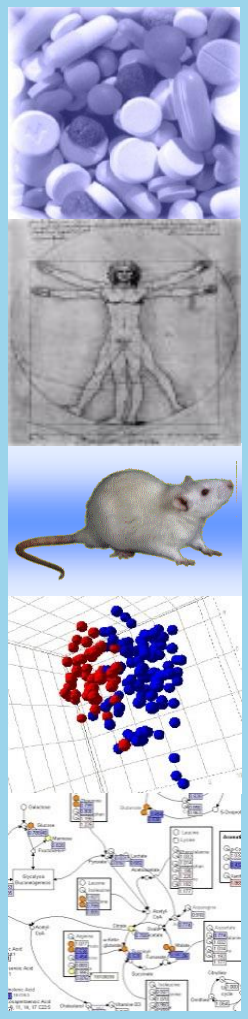

Read Across with Metabolomics for Phenoxy Herbicides a Case Study with MCPP

BASF SE

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

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Introduction: Case Study MCPP

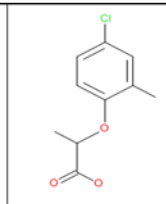
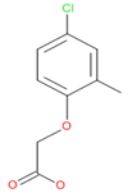
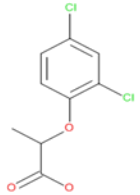
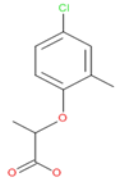
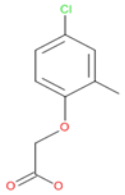
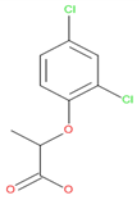
- Purpose: to demonstrate the usefulness of a biology based tool to provide qualitative and quantitative information to improve chemical grouping for read-across purposes.
- !! The case study is not made to substitute any data, because the compounds used in this case have a full, agrochemical, toxicological data base.

- Read-across is proposed to fill a data gap for 90-day oral repeated dose toxicity of the phenoxy herbicide MCPP. The read-across hypothesis is that as a result of their chemical structural similarity and a similar mode of action the toxicological properties of the category members are likely to be similar.
- This read-across is consistent with RAAF Scenarios # 4 – i.e. category approach with a read-across hypothesis based on different compounds which have the same type of effect(s).

MetaMap® Tox: 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%**	

Acute Toxicity and Genotoxicity

	MCPP	2,4-DP	MCPA
Acute oral toxicity (mg/kg bw)	LD50 = 775	LD50 = 567	LD50 = 765
Acute inhalation toxicity (mg/l air)	LC50 > 5.6	LC50 > 2.3	LC50 > 6.3
Acute dermal toxicity (mg/kg bw)	LD50 > 2000	LD50 > 2000	LD50 > 2000
Skin irritation	Irritant	Irritant	Slightly irritant (no classification)
Eye irritation	Strongly irritant	Strongly irritant	Strongly irritant
Skin sensitization	Non sensitizer	Non sensitizer	Non sensitizer
Mutagenicity	Overall negative	Overall negative	Overall negative

Kinetics & Metabolism (for target and source substances)

- Rapid absorption, bioavailability > 90%
- Unchanged parent compound is the major component in the blood
- Metabolism is limited to the production of one or a few minor metabolites
- Rapid elimination through the urine (low dose levels 80 – 90%)
- Fecal elimination accounts for ca. 10% (low) to ca. 20% (high dose)
- Fast elimination is reflected in relatively short and comparable half-lives

→ ADME properties of the target and sources substances are similar

28 day toxicity rats

- Limited data set (note: 28 day study is not a regulatory requirement for crop protection products, the 90 day study is)
- **MCPA:**

  2000 ppm (only dose tested): Reduction in body weight

  Clinical chemistry: → liver and kidney are target organs at a functional level (no pathological changes). Some effects on red blood cell parameters. The testes may be an additional target organ, although the observed changes may be related to the decreased body weights in these young adult animals.

  A NOAEL was not determined in this study

- **2,4DP**:

  No adverse effects at highest dose level tested (500 ppm)

- **MCPP**:

  No adverse effects at highest dose level tested (400 ppm)

  400 ppm: reduced cholesterol levels, increase in urea and creatinine values in female rats.
  Increased kidney weight and increased liver weights.
  These changes were not considered to be adverse in nature

Why Metabolomics / Metabolic Profiling ?

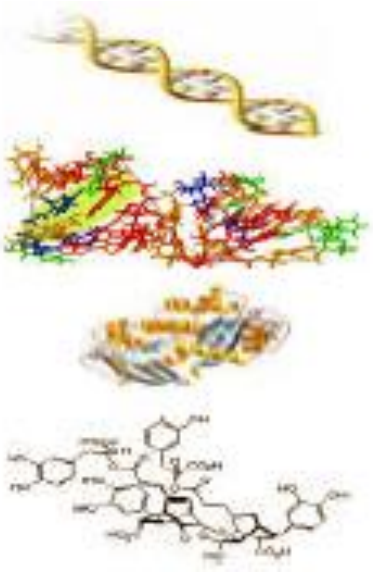
Mostly unknown

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
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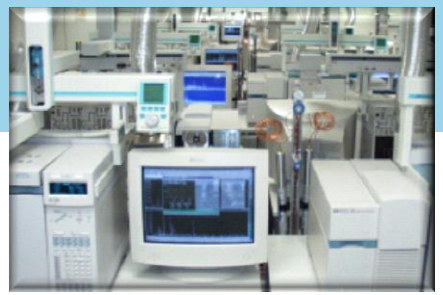
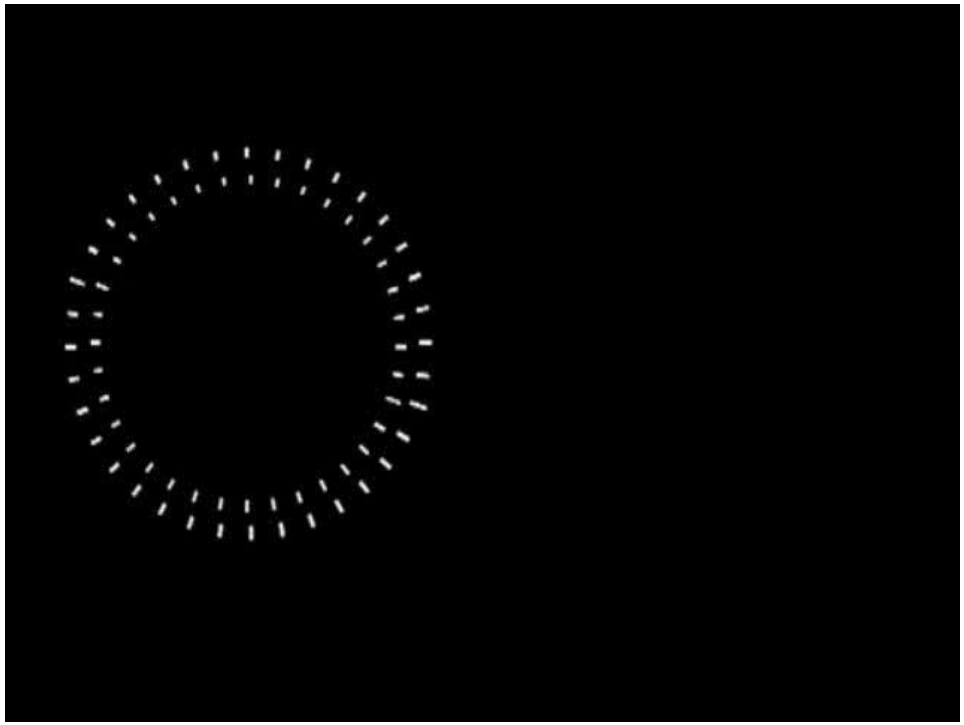
BLOOD PROFILING

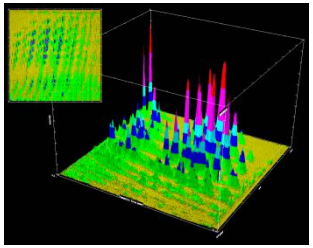


LC-MS/ GC-MS

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)

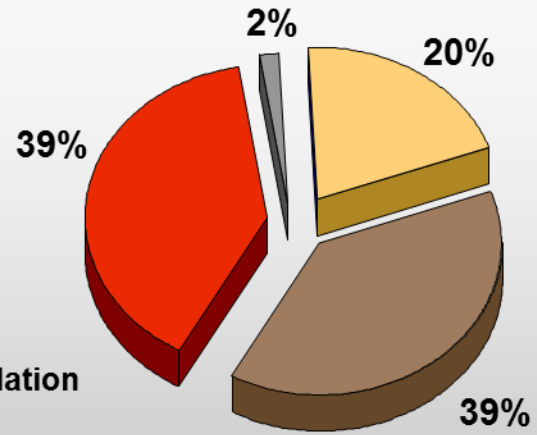
SAMPLE **REFERENCE** **300 Known Metabolites**

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



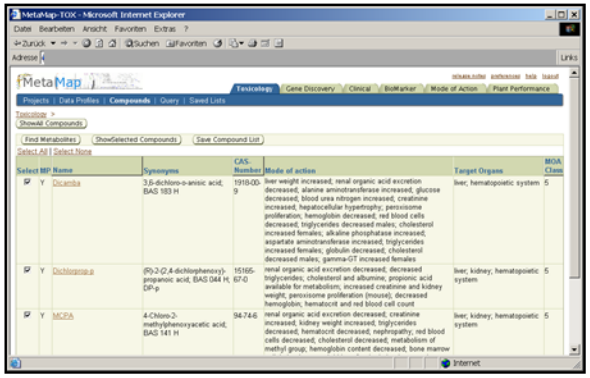
Pattern Creation

- 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)

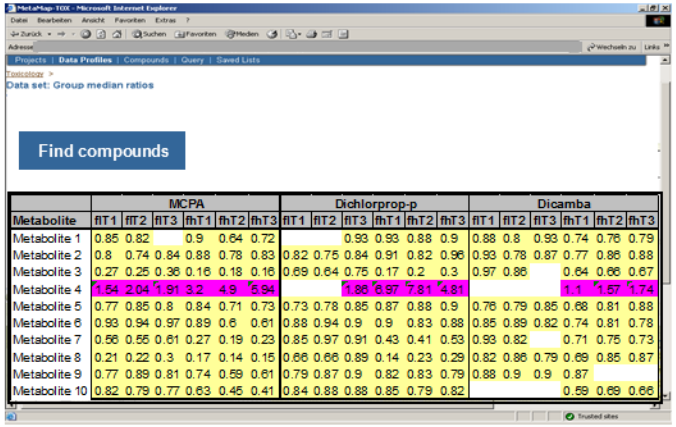
MetaMap™ Tox

Group of substances



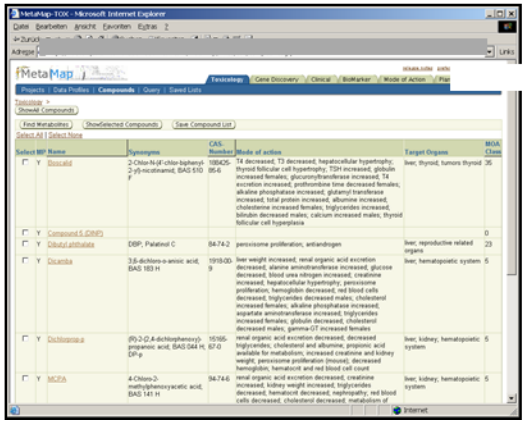
"Find Metabolites"

List of changed metabolites "metabolic profiling"



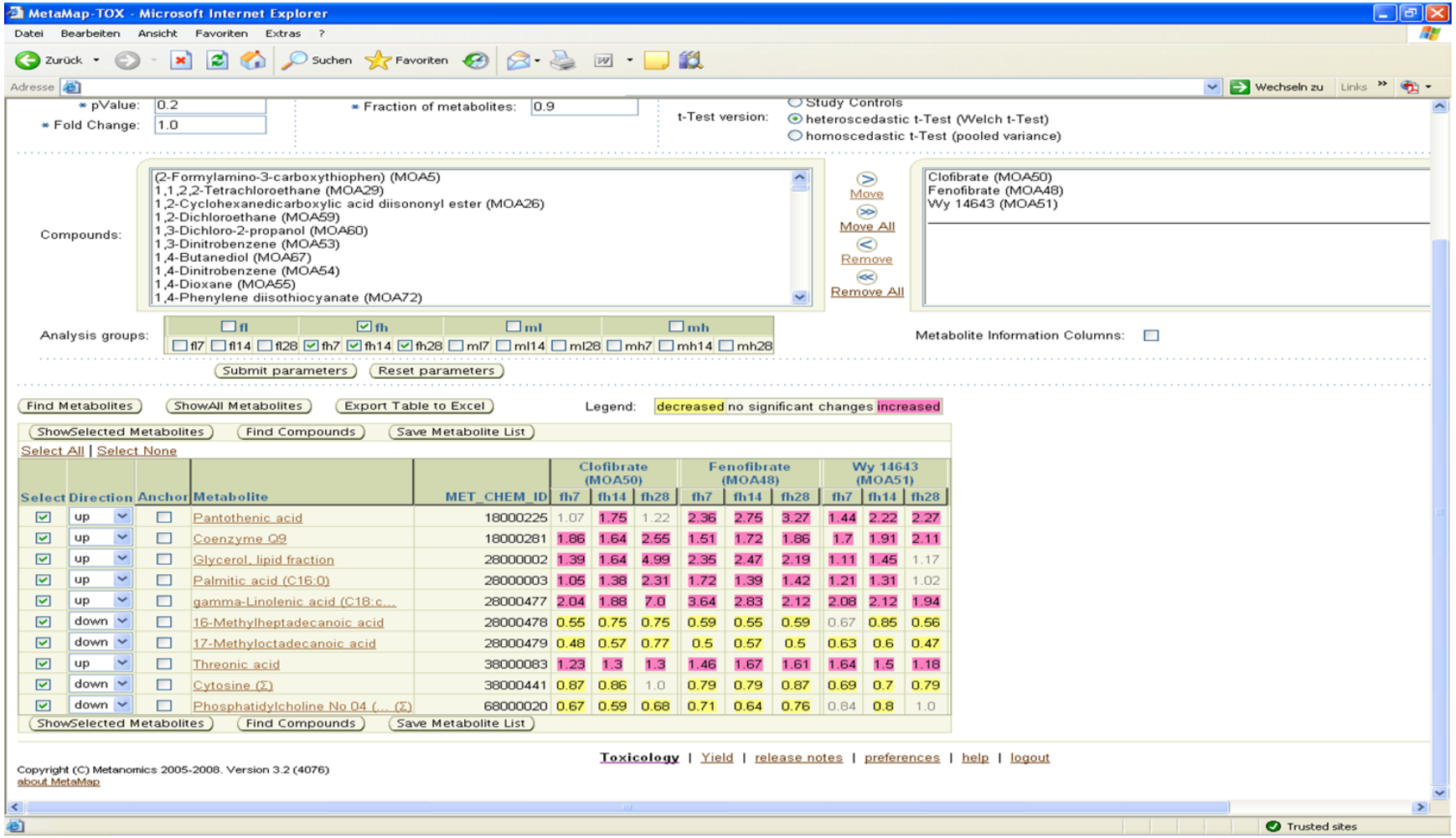
Metabolite	MCPA						Dichlorprop-p						Dicamba					
	fiT1	fiT2	fiT3	fhT1	fhT2	fhT3	fiT1	fiT2	fiT3	fhT1	fhT2	fhT3	fiT1	fiT2	fiT3	fhT1	fhT2	fhT3
Metabolite 1	0.85	0.82		0.9	0.64	0.72			0.93	0.93	0.88	0.9	0.88	0.8	0.93	0.74	0.76	0.79
Metabolite 2	0.8	0.74	0.84	0.88	0.78	0.83	0.82	0.75	0.84	0.91	0.82	0.96	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



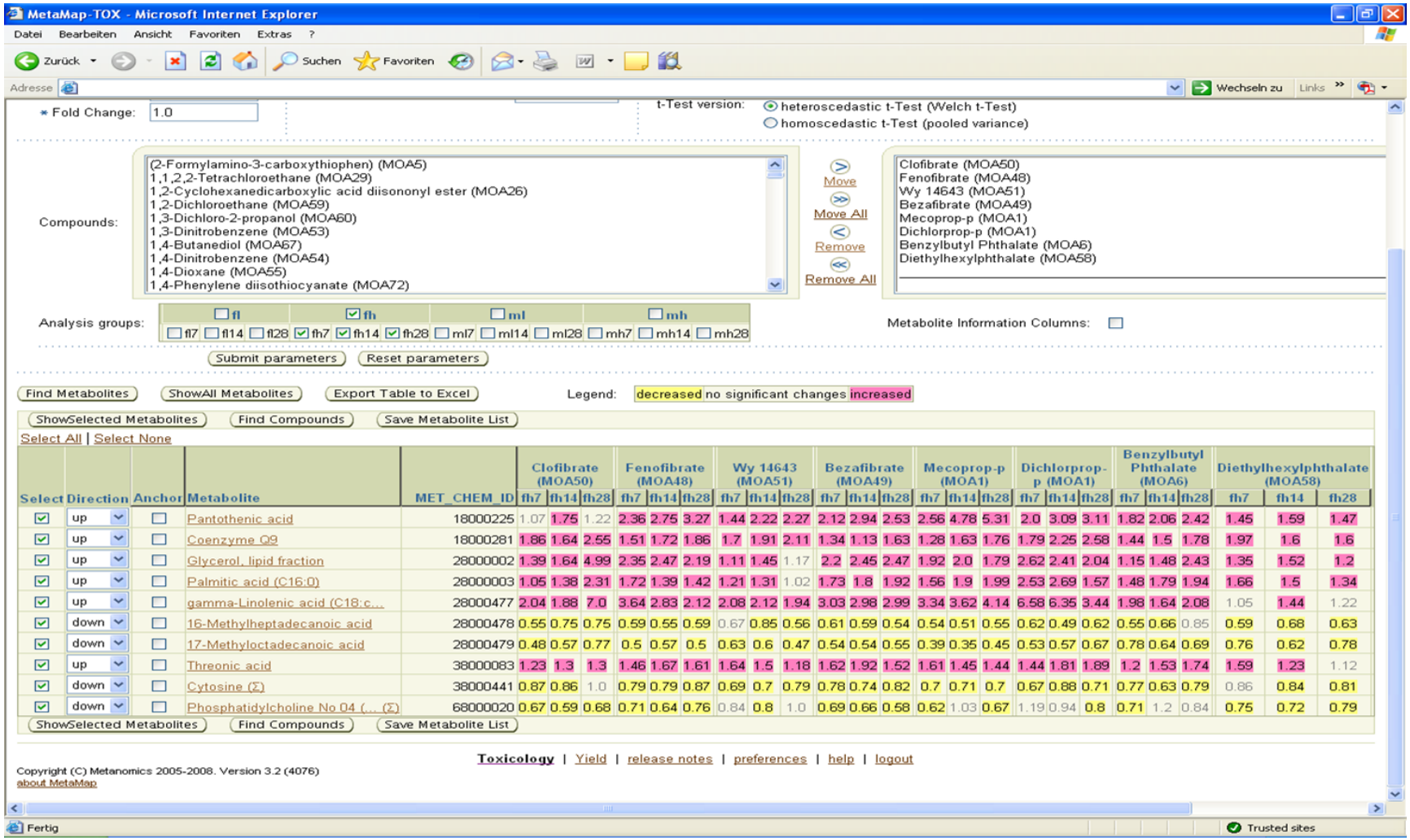
"Find Compounds"

Recognising mode-of-action: peroxisome proliferation



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

Recognising mode-of-action: peroxisome proliferation

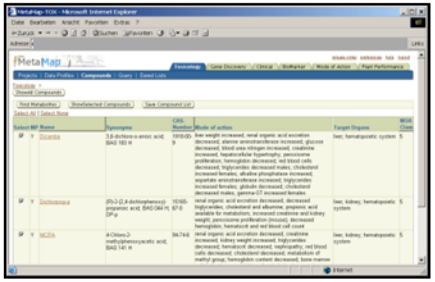


Pairwise Comparison: Clofibrate

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

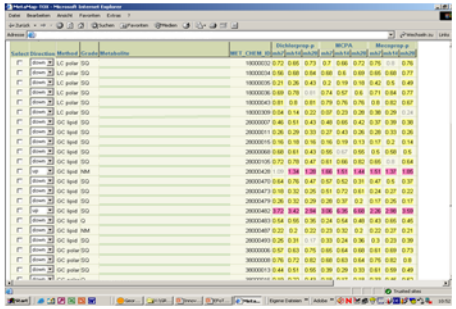
Assessment is based on joint evaluation of Pattern Ranking, TPC and Biochem Interpretation

Test Substance



"Find Metabolites"

Metabolite profile

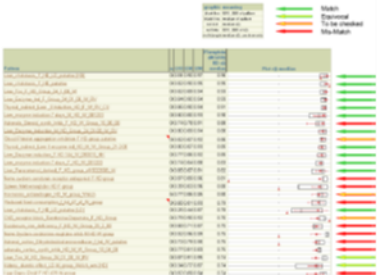


Biochemical Interpretation

"Total Profile Comparison"

"Pattern Ranking"

Statistical Correlation - Ranking based on biological similarity



Similarity of test compound-induced changes compared to toxicity patterns

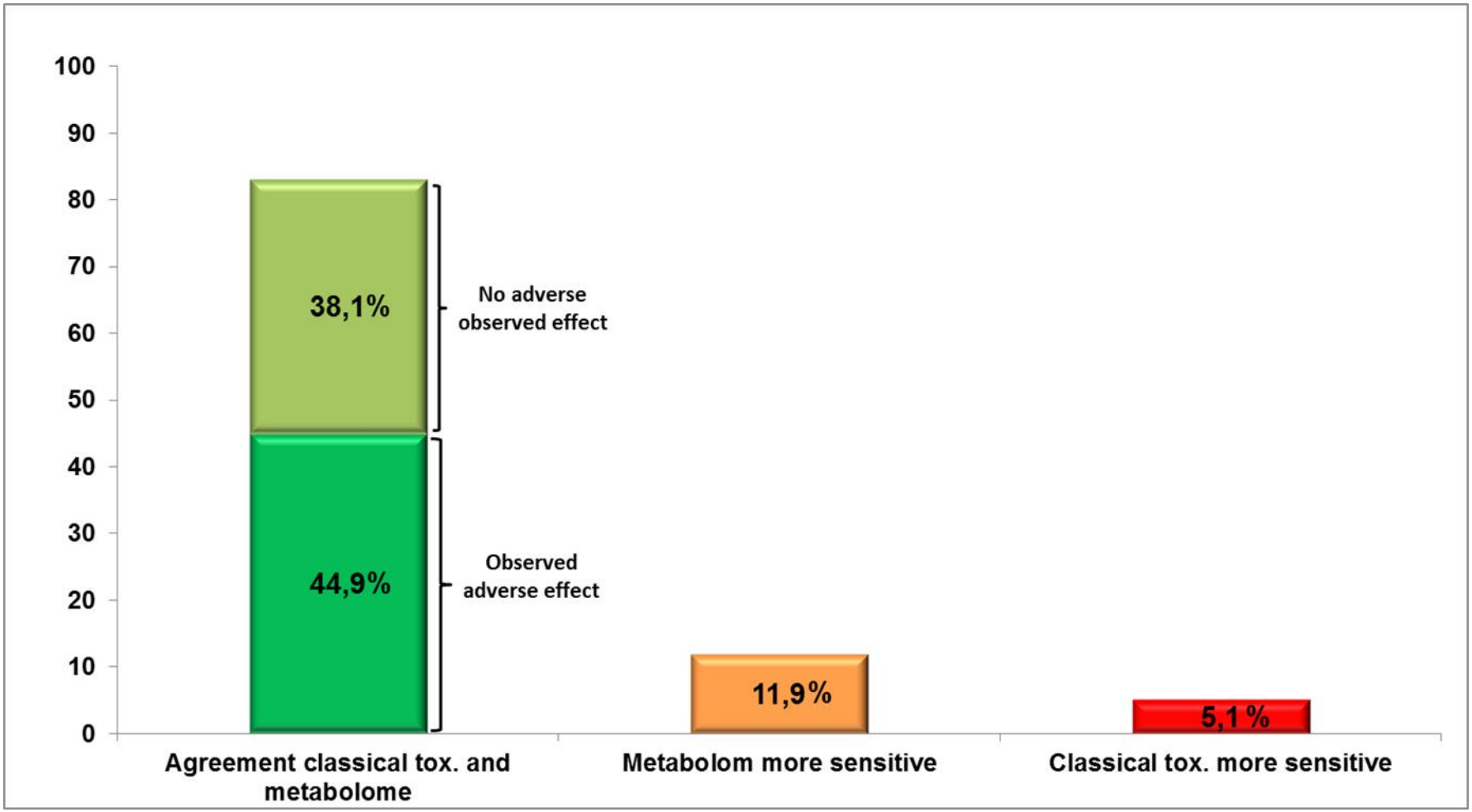
MetaMap®Tox: Assessment of predictivity

- 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:
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:
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

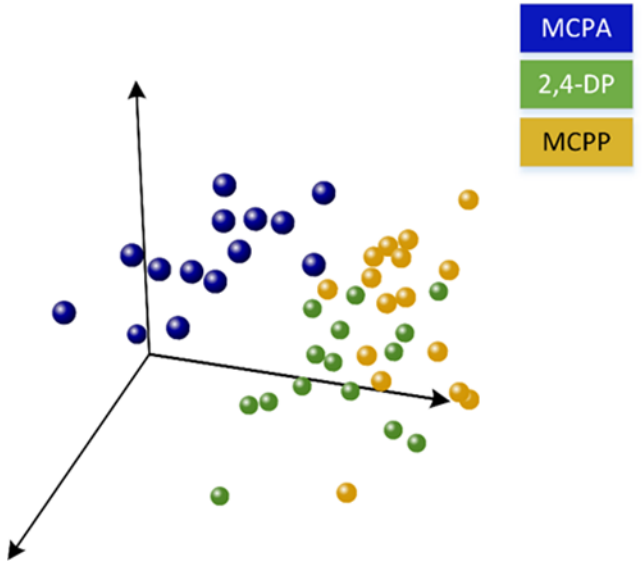
b)

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**

90 day toxicity MCPA (1)

- MCPA was administered to groups of 15 male and 15 female Wistar rats at dietary concentrations of 0, 50, 500 or 2500 ppm
- **2500 ppm:**

  Decreased body weights
  Decrease in hematological parameters (red blood cells, hemoglobin and hematocrit)
  Increase in liver enzymes (alanine amino transferase, alkaline phosphatase and aspartate aminotransferase)

  Histopathology:
  - Liver: cytoplasmic eosinophilia and granular cytoplasm
  - Lung: higher incidence/grading of foam cell accumulations
  - Myeloid atrophy of the hematopoietic marrow
  - Testes: decreased weights, testicular atrophy and atrophy of the seminal vesicles and prostate, aspermia or oligospermia in the epididymides

  Neurofunctional:
  - Decreased value of hindlimb grip strength in females on day 85, decreased foot splay test values in males on day 22 and reduced values ($p < 0.02$) of forelimb grip strength in males on day 50

  The NOAEL was at 500 ppm

90 day toxicity MCPA (2)

- MCPA was administered to rats (15 per sex per dose) at dietary concentrations of 0, 50, 150 or 450 ppm
- 450 ppm:
  - Increased creatinine values (females)
  - Decreased cholesterol and calcium values (males)
  - Increased absolute and relative kidney weights (males)
- NOEL is 150 ppm

90 day toxicity MCPA - conclusion

Study 1

- 2500 ppm body weight development is severely affected
- Target organ liver
- Reduced red blood cell values (bone marrow cells target)
- Effects noted in the functional observation battery and the effects on the male reproductive system may have been secondary to the body weight effects

Study 2

- Target organ: kidney
- NOAEL between 150 and 500 ppm

90 day toxicity 2,4-DP (1)

- 15 Wistar rats/sex/group were dosed in the diet with 0, 100, 500, 2000 (males only) or 3000 ppm (females only)
- High dose:
  Reduced body weight development & food consumption, increased water consumption
  Reduced red blood cells, hemoglobin, and hematocrit values
  Increased alkaline phosphatase activity
  Reduced mean globulin, triglycerides, cholesterol and specific urinary gravity values
  Increased absolute and relative liver and kidney weights
  Liver: decreased fat storage and increased in incidence and severity of cytoplasmic eosinophilia and granular cytoplasm
- NOAEL is 500 ppm

90 day toxicity 2,4-DP (2)

- 10 Wistar rats/sex/group were treated in the diet with 0, 100, 500 or 2500 ppm

- 2500 ppm:
  Reduced body weights. Increased water consumption

  Reduced red blood cell count, hemoglobin concentration and hematocrit. Increased alanine aminotransferase, alkaline phosphatase, urea, creatinine and total bilirubin values
  Reduced globulin, triglyceride (males) and cholesterol (males) values

  Liver weights increased, absence of peripheral fatty infiltration

  Kidneys weights increased at 2500 ppm and 500 ppm (males)

- NOAEL is 500 ppm

90 day toxicity 2,4-DP - conclusion

- 3000 - 2500 ppm
- Reduced body weight development, increased water consumption
- Reduced red blood cell values
- Target organs: liver and kidney

- NOAEL is 500 ppm

Metabolomics Quantitative Aspects (1)

- Sum of the fold changes of commonly significantly changed metabolites ($p < 0.05$) at three time points in rat plasma of four week studies with administration of 2500 ppm and 1000 ppm 2,4-DP, MCPP and MCPA

Dose	2,4-DP		MCPP		MCPA	
	males	females	males	females	males	females
2500 ppm	292	210	292	165	320	185
1000 ppm	29	23	33	23	30	33

→ Overall, the compounds appear to be equally potent
Females have lower values than males
There is a steep dose relationship
Values found at 1000 ppm are only moderately higher than what could be expected from random change

Metabolomics Quantitative Aspects (2)

- Overall profile strength of 2,4-DP, MCPP and MCPA for males and females at 2500 ppm and 1000 ppm. Calculated as the median profile P of all analytes of target treatment, i.e. “rounded down average of absolute medians of t-values”.

Dose	2,4-DP		MCPP		MCPA	
	Males	Females	Males	Females	Males	Females
2500 ppm	2.44	2.29	3.02	2.84	3.01	2.79
1000 ppm	1.14	1.1	1.85	1.57	1.81	1.71

<u>Overall profile strength</u>:

MCPP and MCPA have a similar strength, 2,4-DP is slightly weaker.
The dose response relationship is relatively steep,
2,4-DP low dose - moderate effect / approaching control values (random change values)
MCPP and MCPA – low dose indicative of a clear test substance related effect

Conclusion for read-across, based on Metabolomics

Qualitative assessment

- The source compounds MCPA and 2,4-DP are similar to the target compound MCPP
- The best read-across should be achieved using 2,4-DP which is considered substantially similar to MCPP

Quantitative assessment

- For read-across purposes, we assume the same strength of effects at the high dose level
- MCPP is at least as potent as 2,4-DP and possibly slightly stronger, particularly at the lower dose level
- NOAEL for MCPP may be below the one for 2,4-DP, comparable to that of MCPA (i.e. between 150 – 500 ppm)

90 day toxicity MCPP

- 15 Wistar rats/sex/group were treated at dietary concentrations of 0, 75, 500, 2500 (males only) or 3000 (females only) ppm

- 3000 - 2500 ppm:

  Decreased body weight and food consumption, increased water consumption
  Decreased red blood cell, hemoglobin and hematocrit values
  Increased alkaline phosphatase, alanine aminotransferase (females), urea and creatinine (males)
  Increased transitional epithelial cells in the urine (males)

  Increased relative liver weights and relative kidney weights

  Adrenal glands discoloration (lipid storage, adverse ?)

  Liver: decreased fat storage, bile duct proliferation, cytoplasmic eosinophilia and granular cytoplasm of hepatocytes

- 500 ppm:

  Decreased red blood cell, hemoglobin, and hematocrit levels (males), decrease in fat storage in the liver (males)

- The NOEL was 75 ppm, the US-EPA considers the NOAEL to be 500 ppm

A Comparison of MCPP and 2,4-DP

MCPP (2500/3000 ppm)	2,4-DP (2500 ppm)	Comments
reduced body weight dev.	reduced body weight dev.	
increased water consumption	increased water consumption	
reduced red blood cell values	reduced red blood cell values	
Liver: enzymes, increased weight, pathology	Liver: enzymes, increased weight, pathology	
Kidney: urea, creatinine, increased weight, epithelial cells in urine	Kidney: urea, creatinine, increased weight, reduced specific urinary gravity	
NOEL = 75 ppm NOAEL = 500 ppm (EPA)	NOAEL = 500 ppm	

From QSAR to QBAR
(quantitative biological activity relationships)