

Webinar Programme

Introduction	Mike Penman (LOA)	13:00 (CET)
Read-Across in REACH; application to UVCBs	Dr. David Bell (ECHA, Helsinki)	13:10
Introduction to Metabolomics	Prof. Mark Viant (University of Birmingham, UK)	13:25
Use of Metabolomics for Read-Across	Prof. Bennard Van Ravenzwaay (BASF)	13:45
Break		14:05
Use of Metabolomics to Assess Biological Coherence in UVCB Read-Across and Category Justification		
A. Introduction	Mike Penman (LOA)	14:10
B. Experimental Design and Data Review	Prof. Hennicke Kamp (BASF)	
C. Conclusion	Dr. Martijn Rooseboom (Shell/LOA)	
Discussion moderator	Prof. Mark Viant (University of Birmingham, UK)	15:15
Close		16:00

LOA REACH **CONSORTIUM**

Utility of Metabolomics to Support Read-Across
for UVCB substances under REACH

Webinar

30th November 2021

Use of Metabolomics to Assess Biological Coherence in UVCB Read-Across and Category Justification

A. Introduction

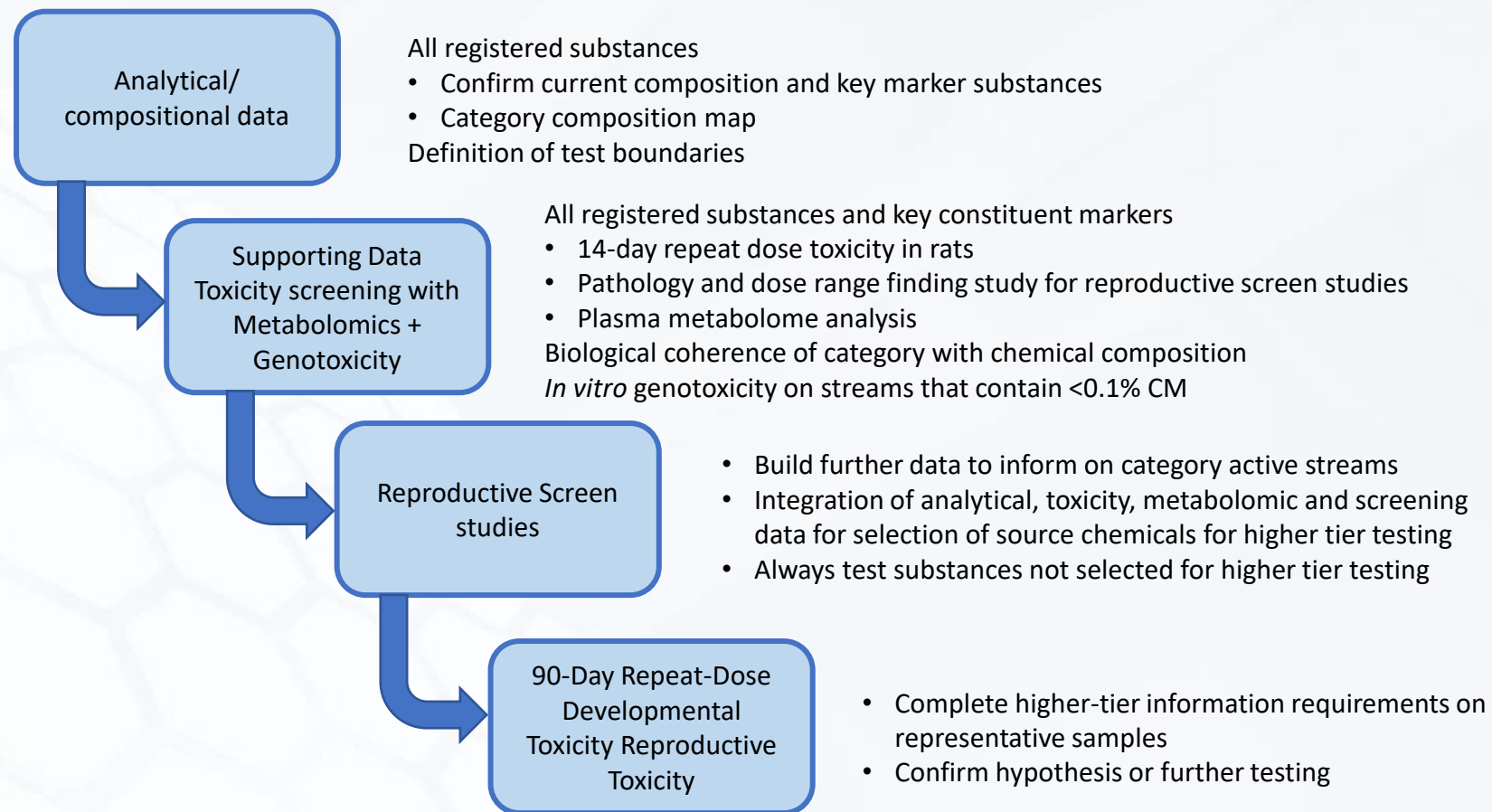
Mike Penman

LOA UVCB categories by C-number and manufacturing process

LOA Category	Category Name	Predominant C Number	Category Manufacturing Process	No. Substances
A	Aliphatic / Cyclic C5 and Higher	C5 to C8	HC streams typ. from a naphtha/pyrolysis gasoline treatment and aromatic extraction unit involving pre-distillation, hydrogenation and extractive distillation processes. Produced after aromatic extraction or (fractionated) distillation of hydrotreated naphtha.	6
E	C5 non-cyclics	C4 to C8	Hydrocarbon streams typ. from the steam cracking process as products of distillation processes. This C5+ cut (typical boiling range of approximately 0 to 75 °C) predominately consist of C5 hydrocarbons. The streams contain significant levels of olefins and diolefins.	6
C	C4, Low 1,3-Butadiene (<0.1%)	C4	Typically produced from the steam-cracking of naphtha and following the extraction of 1,3-butadiene from a C4-rich stream.	5
D	C4, High 1,3-Butadiene (≥0.1%)	C4	Typically produced from the steam-cracking of naphtha as a C4-rich stream.	6
H	High Benzene Naphthas	C5 to C11	Distillation of products from a steam cracking process or by pyrolysis. Predominantly hydrocarbons >C6 and BP range 30°C to 300°C.	26
J	Low Benzene Naphthas	C7 to C13	Distillation of products from a steam cracking process or by pyrolysis. <0.1% benzene.	4
L	Resin Oils and Cyclic Dienes	C5 to C15	Hydrocarbons typ. produced by distillation of products from a steam cracking process. Non-hydrotreated products (Resin Oils) and/or concentrates of (1) DCPD and (2) (MeDCPD).	10*
B	Butylene Oligomers	C4 to C20	Streams obtained by the oligomerisation of butylenes optionally followed by hydrotreating processes. Predominantly C8, C12, C16 and/or C20 hydrocarbons. The streams' constituents boil between 30 and 350 °C and the streams contain less than 0.1% butadiene.	8
G	Fuel Oils	C6 to C21	Hydrocarbon streams typically produced by distillation of products: from a steam cracking process, from an ethylene manufacturing process; residual fraction from these distillation processes, or produced by pyrolysis.	13
K	Other Petroleum Gases	C1 to C5	Hydrocarbon streams containing petroleum gases (alkanes/alkenes) predominantly in the C1-C5 range (with some carbon numbers present at lower levels up to C10) and include some LPGs. The majority of the members of this category contain <0.1% 1,3-butadiene.	29

Test strategy overview for non-CMR categories

Test strategy overview



LOA Streams and Categories

- LOA Working Group - strove to find techniques to help support LOA UVCBs
 - Aim – develop biological evidence to understand UVCB relationships in categories
 - Discussions with academia, CROs, industry experts
 - Biological Coherence Workshop in June 2017 with third parties
 - Examined New Approach Methodologies (NAMs)
 - *in silico*
 - *in vitro*
 - In vitro functional and binding assays –broad data on Biological Molecular Initiating Events (MIEs)
 - Embryonic Stem Cell Testing
 - “Smart” *in vivo*
 - Screening Reproductive and Developmental Effects in Embryos of 3 Non-Mammalian Species
 - ‘Classical’ RDT DRF with additional biological “readout”

Evaluation of an approach for Biological Coherence

- Considerations:

- Biological endpoint coverage and relevance
- Suitability for UVCBs
- Suitability for grouping / selection of candidates for further testing
- Animal use
- Reliability
- Cost
- Timeline

- Conclusions

- *In vitro* screening approaches and DART alternatives are scientifically interesting while currently problematic for regulatory purpose
 - data often potentially insufficiently robust to base read across or test substance selection rationale to meet the REACH information requirements
- Short term *In vivo* studies have a regulatory purpose under REACH
- A 14-day study coupled with additional screening assay believed to be the most promising approach

Rationale for commissioning Metabolomics study

- Well developed technology with reliable partners
- Based upon in vivo 14-day rat oral studies with pathology and clinical chemistry
 - Repeat dose data – information for dose range finding for screening and higher tier tests
- Ability to include major markers in the programme – coherence ?
 - Link to earlier approach (2010)
- Could provide basis for an understanding of the underlying Mode of Action (MoA) and Adverse outcome pathways (AOP)
- Could support category approaches and read across
 - Will report the findings in registration dossiers
- Extensive OECD 422 data also planned on each stream to build the databases and understand relevance for RDT and reproductive effects
- Method could be used to support Biological coherence of other LOA categories
 - if alignment seen between metabolomics and OECD 422

Choice and composition of Samples for a Metabolomics study

- **Category - Resin Oils and Cyclic dienes**
 - (LOA Category L)
 - Hydrocarbons typ. produced by distillation of products from a steam cracking process. Non-hydrotreated products (Resin Oils) and/or concentrates of Dicyclopentadiene (DCPD) and MeDCPD.
 - Complex category defined by composition and Manufacturing process
- **Detailed analysis of substances as well as biological investigation**
 - Quantified “marker” substances

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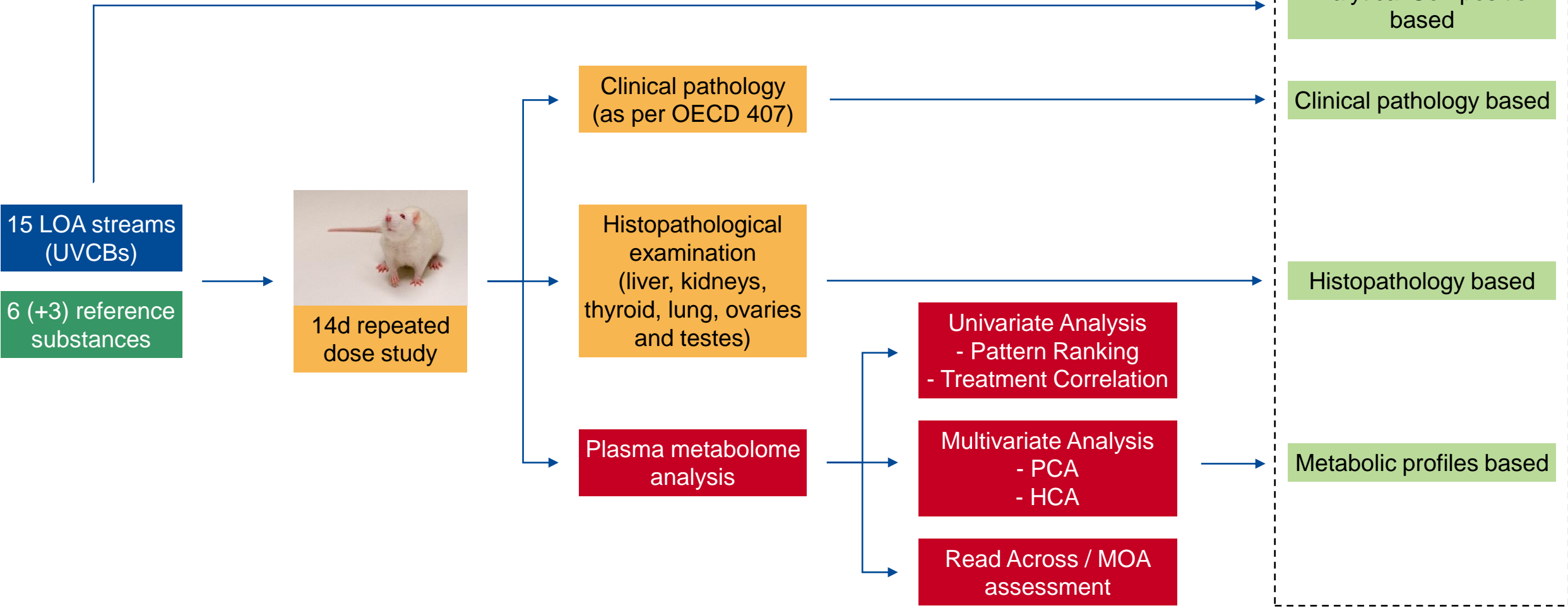
30th November 2021

Use of Metabolomics to Assess Biological Coherence in UVCB Read-Across and Category Justification

B. Experimental Design and Data Review

Prof. Hennicke Kamp (BASF)

Overview



LOA: 14-day Metabolome Study - Design

- Rats Crl:Wi(Han)
 - ▶ Both sexes, 5 animals per dose group
 - Low Dose (LD) and High Dose (HD) groups
 - ▶ Control group - 10 animals per group
- 14 day treatment (oral, gavage in corn oil)
- Plasma metabolome analysis (1 timepoint), including catecholamines and steroids
- Clinical pathology (haematology and blood chemistry in accordance with OECD 407)
- Histopathological examination of liver, kidneys, thyroid, lung, ovaries and testes
- Tissue storage for the purpose of further analysis (not limited to histopathology purposes)

§ - Good Laboratory Practice - §



LOA: 14-day Metabolome Study - Design

Dose levels

→ Based on 7 day study

→ Clinical data

→ Food consumption

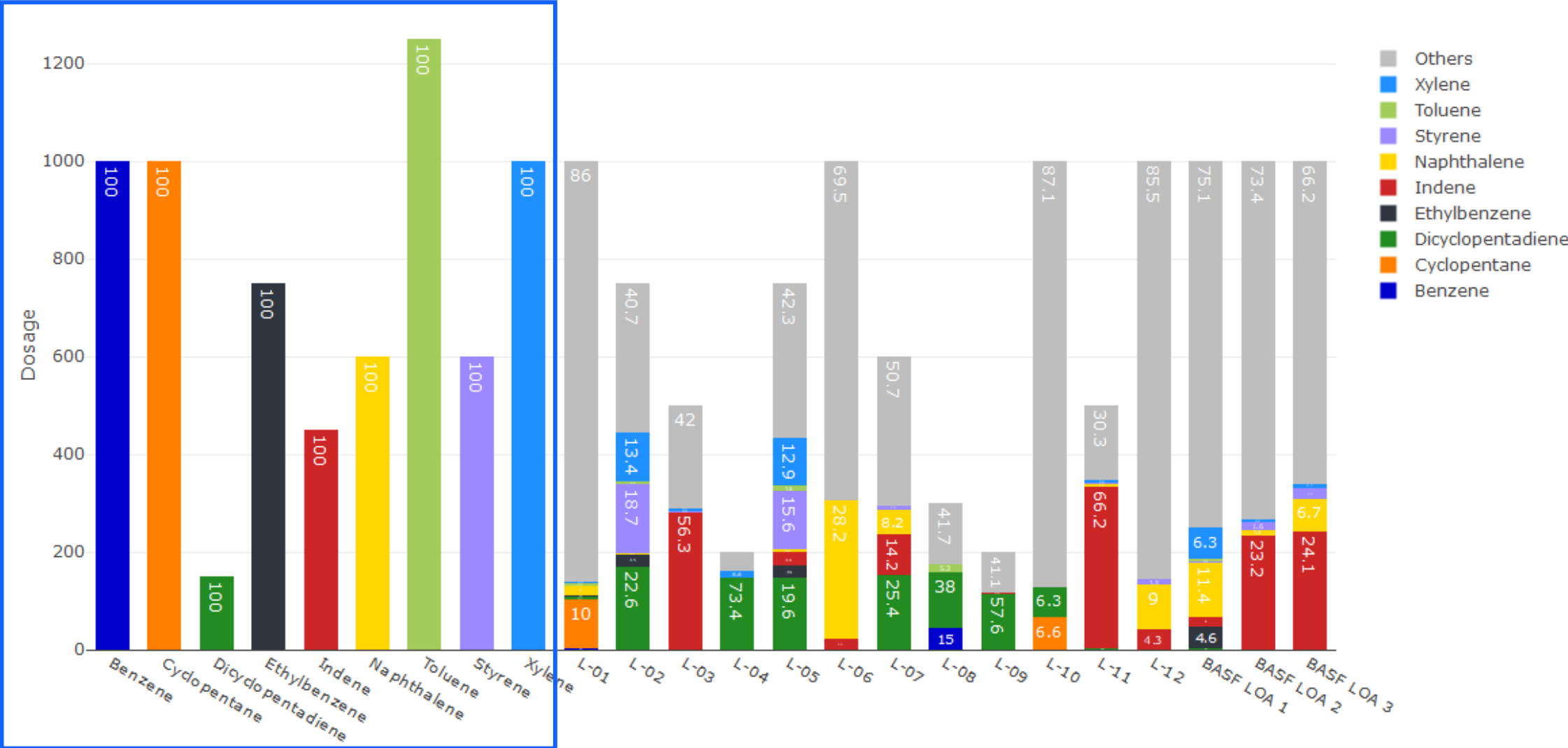
→ Body weight

Stream	Low dose (mg/kg bw)	High dose (mg/kg bw)	Marker	Low dose (mg/kg bw)	High dose (mg/kg bw)
L-01	300	1000	Benzene	300	1000
L-02	250	750	Xylene	300	1000
L-03	150	500	Naphthalene	250	600
L-04	70	200	DCPD*	50	150
L-05	250	750	Cyclohexane	300	1000
L-06	300	1000	Indene	100	450
L-07	200	600	Ethylbenzene**	250	750
L-08	100	300	Toluene**	600	1250
L-09	70	200	Styrene**	200	600
L-10	300	1000			
L-11	150	500			
L-12	300	1000			
BASF LOA 1	300	1000			
BASF LOA 2	300	1000			
BASF LOA 3	300	1000			

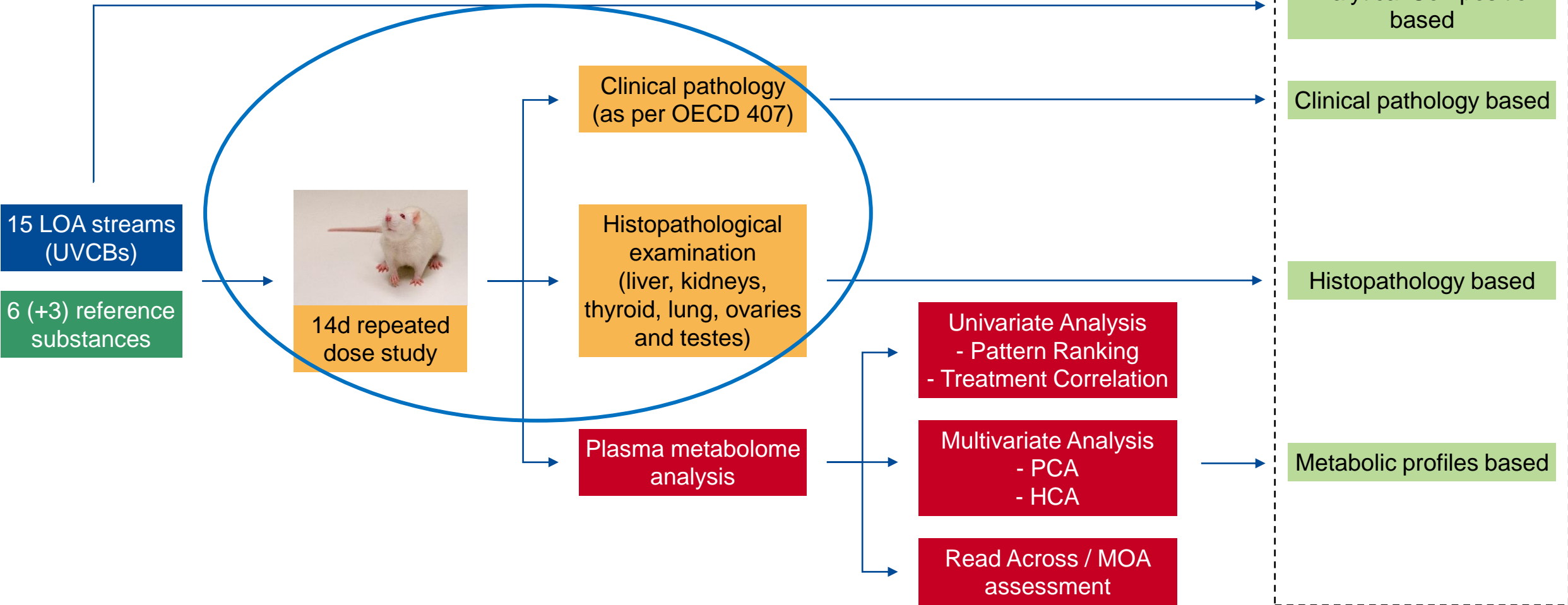
* Dicyclopentadiene

** 28-day studies

Dosage (HD) and % of Marker Substances in Stream composition



Summary Clinical & Pathology Results



14-day Results: Food Consumption, Body Weight & Clinical Data

- Food Consumption
 - ▶ Initial strong reduction in low and high dose groups
- Body weight
 - ▶ Reduction: High Dose up to 16%; Low dose, up to 6%
- Clinical Observations
 - ▶ Semi-closed eyelid, piloerection and apathy observed in almost all high dose groups
 - Dose response observed
 - Not seen (or lesser effect) in L-01, benzene, xylene, cyclopentane groups
- Observations were as expected from test study (except naphthalene)
 - Data give sound basis for dose setting for OECD 422 studies currently being conducted

14-day Results: Clinical Chemistry in High Dose Groups

Organ	Effect	Streams		Markers
		Males	Females	
Red Blood Cells	Increased RBC metabolism RetA ↑	L-1,-2,-3,-5,-6,-7,-10,-12	L-1,-2,-5,-6,-7,-8	Napthalene
	Regenerative anaemia	L-11, BASF LOA-2	L-3,-11,-12 BASF LOA-1, -2, -3	Indene
White Blood Cells	lymphopenia	-	-	Benzene
Liver	Liver cell dysfunction HQT	L-8,-9	L-4,-8,-9	Indene, xylene Dicyclopentadiene, Napthalene
	Liver cell membrane degredation ALT ↑	L-1,-2,-3,-5,-6,-7 BASF LOA-1,-2,-3	L-1,-2,-3,-5,-6,-7 BASF LOA-1,-2,-3	Napthalene
No adverse effect		L-4	L-10	Cyclopentane

RetA: absolute reticulocyte counts
HQT: prothrombin time (Hepatoquick's test)
ALT: alanine aminotransferase

Stream	High dose (mg/kg bw)
L-01	1000
L-02	750
L-03	500
L-04	200
L-05	750
L-06	1000
L-07	600
L-08	300
L-09	200
L-10	1000
L-11	500
L-12	1000
BASF LOA 1	1000
BASF LOA 2	1000
BASF LOA 3	1000

14-day Results: Organ Weight Data

- Liver
 - ▶ High Dose - generally increased (relative and absolute) – except benzene, cyclopentane
 - ▶ Low dose partially
- Thyroid
 - ▶ Increased for some Streams and Markers (relative and absolute)
- Kidney
 - ▶ Increased in males (relative and partly absolute)
- Spleen, heart, adrenals, ovaries, testes, seminal vesicle, epididymides, prostate
 - ▶ Occasional weight changes
 - at least partially related to body weight changes

14-day Results: Histopathology – 1

Overview: dose-response seen for most of the effects observed

■ Liver

- ▶ Hepatocellular hypertrophy (centrilobular, periportal, diffuse)
 - main histological finding - except for
 - Benzene, DCPD, Cyclopentane, Indene, BASF LOA streams in males
 - L-09, Benzene, Cyclopentane, Indene in females
- ▶ Occasionally Kupffer cell hypertrophy/ hyperplasia, single cell necrosis
 - mostly in single animals
 - L-09 (HD) in males; Naphthalene (HD), DCPD (HD) in females
 - only benzene showed single cell necrosis in three female animals of the high dose and 5 female animals of the low dose

■ Thyroid

- ▶ Follicular hypertrophy/ hyperplasia
 - all LOA-streams in males and most LOA-streams in female
 - all BASF-LOA streams, some marker compounds in female animals

14-day Results: Histopathology – 2

Overview: dose-response seen for most observed effects

■ Kidney

- ▶ Eosinophilic droplets in males except for Naphthalene, Indene (confirmed: alpha 2u globulin)
- ▶ Occasionally tubular degeneration/ regeneration in females: Naphthalene, L-03 (single animals)

■ Lung

- ▶ Bronchiolar hypertrophy/ hyperplasia, alveolar histiocytosis, thickened septae (unclear origin)
 - high dose males: → L-03, L-04, L-07, L-10, L-11
 - finding occasionally seen also in control animals
 - only minimal to slight changes
 - considered not adverse (indicative for local irritation, potentially adaptive clearance processes)

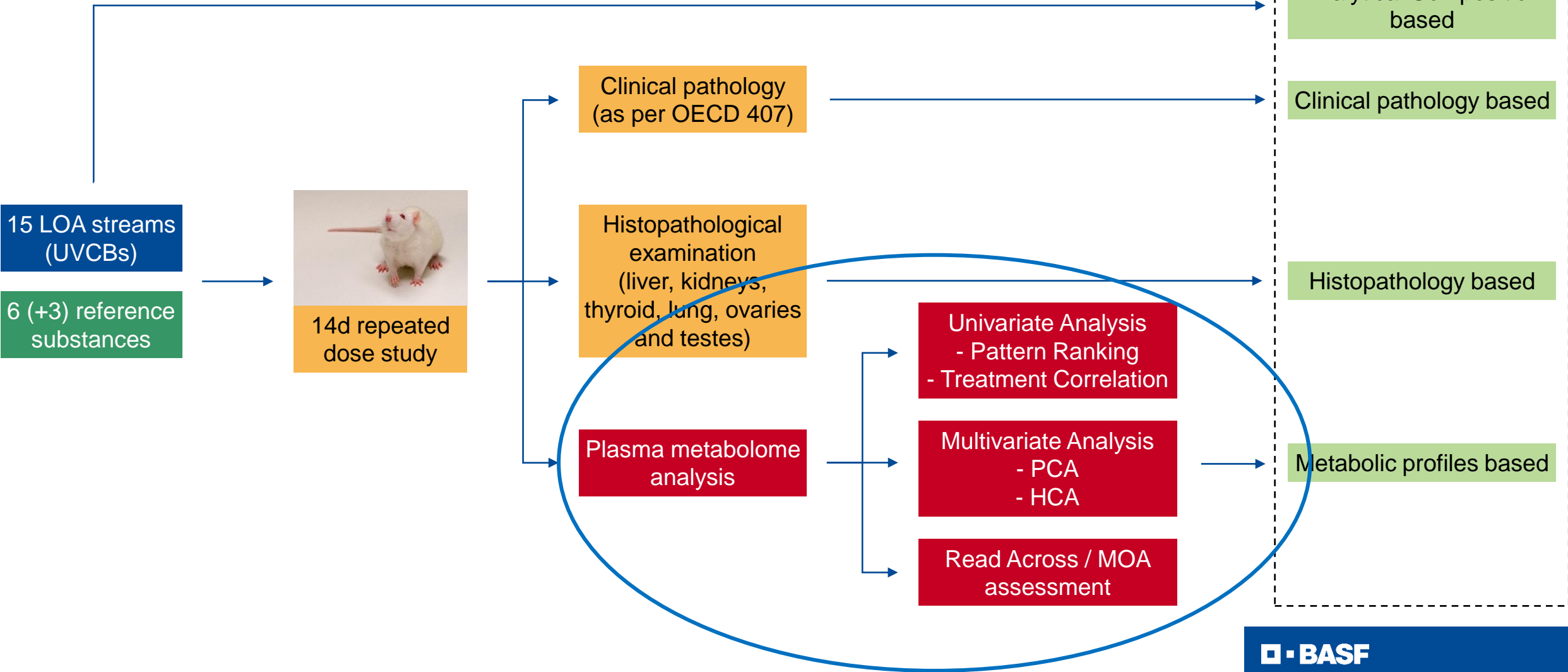
■ Ovaries and Testes

- ▶ No histological changes except for slight reduction in size in two animals diagnosed at necropsy for L-12

14-day Results – Summary classical parameters

- Clinical symptoms, food consumptions and body weight effects observed were as expected from test study (except naphthalene)
- Clinical pathology
 - ▶ Effects on liver and the red blood cell system
- Organ weight
 - ▶ Effects mainly on liver, thyroid (both sexes) and kidney (males).
- Histopathology
 - ▶ Liver and thyroid effects (indicative for enzyme induction) in both sexes
 - ▶ Kidney effects in males (confirmed alpha 2u globulin)

Summary Metabolome Results



14-day Metabolome Study – the Metabolome Data

Overview

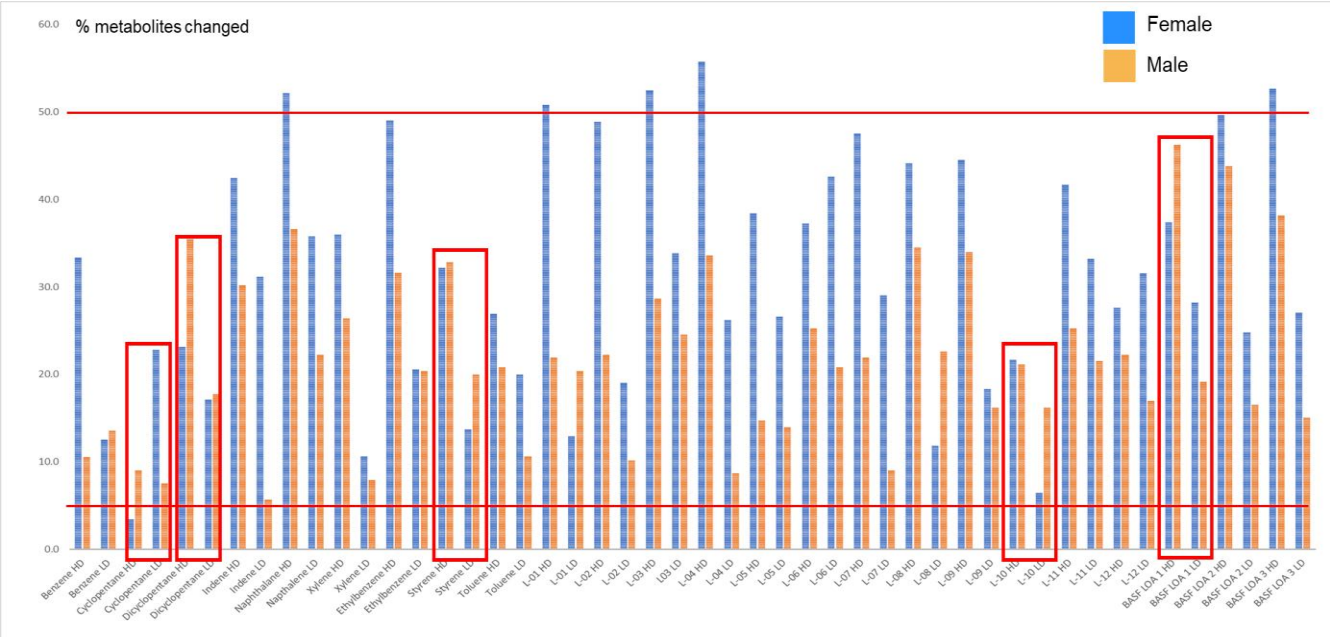
- Significant effects on metabolome
 - ▶ Dose response for all treatments
 - ▶ Effects in females stronger than in males
- Pattern matches indicated primarily liver as target organ
- Treatment correlation shows three clusters of higher similarity
- Multivariate analysis show separating trend for three clusters

Metabolite Changes → Strength of Effects

- Generally dose-dependency observed
 - ▶ (exc. Benzene (m), Cyclopentane (f))






- Response in female animals generally higher compared to males
 - ▶ (exc. Cyclopentane, Dicyclopentadiene, L-10, BASF LOA 1)

- Generally very high response, especially for female animals
 - ▶ (> 50% for Naphthalene, L-01, L-03, L-04, BASF LOA 2 + 3)



Pattern Ranking: Example LOA Stream L-01, HD

Rank no.	Pattern Name	Gender	Median r	Assessment
1	Liver, cholestasis (putative)	Female	0.91077475	95%
2	Liver Toxicity	Female	0.90536355	100%
3	Liver, enzyme induction	Female	0.89080331	96%
4	Liver, paracetamol-like toxicity	Female	0.88672816	86%
5	Liver, enzyme induction d14	Female	0.85687712	96%
6	Thyroid indirect, liver enzyme induction	Female	0.83587564	92%
7	Thyroid indirect, liver enzyme induction	Male	0.83406248	81%
8	Blood platelet aggregation inhibition	Female	0.8331395	78%
9	Liver, paracetamol-like toxicity	Male	0.82475422	88%
10	Bone marrow suppression d28 (putative)	Female	0.82273891	73%
11	Kidney, interstitial nephritis	Male	0.80433547	61%
12	Liver, enzyme induction	Male	0.78154309	72%
13	Anemia, iron deficiency	Female	0.74486341	64%
14	Liver, peroxisome proliferation d28	Female	0.72757798	67%
15	CNS, serotonin reuptake inhibition	Male	0.72532916	80%

	match
	weak match
	equivocal
	inconclusive
	mismatch

Pattern Ranking – Males (Overview)

		Marker Compounds												BASF LOA Streams					LOA Streams																											
		Benzene HD	Benzene LD	Cyclopenta	Cyclopenta	Cyclopenta	Indene HD	Indene LD	Naphthalene	Naphthalene	Xylene HD	Xylene LD	Toluene HD	Toluene LD	Styrene HD	Styrene LD	Ethylbenzene	Ethylbenzene	BASF L1 HD	BASF L1 LD	BASF L2 HD	BASF L2 LD	BASF L3 HD	BASF L3 LD	L-01 HD	L-01 LD	L-02 HD	L-02 LD	L-03 HD	L-03 LD	L-04 HD	L-04 LD	L-05 HD	L-05 LD	L-06 HD	L-06 LD	L-07 HD	L-07 LD	L-08 HD	L-08 LD	L-09 HD	L-09 LD	L-10 HD	L-10 LD	L-11 HD	L-11 LD
Sex	Pattern																																													
Male	Liver toxicity																																													
	Hepatocarcinoma-like toxicity																																													
	Liver, enzyme induction																																													
	Liver, oxidative stress (LD) (putative)																																													
	Liver, oxidative stress d28 (putative)																																													
	Liver, oxidative stress (putative)																																													
	Polypates, short chain																																													
	Thyroid indirect, liver enzyme induction																																													
	kidney interstitial nephritis																																													
	kidney, alpha 2u																																													
	Blood, anemia																																													
	Hormones, steroid dysregulation																																													
	Hormones, androgen receptor agonist																																													
	Adrenal cortex, corticosterone synthesis inhibition																																													
	Testicular toxicity																																													
	immunosuppression d28																																													
	CNS, serotonin reuptake inhibition																																													
	CNS, dopamine antagonist																																													
	Pharma, PDE-V inhibition																																													
	Nervous system sympathetic, noradrenergic increase (putative)																																													
	Hormones, testosterone																																													
	Adrenals, Hypercortisolism d28																																													
	Duodenum, non deficiency																																													
	Nervous system, nicotinic receptor agonist (putative)																																													
	Kidney, diuretic effect																																													
	Liver, peroxisome proliferation d14																																													
Nervous system sympathetic, noradrenergic increase (putative)																																														
Spleen, haemostasis																																														
Antibiotics, fluoroquinolones																																														
Kidney, organic anion transporter inhibition																																														
Hormones, estrogen receptor modulator																																														
Kidney, tubular defect																																														
Kidney, ADH inhibitor d28																																														
Liver, PPAR gamma agonist																																														
Liver, peroxisome proliferation																																														
Liver, peroxisome proliferation d28																																														
Adrenal cortex, dihydrotestosterone reductase (putative)																																														
CNS, dopamine antagonist																																														
Thyroid, hyperthyroidism																																														
CNS, serotonin receptor antagonist																																														
Stress, swim																																														
Thyroid, strong direct inhibition																																														
Kidney, tubular defect																																														
Bone marrow suppression d28																																														
Spleen, haemostasis																																														
Kidney, glomerular tubular defect																																														
Bone marrow suppression, platelet																																														
Neomycine Gentamycine Males HD																																														
Antibiotics, tetracyclines																																														
Lincosamide effects on microbiome																																														
Trazolol pattern M																																														
Blood, aplastic anemia																																														
Stress, swim																																														
Pharma, PDE-V inhibition d28																																														
Nutrition biomarkers (non-fasted)																																														
Nutrition non fasted animals																																														
Spleen, Metformin																																														
Pregnant fetal effects																																														
CNS, serotonin reuptake inhibition																																														
Antibiotics, aminoglycosides (putative)																																														
CNS, GABA receptor agonist d28																																														
Spleen, haemostasis (putative)																																														
Nutrition non fasted animals																																														
Kidney, organic anion transporter inhibition																																														
Eye, WFO inhibition																																														
Antibiotics, fluoroquinolones M group																																														
Nutrition biomarkers (non-fasted)																																														
Adrenals, corticosterone reduction																																														
Kidney, tubular toxicity d28																																														
Bone mineralisation d28																																														
Blood anemia																																														
Adrenals, Corticosterone reduction																																														
Testes toxicity, degeneration																																														
Adrenals, Hypercortisolism d28																																														
Spleen, Metformin																																														
MV, Kidney, Mitochondrial DNA-interaction																																														
MV, Kidney, Lysosomal overload																																														
MV, Liver Enzyme Induction new																																														
MV, Kidney, Covalent protein binding																																														
MV, Liver Enzyme Induction																																														
MV, Peroxisome Proliferators new																																														
MV, Peroxisome Proliferators																																														
Hormones, Sishomone																																														
Kidney, glomerular tubular defect d7 (putative)																																														
Pancreas, insulin secretion (putative)																																														
CNS, serotonin reuptake inhibition																																														
Liver, enzyme induction d7																																														
Liver, enzyme induction d7																																														
Reduced food consumption d7																																														
Systemic, antiproliferative																																														
Liver, peroxisome proliferation d7																																														
Decreased energy metabolism																																														
Reduced food consumption d7																																														
Reduced food consumption d7																																														
Bone marrow suppression d28 (putative)																																														
Vehicle oil																																														
Blood anemia d7																																														
Pancreas, endocrine modulation																																														
Duodenum, non deficiency																																														
CNS, psychoanaleptics																																														

Match	
Weak match	
Equivocal	
Inconclusive	
Mismatch	

Pattern Ranking – Males (Patterns with Matches)

		Marker Substances																BASF LOA Streams							
Sex	Pattern	Benzene HD	Benzene LD	Cyclopentar	Cyclopentar	Dicyclopent	Dicyclopent	Indene HD	Indene LD	Naphthalen	Naphthalen	Xylene HD	Xylene LD	Toluene HD	Toluene LD	Styrene HD	Styrene LD	Ethylbenzer	Ethylbenzer	BASF L1 HD	BASF L1 LD	BASF L2 HD	BASF L2 LD	BASF L3 HD	BASF L3 LD
Male	Liver Toxicity	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Liver, paracetamol-like toxicity	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Liver, enzyme induction	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Liver, oxidative stress (LD) (putative)	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Liver, oxidative stress d28 (putative)	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Liver, oxidative stress (putative)	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Phthalates, short chain	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Thyroid indirect, liver enzyme induction	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	kidney interstitial nephritis	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	kidney, alpha 2u	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Blood, anemia	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Hormones, steroid dysregulation	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Hormones, antiandrogen receptor agonist	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Adrenal cortex, corticosterone synthesis inhibition	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	Testicular toxicity	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match

		LOA Streams																								
Sex	Pattern	L-01 HD	L-01 LD	L-02 HD	L-02 LD	L-03 HD	L-03 LD	L-04 HD	L-04 LD	L-05 HD	L-05 LD	L-06 HD	L-06 LD	L-07 HD	L-07 LD	L-08 HD	L-08 LD	L-09 HD	L-09 LD	L-10 HD	L-10 LD	L-11 HD	L-11 LD	L-12 HD	L-12 LD	
Male	Liver Toxicity	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Liver, paracetamol-like toxicity	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Liver, enzyme induction	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Liver, oxidative stress (LD) (putative)	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Liver, oxidative stress d28 (putative)	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Liver, oxidative stress (putative)	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Phthalates, short chain	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Thyroid indirect, liver enzyme induction	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	kidney interstitial nephritis	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match
	kidney, alpha 2u	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Blood, anemia	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Hormones, steroid dysregulation	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Hormones, antiandrogen receptor agonist	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Adrenal cortex, corticosterone synthesis inhibition	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	
	Testicular toxicity	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	Match	

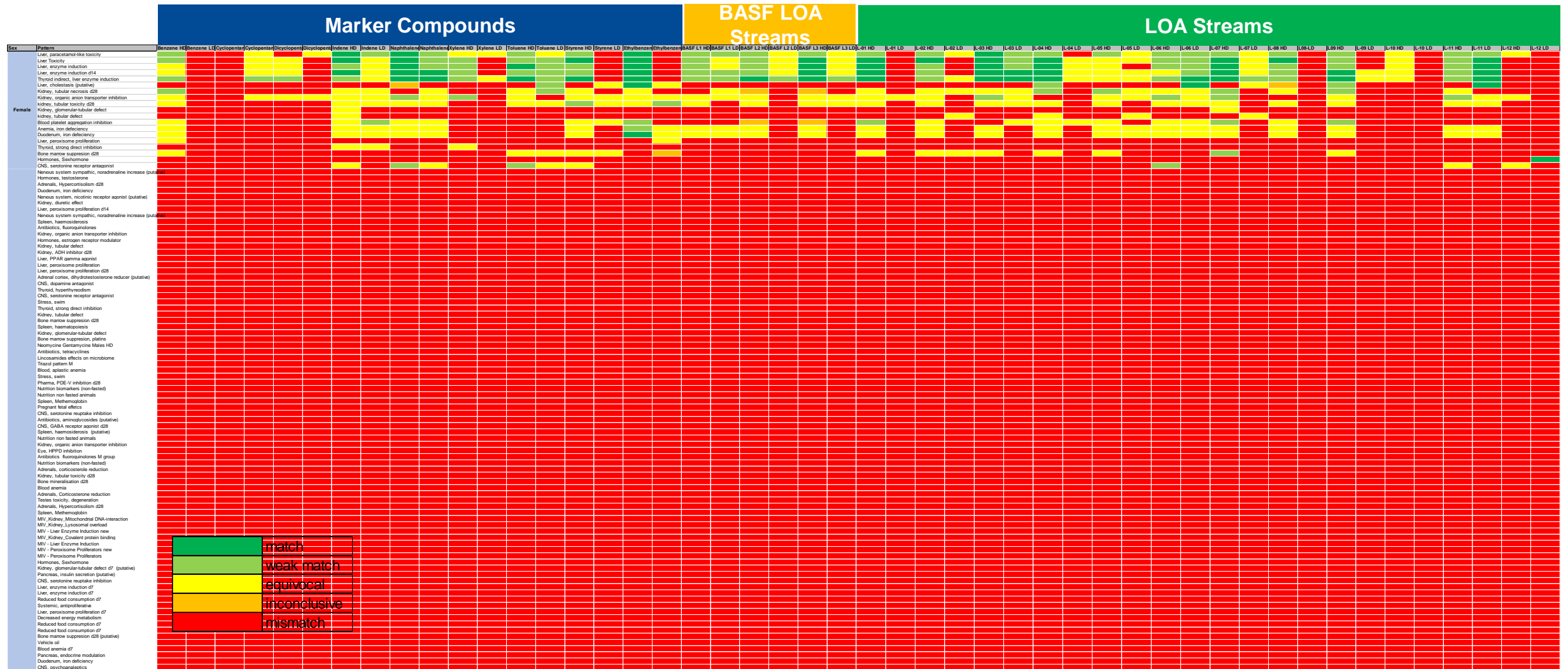
Match	match
Weak match	weak match
Equivocal	equivocal
Inconclusive	inconclusive
Mismatch	mismatch



Pattern Ranking – Males (Conclusions)

- Liver:
 - ▶ Primary Target Organ: mostly enzyme induction, liver toxicity
- Thyroid:
 - ▶ secondary to liver enzyme induction
- Kidney:
 - ▶ Styrene (HD), Ethylbenzene (HD), BASF LOA 1 – 3 (HD + LD for BASF LOA 1), L-08 (HD)
- Hormonal effects: considered not relevant
 - ▶ Dicyclopentadiene (HD) → incidental: no correlate with classical parameters (e.g., organ weight)
 - ▶ L-04 (HD) → incidental: no correlation with classical parameters
 - ▶ L-05 (LD) → incidental: no correlation with classical parameters, no dose-response
- CNS (Serotonin reuptake inhibition): considered not relevant
 - ▶ L-03 (HD) → incidental: no correlation with classical parameters, isolated finding

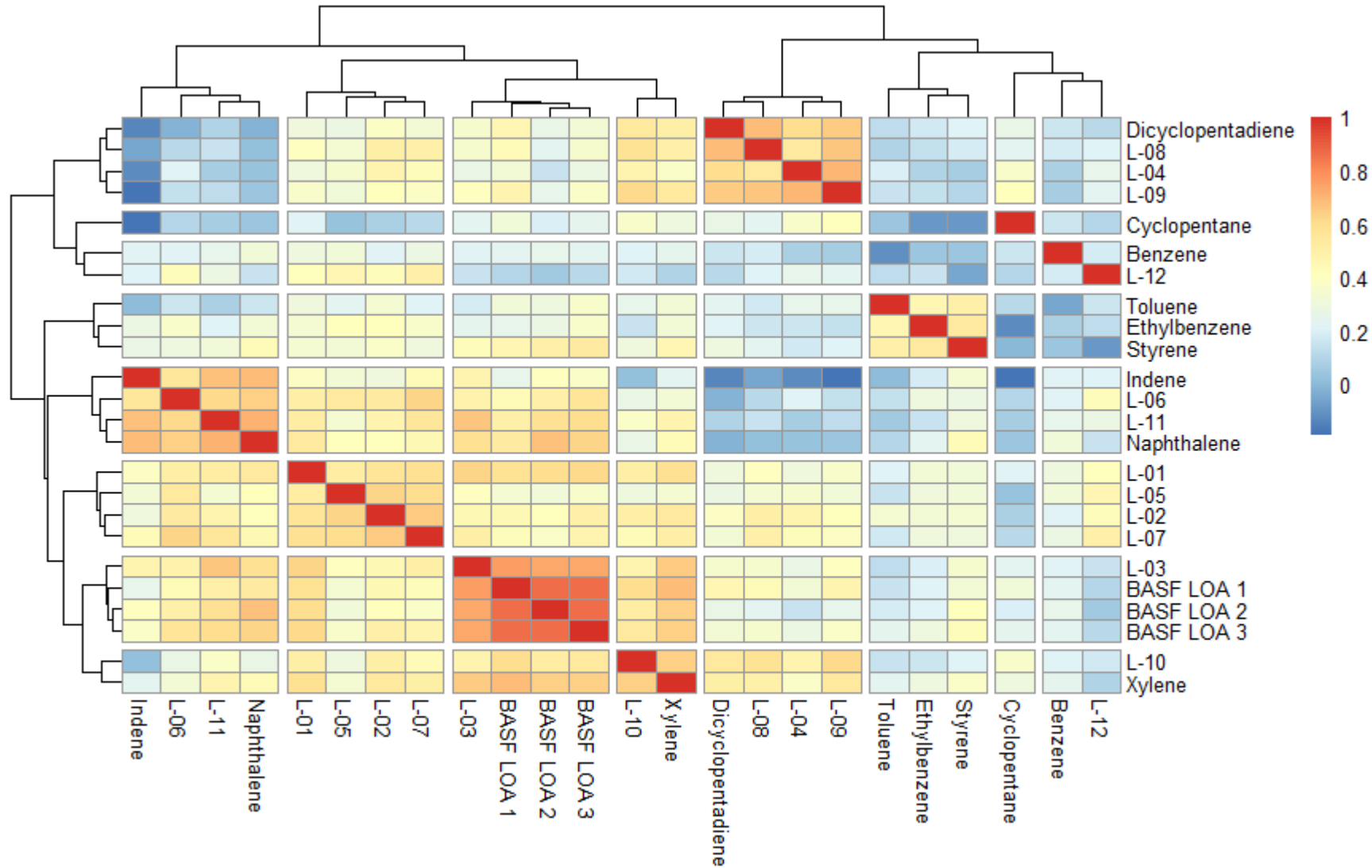
Pattern Ranking – Females (Overview)



Pattern Ranking – Females (Conclusions)

- Liver
 - ▶ Primary Target Organ: mostly enzyme induction
- Thyroid
 - ▶ Secondary to liver enzyme induction
- Kidney
 - ▶ Benzene (HD), Ethylbenzene (HD), Naphthalene (HD), Styrene (HD), Toluene (LD), Xylene (HD), L-03 (HD) – L-07 (HD), L-09 (HD) and L-11
- Blood cell disorders
 - ▶ Indene (LD), Ethylbenzene (HD), L-01 (HD), L-07 (HD), L-09 (HD)
- CNS (serotonin receptor antagonist):
 - ▶ Naphthalene (HD), Toluene (HD), L-06 (HD) → potentially false-positive due to liver effects
- Hormonal effects:
 - ▶ only L-12 (LD) → no dose response - probably false-positive

Treatment Correlation – Clustering, Males

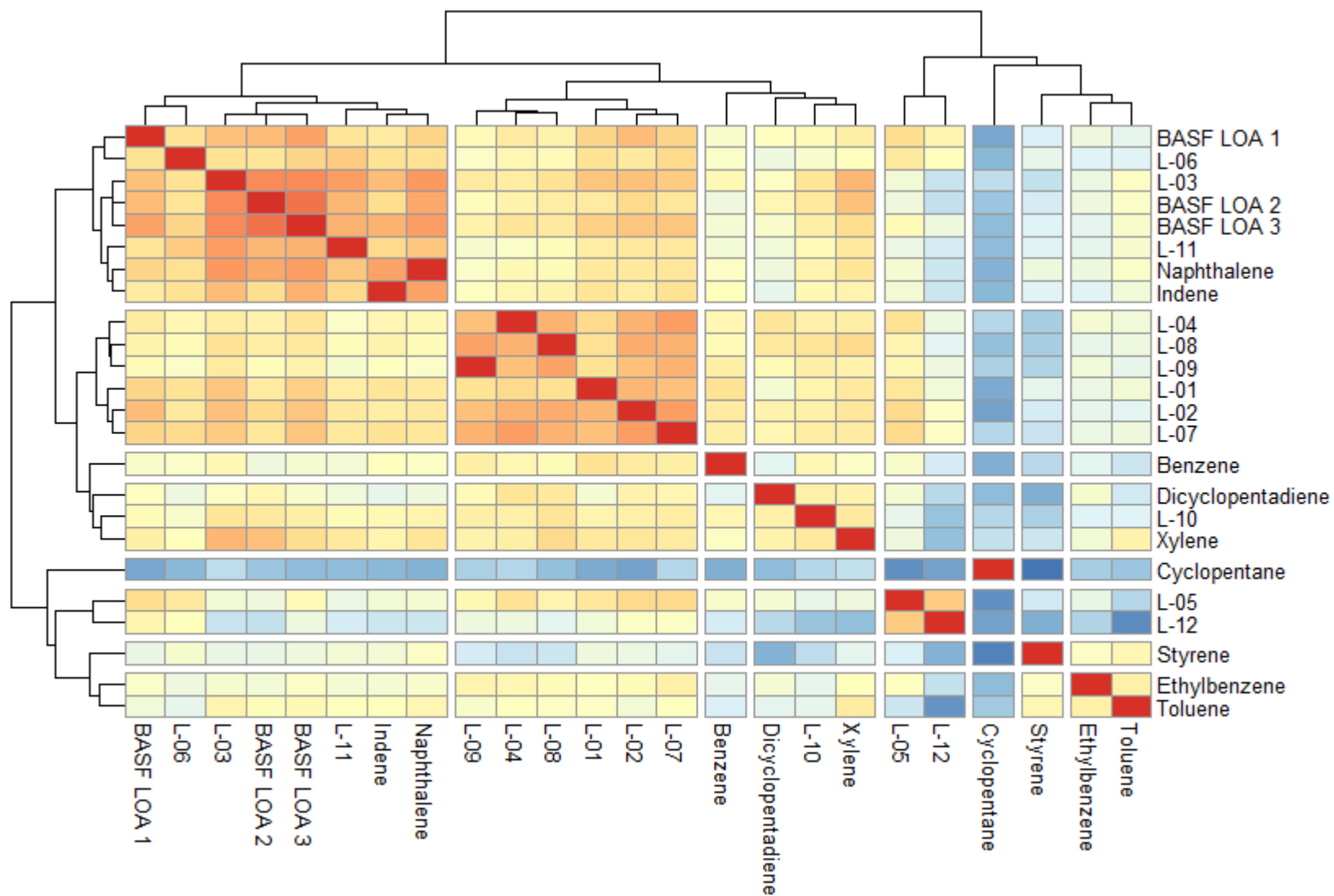


■ Clustering based on correlation coefficients from treatment correlation

■ Clear sub-clusters:

- ▶ DCPD, L-04, L-08, L-09
- ▶ Toluene, Ethylbenzene, Styrene
- ▶ Naphthalene, Indene, L-06, L-11
- ▶ BASF LOA streams, L-03

Treatment Correlation – Clustering, Females



■ Clustering based on correlation coefficients from treatment correlation

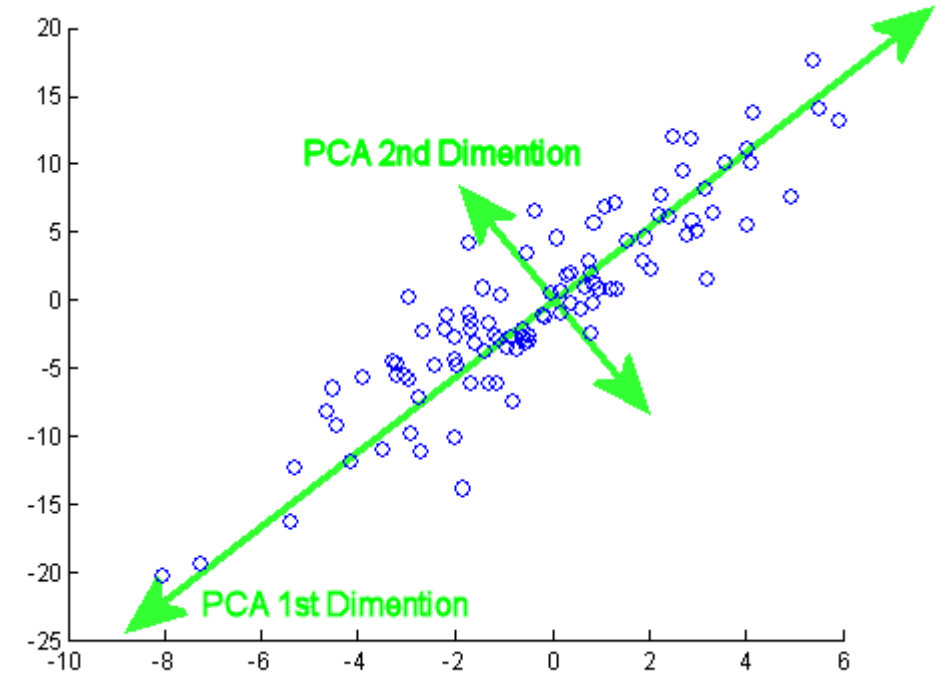
■ Clear sub-clusters:

▶ L-01, L-02, L-04, L-07, L-08, L-09

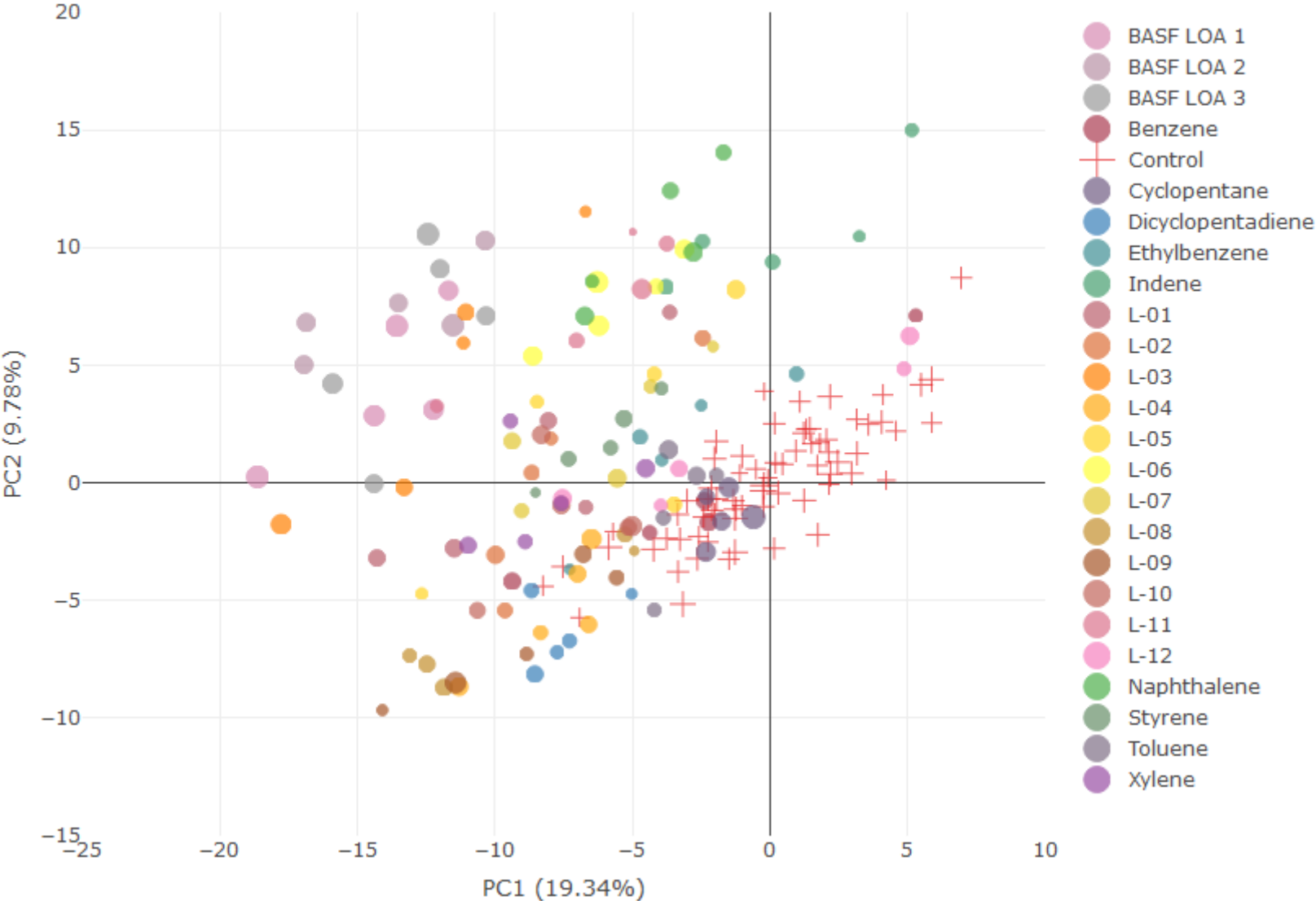
▶ Naphthalene, Indene, BASF LOA streams, L-03, L-06, L-11

Principal Component Analysis - Methodology

- Reduces dimensionality
 - Allows visualization of a high-dimensional data in few (2/3) dimensions
- Linear transformation of the original variables
 - by making a rotation of the multidimensional space
- Retain as much variation as possible
 - in a way to focus on the direction of maximum variation
- Principal components (PC's) are uncorrelated and ordered
 - ▶ First PC has the most information; then second.
- Identify differences: samples can be grouped based on similarity of differences.

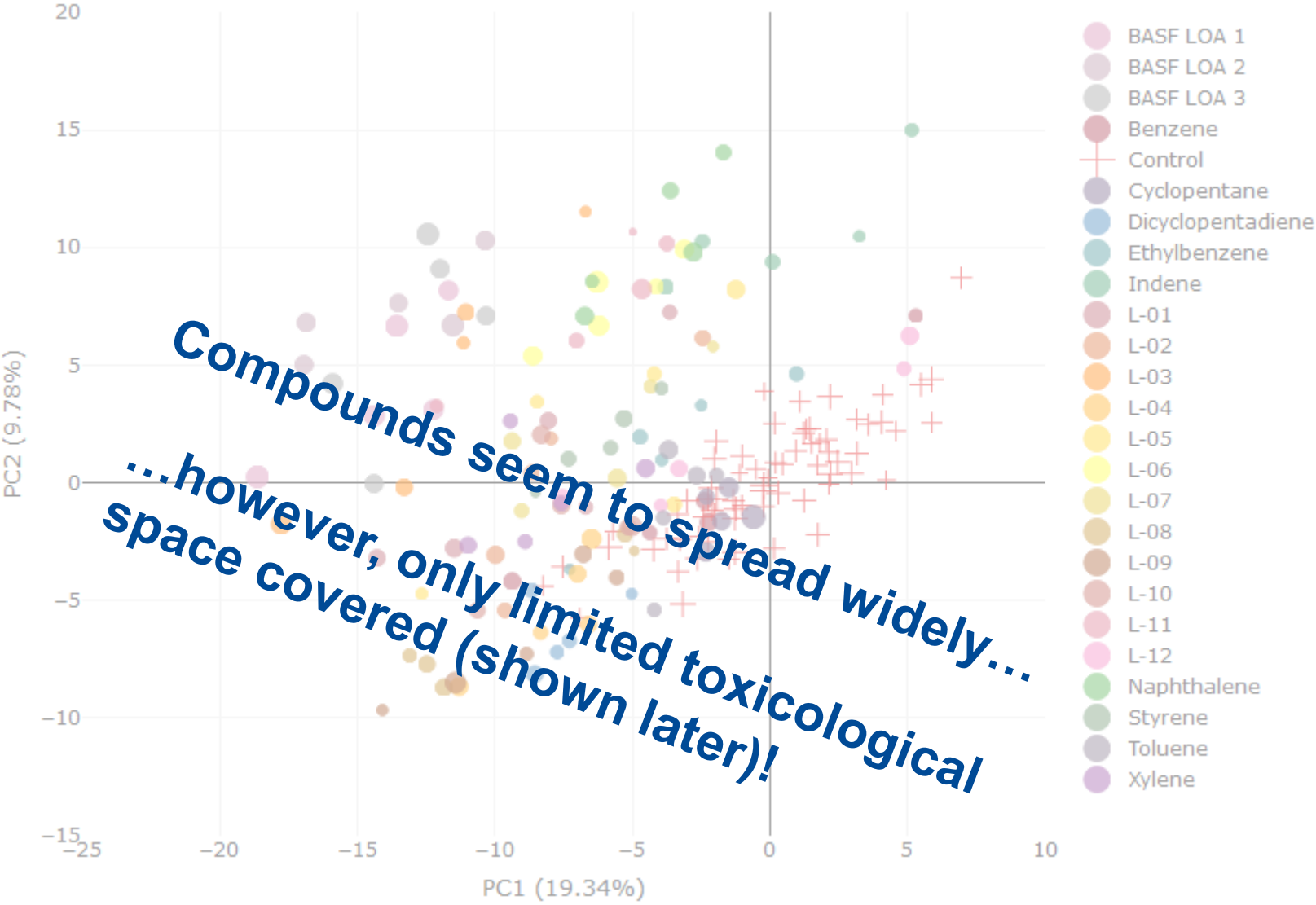


PCA – Males (Controls and HD of All Treatments)



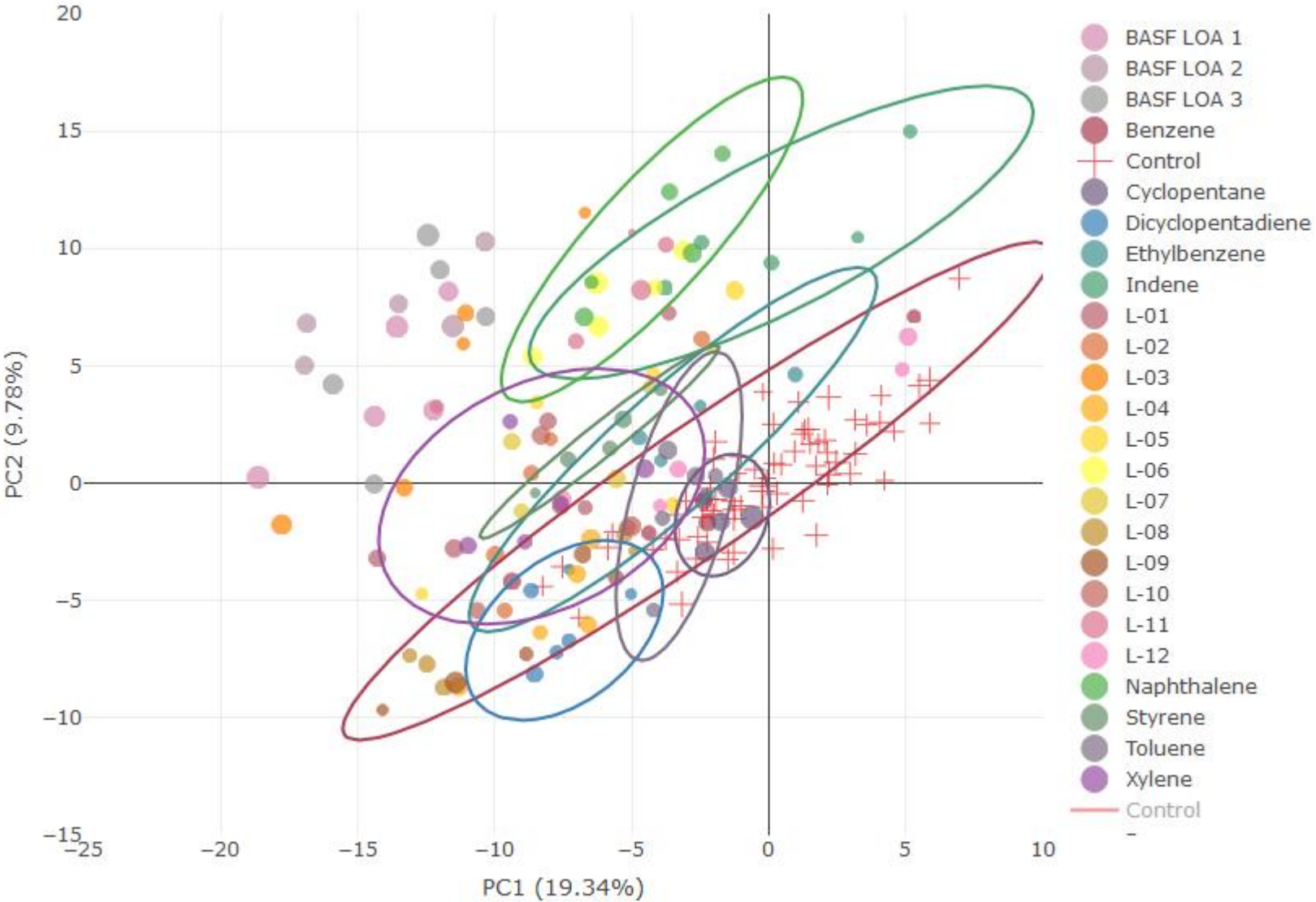
- (Biological) variability induces spread of data points
- Treatment-related effect visible for many streams/ marker compounds
- Some treatment overlap with controls
→ e.g., cyclopentane
- Some treatments are distinguishable from each other
→ e.g., BASF LOA streams vs Naphthalene/ L-06 vs. DCPD/ L-09

PCA – Males (Controls and HD of All Treatments)



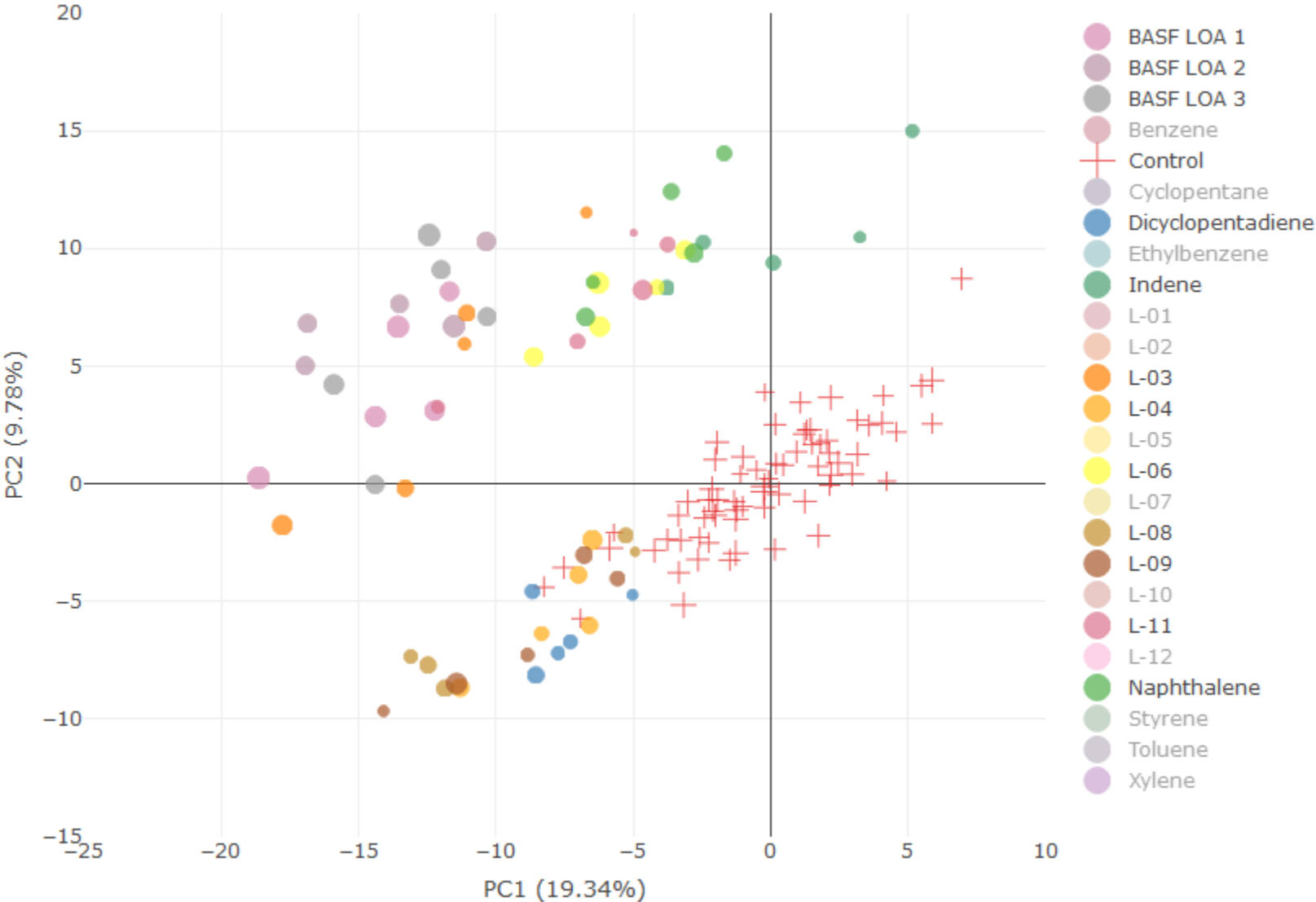
- (Biological) variability induces spread of data points
- Treatment-related effect visible for many streams/ marker compounds
- Some treatment overlap with controls
→ e.g., cyclopentane
- Some treatments are distinguishable from each other
→ e.g., BASF LOA streams vs Naphthalene/ L-06 vs. DCPD/ L-09

PCA – Males (Controls and HD of All Treatments)



- Marker compounds highlighted
- Naphthalene, Indene, Styrene, Xylene, DCPD with clear effect
- Benzene, Toluene, Ethylbenzene close to control (partly overlapping)
- Cyclopentane w/o any effect
- Marker compounds separate into different directions

PCA – Males (Controls and HD of All Treatments)



- **Compounds with highest separation highlighted**
- Tendency for sub-clusters:
 - ▶ DCPD, L-04, L-08, L-09
 - ▶ Naphthalene, Indene, L-06, L-11
 - ▶ BASF LOA streams, L-03
- The latter two sub-clusters closer together

Bootstrapping

5 measured samples per group

Sex	Study Day	Treatment	Meta 1	Meta 2	Meta 3	Meta 4	Meta 5
m	14	T1	A ₁	A ₂	A ₃	A ₄	A ₅
m	14	T1	B ₁	B ₂	B ₃	B ₄	B ₅
m	14	T1	C ₁	C ₂	C ₃	C ₄	C ₅
m	14	T1	D ₁	D ₂	D ₃	D ₄	D ₅
m	14	T1	E ₁	E ₂	E ₃	E ₄	E ₅

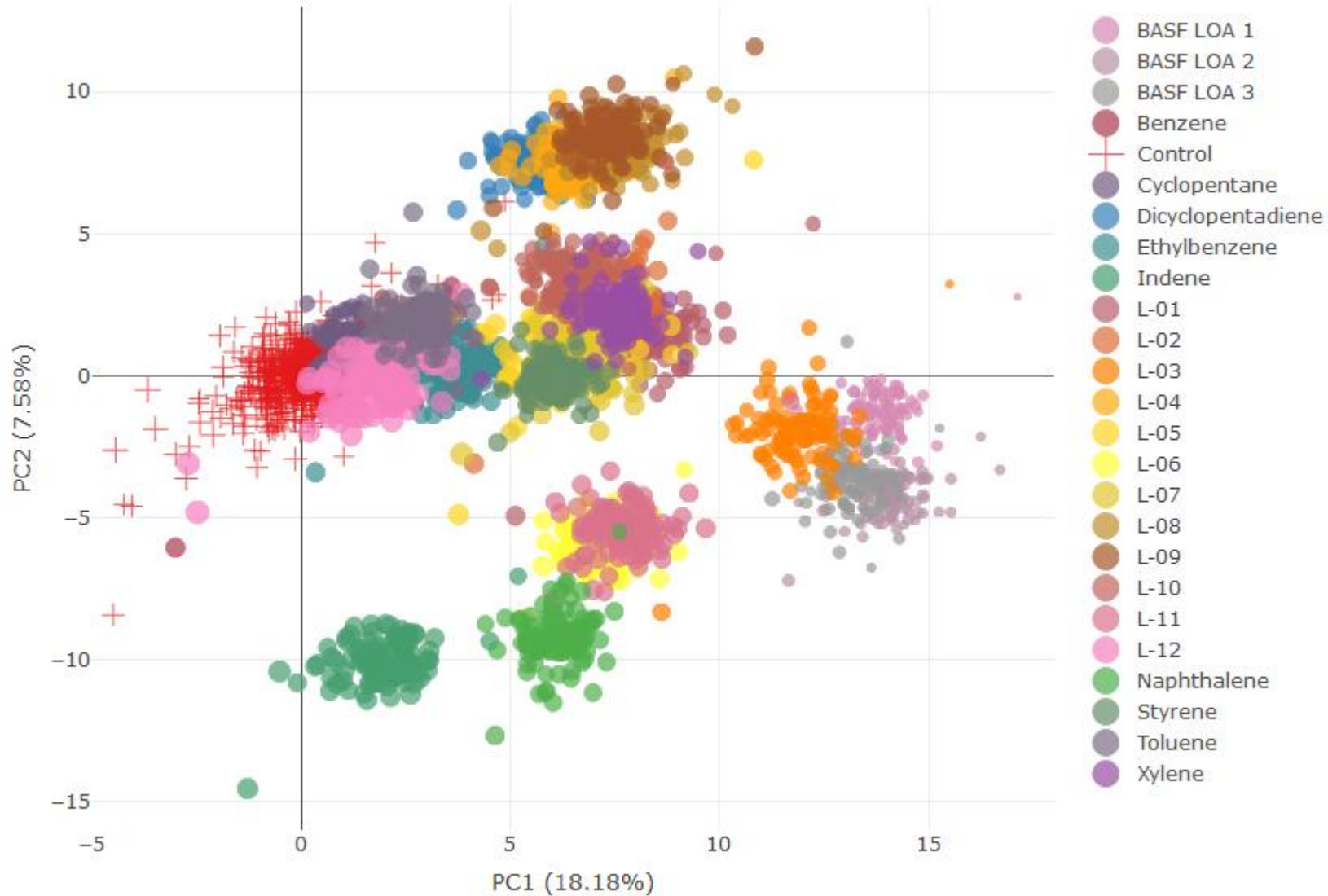


n pseudo samples per group

Sex	Study Day	Treatment	Meta 1	Meta 2	Meta 3	Meta 4	Meta 5
m	14	T1	B ₁	E ₂	E ₃	B ₄	B ₅
m	14	T1	A ₁	C ₂	B ₃	B ₄	E ₅
m	14	T1	B ₁	E ₂	E ₃	D ₄	C ₅
m	14	T1	E ₁	A ₂	E ₃	E ₄	E ₅
m	14	T1	C ₁	B ₂	B ₃	E ₄	E ₅
m	14	T1	D ₁	E ₂	C ₃	A ₄	C ₅
m	14	T1	D ₁	C ₂	A ₃	A ₄	C ₅
m	14	T1	C ₁	A ₂	D ₃	A ₄	E ₅
m	14	T1	E ₁	B ₂	B ₃	D ₄	B ₅
m	14	T1	E ₁	C ₂	D ₃	D ₄	D ₅
...					
m	14	T1	A ₁	C ₂	D ₃	C ₄	B ₅

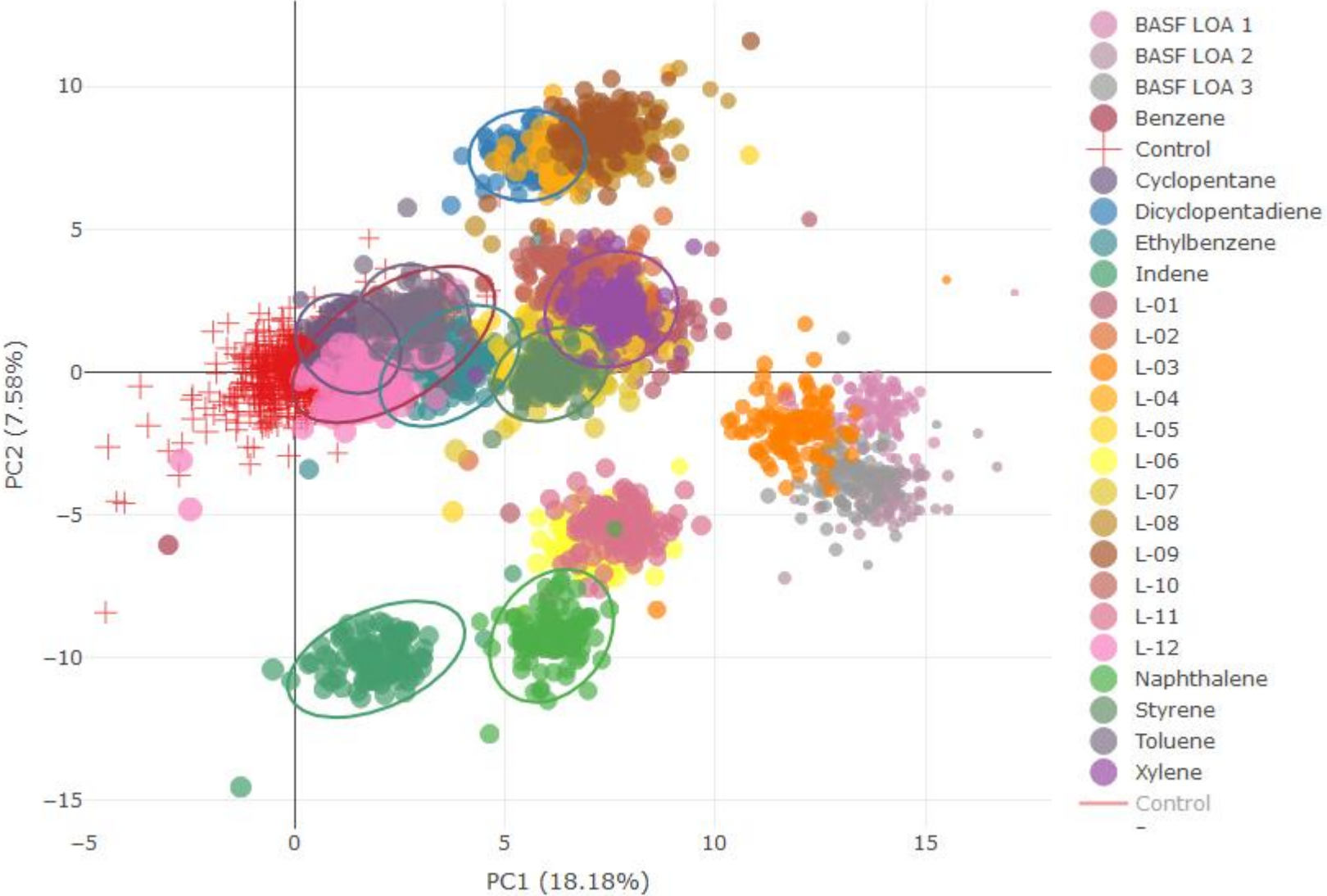
- Random sampling with replacement
- performed independently for each measured metabolite
- within the treatment group (Compound, Dose, Sex, Study day).
- The method provides a way to increase sample numbers.
- Breaks any correlations within treatment group while preserving across groups.
 - ▶ Counters the variation within group and allows comparison across groups.

Bootstrapped PCA – Males



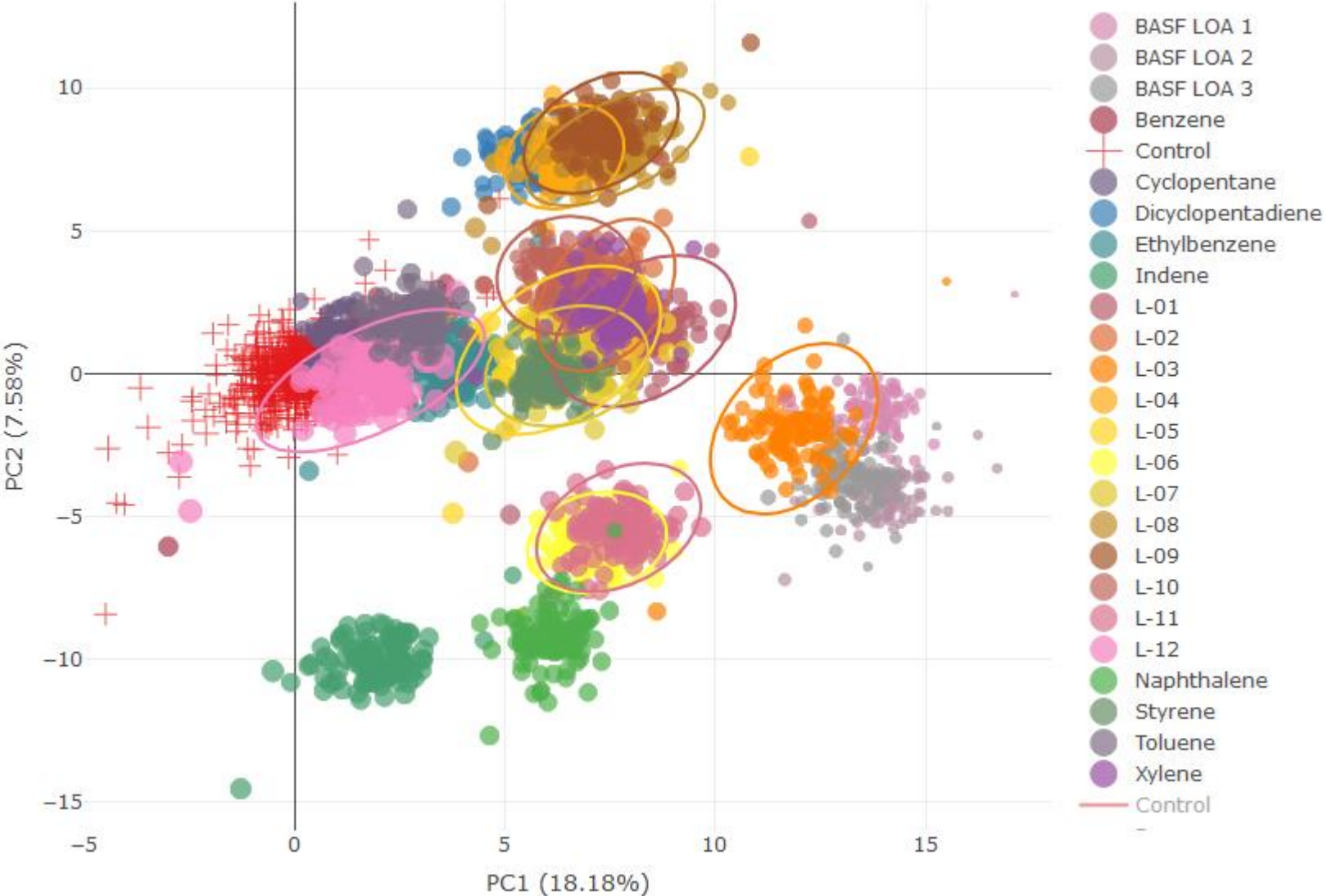
- All compounds: PCA after bootstrapping (100x) + original values
- Clearer tendency for three sub-clusters:
 - ▶ DCPD, L-04, L-08, L-09
 - ▶ Naphthalene, Indene, L-06, L-11
 - ▶ BASF LOA streams, L-03
- Better separation between the latter two sub-clusters
- Potentially fourth sub-cluster in the center of all data points

Bootstrapped PCA – Males



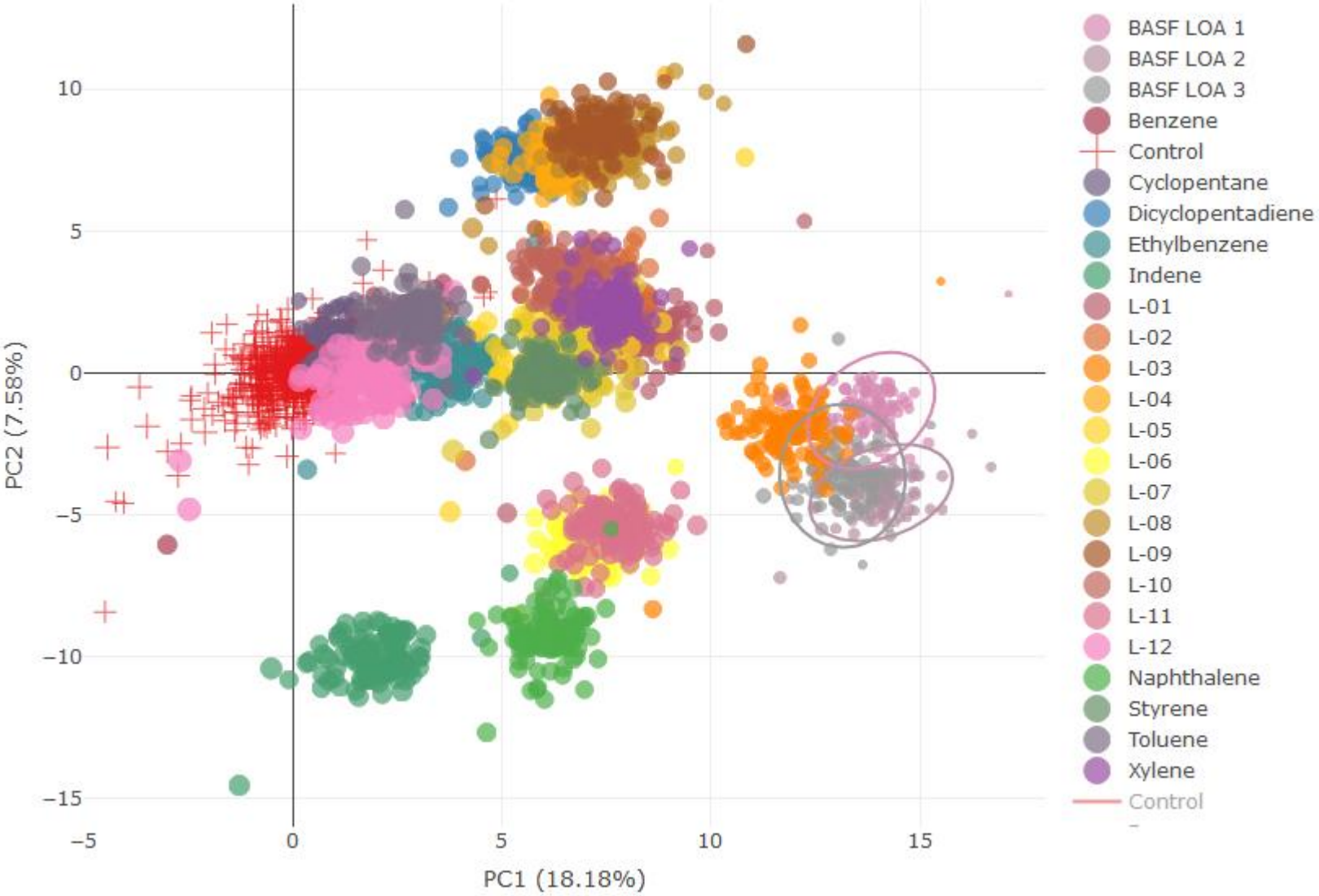
- All compounds: PCA after bootstrapping (100x) + original values
- Marker compounds highlighted

Bootstrapped PCA – Males



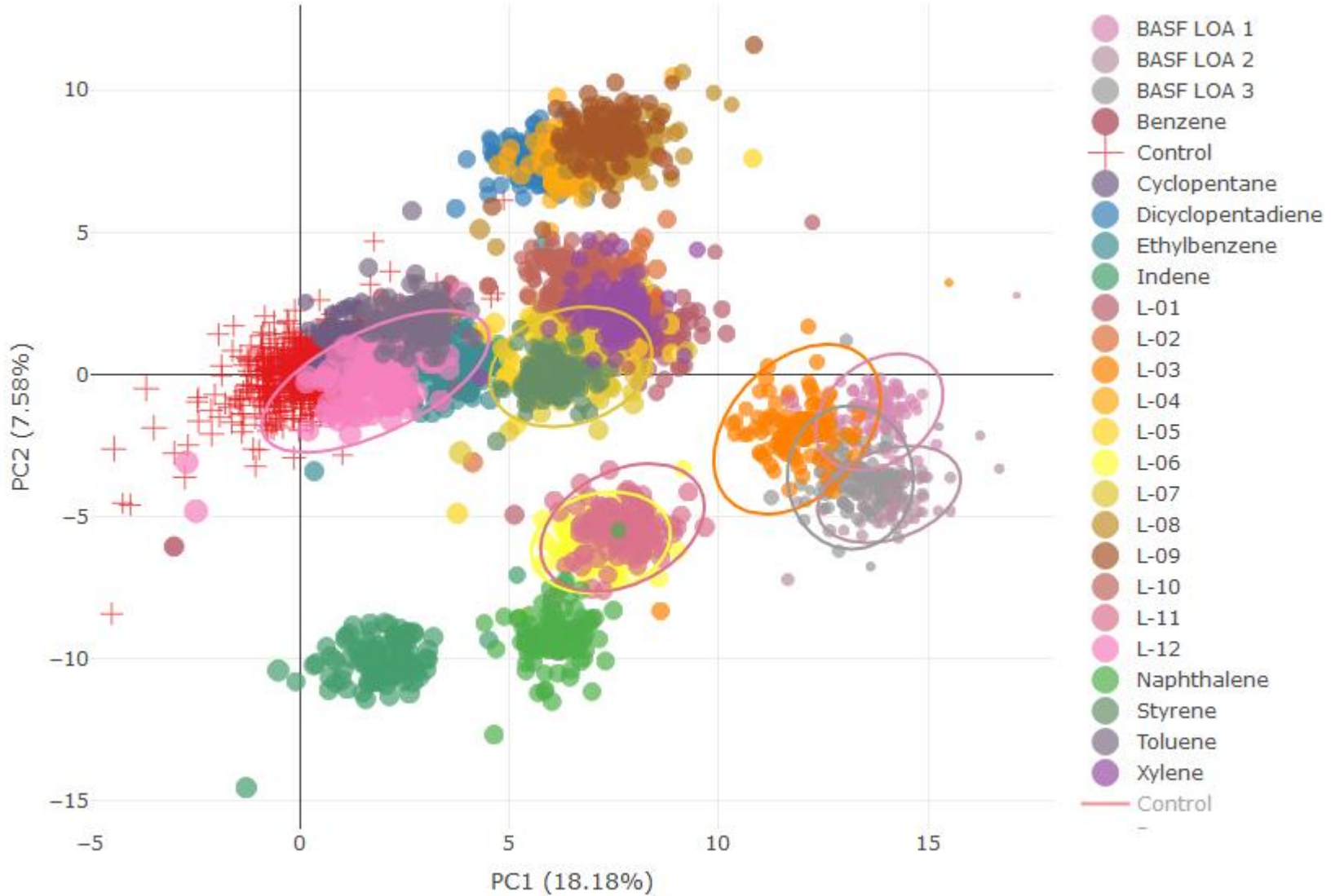
- All compounds: PCA after bootstrapping (100x) + original values
- LOA streams highlighted

Bootstrapped PCA – Males



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

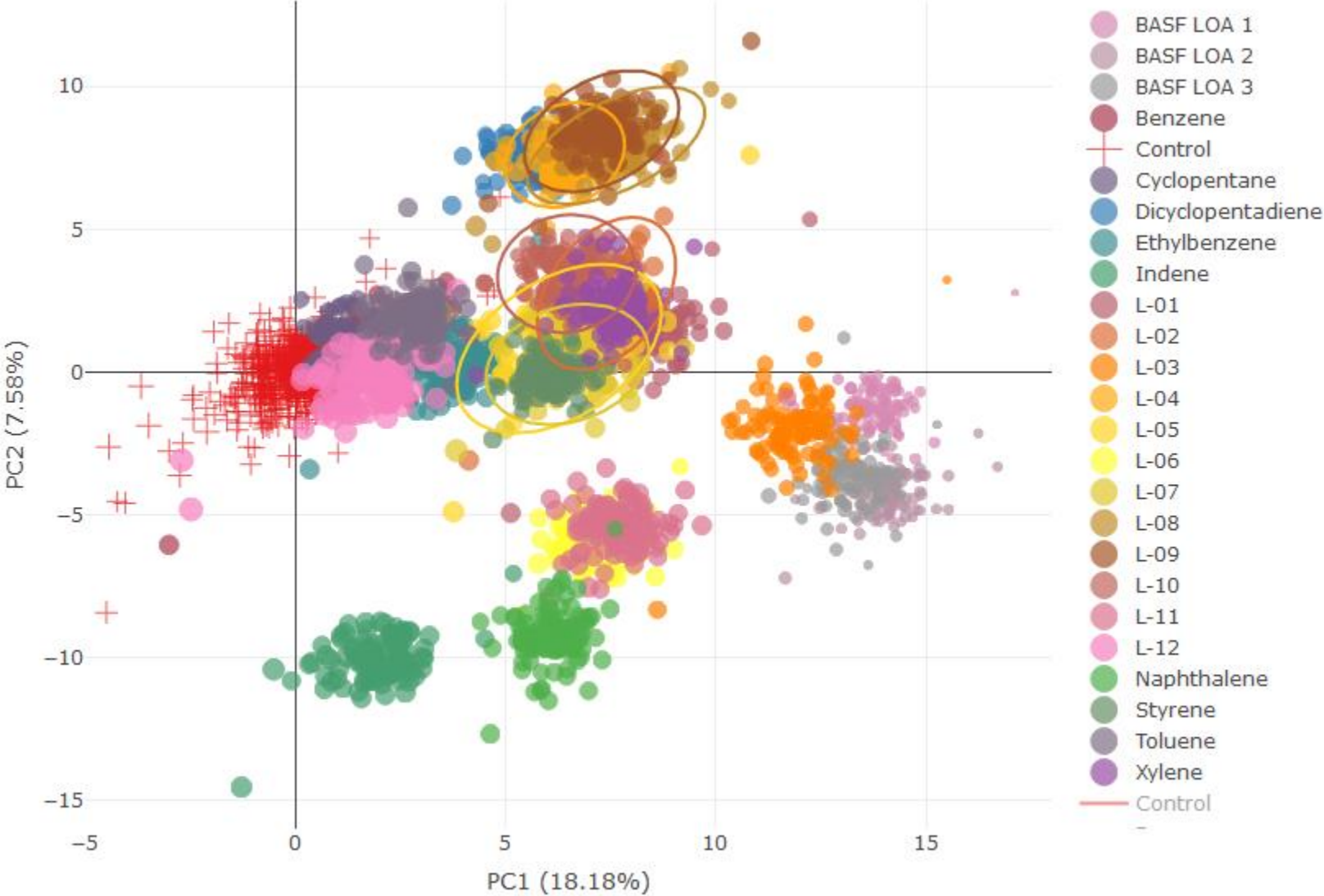
Bootstrapped PCA – Males



■ All compounds: PCA after bootstrapping (100x) + original values

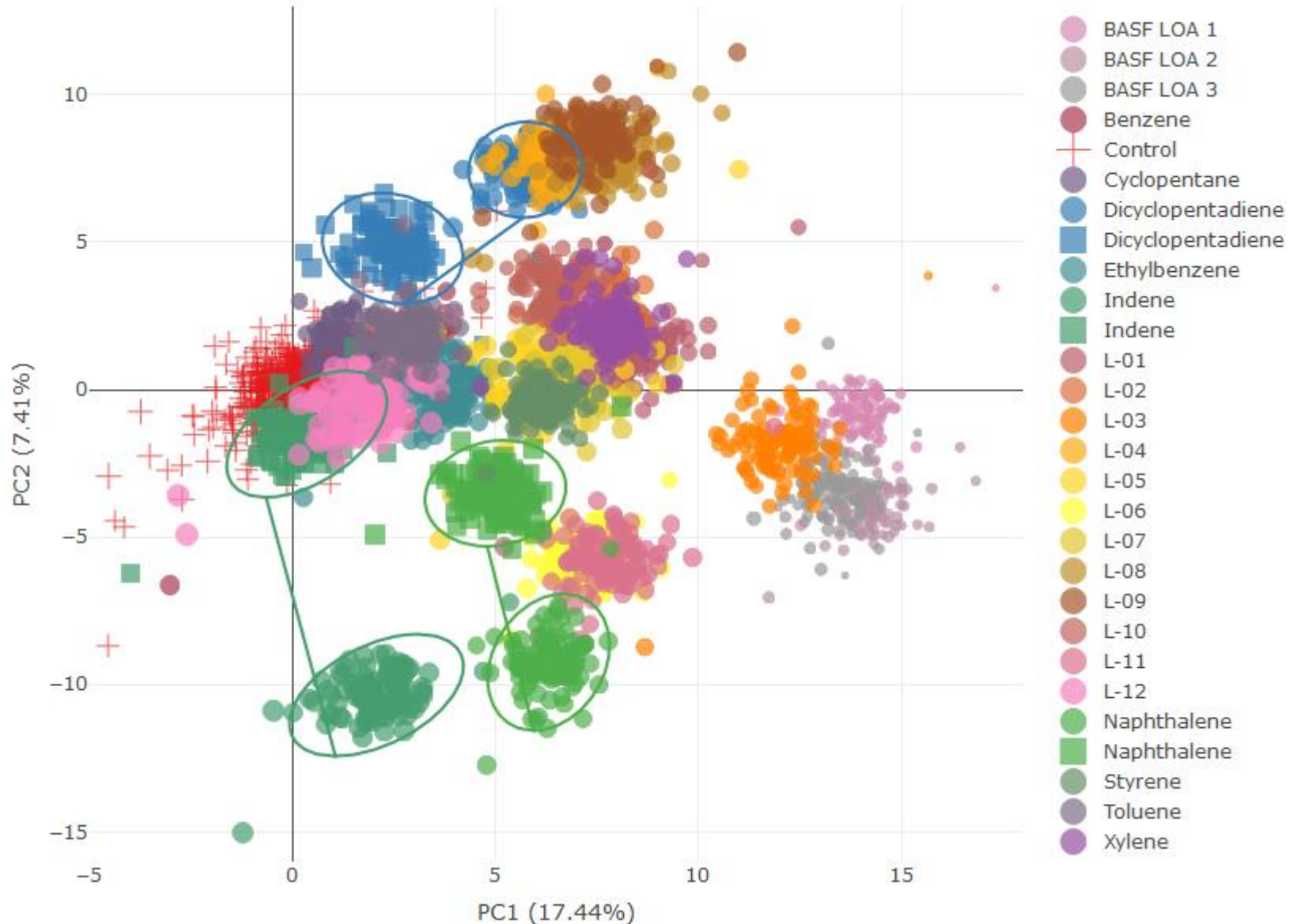
■ Naphthalene-/ Indene-rich streams highlighted

Bootstrapped PCA – Males



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

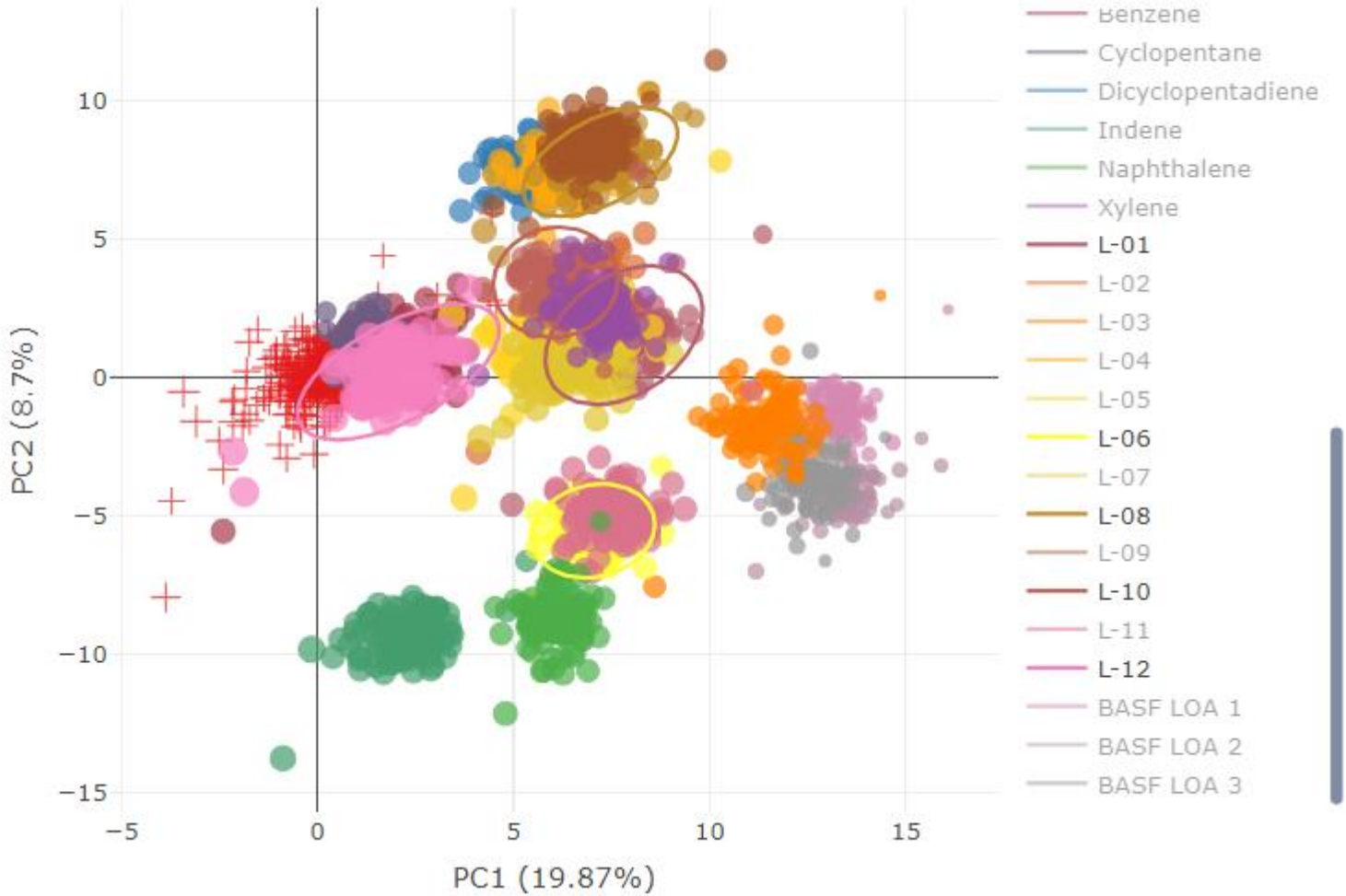
PCA – Males (Bootstrapped – LD Naphthalene, Indene, DCPD)



■ All compounds: PCA after bootstrapping (100x) + original values

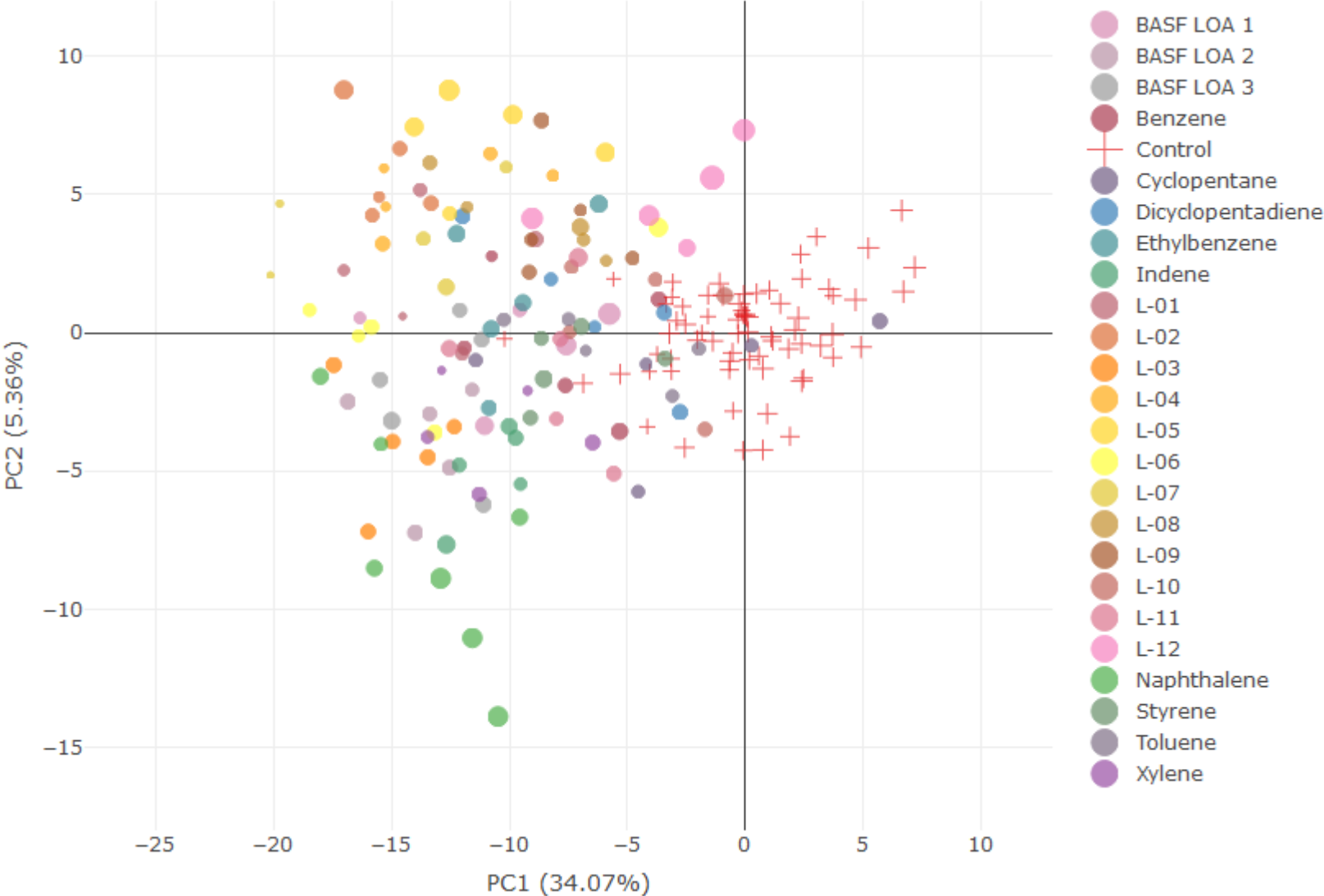
■ Additional display of low dose animals for Naphthalene, Indene, DCPD (squares)

Bootstrapped PCA (Males)



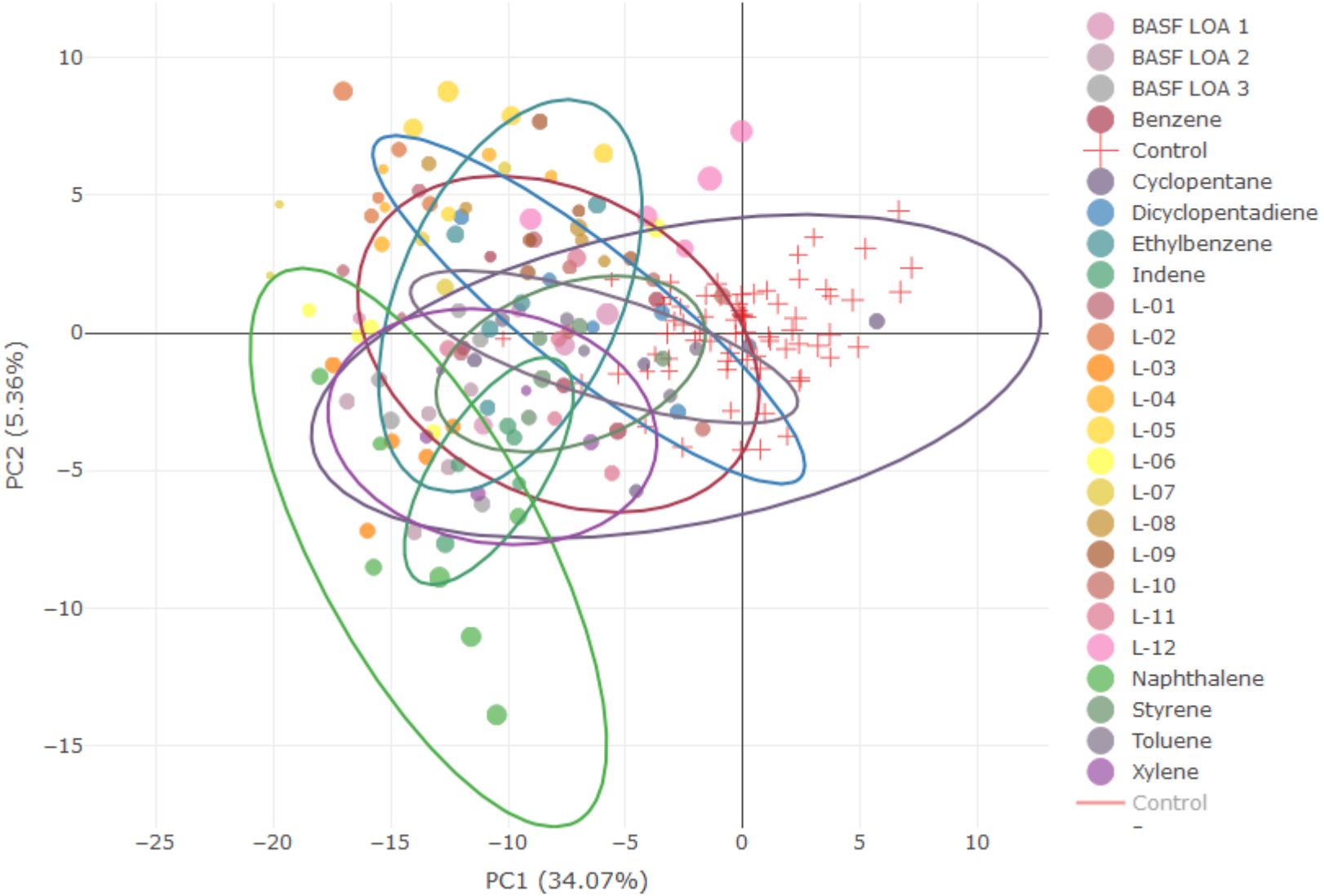
- All compounds: PCA after bootstrapping (100x) + original values
- Streams outside Cat L boundary

PCA – Females (Controls and HD of All Treatments)



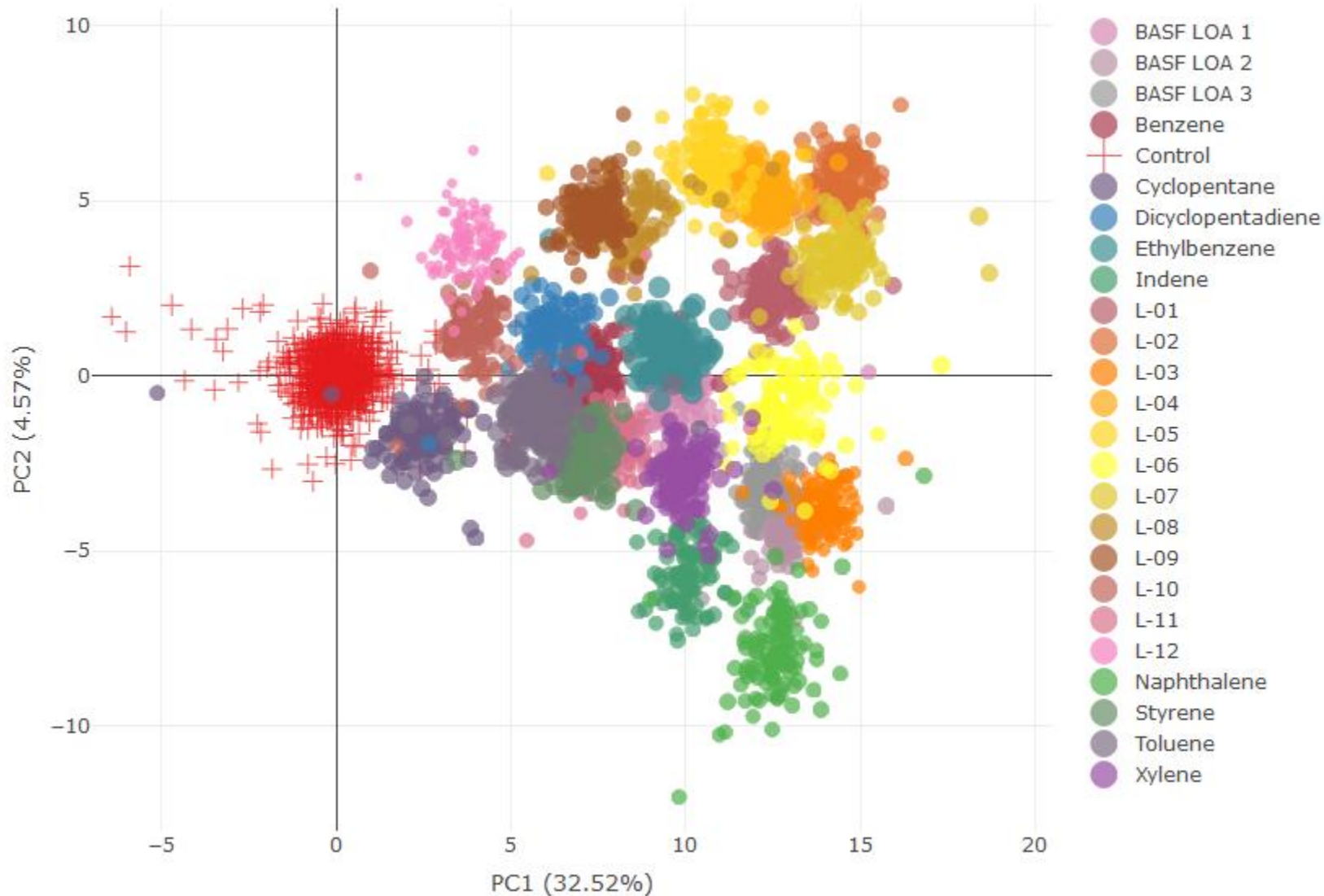
- Biological variability induces spread of data points
- Treatment-related effect visible for many streams/ marker compounds
- Some treatment overlap with controls
→ e.g., cyclopentane
- Some treatments are distinguishable from each other
→ e.g., Naphthalene/ Indene vs. L-05, L-06, L-08, L-09
- Separation less clear as for males

PCA – Females (Controls and HD of All Treatments)



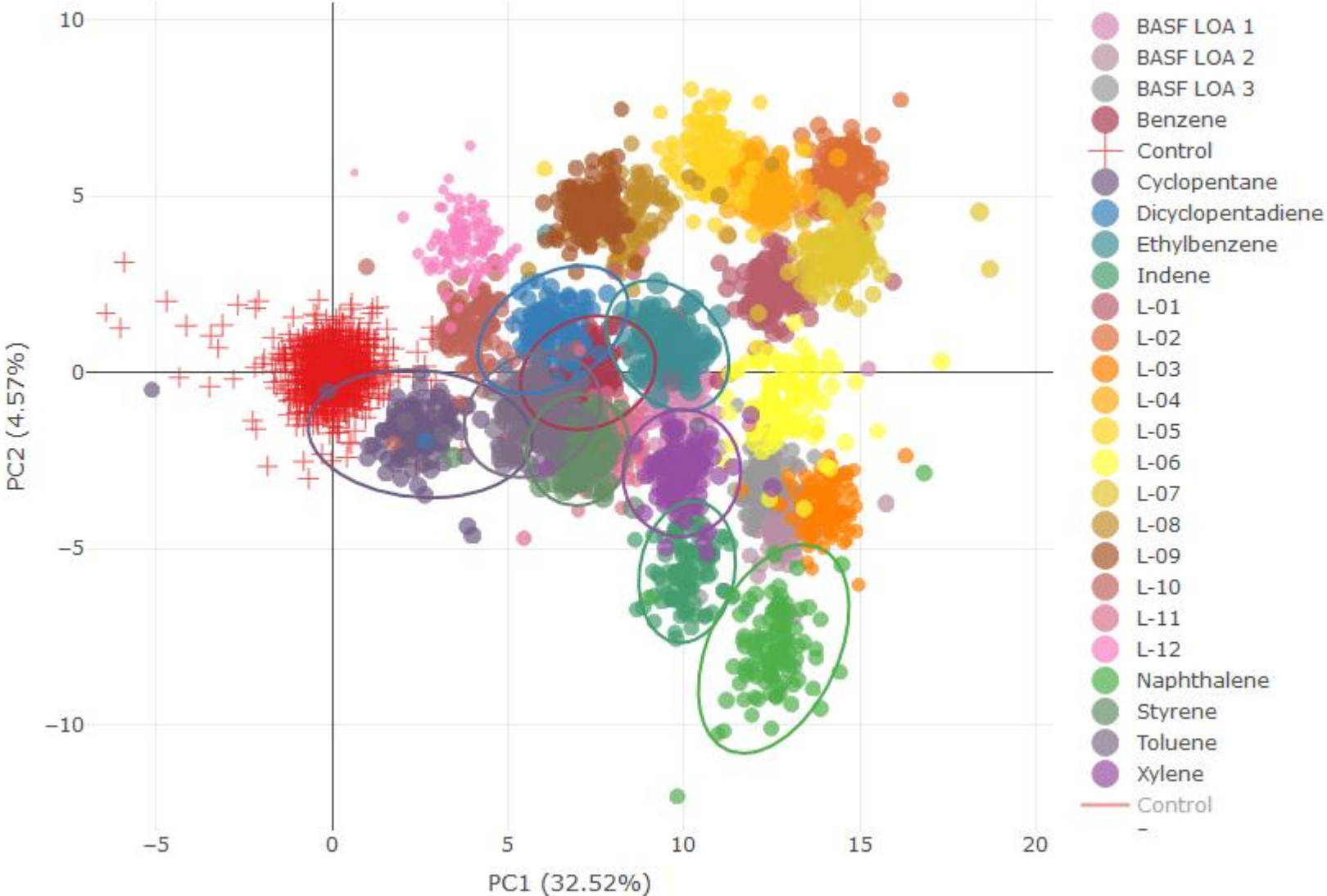
- Marker compounds highlighted
- Naphthalene, Indene, Xylene, Ethylbenzene with clear effect
- Benzene, Styrene, Toluene, DCPD, Cyclopentane close to control (partly overlapping)
- Marker compounds overlaying

Bootstrapped PCA – Females



- **All compounds: PCA after bootstrapping (100x) + original values**
- **Tendency for one or two sub-clusters:**
 - ▶ L-01, L-02, L-04, L-05, L-07, L-08, L-09
 - ▶ Naphthalene, Indene, Xylene, BASF LOA streams, L-03, L-11, L-06
- **Lesser separation as compared to males confirmed**

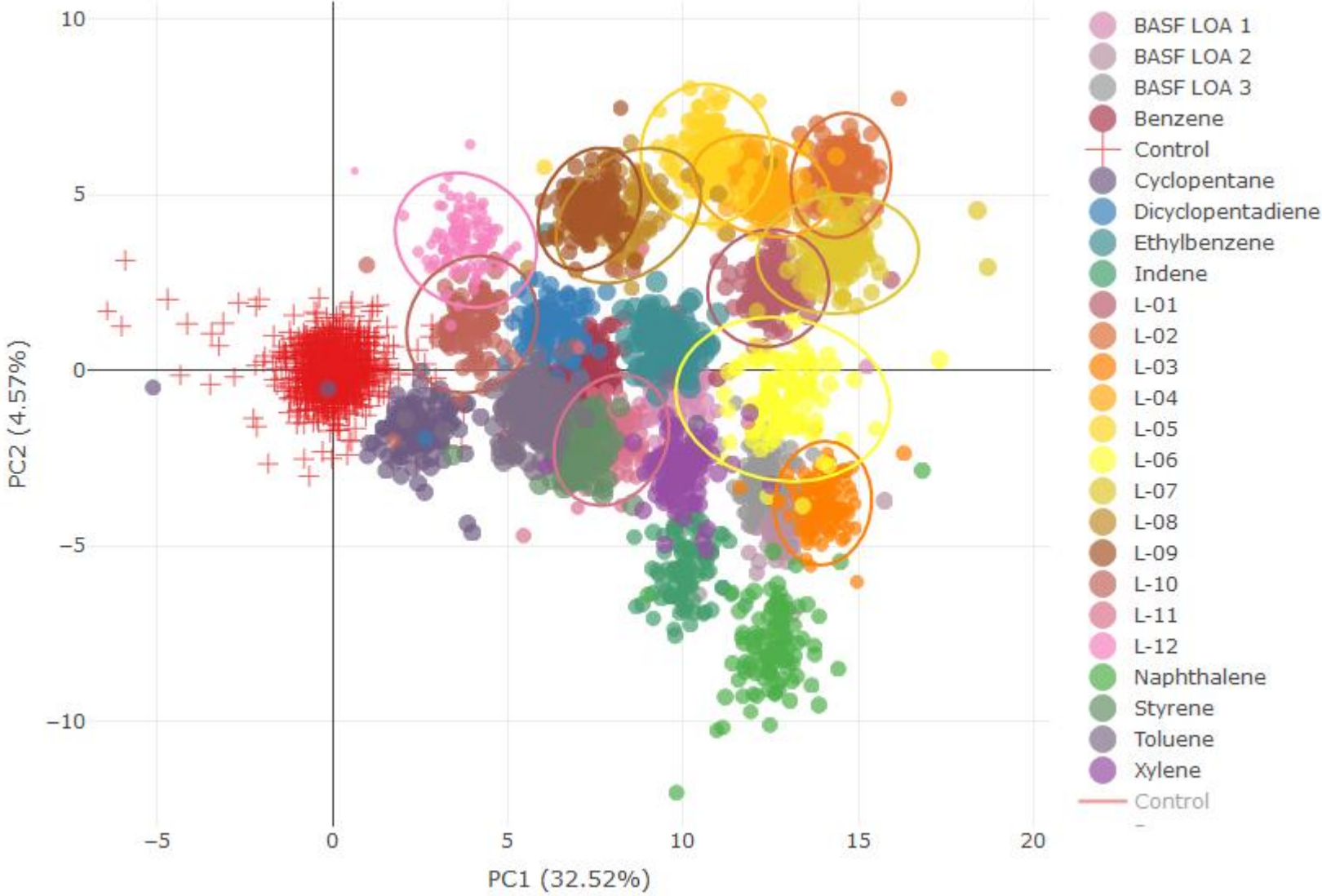
Bootstrapped PCA – Females



■ All compounds: PCA after bootstrapping (100x) + original values

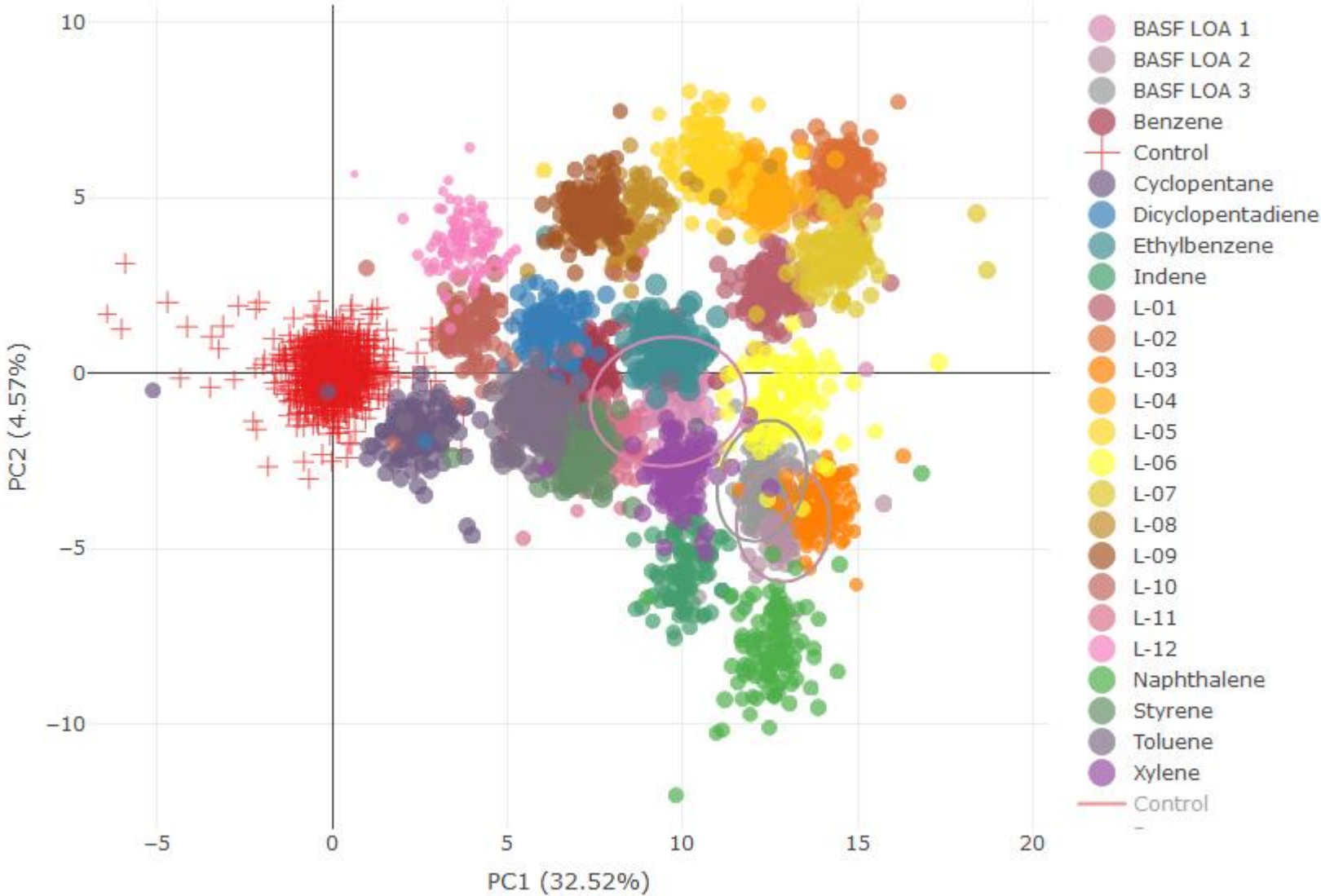
■ Marker compounds highlighted

Bootstrapped PCA – Females



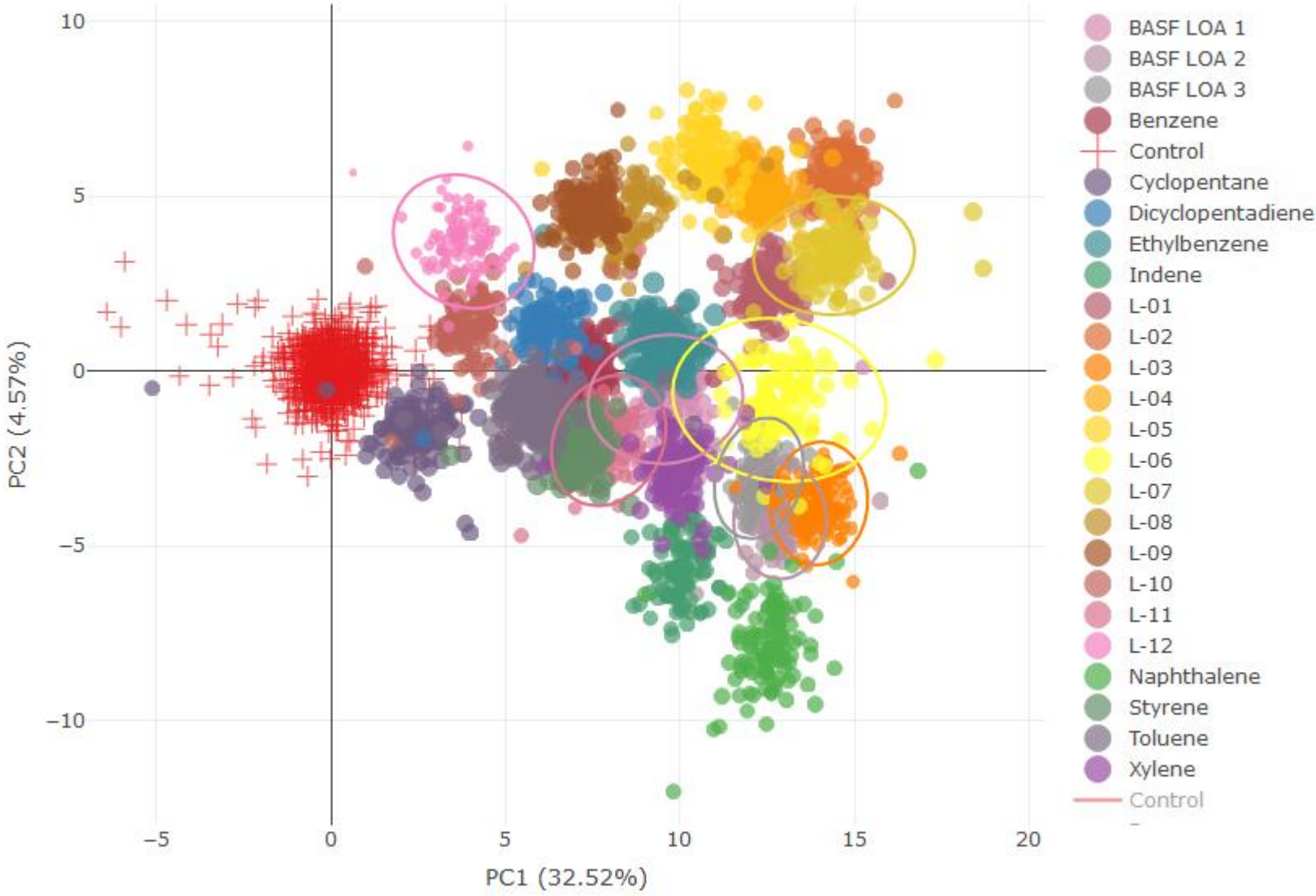
- All compounds: PCA after bootstrapping (100x) + original values
- LOA streams highlighted

Bootstrapped PCA – Females



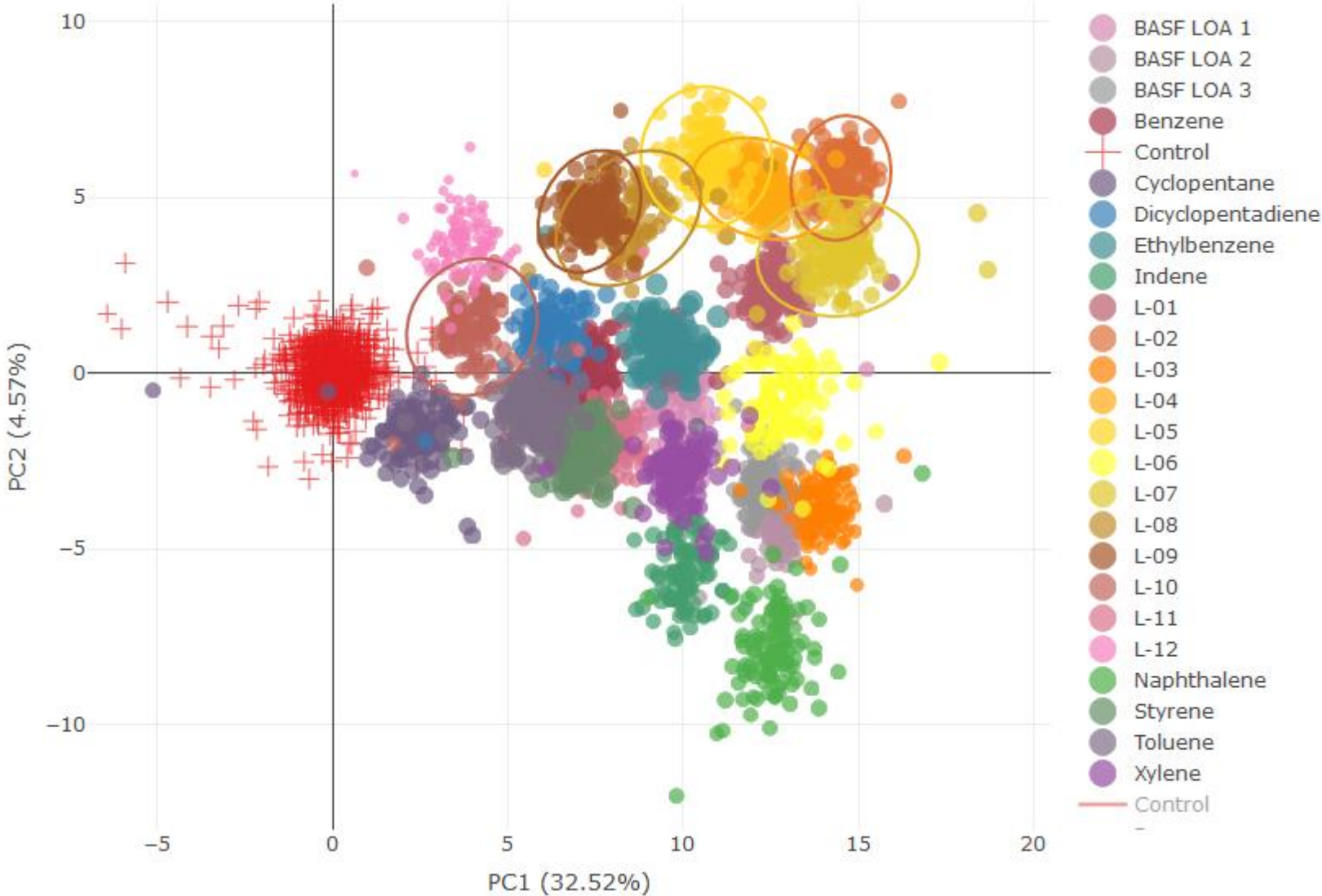
- All compounds: PCA after bootstrapping (100x) + original values
- BASF LOA streams highlighted

Bootstrapped PCA – Females



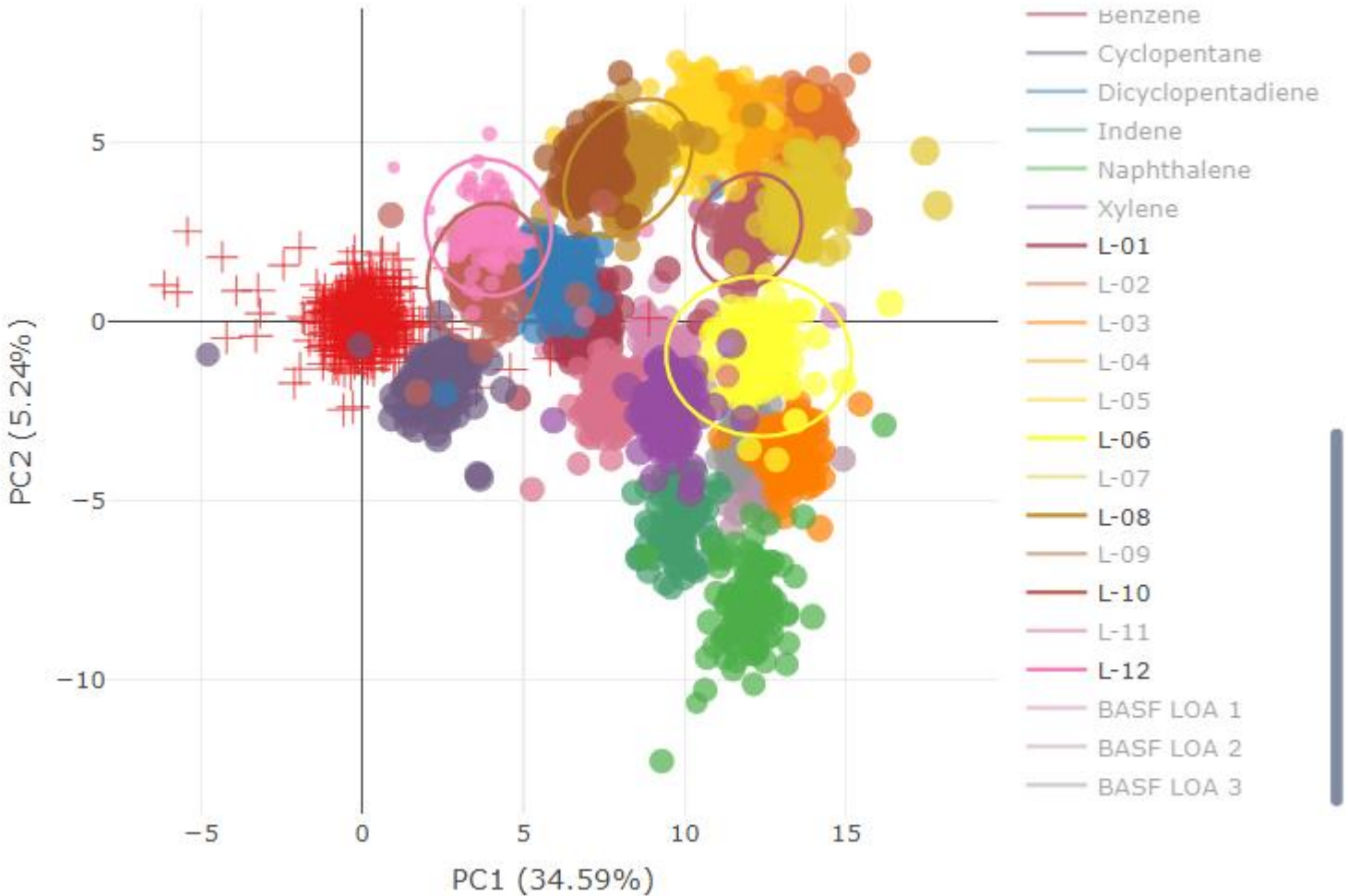
- All compounds: PCA after bootstrapping (100x) + original values
- Naphthalene-/ Indene-rich streams highlighted

Bootstrapped PCA – Females



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

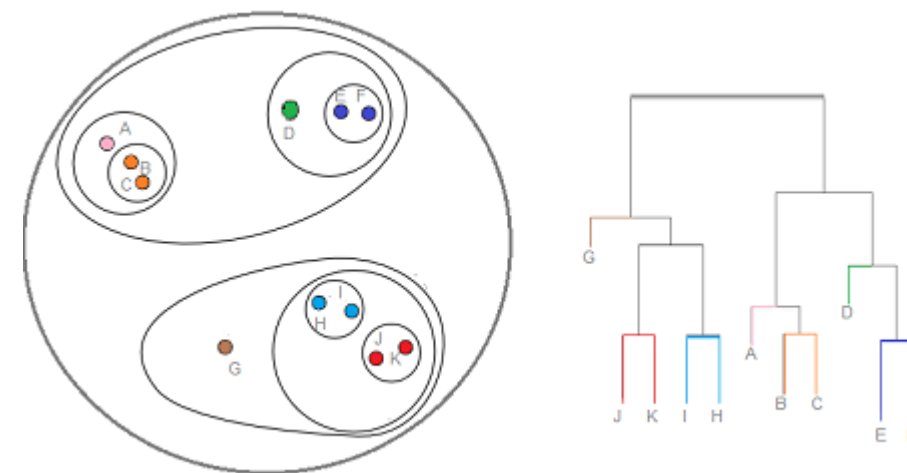
Bootstrapped PCA (Females)



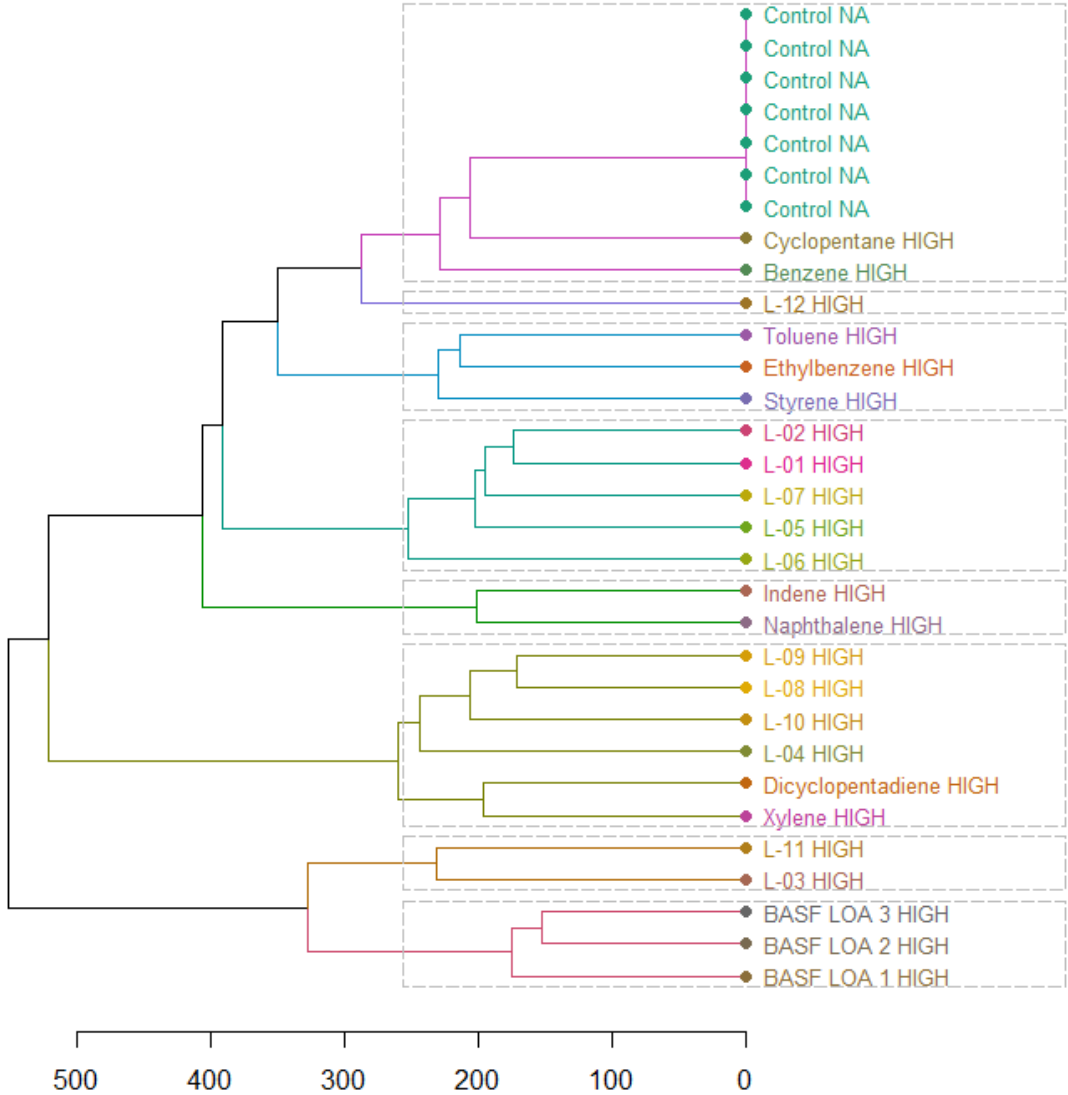
- All compounds: PCA after bootstrapping (100x) + original values
- Streams outside Cat L boundary

Hierarchical Clustering Analysis

- Clusters data
 - Groups similar samples into clusters
- The clusters are based on the distance/similarity of samples
 - based on distances in the large multi-dimensional space
- Finds similar objects: the clusters indicate objects that belong together

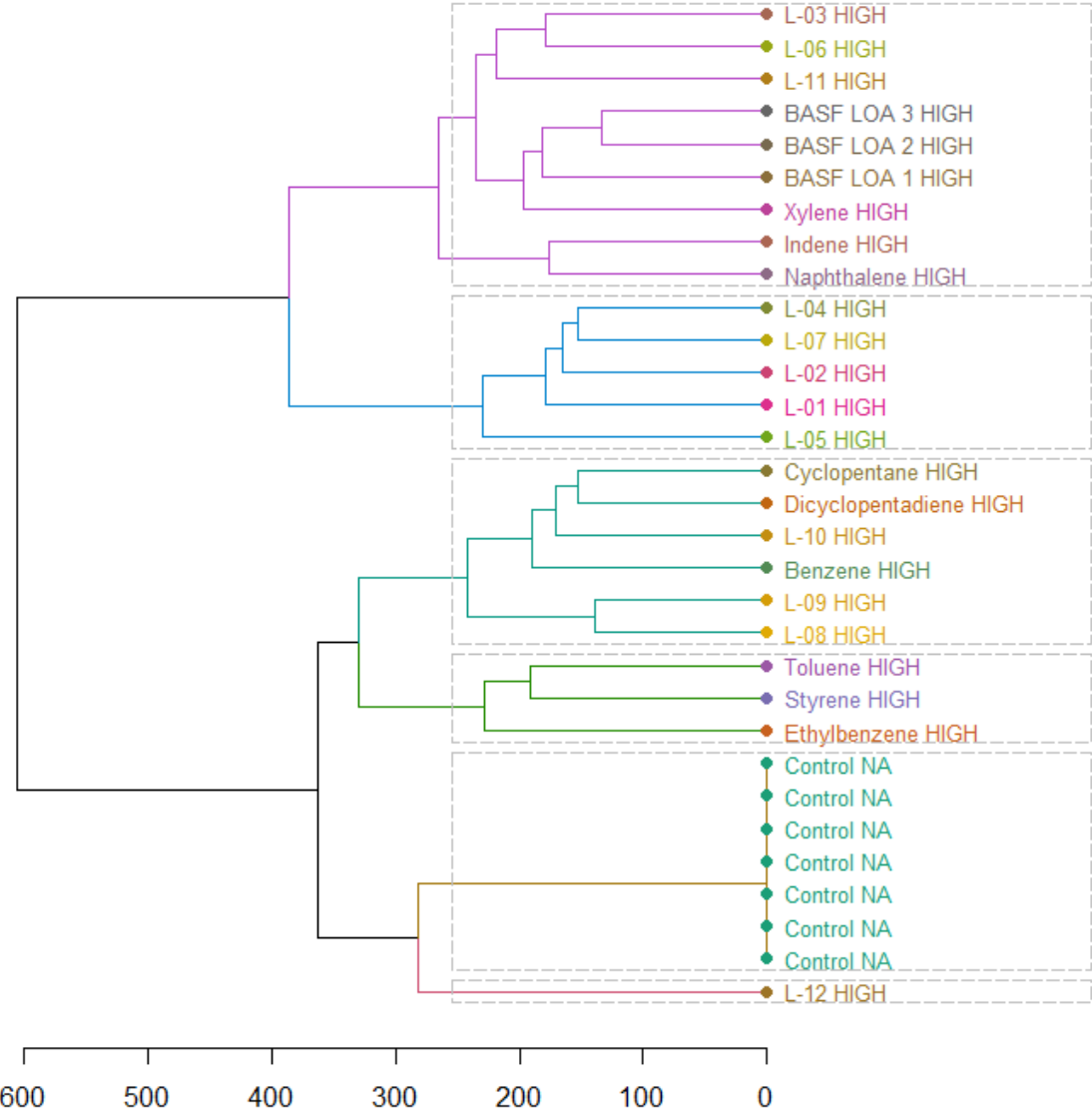


Hierarchical Clustering - Males



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

Hierarchical Clustering - Females



- Controls form a separate cluster; L-12 stands out
- Toluene, Ethylbenzene and Styrene cluster together
- DCPD, L-08, L-09, L-10 form a cluster with Cyclopentane and Benzene
- Naphthalene, Indene, Xylene, the BASF LOA streams as well as L-03, L-06, and L-11 form a larger cluster

Comparison of Clusters

	Males				Females		
	PCA	HCA	TC		PCA	HCA	TC
Indene	Blue	Blue	Blue	Indene	Blue	Blue	Blue
Naphthalene	Blue	Blue	Blue	Naphthalene	Blue	Blue	Blue
L-06	Blue	White	Blue	Xylene	Blue	Light Blue	Yellow
L-11	Blue	Light Green	Blue	L-03	Blue	Light Blue	Blue
L-03	Green	Light Green	Green	L-06	Blue	Light Blue	Blue
BASF LOA 1	Green	Green	Green	L-11	Blue	Light Blue	Blue
BASF LOA 2	Green	Green	Green	BASF LOA 1	Blue	Light Blue	Blue
BASF LOA 3	Green	Green	Green	BASF LOA 2	Blue	Light Blue	Blue
DCPD	Yellow	Yellow	Yellow	BASF LOA 3	Blue	Light Blue	Blue
Xylene	White	Yellow	White	Benzene	White	Yellow	Yellow
L-04	Yellow	Yellow	Yellow	Cyclopentane	White	Yellow	White
L-08	Yellow	Yellow	Yellow	DCPD	White	Yellow	Yellow
L-09	Yellow	Yellow	Yellow	L-01	Yellow	White	White
L-10	White	Yellow	White	L-02	Yellow	White	White
Benzene	White	White	White	L-04	Yellow	White	White
Cyclopentane	White	White	White	L-05	Yellow	White	White
Ethylbenzene	White	White	White	L-07	Yellow	White	White
Styrene	White	White	White	L-08	Yellow	Yellow	White
Toluene	White	White	White	L-09	Yellow	Yellow	White
L-01	White	White	White	L-10	White	Yellow	Yellow
L-02	White	White	White	Ethylbenzene	White	White	White
L-05	White	White	White	Styrene	White	White	White
L-07	White	White	White	Toluene	White	White	White
L-12	White	White	White	L-12	White	White	White

- Colour indicates the different clusters

- ▶ Three clusters in male animals:
 1. Naphthalene-/ Indene-rich (blue)
 2. BASF-LOA + L-03 (green)
 3. DCPD-rich (yellow)

- ▶ Two clusters in female animals:
 1. Naphthalene-/ Indene-rich & BASF-LOA + L-03 (blue)
 2. DCPD-rich (yellow)

The shades represent sub-clusters

- Clusters in males visible homogenously using all techniques

- ▶ PCA: samples grouped based on similarity of differences
- ▶ HCA: similar samples grouped into clusters
- ▶ TC: correlation of metabolome changes between compounds

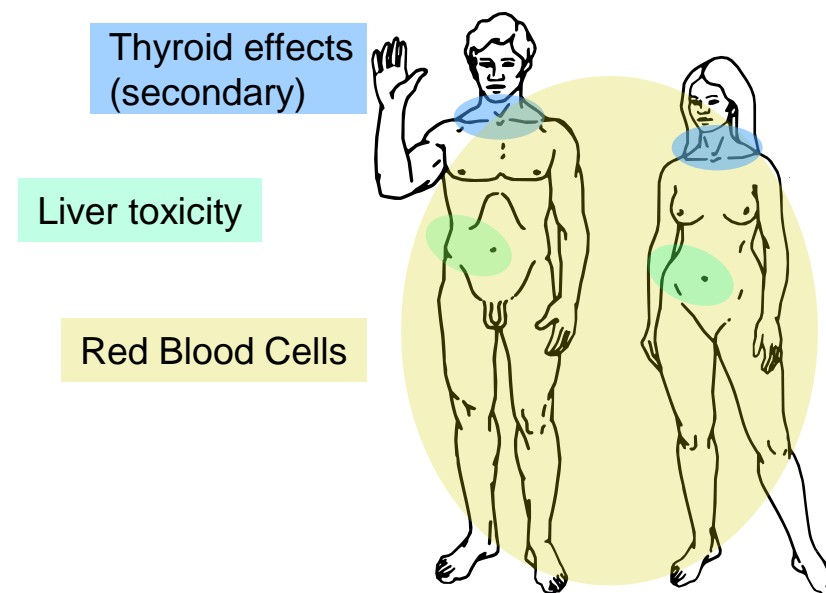
- Clusters in females scatter more; Naphthalene-/ Indene-rich fall together with BASF LOA streams

Conclusion: Target Organs

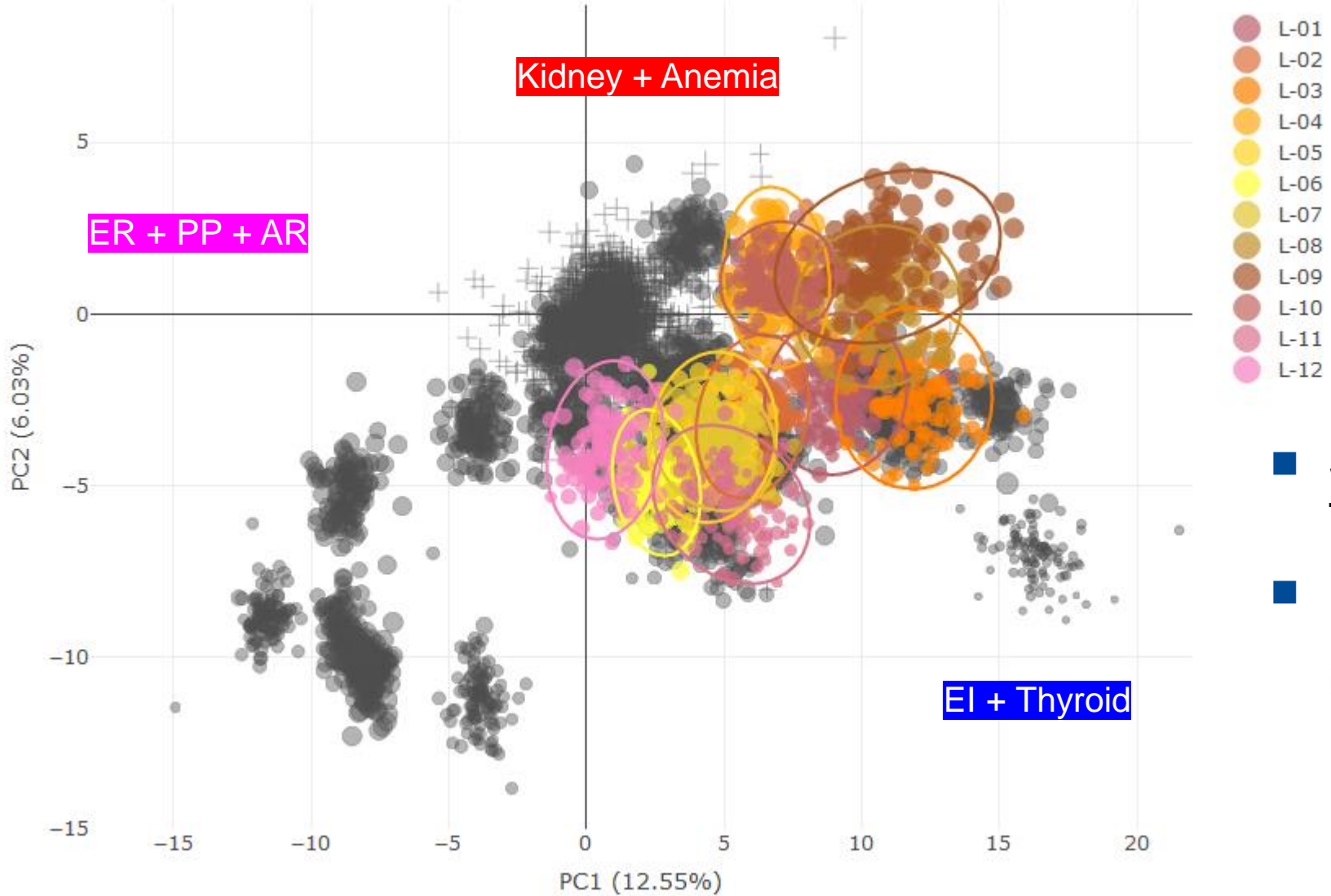
- Liver
 - ▶ Primary Target Organ: mostly enzyme induction, liver toxicity
 - ▶ Organ weight increased, histopathology, liver cell dysfunction, ALT-increases, pattern matches
- Thyroid:
 - ▶ secondary to liver enzyme induction, no changes in thyroxine (T4)
 - ▶ Organ weight increased, histopathology, pattern matches
- Kidney:
 - ▶ alpha 2u globulin accumulation in male rats
 - ▶ Organ weight increased, histopathology incl. immunohistochemistry, pattern matches
- Red blood cells:
 - ▶ Regenerative anaemia
- Other findings considered incidental or secondary

Conclusion: Grouping

- Toxicity data shows relatively homogenous effects: liver, thyroid, red blood cells, kidney (males)
 - ▶ Supported by metabolome patterns, PCA
 - ▶ Metabolome data suggest absence of certain toxicities (e.g., endocrine effects)
- Metabolome data show sub-clustering of LOA Cat L streams
 - ▶ Correlation analysis, bootstrapped PCA, hierarchical clustering
- Toxicity & metabolome effects partly driven by marker compounds (based on the 9 cmpds tested)
 - ▶ the LOA stream toxicity & metabolome effects are not identical to an individual marker substance
 - ▶ No effects observed for LOA streams that were not seen for marker compounds
- BASF LOA streams support the overall (sub-)category approach

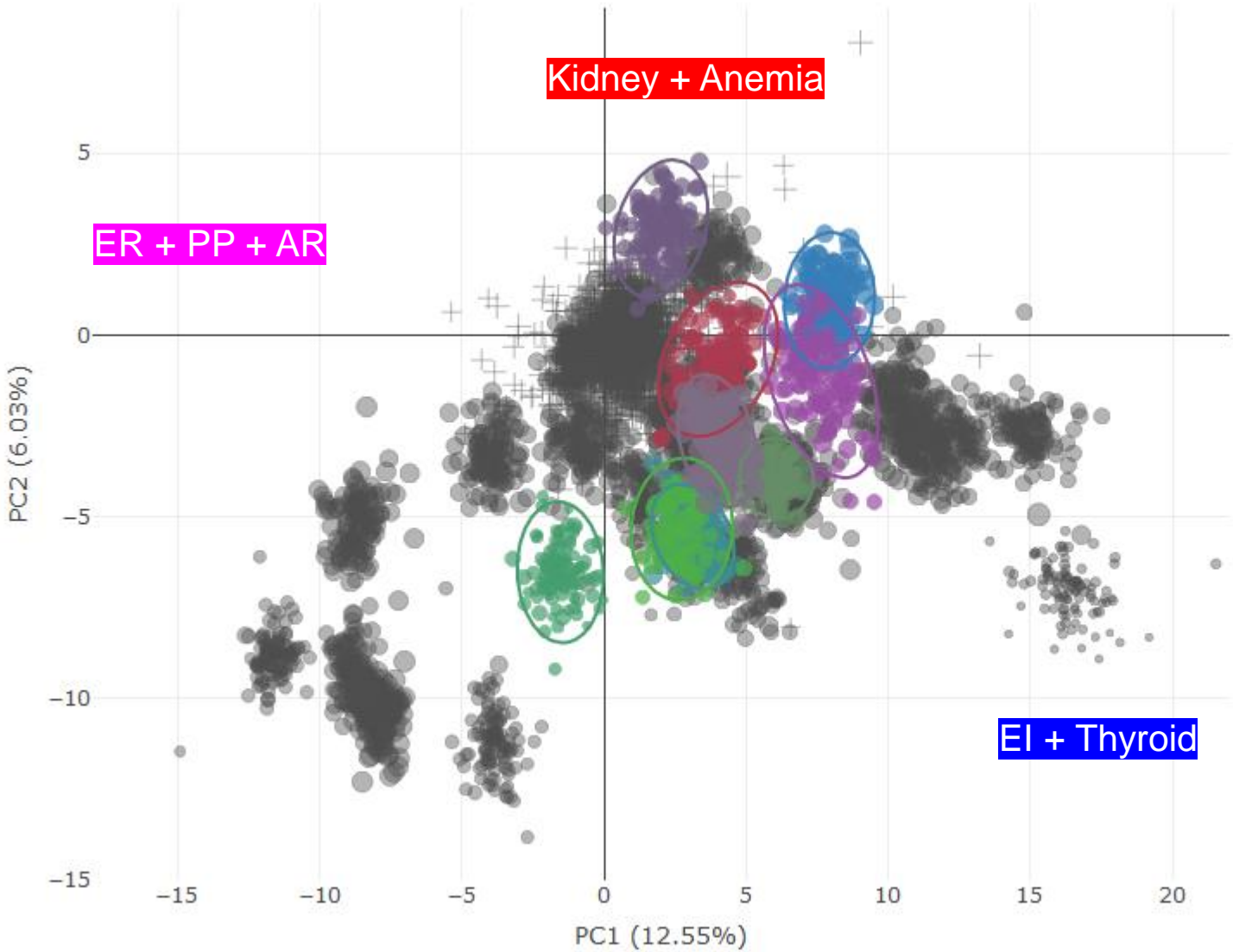


LOA Streams Overlaid



- **Spanning the “universe” of toxicities**
- **Display of LOA streams**
→ overlapping with liver enzyme inducers

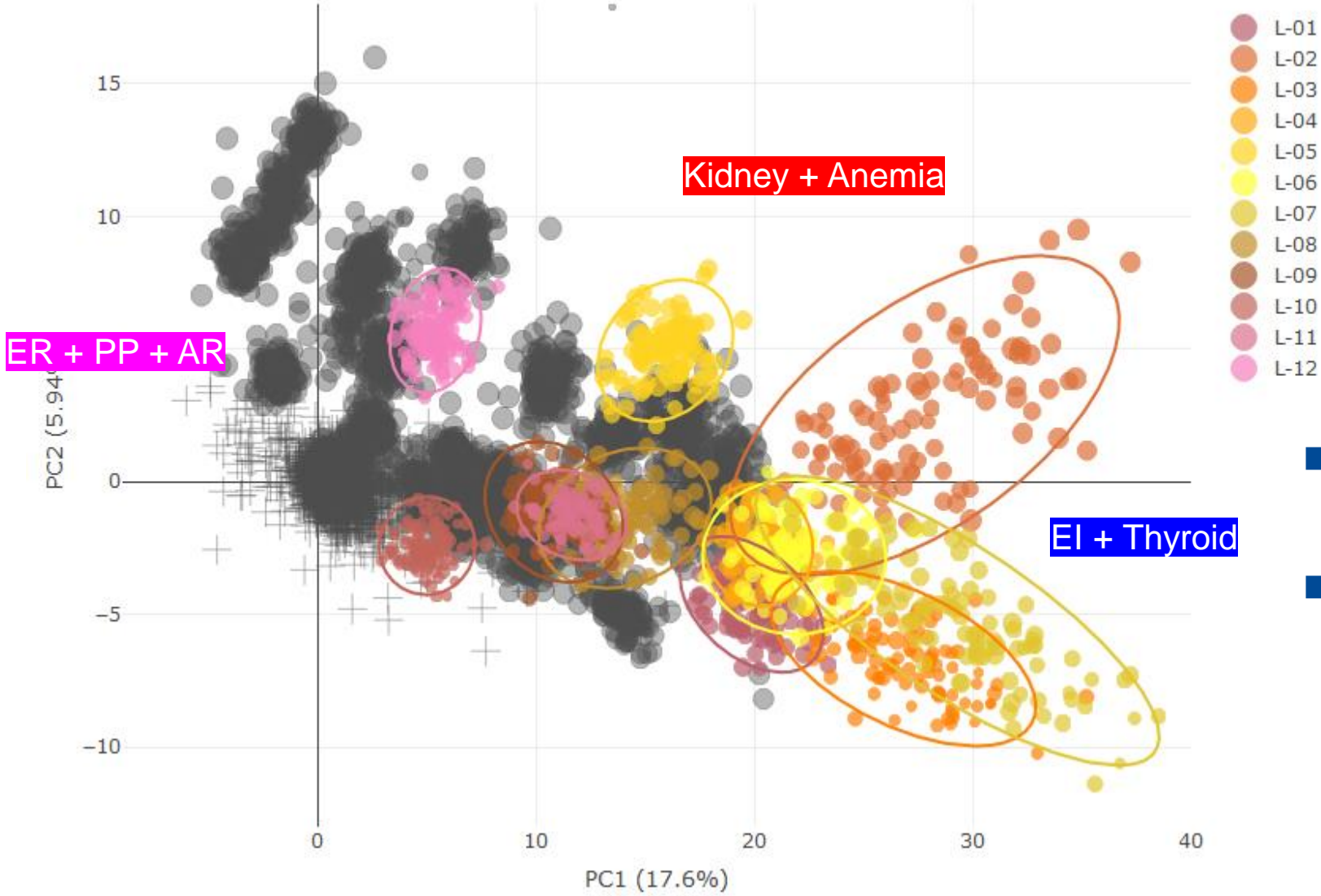
LOA Marker Substances Overlaid



- Benzene
- Cyclopentane
- Dicyclopentadiene
- Ethylbenzene
- Indene
- Naphthalene
- Styrene
- Toluene
- Xylene

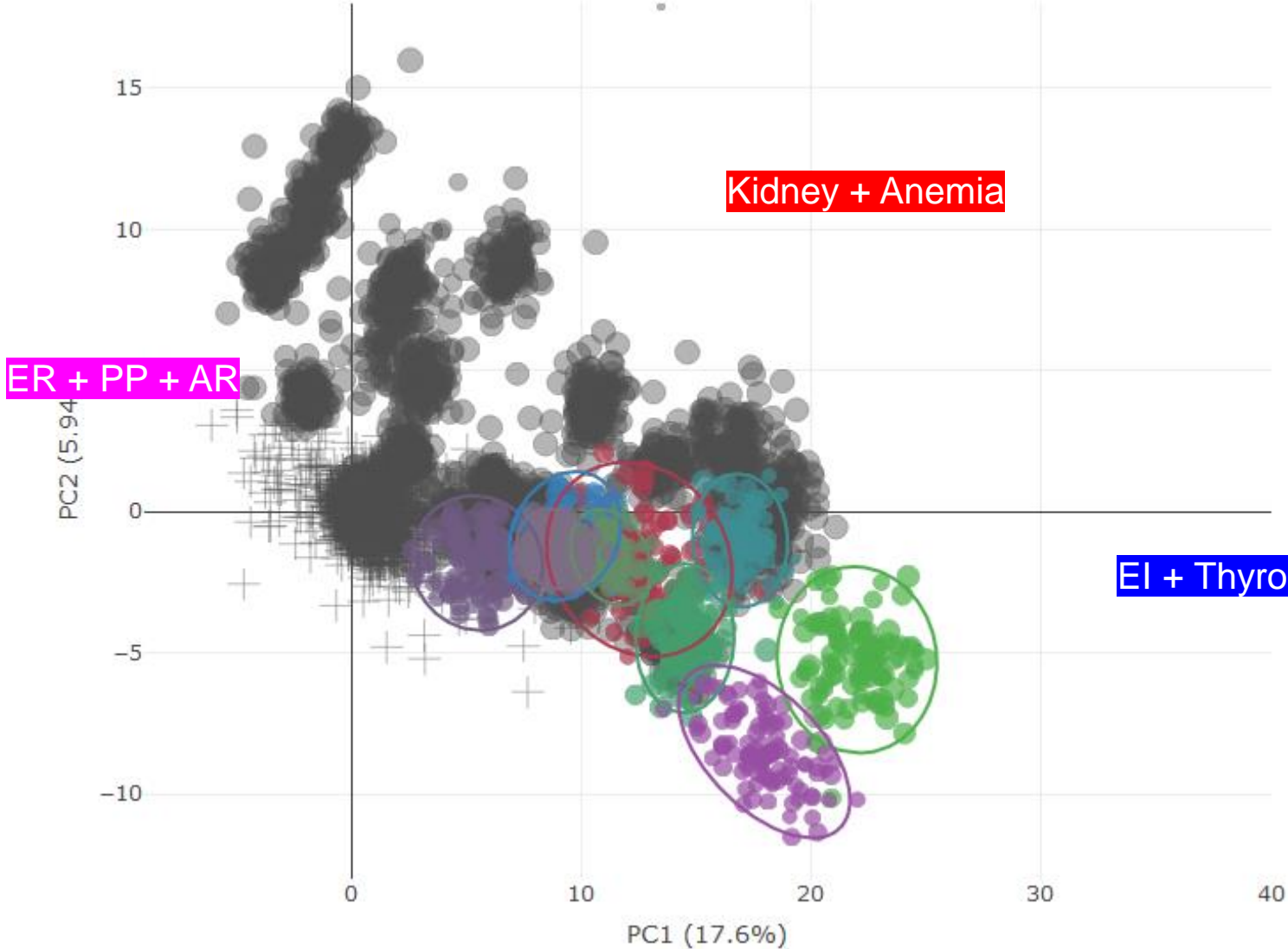
- **Spanning the “universe” of toxicities**
- **Display of marker compounds → overlapping with liver enzyme inducers, except Indene**

LOA Streams Overlaid



- **Spanning the “universe” of toxicities**
- **Display of LOA streams**
→ overlapping with liver enzyme inducers

LOA Marker Substances Overlaid



- Benzene
- Cyclopentane
- Dicyclopentadiene
- Ethylbenzene
- Indene
- Naphthalene
- Styrene
- Toluene
- Xylene

- **Spanning the “universe” of toxicities**
- **Display of marker compounds**
→ overlapping with liver enzyme inducers, except Indene

Webinar Programme

Introduction	Mike Penman (LOA)	13:00 (CET)
Read-Across in REACH; application to UVCBs	Dr. David Bell (ECHA, Helsinki)	13:10
Introduction to Metabolomics	Prof. Mark Viant (University of Birmingham, UK)	13:25
Use of Metabolomics for Read-Across	Prof. Bennard Van Ravenzwaay (BASF)	13:45
Break		14:05
Use of Metabolomics to Assess Biological Coherence in UVCB Read-Across and Category Justification		
A. Introduction	Mike Penman (LOA)	14:10
B. Experimental Design and Data Review	Prof. Hennicke Kamp (BASF)	
C. Conclusion	Dr. Martijn Rooseboom (Shell/LOA)	
Discussion moderator	Prof. Mark Viant (University of Birmingham, UK)	15:15
Close		16:00

LOA REACH CONSORTIUM

Utility of Metabolomics to Support Read-Across
for UVCB substances under REACH

Webinar

30th November 2021

Use of Metabolomics to Assess Biological Coherence in UVCB Read-Across and Category Justification

C. Conclusions

Dr. Martijn Rooseboom (Shell / LOA)

14-day/metabolomics project (Resin Oils and Cyclic Dienes)

Results Interpretation

- Study succeeded as a dose range finder for higher tier tests
- Metabolome profile recognition results
 - Of the 110 toxicity profiles in MetaMap[®] database only ~10% seen in pilot study
- Streams cluster into one area of toxicity and mainly on the liver, i.e. toxicity in the area we might expect for such substances
 - Pathology and clinical chemistry do not generally show unexpected findings
 - No metabolomics patterns seen by UVCB substances that have not also been seen with the tested marker substances
 - No mode of action that is alerting – no endocrine

14-day/metabolomics project (Resin Oils and Cyclic Dienes)

Results Interpretation - 2

- Closer analysis: Each UVCB substance is unique in its chemical composition and metabolomic profile
 - Form one group – a number of sub-groups can be observed
 - Marker findings do not explain the full picture
- Streams for further testing should consider both analytical and metabolomics data
 - Very specific technique – can highlight key pathways – from metabolomics data universe
- Ongoing OECD 422 data on each stream will strengthen the overall analysis and data interpretation

14-day/metabolomics project (Resin Oils and Cyclic Dienes)

Conclusion

- The current study has
 - Demonstrated the utility of Metabolomics to inform on biological coherence for these streams
 - Provided data to assist grouping and read across
 - Assisting the design of further studies (OECD 422, OECD 408 and OECD 414) in the resin oils and cyclic dienes category
 - Facilitated the reduction of animal use without compromising data confidence
- Metabolomics will be considered for other LOA non-CMR categories

Acknowledgments

- LOA Information Requirements Working Group
 - Martijn Rooseboom - Shell (Chair)
 - Neslihan AYGUN Kocabas - Total (Vice-chair)
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 - Frank Fullhammer - BASF
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 - Volker Strauss
 - Dorothee Funk-Weyer

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Discussion

Hosted by Professor Mark Viant (University of Birmingham, UK)

BACK-UP

Pattern Ranking: Example LOA Stream L-01, HD

L-01, HD against pattern for „liver toxicity“
 >90%* of metabolite changes similar → **MATCH**

Regulate	Anchor	Metabolite Name	Analyte ID	L-01 (MOA1) C011_Cohort1) HD			Beta-ionone (MOA4) HD			Caffeine (MOA10) HD			D	
				f7	f14	f28	f7	f14	f28	f7	f14	f28		
✓	↑	↓	Glycerol, lipid fraction	28000002	NA	2.85	2.85	1.66	1.56	1.6	1.6	1.68	1.38	1.
✓	↑	↓	Palmitic acid (C16:0)	28000003	NA	2.14	2.14	1.73	1.61	1.79	1.53	1.77	2.07	1.
✓	↑	↓	Linoleic acid (C18:cis[9,12]2)	28000004	NA	2.47	2.47	1.83	1.81	2	1.64	1.79	1.82	1.
✓	↑	↓	Stearic acid (C18:0)	28000007	NA	2.16	2.16	1.92	1.91	2.03	1.29	1.49	1.87	1.
✓	↑	↓	Arachidonic acid (C20:cis[5,8,11,14,17,20]4)	28000011	NA	2.06	2.06	2.08	1.94	2.29	1.48	1.73	2.35	1.
✓	↑	↓	Docosahexaenoic acid (C22:cis[4,7,10,13,16,19]6)	28000015	NA	1.6	1.6	2.68	2.33	2.45	2.44	2.93	2.44	1.
✓	↑	↓	Cholesterol, total	28000019	NA	1.83	1.83	2.35	2.22	2.73	1.65	1.83	2.48	1.
✓	↑	↓	Glycerol phosphate, lipid fract	28000044	NA	4.01	4.01	2.47	2	2.69	1.42	1.89	2.07	1.
✓	↑	↓	Lignoceric acid (C24:0)	28000052	NA	1.6	1.6	2.77	2.92	3.1	1.68	2.19	2.21	1.
✓	↑	↓	Heptadecanoic acid (C17:0)	28000068	NA	1.83	1.83	1.96	1.45	2.05	1.27	1.74	1.6	1.
✓	↑	↓	Tricosanoic acid (C23:0)	28000072	NA	1.49	1.49	2.63	2.45	2.71	1.59	2.4	2.36	1.
✓	↑	↓	Phosphate, lipid fraction	28000086	NA	1.66	1.66	1.86	1.83	1.92	1.72	1.57	1.82	1.
✓	↑	↓	Behenic acid (C22:0)	28000152	NA	1.53	1.53	2.65	2.71	3.02	1.75	2.15	2.37	1.
✓	↑	↓	4-Hydroxy sphinganine (t18:0, Ph)	28000158	NA	2.97	2.97	2.24	2.02	2.64	1.54	2.93	2.65	1.
✓	↑	↓	Nervonic acid (C24:cis[15]1)	28000159	NA	2.21	2.21	4.26	3.98	4.37	1.49	2.43	2.76	2.
✓	↑	↓	dihomo-gamma-Linolenic acid (C22)	28000482	NA	2.16	2.16	2.49	2.5	2.39	1.48	2.46	2.13	1.
✓	↑	↓	3-O-Methylsphingosine (d18:1) (Σ)	28000489	NA	5.1	5.1	4.47	4.52	4.42	2.28	3.18	3.17	1.
✓	↑	↓	threo-Sphingosine (d18:1) (Σ)	28000491	NA	3.05	3.05	3.31	3.42	3.76	1.87	2.53	2.36	1.
✓	↑	↓	Docosapentaenoic acid (C22:cis[4,7,10,13,16,19]6)	28000493	NA	1.5	1.5	2.03	1.36	1.75	1.89	3.01	2.14	2.
✓	↑	↓	5-O-Methylsphingosine (d18:1) (Σ)	28000494	NA	4.2	4.2	4.84	4.39	4.41	2.3	3.09	3	1.
✓	↑	↓	erythro-Sphingosine (d18:1) (Σ)	28000495	NA	3.31	3.31	3.77	3.66	3.51	2.12	2.7	2.56	1.
✓	↑	↓	Cholesterol, total	28000504	NA	1.82	1.82	2.74	2.54	3.02	1.8	2.62	3.32	1.
✓	↑	↓	Threonic acid	38000083	NA	1.28	1.28	1.48	1.35	2.04	1.37	1.72	1.57	1.
✓	↑	↓	Ceramide (d18:1,C24:1) (Σ)	68000025	NA	1.58	1.58	2.73	2.59	2.62	1.49	1.97	1.97	1.
✓	↑	↓	TAG (C16:1,C16:1) and TAG (C14: (Σ)	68000028	NA	1.15	1.15	1.48	1.02	1.12	1.73	2.21	0.74	2.
✓	↑	↓	TAG (C18:2,C18:2)	68000029	NA	2.76	2.76	1.98	1.84	1.97	1.83	2.5	1.33	2.
✓	↑	↓	TAG (C16:0,C18:2)	68000030	NA	3.07	3.07	2.44	1.93	2.37	1.65	2.64	0.83	2.

*75-90% of metabolite changes similar → **WEAK MATCH**

L-01, HD against pattern for „anemia, iron deficiency“
 < 75% of metabolite changes similar → **EQUIVOCAL**

Analyte ID	L-01 (MOA1) C011_Cohort1) HD			4-Chloroaniline (MOA78) HD			Dimoxystrobin (MOA2) HD			Iron deficient diet (MOA3)		
	f7	f14	f28	f7	f14	f28	f7	f14	f28	f7	f14	f28
18000032	NA	1.01	1.01	1.14	1.33	1.3	1.32	1.2	1.17	1.18	1.17	1.15
18000034	NA	1.08	1.08	1.74	1.64	1.87	1.54	1.25	1.19	1.35	1.21	1.34
18000217	NA	1.69	1.69	1.51	1.94	1.71	1.5	0.93	1.04	1.15	1.24	1.18
18000292	NA	0.92	0.92	6.37	4.24	9.49	12.2	1.96	1	14.98	5.77	5.88
38000006	NA	1.24	1.24	1.88	2.09	1.98	1.5	1.18	1.17	1.33	1.26	1.41
38000008	NA	1.13	1.13	1.17	1.34	1.27	1.11	1.14	1.19	1.37	1.1	1.24
38000012	NA	0.77	0.77	1.38	1.52	1.39	1.56	1.62	1.33	1.45	1.32	1.3
38000052	NA	1.13	1.13	1.24	1.46	1.28	1.36	1.19	1.21	1.08	1.05	1.12
38000079	NA	1.2	1.2	1.2	1.36	1.43	1.2	1.4	1.29	1.18	1.19	1.16
38000443	NA	1.15	1.15	0.73	0.71	1.35	0.49	0.73	0.49	0.2	0.33	0.39
58000020	NA	1.26	1.26	1.12	1.39	0.78	0.55	0.64	0.37	0.42	0.17	0.13
58000023	NA	1.35	1.35	NA	1.58	NA	0.67	1.37	0.89	0.4	0.48	0.53
68000018	NA	1.85	1.85	1.61	1.85	1.87	1.28	1.18	1.06	1.22	1.14	1.24
68000026	NA	1.85	1.85	4.15	2.87	3.39	2.28	2.99	2.85	2.91	3.04	1.92
68000029	NA	2.78	2.78	7.94	4.21	8.29	3.69	3.82	5.11	1.52	3.4	1.63
68000032	NA	3.39	3.39	4.05	4.58	8.62	4.18	3.37	4.49	2.84	3.49	1.44
68000034	NA	0.74	0.74	1.07	1.19	1.1	1.29	1.19	1.14	1.38	1.24	1.5
68000043	NA	3.9	3.9	8.21	4.14	7.26	3.61	2.41	3.9	1.97	2.6	1.07
68000044	NA	3.2	3.2	6.36	4.97	8.42	3.51	3.6	5.25	1.91	3.02	1.74
68000045	NA	0.83	0.83	0.56	0.66	0.37	0.75	0.7	0.52	0.8	0.65	0.84
68000056	NA	1.39	1.39	6.13	5.46	9.18	2.5	3.54	4.13	2.09	3.35	1.75
68000057	NA	3.18	3.18	8.16	4.12	6.7	4.17	5.45	3.93	1.46	3.75	1.26

Potentially leave out

Pattern Ranking: Example LOA Stream L-01, HD

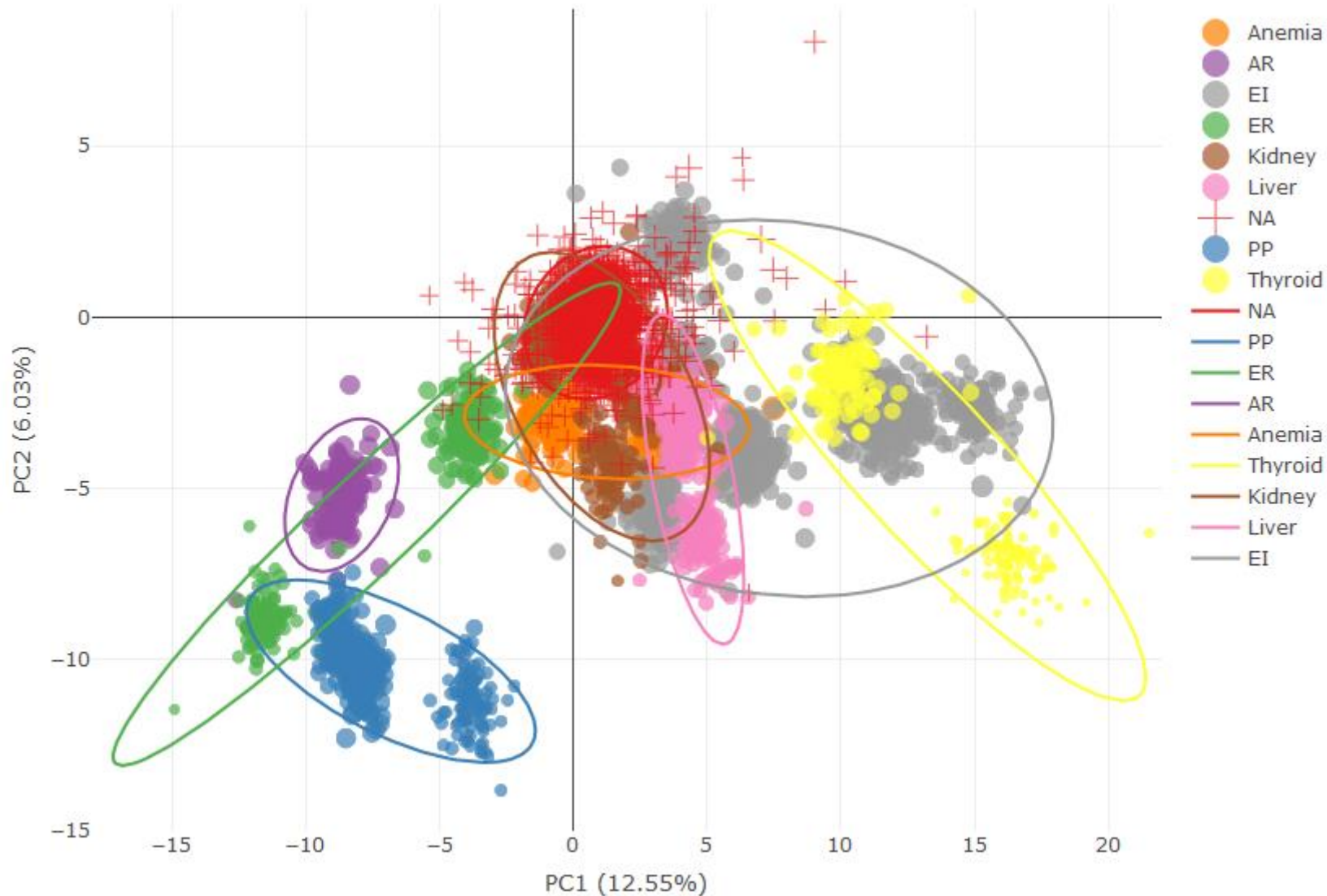
L-01, HD against pattern for „hormones, antiandrogen“ < 50% of metabolite changes similar → **MISMATCH**

L-01, HD against pattern for „hormones, antiandrogen“ Anchor metabolites dissimilar → **MISMATCH**

Potentially leave out

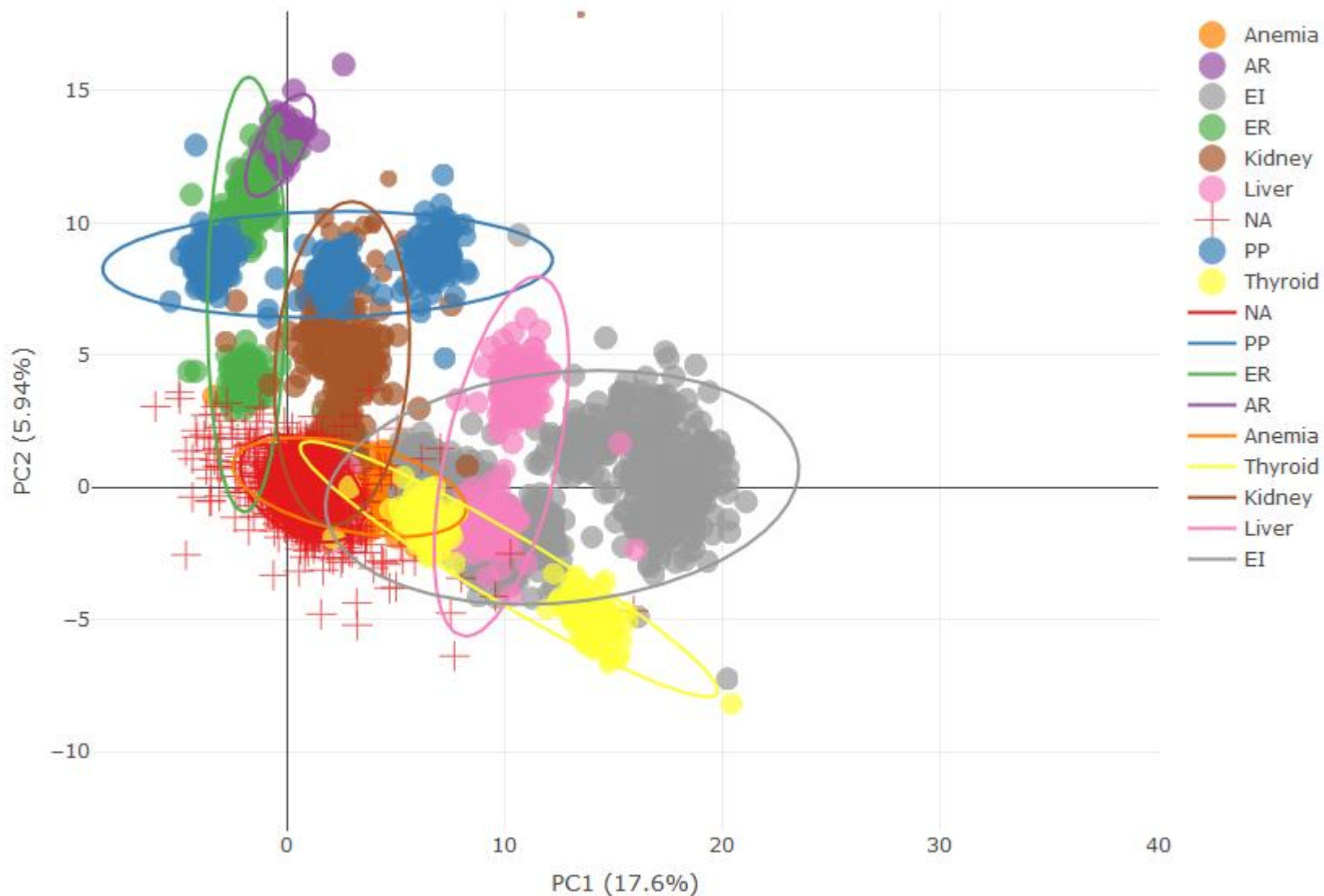
Regulate	Anchor	Metabolite Name	Analyte ID	L-01 (MOA190011_Cohort1) HD			Cyproteron Acetate (MOA53) LD			Flutamide (MOA23) HD			Vinclozolin (MOA22) HD		
				m7	m14	m28	m7	m14	m28	m7	m14	m28	m7	m14	m28
✓	↑	Threonine	18000034	NA	0.97	0.97	1.27	1.58	1.87	1.33	1.24	1.22	1.18	1.08	1.16
✓	↑	myo-Inositol, lipid fraction	28001194	NA	1.58	1.58	1.54	1.75	2.77	1.27	1.03	1.22	1.2	1.28	1.2
✓	↑	Threonine	38000006	NA	1.11	1.11	1.25	1.53	1.89	1.35	1.3	1.13	1.21	1.17	1.02
✓	↓	Glycerol, polar fraction	38000038	NA	1.04	1.04	0.71	0.79	1.11	0.72	0.72	0.89	0.8	0.9	0.79
✓	↑	Glycerol-3-phosphate, polar fra	38000053	NA	1.09	1.09	1.31	1.28	1	0.75	1.08	1.17	1.74	1.18	1.22
✓	↓	trans-4-Hydroxyproline	38000437	NA	0.8	0.8	0.69	0.72	0.84	0.79	0.89	0.75	0.81	0.78	0.86
✓	↑	Glucuronic acid	38000551	NA	1.88	1.88	3.4	3.49	2.89	2.93	2.3	1.73	3.56	2.98	3.51
✓	↑	3-Methoxytyrosine	78000025	NA	1.23	1.23	1.32	1.34	1.27	1.0	1.43	1.59	1.29	1.13	1.15
✓	↑	Androstenedione	128000001	NA	0.9	0.9	4.31	3.12	1.26	30.68	55.72	23.97	3.33	4.15	2.27
✓	↑	Testosterone	128000004	NA	0.73	0.73	4.01	2.47	0.97	14.84	19.61	10.58	2.03	2.83	1.36
✓	↓	Progesterone	128000008	NA	0.81	0.81	1.03	0.42	0.84	0.56	0.4	0.31	0.18	0.22	0.54
✓	↓	21-Hydroxyprogesterone (11-Deox	128000010	NA	0.83	0.83	0.38	0.39	0.04	0.59	0.43	0.21	0.16	0.12	0.17
✓	↓	18-Hydroxy-11-deoxycorticostero	128000012	NA	0.84	0.84	0.04	0.01	0	0.85	0.68	0.07	0.08	0.17	0.23
✓	↓	Corticosterone	128000016	NA	0.93	0.93	0.04	0.01	0	0.87	0.46	0.07	0.27	0.16	0.57

MetaMap[®]Tox Reference Substances with Different MOAs (Males)



- **Spanning the “universe” of toxicities**
- **Metabolome data of reference compounds with different MoAs from MetaMap[®]Tox data base:**
 - ▶ Anemia
 - ▶ Androgens (AR)
 - ▶ Liver enzyme inducers (EI)
 - ▶ Estrogens (ER)
 - ▶ Kidney toxicants
 - ▶ Liver toxicants
 - ▶ Peroxisome proliferators (PP)
 - ▶ Thyroid toxicants
- **Bootstrapped data**

MetaMap[®]Tox Reference Substances with Different MOAs (Females)



- **Spanning the “universe” of toxicities**
- **Metabolome data of reference compounds with different MoAs from MetaMap[®]Tox data base:**
 - ▶ Anemia
 - ▶ Androgens (AR)
 - ▶ Liver enzyme inducers (EI)
 - ▶ Estrogens (ER)
 - ▶ Kidney toxicants
 - ▶ Liver toxicants
 - ▶ Peroxisome proliferators (PP)
 - ▶ Thyroid toxicants
- **Bootstrapped data**