Discussion moderator	**Prof. Mark Viant (University of Birmingham, UK)**	15:15
Close		16:00

LOA Background

- Registration, Evaluation Authorisation of Chemicals (2007)
  - Requirement for all manufactures / importers of substances to submit dossier to European CHemicals Agency
    - Intrinsic properties / Tonnage / Uses / Risk Assessment / Risk Management
- Olefins and Aromatics
  - Many data rich substances with history of regulatory dialogue
  - Many complex production streams of unknown / variable composition

LOA Background

- LOA formed in 2008
  - … to assist its Members with their REACH registrations in a cost-efficient manner by combining financial and human resources
  - … ensure that the documentation produced is fit for the purpose of supporting Member Companies' REACH registrations
- Scope
  - 46 Member companies - 250 Registrant companies
  - 150 substances under management
    - 28 monoconstituents / reaction masses
    - 115 UVCB “streams” from production processes

LOA Streams and Categories

- Basic production process – Steam Cracking of petroleum streams
  - Aim – isolate pure substances for further syntheses
    - The petrochemical building blocks
  - Process - quenching of short hydrocarbons to get pure substances
  - Streams are by-products and intermediates of this process and not the main aim
    - By contrast - Refining and pyrolysis though Catalytic cracking aims to produce streams
  - Generally, not intended for wide dispersive use
  - Tend to have higher levels of identified olefin and aromatic substances which are registered as mono-constituent
    - Able to characterise the majority of constituents
  - Nature of the LOA categories varies considerably depending upon process step

LOA UVCB categories by C-number and manufacturing process

LOA Category	Category Name	Predominant C Number	Category Manufacturing Process	No. Substances
A	Aliphatic / Cyclic C5 and Higher	C5 to C8	HC streams typ. from a naphtha/pyrolysis gasoline treatment and aromatic extraction unit involving pre-distillation, hydrogenation and extractive distillation processes. Produced after aromatic extraction or (fractionated) distillation of hydrotreated naphtha.	6
E	C5 non-cyclics	C4 to C8	Hydrocarbon streams typ. from the steam cracking process as products of distillation processes. This C5+ cut (typical boiling range of approximately 0 to 75 °C) predominately consist of C5 hydrocarbons. The streams contain significant levels of olefins and diolefins.	6
C	C4, Low 1,3-Butadiene (<0.1%)	C4	Typically produced from the steam-cracking of naphtha and following the extraction of 1,3-butadiene from a C4-rich stream.	5
D	C4, High 1,3-Butadiene (≥0.1%)	C4	Typically produced from the steam-cracking of naphtha as a C4-rich stream.	6
H	High Benzene Naphthas	C5 to C11	Distillation of products from a steam cracking process or by pyrolysis. Predominantly hydrocarbons >C6 and BP range 30°C to 300°C.	26
J	Low Benzene Naphthas	C7 to C13	Distillation of products from a steam cracking process or by pyrolysis. <0.1% benzene.	4
L	**Resin Oils and Cyclic Dienes**	**C5 to C15**	**Hydrocarbons typ. produced by distillation of products from a steam cracking process. Non-hydrotreated products (Resin Oils) and/or concentrates of (1) DCPD and (2) (MeCPD).**	**10***
B	Butylene Oligomers	C4 to C20	Streams obtained by the oligomerisation of butylenes optionally followed by hydrotreating processes. Predominantly C8, C12, C16 and/or C20 hydrocarbons. The streams' constituents boil between 30 and 350 °C and the streams contain less than 0.1% butadiene.	8
G	Fuel Oils	C6 to C21	Hydrocarbon streams typically produced by distillation of products: from a steam cracking process, from an ethylene manufacturing process; residual fraction from these distillation processes or produced by pyrolysis.	13
K	Other Petroleum Gases	C1 to C5	Hydrocarbon streams containing petroleum gases (alkanes/alkenes) predominantly in the C1-C5 range (with some carbon numbers present at lower levels up to C10) and include some LPGs. The majority of the members of this category contain <0.1% 1,3-butadiene.	29

LOA Streams and Categories

- Approach in 2010 to meet Information Requirements included marker substances
  - Major mono-constituents that were relatively data rich
  - Drove hazard of the stream and were usual markers of exposure
- Since 2010, appreciation that more data was required on the streams themselves
  - Development of guidance (RAAF) and DART requirements
- Question for complex streams
  - How to show biological coherence as well as Chemical coherence?
    - Support read across with additional data - not replace statutory testing

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Read-across in REACH; application to UVCBs

Utility of Metabolomics to Support Read-Across for UVCB substances under REACH

30 November 2021

David Bell

European Chemicals Agency

Content

- Legal requirements
- Application to UVCBs
    - Similarity for UVCBs
    - RAAF/ multi-constituent RAAF
    - Large category approach

This is not an official position of ECHA. This presentation is intended to be educational, but you should consult ECHA's guidance and the legal text for definitive information. This presentation is not an endorsement of any specific case or approach used for read-across in a specific case.

Annex XI, 1.5

Grouping and read-across

Annex XI, 1.5

- Legal text amended
  - COMMISSION REGULATION (EU) 2021/979 of 17 June 2021
  - “This Regulation shall enter into force on the twentieth day following that of its publication in the Official Journal of the European Union.
  - It shall apply from 8 January 2022.”
- See ECHA news release and the Regulation
  - Guidance update anticipated in December 2021

Legal text- highlights

Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of **structural similarity**, may be considered as a group, or category, of substances. Application of the group concept requires that physicochemical properties, **human health effects and environmental effects or environmental fate may be predicted** from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach). This avoids the need to test every substance for every endpoint.

…

Structural similarity for UVCB substances shall be established on the basis of similarities in the structures of the constituents, together with the concentration of these constituents and variability in the concentration of these constituents. If it can be demonstrated that the identification of all individual constituents is not technically possible or impractical, the structural similarity may be demonstrated by other means, to enable a quantitative and qualitative comparison of the actual composition between substances. …

Legal text- highlights II

…

In all cases, **results shall fulfil all of the following conditions**:

— be adequate for the purpose of classification and labelling and/or risk assessment,

— have adequate and reliable coverage of the key parameters addressed in the corresponding study that shall normally be performed for a particular information requirement,

— cover an exposure duration comparable to or longer than the corresponding study that shall normally be performed for a particular information requirement if exposure duration is a relevant parameter.

…

Application to UVCBs

Structural similarity for UVCBs

- “Structural similarity for UVCB substances shall be established on the basis of similarities in the structures of the constituents, together with the concentration of these constituents and variability in the concentration of these constituents.

  If it can be demonstrated that the identification of all individual constituents is not technically possible or impractical, the structural similarity may be demonstrated by other means, to enable a quantitative and qualitative comparison of the actual composition between substances.”

  - No or inadequate information on constituents = failure
  - Requirement for information on concentration and variability of constituents
  - For complex UVCBs, impractical to identify and quantify all individual constituents
  - Case-specific considerations.
    - Structural similarity is for the purpose of demonstrating that you can predict human health or ENV properties, and must be fit for that purpose
    - ECHA has accepted e.g. detailed 2-D GC plus specific constituent identification

RAAF/ multi-constituent RAAF

- How ECHA assesses read-across
  - Read-across assessment framework (RAAF-link)
  - UVCB/multi-constituent RAAF (link)
- Key issues
    - Category or analogue approach
    - (Bio)transformation to common compound(s) or Different compounds have the same type of effect(s)
    - Check list of scientific issues that need addressing for each scenario

Generic category approach-HH

- RAAF scenario 4 or 6
  - Sufficient data (compositional data, bridging data and higher-tier data) to provide confidence in a prediction based on similar/ regular pattern of biological activity
  - Analytical and bridging data must support the read-across
    - Case-specific assessment

Thank you!

david.bell@echa.europa.eu

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UNIVERSITY OF BIRMINGHAM

Introduction to Metabolomics

Mark Viant

Professor of Metabolomics, University of Birmingham, UK

LOA Webinar

30th November 2021

Four take-home messages

- Metabolic biomarkers are already used as ‘endpoints’ in OECD Test Guidelines to indicate mode-of-action
- Metabolic biomarkers typically occur downstream in the molecular cascade that follows chemical exposure, hence they can be good predictors of phenotype (i.e., apical endpoints)
- Metabolomics is simply a technology that measures multiple metabolic biomarkers simultaneously; it generates data that is typically analysed using multivariate statistics
- Metabolomics can be used for grouping because chemicals that act via the same MoA should induce similar metabolic biomarkers.

Metabolic biomarkers can predict MoA (1)

408 OECD/OCDE

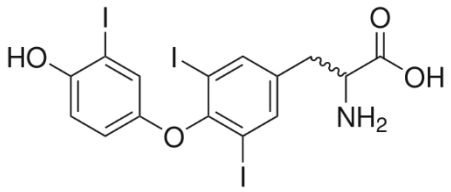
OECD GUIDELINE FOR THE TESTING OF CHEMICALS

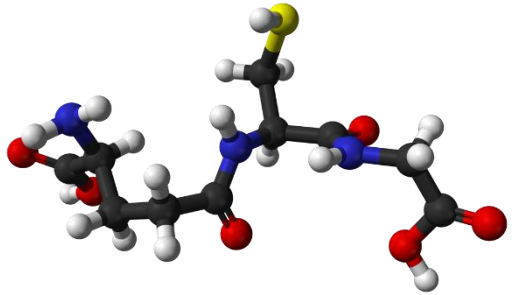
Repeated Dose 90-day Oral Toxicity Study in Rodents

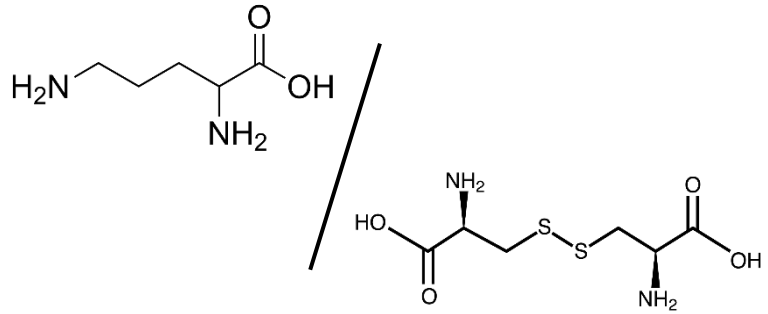
4. The revised Guideline places additional emphasis on endocrine endpoints to combine with the existing sensitivity to neurological and immunological and reproductive effects. The need for careful clinical observations of the animals, so as to obtain as much information as possible, is also stressed. Required endpoints include the measurement of thyroxine (T4), triiodothyronine (T3) thyroid stimulating hormone (TSH) and thyroid gland weight, which are responsive to thyroid pathway perturbation (2). In addition, serum total

Serum/Plasma Hormone Analyses	
Thyroxine (T4)	FSH
TSH	LH
T3	Oestradiol
	Testosterone

Metabolic biomarkers can predict MoA (2)







T3 or T4

GLUCOSE

GLUTATHIONE

ORNITHINE / CYSTINE

Stemina
BIOMARKER DISCOVERY

devTOX qP quickPREDICT

devTOX *quick*Predict™ is a biomarker-based, human, *in vitro* assay that predicts developmental toxicity. It utilizes key biomarkers identified using Stemina's metabolomics platform.

Conceptually - lets combine these metabolic biomarkers into a biomarker panel to predict multiple MoAs

T3 or T4

Targeted assay measuring metabolic biomarkers for one MoA

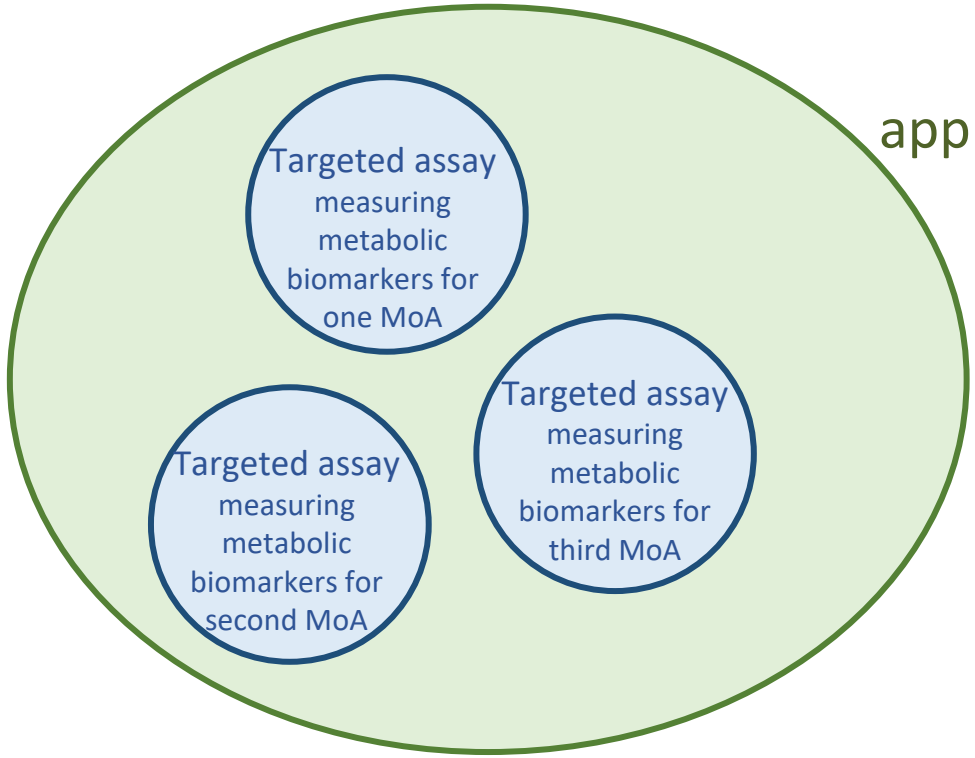
Targeted assay measuring metabolic biomarkers for second MoA

Targeted assay measuring metabolic biomarkers for third MoA

Stemina BIOMARKER DISCOVERY

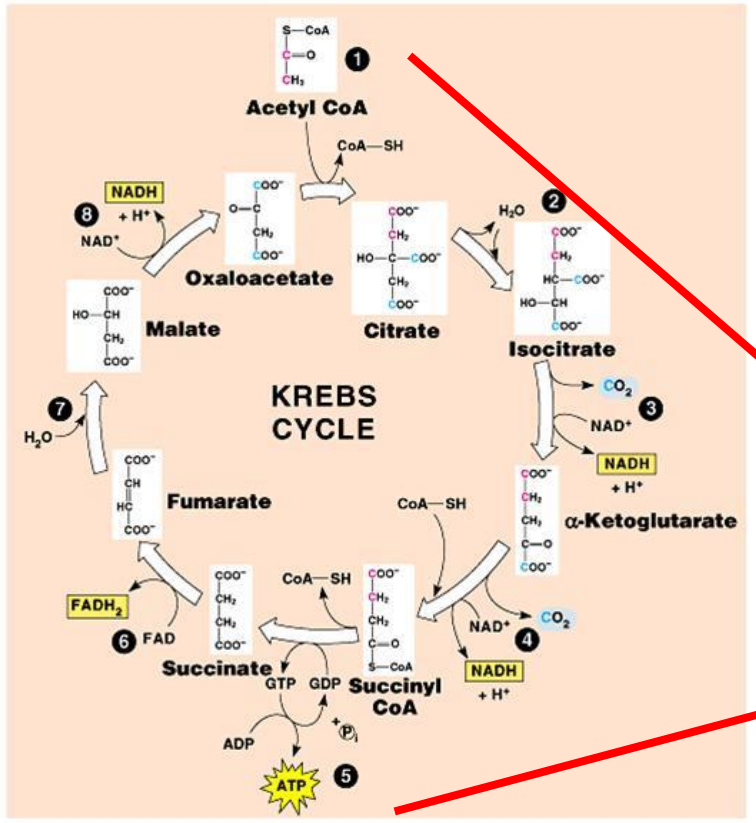
devTOX qP quickPREDICT

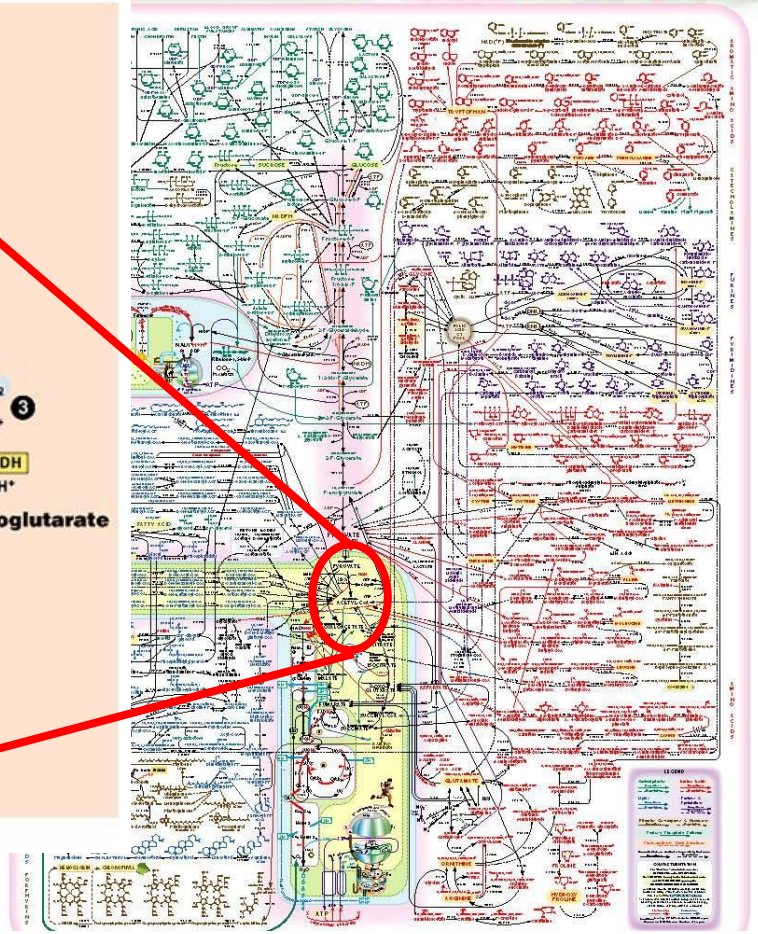
Conceptually - lets combine these metabolic biomarkers into a biomarker panel to predict multiple MoAs



This is the BASF *Metabolic Profiling* approach, they are measuring the green 'biomarker panel'

Metabolic Pathways

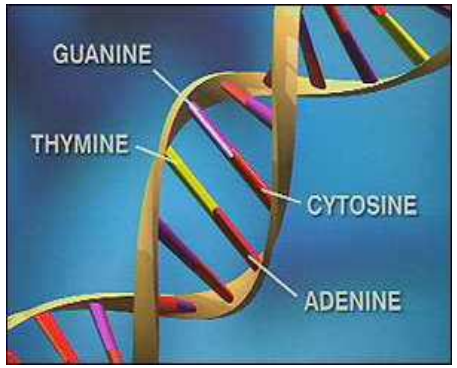


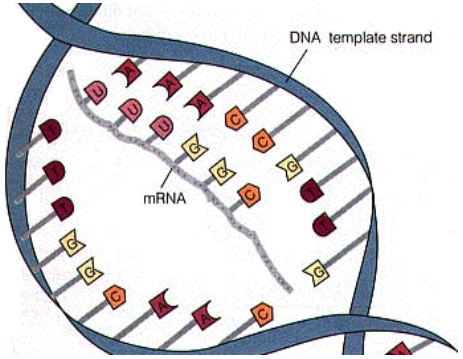


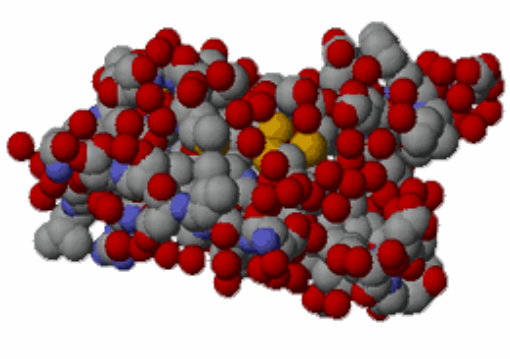
- Amino acids
- Carbohydrates
- Lipids
- Steroids
- Nucleotides
- 1,000's metabolites in a 'metabolome'

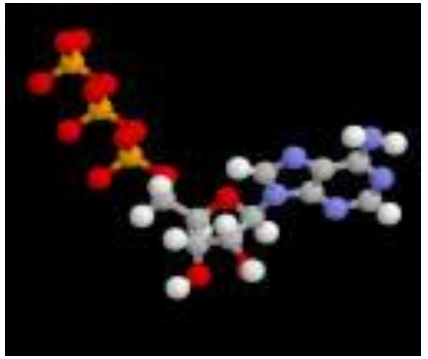
Metabolic profiling: Measurement of the endogenous metabolic responses of a biological system (to a chemical); not the metabolites of the exposure chemical

Central dogma of molecular biology (simplified view)





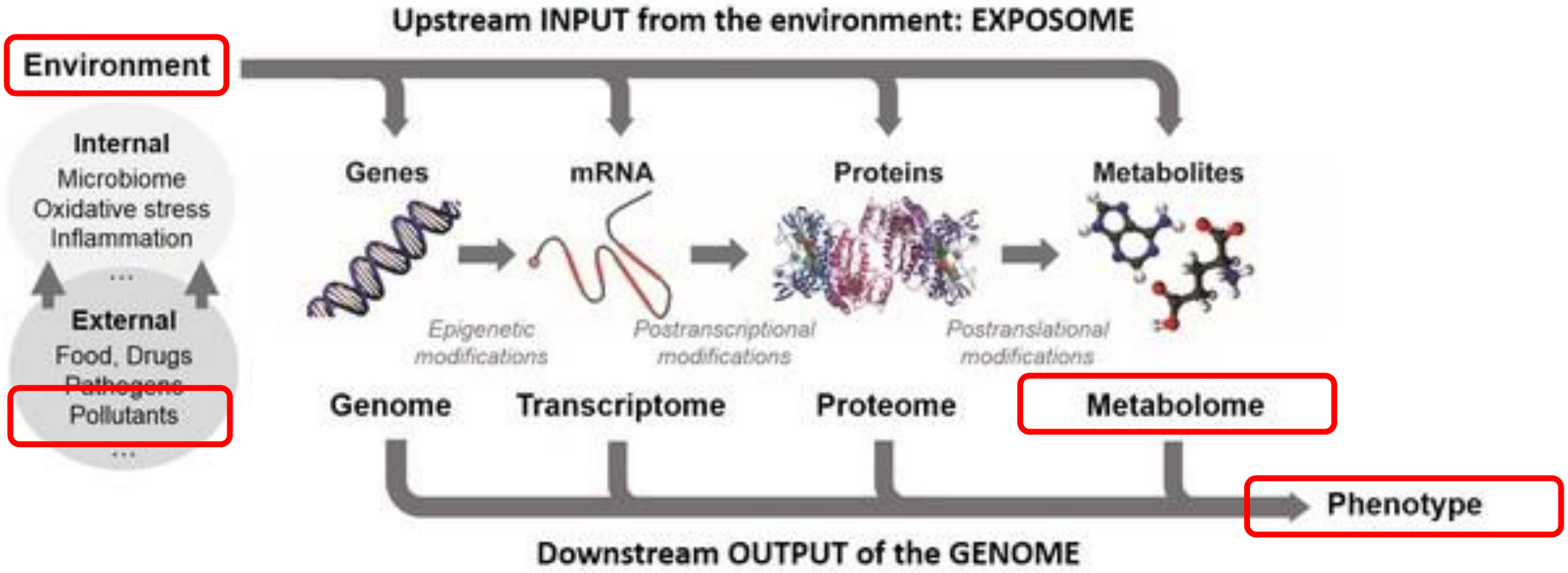




DNA → Genes (mRNA) → Proteins ⟲ Endogenous metabolites

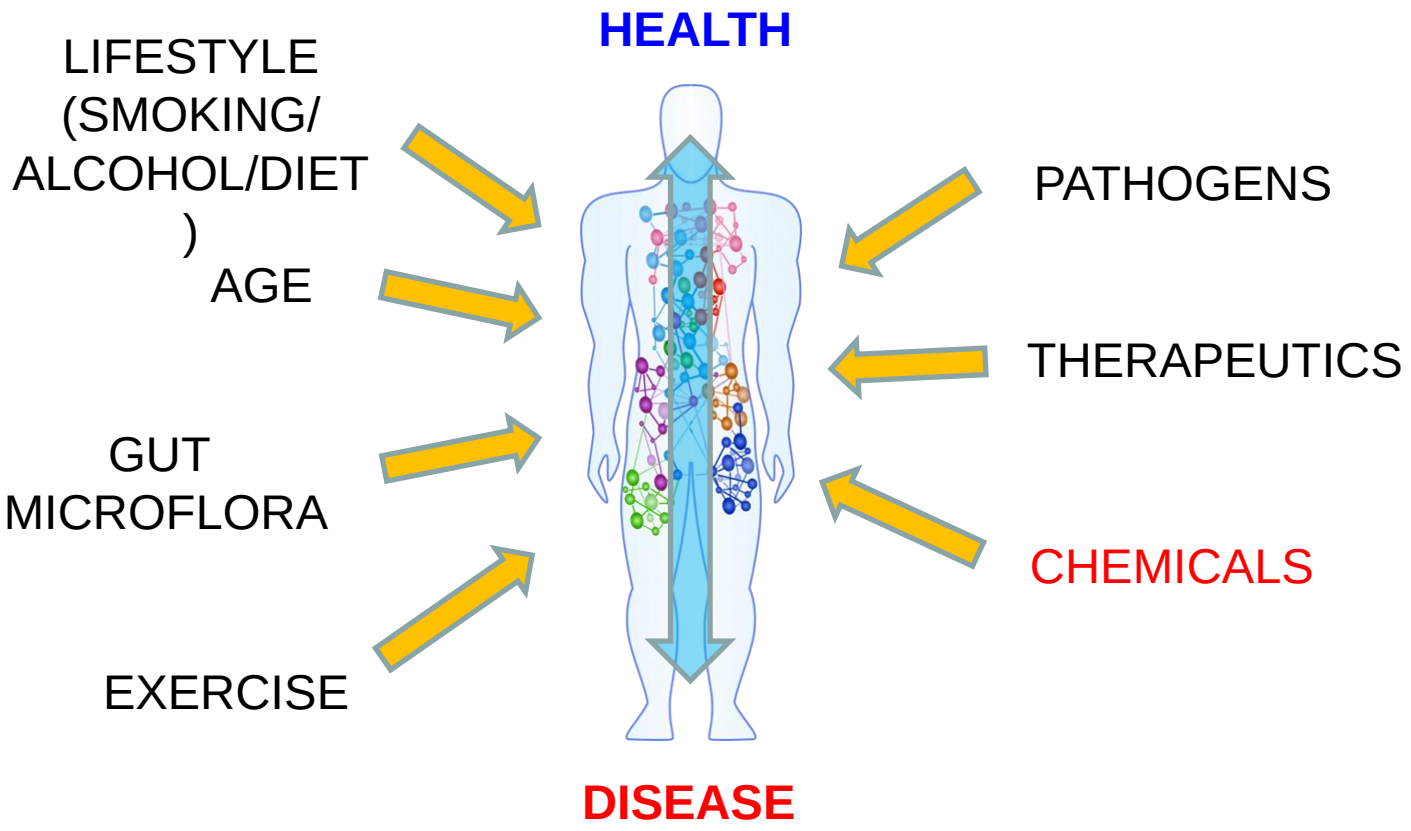
Transcription | Translation | Enzyme activity

Why focus on metabolic biomarkers?



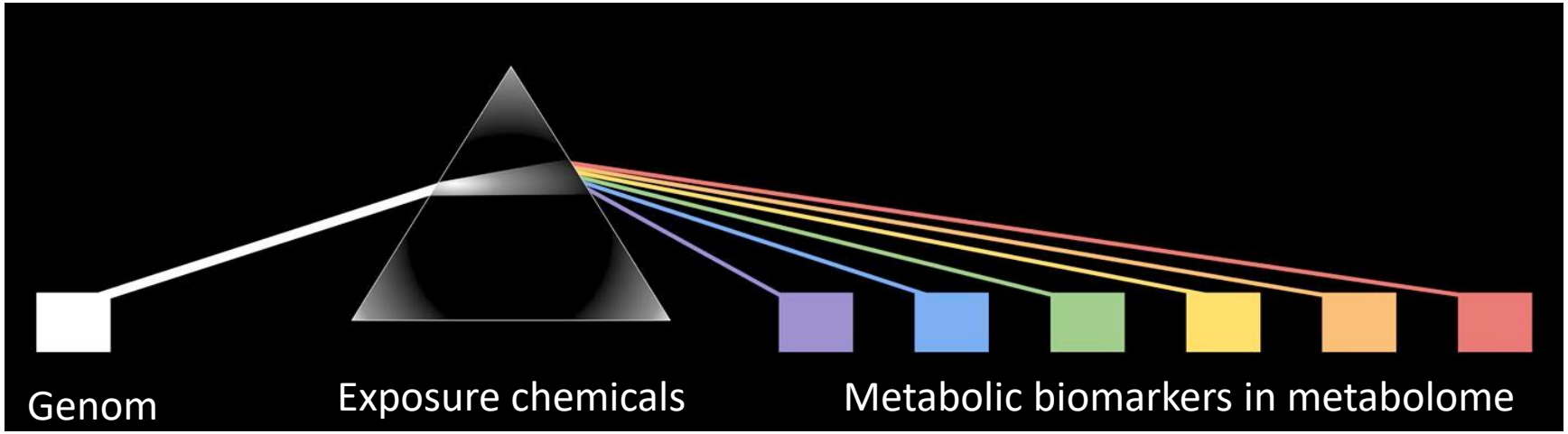
How is this relevant to grouping / read-across?

Metabolic biomarkers (in the metabolome) are condition dependent



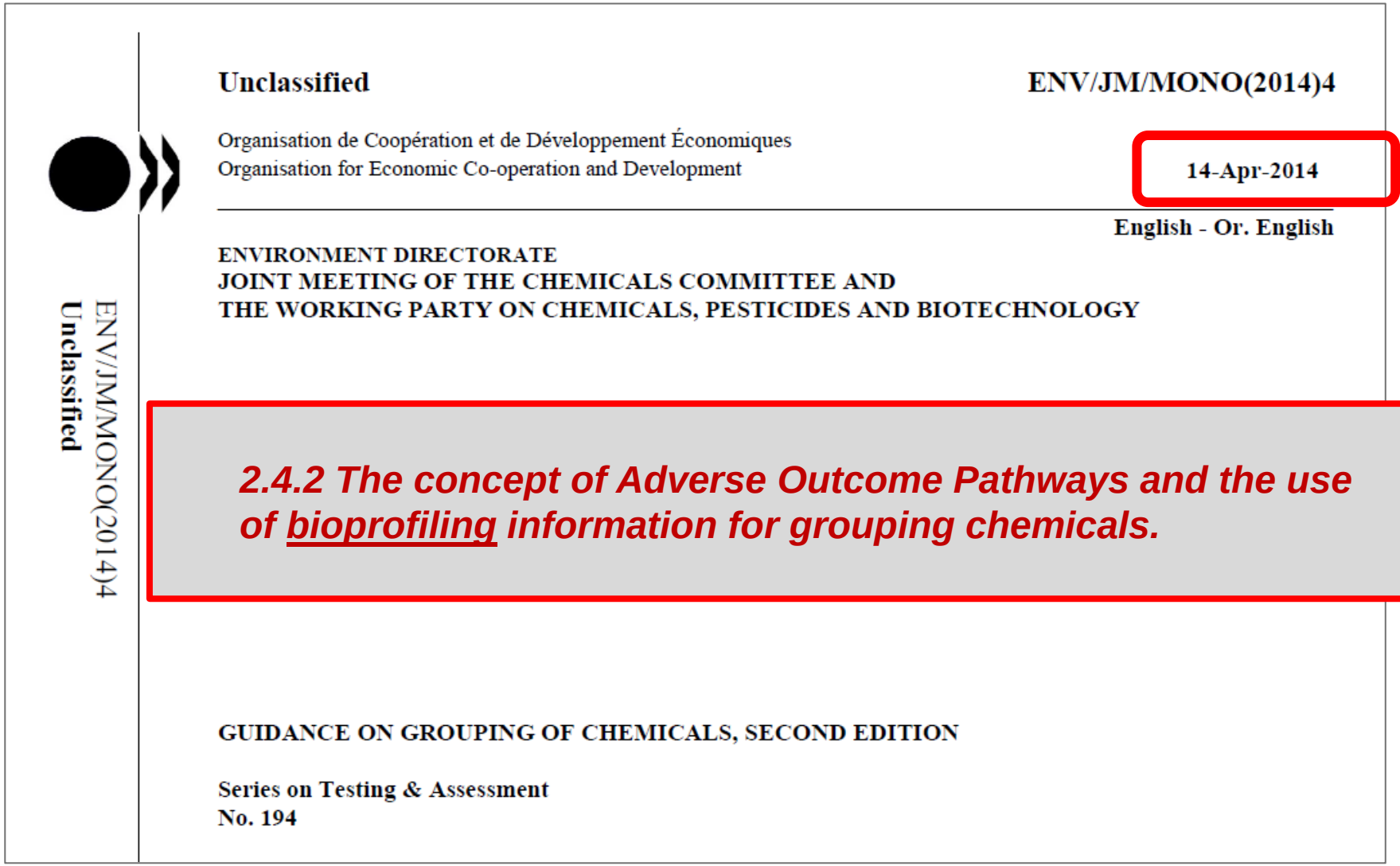
Metabolic fingerprint

Metabolic biomarkers (in the metabolome) are condition dependent



- Chemicals acting via different MoAs will perturb different sets of metabolic biomarkers
- Chemicals acting via the same MoA should perturb similar metabolic biomarkers

ot new: Existing OECD Guidance for G/RAx



Unclassified

ENV/JM/MONO(2014)4

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

14-Apr-2014

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

2.4.2 The concept of Adverse Outcome Pathways and the use of bioprofiling information for grouping chemicals.

GUIDANCE ON GROUPING OF CHEMICALS, SECOND EDITION

Series on Testing & Assessment
No. 194

ENV/JM/MONO(2014)4
Unclassified

Deficiencies in Use of Grouping / Read-Across

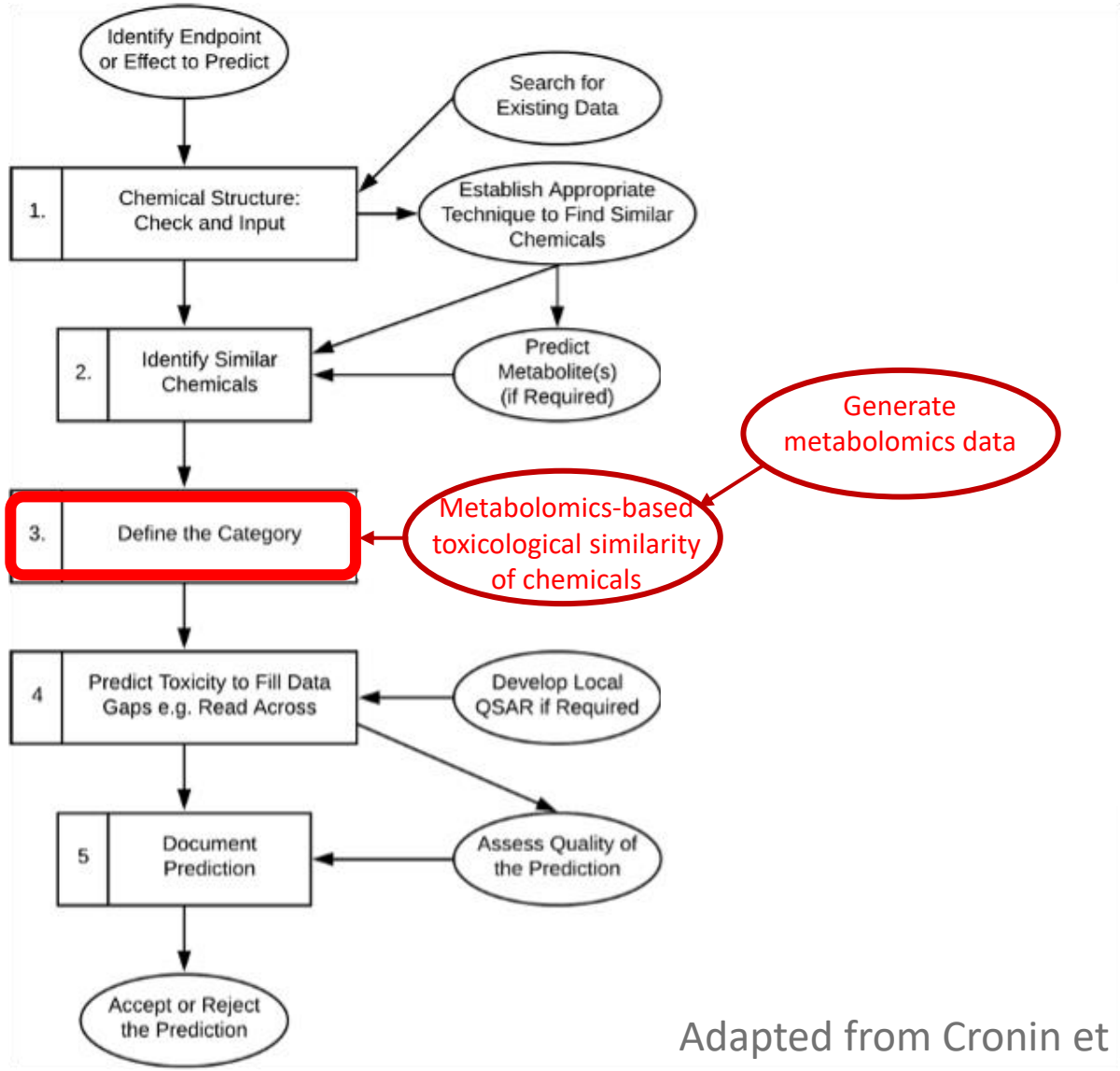
- ECHA report - *The use of alternatives to testing on animals for the REACH Regulation* June 2020

  https://echa.europa.eu/documents/10162/0/alternatives_test_animals_2020_en.pdf

- Most common shortcomings include (from list of 6):
  - lack of, or low quality of, supporting data;
  - shortcomings in the hypothesis and justification of the toxicological prediction.

- "To increase the robustness and regulatory acceptance for high-tier human health endpoints, additional data is needed, particularly related to toxicological mechanisms and ADME properties."

How could molecular biomarkers be added to the multi-step process of G/RAx?

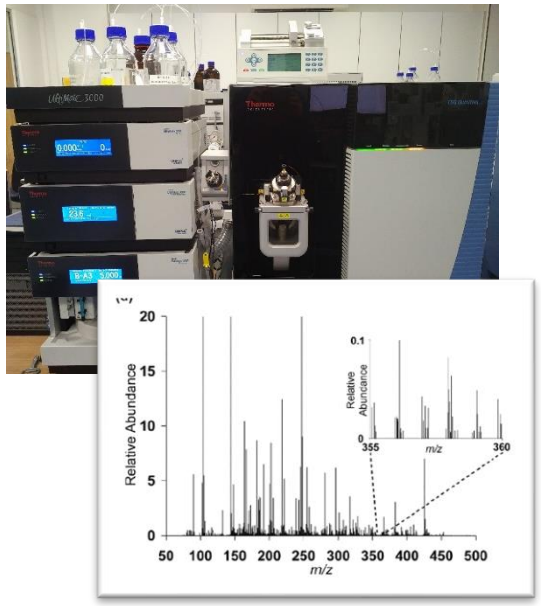


Adapted from Cronin et al. (2013)

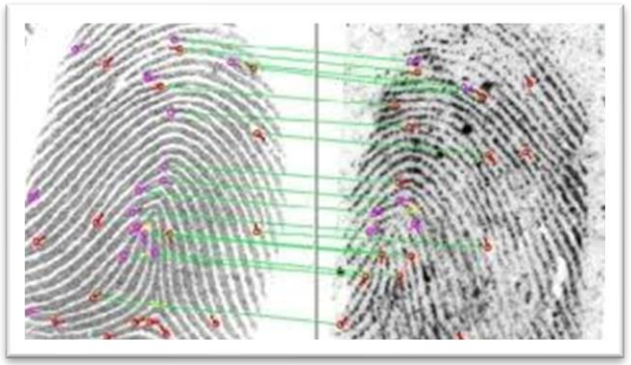
How can we measure “toxicological similarity” (or differences) using metabolomics data?

- Univariate statistical analysis deals with one metabolite at a time, but BASF are measuring ca. 270 metabolites simultaneously
- Multivariate statistical analysis can handle the analysis of all the measured metabolites
- Multivariate analysis takes into account the interactions between metabolites
- PCA, HCA

Principal components analysis (PCA)



GC-MS, LC-MS/MS

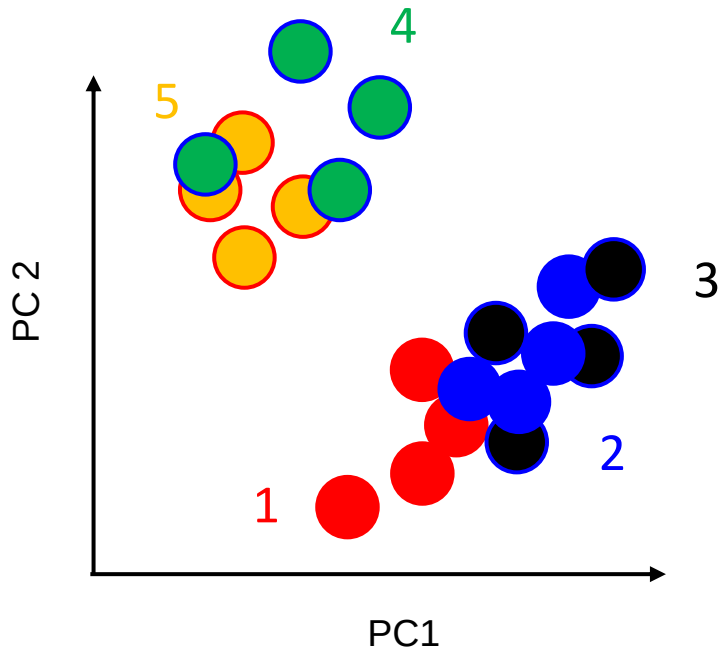


Response to chemical 1

Response to chemical 2… 5

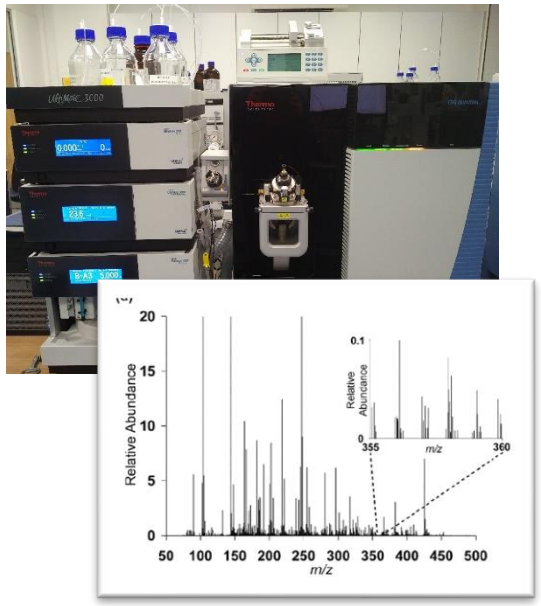
Determine which metabolites show the greatest variation across all the samples

PCA scores plot



Visualise the similarities/differences between all 5 chemicals in the study

Hierarchical cluster analysis (HCA)



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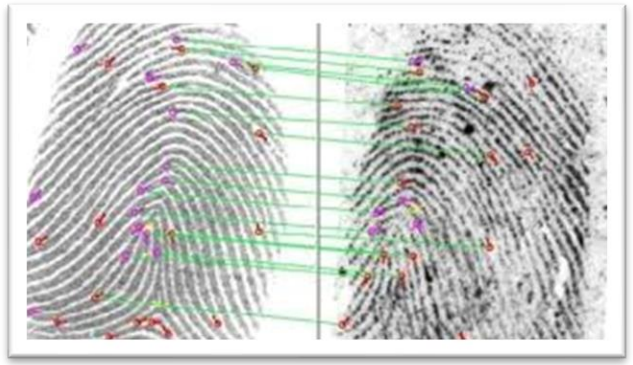
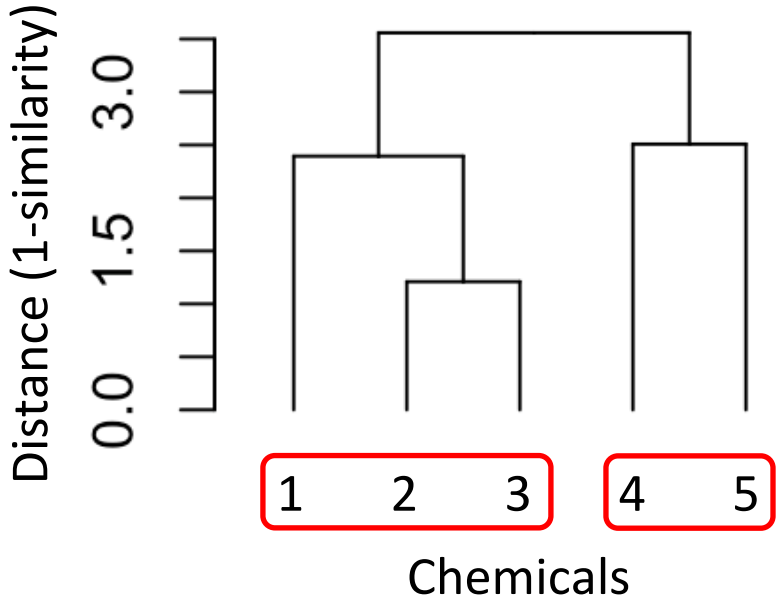


Response to chemical 1

Response to chemical 2… 5

Calculate similarity of the metabolite fingerprints between each pair of chemicals

Dendrogram



Visualise the similarities/differences between all 5 chemicals in the study

Four take-home messages

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- Metabolic biomarkers typically occur downstream in the molecular cascade that follows chemical exposure, hence they can be good predictors of phenotype (i.e., apical endpoints)
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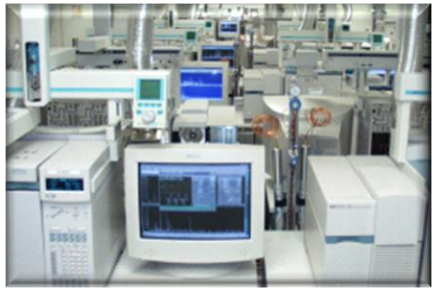
Use of metabolomics for Read-Across

- an introduction -

Prof. Dr. Bennard van Ravenzwaay

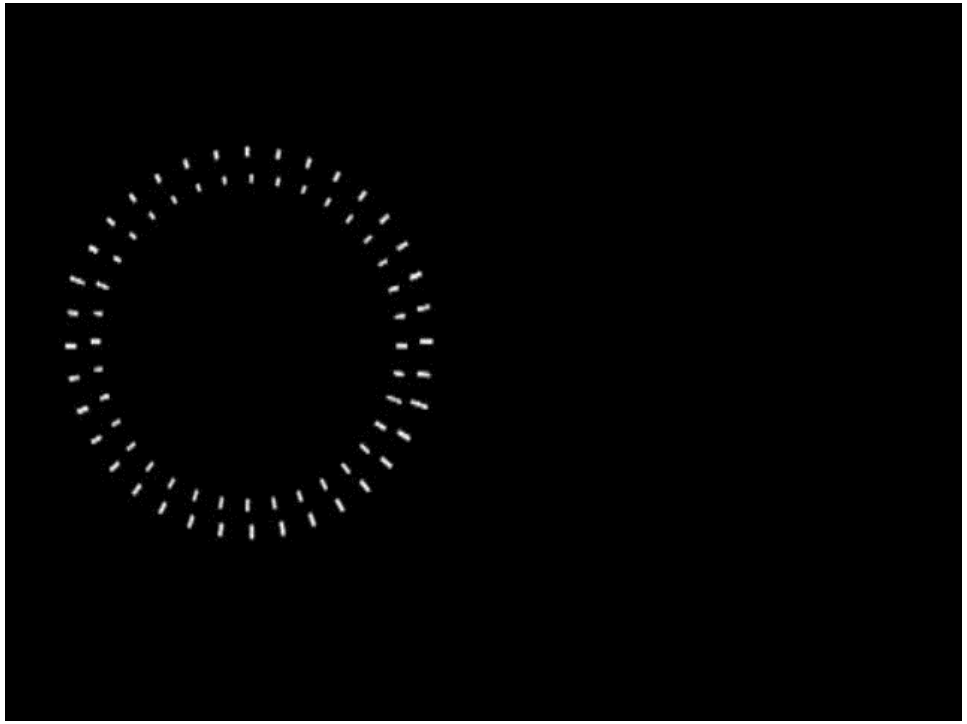
The Use of MetaMap®Tox

BLOOD PROFILING

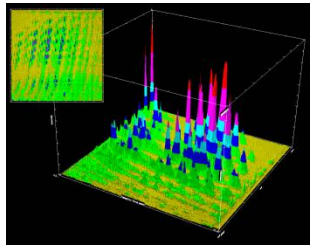


LC-MS/ GC-MS

MetaMap®Tox



Metabolite
Tryptophan
Arginine
Tyrosine
Thyroxine (T4)
Linolenic acid (C18:cis[9,1...
alpha-Tocopherol
Lignoceric acid (C24:0)
Campesterol
Tricosanoic acid (C23:0)
Phytosphingosine
14-Methyl-Pentadecanoic aci...
17-Methyloctadecanoic acid
Eicosatrienoic acid (C20:3)...
O-Methylsphingosine No1 (pl...
O-Methylsphingosine No2 (pl...
erythro-Sphingosine
Cholesterol
5-Oxoproline
Citrate
Glutamate
Creatinine
Sphingomyelin No 01 (putative)
Sphingomyelin (d18:1, C16:0...



Total Metabolome Signature (9000 analyte signals)

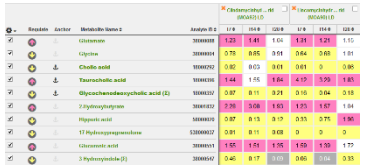
SAMPLE

REFERENCE

300 Known Metabolites

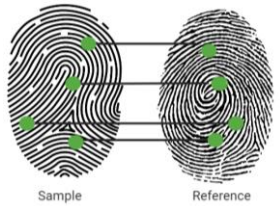
MetaMap®Tox: Reduction through Refinement
Blood metabolome analysis in short-term studies

Mode of action determination



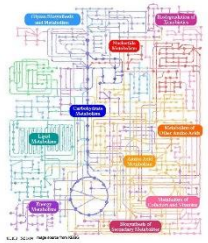
> 110 MoA patterns established

Compound Metabolome Comparison



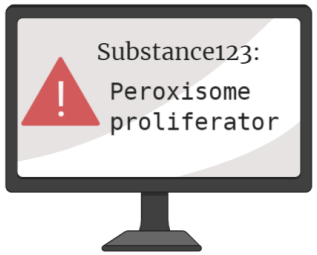
Ranking of (new) compound's metabolome against metabolome of 1000 substances

Biochemical pathway analysis



Identify molecular mechanisms of toxicity

Predictivity rate: 80%



Sensitivity: as classical toxicology

BASF
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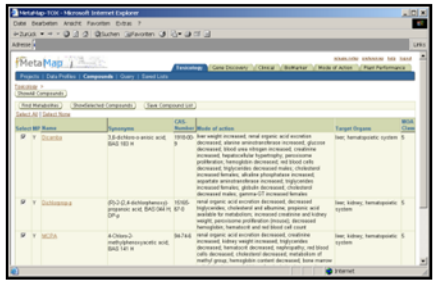
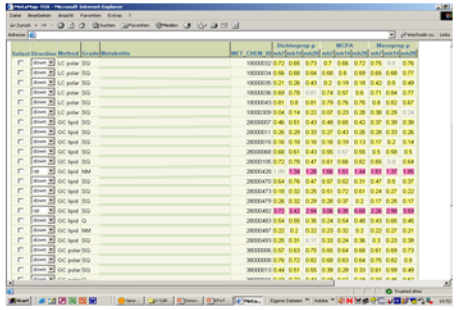
MetaMap®Tox: Reference Data Base

Test Substance



"Find Metabolites"

Metabolite profile



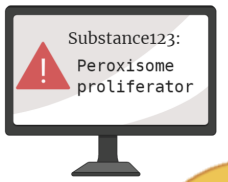
"Profile Comparison"

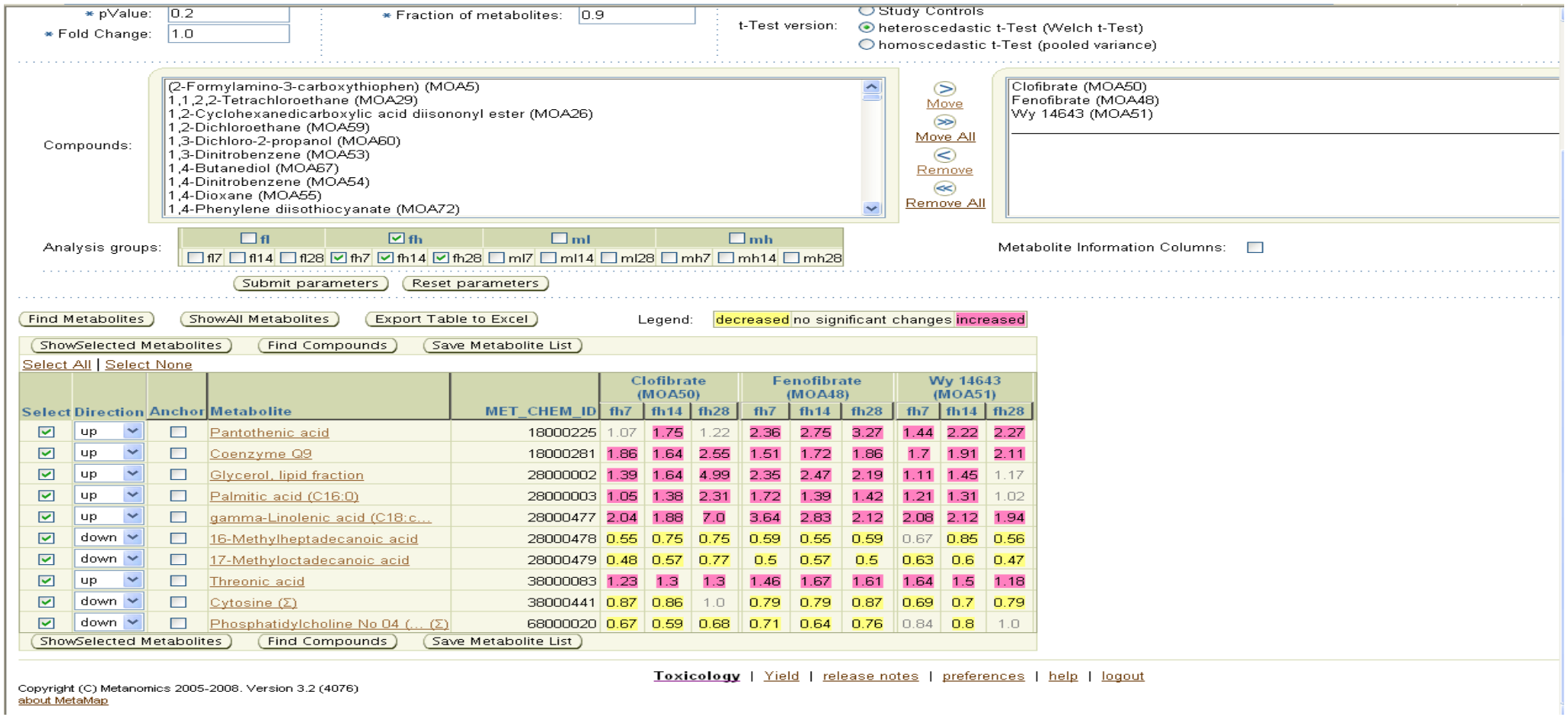
Statistical Correlation - Ranking based on biological similarity

"Pattern Ranking"

Similarity of test compound-induced changes compared to toxicity patterns

Recognising mode-of-action: peroxisome proliferation

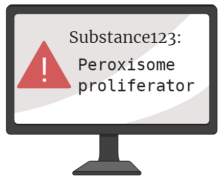




Select	Direction	Anchor	Metabolite	MET_CHEM_ID	Clofibrate (MOA50) fh7	Clofibrate (MOA50) fh14	Clofibrate (MOA50) fh28	Fenofibrate (MOA48) fh7	Fenofibrate (MOA48) fh14	Fenofibrate (MOA48) fh28	Wy 14643 (MOA51) fh7	Wy 14643 (MOA51) fh14	Wy 14643 (MOA51) fh28
☑	up	☐	Pantothenic acid	18000225	1.07	1.75	1.22	2.36	2.75	3.27	1.44	2.22	2.27
☑	up	☐	Coenzyme Q9	18000281	1.86	1.64	2.55	1.51	1.72	1.86	1.7	1.91	2.11
☑	up	☐	Glycerol, lipid fraction	28000002	1.39	1.64	4.99	2.35	2.47	2.19	1.11	1.45	1.17
☑	up	☐	Palmitic acid (C16:0)	28000003	1.05	1.38	2.31	1.72	1.39	1.42	1.21	1.31	1.02
☑	up	☐	gamma-Linolenic acid (C18:c...	28000477	2.04	1.88	7.0	3.64	2.83	2.12	2.08	2.12	1.94
☑	down	☐	16-Methylheptadecanoic acid	28000478	0.55	0.75	0.75	0.59	0.55	0.59	0.67	0.85	0.56
☑	down	☐	17-Methyloctadecanoic acid	28000479	0.48	0.57	0.77	0.5	0.57	0.5	0.63	0.6	0.47
☑	up	☐	Threonic acid	38000083	1.23	1.3	1.3	1.46	1.67	1.61	1.64	1.5	1.18
☑	down	☐	Cytosine (Σ)	38000441	0.87	0.86	1.0	0.79	0.79	0.87	0.69	0.7	0.79
☑	down	☐	Phosphatidylcholine No 04 (... (Σ)	68000020	0.67	0.59	0.68	0.71	0.64	0.76	0.84	0.8	1.0

Recognising mode-of-action: peroxisome proliferation

Compounds found



* Fold Change: 1.0

t-Test version: heteroscedastic t-Test (Welch t-Test) / homoscedastic t-Test (pooled variance)

Compounds:

(2-Formylamino-3-carboxythiophen) (MOA5)
1,1,2,2-Tetrachloroethane (MOA29)
1,2-Cyclohexanedicarboxylic acid diisononyl ester (MOA26)
1,2-Dichloroethane (MOA59)
1,3-Dichloro-2-propanol (MOA60)
1,3-Dinitrobenzene (MOA53)
1,4-Butanediol (MOA67)
1,4-Dinitrobenzene (MOA54)
1,4-Dioxane (MOA55)
1,4-Phenylene diisothiocyanate (MOA72)

Move / Move All / Remove / Remove All

Clofibrate (MOA50)
Fenofibrate (MOA48)
Wy 14643 (MOA51)
Bezafibrate (MOA49)
Mecoprop-p (MOA1)
Dichlorprop-p (MOA1)
Benzylbutyl Phthalate (MOA6)
Diethylhexylphthalate (MOA58)

Analysis groups: fl | fh | ml | mh
fl7 | fl14 | fl28 | fh7 | fh14 | fh28 | ml7 | ml14 | ml28 | mh7 | mh14 | mh28

Metabolite Information Columns:

Submit parameters | Reset parameters

Find Metabolites | ShowAll Metabolites | Export Table to Excel | Legend: decreased / no significant changes / increased

ShowSelected Metabolites | Find Compounds | Save Metabolite List

Select All | Select None

Select	Direction	Anchor	Metabolite	MET_CHEM_ID	Clofibrate (MOA50)			Fenofibrate (MOA48)			Wy 14643 (MOA51)			Bezafibrate (MOA49)			Mecoprop-p (MOA1)			Dichlorprop-p (MOA1)			Benzylbutyl Phthalate (MOA6)			Diethylhexylphthalate (MOA58)		
					fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28
☑	up	☐	Pantothenic acid	18000225	1.07	1.75	1.22	2.36	2.75	3.27	1.44	2.22	2.27	2.12	2.94	2.53	2.56	4.78	5.31	2.0	3.09	3.11	1.82	2.06	2.42	1.45	1.59	1.47
☑	up	☐	Coenzyme Q9	18000281	1.86	1.64	2.55	1.51	1.72	1.86	1.7	1.91	2.11	1.34	1.13	1.63	1.28	1.63	1.76	1.79	2.25	2.58	1.44	1.5	1.78	1.97	1.6	1.6
☑	up	☐	Glycerol, lipid fraction	28000002	1.39	1.64	4.99	2.35	2.47	2.19	1.11	1.45	1.17	2.2	2.45	2.47	1.92	2.0	1.79	2.62	2.41	2.04	1.15	1.48	2.43	1.35	1.52	1.2
☑	up	☐	Palmitic acid (C16:0)	28000003	1.05	1.38	2.31	1.72	1.39	1.42	1.21	1.31	1.02	1.73	1.8	1.92	1.56	1.9	1.99	2.53	2.69	1.57	1.48	1.79	1.94	1.66	1.5	1.34
☑	up	☐	gamma-Linolenic acid (C18:c...	28000477	2.04	1.88	7.0	3.64	2.83	2.12	2.08	2.12	1.94	3.03	2.98	2.99	3.34	3.62	4.14	6.58	6.35	3.44	1.98	1.64	2.08	1.05	1.44	1.22
☑	down	☐	16-Methylheptadecanoic acid	28000478	0.55	0.75	0.75	0.59	0.55	0.59	0.67	0.85	0.56	0.61	0.59	0.54	0.54	0.51	0.55	0.62	0.49	0.62	0.55	0.66	0.85	0.59	0.68	0.63
☑	down	☐	17-Methyloctadecanoic acid	28000479	0.48	0.57	0.77	0.5	0.57	0.5	0.63	0.6	0.47	0.54	0.54	0.55	0.39	0.35	0.45	0.53	0.57	0.67	0.78	0.64	0.69	0.76	0.62	0.78
☑	up	☐	Threonic acid	38000083	1.23	1.3	1.3	1.46	1.67	1.61	1.64	1.5	1.18	1.62	1.92	1.52	1.61	1.45	1.44	1.44	1.81	1.89	1.2	1.53	1.74	1.59	1.23	1.12
☑	down	☐	Cytosine (Σ)	38000441	0.87	0.86	1.0	0.79	0.79	0.87	0.69	0.7	0.79	0.78	0.74	0.82	0.7	0.71	0.7	0.67	0.88	0.71	0.77	0.63	0.79	0.86	0.84	0.81
☑	down	☐	Phosphatidylcholine No 04 (... (Σ)	68000020	0.67	0.59	0.68	0.71	0.64	0.76	0.84	0.8	1.0	0.69	0.66	0.58	0.62	1.03	0.67	1.19	0.94	0.8	0.71	1.2	0.84	0.75	0.72	0.79

ShowSelected Metabolites | Find Compounds | Save Metabolite List

Toxicology | Yield | release notes | preferences | help | logout

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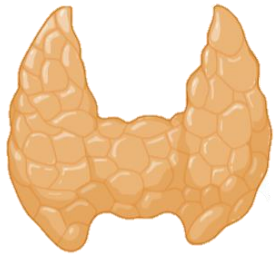
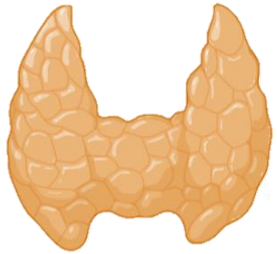
about MetaMap



Thyroid: Direct Effect: ETU & PTU

Select	Direction	Method	Grade	Metabolite	MET_CHEM_ID	Ethylenethiourea (MOA58)			6-Propyl-2-thiouracil (MOA24)		
						mh7	mh14	mh28	mh7	mh14	mh28
☐	up	LC lipid	SQ	alpha-Tocopherol	18000217	1.74	1.35	1.5	1.04	1.27	1.12
☐	down	LC lipid	SQ	18-Hydroxycorticosterone	18000285	1.0	0.73	0.35	1.46	0.45	0.54
☐	down	LC lipid	SQ	18-Hydroxycorticosterone	18000288	1.01	0.59	0.33	1.53	0.44	0.46
☐	down	LC polar	SQ	3-Hydroxybutyric acid	18000293	1.08	0.8	0.74	1.0	0.81	0.77
☐	down	LC polar	SQ	Thyroxine	18000309	0.28	0.28	0.71	0.07	0.07	0.08
☐	up	GC lipid	SQ	alpha-Tocopherol	28000018	2.59	2.52	3.26	1.18	1.18	1.14
☐	up	GC lipid	SQ	Cholesterol	28000019	1.97	1.61	2.28	1.3	1.0	1.01
☐	up	GC lipid	SQ	Lignoceric acid (C24:0)	28000052	2.04	1.56	1.98	1.29	1.22	1.2
☐	up	GC lipid	SQ	Campesterol	28000053	1.67	1.39	1.78	1.04	1.19	1.34
☐	up	GC lipid	SQ	Behenic acid (C22:0)	28000152	2.04	1.74	2.04	1.07	1.01	1.44
☐	up	GC lipid	SQ	Nervonic acid (C24:1)	28000159	2.07	1.61	2.35	1.03	1.02	1.21
☐	down	GC lipid	SQ	16-Methylheptadecanoic acid	28000478	0.73	0.84	0.77	0.85	0.61	0.5
☐	up	GC lipid	SQ	putative Eicosatrienoic acid ME (C20:3 ME)	28000482	3.87	2.09	3.01	1.0	1.45	1.47
☐	up	GC lipid	NM	Sphingolipids	28000489	2.77	2.28	3.24	1.52	1.44	1.88
☐	up	GC lipid	NM	Sphingolipids	28000491	2.42	1.94	2.03	1.34	1.29	1.5
☐	up	GC lipid	NM	Sphingolipids	28000494	2.75	2.59	2.76	1.5	1.39	1.91
☐	up	GC lipid	SQ	Sphingolipids	28000495	2.3	2.24	2.08	1.46	1.32	1.47
☐	up	GC lipid	SQ	Cholesterol	28000503	2.49	1.99	2.5	1.27	1.34	1.16
☐	up	GC lipid	SQ	Cholesterol	28000504	1.52	2.07	2.5	1.3	1.03	1.16
☐	down	GC polar	SQ	Citrate	38000012	0.93	0.79	0.64	0.89	0.74	0.64
☐	up	GC polar	SQ	Tyrosine	38000160	1.17	1.2	1.04	1.03	1.17	1.21
☐	down	GC polar	SQ	3-Hydroxybutyric acid	38000393	1.15	0.67	0.54	0.89	0.88	0.69

BASF
We create chemistry



Thyroid: Direct Effect
Found: Methimazole & Metiram

Select	Direction	Method	Grade	Metabolite	MET_CHEM_ID	Ethylenethiourea (MOA58)			6-Propyl-2-thiouracil (MOA24)			Methimazole (MOA51)			Metiram (MOA20)		
						mh7	mh14	mh28	mh7	mh14	mh28	mh7	mh14	mh28	mh7	mh14	mh28
☐	up	LC lipid	SQ	alpha-Tocopherol	18000217	1.74	1.35	1.5	1.04	1.27	1.12	2.26	3.65	2.04	1.41	NA	NA
☐	down	LC lipid	SQ	18-Hydroxycorticosterone	18000285	1.0	0.73	0.35	1.46	0.45	0.54	0.38	0.82	0.24	0.24	NA	NA
☐	down	LC lipid	SQ	18-Hydroxycorticosterone	18000288	1.01	0.59	0.33	1.53	0.44	0.46	0.48	0.89	0.24	0.24	NA	NA
☐	down	LC polar	SQ	3-Hydroxybutyric acid	18000293	1.08	0.8	0.74	1.0	0.81	0.77	0.77	0.53	0.46	0.77	NA	NA
☐	down	LC polar	SQ	Thyroxine	18000309	0.28	0.28	0.71	0.07	0.07	0.08	0.12	0.05	0.02	0.49	NA	NA
☐	up	GC lipid	SQ	alpha-Tocopherol	28000018	2.59	2.52	3.26	1.18	1.18	1.14	1.76	2.66	2.18	1.56	NA	NA
☐	up	GC lipid	SQ	Cholesterol	28000019	1.97	1.61	2.28	1.3	1.0	1.01	1.69	1.93	1.56	1.47	NA	NA
☐	up	GC lipid	SQ	Lignoceric acid (C24:0)	28000052	2.04	1.56	1.98	1.29	1.22	1.2	2.12	2.82	2.16	1.43	NA	NA
☐	up	GC lipid	SQ	Campesterol	28000053	1.67	1.39	1.78	1.04	1.19	1.34	1.43	1.6	1.46	1.09	NA	NA
☐	up	GC lipid	SQ	Behenic acid (C22:0)	28000152	2.04	1.74	2.04	1.07	1.01	1.44	2.02	3.03	3.01	1.38	NA	NA
☐	up	GC lipid	SQ	Nervonic acid (C24:1)	28000159	2.07	1.61	2.35	1.03	1.02	1.21	2.59	3.0	2.23	1.69	NA	NA
☐	down	GC lipid	SQ	16-Methylheptadecanoic acid	28000478	0.73	0.84	0.77	0.85	0.61	0.5	1.21	0.73	0.47	0.86	NA	NA
☐	up	GC lipid	SQ	putative Eicosatrienoic acid ME (C20:3 ME)	28000482	3.87	2.09	3.01	1.0	1.45	1.47	2.38	3.22	2.02	1.3	NA	NA
☐	up	GC lipid	NM	Sphingolipids	28000489	2.77	2.28	3.24	1.52	1.44	1.88	3.47	3.85	4.55	1.77	NA	NA
☐	up	GC lipid	NM	Sphingolipids	28000491	2.42	1.94	2.03	1.34	1.29	1.5	3.02	3.78	4.3	1.6	NA	NA
☐	up	GC lipid	NM	Sphingolipids	28000494	2.75	2.59	2.76	1.5	1.39	1.91	3.57	4.15	3.4	1.78	NA	NA
☐	up	GC lipid	SQ	Sphingolipids	28000495	2.3	2.24	2.08	1.46	1.32	1.47	2.92	3.71	3.47	1.63	NA	NA
☐	up	GC lipid	SQ	Cholesterol	28000503	2.49	1.99	2.5	1.27	1.34	1.16	1.86	2.33	1.65	1.29	NA	NA
☐	up	GC lipid	SQ	Cholesterol	28000504	1.52	2.07	2.5	1.3	1.03	1.16	1.92	2.12	2.25	1.41	NA	NA
☐	down	GC polar	SQ	Citrate	38000012	0.93	0.79	0.64	0.89	0.74	0.64	0.69	0.69	0.73	0.88	NA	NA
☐	up	GC polar	SQ	Tyrosine	38000160	1.17	1.2	1.04	1.03	1.17	1.21	1.3	1.44	1.24	1.09	NA	NA
☐	down	GC polar	SQ	3-Hydroxybutyric acid	38000393	1.15	0.67	0.54	0.89	0.88	0.69	0.74	0.47	0.48	0.52	NA	NA

BASF
We create chemistry

Thyroid direct: L-Thyroxine

Inverse pattern

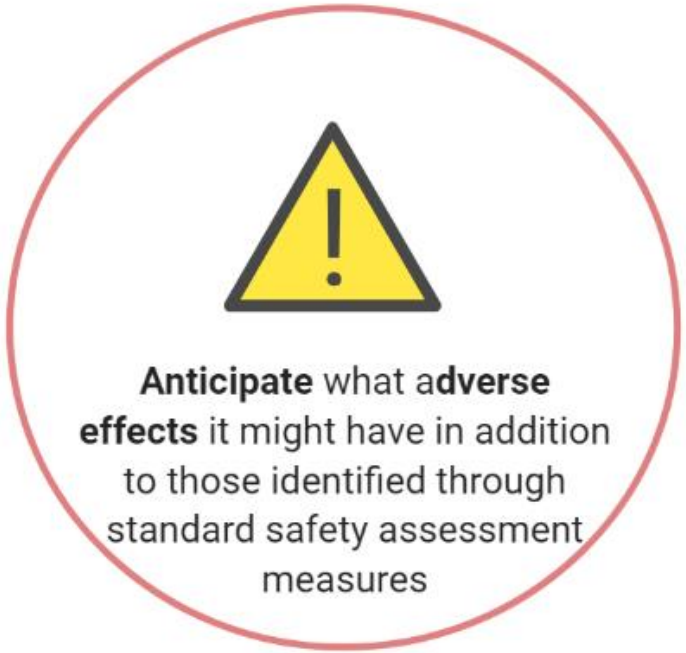
tion	Method	Grade	Metabolite	MET_CHEM_ID	Ethylenethiourea (MOA58)			6-Propyl-2-thiouracil (MOA24)			Methimazole (MOA51)			Metiram (MOA20)			L-thyroxine (MOA9)		
					mh7	mh14	mh28	mh7	mh14	mh28	mh7	mh14	mh28	mh7	mh14	mh28	mh7	mh14	mh28
	LC lipid	SQ	alpha-Tocopherol	18000217	1.74	1.35	1.5	1.04	1.27	1.12	2.26	3.65	2.04	1.41	NA	NA	0.73	0.88	0.93
	LC lipid	SQ	18-Hydroxycorticosterone	18000285	1.0	0.73	0.35	1.46	0.45	0.54	0.38	0.82	0.24	0.24	NA	NA	1.06	2.65	1.33
	LC lipid	SQ	18-Hydroxycorticosterone	18000288	1.01	0.59	0.33	1.53	0.44	0.46	0.48	0.89	0.24	0.24	NA	NA	0.86	2.71	1.09
	LC polar	SQ	3-Hydroxybutyric acid	18000293	1.08	0.8	0.74	1.0	0.81	0.77	0.77	0.53	0.46	0.77	NA	NA	0.9	1.21	0.7
	LC polar	SQ	Thyroxine	18000309	0.28	0.28	0.71	0.07	0.07	0.08	0.12	0.05	0.02	0.49	NA	NA	5.52	3.62	3.76
	GC lipid	SQ	alpha-Tocopherol	28000018	2.59	2.52	3.26	1.18	1.18	1.14	1.76	2.66	2.18	1.56	NA	NA	1.01	0.85	0.75
	GC lipid	SQ	Cholesterol	28000019	1.97	1.61	2.28	1.3	1.0	1.01	1.69	1.93	1.56	1.47	NA	NA	0.98	0.67	0.92
	GC lipid	SQ	Lignoceric acid (C24:0)	28000052	2.04	1.56	1.98	1.29	1.22	1.2	2.12	2.82	2.16	1.43	NA	NA	0.78	0.68	0.9
	GC lipid	SQ	Campesterol	28000053	1.67	1.39	1.78	1.04	1.19	1.34	1.43	1.6	1.46	1.09	NA	NA	1.21	0.83	0.91
	GC lipid	SQ	Behenic acid (C22:0)	28000152	2.04	1.74	2.04	1.07	1.01	1.44	2.02	3.03	3.01	1.38	NA	NA	0.9	0.81	1.02
	GC lipid	SQ	Nervonic acid (C24:1)	28000159	2.07	1.61	2.35	1.03	1.02	1.21	2.59	3.0	2.23	1.69	NA	NA	0.87	0.66	0.89
	GC lipid	SQ	16-Methylheptadecanoic acid	28000478	0.73	0.84	0.77	0.85	0.61	0.5	1.21	0.73	0.47	0.86	NA	NA	1.1	0.83	1.08
	GC lipid	SQ	putative Eicosa[illegible]	28000482	3.87	2.09	3.01	1.0	1.45	1.47	2.38	3.22	2.02	1.3	NA	NA	1.0	0.86	1.08
	GC lipid	NM	Sphingolipids	28000489	2.77	2.28	3.24	1.52	1.44	1.88	3.47	3.85	4.55	1.77	NA	NA	0.79	0.55	0.81
	GC lipid	NM	Sphingolipids	28000491	2.42	1.94	2.03	1.34	1.29	1.5	3.02	3.78	4.3	1.6	NA	NA	0.79	0.68	0.91
	GC lipid	NM	Sphingolipids	28000494	2.75	2.59	2.76	1.5	1.39	1.91	3.57	4.15	3.4	1.78	NA	NA	0.79	0.45	0.82
	GC lipid	SQ	Sphingolipids	28000495	2.3	2.24	2.08	1.46	1.32	1.47	2.92	3.71	3.47	1.63	NA	NA	0.79	0.58	0.82
	GC lipid	SQ	Cholesterol	28000503	2.49	1.99	2.5	1.27	1.34	1.16	1.86	2.33	1.65	1.29	NA	NA	0.87	0.58	0.64
	GC lipid	SQ	Cholesterol	28000504	1.52	2.07	2.5	1.3	1.03	1.16	1.92	2.12	2.25	1.41	NA	NA	0.85	0.66	0.83
	GC polar	SQ	Citrate	38000012	0.93	0.79	0.64	0.89	0.74	0.64	0.69	0.69	0.73	0.88	NA	NA	1.06	1.12	1.41
	GC polar	SQ	Tyrosine	38000160	1.17	1.2	1.04	1.03	1.17	1.21	1.3	1.44	1.24	1.09	NA	NA	0.99	1.01	1.13
	GC polar	SQ	3-Hydroxybutyric acid	38000393	1.15	0.67	0.64	0.89	0.88	0.69	0.74	0.47	0.48	0.62	NA	NA	1.05	1.62	1.01

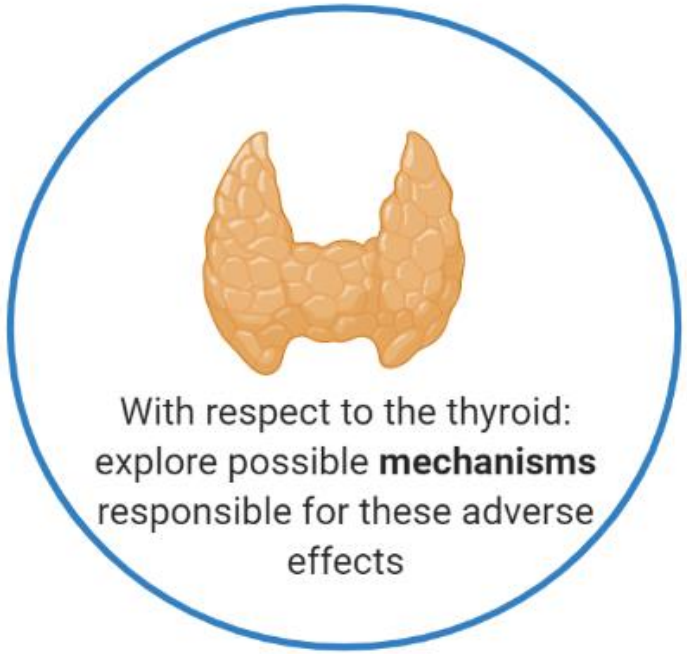
MetaMap®Tox Case Study

Thyroid Direct/Indirect Effects

Objectives

Applying the unique strengths of MetaMap®Tox for drug development





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MetaMap®Tox Case Study
Thyroid Indirect Effects

Metabolite Profile vs. “Thyroid Indirect”

Submit parameters | Reset parameters

Find Metabolites | ShowAll Metabolites | Export Table to Excel | Legend: decreased | no significant changes | increased

ShowSelected Metabolites | Find Compounds | Save Metabolite List

Select All | Select None

Save Metabolite List

Select	Direction	Anchor	Metabolite	MET_CHEM_ID	Amiodarone (MOA33)			2-Methylimidazole (MOA16)			Aroclor 1254 (MOA61)			Beta-ionone (MOA4)			Boscalid (MOA11)			
					fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fl
☑	up	☐	Palmitic acid (C16:0)		1.29	1.18	1.25	1.21	1.3	1.44	1.16	1.27	1.19	1.73	1.61	1.79	1.23	1.19	1.08	1.
☑	up	☐	Linoleic acid (C18:cis[9,12]2)		1.35	1.11	1.06	1.19	1.27	1.21	1.34	1.34	1.54	1.83	1.81	2.0	1.33	1.35	1.27	1.
☑	up	☐	Stearic acid (C18:0)		1.27	1.25	1.26	0.99	1.02	1.17	1.34	1.54	1.91	1.92	1.91	2.03	1.71	1.61	1.56	0.
☑	up	☐	Arachidonic acid (C20:cis[5...		1.46	1.37	1.41	1.04	1.2	1.36	1.25	1.48	1.53	2.08	1.94	2.29	1.76	1.6	1.53	0.
☑	up	☐	Docosahexaenoic acid (C22:c...		1.56	1.39	1.21	1.37	1.38	1.27	1.22	1.69	1.74	2.68	2.33	2.45	1.59	1.4	1.33	1.
☑	up	☐	Cholesterol, total		1.47	1.5	1.58	1.14	1.41	1.55	1.43	1.45	1.53	2.35	2.22	2.73	1.74	1.47	1.47	1
☑	up	☐	Glycerol phosphate, lipid f...		1.41	1.32	1.42	1.21	1.29	1.42	1.31	1.68	1.89	2.47	2.0	2.69	1.64	1.52	1.37	0.
☑	up	☐	Dodecanol		1.81	1.72	1.25	1.02	1.11	1.18	1.32	1.13	1.25	1.71	1.52	1.64	1.24	1.73	1.59	1.
☑	up	☐	Heptadecanoic acid (C17:0)		1.28	1.16	1.15	1.18	1.18	1.41	1.42	1.07	1.44	1.96	1.45	2.05	1.32	1.44	1.22	0.
☑	up	☐	Eicosanoic acid (C20:0)		0.93	1.09	1.22	1.12	1.35	1.2	2.01	2.6	1.49	2.3	2.0	2.42	1.78	1.07	1.65	0.
☑	up	☐	myo-Inositol-2-phosphate, I...		1.43	1.48	1.24	1.68	1.63	1.66	2.11	1.77	1.26	5.39	3.32	5.23	2.13	2.36	1.9	1.
☑	up	☐	Behenic acid (C22:0)		1.36	1.16	1.47	1.35	1.27	1.36	1.71	1.55	1.25	2.65	2.71	3.02	1.83	1.45	1.68	1
☑	up	☐	Phytosphingosine, total		1.32	1.3	1.49	1.28	1.66	1.91	1.84	2.29	1.96	2.24	2.02	2.64	1.49	1.62	1.58	0.
☑	up	☐	Nervonic acid (C24:cis[15]1)		1.78	1.86	1.43	1.11	1.52	1.74	1.2	1.13	1.31	4.26	3.98	4.37	2.34	1.77	2.08	1.
☑	up	☐	gamma-Linolenic acid (C18:c...		1.56	1.26	1.3	0.83	1.55	1.96	1.62	1.34	1.37	2.55	3.06	3.02	1.24	1.69	1.43	0.
☑	up	☐	dihomo-gamma-Linolenic acid...		1.41	1.08	1.16	0.88	1.42	1.26	1.71	1.56	2.35	2.49	2.5	2.39	1.7	1.81	1.57	1.
☑	up	☐	3-O-Methylsphingosine (Σ)		1.6	1.8	1.72	1.5	1.63	1.94	1.22	2.1	1.68	4.47	4.52	4.42	2.15	2.05	2.19	1.
☑	up	☐	threo-Sphingosine (Σ)		1.34	1.52	1.45	1.33	1.62	1.76	1.32	1.59	1.72	3.31	3.42	3.76	2.0	1.95	1.94	1.
☑	up	☐	5-O-Methylsphingosine (Σ)		1.51	1.91	1.64	1.38	1.62	1.79	1.26	2.31	1.58	4.84	4.39	4.41	2.63	2.05	2.33	1.
☑	up	☐	erythro-Sphingosine (Σ)		1.52	1.71	1.46	1.38	1.61	1.68	1.16	1.91	1.37	3.77	3.66	3.51	2.22	1.81	2.19	1.
☑	up	☐	Cholesterol, total		1.91	1.54	1.75	1.0	1.33	1.61	1.38	1.94	1.55	3.27	2.75	3.95	1.84	1.77	1.55	1.
☑	up	☐	Cholesterol, total		1.67	1.6	1.7	1.19	1.36	1.54	1.46	1.6	1.45	2.84	2.39	3.33	2.44	1.82	1.97	1.
☑	up	☐	Cholesterol, total		1.54	1.32	1.71	1.21	1.39	1.59	1.51	1.63	1.46	2.74	2.54	3.02	2.04	1.58	1.62	1

This display compares the pattern of metabolites with altered levels for Amiodarone with that of known liver enzyme inducers (i.e., an *indirect effect)*

➔ Profile Comparison – ranking with reference compounds based on similarity of metabolite profiles

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MetaMap®Tox Case Study
Thyroid Direct Effects

Metabolite Profile vs. “Thyroid Direct”

Submit parameters | Reset parameters

Find Metabolites | ShowAll Metabolites | Export Table to Excel | Legend: decreased | no significant changes | increased

ShowSelected Metabolites | Find Compounds | Save Metabolite List

Select All | Select None

Select	Direction	Anchor	Metabolite	MET_CHEM_ID	Amiodarone (MOA33)			6-Propyl-2-thiouracil (MOA24)			Methimazole (MOA51)		
					fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28
☑	down	☐	Thyroxine (T4)	18000309	3.35	0.93	1.45	0.66	0.12	0.35	0.22	0.26	0.1
☑	up	☐	Docosahexaenoic acid (C22:c...	28000015	1.56	1.39	1.21	1.27	1.11	0.83	2.07	1.37	1.24
☑	up	☐	Tricosanoic acid (C23:0)	28000072	1.48	1.4	1.52	1.45	1.31	1.36	2.05	2.55	1.96
☑	up	☐	Behenic acid (C22:0)	28000152	1.36	1.16	1.47	1.33	1.28	1.2	2.17	2.72	2.01
☑	up	☐	threo-Sphingosine (Σ)	28000491	1.34	1.52	1.45	1.36	1.36	1.33	2.48	3.19	2.41
☑	up	☐	5-O-Methylsphingosine (Σ)	28000494	1.51	1.91	1.64	1.4	1.36	1.22	2.86	4.18	3.07
☑	up	☐	erythro-Sphingosine (Σ)	28000495	1.52	1.71	1.46	1.47	1.28	1.24	2.56	3.22	2.43
☑	down	☐	Pyruvate (Σ)	38000002	0.68	0.6	0.53	0.88	0.65	0.77	0.6	0.56	0.78
☑	up	☐	Glycine	38000004	1.0	1.02	0.93	1.13	1.08	1.05	1.14	1.14	1.28
☑	down	☐	Citrate (Σ)	38000012	0.96	0.89	0.82	0.87	0.69	0.66	0.83	0.76	0.84
☑	down	☐	Ketoleucine	58000021	0.73	0.91	0.81	1.05	0.8	0.89	0.86	0.8	0.78
☑	up	☐	Lysophosphatidylcholine (C1...	68000001	1.05	1.06	1.0	1.07	1.31	1.26	1.52	1.57	1.31
☑	up	☐	Sphingomyelin (d18:1,C16:0)	68000008	1.34	1.28	1.29	1.01	1.46	1.62	1.89	2.54	1.77
☑	up	☐	Phosphatidylcholine (C16:1,...	68000010	1.32	1.36	1.06	1.49	1.37	1.09	1.89	2.4	2.21
☑	up	☐	Phosphatidylcholine (C18:2,... (Σ)	68000011	1.18	1.18	1.31	1.2	1.17	1.17	1.39	1.25	1.33
☑	up	☐	Sphingomyelin (d18:1,C16:0)	68000046	1.2	1.15	1.09	1.26	1.33	1.29	1.45	1.44	1.31
☑	up	☐	Sphingomyelin (d18:1,C24:0)	68000054	1.21	1.11	1.35	1.22	1.42	1.08	1.54	1.8	1.63

ShowSelected Metabolites | Find Compounds | Save Metabolite List

This display compares the pattern of metabolites with altered levels for Amiodarone with that of drugs known to directly alter thyroid function

➔ Profile Comparison – ranking with reference compounds based on similarity of metabolite profiles

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Thyroid Direct Effects

Metabolite Profile vs. “Thyroid Direct”

Submit parameters | Reset parameters

Find Metabolites | ShowAll Metabolites | Export Table to Excel | Legend: decreased | no significant changes | increased

ShowSelected Metabolites | Find Compounds | Save Metabolite List

Select All | Select None

Select	Direction	Anchor	Metabolite	MET_CHEM_ID	Amiodarone (MOA33)			6-Propyl-2-thiouracil (MOA24)			Methimazole (MOA51)		
					fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28
☑	down	☐	Thyroxine (T4)	18000309	3.35	0.93	1.45	0.66	0.12	0.35	0.22	0.26	0.1
☑	up	☐	Docosahexaenoic acid (C22:c...	28000015	1.56	1.39	1.21	1.27	1.11	0.83	2.07	1.37	1.24
☑	up	☐	Tricosanoic acid (C23:0)	28000072	1.48	1.4	1.52	1.45	1.31	1.36	2.05	2.55	1.96
☑	up	☐	Behenic acid (C22:0)	28000152	1.36	1.16	1.47	1.33	1.28	1.2	2.17	2.72	2.01
☑	up	☐	threo-Sphingosine (Σ)	28000491	1.34	1.52	1.45	1.36	1.36	1.33	2.48	3.19	2.41
☑	up	☐	5-O-Methylsphingosine (Σ)	28000494	1.51	1.91	1.64	1.4	1.36	1.22	2.86	4.18	3.07
☑	up	☐	erythro-Sphingosine (Σ)	28000495	1.52	1.71	1.46	1.47	1.28	1.24	2.56	3.22	2.43
☑	down	☐	Pyruvate (Σ)	38000002	0.68	0.6	0.53	0.88	0.65	0.77	0.6	0.56	0.78
☑	up	☐	Glycine	38000004	1.0	1.02	0.93	1.13	1.08	1.05	1.14	1.14	1.28
☑	down	☐	Citrate (Σ)	38000012	0.96	0.89	0.82	0.87	0.69	0.66	0.83	0.76	0.84
☑	down	☐	Ketoleucine	58000021	0.73	0.91	0.81	1.05	0.8	0.89	0.86	0.8	0.78
☑	up	☐	Lysophosphatidylcholine (C1...	68000001	1.05	1.06	1.0	1.07	1.31	1.26	1.52	1.57	1.31
☑	up	☐	Sphingomyelin (d18:1,C16:0)	68000008	1.34	1.28	1.29	1.01	1.46	1.62	1.89	2.54	1.77
☑	up	☐	Phosphatidylcholine (C16:1,...	68000010	1.32	1.36	1.06	1.49	1.37	1.09	1.89	2.4	2.21
☑	up	☐	Phosphatidylcholine (C18:2,... (Σ)	68000011	1.18	1.18	1.31	1.2	1.17	1.17	1.39	1.25	1.33
☑	up	☐	Sphingomyelin (d18:1,C16:0)	68000046	1.2	1.15	1.09	1.26	1.33	1.29	1.45	1.44	1.31
☑	up	☐	Sphingomyelin (d18:1,C24:0)	68000054	1.21	1.11	1.35	1.22	1.42	1.08	1.54	1.8	1.63

ShowSelected Metabolites | Find Compounds | Save Metabolite List

This display compares the pattern of metabolites with altered levels for Amiodarone with that of drugs known to directly alter thyroid function

Note, however, that Amiodarone increases the level of the thyroid hormone thyroxine

➔ Profile Comparison – ranking with reference compounds based on similarity of metabolite profiles

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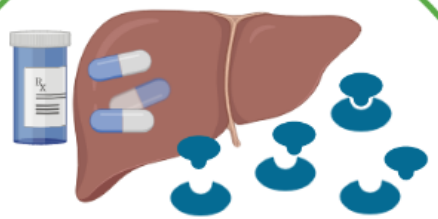
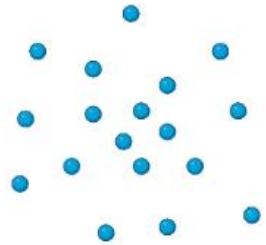
MetaMap®Tox Case Study

Thyroid Direct/Indirect Effects

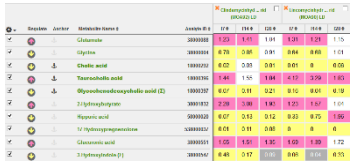
Conclusions



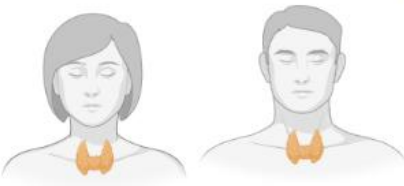
Amiodarone induces liver enzyme activities in rats with resulting indirect thyroid effects



Amiodarone may also have direct effects on thyroid hormone function



Based on metabolome data two possible MoA for direct effects were proposed (inhibition of deionidase was later confirmed by mechanistic investigations)



In clinical studies thyroid effects in humans were observed *(Cohen-Lehman, J. et al. Nat Rev Endocrinol, 2010)*

➔ Thus the clinical observations could have been anticipated using MetaMap®Tox

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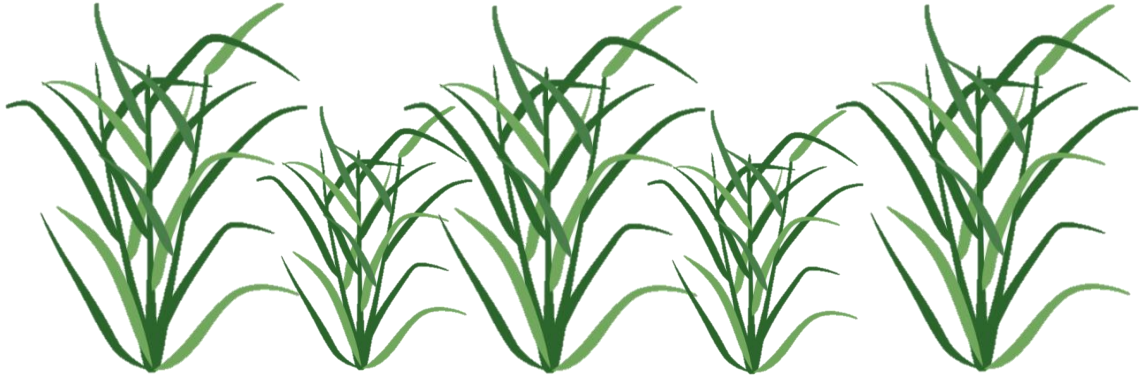
HPPD inhibitors: metabolites connection

			BAS 6H HD			Meso HD			NTBC HD			Top HD		
Metabolite	Direction	Subclass	m7	m14	m28	m7	m14	m28	m7	m14	m28	m7	m14	m28
Threonine	up	Amino acids, neutral	**1,16**	**1,20**	**1,50**	**1,55**	**1,55**	**1,49**	**1,36**	**1,51**	**1,54**	**1,29**	**1,17**	**1,35**
Citrulline	down	Urea cycle and related	0,73	0,72	0,80	0,83	0,70	0,68	0,84	0,88	0,83	0,72	0,72	0,80
Phenylalanine	down	Amino acids, aromatic	0,79	0,90	0,82	0,82	0,87	0,87	0,78	0,77	1,02	0,86	0,79	0,89
Tyrosine	up	Amino acids, aromatic	**8,98**	**7,65**	**10,02**	**22,33**	**22,69**	**24,73**	**25,56**	**27,19**	**28,32**	**23,03**	**21,00**	**26,70**
4-Hydroxyphenylpyruvate	up	Tyrosine metabolism	**31,22**	**34,78**	**25,05**	535,51	313,40	NA	**364,14**	**435,77**	**366,39**	NA	995,98	1139,71
Threonine	up	Amino acids, neutral	**1,23**	**1,19**	1,39	**1,47**	**1,35**	**1,42**	**1,33**	**1,52**	**1,47**	**1,32**	**1,19**	**1,38**
5-Oxoproline	down	Amino acid metabolites	0,50	0,58	0,54	0,56	0,54	0,55	0,60	0,61	0,60	0,50	0,56	0,52
Lysine	up	Amino acids, basic	**1,23**	**1,30**	**1,31**	**1,88**	**1,65**	**1,76**	1,12	**1,40**	**1,46**	**1,25**	**1,21**	**1,36**
Glutamine	down	Amino acids, basic	0,41	0,44	0,41	0,44	0,43	0,43	0,55	0,57	0,58	0,41	0,44	0,41
Phenylalanine	down	Amino acids, aromatic	0,90	0,83	0,83	0,88	0,92	0,83	0,78	0,78	0,94	0,86	0,88	0,84
Tyrosine	up	Amino acids, aromatic	**21,12**	**18,86**	**14,45**	**44,31**	**28,31**	**34,44**	**56,14**	**58,08**	**52,56**	**48,44**	**34,20**	**35,17**



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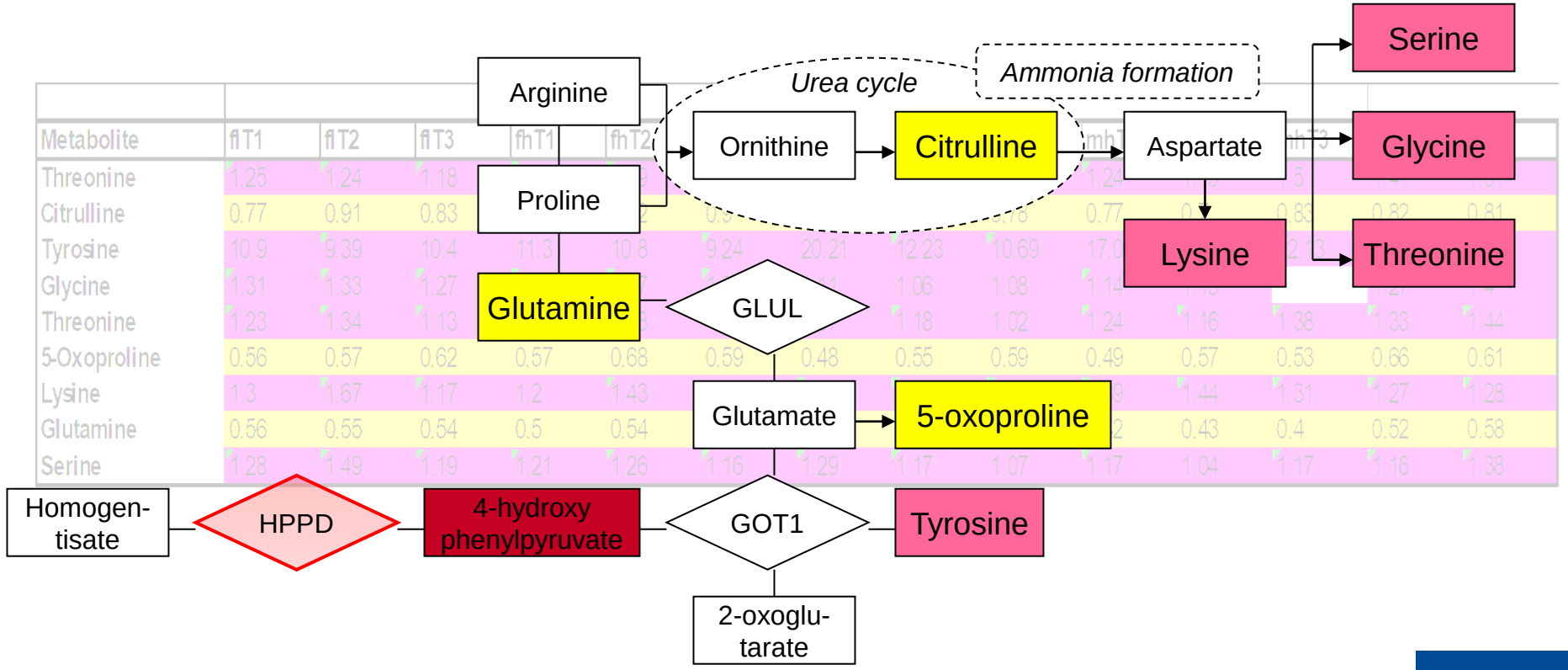
HPPD inhibitors: metabolites connection



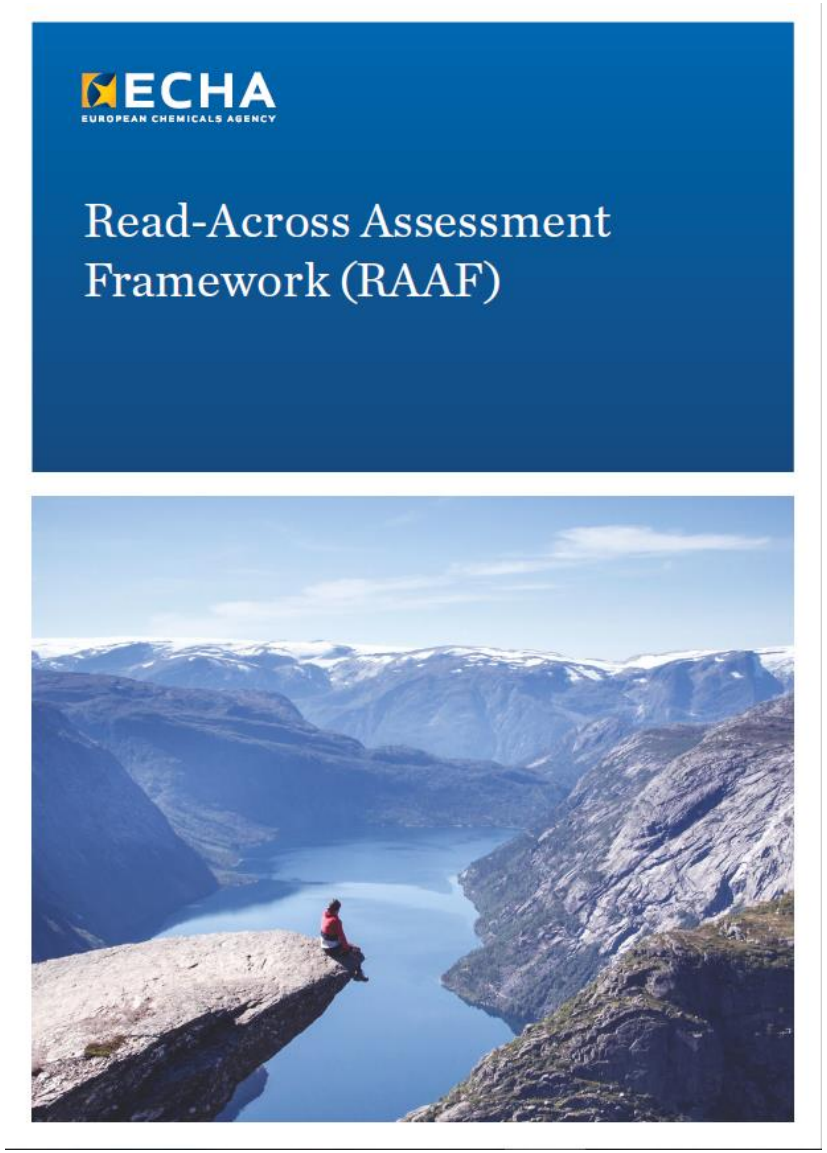
Profile Comparison: the entire metabolome of compound X is compared with that of 1000 other substances.

Here X = MCPA

	Pearson		Spearman		Norm. vectorprodcut	
	r	rank	r	rank	r	rank
MCPA	1	1	1	1	1	1
MCPA [Han:Rcc:WIST(SPF)]	0.821	2	0.831	2	0.775	3
2,4-D (MOA22)	0.813	3	0.779	5	0.799	2
MCPA [F-344/Crl]	0.807	4	0.764	6	0.735	5
MCPA [Crl:WI(Han)]	0.787	5	0.789	4	0.768	4
MCPA [Crl:CD(R) (Sprague Dawly)]	0.784	6	0.791	3	0.733	6
Dichlorprop-p	0.724	7	0.633	7	0.727	7
Mecoprop-p	0.709	8	0.624	8	0.706	8
Pentachlorophenol	0.572	9	0.529	9	0.588	9
Fenofibrate	0.556	10	0.422	15	0.535	10
Mecoprop-p	0.513	11	0.449	13	0.448	12
Mecoprop-p FyAn	0.498	12	0.503	10	0.392	18
Mecoprop-p FyAy	0.49	13	0.389	20	0.453	11
Probenecid	0.486	14	0.393	18	0.406	16
Clofibrate	0.457	15	0.423	16	0.424	15
Dicamba	0.454	16	0.486	12	0.434	14

Read across

"... read-across is regarded as a technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s) (source substance(s))."

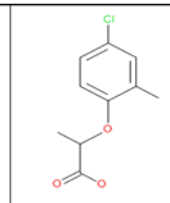
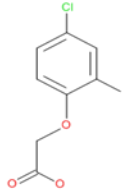
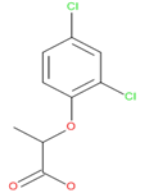
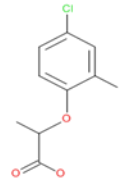
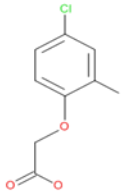
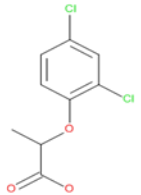


Case Study Phenoxy herbicides

- Target Substance:
  MCPP (Mecoprop-P)
- Source Substancen:
  2,4-DP (Dichlorprop-P)
  MCPA
- Structurally similar

is read-across possible ?
which is the best source compound ?

Tanimoto scores

		 MCPP	 MCPA	 2,4-DP
	MCPP		**75.0%**	**96.0%**
	MCPA	**75.0%**		**77.4%**
	2,4-DP	**96.0%**	**77.4%**	

Case Study Phenoxy herbicides

Metabolite	2,4-DP			MCPA			MCPP		
	m7	m14	m28	m7	m14	m28	m7	m14	m28
16-Methylheptadecanoic acid	0,24	0,31	0,41	0,23	0,33	0,18	0,23	0,25	0,21
17-Methyloctadecanoic acid	0,22	0,34	0,30	0,29	0,35	0,20	0,16	0,24	0,16
3-Hydroxyindole	3,70	3,54	3,94	1,95	2,58	2,93	2,59	2,56	1,94
Arachidonic acid (C20:cis[5,8,11,14]4)	0,20	0,29	0,41	0,27	0,42	0,26	0,28	0,34	0,26
Arginine	0,74	0,80	0,68	0,79	0,73	0,76	0,78	0,82	0,67
Asparagine	0,62	0,74	0,66	0,75	0,59	0,74	0,74	0,72	0,72
Cholesterylester C20:4	0,21	0,21	0,35	0,57	0,29	0,33	0,29	0,33	0,44
Cytosine	0,44	0,62	0,69	0,63	0,60	0,60	0,73	0,73	0,66
dihomo-gamma-Linolenic acid (C20:cis[8,11,14]3)	3,67	3,48	2,79	3,87	6,34	8,21	2,58	2,99	3,44
Docosahexaenoic acid (C22:cis[4,7,10,13,16,19]6)	0,15	0,21	0,23	0,15	0,20	0,09	0,17	0,24	0,15
Docosapentaenoic acid (C22:cis[7,10,13,16,19]5)	0,23	0,21	0,16	0,15	0,25	0,13	0,20	0,30	0,21
Glucuronic acid	6,79	5,82	3,32	3,06	2,88	3,87	4,49	3,48	2,27
Ketoleucine	0,57	0,62	0,62	0,39	0,26	0,34	0,72	0,79	0,57
Lysine	0,44	0,52	0,56	0,40	0,30	0,33	0,57	0,60	0,50
Lyso PE (C22:0) (putative)	0,24	0,21	0,28	0,38	0,28	0,29	0,20	0,20	0,18
Lysophosphatidylcholine (C17:0)	0,43	0,35	0,35	0,59	0,54	0,35	0,43	0,34	0,24
Lysophosphatidylcholine (C18:0)	0,77	0,78	0,83	0,81	0,83	0,73	0,75	0,78	0,77
Lysophosphatidylcholine (C18:2)	1,28	1,47	1,05	1,54	1,40	1,39	1,38	1,40	1,24
Methionine	0,76	0,73	0,81	0,66	0,59	0,64	0,72	0,82	0,80
PC No 04 (putative)	0,28	0,37	0,30	0,42	0,44	0,36	0,30	0,40	0,34
Phosphatidylcholine (C16:0,C20:4)	0,71	0,74	0,77	0,63	0,80	0,62	0,62	0,67	0,64
Phosphatidylcholine (C16:0,C20:5)	1,48	1,51	1,19	1,73	1,82	2,11	1,43	1,20	1,22
Phosphatidylcholine (C16:0,C22:6)	0,46	0,44	0,50	0,37	0,45	0,34	0,40	0,39	0,38
Phosphatidylcholine (C18:0,C20:3)	0,53	0,46	0,53	0,49	0,82	0,48	0,37	0,47	0,38
Phosphatidylcholine (C18:0,C20:4)	0,36	0,40	0,51	0,36	0,55	0,24	0,32	0,41	0,38
Phosphatidylcholine (C18:0,C22:6)	0,34	0,38	0,41	0,30	0,30	0,18	0,29	0,33	0,30
Phosphatidylcholine No 02	0,43	0,37	0,39	0,53	0,56	0,51	0,41	0,41	0,35
Proline	0,69	0,72	0,77	0,63	0,51	0,52	0,66	0,72	0,64
Pseudouridine	1,14	1,58	1,39	1,31	1,49	1,41	1,17	1,43	1,32
Stearic acid (C18:0)	0,34	0,50	0,45	0,48	0,67	0,43	0,36	0,39	0,38
TAG (putative)	0,64	0,54	0,46	0,35	0,59	0,36	0,32	0,35	0,40
Threonine	0,56	0,68	0,82	0,68	0,63	0,69	0,65	0,68	0,77
Tryptophan	0,21	0,24	0,45	0,20	0,19	0,18	0,33	0,50	0,49
Unknown lipid (68000033)	0,58	0,56	0,67	0,45	0,49	0,42	0,57	0,54	0,56
Unknown lipid (68000034)	0,37	0,30	0,38	0,31	0,26	0,22	0,39	0,38	0,33
Unknown lipid (68000052)	0,31	0,33	0,48	0,31	0,42	0,22	0,29	0,31	0,29

Mode of action	2,4-DP	MCPA	MCPP
Liver peroxisome proliferation			
Liver fibrate phthalate and phenoxy			
Reduced feed consumption	-		
Kidney inhibition weak org. acids			
Phthalates long chain			
Liver PPAR alpha agonist			
Liver oxidative stress		-	

Match
Weak Match
Equivocal
Missmatch

- Very good overlap of metabolic profiles
- Common target organs: Liver & Kidney

Case Study Phenoxy herbicides

a)

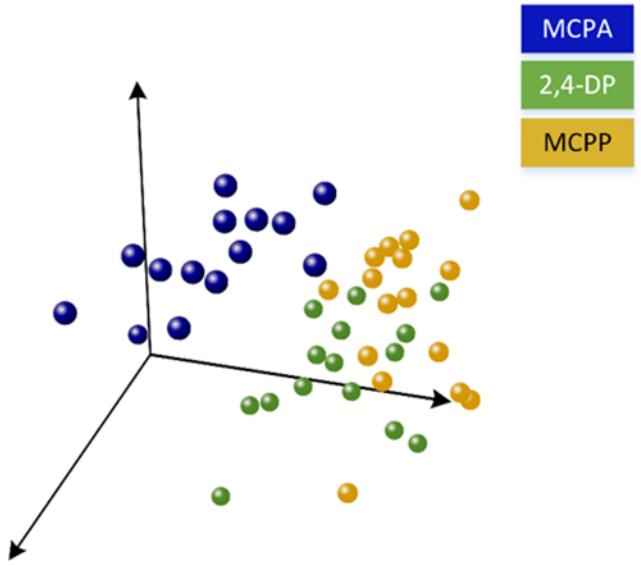
Metabolite	MCPP			2,4-DP			MCPA		
	m7	m14	m28	m7	m14	m28	m7	m14	m28
3-Indoxylsulfate	4,14	2,10	3,00	5,58	3,28	3,25	0,72	1,53	1,92
3-Methoxytyrosine	1,33	1,35	1,76	1,22	1,32	1,35	1,08	1,19	1,84
alpha-Tocopherol	0,56	0,65	0,59	0,70	0,63	0,68	0,93	1,09	0,98
beta-Sitosterol	0,24	0,34	0,23	0,37	0,30	0,31	0,65	1,03	0,74
Campesterol	0,30	0,36	0,23	0,31	0,29	0,32	0,68	1,04	0,99
Cholesterol, total	0,44	0,50	0,45	0,38	0,48	0,52	0,67	0,90	0,73
Ethanolamine plasmalogen (C39:4)	0,49	0,54	0,52	0,62	0,52	0,48	0,72	0,84	0,72
Galactose, lipid fraction	0,52	0,51	0,56	0,62	0,45	0,65	0,65	0,90	0,86
Indole-3-acetic acid	0,49	0,65	0,64	0,31	0,41	0,63	0,52	0,90	1,18
myo-Inositol, lipid fraction	0,56	0,55	0,56	0,45	0,53	0,61	0,54	0,92	0,76
myo-Inositol-2-phosphate, lipid fraction	0,18	0,22	0,25	0,27	0,21	0,32	0,30	0,61	0,52
Myristic acid (C14:0)	0,61	0,81	0,58	0,61	0,72	0,44	0,53	0,71	0,81
Pantothenic acid	3,57	4,54	4,58	2,45	3,34	3,73	0,92	1,41	0,86
Phosphate, lipid fraction	0,64	0,74	0,67	0,64	0,69	0,62	0,75	1,01	0,80
Sphingomyelin (d18:1,C16:0)	0,75	0,85	0,76	0,76	0,80	0,75	1,27	1,26	1,33
Threonic acid	1,40	1,07	1,36	1,78	1,34	1,63	0,99	1,14	1,13
Unknown lipid (28000473)	0,23	0,27	0,21	0,17	0,32	0,30	0,50	0,77	0,60

b)

Metabolite	MCPP			MCPA			2,4-DP		
	m7	m14	m28	m7	m14	m28	m7	m14	m28
5-Oxoproline	0,98	0,81	0,78	0,66	0,69	0,69	0,97	0,99	1,03
Alanine	0,67	0,71	0,67	0,68	0,77	0,83	0,81	0,84	0,97
Deoxyribonucleic acids, total	0,81	0,82	0,70	0,94	0,87	0,77	0,50	0,78	0,72
Ethanolamine plasmalogen (C39:5)	0,52	0,56	0,50	0,57	0,67	0,60	0,69	0,29	0,60
Heptadecanoic acid (C17:0)	0,52	0,57	0,44	0,53	0,70	0,54	0,60	0,59	0,49
Isopalmitic acid (C16:0)	0,39	0,46	0,27	0,48	0,47	0,25	0,41	0,77	0,47
Tyrosine	0,74	0,89	0,76	0,87	0,77	0,87	0,89	0,94	0,89
Uracil	0,75	0,83	0,71	0,79	0,88	0,75	0,84	0,88	1,07
Uric acid	0,72	0,79	0,71	0,76	0,85	0,61	1,23	0,99	1,52

Total Profile comparison:

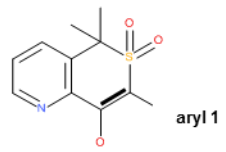
Best Match with 2,4-DP



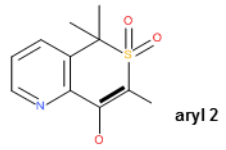
- **2,4-DP is the best read-across source substance**

van Ravenzwaay, B., Sperber, S., Lemke, O., Fabian, E., Faulhammer, F., Kamp, H., Mellert, W., Strauss, V., Strigun, A., Peter, E., Spitzer, M., Walk, T., 2016. Metabolomics as read-across tool: A case study with phenoxy herbicides. Regul. Toxicol. Pharmacol. RTP 81, 288–304.

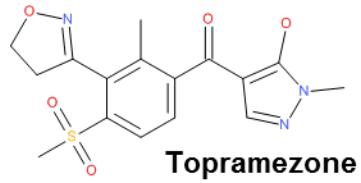
Example 2: HPPD-Inhibitors



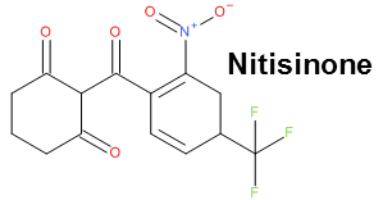
Coumarone 1



Coumarone 2



Topramezone



Reference: Topramezone, females, HD		
Treatment	Pearson correlation	
	r	rank
Topramezone, LD	0.895	1
Coumarone 4, LD	0.892	2
Nitisinone, HD	0.886	3
Topramezone, 2nd study, HD	0.886	4
Coumarone 3, LD	0.867	5
Coumarone 4, HD	0.866	6
Coumarone 3, HD	0.858	7
Nitisinone, LD	0.851	8
Coumarone 1, LD	0.862	9
Coumarone 2, LD	0.841	10

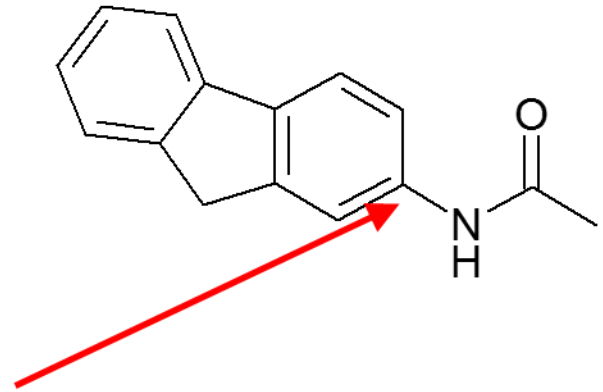
Tanimoto similarity using MACCS keys			
	Coumarone 1	Coumarone 2	Nitisinone
Coumarone 2	81.5%		
Nitisinone	48.1%	42.1%	
Topramezone	32.9%	27.8%	36.3%

Metabolite	Coumarone 1	Coumarone 2	Topramezone	Nitisinone
4-Hydroxyphenylpyruvate	148.43	209.61	317.76	293.51
5-Oxoproline	0.60	0.63	0.59	0.55
Citrulline	1.15	1.14	0.63	0.78
Glutamine	0.69	0.59	0.41	0.46
Glycine	1.34	2.22	1.30	1.33
Lysine	1.24	1.32	1.33	1.43
Methionine	1.26	1.19	1.14	1.28
Serine	1.35	1.52	1.27	1.48
Threonine	1.48	1.38	1.25	1.83
Tyrosine	35.82	42.23	44.38	50.64

BASF
We create chemistry

Example 3: 2- and 4-Acetylaminofluorene

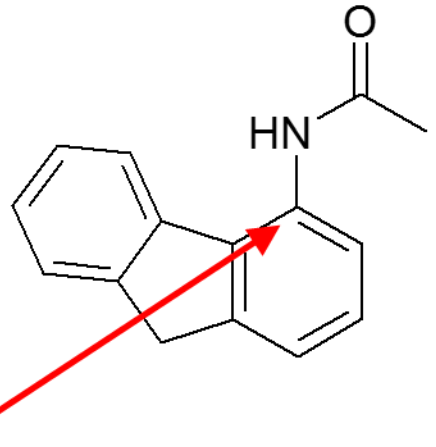
Tanimoto score: 63%



2-Acetylaminofluorene

- strong liver enzyme inducer
- liver carcinogen

- immune suppressant
- bladder carcinogen



4-Acetylaminofluorene

- **slight** liver enzyme inducer
- **no** liver carcinogen

- lipid accumulation in liver
- immune suppressant

Example 3: 2 and 4-Acetylaminofluorene Metabolome patterns

Metabolite profile compared to other **liver enzyme inducers (and liver cell carcinogens)**

Compound	2-Acetylaminofluorene			4-Acetylaminofluorene			Pentachlorobenzene			Cyproteron Acetate		
	day 7	day 14	day 28	day 7	day 14	day 28	day 7	day 14	day 28	day 7	day 14	day 28
3-O-Methylsphingosine (d18:1)	1.56	**1.36**	1.45	1.16	1.33	1.33	1.42	**1.76**	**1.74**	**3.26**	1.75	**2.49**
4-Hydroxysphinganine (t18:0, Phytosphingosine)	0.86	**1.28**	1.25	1.10	1.46	1.23	1.10	**1.40**	**1.32**	**1.99**	1.47	**1.96**
5-O-Methylsphingosine (d18:1)	**1.44**	**1.37**	**1.46**	1.21	1.47	1.40	**1.46**	**1.92**	**1.66**	**2.98**	**2.13**	**2.57**
Arachidonic acid (C20:cis[5,8,11,14]4)	**1.18**	**1.20**	1.14	1.22	1.22	1.28	**1.27**	**1.89**	**1.50**	**1.40**	0.98	**1.89**
Behenic acid (C22:0)	0.97	**1.20**	**1.39**	1.15	1.11	1.18	**1.22**	**1.64**	**1.44**	**1.70**	1.51	**2.11**
Cholesterol, total	1.19	**1.32**	1.01	**1.31**	1.25	1.38	**1.23**	**1.64**	**1.62**	**1.82**	**1.62**	**2.51**
Cholesterolester, total	**1.14**	**1.12**	**1.08**	1.10	1.13	1.09	**1.45**	**2.08**	**1.60**	**1.15**	**1.07**	**1.10**
dihomo-gamma-Linolenic acid (C20:cis[8,11,14]3)	**1.44**	1.22	1.27	1.08	1.20	1.12	**2.19**	**3.89**	**2.94**	**3.24**	**2.41**	**1.85**
Docosahexaenoic acid (C22:cis[4,7,10,13,16,19]6)	**1.14**	**1.37**	1.34	1.14	1.56	1.51	**1.73**	**2.42**	**1.59**	**1.55**	1.06	**1.81**
Dodecanol	1.03	**1.23**	**1.38**	1.50	1.02	1.18	**1.21**	**2.11**	**1.58**	**1.84**	**1.43**	**1.60**
Eicosanoic acid (C20:0)	1.03	1.17	**1.19**	0.96	1.45	1.27	1.37	**2.61**	**1.90**	1.57	**1.34**	**1.65**
erythro-Sphingosine (d18:1)	**1.56**	**1.26**	**1.34**	1.34	1.32	1.28	1.30	**1.71**	**1.62**	**2.59**	**1.85**	**2.36**
Galactose, lipid fraction	0.98	**1.12**	1.16	1.01	1.08	1.01	**1.16**	**1.54**	**1.32**	**1.26**	1.12	**1.43**
gamma-Linolenic acid (C18:cis[6,9,12]3)	1.14	**1.59**	**1.58**	1.34	**1.23**	**1.45**	1.73	**4.42**	**3.02**	**2.91**	1.15	**2.14**
Glycerol phosphate, lipid fraction	**1.40**	**1.35**	**1.28**	1.35	1.34	1.07	**1.29**	**1.81**	**1.51**	**1.93**	**1.60**	**2.01**
Glycerol, lipid fraction	**1.62**	**1.97**	**1.47**	1.20	**1.26**	1.17	**2.40**	**8.03**	**3.33**	**2.23**	**1.37**	**1.68**
Heptadecanoic acid (C17:0)	1.09	**1.31**	1.01	1.29	**1.39**	**1.34**	**1.27**	**1.81**	**1.34**	**1.65**	**1.13**	**1.63**
Lignoceric acid (C24:0)	1.07	**1.22**	**1.24**	**1.12**	1.19	1.14	1.39	**1.60**	**1.75**	1.26	1.00	**2.02**
Linoleic acid (C18:cis[9,12]2)	**1.37**	**1.45**	**1.38**	1.16	**1.24**	1.27	**2.11**	**5.23**	**2.69**	**2.96**	**2.07**	**1.70**
myo-Inositol-2-phosphate, lipid fraction	1.13	1.35	**1.21**	**1.25**	1.28	1.28	**1.86**	**3.50**	**1.81**	1.22	1.03	**1.93**
Nervonic acid (C24:cis[15]1)	1.19	1.43	**1.46**	**1.56**	1.21	1.33	0.97	**1.55**	**1.46**	**5.05**	**2.51**	**4.42**
Palmitic acid (C16:0)	**1.29**	**1.37**	**1.42**	1.21	**1.16**	1.25	**1.59**	**3.46**	**1.86**	**2.19**	**1.82**	**2.09**
Phosphate, lipid fraction	**1.19**	**1.20**	**1.14**	1.05	1.15	**1.32**	**1.29**	**1.71**	**1.33**	**1.69**	**1.37**	**1.64**
Phosphatidylcholine (C18:0,C18:1)	**1.08**	1.18	**1.31**	**1.11**	**1.12**	1.05	**1.28**	**1.72**	**1.51**	**1.72**	**1.24**	**1.28**
Phosphatidylcholine (C18:1,C18:2)	**1.09**	1.10	**1.20**	1.02	1.01	1.05	1.20	**1.26**	1.13	**1.40**	**1.13**	**1.27**
Sphingomyelin (d18:1,C16:0)	1.00	1.02	**1.03**	1.06	1.05	1.02	1.11	**1.18**	**1.20**	**1.10**	**1.09**	1.08
Sphingomyelin (d18:1,C24:0)	**1.07**	1.13	**1.23**	1.10	1.06	1.03	1.08	**1.33**	1.07	**1.28**	0.97	1.12
Stearic acid (C18:0)	**1.16**	**1.19**	**1.15**	1.24	1.23	**1.25**	**1.30**	**1.87**	**1.65**	**1.21**	0.93	**1.52**
threo-Sphingosine (d18:1)	**1.24**	**1.22**	**1.43**	1.06	1.28	1.31	1.28	**1.44**	**1.43**	**1.85**	**1.64**	**2.27**
Tricosanoic acid (C23:0)	1.02	**1.20**	1.35	**1.22**	**1.39**	1.39	1.18	**1.46**	**1.45**	0.95	0.56	**1.50**

2-AAF has a very low overall match with 4-AAF: rank 1443

Thank you very much for your attention

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