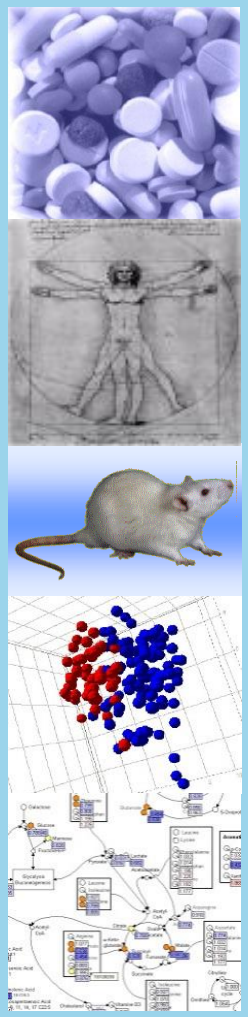


Read Across with Metabolomics for Phenoxy Herbicides

BASF SE

Prof. Dr. Bennard van Ravenzwaay, BASF, Ludwigshafen, Germany
Experimental Toxicology and Ecology

BASF
We create chemistry

Why Metabolomics / Metabolic Profiling ?

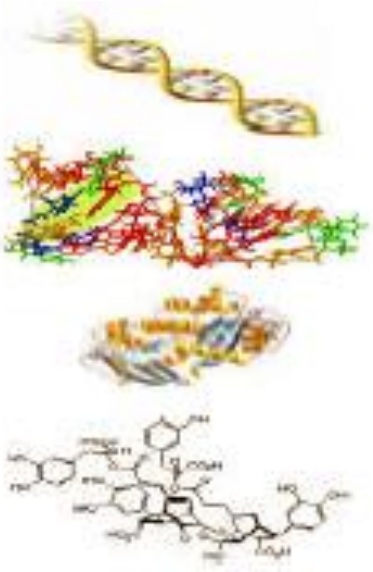
Mostly unknown

DNA
40,000 genes

RNA
150,000 transcripts

Proteins
1,000,000 proteins

Metabolites
2,500 metabolites



Genomics

Transcriptomics

Proteomics

Metabolomics

Mostly known

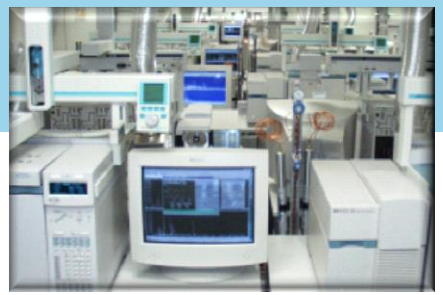
Advantage
Single matrix (blood)

Closer to classical toxicology

The Use of MetaMap®Tox

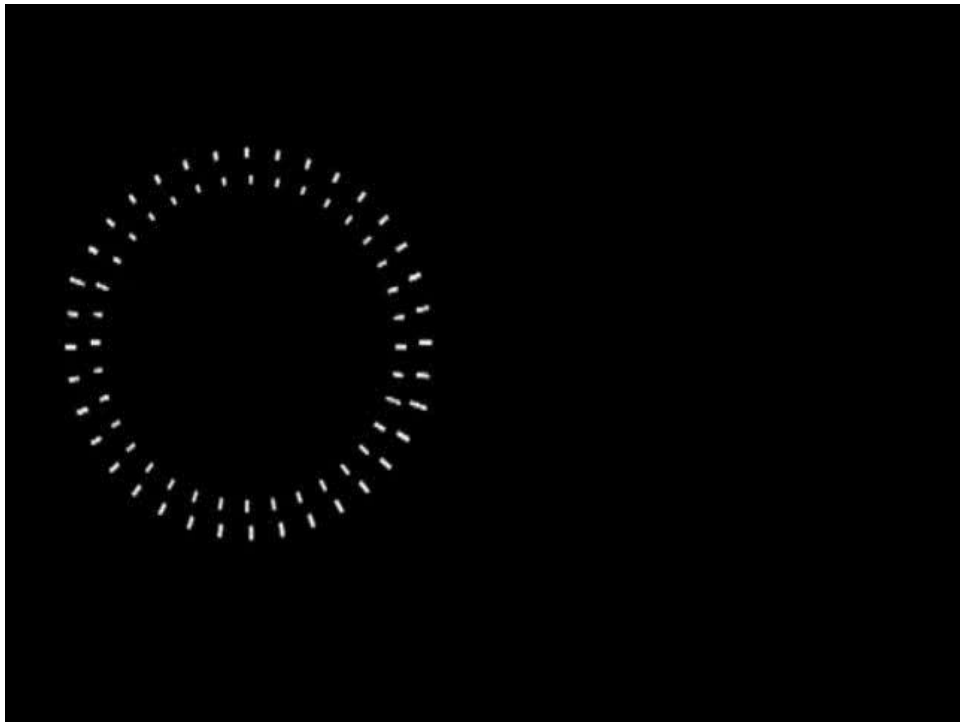
BASF
We create chemistry

BLOOD PROFILING

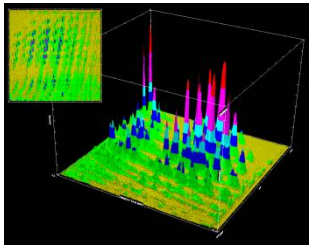


LC-MS/ GC-MS

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)

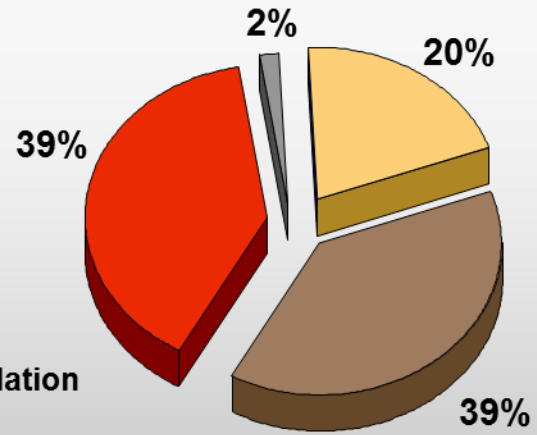
SAMPLE **REFERENCE** **300 Known Metabolites**

MetaMap®Tox: Reference Data Base

- Crl:Wi(Han) Rats
- controls, two dose groups
- male, female
- 4 weeks studies
- Plasma sampling d7, d14, d28

- > 500 reference compounds
- Ca. 800 compounds tested
- > 120 specific metabolite patterns

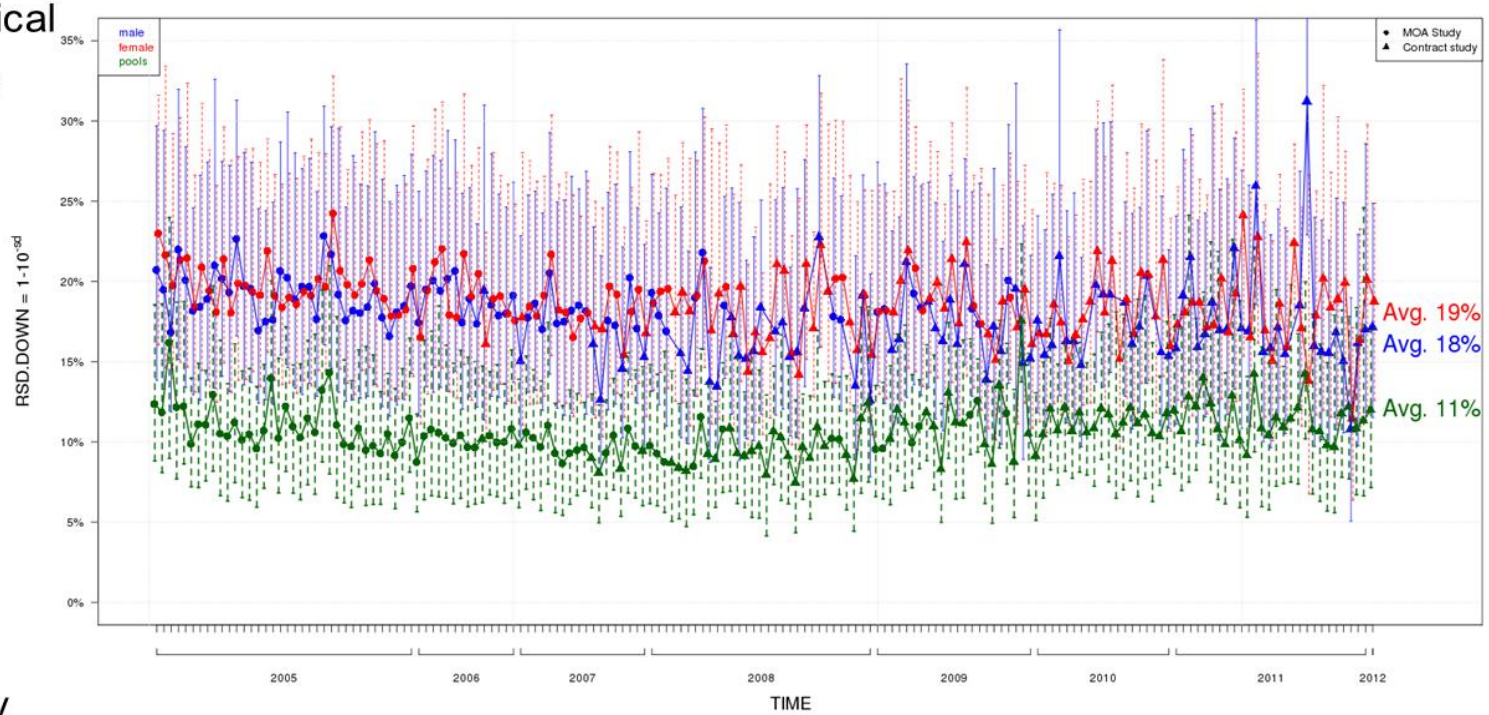
- Liver
- Kidney
- Adrenals
- Thyroid
- Testes
- Ovaries
- CNS
- Endocrine modulation
- Duodenum
- Nervous system
- Blood
- Bone
- Eye



MetaMap®Tox: Reproducibility and Robustness

Technical and biological variation of rat plasma samples 2004 to 2012

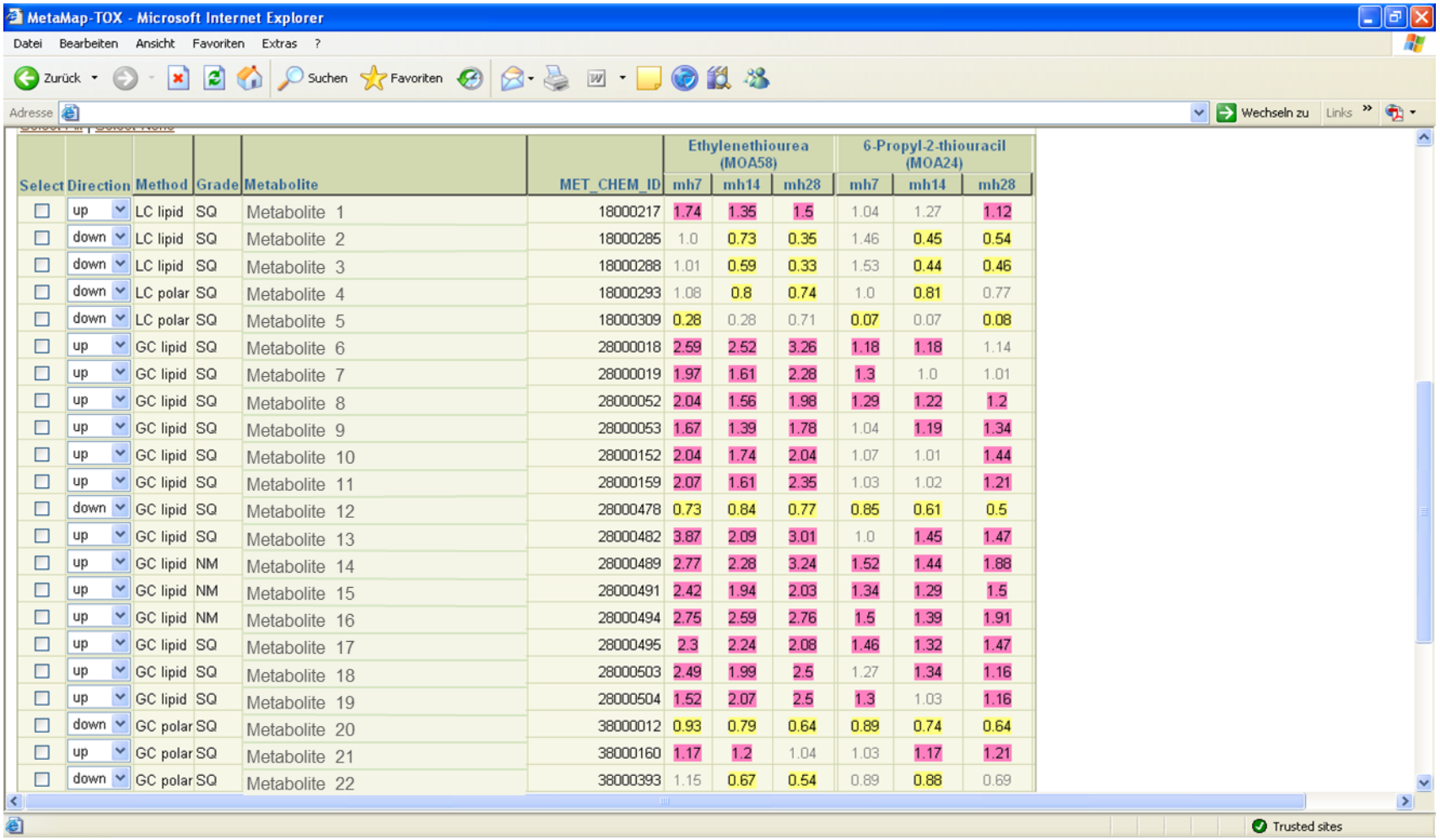
- > 5000 biological control samples



- High accuracy
  - mean technical variation 11%
  - mean biological variation ~ 18% (m & f comparable)

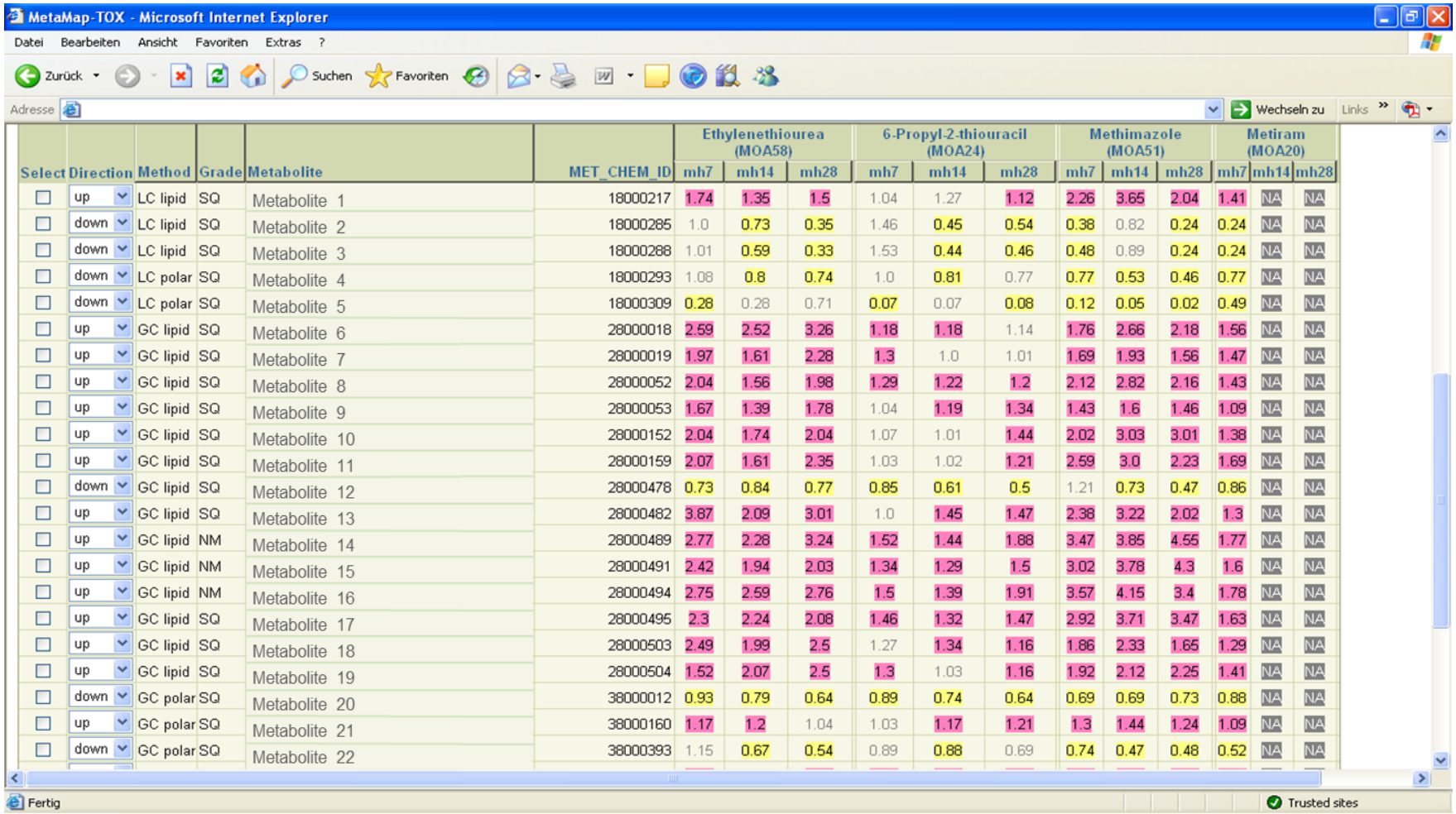
Vartech < Varbiol

Thyroid: Direct Effect: ETU & PTU



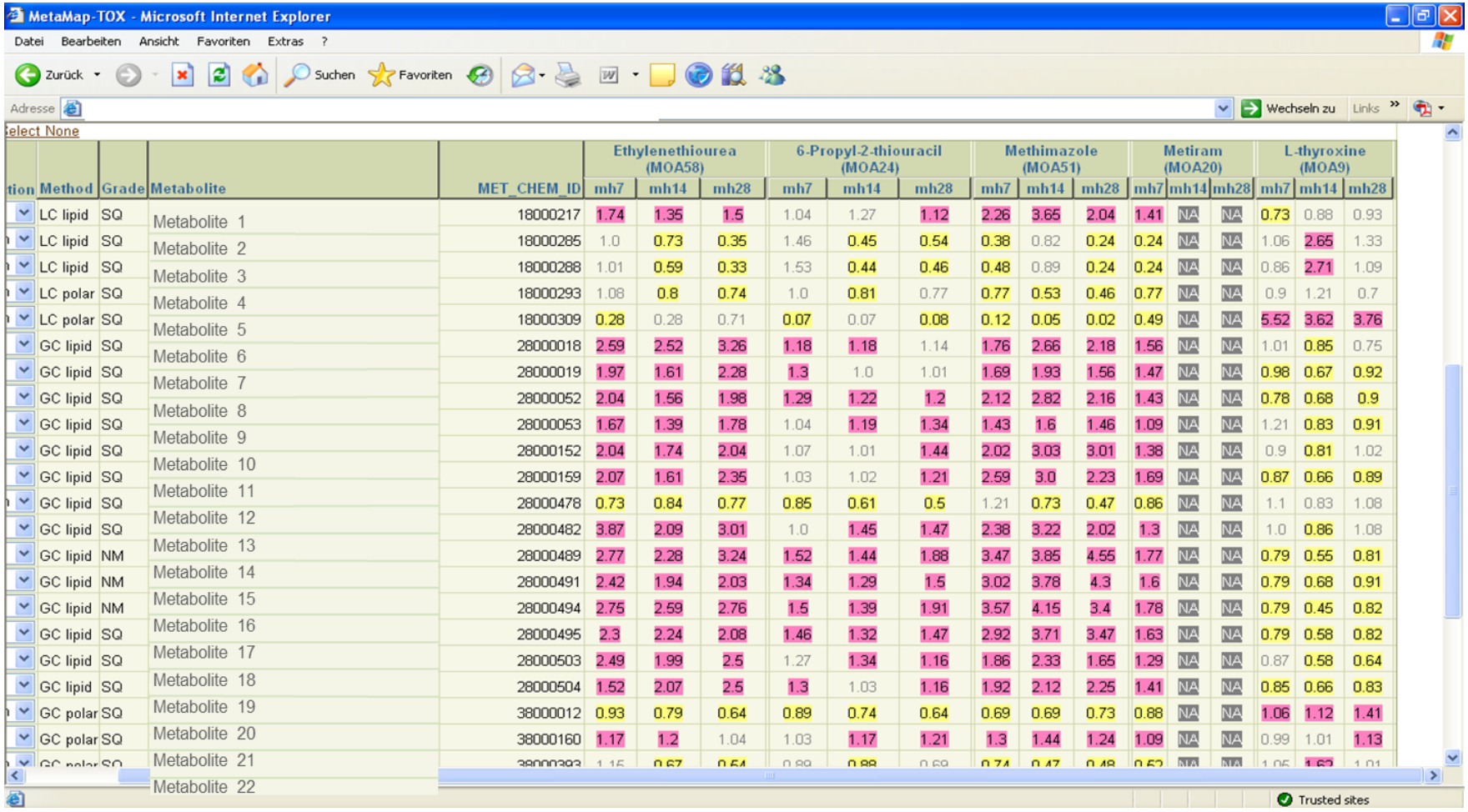
Select	Direction	Method	Grade	Metabolite	MET_CHEM_ID	Ethylenethiourea (MOA58)			6-Propyl-2-thiouracil (MOA24)		
						mh7	mh14	mh28	mh7	mh14	mh28
☐	up	LC lipid	SQ	Metabolite 1	18000217	1.74	1.35	1.5	1.04	1.27	1.12
☐	down	LC lipid	SQ	Metabolite 2	18000285	1.0	0.73	0.35	1.46	0.45	0.54
☐	down	LC lipid	SQ	Metabolite 3	18000288	1.01	0.59	0.33	1.53	0.44	0.46
☐	down	LC polar	SQ	Metabolite 4	18000293	1.08	0.8	0.74	1.0	0.81	0.77
☐	down	LC polar	SQ	Metabolite 5	18000309	0.28	0.28	0.71	0.07	0.07	0.08
☐	up	GC lipid	SQ	Metabolite 6	28000018	2.59	2.52	3.26	1.18	1.18	1.14
☐	up	GC lipid	SQ	Metabolite 7	28000019	1.97	1.61	2.28	1.3	1.0	1.01
☐	up	GC lipid	SQ	Metabolite 8	28000052	2.04	1.56	1.98	1.29	1.22	1.2
☐	up	GC lipid	SQ	Metabolite 9	28000053	1.67	1.39	1.78	1.04	1.19	1.34
☐	up	GC lipid	SQ	Metabolite 10	28000152	2.04	1.74	2.04	1.07	1.01	1.44
☐	up	GC lipid	SQ	Metabolite 11	28000159	2.07	1.61	2.35	1.03	1.02	1.21
☐	down	GC lipid	SQ	Metabolite 12	28000478	0.73	0.84	0.77	0.85	0.61	0.5
☐	up	GC lipid	SQ	Metabolite 13	28000482	3.87	2.09	3.01	1.0	1.45	1.47
☐	up	GC lipid	NM	Metabolite 14	28000489	2.77	2.28	3.24	1.52	1.44	1.88
☐	up	GC lipid	NM	Metabolite 15	28000491	2.42	1.94	2.03	1.34	1.29	1.5
☐	up	GC lipid	NM	Metabolite 16	28000494	2.75	2.59	2.76	1.5	1.39	1.91
☐	up	GC lipid	SQ	Metabolite 17	28000495	2.3	2.24	2.08	1.46	1.32	1.47
☐	up	GC lipid	SQ	Metabolite 18	28000503	2.49	1.99	2.5	1.27	1.34	1.16
☐	up	GC lipid	SQ	Metabolite 19	28000504	1.52	2.07	2.5	1.3	1.03	1.16
☐	down	GC polar	SQ	Metabolite 20	38000012	0.93	0.79	0.64	0.89	0.74	0.64
☐	up	GC polar	SQ	Metabolite 21	38000160	1.17	1.2	1.04	1.03	1.17	1.21
☐	down	GC polar	SQ	Metabolite 22	38000393	1.15	0.67	0.54	0.89	0.88	0.69

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	Metabolite 1	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	Metabolite 2	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	Metabolite 3	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	Metabolite 4	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	Metabolite 5	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	Metabolite 6	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	Metabolite 7	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	Metabolite 8	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	Metabolite 9	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	Metabolite 10	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	Metabolite 11	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	Metabolite 12	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	Metabolite 13	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	Metabolite 14	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	Metabolite 15	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	Metabolite 16	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	Metabolite 17	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	Metabolite 18	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	Metabolite 19	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	Metabolite 20	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	Metabolite 21	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	Metabolite 22	38000393	1.15	0.67	0.54	0.89	0.88	0.69	0.74	0.47	0.48	0.52	NA	NA

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	Metabolite 1	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	Metabolite 2	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	Metabolite 3	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	Metabolite 4	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	Metabolite 5	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	Metabolite 6	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	Metabolite 7	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	Metabolite 8	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	Metabolite 9	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	Metabolite 10	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	Metabolite 11	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	Metabolite 12	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	Metabolite 13	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	Metabolite 14	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	Metabolite 15	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	Metabolite 16	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	Metabolite 17	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	Metabolite 18	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	Metabolite 19	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	Metabolite 20	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	Metabolite 21	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	Metabolite 22	38000393	1.15	0.67	0.64	0.89	0.88	0.69	0.74	0.47	0.48	0.52	NA	NA	1.05	1.62	1.01

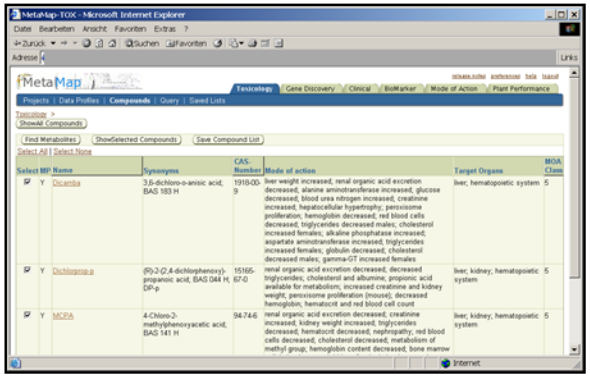
Pattern Creation

- Need at least 4 compounds sharing a MoA
- Determine common metabolites with 3 compounds, separated by sex, for at least two time points
- Pattern must find 4th compound (90% of metabolites correct)
- Pattern must be selective at 80% (should not find compounds that do not have the MoA)

- Validation: If more same MoA compounds are available: pattern must remain sensitive and selective (80% of metabolites correct)

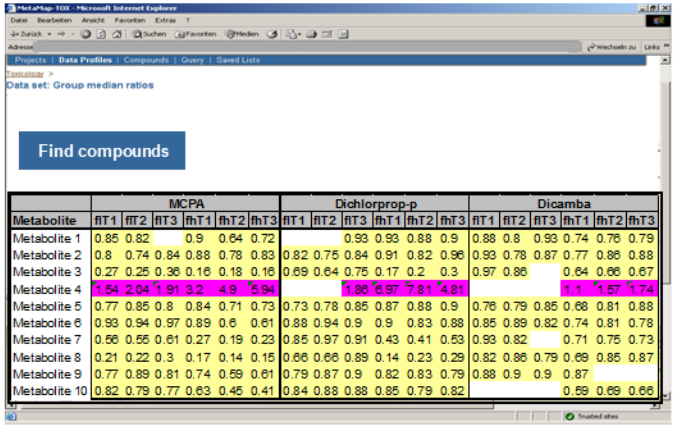
MetaMap™ Tox

Group of substances



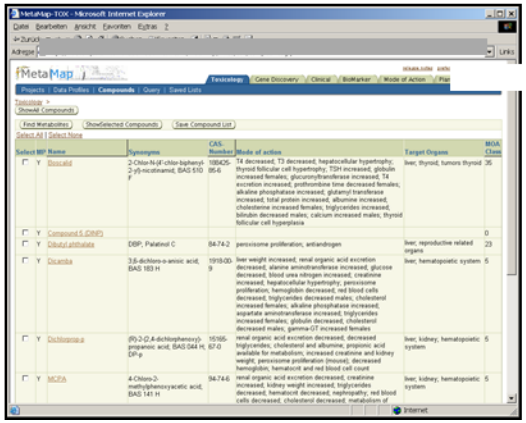
"Find Metabolites"

List of changed metabolites "metabolic profiling"



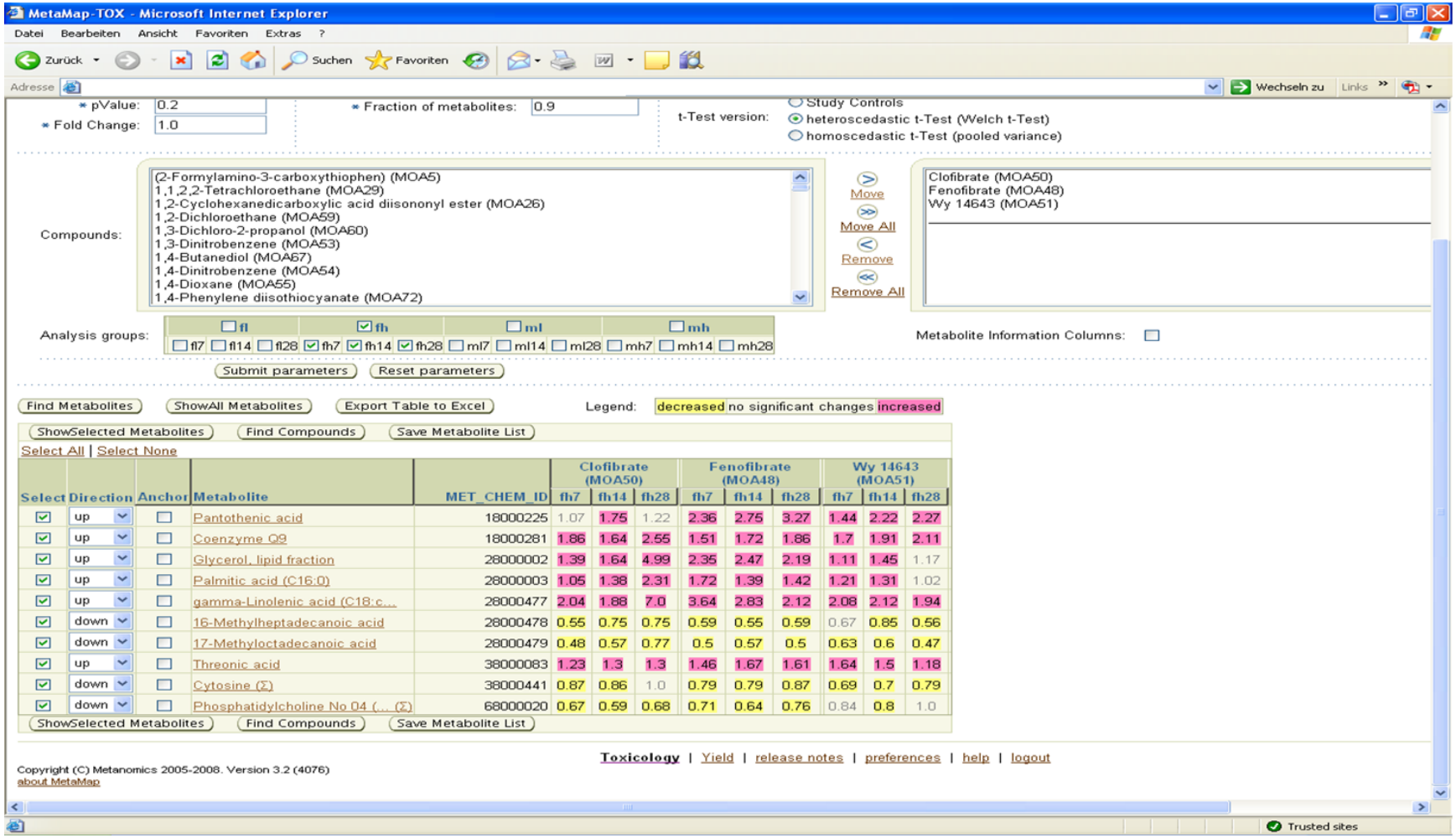
Metabolite	MCPA						Dichlorprop-p						Dicamba					
	fiT1	fiT2	fiT3	fhT1	fhT2	fhT3	fiT1	fiT2	fiT3	fhT1	fhT2	fhT3	fiT1	fiT2	fiT3	fhT1	fhT2	fhT3
Metabolite 1	0.85	0.82		0.9	0.64	0.72			0.93	0.93	0.88	0.9	0.88	0.8	0.93	0.74	0.76	0.79
Metabolite 2	0.8	0.74	0.84	0.88	0.78	0.83	0.82	0.75	0.84	0.91	0.82	0.96	0.93	0.78	0.87	0.77	0.86	0.88
Metabolite 3	0.27	0.25	0.36	0.16	0.18	0.16	0.69	0.64	0.75	0.17	0.2	0.3	0.97	0.86		0.64	0.66	0.67
Metabolite 4	1.54	2.04	1.91	3.2	4.9	5.94			1.86	6.97	7.81	4.81				1.1	1.57	1.74
Metabolite 5	0.77	0.85	0.8	0.84	0.71	0.73	0.73	0.78	0.85	0.87	0.88	0.9	0.76	0.79	0.85	0.68	0.81	0.88
Metabolite 6	0.93	0.94	0.97	0.89	0.6	0.61	0.88	0.94	0.9	0.9	0.83	0.88	0.85	0.89	0.82	0.74	0.81	0.78
Metabolite 7	0.56	0.55	0.61	0.27	0.19	0.23	0.85	0.97	0.91	0.43	0.41	0.53	0.93	0.82		0.71	0.75	0.73
Metabolite 8	0.21	0.22	0.3	0.17	0.14	0.15	0.66	0.66	0.89	0.14	0.23	0.29	0.82	0.86	0.79	0.69	0.85	0.87
Metabolite 9	0.77	0.89	0.81	0.74	0.59	0.61	0.79	0.87	0.9	0.82	0.83	0.79	0.88	0.9	0.9	0.87		
Metabolite 10	0.82	0.79	0.77	0.63	0.45	0.41	0.84	0.88	0.88	0.85	0.79	0.82				0.59	0.69	0.66

Group of substances with similar metabolic profiling

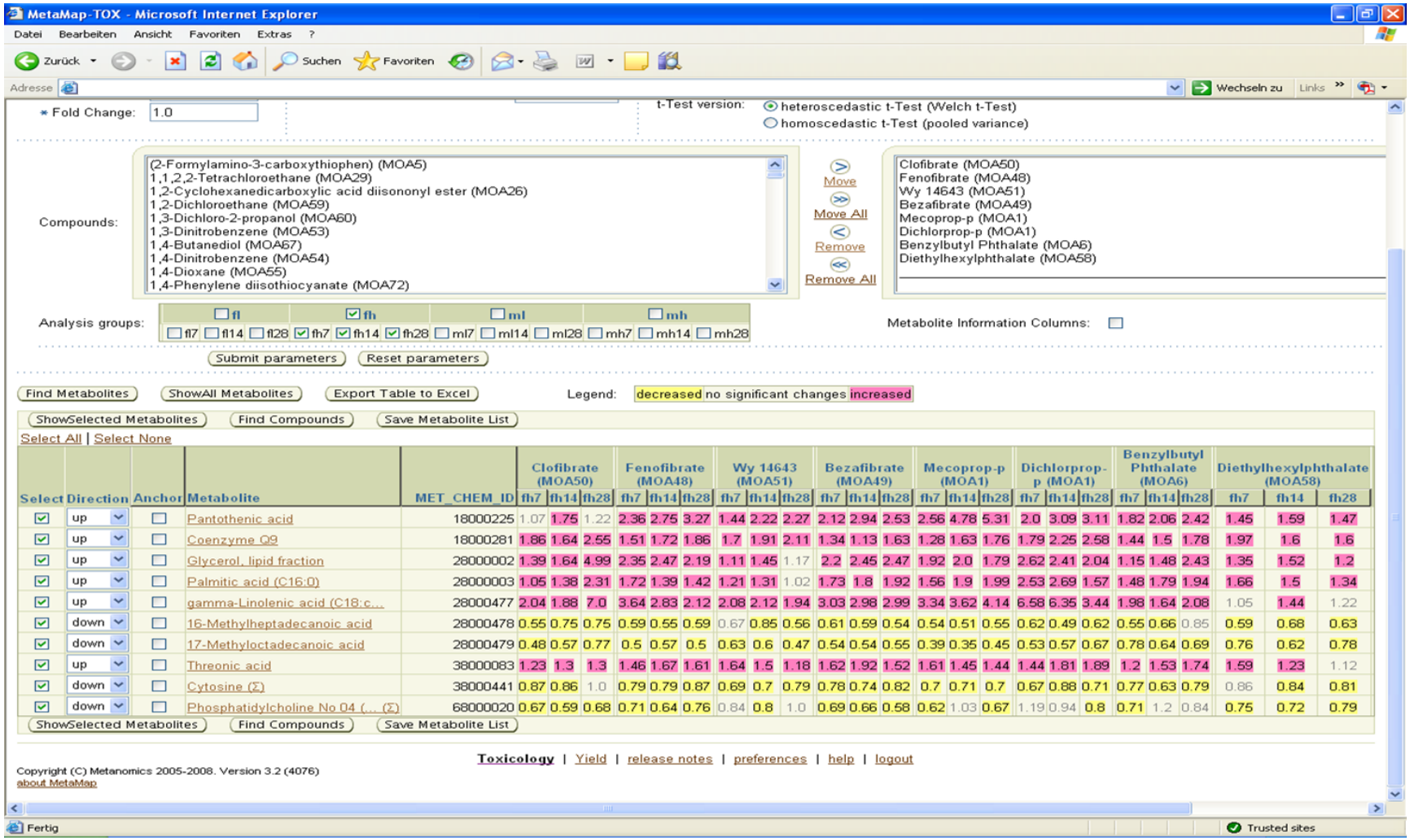


"Find Compounds"

Recognising mode-of-action: peroxisome proliferation



BASF
We create chemistry

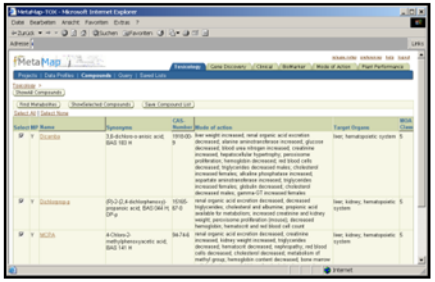


Pairwise Comparison: Clofibrate

Clofibrate (MOA50)	Pearson			Spearman		
Treatment	rp	p	rank	rs	p	rank
Clofibrate (MOA50)	1,00	0,00	**1**	1,00	0,00	1
Fenofibrate (MOA48)	0,79	0,00	**2**	0,70	0,00	3
Mecoprop-p (MOA58)	0,78	0,00	**3**	0,66	0,00	5
Pravastatin + Fenofibrat (MOAFW05)	0,73	0,00	**4**	0,63	0,00	11
Bezafibrate (MOA49)	0,72	0,00	**5**	0,62	0,00	14
Diethylhexylphthalate (MOA28)	0,71	0,00	**6**	0,61	0,00	16
Wy 14643 (MOA51)	0,71	0,00	**7**	0,64	0,00	9
Atorvastatin + Fenofibrat (MOAFW05)	0,70	0,00	**8**	0,63	0,00	10
Diethylhexylphthalate + Dibutyl phthalate	0,70	0,00	**9**	0,64	0,00	7
Diisopentylphthalate (MOA8)	0,70	0,00	**10**	0,62	0,00	13
Benzylbutyl Phthalate (MOA6)	0,68	0,00	**11**	0,56	0,00	21
Oxaliplatin (MOA69)	0,00	0,96	**544**	0,07	0,17	430
2-Butanone oxime (MOA18)	0,00	0,96	**545**	0,02	0,68	513
Bisphenol A (60 Kcal fat diet) (MOAFW04)	0,00	0,97	**546**	0,05	0,37	467
Ethyl benzene (MOA56)	0,00	0,98	**547**	-0,02	0,76	520
3-Chloro-1,2-propanediol (MOA56)	0,00	0,99	**548**	-0,09	0,08	388

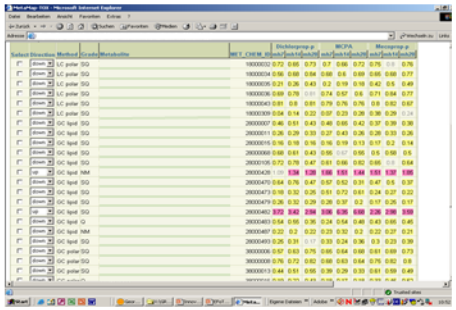
Assessment is based on joint evaluation of Pattern Ranking, TPC and Biochem Interpretation

Test Substance



"Find Metabolites"

Metabolite profile



Biochemical Interpretation

"Total Profile Comparison"

"Pattern Ranking"

Statistical Correlation - Ranking based on biological similarity

Similarity of test compound-induced changes compared to toxicity patterns

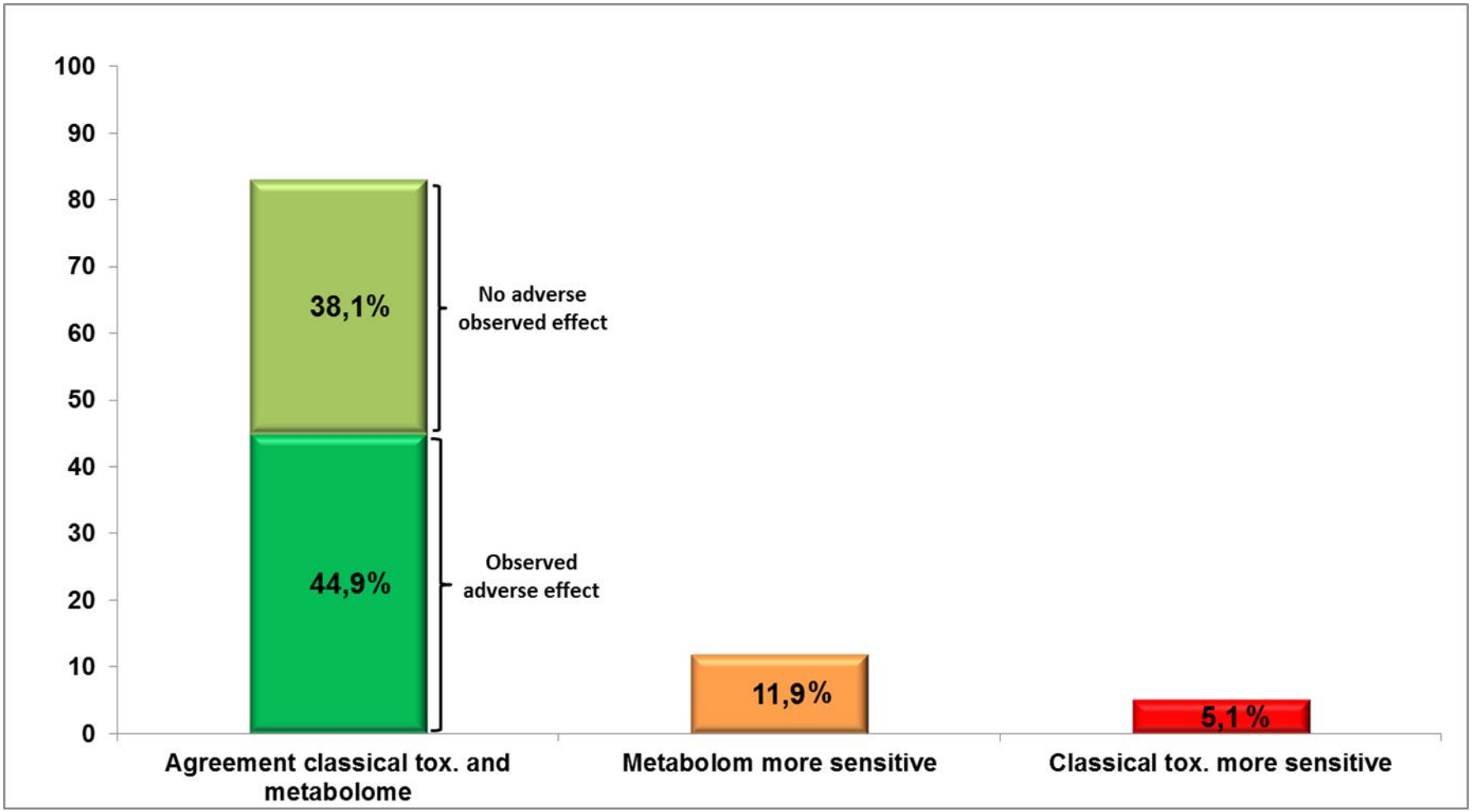
MetaMap®Tox: Assessment of predictivity

- Evaluation of MetaMap®Tox predictions against histopathology outcome

	Histopathology: positive	Histopathology: negative	Sum
MetaMap®Tox: positive	19 (83%)	3 (16%)	22
MetaMap®Tox: negative	4 (17 %)	16 (84%)	20
Sum	23	19	42

- Current Status (n=42): > 80 % correct predictions

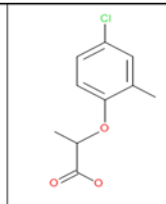
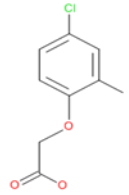
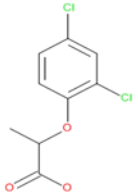
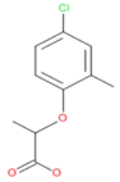
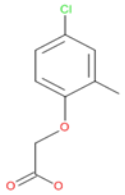
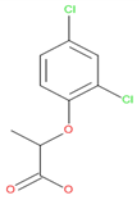
MetaMap®Tox: sensitivity analysis
A NOAEL analysis (N =120), based on routine studies



MetaMap™ Tox: Example 1
Phenoxy herbicides - Structures

- Target substance:
  MCPP (Mecoprop-P)
- Source substances:
  2,4-DP (Dichlorprop-P)
  MCPA
- Structurally similar
  - MCPP vs MCPA: methyl and chlorine substituent in the 2,4-position
  - MCPP vs 2,4-DP: phenoxy-propionic acids

Tanimoto scores

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

MetaMap™ Tox: Example 1
Phenoxy herbicides – Metabolic communalities

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		-	

- Very good overlap of metabolic profiles
- Common target organs: Liver and kidneys

MetaMap™ Tox: Example 1
Phenoxy herbicides – Best read-across option

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

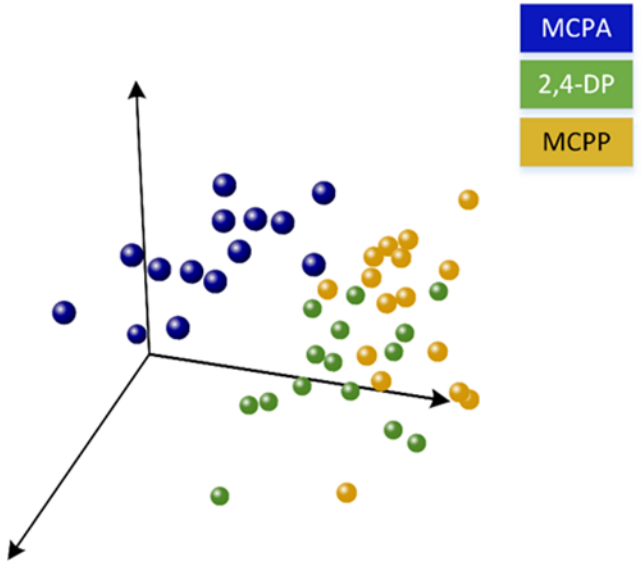
b)

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

Pair-wise (total profile) comparison:

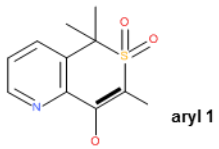
Best match with 2,4-DP (pearson r = 0.817), MCPA rank 25 (r =0,58). In between PPAR alpha agonists.

The PCA analysis of the 3 compounds demonstrates the same: 2,4-DP is closer to MCPP than MCPA

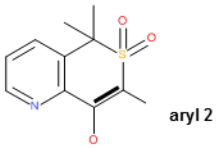


- **2,4-DP is the best read-across source substance**

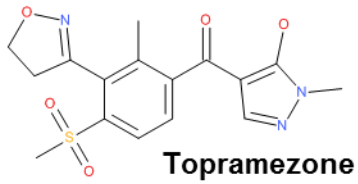
Example 2: HPPD-Inhibitors



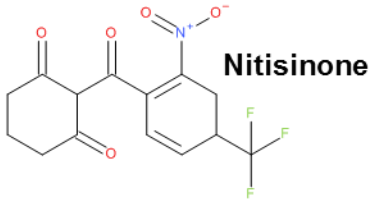
Coumarone 1



Coumarone 2



Topramezone



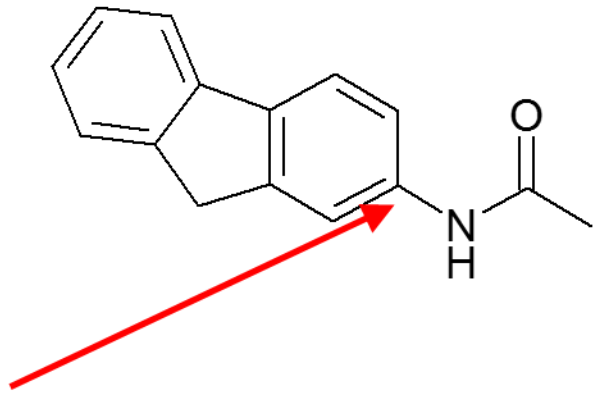
Reference: Topramezone, females, HD		
Treatment	Pearson correlation	
	r	rank
Topramezone, LD	0.895	1
Coumarone 4, LD	0.892	2
Nitisinone, HD	0.886	3
Topramezone, 2nd study, HD	0.886	4
Coumarone 3, LD	0.867	5
Coumarone 4, HD	0.866	6
Coumarone 3, HD	0.858	7
Nitisinone, LD	0.851	8
Coumarone 1, LD	0.862	9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

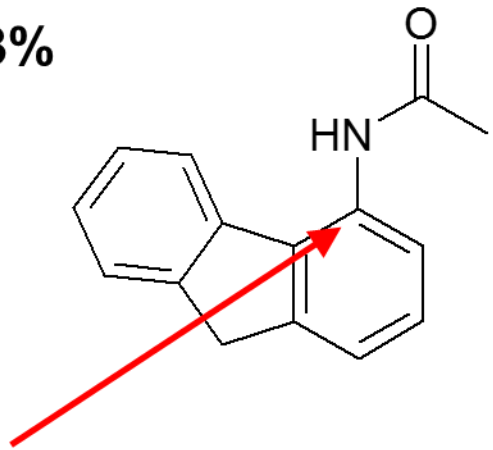
Tanimoto similarity using MACCS keys			
	Coumarone 1	Coumarone 2	Nitisinone
Coumarone 2	81.5%		
Nitisinone	48.1%	42.1%	
Topramezone	32.9%	27.8%	36.3%

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

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**	**1.25**	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	1.32	1.28	**1.44**	**1.43**	**1.85**	**1.64**	**2.27**
Tricosanoic acid (C23:0)	1.02	**1.20**	1.35	**1.24**	**1.39**	1.29	1.18	**1.46**	**1.45**	0.95	0.56	**1.50**

Example 3 : 2- and 4-Acetylaminofluorene Pair-wise (total) Profile Comparison

2-AAF

Treatment
2-Acetylaminofluorene (MOA65)
Treatment 433 (MOA65)
Treatment 386 (MOA69)
Treatment 382 (MOA44)
Treatment 213 (MOA71)
Treatment 209 (MOA35)
Treatment 253 (MOA65)
Treatment 441 (MOA58)
Treatment 185 (MOA69)
Treatment 404 (MOA52)

4-AAF

Treatment
4-Acetylaminofluorene (MOA64)
Treatment 424 (MOA64)
Treatment 493 (MOA64)
Treatment 345 (MOA12)
Treatment 378 (MOA64)
Treatment 368 (MOA32)
Treatment 239 (MOA60)
Treatment 144 (MOA63)
Treatment 320 (MOA72)
Treatment 209 (MOA35)

2-AAF and 4-AAF rank best with completely different compounds
2-AAF has a very low overall match with 4-AAF – rank 1443

Pattern identification different
Pair-wise comparison different
Metabolomics conclusion: 2-AAF and 4-AAF are biologically unequal

REACH: chemical grouping and read across

CONCLUSIONS

‘Omics technologies provide important data useful in read across

Biology based data should be more relevant than structure based data

From QSAR to QBAR
(quantitative biological activity relationships)