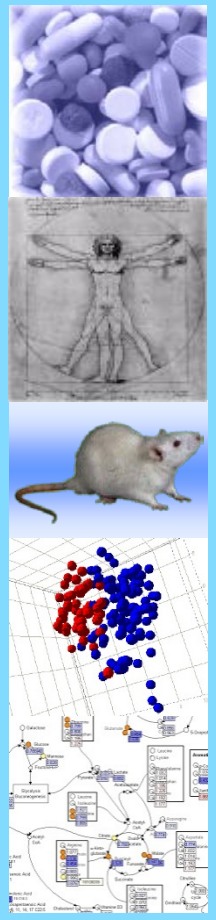

The use of Metabolomics In Toxicology

BASF SE
metanomics

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

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Why Metabolomics / Metabolic Profiling ?

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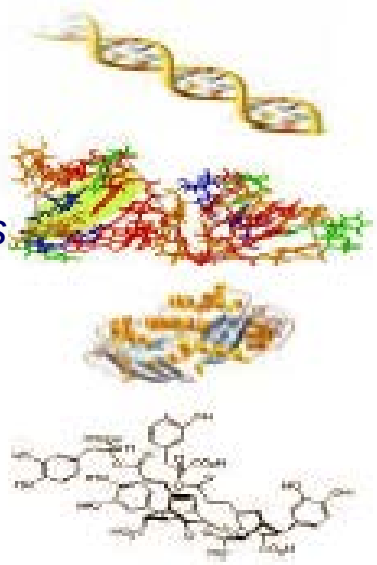
Mostly unknown

DNA
ten-thousands genes

RNA
hundert-thousands transcripts

Proteins
millions proteins

Metabolites
thousands metabolites



Genomics

Transcriptomics

Proteomics

Metabolomics

Mostly known

Advantage
Single matrix (blood)

Closer to classical toxicology

Three Profiling Data Sets from "One Analysis"

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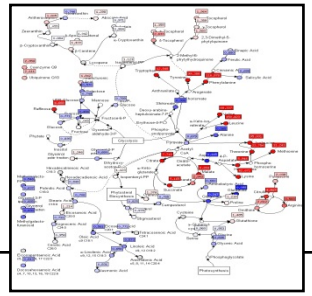
SAMPLE
ca. 100 µl plasma

Target Metabolites

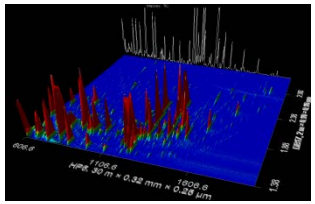
- **Currently 300** per sample matrix
- Structure ID established
- Sensitive detection at actual levels
- Absolute quantitation possible



Metabolite Structure Identification

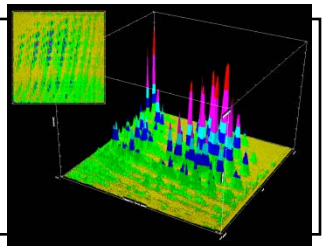
Known-Unknown Metabolites

- Additional ca. **500** compounds
- Metabolite ID not final but indexed
- Relative concentrations reliably measured

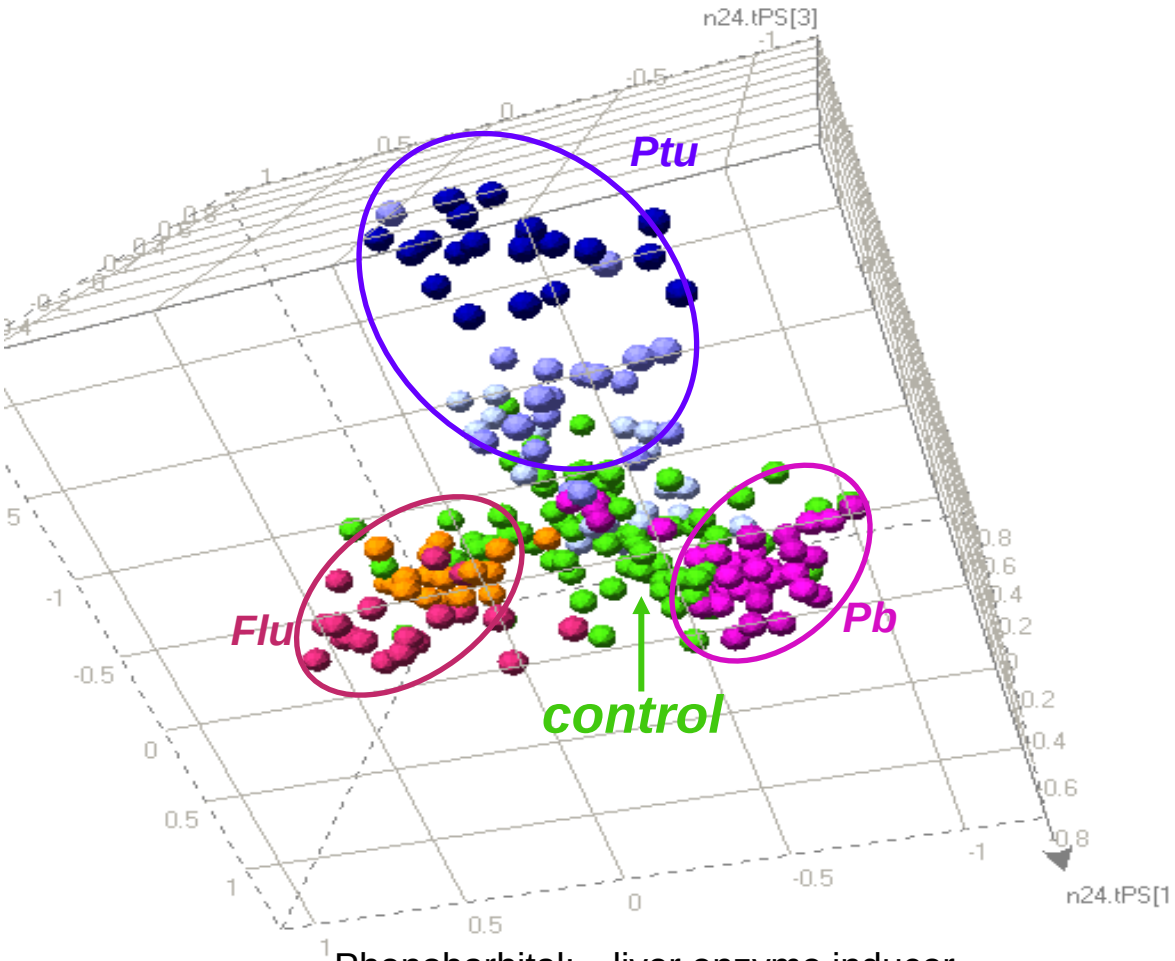


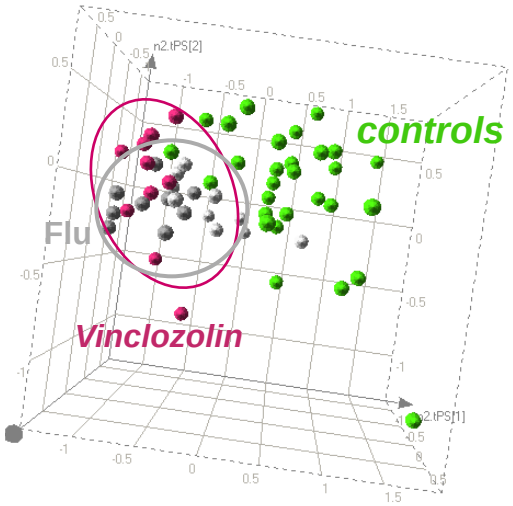
Total Metabolome Signature

- **Up to 9.000** analyte signals per sample
- Directly used in Data Mining
- Based on original 3D MS data sets



Results Toxicology: MoA Identification





☺ MoA Identification

☺ MoA Classification

☺ Dose-response relationship

Phenobarbital: liver enzyme inducer
Propylthiouracil: thyroid hormone formation blocker
Flutamide: anti-androgenic

Study design for the MetaMap™Tox database

- Four weeks studies with Crl:Wi(Han) rats
- Control, low (pharmacological) dose and high (toxicological) dose group
- Blood sampling d7, d14, d28

MetaMap®Tox – Identification of MoA / Toxicity

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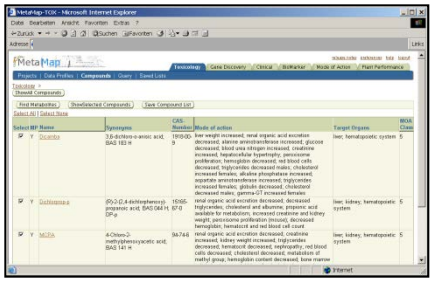
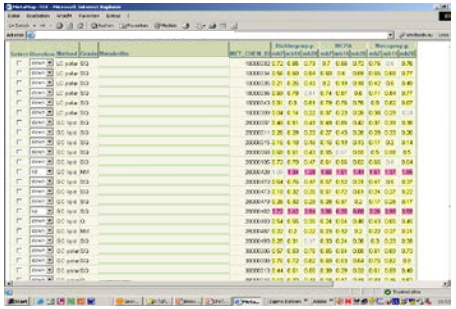
Test Substance



"Find Metabolites"

Metabolite profile



"Total Profile Comparison"

"Pattern Ranking"

Statistical Correlation - Ranking based on biological similarity

Similarity of test compound-induced changes compared to toxicity patterns

Conclusions: drawn on joint evaluation of Pattern Ranking, TPC and Biochem Interpretation

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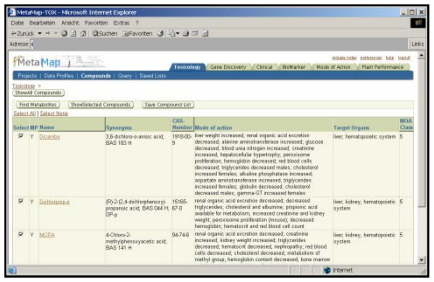
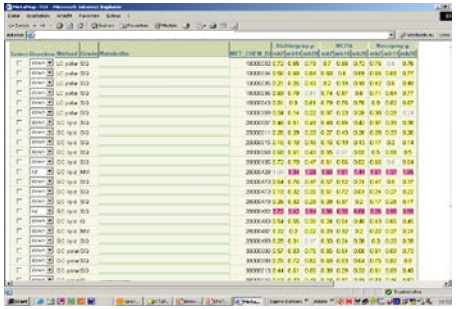
Test Substance



"Find Metabolites"

Metabolite profile



Biochemical Interpretation

"Total Profile Comparison"

"Pattern Ranking"

Treatment
2-Acetylaminofluorene
Treatment 433
Treatment 386
Treatment 382
Treatment 213
Treatment 209
Treatment 253

Statistical Correlation - Ranking based on biological similarity

Similarity of test compound-induced changes compared to toxicity patterns

The MetaMap®Tox Data Base

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Display of metabolites

Select	Direction	Anchor	Compound Name	MET_CHEM_ID	m7	m14	m28
	up		gamma-Linolenic acid (C18:c...	28000477	1.93	1.36	2.02
	down		16-Methylheptadecanoic acid	28000478	0.94	0.79	0.71
	down		17-Methyloctadecanoic acid	28000479	1.16	0.75	0.66
	up		dihomo-gamma-Linolenic acid...	28000482	1.55	1.46	2.58
	down		Eicosaenoic acid (C20:1) No 02	28000485	0.63	0.46	0.26
	up		threo-Sphingosine (d18:1) (Σ)	28000491	1.39	0.95	1.38
	down		Elaidic acid (C18:trans[9]1)	28000830	0.97	0.8	0.6
	up		myo-Inositol, lipid fraction	28001194	1.27	1.03	1.22
	up		Alanine	38000003	1.5	1.49	1.14
	up		Glycine	38000004	1.22	1.25	1.17
	up		Threonine	38000006	1.35	1.3	1.13
	up		Lysine	38000013	1.2	1.3	1.28
	up		Glucose (Σ)	38000037	1.43	1.17	0.95
	down		Glycerol, polar fraction	38000038	0.72	0.72	0.89
	up		Ornithine (Σ)	38000046	1.09	1.24	1.21
	up		Serine	38000052	1.47	1.52	1.18
	up		Proline	38000079	1.18	1.37	1.1
	up		Ascorbic acid (Σ)	38000159	1.38	1.2	1.1
	down		3-Hydroxybutyrate	38000393	0.52	0.61	0.77
	down		Ribal (Σ)	38000407	0.6	0.69	0.72
	down		trans-4-Hydroxyproline	38000437	0.79	0.89	0.75
	down		Cytosine (Σ)	38000441	0.59	0.73	0.75
	up		Arginine	38000459	1.18	1.26	1.09

The MetaMap®Tox Data Base

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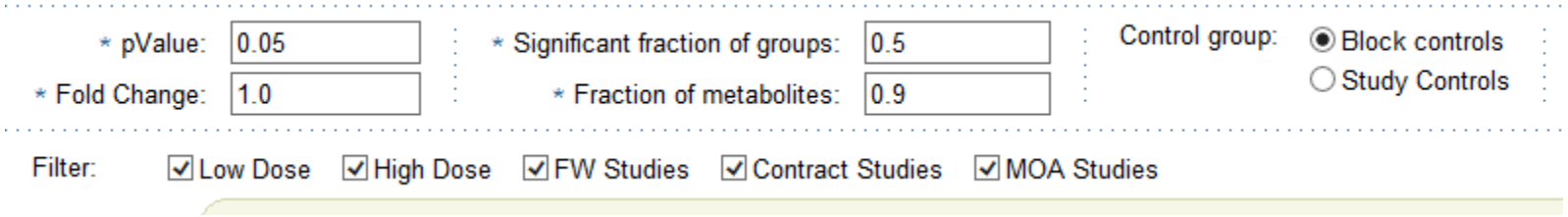
Some selected features:



Thyroid: Direct Effect: ETU & PTU

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MetaMap-TOX - Microsoft Internet Explorer

Select	Direction	Method	Grade	Metabolite	MET_CHEM_ID	Ethylenethiourea (MOA58) mh7	mh14	mh28	6-Propyl-2-thiouracil (MOA24) 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

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MetaMap-TOX - Microsoft Internet Explorer

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

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MetaMap-TOX - Microsoft Internet Explorer

Select None

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	0.54	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 (unless tox indicates that MoA can only be expected at 1 time point)
- Pattern must find 4th compound (at 90%) – adjust pattern if necessary
- If more same MoA compounds are available:
  pattern must be sensitive (find additional compounds sharing MoA) at 80% - adjust pattern if necessary
- Pattern must be selective at 80% (should not find compounds that do not have the MoA)

Pattern Verification

- With increasing numbers of compounds sharing the MoA; pattern must remain sensitive at 80% - adjust pattren if necessary
- If possible confirm pattern biochemically (include anchor metabolites)
- If possible use antagonist to MoA – metabolite direction should be oposite

Recognising mode-of-action: peroxisome proliferation

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Datei Bearbeiten Ansicht Favoriten Extras ?

Zurück Suchen Favoriten

Adresse Wechseln zu Links

* 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: ☐

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Legend: decreased | no significant changes | increased

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Select All | Select None

Select	Direction	Anchor	Metabolite	MET_CHEM_ID	Clofibrate (MOA50)			Fenofibrate (MOA48)			Wy 14643 (MOA51)		
					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
☑	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

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Recognising mode-of-action: peroxisome proliferation

Compounds found

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* 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

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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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Modes of action (MOA) patterns

- **Liver**
  - Enzyme induction
  - Peroxisome proliferation
  - Liver toxicity
- **Nervous system**
  - Dopamine agonism/antagonism
  - Noradrenaline agonism
  - Acetyl-cholinesterase inhibition
  - Nicotinic receptor agonist
- **Kidney**
  - Tubular toxicity
  - Organic anion transporter inhibition
- **Adrenals**
  - Synthesis inhibition
- **Testes**
  - Impaired spermatogenesis
- **Ovaries**
  - Estrogenic receptor modulation
- **Endocrine modulation**
  - Aromatase inhibition
  - Anti-androgenic effect
  - Estrogenic effects
- **Thyroid**
  - Direct: hormone synthesis inhibition
  - Indirect: increased metabolism
- **Blood**
  - Porphyrine synthese inhibition
  - Aplastische anemia
  - Hemolytic anemia
  - Platelet aggregation inhibition
- **Bone**
  - Osteoblast inhibition
  - Mineralisation
- **Dodenum**
- **Eye**
- **Stress**
- **.....**

Pattern Ranking

Example: Vinclozoline

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Pattern	n	Q10	Q50	Q90	Vinclozolin (MOA22) r (i) median	Plot r(i) median
Liver_Tox_F_HD_Group_24_1_08_W	28	0.78	0.89	0.94	0.92	
Liver_Enzyme_Ind_F_Group_24_01_08_W_RV	38	0.82	0.90	0.94	0.92	
Adrenals_Steroid_synth_Inhib_F_HD_W_Group_15_05_08	3	0.78	0.79	0.91	0.90	
Liver_Enzyme_Induction_M_HD_Group_24_01-08_W_RV	50	0.80	0.90	0.94	0.86	
Thyroid_indirect_Liver_II Induction_HD_F_W_RV_CU	13	0.84	0.90	0.94	0.85	
adrenals_cortex_synth_inhib_HD_M_W_Group_10_04_08	3	0.71	0.78	0.85	0.85	
Thyroid_indirect_Liver II enzyme ind_HD_M_W_Group_21-2-08	24	0.81	0.88	0.93	0.84	
Hormones_antiandrogen_HD_M_group_Welch_wm	6	0.75	0.86	0.93	0.83	
hormones_antiandrogen_receptor_antagonist_prostate_HD_M_W_Group_19_06_08	3	0.67	0.77	0.79	0.79	
Eye_HPPD_Inhib_M_HD_W_Group_15_05_08	4	0.82	0.84	0.98	0.26	
Liver_Peroxi_Prolif_M_HD_Group_24_01_08_W_RV	20	0.80	0.89	0.95	0.04	
Hormones_estrogen_M_HD_W_Group_28_08_08	3	0.81	0.87	0.88	-0.06	
Kidney_inhib_weak_org_acid_M_HD_W_Group_21_2_08	6	0.70	0.78	0.90	-0.09	
Hormones Estrogen Rez. Modulator F HD Group Str 24 01 08 W	2	0.84	0.84	0.84	-0.11	
Eye_HPPD_Inhib_F_HD_W_Group_15_05_08	4	0.81	0.94	0.98	-0.15	
Blood porphyrin inhibition m HD group 24 01 08 Str W	2	0.88	0.88	0.88	-0.26	
Hormones_Estrogens_F_HD_W_Group	3	0.83	0.84	0.90	-0.31	

graphic	meaning
black box	Q10..Q90 of pattern
black line	median of pattern
red dot	median r(i)
red ticks	Q10..Q90 of r(i)

Biochemical analysis

Evaluate metabolite changes from a biochemical perspective

→ Confirmation of MoA (for pattern development)
→ Establish anchor metabolites (enhances selectivity)

→ Determination of MoA (for new compounds)

A metabolomics pathway analysis for direct thyroid toxicity for male rats

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Select	Direction	Compound Name	Class	Subclass	Methimazole (MOA51) HD			Ethylenethiourea (MOA58) HD			6-Propyl-2-thiouracil (MOA24) HD		
					m7	m14	m28	m7	m14	m28	m7	m14	m28
☑	down	5-Oxoproline (Σ)	Amino acids related	Amino acid metabolites	0.93	0.83	0.71	0.88	0.91	0.93	0.9	0.89	0.79
☑	down	Glutamate	Amino acids	Amino acids, acidic	0.61	0.57	0.46	1.07	0.72	0.71	0.78	0.78	0.65
☑	down	Glutamate	Amino acids	Amino acids, acidic	0.74	0.49	0.4	1.15	0.82	0.77	1.02	0.7	0.49
☑	up	Tryptophan	Amino acids	Amino acids, aromatic	1.14	1.18	0.96	1.12	1.24	1.26	1.28	1.36	1.24
☑	up	Tyrosine	Amino acids	Amino acids, aromatic	1.28	1.38	1.23	1.03	1.08	1.11	1.19	1.27	1.45
☑	up	Arginine	Amino acids	Amino acids, basic	1.09	1.09	1.14	1.24	1.26	1.23	1.09	1.1	1.35
☑	down	Citrate	Energy metabolism and related	Citrate cycle	0.69	0.69	0.73	0.86	0.81	0.64	0.92	0.73	0.66
☑	down	Creatinine (Σ)	Amino acids related	Creatine metabolism	0.24	0.37	0.98	0.64	0.72	0.68	0.53	0.66	0.7
☑	down	DAG (C18:1,C18:2)	Complex lipids, fatty acids and related	Diacylglycerols	0.59	0.7	0.66	0.96	0.86	0.69	0.77	0.69	0.57
☑	up	Campesterol	Miscellaneous	Diet related	1.42	1.58	1.44	1.64	1.59	1.69	1.12	1.17	1.3
☑	down	17-Methyloctadecanoic acid	Complex lipids, fatty acids and related	Fatty acids, branched	1.36	0.92	0.45	0.96	0.83	0.89	1.08	0.67	0.7
☑	down	Linolenic acid (C18:cis[9,1...	Complex lipids, fatty acids and related	Fatty acids, poly-unsaturated	0.7	0.8	0.7	0.91	0.84	0.68	0.78	0.61	0.57
☑	up	dihomo-gamma-Linolenic acid...	Complex lipids, fatty acids and related	Fatty acids, poly-unsaturated	2.38	3.18	2.09	2.06	2.17	2.03	1.04	1.33	1.5
☑	up	Lignoceric acid (C24:0)	Complex lipids, fatty acids and related	Fatty acids, saturated	2.12	2.82	2.16	1.94	1.66	2.07	1.28	1.12	1.16
☑	up	Tricosanoic acid (C23:0)	Complex lipids, fatty acids and related	Fatty acids, saturated	1.93	2.69	2.5	1.67	2.01	2.39	1.28	1.1	1.55
☑	down	Isopalmitic acid (C16:0)	Complex lipids, fatty acids and related	Fatty acids, saturated	1.13	0.83	0.52	0.97	0.96	0.77	0.96	0.83	0.62
☑	down	Thyroxine (T4)	Hormones, signal substances and related	Other hormones, signal substances and related	0.11	0.04	0.02	0.43	0.43	0.48	0.07	0.07	0.08
☑	up	4-Hydroxysphinganine (t18:0...	Complex lipids, fatty acids and related	Sphingolipids	2.0	3.12	3.11	1.59	1.53	2.11	1.18	1.13	1.24
☑	up	3-O-Methylsphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	3.59	3.98	4.59	2.24	2.37	2.96	1.6	1.4	1.92
☑	up	threo-Sphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	2.91	3.72	4.28	1.98	1.94	2.26	1.31	1.24	1.41
☑	up	5-O-Methylsphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	3.41	4.75	4.37	2.33	2.15	2.84	1.51	1.2	1.79
☑	up	erythro-Sphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	2.91	3.71	3.44	2.21	2.09	2.63	1.46	1.3	1.47
☑	up	Sphingomyelin (d18:2,C16:0)	Complex lipids, fatty acids and related	Sphingomyelins	1.28	1.06	0.88	1.72	1.85	1.53	0.96	1.15	1.63
☑	up	Sphingomyelin (d18:1,C16:0)	Complex lipids, fatty acids and related	Sphingomyelins	2.24	2.27	1.92	1.65	1.69	1.79	1.35	1.35	1.71
☑	up	Sphingomyelin (d18:1,C16:0)	Complex lipids, fatty acids and related	Sphingomyelins	1.27	1.21	1.21	1.1	1.06	1.35	0.94	1.19	1.57
☑	down	TAG (C18:2,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.4	0.32	0.35	0.61	0.87	0.61	0.47	0.18	0.14
☑	down	TAG (C16:0,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.49	0.44	0.48	0.74	0.76	0.7	0.52	0.21	0.2
☑	down	TAG (C18:1,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.41	0.3	0.35	0.95	0.76	0.69	0.44	0.22	0.21
☑	down	TAG (C16:0,C16:1)	Complex lipids, fatty acids and related	Triacylglycerols	0.37	0.28	0.2	0.45	0.48	0.4	0.64	0.33	0.31
☑	down	TAG (C18:2,C18:3)	Complex lipids, fatty acids and related	Triacylglycerols	0.57	0.34	0.63	0.9	0.67	0.69	0.39	0.18	0.24
☑	down	TAG (C18:2,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.35	0.28	0.31	0.69	0.71	0.58	0.42	0.21	0.16

A metabolomics pathway analysis for direct thyroid toxicity for male rats

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Select	Direction	Compound Name	Class	Subclass	Methimazole (MOA51) HD			Ethylenethiourea (MOA58) HD			6-Propyl-2-thiouracil (MOA24) HD		
					m7	m14	m28	m7	m14	m28	m7	m14	m28
☑	down	5-Oxoproline (Σ)	Amino acids related	Amino acid metabolites	0.93	0.83	0.71	0.88	0.91	0.93	0.9	0.89	0.79
☑	down	Glutamate	Amino acids	Amino acids, acidic	0.61	0.57	0.46	1.07	0.72	0.71	0.78	0.78	0.65
☑	down	Glutamate	Amino acids	Amino acids, acidic	0.74	0.49	0.4	1.15	0.82	0.77	1.02	0.7	0.49
☑	up	Tryptophan	Amino acids	Amino acids, aromatic	1.14	1.18	0.96	1.12	1.24	1.26	1.28	1.36	1.24
☑	up	Tyrosine	Amino acids	Amino acids, aromatic	1.28	1.38	1.23	1.03	1.08	1.11	1.19	1.27	1.45
☑	up	Arginine	Amino acids	Amino acids, basic	1.09	1.09	1.14	1.24	1.26	1.23	1.09	1.1	1.35
☑	down	Citrate	Energy metabolism and related	Citrate cycle	0.69	0.69	0.73	0.86	0.81	0.64	0.92	0.73	0.66
☑	down	Creatinine (Σ)	Amino acids related	Creatine metabolism	0.24	0.37	0.98	0.64	0.72	0.68	0.53	0.66	0.7
☑	down	DAG (C18:1,C18:2)	Complex lipids, fatty acids and related	Diacylglycerols	0.59	0.7	0.66	0.96	0.86	0.69	0.77	0.69	0.57
☑	up	Campesterol	Miscellaneous	Diet related	1.42	1.58	1.44	1.64	1.59	1.69	1.12	1.17	1.3
☑	down	17-Methyloctadecanoic acid	Complex lipids, fatty acids and related	Fatty acids, branched	1.36	0.92	0.45	0.96	0.83	0.89	1.08	0.67	0.7
☑	down	Linolenic acid (C18:cis[9,1...	Complex lipids, fatty acids and related	Fatty acids, poly-unsaturated	0.7	0.8	0.7	0.91	0.84	0.68	0.78	0.61	0.57
☑	up	dihomo-gamma-Linolenic acid...	Complex lipids, fatty acids and related	Fatty acids, poly-unsaturated	2.38	3.18	2.09	2.06	2.17	2.03	1.04	1.33	1.5
☑	up	Lignoceric acid (C24:0)	Complex lipids, fatty acids and related	Fatty acids, saturated	2.12	2.82	2.16	1.94	1.66	2.07	1.28	1.12	1.16
☑	up	Tricosanoic acid (C23:0)	Complex lipids, fatty acids and related	Fatty acids, saturated	1.93	2.69	2.5	1.67	2.01	2.39	1.28	1.1	1.55
☑	down	Isopalmitic acid (C16:0)	Complex lipids, fatty acids and related	Fatty acids, saturated	1.13	0.83	0.52	0.97	0.96	0.77	0.96	0.83	0.62
☑	down	Thyroxine (T4)	Hormones, signal substances and related	Other hormones, signal substances and related	0.11	0.04	0.02	0.43	0.43	0.48	0.07	0.07	0.08
☑	up	4-Hydroxysphinganine (t18:0...	Complex lipids, fatty acids and related	Sphingolipids	2.0	3.12	3.11	1.59	1.53	2.11	1.18	1.13	1.24
☑	up	3-O-Methylsphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	3.59	3.98	4.59	2.24	2.37	2.96	1.6	1.4	1.92
☑	up	threo-Sphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	2.91	3.72	4.28	1.98	1.94	2.26	1.31	1.24	1.41
☑	up	5-O-Methylsphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	3.41	4.75	4.37	2.33	2.15	2.84	1.51	1.2	1.79
☑	up	erythro-Sphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	2.91	3.71	3.44	2.21	2.09	2.63	1.46	1.3	1.47
☑	up	Sphingomyelin (d18:2,C16:0)	Complex lipids, fatty acids and related	Sphingomyelins	1.28	1.06	0.88	1.72	1.85	1.53	0.96	1.15	1.63
☑	up	Sphingomyelin (d18:1,C16:0)	Complex lipids, fatty acids and related	Sphingomyelins	2.24	2.27	1.92	1.65	1.69	1.79	1.35	1.35	1.71
☑	up	Sphingomyelin (d18:1,C16:0)	Complex lipids, fatty acids and related	Sphingomyelins	1.27	1.21	1.21	1.1	1.06	1.35	0.94	1.19	1.57
☑	down	TAG (C18:2,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.4	0.32	0.35	0.61	0.87	0.61	0.47	0.18	0.14
☑	down	TAG (C16:0,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.49	0.44	0.48	0.74	0.76	0.7	0.52	0.21	0.2
☑	down	TAG (C18:1,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.41	0.3	0.35	0.95	0.76	0.69	0.44	0.22	0.21
☑	down	TAG (C16:0,C16:1)	Complex lipids, fatty acids and related	Triacylglycerols	0.37	0.28	0.2	0.45	0.48	0.4	0.64	0.33	0.31
☑	down	TAG (C18:2,C18:3)	Complex lipids, fatty acids and related	Triacylglycerols	0.57	0.34	0.63	0.9	0.67	0.69	0.39	0.18	0.24
☑	down	TAG (C18:2,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.35	0.28	0.31	0.69	0.71	0.58	0.42	0.21	0.16

A metabolomics pathway analysis for direct thyroid toxicity for male rats

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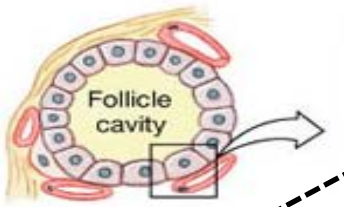
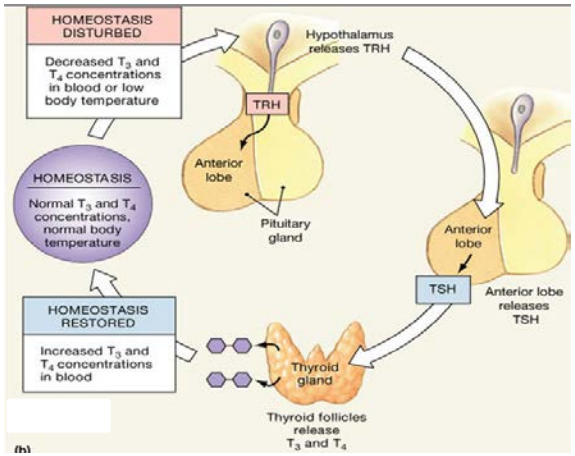
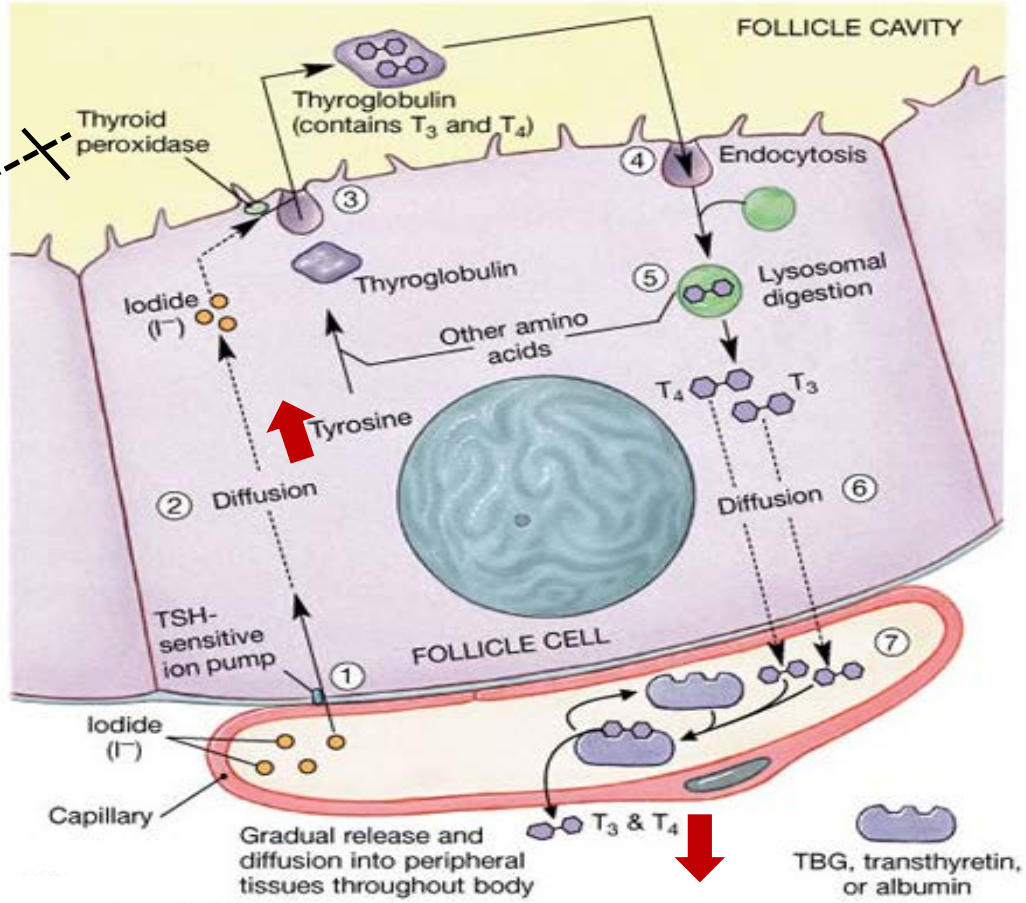


Thioamides

Thyroid system





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A metabolomics pathway analysis for direct thyroid toxicity for male rats

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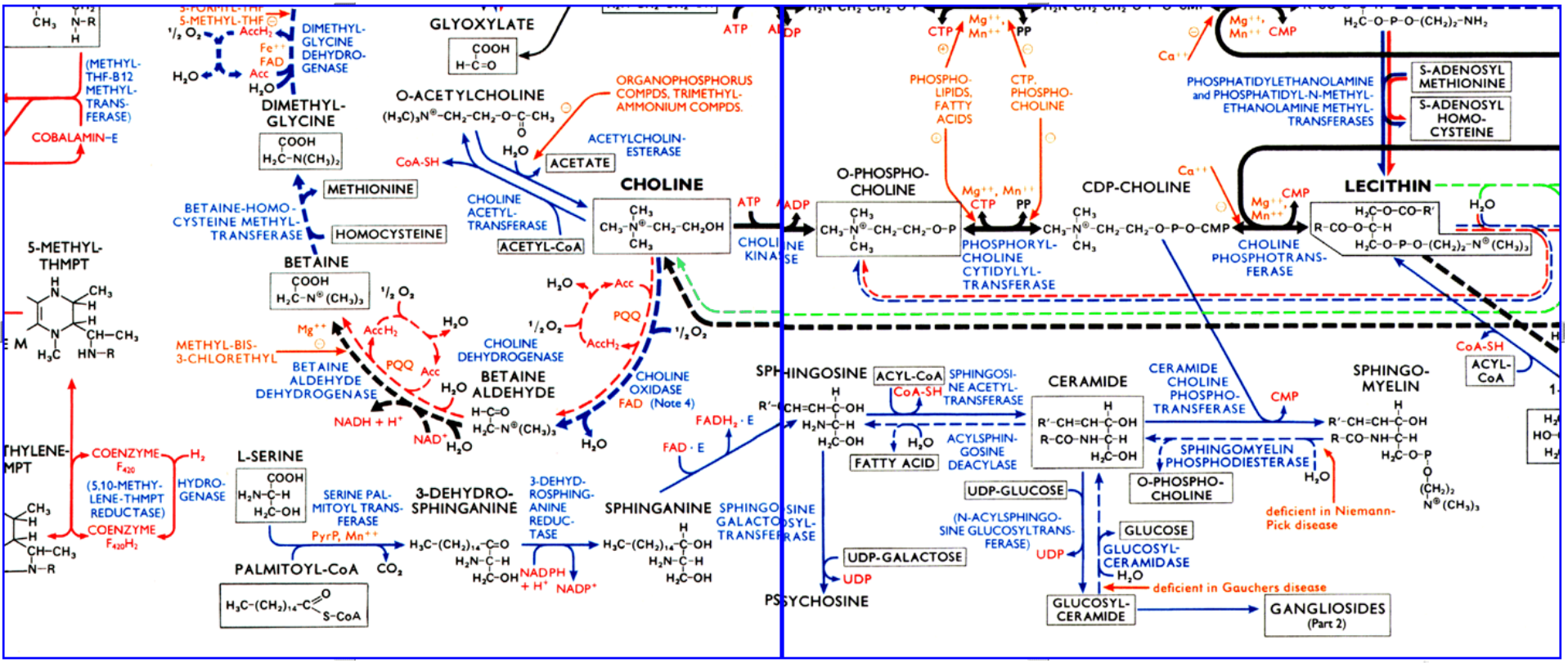
Select	Direction	Compound Name	Class	Subclass	Methimazole (MOA51) HD			Ethylenethiourea (MOA58) HD			6-Propyl-2-thiouracil (MOA24) HD		
					m7	m14	m28	m7	m14	m28	m7	m14	m28
☑	down	5-Oxoproline (Σ)	Amino acids related	Amino acid metabolites	0.93	0.83	0.71	0.88	0.91	0.93	0.9	0.89	0.79
☑	down	Glutamate	Amino acids	Amino acids, acidic	0.61	0.57	0.46	1.07	0.72	0.71	0.78	0.78	0.65
☑	down	Glutamate	Amino acids	Amino acids, acidic	0.74	0.49	0.4	1.15	0.82	0.77	1.02	0.7	0.49
☑	up	Tryptophan	Amino acids	Amino acids, aromatic	1.14	1.18	0.96	1.12	1.24	1.26	1.28	1.36	1.24
☑	up	Tyrosine	Amino acids	Amino acids, aromatic	1.28	1.38	1.23	1.03	1.08	1.11	1.19	1.27	1.45
☑	up	Arginine	Amino acids	Amino acids, basic	1.09	1.09	1.14	1.24	1.26	1.23	1.09	1.1	1.35
☑	down	Citrate	Energy metabolism and related	Citrate cycle	0.69	0.69	0.73	0.86	0.81	0.64	0.92	0.73	0.66
☑	down	Creatinine (Σ)	Amino acids related	Creatine metabolism	0.24	0.37	0.98	0.64	0.72	0.68	0.53	0.66	0.7
☑	down	DAG (C18:1,C18:2)	Complex lipids, fatty acids and related	Diacylglycerols	0.59	0.7	0.66	0.96	0.86	0.69	0.77	0.69	0.57
☑	up	Campesterol	Miscellaneous	Diet related	1.42	1.58	1.44	1.64	1.59	1.69	1.12	1.17	1.3
☑	down	17-Methyloctadecanoic acid	Complex lipids, fatty acids and related	Fatty acids, branched	1.36	0.92	0.45	0.96	0.83	0.89	1.08	0.67	0.7
☑	down	Linolenic acid (C18:cis[9,1...	Complex lipids, fatty acids and related	Fatty acids, poly-unsaturated	0.7	0.8	0.7	0.91	0.84	0.68	0.78	0.61	0.57
☑	up	dihomo-gamma-Linolenic acid...	Complex lipids, fatty acids and related	Fatty acids, poly-unsaturated	2.38	3.18	2.09	2.06	2.17	2.03	1.04	1.33	1.5
☑	up	Lignoceric acid (C24:0)	Complex lipids, fatty acids and related	Fatty acids, saturated	2.12	2.82	2.16	1.94	1.66	2.07	1.28	1.12	1.16
☑	up	Tricosanoic acid (C23:0)	Complex lipids, fatty acids and related	Fatty acids, saturated	1.93	2.69	2.5	1.67	2.01	2.39	1.28	1.1	1.55
☑	down	Isopalmitic acid (C16:0)	Complex lipids, fatty acids and related	Fatty acids, saturated	1.13	0.83	0.52	0.97	0.96	0.77	0.96	0.83	0.62
☑	down	Thyroxine (T4)	Hormones, signal substances and related	Other hormones, signal substances and related	0.11	0.04	0.02	0.43	0.43	0.48	0.07	0.07	0.08
☑	up	4-Hydroxysphinganine (t18:0...	Complex lipids, fatty acids and related	Sphingolipids	2.0	3.12	3.11	1.59	1.53	2.11	1.18	1.13	1.24
☑	up	3-O-Methylsphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	3.59	3.98	4.59	2.24	2.37	2.96	1.6	1.4	1.92
☑	up	threo-Sphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	2.91	3.72	4.28	1.98	1.94	2.26	1.31	1.24	1.41
☑	up	5-O-Methylsphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	3.41	4.75	4.37	2.33	2.15	2.84	1.51	1.2	1.79
☑	up	erythro-Sphingosine (d18:1) (Σ)	Complex lipids, fatty acids and related	Sphingolipids	2.91	3.71	3.44	2.21	2.09	2.63	1.46	1.3	1.47
☑	up	Sphingomyelin (d18:2,C16:0)	Complex lipids, fatty acids and related	Sphingomyelins	1.28	1.06	0.88	1.72	1.85	1.53	0.96	1.15	1.63
☑	up	Sphingomyelin (d18:1,C16:0)	Complex lipids, fatty acids and related	Sphingomyelins	2.24	2.27	1.92	1.65	1.69	1.79	1.35	1.35	1.71
☑	up	Sphingomyelin (d18:1,C16:0)	Complex lipids, fatty acids and related	Sphingomyelins	1.27	1.21	1.21	1.1	1.06	1.35	0.94	1.19	1.57
☑	down	TAG (C18:2,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.4	0.32	0.35	0.61	0.87	0.61	0.47	0.18	0.14
☑	down	TAG (C16:0,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.49	0.44	0.48	0.74	0.76	0.7	0.52	0.21	0.2
☑	down	TAG (C18:1,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.41	0.3	0.35	0.95	0.76	0.69	0.44	0.22	0.21
☑	down	TAG (C16:0,C16:1)	Complex lipids, fatty acids and related	Triacylglycerols	0.37	0.28	0.2	0.45	0.48	0.4	0.64	0.33	0.31
☑	down	TAG (C18:2,C18:3)	Complex lipids, fatty acids and related	Triacylglycerols	0.57	0.34	0.63	0.9	0.67	0.69	0.39	0.18	0.24
☑	down	TAG (C18:2,C18:2)	Complex lipids, fatty acids and related	Triacylglycerols	0.35	0.28	0.31	0.69	0.71	0.58	0.42	0.21	0.16

A metabolomics pathway analysis for direct thyroid toxicity for male rats



Adapted from Hannun 2002 and Wang 2010

ExPASy, 2013

Total Pattern Comparison
(pairwise comparison)

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Compare the total metabolome of compound X with all of the other metabolomes available in the data base

Establish a ranking according to similarity

Evaluate toxicity for best matches (e.g. > 95 percentile);
look for common denominators within this group

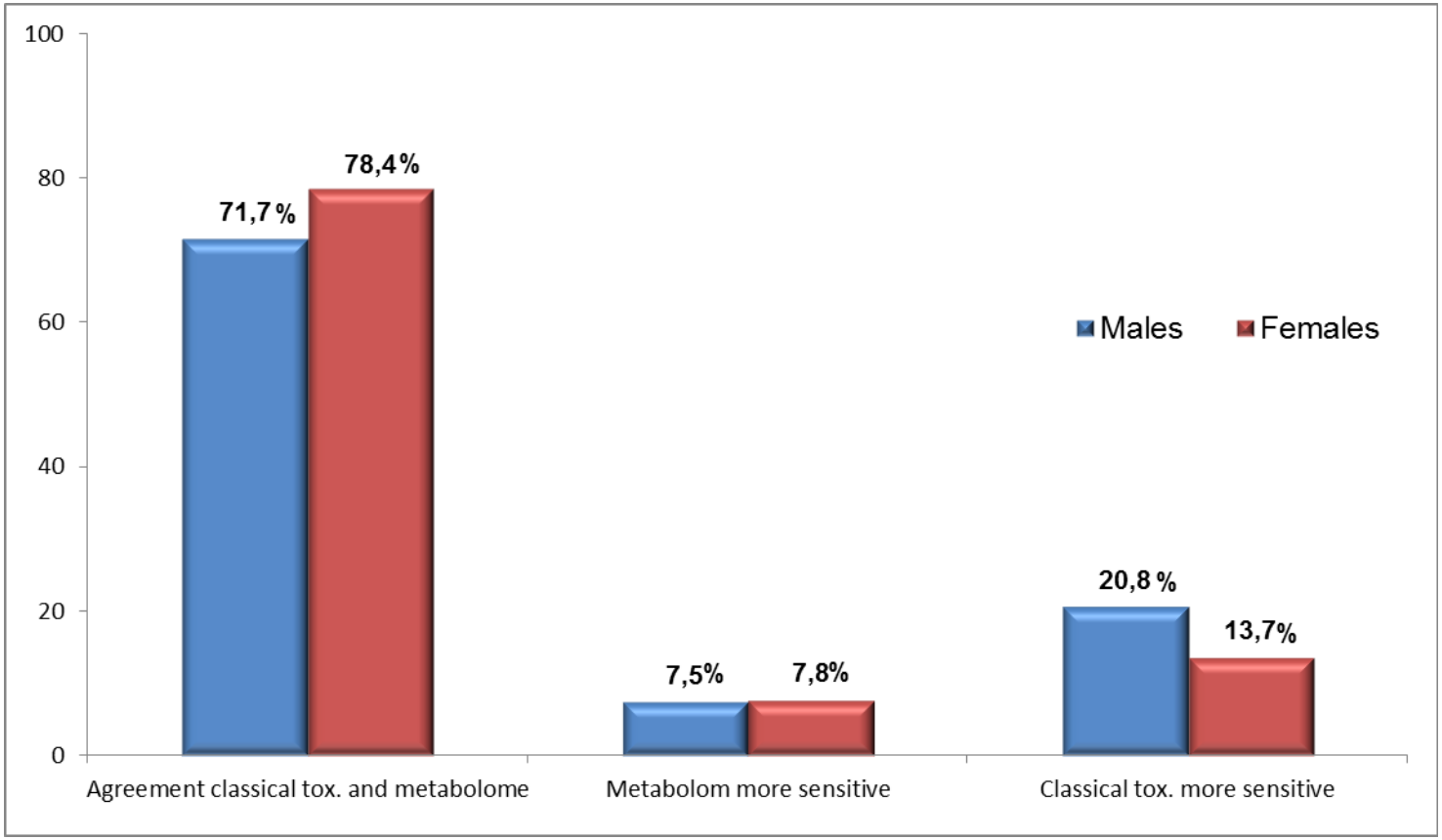
Pairwise Comparison

Example 2: Clofibrate

Clofibrate (MOA50)	Pearson			Spearman			norm. vector product		
Treatment	rp	p	rank	rs	p	rank	nvp	p	rank
Clofibrate (MOA50)	1,00	0,00	**1**	1,00	0,00	1	1,00	0,00	1
Fenofibrate (MOA48)	0,79	0,00	**2**	0,70	0,00	3	0,80	0,00	2
Mecoprop-p (MOA58)	0,78	0,00	**3**	0,66	0,00	5	0,76	0,00	4
Pravastatin + Fenofibrat (MOAFW05)	0,73	0,00	**4**	0,63	0,00	11	0,73	0,00	7
Bezafibrate (MOA49)	0,72	0,00	**5**	0,62	0,00	14	0,70	0,00	12
Diethylhexylphthalate (MOA28)	0,71	0,00	**6**	0,61	0,00	16	0,69	0,00	14
Wy 14643 (MOA51)	0,71	0,00	**7**	0,64	0,00	9	0,75	0,00	5
Atorvastatin + Fenofibrat (MOAFW05)	0,70	0,00	**8**	0,63	0,00	10	0,74	0,00	6
Diethylhexylphthalate + Dibutyl phthalate	0,70	0,00	**9**	0,64	0,00	7	0,66	0,00	18
Diisopentylphthalate (MOA8)	0,70	0,00	**10**	0,62	0,00	13	0,70	0,00	11
Benzylbutyl Phthalate (MOA6)	0,68	0,00	**11**	0,56	0,00	21	0,71	0,00	10
Oxaliplatin (MOA69)	0,00	0,96	**544**	0,07	0,17	430	0,04	0,27	458
2-Butanone oxime (MOA18)	0,00	0,96	**545**	0,02	0,68	513	-0,15	0,00	277
Bisphenol A (60 Kcal fat diet) (MOAFW04)	0,00	0,97	**546**	0,05	0,37	467	0,07	0,08	393
Ethyl benzene (MOA56)	0,00	0,98	**547**	-0,02	0,76	520	-0,04	0,31	463
3-Chloro-1,2-propanediol (MOA56)	0,00	0,99	**548**	-0,09	0,08	388	0,10	0,02	341

MetaMap®Tox: Sensitivity

Gender specific evaluation of NOAEL values for metabolomics versus classical toxicology (n=104; 53 males, 51 females)



MetaMap®Tox: Predictivity (for adverse effects)

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85 % correct predictions

males

	Histopathology: pos.	Histopathology: neg.	Sum
MetaMap®Tox: positive	**36 (84%)**	5 (14%)	41
MetaMap®Tox: negative	7 (16 %)	30 (86%)	37
Sum	43	35	78

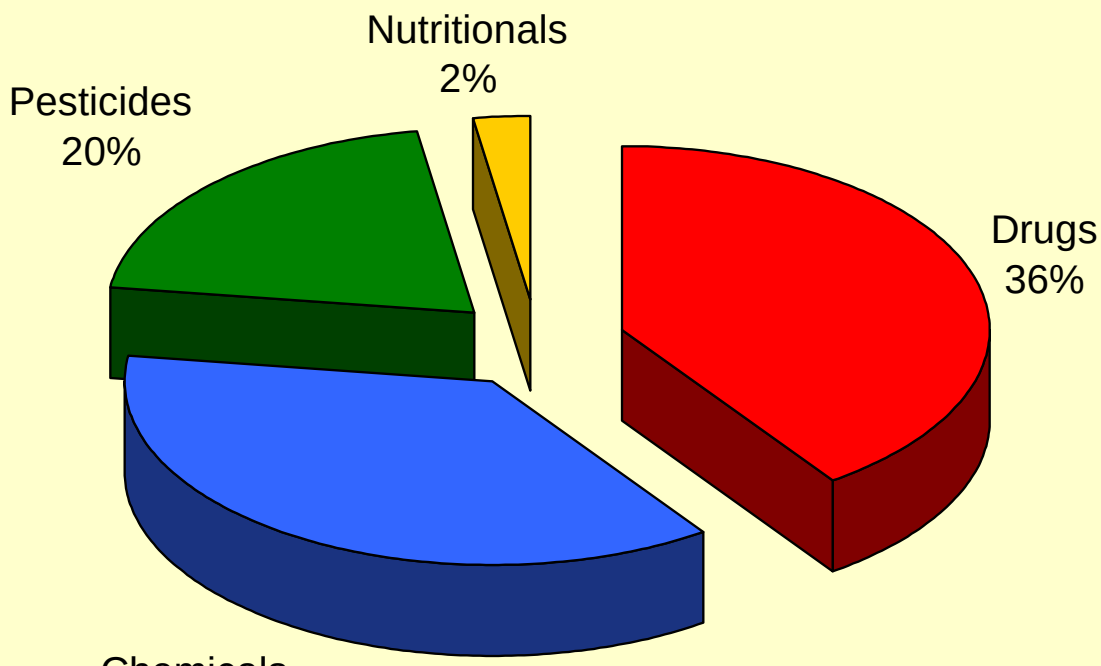
females

	Histopathology: pos.	Histopathology: neg.	Sum
MetaMap®Tox: positive	**36 (86%)**	6 (17%)	42
MetaMap®Tox: negative	6 (14 %)	30 (83%)	36
Sum	42	36	78

„Big Data“

- **Collect and assess control groups over time**
- **Include exact repeats of different treatments regularly**
- **Use collective of all data (particularly controls) to ask the question „WHAT IS NORMAL ?“**

Compounds included in the MetaMap®Tox database



> 650 compounds
> 85 standard control groups

Metabolite Profiling Expertise
➔ Reproducibility & Robustness

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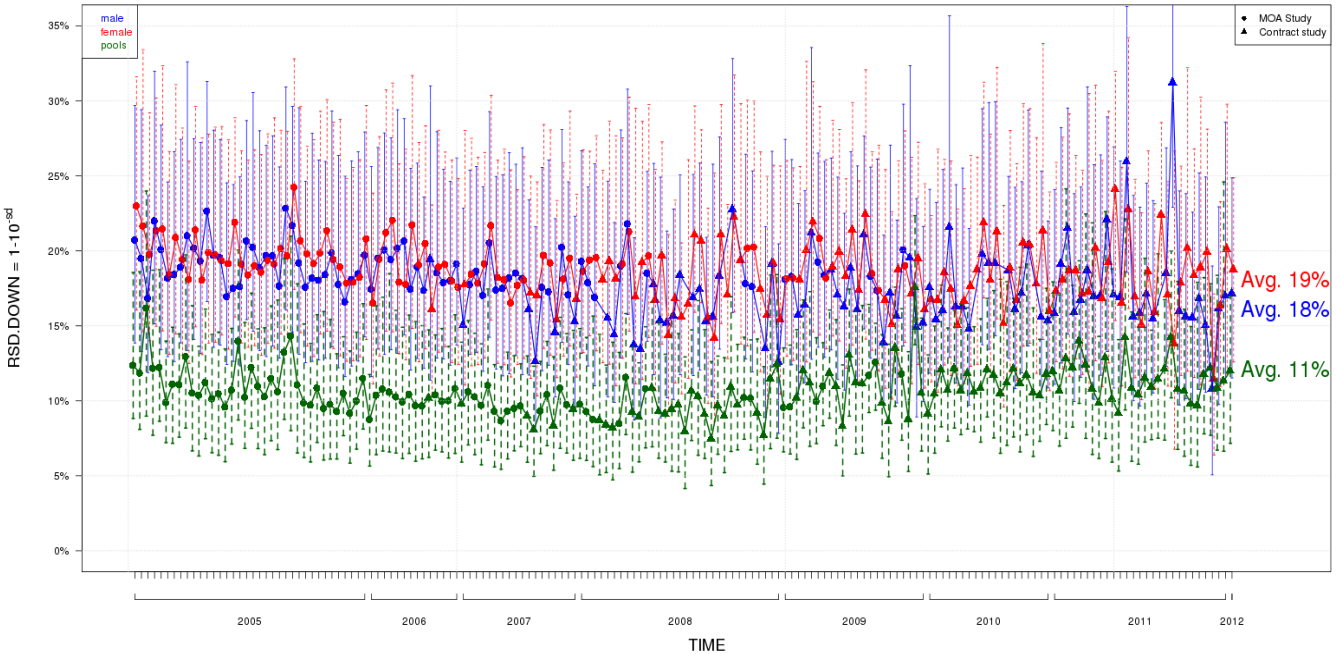
Technical and biological variation of rat plasma samples 2004 to 2012

- > 5000 biological control samples



- **High accuracy**

mean technical variation 11% Vartech < Varbiol

´ mean biological variation ~ 18% (m & f comparable)

Estradiol and other estrogenic compounds

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MetaMap-TOX - Microsoft Internet Explorer

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	Method	Grade	Metabolite	Class	MET_CHEM_ID	17-alpha-Ethynylestradiol (MOA13)			17-alpha-Ethynylestradiol (MOA58)			Diethylstilboestrol dipropionate (MOA8)			Trenbolone (MOA53)		
							fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28
☑	up	GC polar	SQ	Metabolite 1	Amino acids	38000006	1.27	1.37	1.38	1.49	2.0	1.31	1.26	1.4	1.47	0.98	0.89	1.07
☑	up	GC polar	SQ	Metabolite 2	Carbohydrates	38000028	1.44	1.39	1.45	1.46	1.44	1.42	1.12	1.21	1.13	1.08	1.22	1.19
☑	up	GC polar	SQ	Metabolite 3	Amino acids	38000052	1.15	1.22	1.22	1.18	1.38	1.09	1.08	1.11	1.2	0.99	0.87	1.09
☑	down	GC polar	SQ	Metabolite 4	Fatty acids	38000085	0.7	0.74	1.09	0.5	0.33	0.68	0.72	0.96	1.38	0.61	0.58	0.61
☑	down	GC polar	SQ	Metabolite 5	Amino acids	38000160	0.78	0.69	0.76	0.78	0.67	0.79	0.86	0.94	0.84	0.9	0.84	0.86
☑	down	LC polar	SQ	Metabolite 6	Carboxylic acids	58000020	0.95	0.68	0.31	0.6	0.98	0.54	0.55	1.13	1.11	0.24	0.3	0.3
☑	down	LC lipid	SQ	Metabolite 7	Phospholipids	68000006	0.83	0.89	0.91	0.71	0.78	0.95	0.95	0.96	1.0	0.95	0.95	0.98
☑	down	LC lipid	SQ	Metabolite 8	Sphingolipids	68000007	0.18	0.3	0.22	0.24	0.5	1.22	0.67	0.71	0.58	0.57	0.45	0.68
☑	down	LC lipid	SQ	Metabolite 9	Sphingolipids	68000008	0.21	0.35	0.29	0.24	0.43	1.11	0.72	0.59	0.73	0.64	0.43	0.76
☑	down	LC lipid	SQ	Metabolite 10		68000009	0.12	0.27	0.24	0.2	0.27	0.66	0.74	0.65	0.37	0.62	0.47	0.58
☑	down	LC lipid	SQ	Metabolite 11	Phospholipids	68000015	0.21	0.41	0.42	0.33	0.44	0.59	0.7	0.75	0.67	0.63	0.45	0.75
☑	down	LC lipid	SQ	Metabolite 12	Phospholipids	68000018	0.19	0.32	0.38	0.32	0.4	0.72	0.91	0.93	0.87	0.64	0.54	0.78
☑	down	LC lipid	SQ	Metabolite 13	Phospholipids	68000020	0.19	0.62	0.39	0.2	0.48	1.26	0.81	1.02	1.09	0.51	0.61	0.84
☑	down	LC lipid	SQ	Metabolite 14		68000022	0.15	0.34	0.37	0.15	0.59	1.14	0.67	0.81	0.79	0.51	0.46	0.64
☑	down	LC lipid	SQ	Metabolite 15	Tocopherols	68000023	0.19	0.26	0.29	0.11	0.47	0.86	0.97	0.72	0.7	0.61	0.47	0.83
☑	down	LC lipid	SQ	Metabolite 16		68000024	0.46	0.28	0.44	0.18	0.7	1.08	1.06	0.78	0.69	0.93	0.73	1.0
☑	up	LC lipid	SQ	Metabolite 17	Lipids	68000029	1.85	3.16	1.98	1.46	2.53	5.56	1.04	1.49	1.68	1.61	2.21	2.71
☑	down	LC lipid	SQ	Metabolite 18	Phospholipids	68000036	0.39	0.51	0.55	0.38	0.67	0.84	0.78	0.91	0.82	0.61	0.67	1.01
☑	down	LC lipid	SQ	Metabolite 19	Phospholipids	68000038	0.34	0.42	0.48	0.3	0.5	0.79	0.91	1.06	1.01	0.61	0.51	0.95
☑	down	LC lipid	SQ	Metabolite 20	Lipids	68000045	0.24	0.17	0.31	0.29	0.3	0.15	0.9	1.13	0.92	0.54	0.45	0.56
☑	down	LC lipid	SQ	Metabolite 21	Phospholipids	68000047	0.25	0.48	0.38	0.3	0.54	0.71	0.67	0.7	0.69	0.87	0.74	0.85
☑	down	LC lipid	SQ	Metabolite 22	Phospholipids	68000055	0.28	0.42	0.5	0.38	0.51	0.87	0.94	0.88	0.89	0.72	0.74	0.71
☑	up	LC lipid	SQ	Metabolite 23	Lipids	68000057	2.35	5.85	3.25	1.38	2.92	4.97	1.68	1.5	1.76	1.48	2.26	2.48
☑	up	LC lipid	SQ	Metabolite 24	Lipids	68000058	1.52	2.65	1.52	1.13	2.22	6.2	0.94	1.07	1.92	1.92	2.39	3.06
☑	down	LC catecholamine	SQ	Metabolite 25	Catechol amines	78000014	1.03	0.67	0.56	0.63	0.41	0.83	0.93	0.76	0.83	0.88	1.09	0.47

ShowSelected Metabolites | Find Compounds | Save Metabolite List

Estradiol: estrogenic pattern finds genistein

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MetaMap-TOX - Microsoft Internet Explorer

Selected Metabolites | Find Compounds | Save Metabolite List

All | Select None

Direction	Method	Grade	Metabolite	Class	MET_CHEM_ID	17-alpha-Ethynylestradiol (MOA13)			17-alpha-Ethynylestradiol (MOA58)			Diethylstilboestrol dipropionate (MOA8)			Trenbolone (MOA53)			Genistein (MOA66)		
						fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28	fh7	fh14	fh28
up	GC polar	SQ	Metabolite 1	Amino acids	38000006	1.27	1.37	1.38	1.49	2.0	1.31	1.26	1.4	1.47	0.98	0.89	1.07	0.91	1.12	1.19
up	GC polar	SQ	Metabolite 2	Carbohydrates	38000028	1.44	1.39	1.45	1.46	1.44	1.42	1.12	1.21	1.13	1.08	1.22	1.19	1.07	1.19	1.04
up	GC polar	SQ	Metabolite 3	Amino acids	38000052	1.15	1.22	1.22	1.18	1.38	1.09	1.08	1.11	1.2	0.99	0.87	1.09	0.88	0.9	0.96
down	GC polar	SQ	Metabolite 4	Fatty acids	38000085	0.7	0.74	1.09	0.5	0.33	0.68	0.72	0.96	1.38	0.61	0.58	0.61	0.66	0.82	0.69
down	GC polar	SQ	Metabolite 5	Amino acids	38000160	0.78	0.69	0.76	0.78	0.67	0.79	0.86	0.94	0.84	0.9	0.84	0.86	0.92	0.89	0.88
down	LC polar	SQ	Metabolite 6	Carboxylic acids	58000020	0.95	0.68	0.31	0.6	0.98	0.54	0.55	1.13	1.11	0.24	0.3	0.3	0.31	0.32	0.38
down	LC lipid	SQ	Metabolite 7	Phospholipids	68000006	0.83	0.89	0.91	0.71	0.78	0.95	0.95	0.96	1.0	0.95	0.95	0.98	0.87	0.86	0.96
down	LC lipid	SQ	Metabolite 8	Sphingolipids	68000007	0.18	0.3	0.22	0.24	0.5	1.22	0.67	0.71	0.58	0.57	0.45	0.68	0.36	0.42	0.76
down	LC lipid	SQ	Metabolite 9	Sphingolipids	68000008	0.21	0.35	0.29	0.24	0.43	1.11	0.72	0.59	0.73	0.64	0.43	0.76	0.36	0.4	0.88
down	LC lipid	SQ	Metabolite 10		68000009	0.12	0.27	0.24	0.2	0.27	0.66	0.74	0.65	0.37	0.62	0.47	0.58	0.39	0.44	0.6
down	LC lipid	SQ	Metabolite 11	Phospholipids	68000015	0.21	0.41	0.42	0.33	0.44	0.59	0.7	0.75	0.67	0.63	0.45	0.75	0.46	0.58	0.6
down	LC lipid	SQ	Metabolite 12	Phospholipids	68000018	0.19	0.32	0.38	0.32	0.4	0.72	0.91	0.93	0.87	0.64	0.54	0.78	0.52	0.62	0.63
down	LC lipid	SQ	Metabolite 13	Phospholipids	68000020	0.19	0.62	0.39	0.2	0.48	1.26	0.81	1.02	1.09	0.51	0.61	0.84	0.46	0.86	0.87
down	LC lipid	SQ	Metabolite 14		68000022	0.15	0.34	0.37	0.15	0.59	1.14	0.67	0.81	0.79	0.51	0.46	0.64	0.41	0.6	0.78
down	LC lipid	SQ	Metabolite 15	Tocopherols	68000023	0.19	0.26	0.29	0.11	0.47	0.86	0.97	0.72	0.7	0.61	0.47	0.83	0.47	0.47	0.59
down	LC lipid	SQ	Metabolite 16		68000024	0.46	0.28	0.44	0.18	0.7	1.08	1.06	0.78	0.69	0.93	0.73	1.0	0.44	0.6	0.79
up	LC lipid	SQ	Metabolite 17	Lipids	68000029	1.85	3.16	1.98	1.46	2.53	5.56	1.04	1.49	1.68	1.61	2.21	2.71	2.04	2.02	1.66
down	LC lipid	SQ	Metabolite 18	Phospholipids	68000036	0.39	0.51	0.55	0.38	0.67	0.84	0.78	0.91	0.82	0.61	0.67	1.01	0.59	0.68	0.86
down	LC lipid	SQ	Metabolite 19	Phospholipids	68000038	0.34	0.42	0.48	0.3	0.5	0.79	0.91	1.06	1.01	0.61	0.51	0.95	0.5	0.72	0.79
down	LC lipid	SQ	Metabolite 20	Lipids	68000045	0.24	0.17	0.31	0.29	0.3	0.15	0.9	1.13	0.92	0.54	0.45	0.56	0.32	0.55	0.4
down	LC lipid	SQ	Metabolite 21	Phospholipids	68000047	0.25	0.48	0.38	0.3	0.54	0.71	0.67	0.7	0.69	0.87	0.74	0.85	0.54	0.52	0.56
down	LC lipid	SQ	Metabolite 22	Phospholipids	68000055	0.28	0.42	0.5	0.38	0.51	0.87	0.94	0.88	0.89	0.72	0.74	0.71	0.72	0.77	0.81
up	LC lipid	SQ	Metabolite 23	Lipids	68000057	2.35	5.85	3.25	1.38	2.92	4.97	1.68	1.5	1.76	1.48	2.26	2.48	3.57	3.16	3.01
up	LC lipid	SQ	Metabolite 24	Lipids	68000058	1.52	2.65	1.52	1.13	2.22	6.2	0.94	1.07	1.92	1.92	2.39	3.06	1.92	1.95	2.11
down	LC catecholamine	SQ	Metabolite 25	Catechol amines	78000014	1.03	0.67	0.56	0.63	0.41	0.83	0.93	0.76	0.83	0.88	1.09	0.47	NA	NA	NA

Selected Metabolites | Find Compounds | Save Metabolite List

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Estradiol: quality control

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MetaMap-TOX - Microsoft Internet Explorer

ShowSelected Metabolites | Find Compounds | Save Metabolite List

Select All | Select None

Select	Direction	Method	Grade	Metabolite	Class	MET_CHEM_ID	17-alpha-Ethynylestradiol (MOA13)			17-alpha-Ethynylestradiol (MOA58)		
							fh7	fh14	fh28	fh7	fh14	fh28
☑	up	GC polar	SQ	Metabolite 1	Amino acids	38000006	1.27	1.37	1.38	1.49	2.0	1.31
☑	up	GC polar	SQ	Metabolite 2	Carbohydrates	38000028	1.44	1.39	1.45	1.46	1.44	1.42
☑	up	GC polar	SQ	Metabolite 3	Amino acids	38000052	1.15	1.22	1.22	1.18	1.38	1.09
☑	down	GC polar	SQ	Metabolite 4	Fatty acids	38000085	0.7	0.74	1.09	0.5	0.33	0.68
☑	down	GC polar	SQ	Metabolite 5	Amino acids	38000160	0.78	0.69	0.76	0.78	0.67	0.79
☑	down	LC polar	SQ	Metabolite 6	Carboxylic acids	58000020	0.95	0.68	0.31	0.6	0.98	0.54
☑	down	LC lipid	SQ	Metabolite 7	Phospholipids	68000006	0.83	0.89	0.91	0.71	0.78	0.95
☑	down	LC lipid	SQ	Metabolite 8	Sphingolipids	68000007	0.18	0.3	0.22	0.24	0.5	1.22
☑	down	LC lipid	SQ	Metabolite 9	Sphingolipids	68000008	0.21	0.35	0.29	0.24	0.43	1.11
☑	down	LC lipid	SQ	Metabolite 10		68000009	0.12	0.27	0.24	0.2	0.27	0.66
☑	down	LC lipid	SQ	Metabolite 11	Phospholipids	68000015	0.21	0.41	0.42	0.33	0.44	0.59
☑	down	LC lipid	SQ	Metabolite 12	Phospholipids	68000018	0.19	0.32	0.38	0.32	0.4	0.72
☑	down	LC lipid	SQ	Metabolite 13	Phospholipids	68000020	0.19	0.62	0.39	0.2	0.48	1.26
☑	down	LC lipid	SQ	Metabolite 14		68000022	0.15	0.34	0.37	0.15	0.59	1.14
☑	down	LC lipid	SQ	Metabolite 15	Tocopherols	68000023	0.19	0.26	0.29	0.11	0.47	0.86
☑	down	LC lipid	SQ	Metabolite 16		68000024	0.46	0.28	0.44	0.18	0.7	1.08
☑	up	LC lipid	SQ	Metabolite 17	Lipids	68000029	1.85	3.16	1.98	1.46	2.53	5.56
☑	down	LC lipid	SQ	Metabolite 18	Phospholipids	68000036	0.39	0.51	0.55	0.38	0.67	0.84
☑	down	LC lipid	SQ	Metabolite 19	Phospholipids	68000038	0.34	0.42	0.48	0.3	0.5	0.79
☑	down	LC lipid	SQ	Metabolite 20	Lipids	68000045	0.24	0.17	0.31	0.29	0.3	0.15
☑	down	LC lipid	SQ	Metabolite 21	Phospholipids	68000047	0.25	0.48	0.38	0.3	0.54	0.71
☑	down	LC lipid	SQ	Metabolite 22	Phospholipids	68000055	0.28	0.42	0.5	0.38	0.51	0.87
☑	up	LC lipid	SQ	Metabolite 23	Lipids	68000057	2.35	5.85	3.25	1.38	2.92	4.97
☑	up	LC lipid	SQ	Metabolite 24	Lipids	68000058	1.52	2.65	1.52	1.13	2.22	6.2
☑	down	LC catecholamine	SQ	Metabolite 25	Catechol amines	78000014	1.03	0.67	0.56	0.63	0.41	0.83

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about MetaMap

Exact Repeats: quality control and more

Evaluation of 10 exact repeats

Question: at which „p“ level do we find the best balance between most matches and least mismatches ?

Exact Repeats: quality control and more

Evaluation of 10 exact repeats

Question: at which „p“ level do we find the best balance between most matches and least mismatches ?

Anwer: p = 0.1

Note: an earlier simulation of the data base (measured variability for individual metabolites) and a pre-defined „true pattern“ provided the same result

Standard Control Groups

MetaMap®Tox contains > 80 standard control groups

Each made up of 10 males and 10 females
Data: d7, 14 and 28

For each control goup compare a defined „control“ group (e.g. animals 1 – 5) to a defined „treatment“ group (animals 6 – 10)

What is normal ?

Distribution of significant changes in controls

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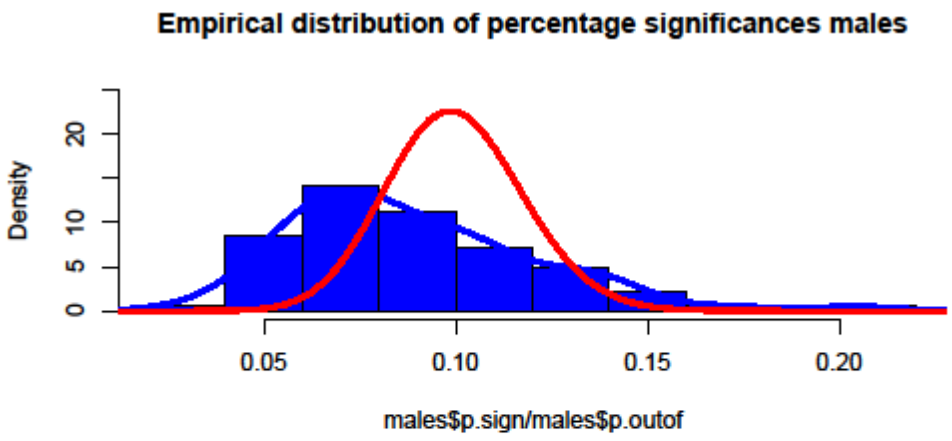
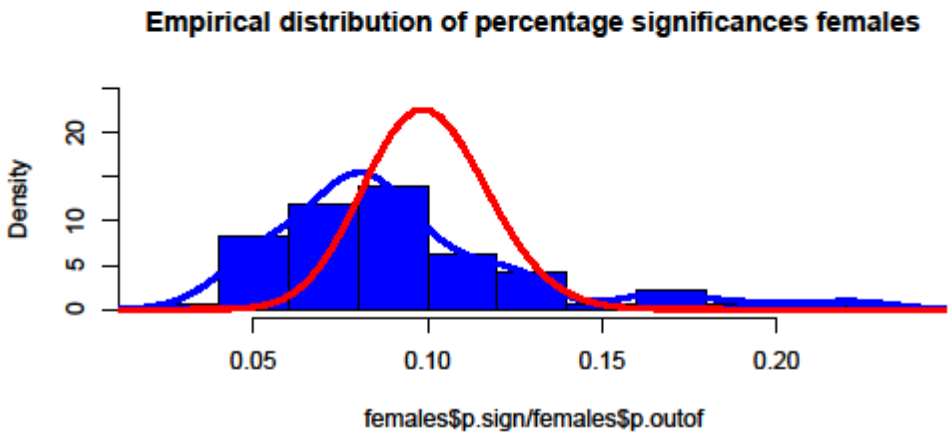
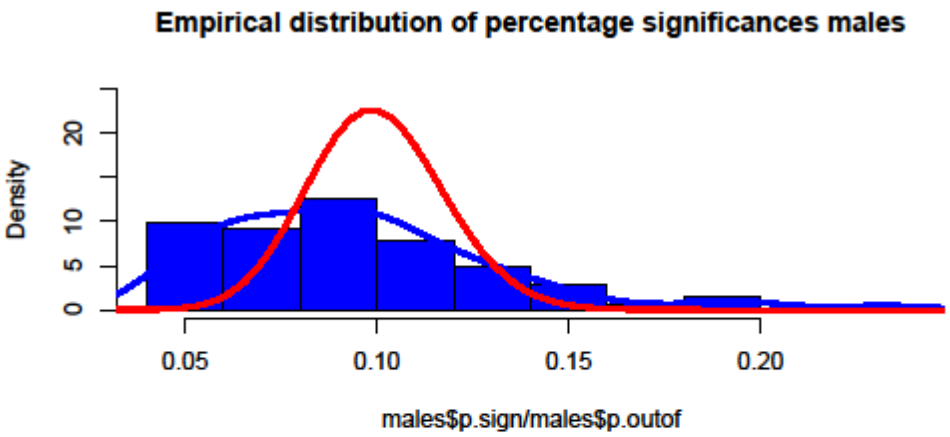
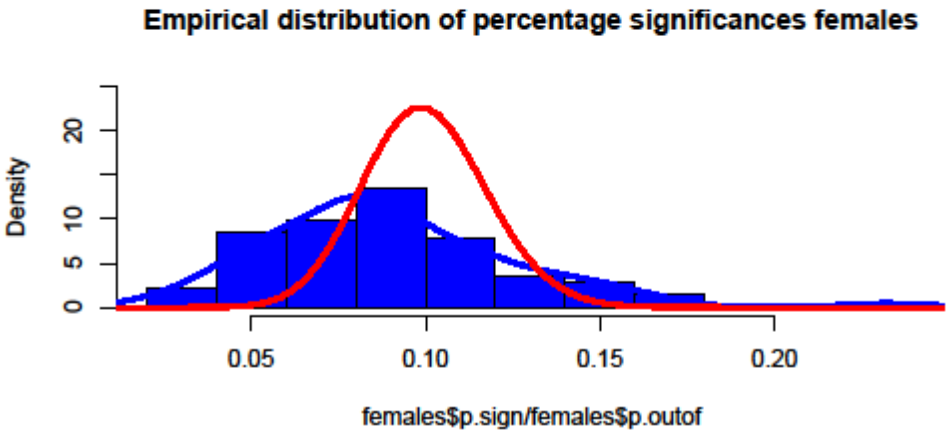
Day 14:





Day 28





What is normal ?

A reality check for each experiment !

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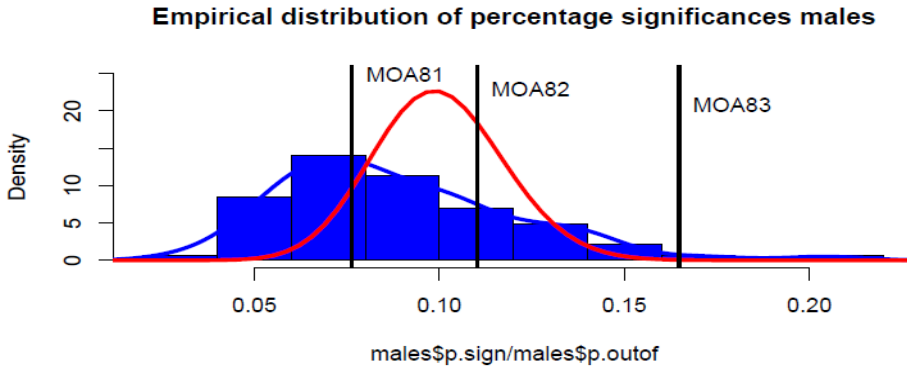
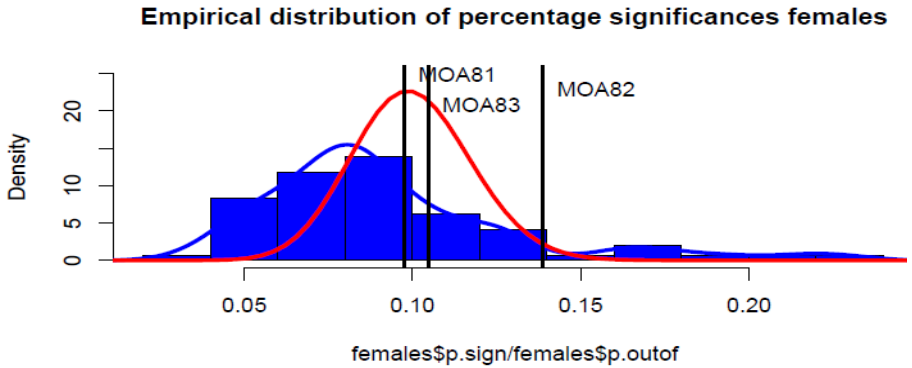
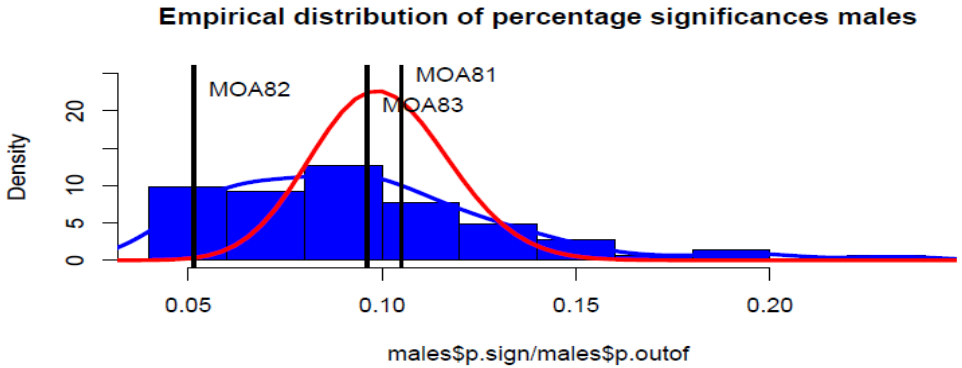
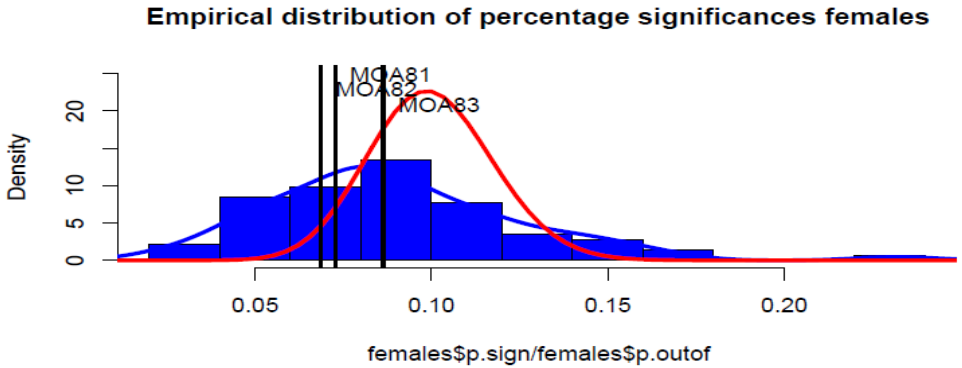
Day 14:





Day 28:





More statistics

Calculate summary statistics of the standard deviations of each metabolite over all studies:

- Min
- 5% Quantile
- 25% Quantile
- Median
- Mean
- 75% Quantile
- 95% Quantile
- Max

Location parameters

- Standard deviation (of standard deviations)
- MAD (Median Absolute Deviation)
- IQR (75% Quantile – 25% Quantile)

Scale parameters

- MAD/Median
- IQR/Median

Relative scale parameters

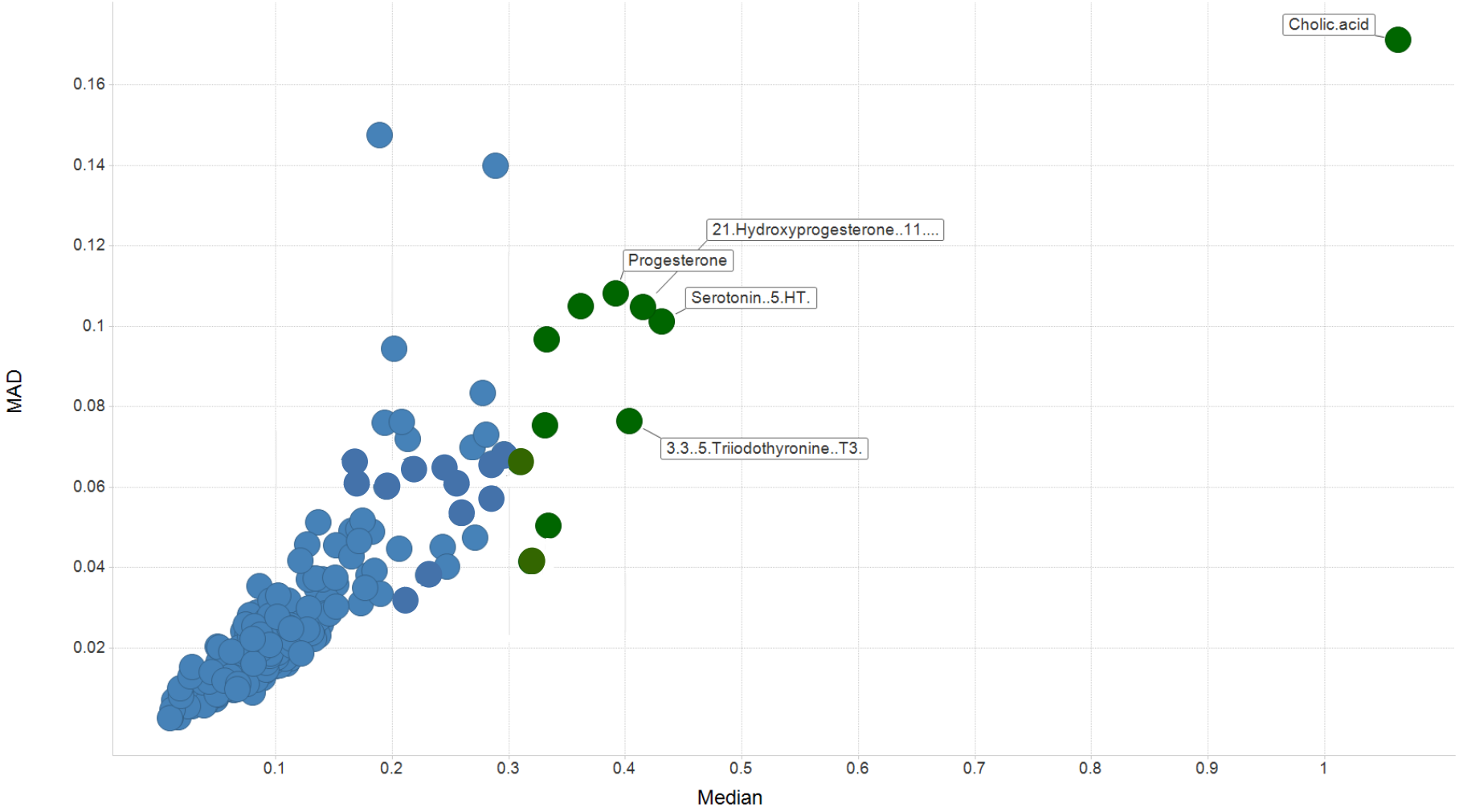
- (75% Quantile - Median) / (Median – 25% Quantile)

Skewness parameter

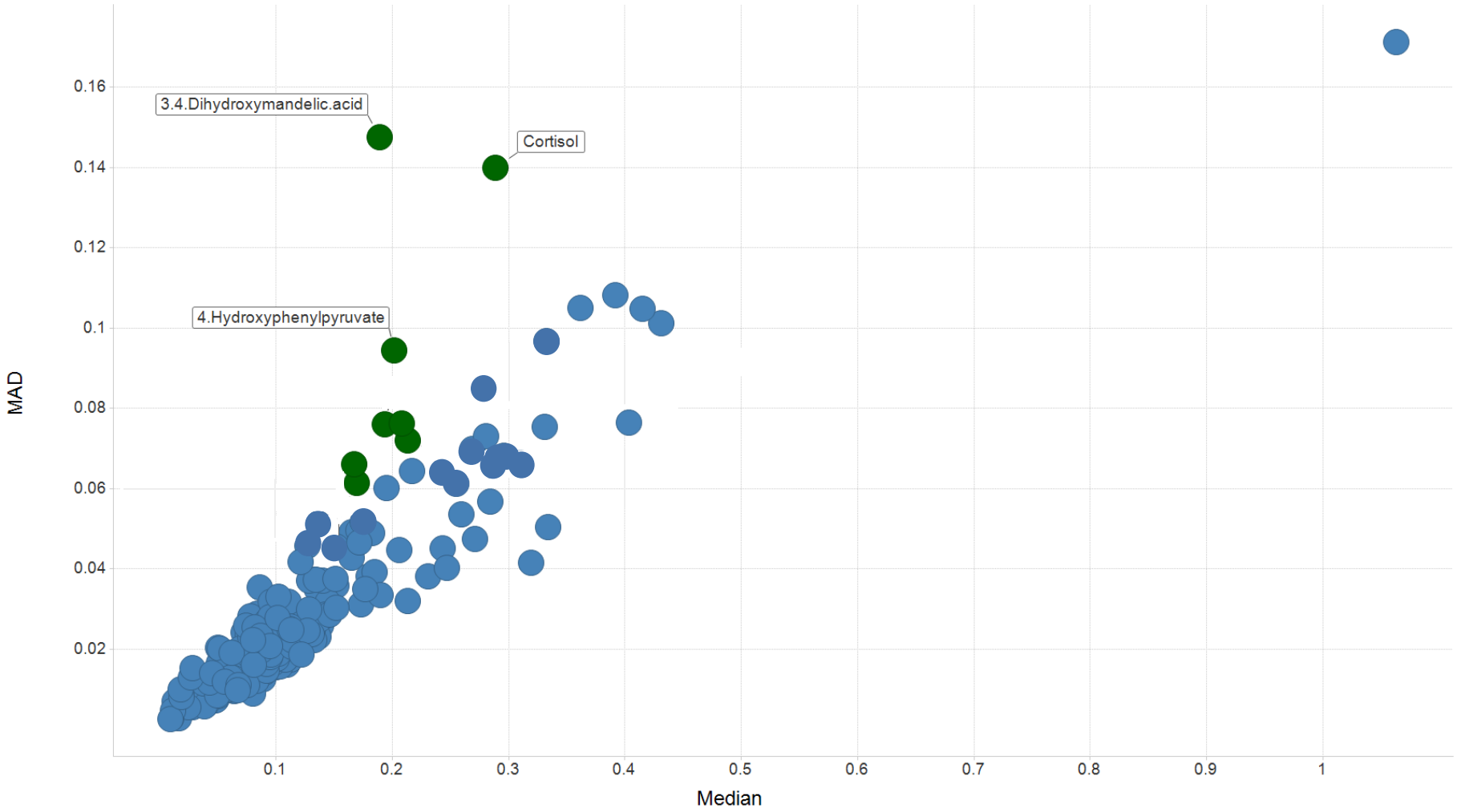
→ summary table with 297 x 16 parameters*

(* for controls 85 x 10 x 2 x 3……)

Metabolites with a high median standard deviation



Metabolites with a relatively wide spread of the standard deviation



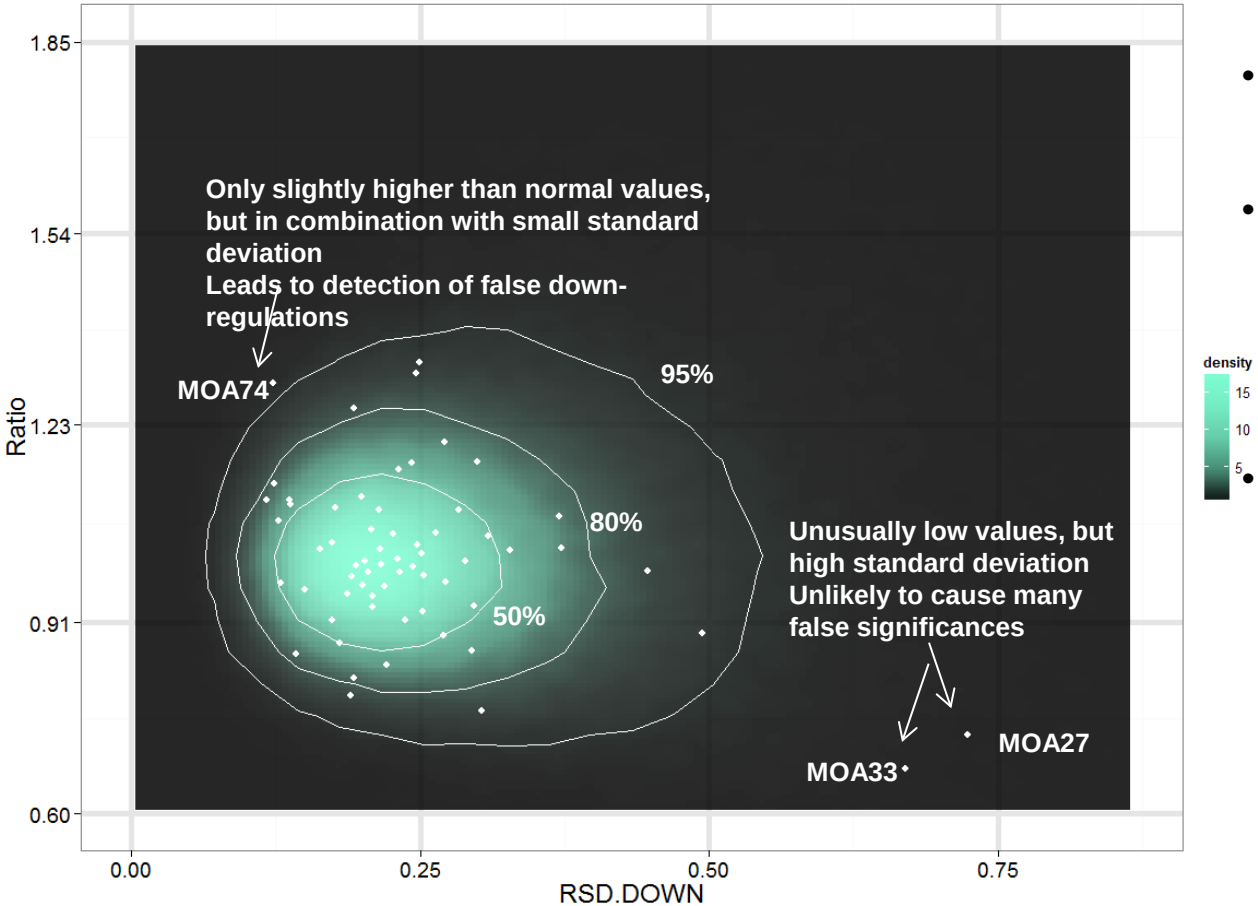
What is normal?

Use <u>all</u> of the control data to define the normal range for metabolite values in untreated controls

1) Check if concurrent control is normal

2) Determine if statistically significant changes are in or outside control range

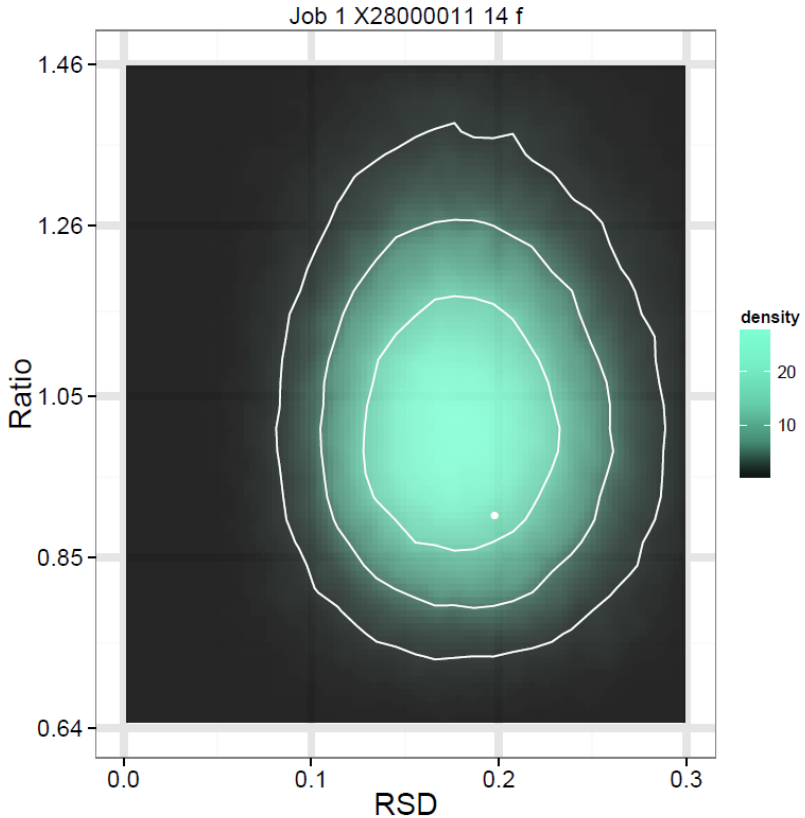
Meta-Analysis – Results Distribution vs. actually observed levels for controls



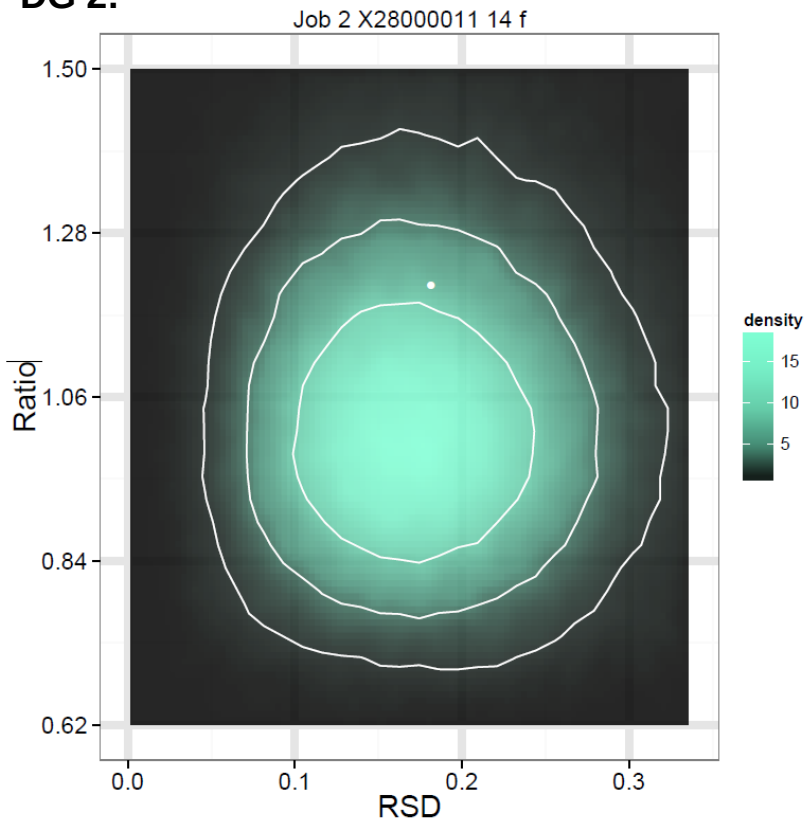
- White dots are MOA-studies
- Percentages indicate how many of the studies should lie within
- Since this is a joint distribution of RSD.DOWN and Ratio abnormal values can be indicated by a highly abnormal Ratio or RSD.DOWN or both
- Application: Detect unusual controls

Example Query MOA 84 Plots

DG 0:



DG 2:



Both points are in their respective clouds, one is a bit low the other one high. In combination the difference turns out significant. BUT: both values are within the normal control distribution

Summary

1) Combined evaluation of different approaches to the data to draw a final conclusion

2) Collect and continuously evaluate your control values (particularly if you are working with multiple labs)
3) → check if your concurrent control is normal
4) → check if stat. sign. changes outside control range

5) Regularly (exactly) repeat experiments

6) Make best use of statistical approaches….but trust in biology

7) Expect the unexpected…….

MetaTox Group

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Dr. Oliver Schmitz

THANK YOU VERY MUCH FOR YOUR ATTENTION

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