



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

Introduction: Case Study MCPP

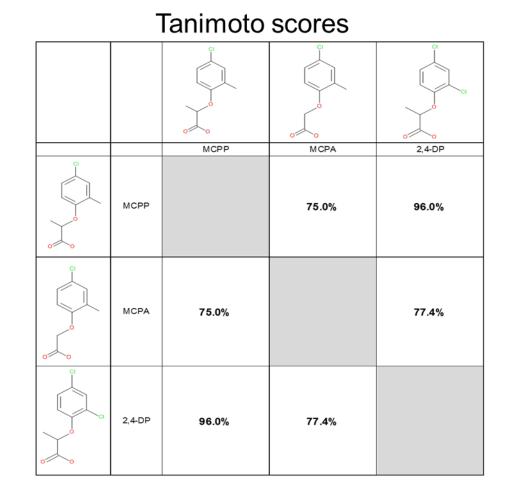
- Purpose: to demonstrate the usefulness of a biology based tool to provide qualitative and quantitative information to improve chemical grouping for readacross purposes.
- If the case study is not made to substitute any data, because the compounds used in this case have a full, agrochemical, toxicological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

We create chemistry

D = BASF We create chemistry

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 2,4position
 - MCPP vs 2,4-DP: phenoxypropionic acids



Acute Toxicity and Genotoxicity

BASF We create chemistry

	МСРР	2,4-DP	МСРА
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

L BASF e create chemistry (for ta

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

 \rightarrow 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:

We create chemistry

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

Clinical chemistry: \rightarrow 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 ?

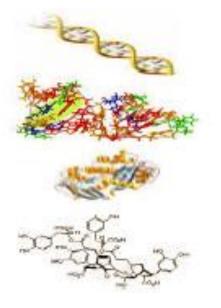
Mostly unknown

DNA 40,000 genes

RNA 150,000 transcripts

Proteins 1,000,000 proteins

Metabolites 2,500 metabolites



Genomics

Transcriptomics

Proteomics

Metabolomics

Advantage Single matrix (blood)

Mostly known

Closer to classical toxicology

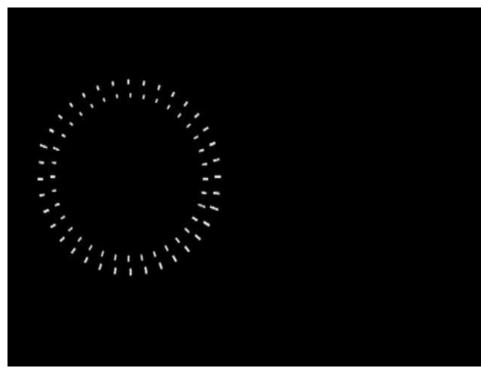
The Use of MetaMap®Tox



BLOOD PROFILING

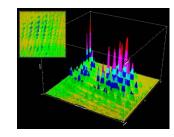






LC-MS/ GC-MS

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

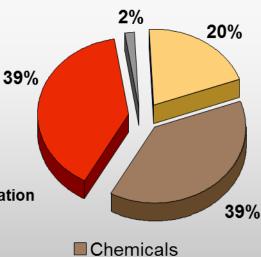
We create chemistry



- 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



Pharmaceuticals

Agrochemicals

Nutritionals

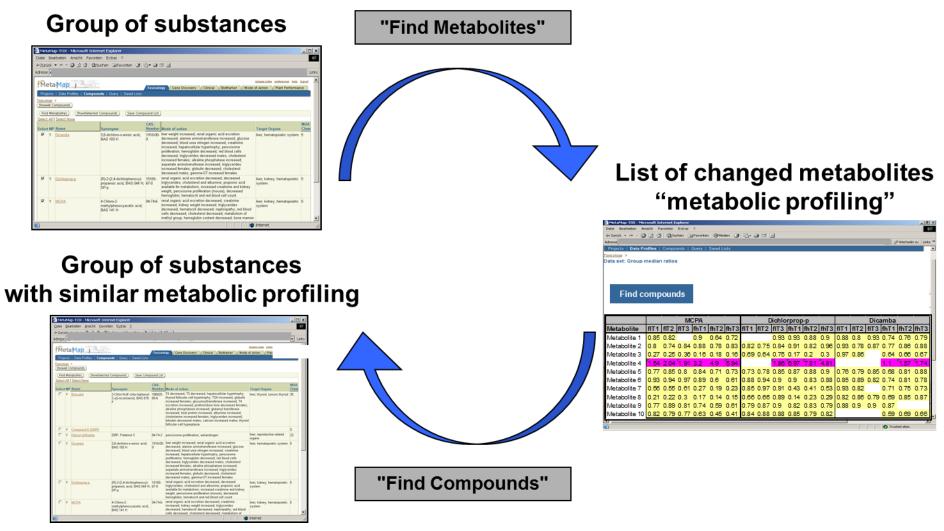


- Need at least 4 compounds sharing a MoA
- Determine common metabolites with 3 compounds, separated by sex, for at least two time points
- Pattern must find 4th compound (90% of metabolites correct)
- Pattern must be selective at 80% (should not find compounds that do not have the MoA)

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



MetaMap[™] Tox



Recognising mode-of-action:

We create chemistry

peroxisome proliferation

	Microsoft Internet Explorer										
atei Bearbeiten A	Ansicht Favoriten Extras ?										
🔁 Zurück 🝷 🕥	🕞 🛋 🛃 🌈 Suchen 🤺 F	avoriten 🧭 🔗 🍑	W -	🛍							
resse 顲										Vechseln zu Lir	nks » 🐑
★ pValue:	0.2 • Fracti	on of metabolites: 0.9					udy Contro				
🛪 Fold Change:	1.0			t-lest	version:				t (Welch t-Test) (pooled variance)		
						Onor	moscedas	tic t-rest	(pooled vanance)		
	(2-Formylamino-3-carboxythiophen) (N	40.45)				~			ofibrate (MOA50)		
	1,1,2,2-Tetrachloroethane (MOA29)	,				8	Nove	Fe	nofibrate (MOA48)		
	1,2-Cyclohexanedicarboxylic acid diis 1,2-Dichloroethane (MOA59)	ononyl ester (MOA26)					\gg	W3	/ 14643 (MOA51)		
Compounds:	1,3-Dichloro-2-propanol (MOA60)						Move A	ш —			
	1,3-Dinitrobenzene (MOA53) 1,4-Butanediol (MOA67)						Remove				
	1,4-Dinitrobenzene (MOA54)						<u>Remove</u>	2			
	1,4-Dioxane (MOA55) 1,4-Phenylene diisothiocyanate (MOA	72)				~	Remove	All			
								L.			
Analysis groups	: fi fi h fi7 fi14 fi28 √ fh7 √ fh14			mh .				Meta	bolite Information Columns: 🛛 📃]	
			niza 🗖 mn	/ 🗖 mn 14	mn28						
	(Submit parameters) (Res	et parameters									
Find Metabolites	ShowAll Metabolites Export T	able to Excel	Legend:	decrease	d no sign	ificant c	:hanges <mark>in</mark>	creased			
ShowSelected Me		Save Metabolite List)									
·		Save Metabolite List	Clafibrata		onofibra	to I	10/1-1	4642	-		
·		Save Metabolite List)	Clofibrate (MOA50)	F	enofibra (MOA48		Wy 1 (MO/				
Select All Select I		Save Metabolite List) MET_CHEM_ID fh	(MOA50)	F h28 fh7)		A51)			
Select All Select	None		(MOA50) 7 fh14 fl		(MOA48)	(MOA	451) 4 fh28			
Select All Select	None Anchor Metabolite	MET_CHEM_ID fb	(MOA50) 7 fh14 fi 07 <mark>1.75</mark> 1	h28 fh7	(MOA48 fh14) fh28	(MOA fh7 fh1	451) 4 fh28 2 2.27			
Select All Select Select Direction A	None Anchor Metabolite Pantothenic acid	MET_CHEM_ID fh 18000225 1.0	(MOA50) 7 fh14 ff 07 1.75 1 36 1.64 2	h28 fh7	(MOA48 fh14 2.75) fh28 3.27 1.86	(MOA fh7 fh1 1.44 2.2	451) 4 fh28 2 2.27 1 2.11			
Select All Select Select Direction A V up V V up V	None Anchor Metabolite Pantothenic acid Coenzyme Q9	MET_CHEM_ID 160 18000225 1.0 18000281 1.5	(MOA50) 7 fh14 fl 07 1.75 1 36 1.64 2 39 1.64 4	h28 fh7 .22 2.36 2.55 1.51	(MOA48 fh14 2.75 1.72) fh28 3.27 1.86 2.19	(MOA fh7 fh1 1.44 2.2 1.7 1.9	 4 fh28 2 2.27 1 2.11 5 1.17 			
Select All Select 1 Select Direction A V up V V up V V up V	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Slycerol, lipid fraction	MET_CHEM_ID 110 18000225 1.0 18000281 18 2800002 13	(MOA50) 7 fh14 ft 07 1.75 1 36 1.64 2 39 1.64 4 05 1.38 2	h28 fh7 1.22 2.36 2.55 1.51 1.99 2.35	(MOA48 fh14 2.75 1.72 2.47	fh28 3.27 1.86 2.19 1.42	(MOA fh7 fh1 1.44 2.2 1.7 1.9 1.11 1.4	4 fh28 2 2.27 1 2.11 5 1.17 1 1.02			
Select All Select All Select All Select Direction A Up Up V Up V Up V Up V Up V Up V	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol, lipid fraction Palmitic acid (C16:0)	MET_CHEM_ID 11/0 18000225 1.0 19000281 15 28000002 15 28000003 10	(MOA50) 7 fh14 ff 36 1.64 2 39 1.64 4 35 1.38 2 44 1.88	h28 fh7 .22 2.36 2.55 1.51 1.99 2.35 2.31 1.72	(MOA48 fh14 2.75 1.72 2.47 1.39	fh28 3.27 1.86 2.19 1.42 2.12	(MO/ fh7 fh1 1.44 2.2 1.7 1.9 1.11 1.4 1.21 1.3	A51) 4 fh28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94			
Select All Select All Select All Select All Select Direction A U Up V Up V U Up V U Up V U Up V U Up V U Up V U Up V	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol, lipid fraction Palmitic acid (C16:0) gamma-Linolenic acid (C18:c	MET_CHEM_ID fbi 18000225 1.0 18000281 1.8 28000002 1.3 28000003 1.0 280000477 2.0	(MOA50) 7 1014 10 7 1.75 1 36 1.64 2 39 1.64 4 35 1.38 2 34 1.88 1 35 0.75 0	h28 fh7 .22 2.36 2.55 1.51 1.99 2.35 2.31 1.72 7.0 3.64	(MOA48) fh14 2.75 1.72 2.47 1.39 2.83	fh28 3.27 1.86 2.19 1.42 2.12 0.59	(MOA 107 101 1.44 2.2 1.7 1.9 1.11 1.4 1.21 1.3 2.08 2.1	A51) 4 fh28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94 5 0.56			
Select All Select All elect Direction A Up V Up V V Up V V Up V V V V Up V V V V V V V V V V V V V V V V V V V	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol, lipid fraction Palmitic acid (C16:0) gamma-Linolenic acid (C18:c 16-Methylheptadecanoic acid	MET_CHEM_ID fbi 18000225 1.0 18000281 1.8 28000002 1.3 2800003 1.0 28000477 2.0 28000478 0.6	(MOA50) 7 10.14 10 17 1.75 1 36 1.64 2 39 1.64 4 15 1.38 2 34 1.88 5 5 0.75 0 48 0.57 0	h28 fh7 1.22 2.36 2.55 1.51 1.99 2.35 2.31 1.72 7.0 3.64 0.75 0.59	(MOA48) fh14 2.75 1.72 2.47 1.39 2.83 0.55	fh28 3.27 1.86 2.19 1.42 2.12 0.59 0.5	(MOA 1.7 1.11 1.44 2.2 1.7 1.9 1.11 1.4 1.21 1.3 2.08 2.1 0.67 0.8	4 fh28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94 5 0.56 6 0.47			
Select All Select A v up v up v	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol, lipid fraction Palmitic acid (C16:0) gamma-Linolenic acid (C18:c 16-Methylheptadecanoic acid 17-Methyloctadecanoic acid	MET_CHEM_ID fb. 18000225 1.0 18000281 1.8 28000002 1.3 2800003 1.0 28000477 2.0 28000478 0.6 28000479 0.4	(MOA50) 7 fh14 fl 7 fh14 fl 17 1.75 1 16 1.64 2 19 1.64 4 15 1.38 2 14 1.88 1 15 0.75 0 18 0.57 0 13 1.3 1	h28 fh7 1.22 2.36 2.55 1.51 1.99 2.35 2.31 1.72 7.0 3.64 0.75 0.59 0.77 0.5	(MOA48 fh14 2.75 1.72 2.47 1.39 2.83 0.55 0.57	fh28 fh28 3.27 1.86 2.19 1.42 2.12 0.59 0.5 1.61	(MOA 107 101 1.44 2.2 1.7 1.9 1.11 1.4 1.21 1.3 2.08 2.1 0.67 0.8 0.67 0.8	AST 4 fh28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94 5 0.56 6 0.47 5 1.18			
Select All Select All Up V Up V Up V Up V Up V Up V Up V	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol, lipid fraction Palmitic acid (C16:0) gamma-Linolenic acid (C18:c 16-Methylheptadecanoic acid 17-Methyloctadecanoic acid Threonic acid	MET_CHEM_ID fb. 18000225 1.0 18000231 1.8 2800002 1.3 2800003 1.0 28000477 2.0 28000478 0.4 3800083 1.2 3800083 1.2 38000441 0.6	(MOA50) 7 h14 f 107 1.75 1 36 1.64 2 39 1.64 4 39 1.64 4 39 1.88 3 35 0.75 0 18 0.57 0 31 1.3 1 37 0.86	h28 fh7 1.22 2.36 2.55 1.51 1.99 2.35 2.31 1.72 7.0 3.64 0.75 0.59 0.77 0.5 1.3 1.46 1.0 0.79	(MOA48 fh14 2.75 1.72 2.47 1.39 2.83 0.55 0.57 1.67 0.79	fh28 3.27 1.86 2.19 1.42 2.12 0.59 0.51 1.61 0.87	(1) 0 / 1) 1) 1 / 2 / 2 1, 1 / 2 / 2 1, 1 / 3 1, 1 / 4 1, 2 / 3 1, 2 / 4 1, 2 / 4 1, 2 / 4 1, 3 / 4 1, 3 / 4 1, 3 / 4 1, 4 / 4 1, 3 / 4 1, 4 /	AS fh28 4 fh28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94 5 0.56 5 0.477 5 1.18 7 0.79			
Select All Select A v up v v up v	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol.lipid fraction Palmitic acid (C16:0) gamma-Linolenic acid (C18:c 15-Methylheptadecanoic acid 17-Methyloctadecanoic acid Threonic acid Cytosine (Σ) Phosphatidylcholine No 04 ((2)	MET_CHEM_ID fb. 18000225 1.0 18000231 1.8 2800002 1.3 2800003 1.0 28000477 2.0 28000478 0.4 3800083 1.2 3800083 1.2 38000441 0.6	(MOA50) 7 h14 f 107 1.75 1 36 1.64 2 39 1.64 4 39 1.64 4 39 1.88 3 35 0.75 0 18 0.57 0 31 1.3 1 37 0.86	h28 fh7 1.22 2.36 2.55 1.51 1.99 2.35 2.31 1.72 7.0 3.64 0.75 0.59 0.77 0.5 1.3 1.46 1.0 0.79	(MOA48 fh14 2.75 1.72 2.47 1.39 2.83 0.55 0.57 1.67 0.79	fh28 3.27 1.86 2.19 1.42 2.12 0.59 0.51 1.61 0.87	(NO fh7 fh1 fh7 fh1 fh4 2.2 fh7 fh1 fh4 2.2 fh7 fh1 fh4 fh4 fh4 fh4 fh4 fh4 fh4 fh4 fh4 fh4	AS fh28 4 fh28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94 5 0.56 5 0.477 5 1.18 7 0.79			
Select All Select A v up v v up v	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol.lipid fraction Palmitic acid (C16:0) gamma-Linolenic acid (C18:c 15-Methylheptadecanoic acid 17-Methyloctadecanoic acid Threonic acid Cytosine (Σ) Phosphatidylcholine No 04 ((2)	MET_CHEM_ID fbi 18000225 1.0 18000225 1.0 28000002 1.3 28000002 1.3 28000047 2.0 28000478 0.4 28000479 0.4 38000478 1.2 38000478 0.4 38000478 0.4 38000478 0.6 28000479 0.4 38000441 0.8 2 6800020 0.6	(MOA50) 7 10.14 11 77 10.125 1 78 1.75 1 79 1.64 2 99 1.64 2 99 1.64 2 94 1.88 1 95 0.75 0 18 0.57 0 13 1.3 1 37 0.86 37 70 0.59 0	Image: heat state Image: heat state 1.22 2.36 2.55 1.51 1.92 2.35 2.31 1.72 7.00 3.64 0.75 0.59 0.77 0.5 1.3 1.46 1.0 0.79 0.68 0.71	(MOA48 h14 2.75 1.72 2.47 1.39 2.83 0.55 0.57 1.67 0.79 0.64	fh28 j 3.27 1.86 2.19 1.42 2.12 0.59 0.5 1.61 0.87 0.76	(hio) 1h7 1h1 1.44 2.2 1.7 1.9 1.11 1.4 1.21 1.3 2.08 2.1 0.63 0.8 0.69 0.3 0.69 0.3 0.84 0.8	AST 4 fb28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94 5 0.56 6 0.47 5 1.18 7 0.79 8 1.0			
Select All Select A v up v v down v v down v v down v	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol. lipid fraction Palmitic acid (C18:0) gamma-Linolenic acid (C18:c 15-Methylheptadecanoic acid 17-Methyloctadecanoic acid Threonic acid Cytosine (E) Phosphatidylcholine No 04 ((Setabolites) (Find Compounds) (Setabolites)	MET_CHEM_ID fbi 18000225 1.0 18000225 1.0 28000002 1.3 28000002 1.3 28000047 2.0 28000478 0.4 28000479 0.4 38000478 1.2 38000478 0.4 38000478 0.4 38000478 0.6 28000479 0.4 38000441 0.8 2 6800020 0.6	(MOA50) 7 10.14 11 77 10.125 1 78 1.75 1 79 1.64 2 99 1.64 2 99 1.64 2 94 1.88 1 95 0.75 0 18 0.57 0 13 1.3 1 37 0.86 37 70 0.59 0	Image: heat state Image: heat state 1.22 2.36 2.55 1.51 1.92 2.35 2.31 1.72 7.00 3.64 0.75 0.59 0.77 0.5 1.3 1.46 1.0 0.79 0.68 0.71	(MOA48 h14 2.75 1.72 2.47 1.39 2.83 0.55 0.57 1.67 0.79 0.64	fh28 j 3.27 1.86 2.19 1.42 2.12 0.59 0.5 1.61 0.87 0.76	(hio) 1h7 1h1 1.44 2.2 1.7 1.9 1.11 1.4 1.21 1.3 2.08 2.1 0.63 0.8 0.69 0.3 0.69 0.3 0.84 0.8	AST 4 fb28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94 5 0.56 6 0.47 5 1.18 7 0.79 8 1.0	I help I logout		
Select All Select A v up v v down v v down v v down v	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol.lipid fraction Palmitic acid (C16:0) gamma-Linolenic acid (C18:c 15-Methylheptadecanoic acid 17-Methyloctadecanoic acid Threonic acid Cytosine (Σ) Phosphatidylcholine No 04 ((2)	MET_CHEM_ID fbi 18000225 1.0 18000225 1.0 28000002 1.3 28000002 1.3 28000047 2.0 28000478 0.4 28000479 0.4 38000478 1.2 38000478 0.4 38000478 0.4 38000478 0.6 28000479 0.4 38000441 0.8 2 6800020 0.6	(MOA50) 7 10.14 11 77 10.125 1 78 1.75 1 79 1.64 2 99 1.64 2 99 1.64 2 94 1.88 1 95 0.75 0 18 0.57 0 13 1.3 1 37 0.86 37 70 0.59 0	Image: heat state Image: heat state 1.22 2.36 2.55 1.51 1.92 2.35 2.31 1.72 7.00 3.64 0.75 0.59 0.77 0.5 1.3 1.46 1.0 0.79 0.68 0.71	(MOA48 h14 2.75 1.72 2.47 1.39 2.83 0.55 0.57 1.67 0.79 0.64	fh28 j 3.27 1.86 2.19 1.42 2.12 0.59 0.5 1.61 0.87 0.76	(hio) 1h7 1h1 1.44 2.2 1.7 1.9 1.11 1.4 1.21 1.3 2.08 2.1 0.63 0.8 0.69 0.3 0.69 0.3 0.84 0.8	AST 4 fb28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94 5 0.56 6 0.47 5 1.18 7 0.79 8 1.0	help logout		
Select All Select A v up v v down v v down v v ShowSelected Me	None Anchor Metabolite Pantothenic acid Coenzyme Q9 Glycerol. lipid fraction Palmitic acid (C18:0) gamma-Linolenic acid (C18:c 15-Methylheptadecanoic acid 17-Methyloctadecanoic acid Threonic acid Cytosine (E) Phosphatidylcholine No 04 ((Setabolites) (Find Compounds) (Setabolites)	MET_CHEM_ID fbi 18000225 1.0 18000225 1.0 28000002 1.3 28000002 1.3 28000047 2.0 28000478 0.4 28000479 0.4 38000478 1.2 38000478 0.4 38000478 0.4 38000478 0.6 28000479 0.4 38000441 0.8 2 6800020 0.6	(MOA50) 7 10.14 11 77 10.125 1 78 1.75 1 79 1.64 2 99 1.64 2 99 1.64 2 94 1.88 1 95 0.75 0 18 0.57 0 13 1.3 1 37 0.86 37 70 0.59 0	Image: heat state Image: heat state 1.22 2.36 2.55 1.51 1.92 2.35 2.31 1.72 7.00 3.64 0.75 0.59 0.77 0.5 1.3 1.46 1.0 0.79 0.68 0.71	(MOA48 h14 2.75 1.72 2.47 1.39 2.83 0.55 0.57 1.67 0.79 0.64	fh28 j 3.27 1.86 2.19 1.42 2.12 0.59 0.5 1.61 0.87 0.76	(hio) 1h7 1h1 1.44 2.2 1.7 1.9 1.11 1.4 1.21 1.3 2.08 2.1 0.63 0.8 0.69 0.3 0.69 0.3 0.84 0.8	AST 4 fb28 2 2.27 1 2.11 5 1.17 1 1.02 2 1.94 5 0.56 6 0.47 5 1.18 7 0.79 8 1.0	help logout		

Recognising mode-of-action:

We create chemistry

peroxisome proliferation

MetaMap-TOX - Microsoft Internet Explorer	
Datei Bearbeiten Ansicht Favoriten Extras ?	
🔇 Zurück 🝷 🕥 - 💌 😰 🏠 🔎 Suchen 🚽	🛛 Favoriten 🚱 🔗 + چ 🔟 👻 🛄
Adresse 🚳	Vechseln zu Links » 👘 🗸
* Fold Change: 1.0	t-Test version:
	O homoscedastic t-Test (pooled variance)
(2-Formylamino-3-carboxythiophen 1,1,2,2-Tetrachloroethane (MOA29)	
1,2-Cyclohexanedicarboxylic acid 1,2-Dichloroethane (MOA59)	diisononyl ester (MOA26) Wy 14643 (MOA51) Bezafibrate (MOA49)
Compounds 1,3-Dichloro-2-propanol (MOA60)	Move All Mecoprop-p (MOA1)
1,3-Dinitrobenzene (MOA53) 1,4-Butanediol (MOA57)	Control Contro
1,4-Dinitrobenzene (MOA54) 1,4-Dioxane (MOA55)	Control Contro
1,4-Phenylene diisothiocyanate (M	10A72) Remove All
☐ fl ♥ f	
Analysis groups:	14 ♥ fh28 □ ml7 □ ml14 □ ml28 □ mh7 □ mh14 □ mh28
Submit parameters)	Reset parameters)
(Find Metabolites) (ShowAll Metabolites) (Expor	rt Table to Excel Legend: decreased no significant changes increased
ShowSelected Metabolites Find Compounds	(Save Metabolite List)
Select All Select None	Benzylbutyl
	Clofibrate Fenofibrate Wy 14643 Bezafibrate Mecoprop-p Dichlorprop- Phthalate Diethylhexylphthalate
Select Direction Anchor Metabolite	(MOA50) (MOA48) (MOA51) (MOA49) (MOA1) p (MOA1) (MOA6) (MOA58) MET_CHEM_ID_fh7_fh14_fh28_f
Up V Dentothenic 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 💌 🗋 <u>Coenzyme Q9</u>	18000281 1.86 1.64 2.55 1.51 1.72 1.66 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
🔽 💶 🔽 🔲 Glycerol, lipid fraction	28000002 139 164 499 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
🔽 💶 🔽 🔲 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
🗹 💵 🔽 🔲 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	
To-wetrymeptadecanoic act	
down V 17-Methyloctadecanoic acid	d 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 0.55 0.62 0.49 0.62 0.55 0.66 0.68 0.63 0.63 28000479 0.48 0.57 0.77 0.5 0.63 0.64 0.59 0.54 0.54 0.55 0.62 0.49 0.62 0.55 0.66 0.63 0.68 0.63 28000479 0.48 0.57 0.77 0.5 0.63 0.65 0.55 0.55 0.56 0.63 0.57 0.78 0.64 0.69 0.68 0.63
Image: down in the second s	d 28000478 0.55 0.75 0.75 0.59 0.55 0.67 0.85 0.61 0.99 0.54 0.54 0.55 0.62 0.49 0.62 0.55 0.66 0.65 0.63 0.63 0.63 0.64 0.54 0.55 0.62 0.49 0.62 0.55 0.66 0.65 0.63 0.63 0.63 0.63 0.64 0.54 0.55 0.52 0.57 0.67 0.56 0.62 0.76 0.76 0.76 0.76 0.76 0.66 0.65 0.63 0.64 0.54 0.54 0.55 0.52 0.57 0.67 0.66 0.62 0.76
 ✓ down ✓ □ 17-Methyloctadecanoic acid ✓ up ✓ □ Threonic acid ✓ down ✓ □ Cytosine (Σ) 	d 28000478 0.55 0.75 0.75 0.55 0.55 0.57 0.55 0.56
✓ down ✓ 17-Methyloctadecanoic acid ✓ up ✓ Threonic acid ✓ down ✓ Cytosine (Σ) ✓ down ✓ Phosphatidylcholine No 04 ()	a 28000478 0.55 0.75 0.75 0.55 0.57
✓ down ✓ 17-Methyloctadecanoic acid ✓ up ✓ Threenic acid ✓ down ✓ Cytosine (Σ)	d 28000478 0.55 0.75 0.75 0.55 0.55 0.57 0.55 0.56
✓ down ✓ 17-Methyloctadecanoic acid ✓ up ✓ Threonic acid ✓ down ✓ Cytosine (Σ) ✓ down ✓ Phosphatidylcholine No 04 (. (ShowSelected Metabolites) Find Compounds)	a 28000478 0.55 0.75 0.75 0.55 0.57
✓ down ✓ 17-Methyloctadecanoic acid ✓ Up ✓ Threonic acid ✓ down ✓ Cytosine (Σ) ✓ down ✓ Phosphatidylcholine No 04 (.)	a 2800478 0.55 0.75 0.75 0.55 0.55 0.57 0.55 0.57 0.55 0.57 0.55 0.57 0.55 0.57 0.55 0.57
down 17-Methyloctadecanoic acid up Threonic acid down Cytosine (2) down Phosphatidylcholine No 04 (, ShowSelected Metabolites) (Find Compounds) Copyright (C) Metanomics 2005-2008. Version 3.2 (4076)	a 2800478 0.55 0.75 0.75 0.55 0.55 0.57 0.55 0.57 0.55 0.57 0.55 0.57 0.55 0.57 0.55 0.57



Pairwise Comparison: Clofibrate

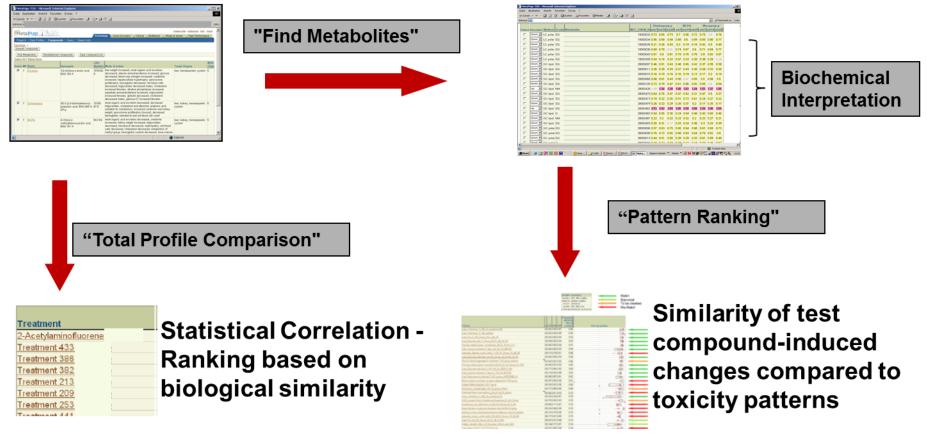
Clofibrate (MOA50)	Pearson	l		Spearm	an	
Treatment	rp	р	rank	rs	р	rank
Clofibrate (MOA50)	1,00	0,00	1	1,00	0,00	1
Fenofibrate (MOA48)	0,79	0,00	2	0,70	0,00	3
Mecoprop-p (MOA58)	0,78	0,00	3	0,66	0,00	5
Pravastatin + Fenofibrat (MOAFW05)	0,73	0,00	4	0,63	0,00	11
Bezafibrate (MOA49)	0,72	0,00	5	0,62	0,00	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

D BASF We create chemistry

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

Metabolite profile

Test Substance



D = BASF We create chemistry

MetaMap[®]Tox: Assessment of predictivity

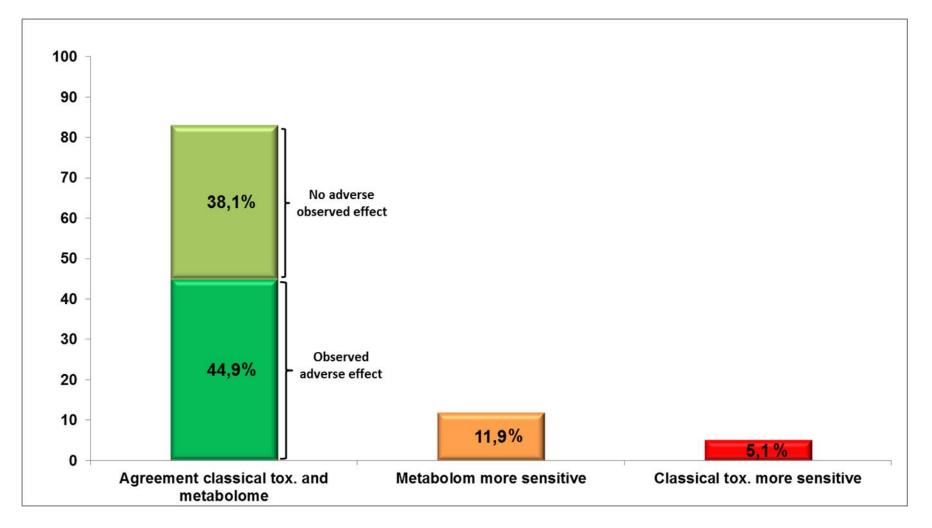
Evaluation of MetaMap®Tox predictions against histopathology outcome

	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



BASF We create chemistry

MetaMap[®] Tox: Phenoxy herbicides – Metabolic communalities

		2,4-DP			MCPA			MCPP	
Metabolite	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	0.20	0.29	0.41	0.27	0.42	0.26	0.28	0.34	0,26
(C20:cis[5,8,11,14]4)		· · · ·	1	1			1	1	
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
LysophosphatidyIcholine (C17:0)	0,43	0,35	0,35	0,59	0,54	0,35	0,43	0,34	0,24
LysophosphatidyIcholine (C18:0)	0,77	0,78	0,83	0,81	0,83	0,73	0,75	0,78	0,77
LysophosphatidyIcholine (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	МСРА	МСРР
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

We create chemistry

MetaMap[®] Tox: Phenoxy herbicides – Best read-across option

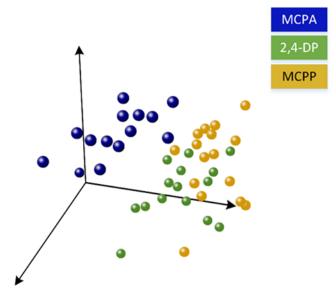
Martin - Par		MCPP						MCPA		
Metabolite	m7	m14	m28	m7	2,4-DP m14	m28	m7	m14	m28	
3-Indoxylsulfate	4,14	2,10	3,00	5,58	3,28	3,25	0,72	1,53	1,92	
3-Methoxytyrosine	1,33	1,35	1,76	1,22	1,32	1,35	1,08	1,19	1,84	
alpha-Tocopherol	0,56	0,65	0,59	0,70	0,63	0,68	0,93	1,09	0,98	
beta-Sitosterol	0,24	0,34	0,23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

	Metabolite		MCPP			MCPA		2,4-DP		
b)	Wetabolite	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.

<u>The PCA analysis</u> 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:

We create chemistry

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:

BASE

We create chemistry

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:

We create chemistry

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:

We create chemistry

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

BASE

We create chemistry

- 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</p>

Dose	2,4	2,4-DP		PP	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

BASE

We create chemistry

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".

	2,4-DP		МС	PP	МСРА		
Dose	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	

Overall profile strength:

We create chemistry

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:

We create chemistry

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

BASF We create chemistry

MCPP (2500/3000 ppm)	2,4-DP (2500 ppm)	Comments
reduced body weight dev.	reduced body weight dev.	\checkmark
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)

D-BASF

We create chemistry

