Use of metabolomics for Read-Across

Prof. Dr. Bennard van Ravenzwaay

Internal

What is the Metabolome?

"The metabolome refers to the complete set of small-molecule chemicals found within a biological sample."

- Amino acids
- Lipids and fatty acids
- Carbohydrates and sugars
- Organic acids
- Hormones
- Vitamines und cofactors

Making sense of the soup

Oliver, SG; Winson MK; Kell DB; Baganz F (1998). "Systematic functional analysis of the yeast genome". Trends Biotechnol. 16 (9): 373–8
Kell, D.B., 2004. Metabolomics and systems biology: making sense of the soup. Curr Opin Microbiol 7, 296–307.

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Why Metabolomics / Metabolic Profiling ?

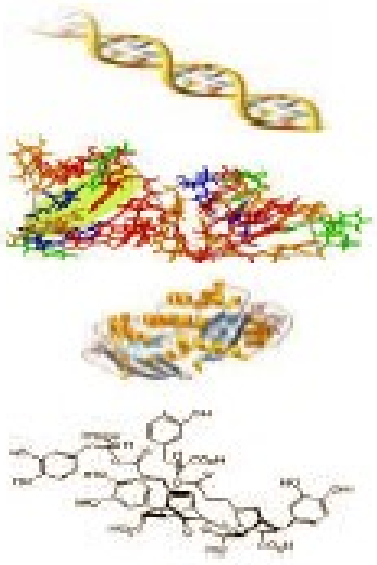
Mostly unknown

DNA
40,000 genes

RNA
150,000 transcripts

Proteins
1,000,000 proteins

Metabolites
2,500 metabolites



Genomics

Transcriptomics

Proteomics

Metabolomics

Mostly known

Advantage
Single matrix (blood)

Closer to classical toxicology

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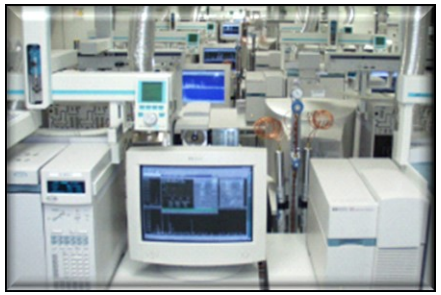
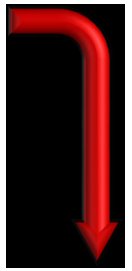
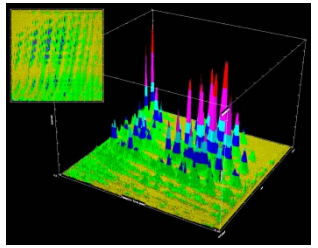
The Use of MetaMap®Tox

BLOOD PROFILING



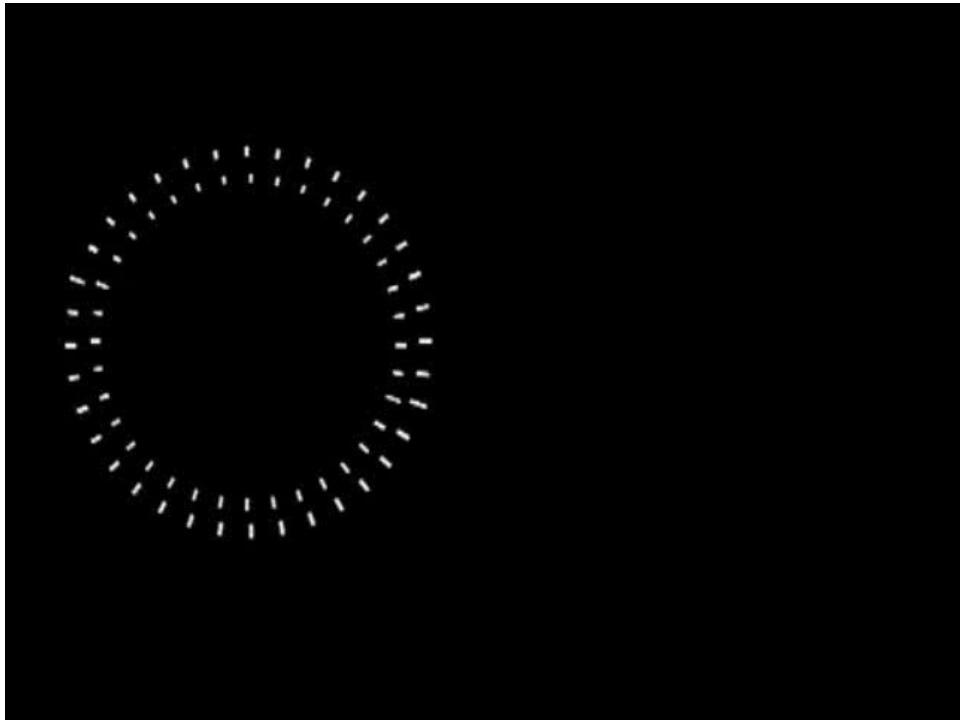
LC-MS/ GC-MS





Total Metabolome Signature (9000 analyte signals)

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...

SAMPLE **REFERENCE** **300 Known Metabolites**

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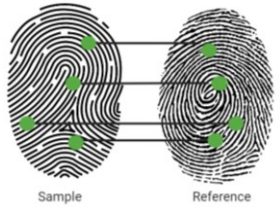
MetaMap®Tox: Reduction through Refinement
Blood metabolome analysis in short-term studies

Mode of action determination

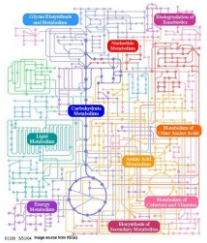
> 110 MoA patterns established

Compound Metabolome Comparison



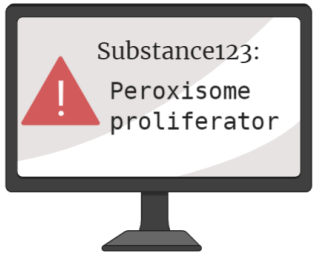
Ranking of (new) compound's metabolome against metabolome of 1000 substances

Biochemical pathway analysis



Identify molecular mechanisms of toxicity

Predictivity rate: 80%



Sensitivity: as classical toxicology

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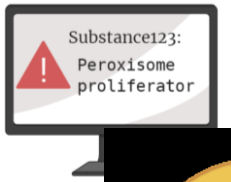
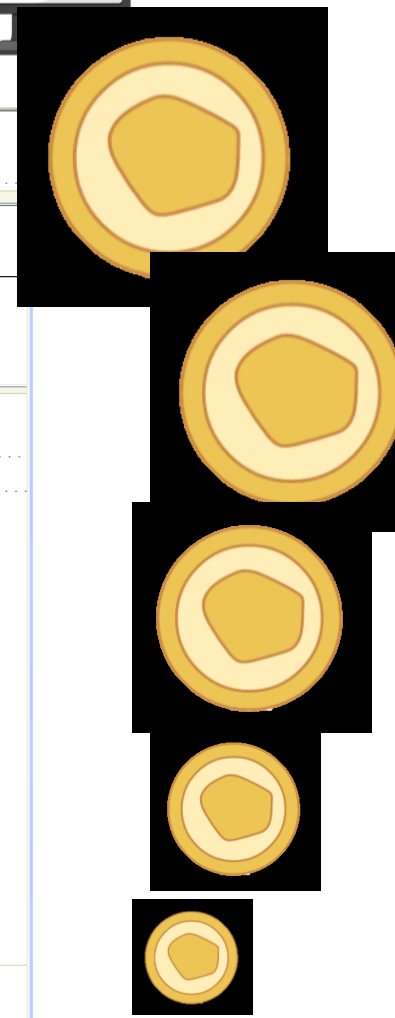
Recognising mode-of-action: peroxisome proliferation





* pValue: 0.2
* Fold Change: 1.0
* Fraction of metabolites: 0.9
t-Test version: Study Controls / 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)

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) 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

ShowSelected Metabolites | Find Compounds | Save Metabolite List

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

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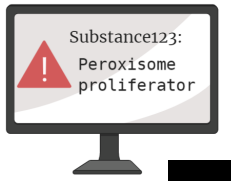
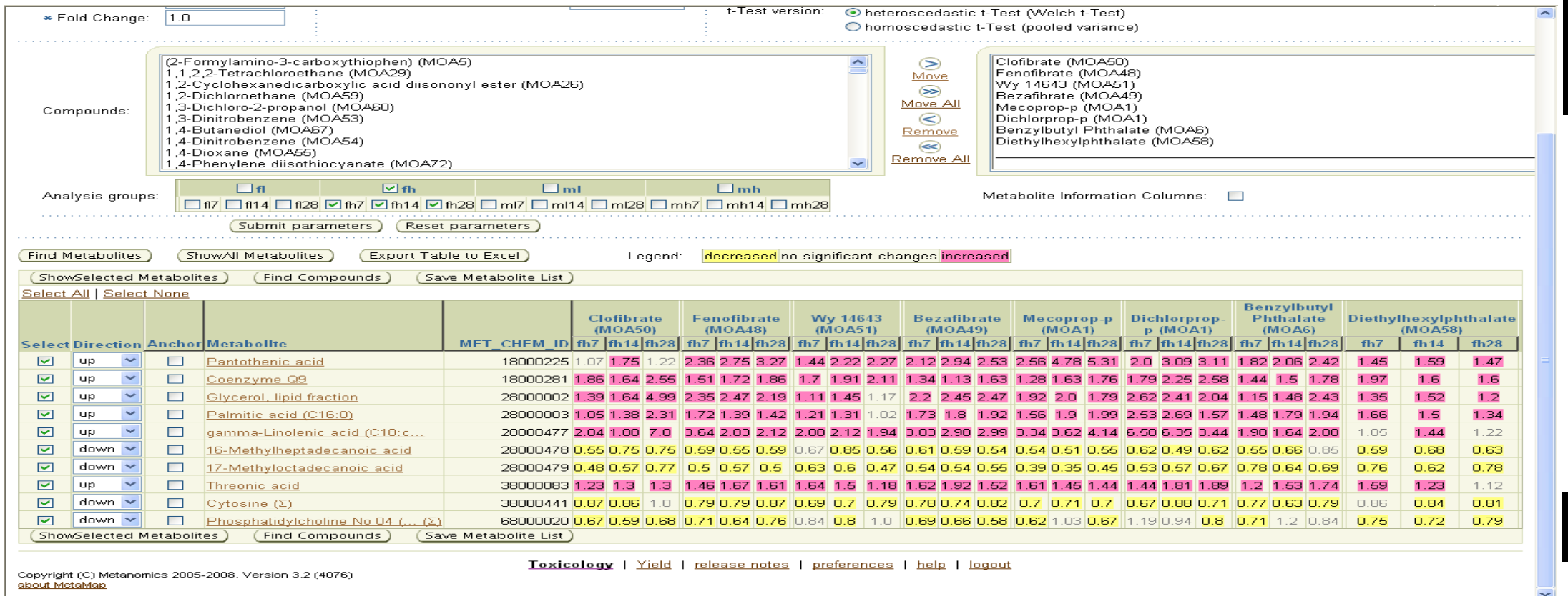
about MetaMap

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:

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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

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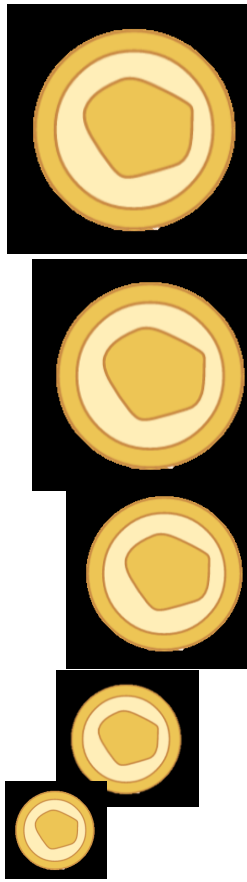
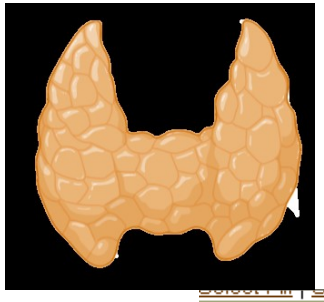
about MetaMap



BASF We create chemistry



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

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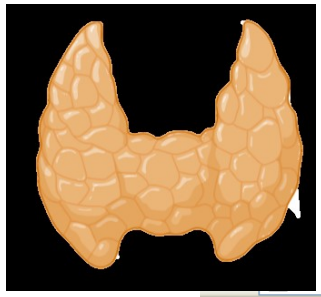

Thyroid: Direct Effect
Found: Methimazole & Metiram

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

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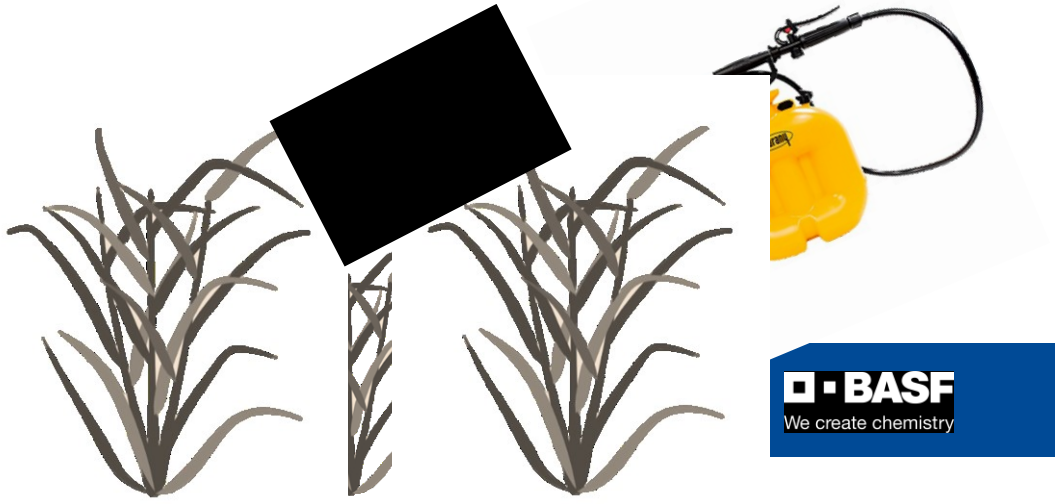
HPPD inhibitors: metabolites connection

			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	[illegible]	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**	[illegible]	[illegible]	**34,20**	**35,17**



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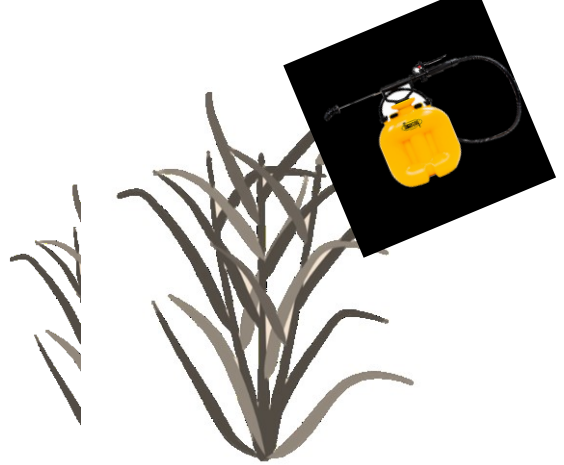
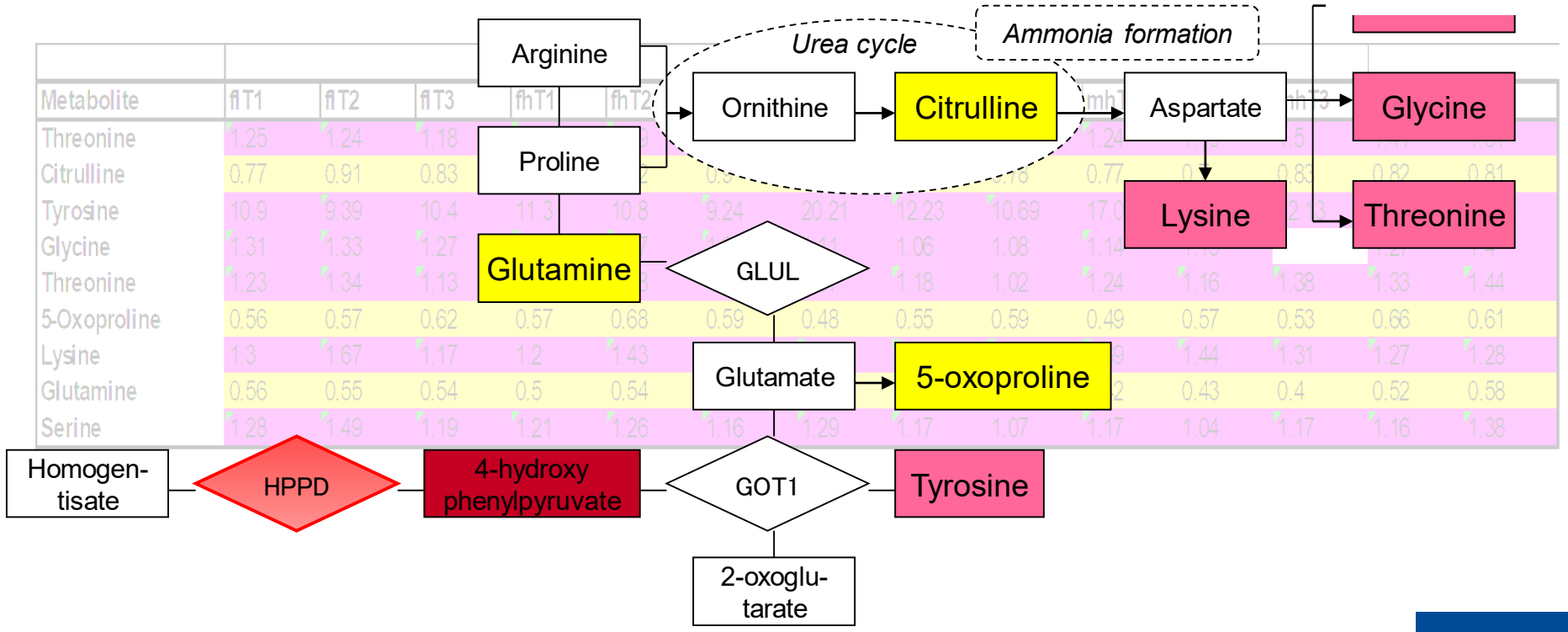
HPPD inhibitors: metabolites connecti





Metabolite	flT1	flT2	flT3	fhT1	fhT2								
Threonine	1.25	1.24	1.18										
Citrulline	0.77	0.91	0.83										
Tyrosine	10.9	9.39	10.4	11.3	10.8	9.24	20.21	12.23	10.69				
Glycine	1.31	1.33	1.27					1.06	1.08				
Threonine	1.23	1.34	1.13					1.18	1.02	1.24	1.16	1.38	1.33	1.44
5-Oxoproline	0.56	0.57	0.62	0.57	0.68	0.59	0.48	0.55	0.59	0.49	0.57	0.53	0.66	0.61
Lysine	1.3	1.67	1.17	1.2	1.43						1.44	1.31	1.27	1.28
Glutamine	0.56	0.55	0.54	0.5	0.54						0.43	0.4	0.52	0.58
Serine	1.28	1.49	1.19	1.21	1.26	1.16	1.29	1.17	1.07	1.17	1.04	1.17	1.16	1.38

Profile or Pairwise 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

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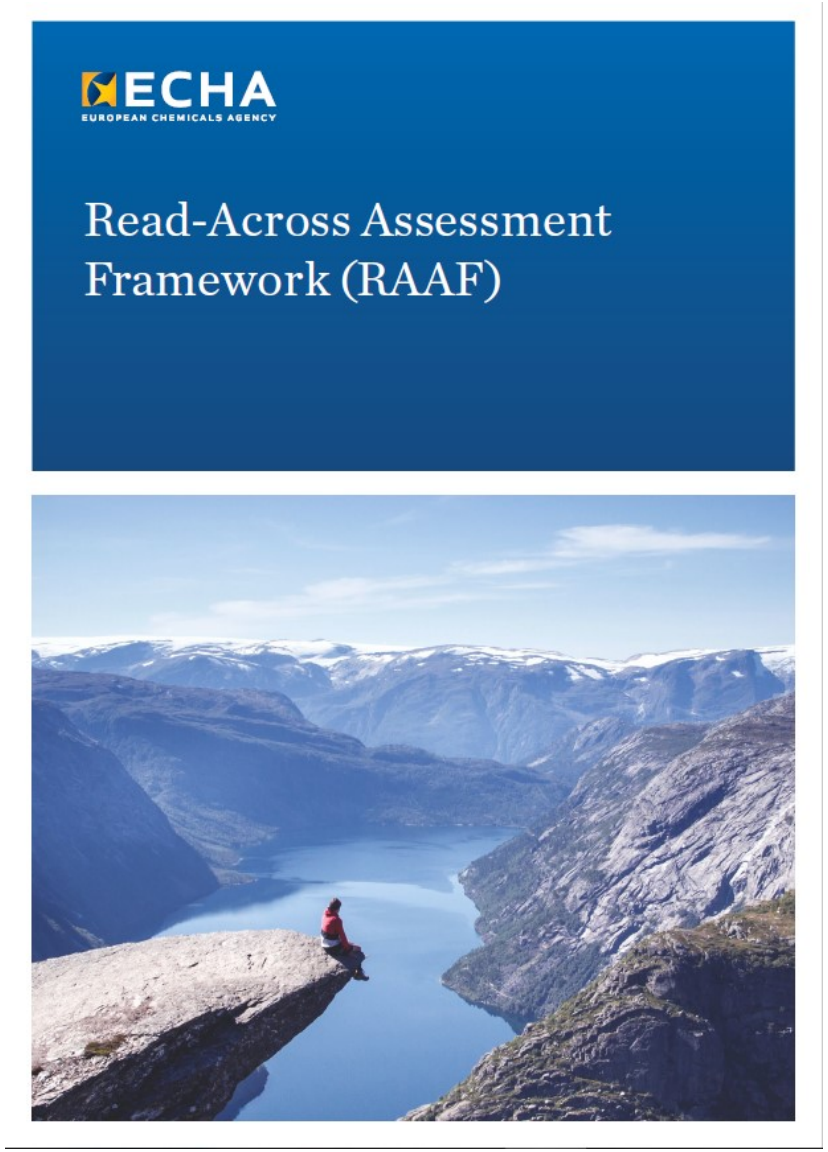
Read across

"... 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))."



Internal

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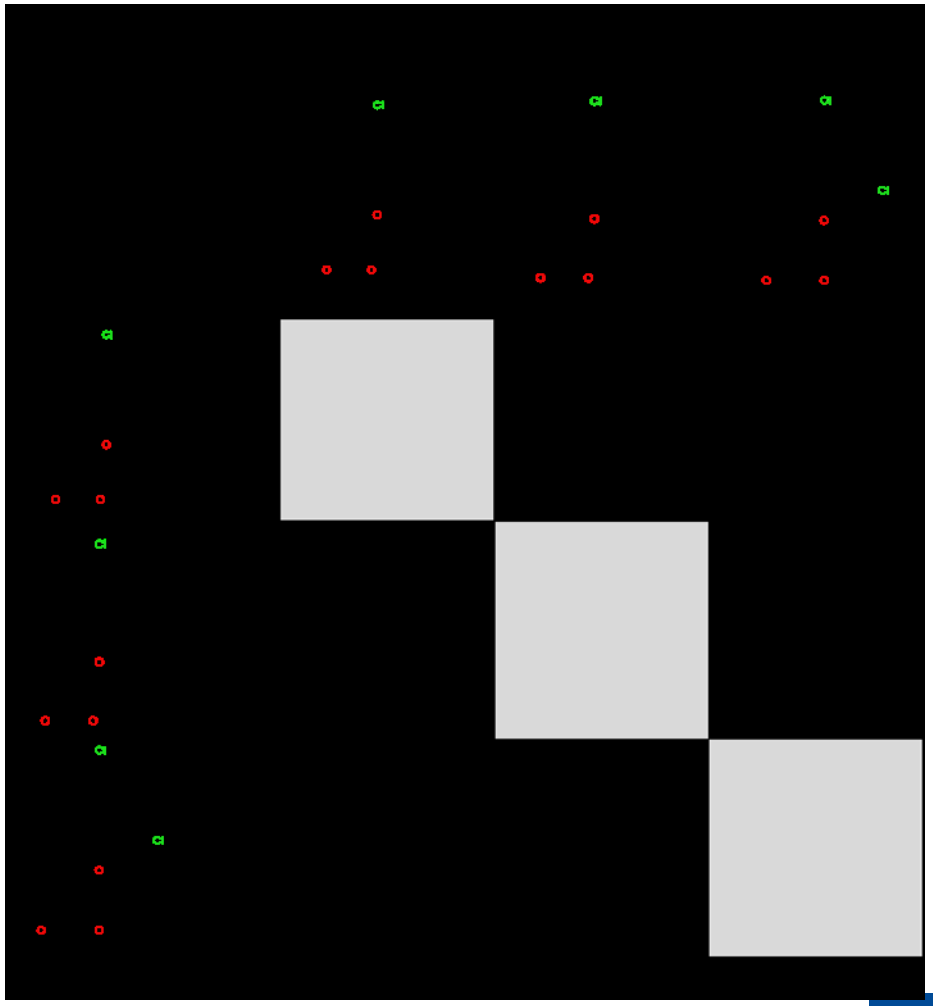
Case Study Phenoxy herbicides

- 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 ?



Internal

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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

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Case Study Phenoxy herbicides

a)

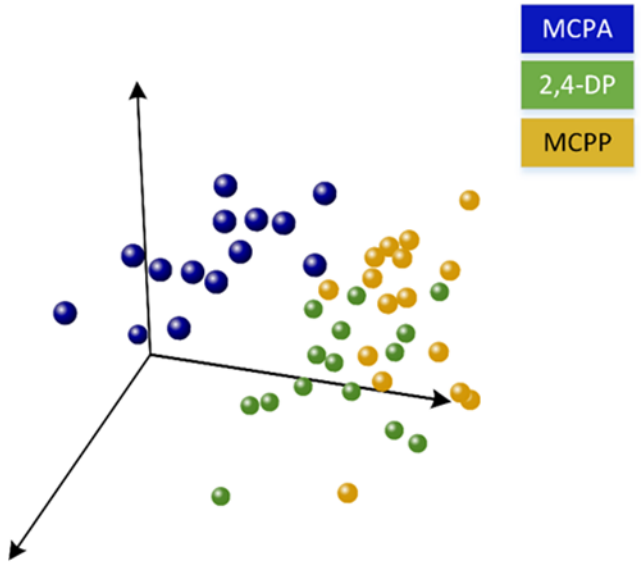
Metabolite	MCPP			2,4-DP			MCPA		
	m7	m14	m28	m7	m14	m28	m7	m14	m28
3-Indoxylsulfate	4,14	2,10	3,00	5,58	3,28	3,25	0,72	1,53	1,92
3-Methoxytyrosine	1,33	1,35	1,76	1,22	1,32	1,35	1,08	1,19	1,84
alpha-Tocopherol	0,56	0,65	0,59	0,70	0,63	0,68	0,93	1,09	0,98
beta-Sitosterol	0,24	0,34	0,23	0,37	0,30	0,31	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.

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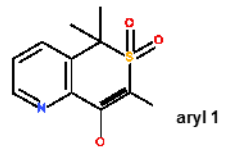
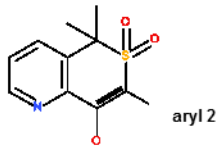
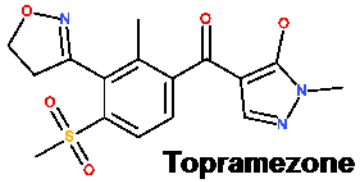
Example 2: HPPD-Inhibitors



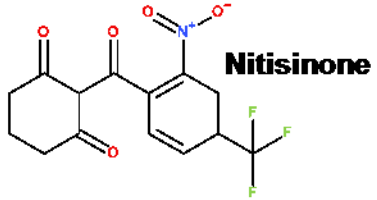
Coumarone 1



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

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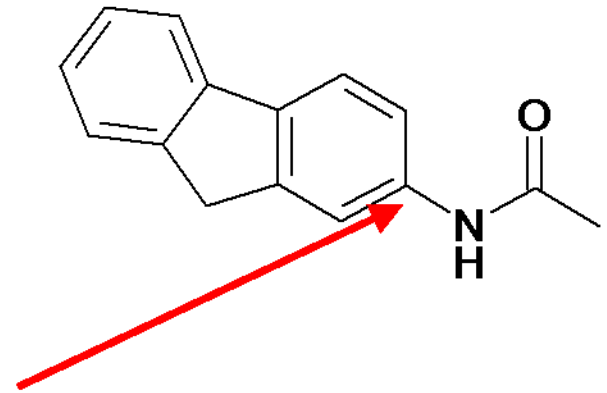
Example 3: 2- and 4-Acetylaminofluorene

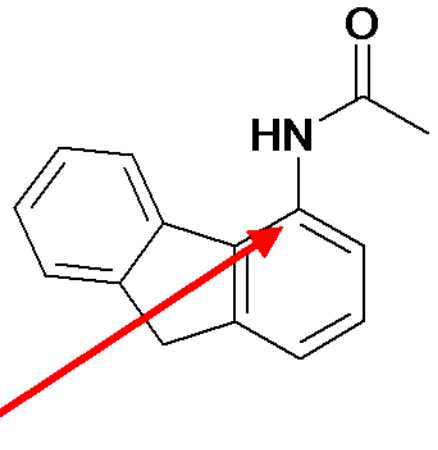
Tanimoto score: 63%



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

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Example 3: 2 and 4-Acetylaminofluorene Metabolome patterns

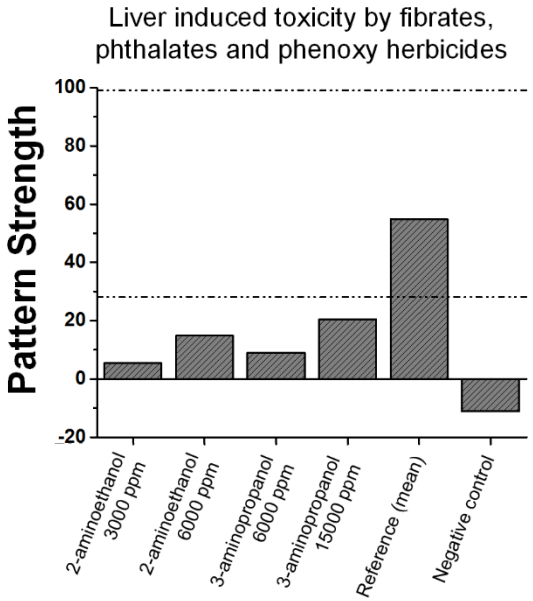
Metabolite profile compared to other **liver enzyme inducers (and liver cell carcinogens)**

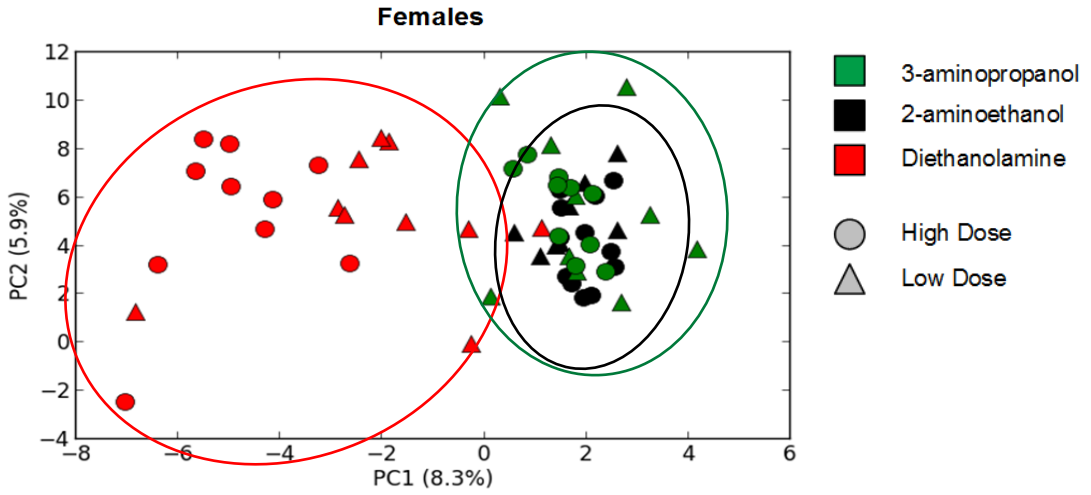
Compound	2-Acetylaminofluorene			4-Acetylaminofluorene			Pentachlorobenzene			Cyproteron Acetate		
	day 7	day 14	day 28	day 7	day 14	day 28	day 7	day 14	day 28	day 7	day 14	day 28
3-O-Methylsphingosine (d18:1)	1.56	**1.36**	1.45	1.16	1.33	1.33	1.42	**1.76**	**1.74**	**3.26**	1.75	**2.49**
4-Hydroxysphinganine (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**	**1.40**	1.23	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**

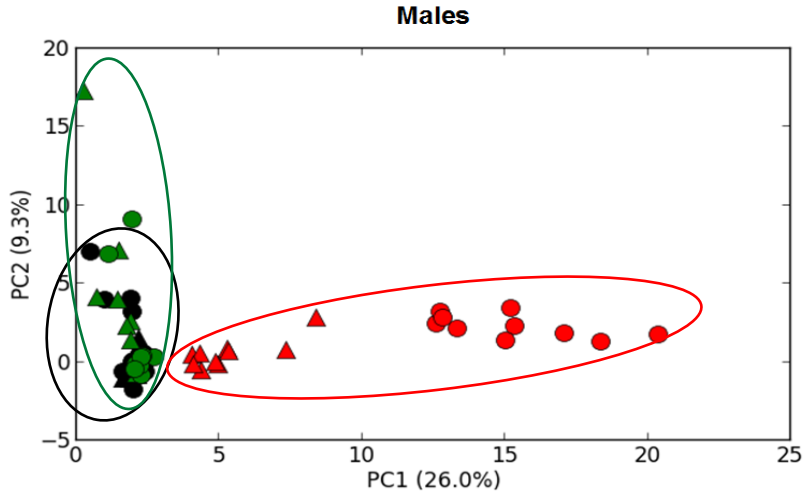
2-AAF has a very low overall match with 4-AAF: rank 1443

Regulatory Read-Across: 3-Aminopropanol and 2-Aminoethanol

Treatment	Strength	Pearson r	Pearson rank
3-aminopropanol HD	1.26	1	
3-aminopropanol LD	1.02	0.697	1
2-aminoethanol LD	0.95	0.638	2
2-aminoethanol HD	0.93	0.583	3
Zearalenone HD	1.74	0.569	4
D(+)-Galactosamine hydrochloride LD	2.19	0.560	5
Zearalenone LD	1.38	0.549	6
BASF LD	1.67	0.543	7
BASF substance 4 HD	2.66	0.514	8
17-alpha-Methyltestosterone HD	3.44	0.503	10







S. Sperber, M. Wahl, F. Berger, H. Kamp, O. Lemke, V. Starck, T. Walk, M. Spitzer, B.v Ravenzwaay, 2019. Metabolomics as read-across tool: An example with 3-aminopropanol and 2-aminoethanol. Regul. Toxicol. Pharmacol., ISSN 0273-2300, https://doi.org/10.1016/j.yrtph.2019.104442

Internal

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Read-Across and UVCBs

Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (**UVCB** Substance)

RAAF

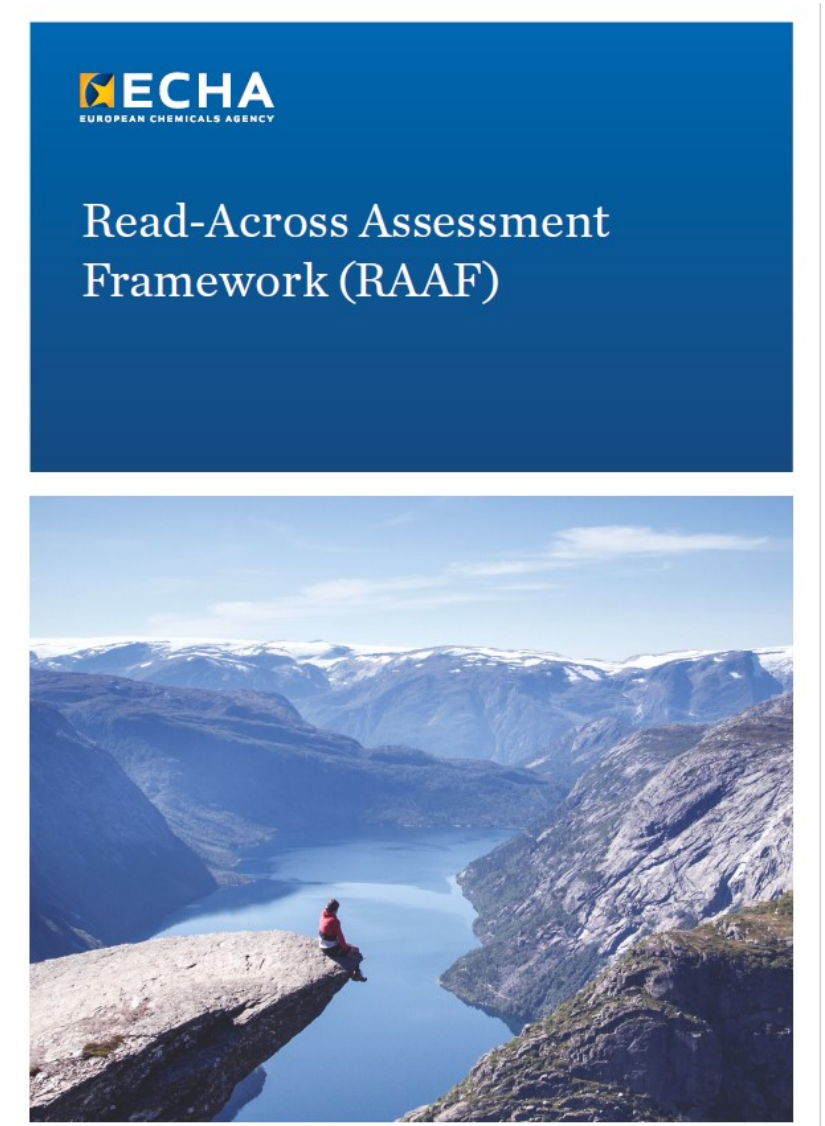
§ 4.2 PREPARATORY ASSESSMENT

"A fundamental aspect of read-across is structural similarity. Chemical composition, including structural information should be well defined."

→ UVCBs cannot be used for read across (?)

! There are many UVCBs !

Could metabolomics provide a solution ?



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LOA: 14 Day Metabolome Study - Background

Testing Program for 12 Lower Olefines and Aromatics-streams

- UVCBs, steamcracker products
- 12 streams collected by different members of the LOA-consortium
- Analytics conducted for all streams
- Additionally: 6 marker substances
  → benzene, xylene, naphthalene, dicyclopentadiene, cyclopentane, indene
- Three marker substances already available in BASF data base MetaMap®Tox
  → toluene, ethylbenzene, styrene
- Three additional BASF LOA streams tested

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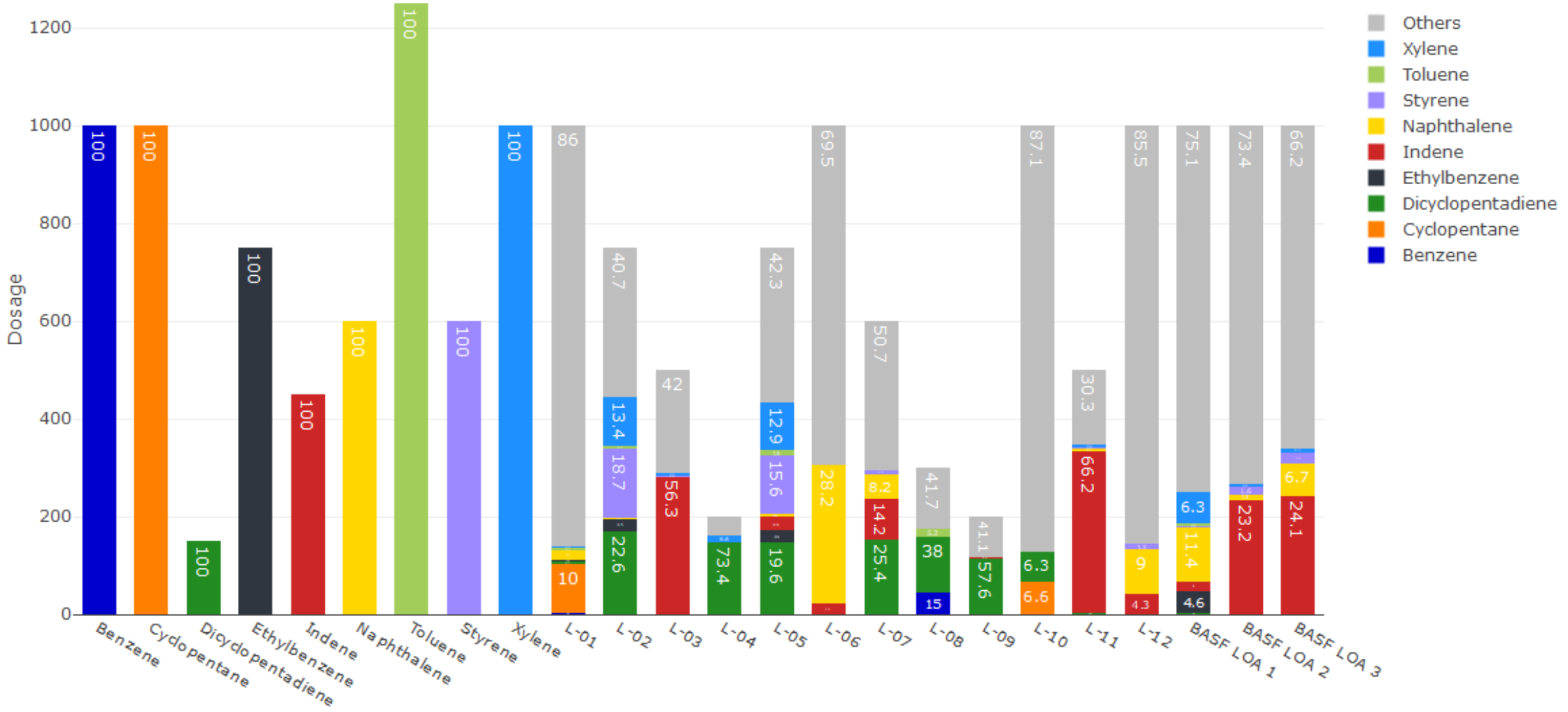
Stream Composition – Comparison with HD of Markers

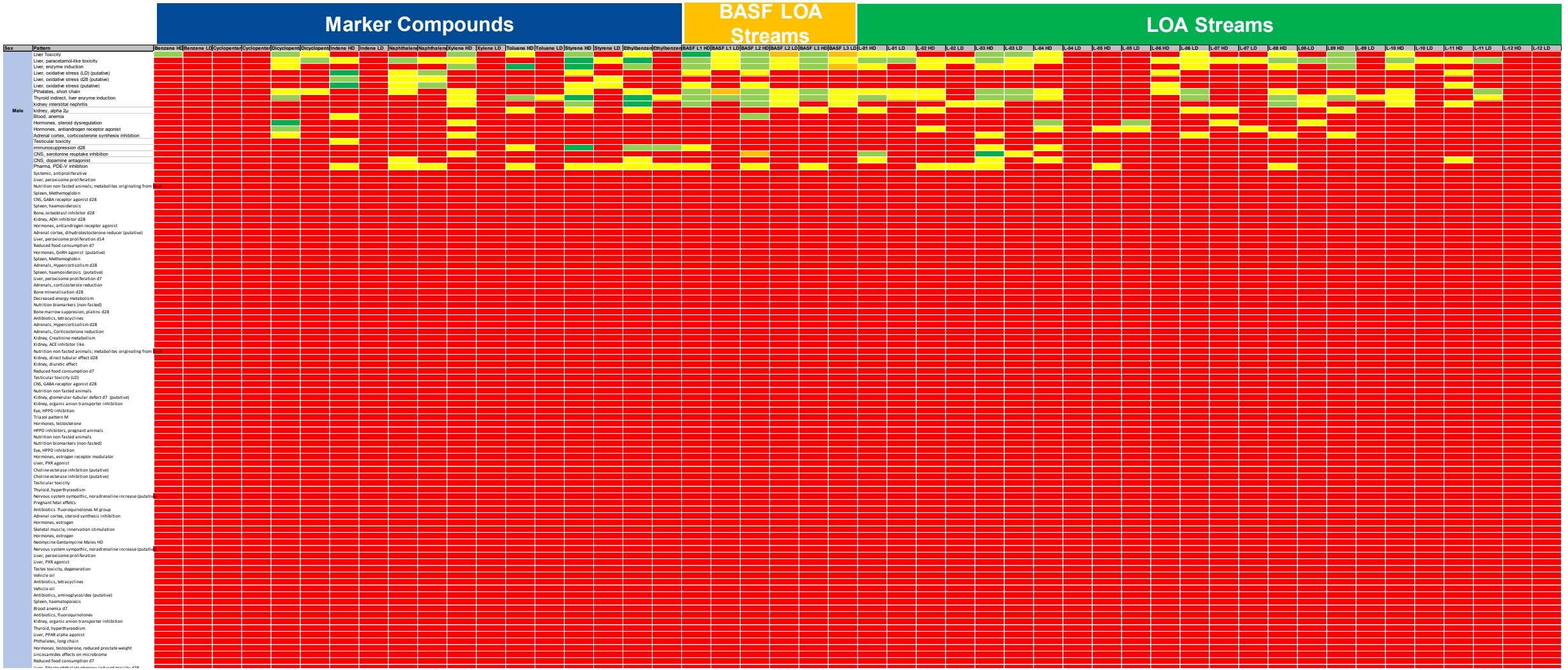


The numbers overlaid on the bars are the percentage amount of the marker substance in the stream.

Internal

Pattern Ranking – Males (Overview)

	match
	weak match
	equivocal
	inconclusive
	mismatch



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Pattern Ranking – Males (Patterns with Matches)

	match
	weak match
	equivocal
	inconclusive
	mismatch

		Marker Compounds																		BASF LOA Streams					
Sex	Pattern	Benzene HD	Benzene LD	Cyclopentan	Cyclopentan	Dicyclopenta	Dicyclopenta	Indene HD	Indene LD	Naphthalene	Naphthalene	Xylene HD	Xylene LD	Toluene HD	Toluene LD	Styrene HD	Styrene LD	Ethylbenzen	Ethylbenzen	BASF L1 HD	BASF L1 LD	BASF L2 HD	BASF L2 LD	BASF L3 HD	BASF L3 LD
Male	Liver Toxicity																								
	Liver, paracetamol-like toxicity																								
	Liver, enzyme induction																								
	Liver, oxidative stress (LD) (putative)																								
	Liver, oxidative stress d28 (putative)																								
	Liver, oxidative stress (putative)																								
	Pthalates, short chain																								
	Thyroid indirect, liver enzyme induction																								
	kidney interstital nephritis																								
	kidney, alpha 2µ																								
	Blood, anemia																								
	Hormones, steroid dysregulation																								
	Hormones, antiandrogen receptor agonist																								
	Adrenal cortex, corticosterone synthesis inhibition																								
	Testicular toxicity																								
	immunosuppression d28																								
	CNS, serotonine reuptake inhibition																								
	CNS, dopamine antagonist																								
	Pharma, PDE-V inhibition																								

		LOA Streams																							
Sex	Pattern	L-01 HD	L-01 LD	L-02 HD	L-02 LD	L-03 HD	L-03 LD	L-04 HD	L-04 LD	L-05 HD	L-05 LD	L-06 HD	L-06 LD	L-07 HD	L-07 LD	L-08 HD	L08-LD	L09 HD	L-09 LD	L-10 HD	L-10 LD	L-11 HD	L-11 LD	L-12 HD	L-12 LD
Male	Liver Toxicity																								
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	Thyroid indirect, liver enzyme induction																								
	kidney interstital nephritis																								
	kidney, alpha 2µ																								
	Blood, anemia																								
	Hormones, steroid dysregulation																								
	Hormones, antiandrogen receptor agonist																								
	Adrenal cortex, corticosterone synthesis inhibition																								
	Testicular toxicity																								
	immunosuppression d28																								
	CNS, serotonine reuptake inhibition																								
	CNS, dopamine antagonist																								
	Pharma, PDE-V inhibition																								

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Treatment Correlation

Example DCPD, Females:

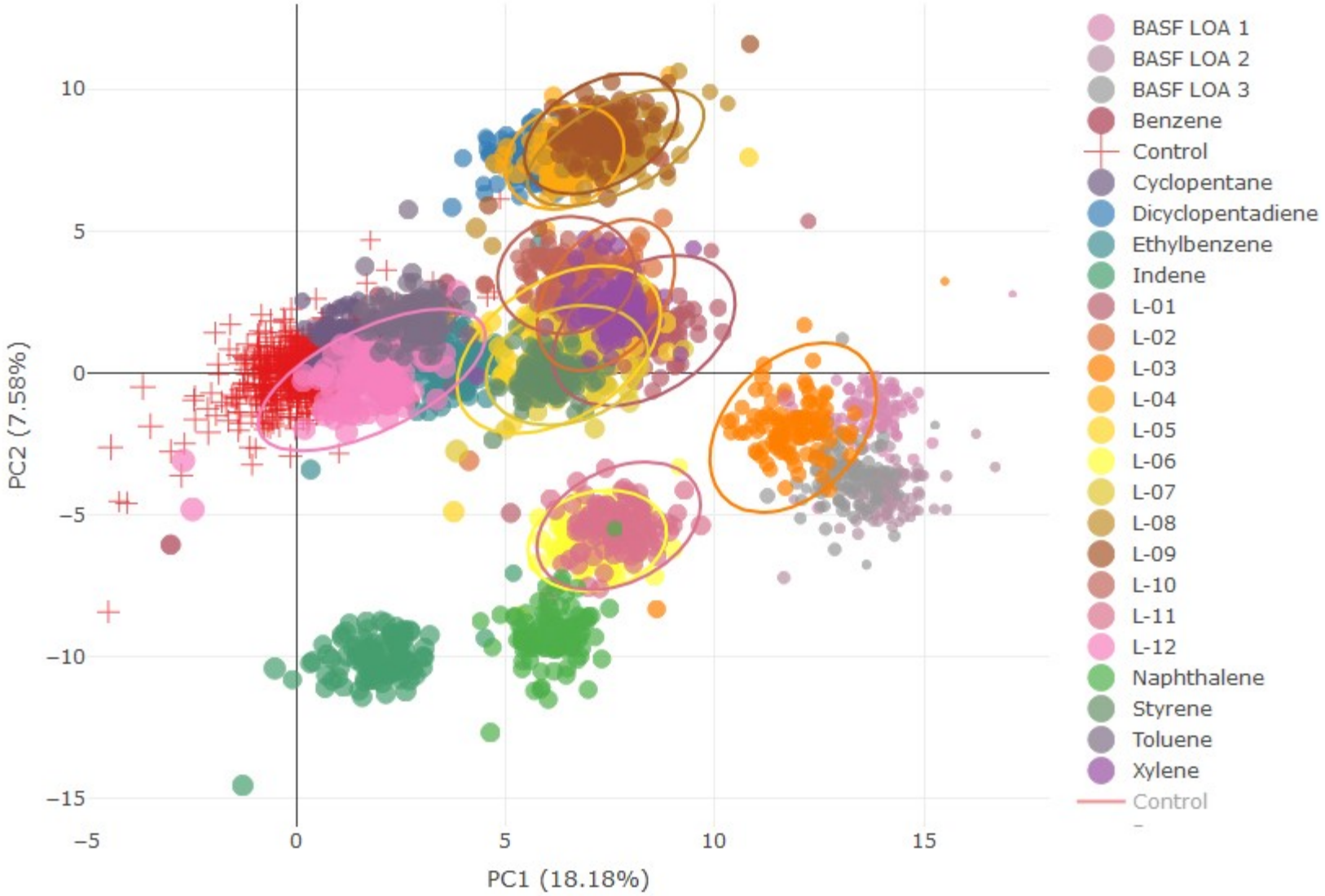
→ **Green colour indicates *relevant* DCPD-content in LOA stream**

→ **Yellow colour indicates low DCPD-content in stream**

			Pearson	Pearson	Pearson	Spearman	Spearman	Spearman
Treatment	Strengt	n	r	p	rank	r	p	rank
Dicyclopentadiene (MOA19C011_Cohort3) HD	1.87	229	1	0		1	0	
L-04 (MOA19C011_Cohort1) HD	3.49	228	0.643493	4.77E-28	1	0.643259	5.06E-28	2
L-08 (MOA19C011_Cohort2) HD	2.74	228	0.64341	4.87E-28	2	0.664787	1.88E-30	1
L-04 (MOA19C011_Cohort1) LD	1.69	228	0.627453	2.33E-26	3	0.559351	3.61E-20	9
L-07 (MOA19C011_Cohort1) LD	2.05	228	0.612325	7.47E-25	4	0.556301	6.35E-20	11
L-10 (MOA19C011_Cohort2) HD	1.78	229	0.586777	1.4E-22	5	0.570561	3.55E-21	5
L-02 (MOA19C011_Cohort1) HD	3.57	229	0.581323	4.24E-22	6	0.580362	5.14E-22	4
L-09 (MOA19C011_Cohort2) HD	2.9	229	0.569105	4.7E-21	7	0.570419	3.65E-21	6
Xylene (MOA19C011_Cohort3) HD	2.67	229	0.560699	2.32E-20	11	0.614395	3.7E-25	3
L-07 (MOA19C011_Cohort1) HD	3.25	228	0.555673	7.12E-20	12	0.566138	1.01E-20	8
Dicyclopentadiene (MOA19C011_Cohort3) LD	1.42	229	0.546037	3.39E-19	13	0.556507	5.07E-20	10
L-09 (MOA19C011_Cohort2) LD	1.49	228	0.541906	8.4E-19	14	0.52585	1.3E-17	16
L-11 (MOA19C011_Cohort2) LD	2.18	228	0.538462	1.53E-18	16	0.553222	1.11E-19	12
L-06 (MOA19C011_Cohort1) LD	2.96	228	0.521873	2.51E-17	18	0.55126	1.59E-19	14
L-03 (MOA19C011_Cohort2) HD	4.61	228	0.510304	1.61E-16	21	0.519596	3.64E-17	19
L-02 (MOA19C011_Cohort1) LD	1.77	229	0.507656	2.1E-16	23	0.41962	3.51E-11	93
L-08 (MOA19C011_Cohort2) LD	1.17	228	0.485279	7.15E-15	31	0.371733	6.99E-09	212
L-03 (MOA19C011_Cohort2) LD	2.34	229	0.468459	6.82E-14	39	0.485596	5.96E-15	28
L-05 (MOA19C011_Cohort1) LD	1.91	229	0.457413	3.06E-13	48	0.452799	5.63E-13	51
Naphthalene (MOA19C011_Cohort3) LD	2.54	229	0.457406	3.06E-13	49	0.506384	2.56E-16	23
L-05 (MOA19C011_Cohort1) HD	2.9	229	0.452713	5.69E-13	54	0.442317	2.18E-12	64
L-01 (MOA19C011_Cohort1) HD	3.32	229	0.448552	9.8E-13	60	0.519826	2.99E-17	18
L-06 (MOA19C011_Cohort1) HD	2.8	228	0.4457	1.58E-12	64	0.428926	1.28E-11	87
Naphthalene (MOA19C011_Cohort3) HD	3.63	229	0.435904	4.88E-12	72	0.444865	1.58E-12	63

Internal

Bootstrapped PCA – Males



- **All compounds: PCA after bootstrapping (100x) + original values**
- LOA streams highlighted

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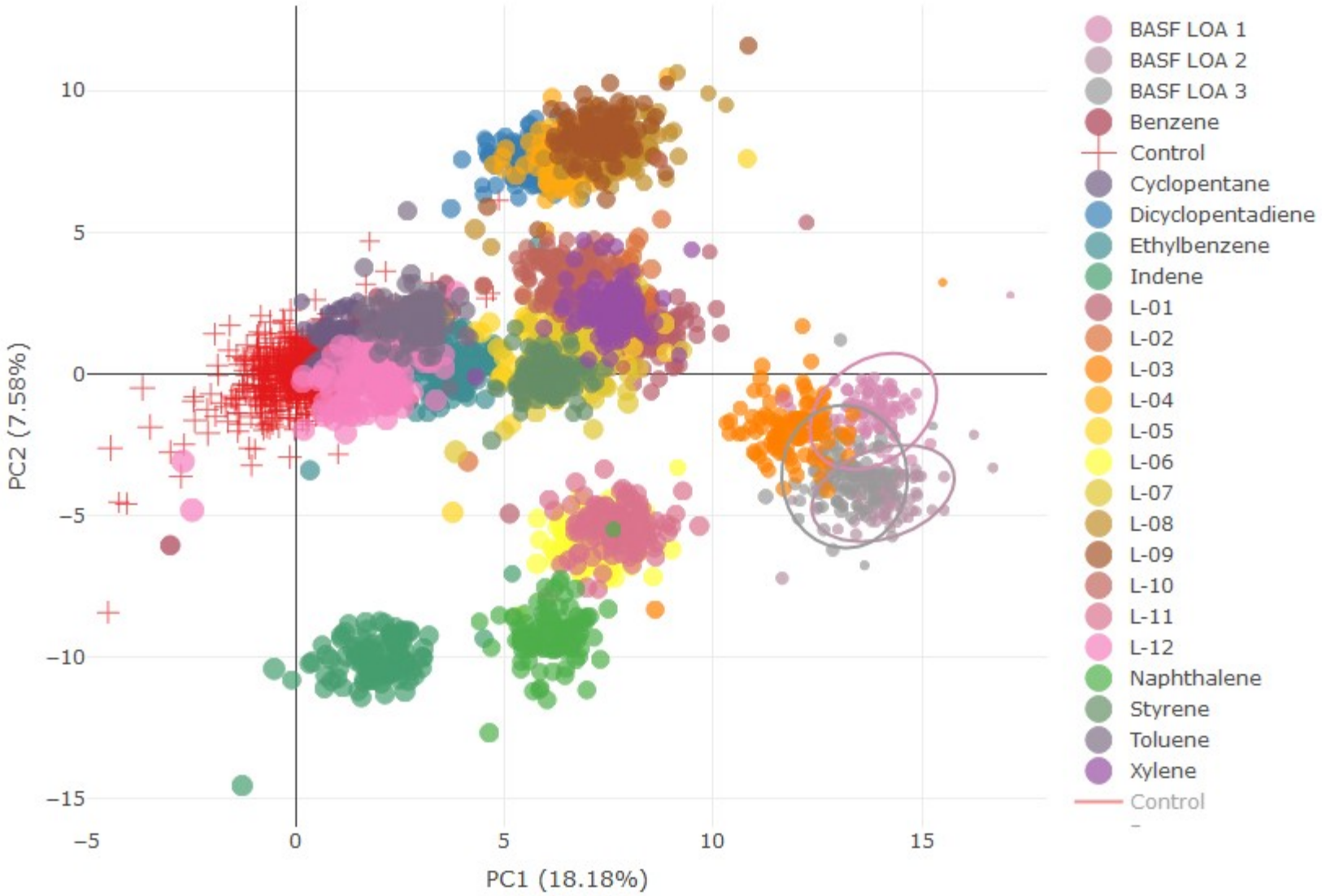
Bootstrapped PCA – Males



- **All compounds: PCA after bootstrapping (100x) + original values**
- BASF LOA streams highlighted

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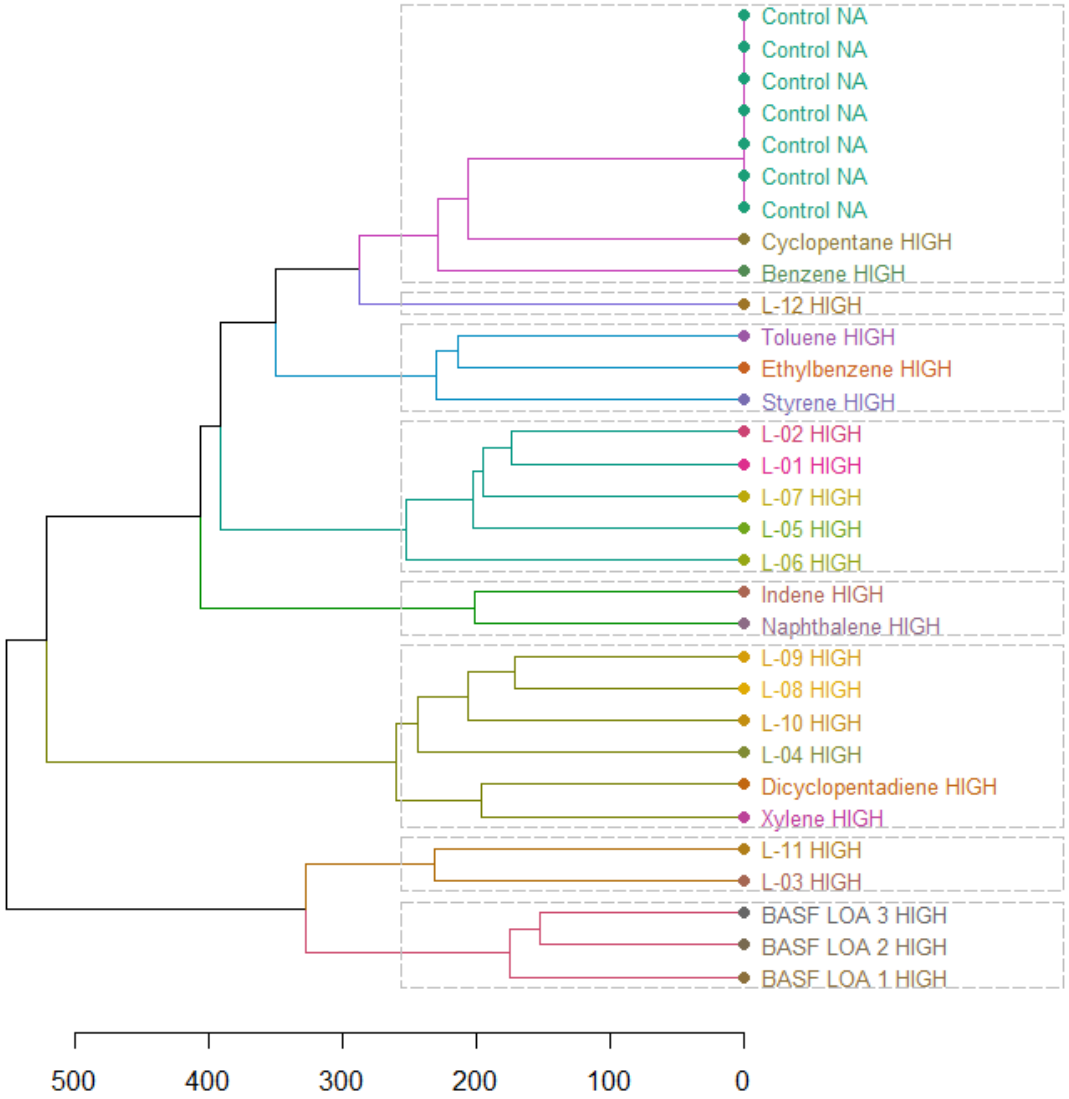
Hierarchical Clustering - Males



- Benzene, Cyclopentane cluster close to controls; L-12 stands out
- Toluene, Ethylbenzene and Styrene cluster together
- DCPD, L-04, L-08, L-09, L-10 form a cluster with Xylene
- Naphthalene/ Indene as well as L-03, L-11 form separate clusters
- BASF LOA streams cluster together, close to L-03 and L-11

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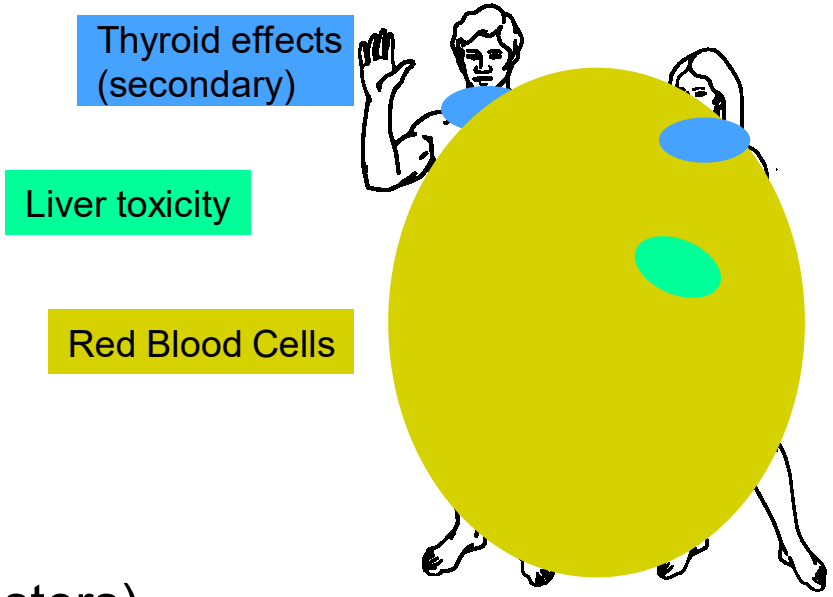
LOA conclusions



- Toxicity / Patholgy data shows relatively homogenous effects: liver, thyroid, red blood cells, kidney (males)
  - Supported by metabolome patterns, PCA
  - Metabolome data also indicates absence of many toxicities
- Metabolome data show subclustering of LOA Cat L streams (3 clusters)
  - Correlation analysis, bootstrapped PCA
- BASF LOA streams form a further subcategory, supporting the overall (sub-)category approach

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REACH: chemical grouping and read across

CONCLUSIONS

Metabolomics provide important data useful to improve the quality of read across

Biology based based read across may be more accurate than structure based data

For UVCBs, metabolomics may be the solution provider to justify read across

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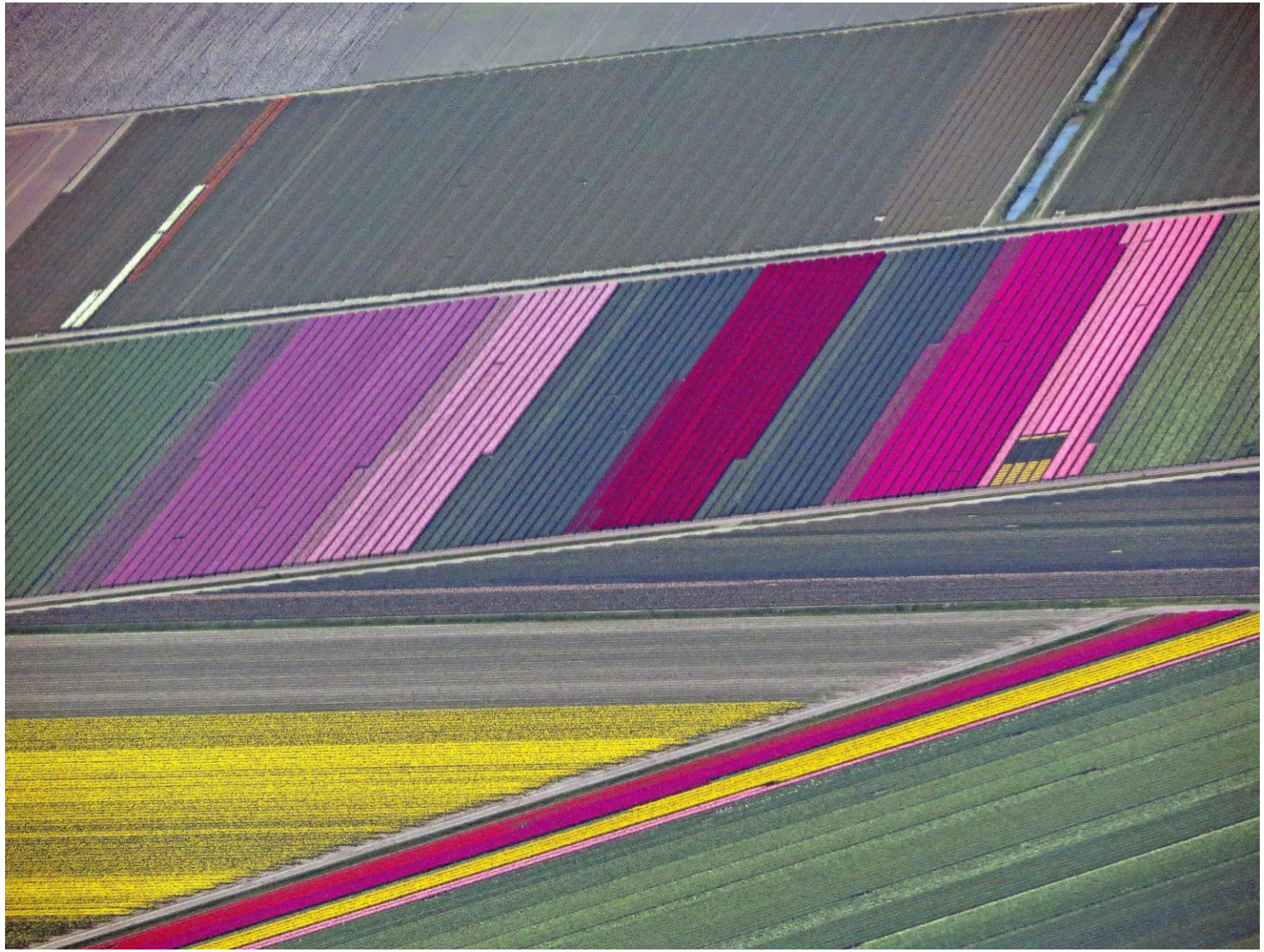
Thank you very much for your attention



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