

Toxicity prediction based on MoA knowledge

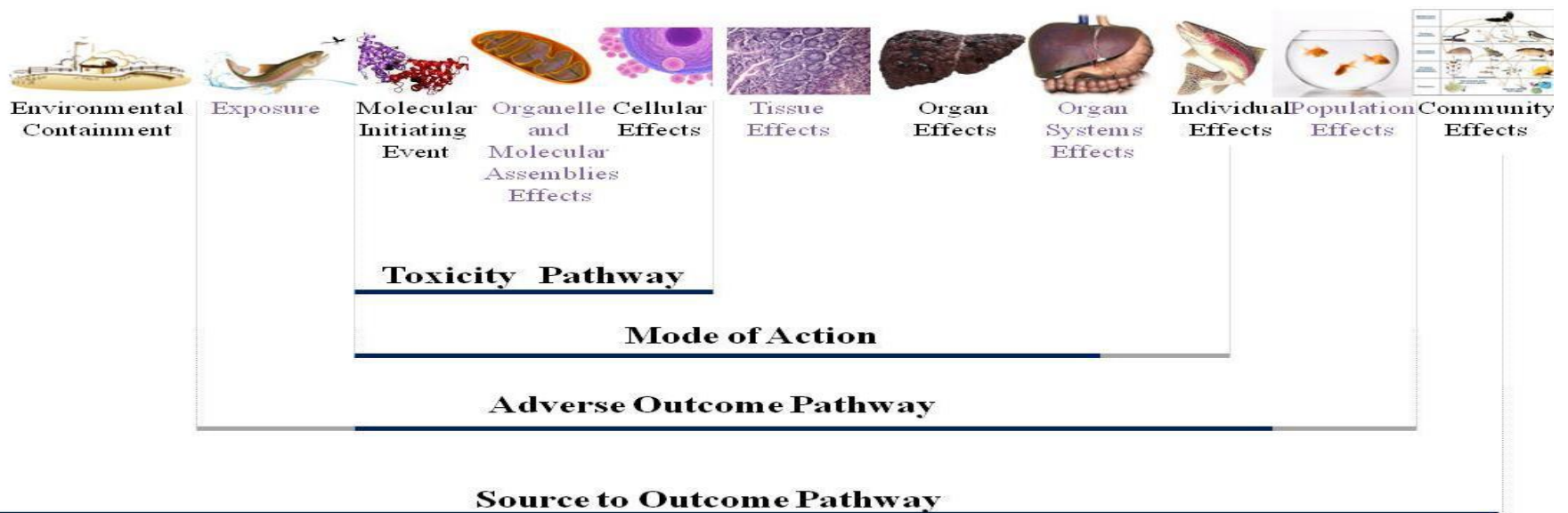
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SEURAT-1 Summer School, 08-10 June 2014

Mode of action (MoA)

The sequence of key events and cellular and biochemical events (measurable parameters) , starting with the interaction of an agent with the target cell, through functional and anatomical changes, resulting in cancer or other adverse health effects.



Seurat-1 Project



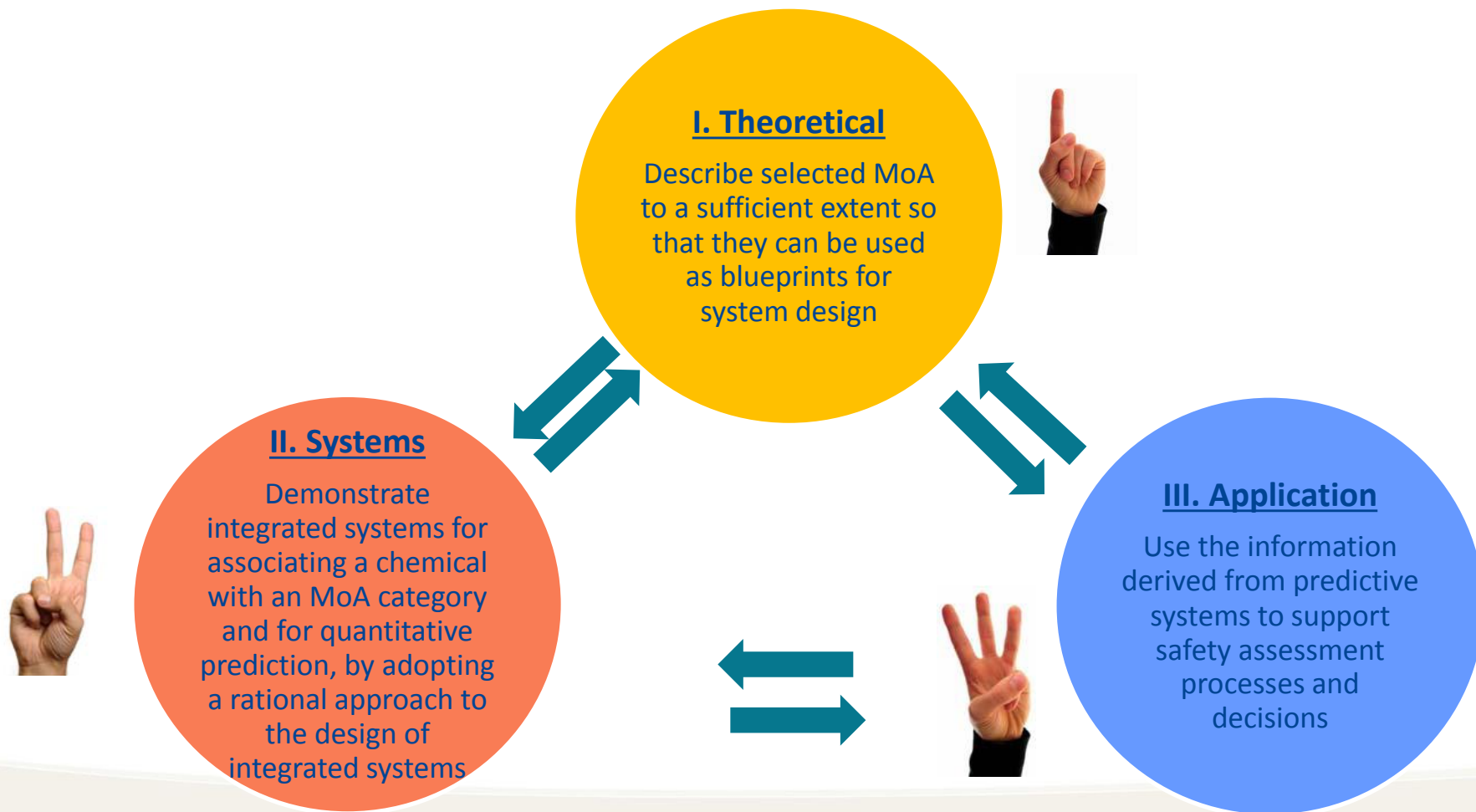
Strategy:

The Seurat strategy is to adopt a toxicological mode-of-action framework to describe how any substance may adversely affect human health, and to use this knowledge to develop complementary theoretical, computational and experimental models that predict quantitative points of departure for safety assessment.

Objectives:

1. Formulate and implement a research strategy based on generating and applying knowledge of mode of action
2. Develop highly innovative tools and methodology that can ultimately support regulatory safety assessment
3. Demonstrate proof of concept at multiple levels – theoretical, systems, and application
4. Provide the blueprint for expanding and applicability domains – chemical, toxicological and regulatory

Demonstrate proof of concept at multiple levels – theoretical, systems, and application



MoA-based ITS: essential elements for toxicity prediction

Prediction Goal

MoA

Methods

The combination of these 3 elements builds an Integrated Testing Strategy to predict the potential toxicity of a chemical.

Design MoA-based ITS: reasoning process

Which toxicity (AO) should be predicted?

Target organ toxicity? Organelle toxicity as mitochondrial toxicity? Repeated dose toxicity?

For the specific AO to be predicted, is there an available MoA?

E.g. liver steatosis has many MoAs

To what extent is the MoA being described?

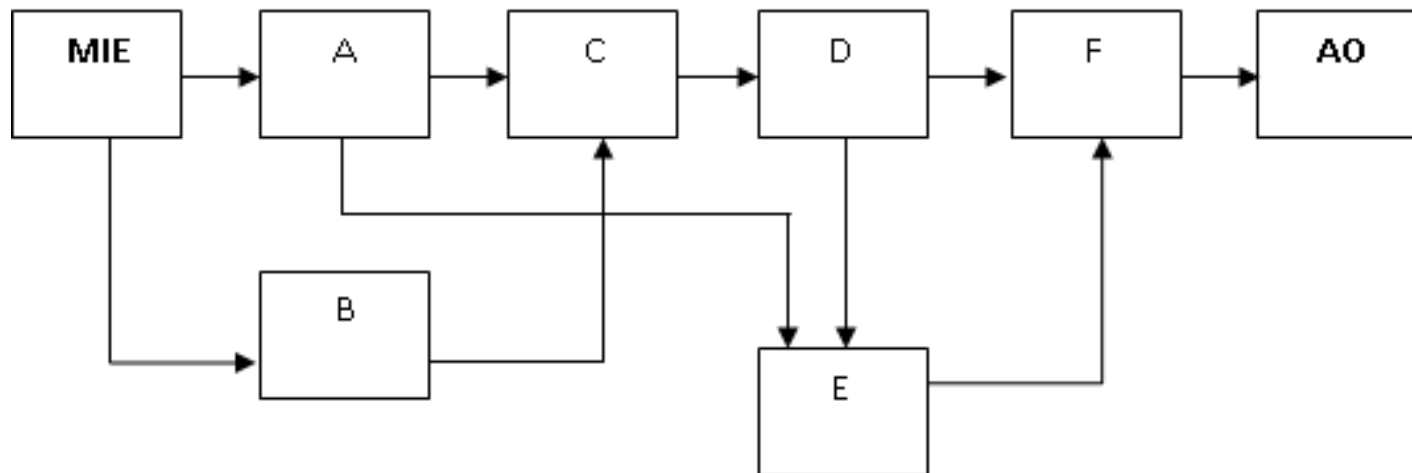
The MoA should be adequately characterised even if no complete: minimum requirements MIE, intermediate event and AO

What is the minimum set of essential event/s of the MoA to be observed in order to predict the chemical's toxicity?

Can each of selected essential events be measured with the available methods?

We are more interested if an event can be measured than how it can be measured

Design MoA-based ITS: example_1



- There is a strong evidence on the accuracy and robustness of this MoA supported by a large results in peer-reviewed literature
- The MIE, event A and event E are considered to be the essential events to be observed in order to predict the toxicity
- We need to evaluate if the selected events can be measured with available *in vitro/in silico* methods

Design MoA-based ITS: example_2

PREDICTION GOAL		Methods						
		1	2	3	4	5	6	7
		Can the event be measured with the available methods?						
MoA events	MIE	yes						
	A		yes					
	B			no				
	C				?			
	D					yes		
	E						yes	
	F							yes

- We can measure all the events essential to associate a chemical with this toxicological MoA and we can combine together the relevant methods to build a reliable Integrated Testing Strategy.

Take home messages

- The mechanistic understating of an AO improves the design of reliable ITS by identifying the key events that need to be measured to associate a chemical with a toxicity. This information can also be used to select the most suitable *in vitro* cell model.
- The description of a MoA should be as accurate as possible but we do not require a fully defined MoA to start designing an ITS and make a toxicity prediction.

Practical example 1

Possible Use of Mode of Action (MoA) in Chemical Category Development

Developing a MoA-based chemical category to support the weight of evidence approach to ED identification - a case study with phthalate esters

Current definition of chemical category

•A chemical category is a group of chemicals whose physico-chemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristic).

OECD, SERIES ON TESTING AND ASSESSMENT Number 80, GUIDANCE ON GROUPING OF CHEMICALS

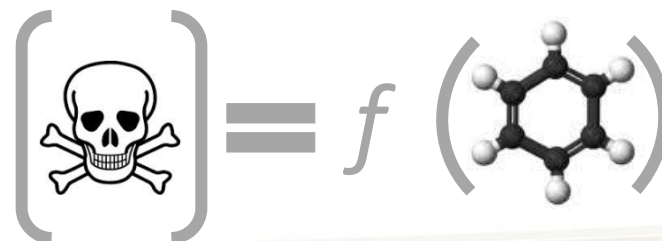
Use of chemical category for toxicity prediction

Read-across to extrapolating toxicity data from data-rich to data-poor chemicals in order to fill data gap.

	Chemical 1	Chemical 2	Chemical 3	Chemical 4
Property 1	●	○	●	○
Property 2	●	○	●	●
Property 3	○	●	●	○

● Reliable data points ○ Missing data points

(Q)SAR to predict properties from knowledge of chemical structure



Develop a chemical category

- In general, chemical categories are formed based on chemical similarity.
- The easiest and most intuitive way of forming a category is to group members of the same chemical class (e.g. phthalates, parabens, allyl esters, etc.) having available toxicity data.

**Structurally similar
chemicals**

Not always have



**Same toxicological
properties**

Existing chemical categories

OECD Existing Chemicals Database

Click on an item ...

- Home
- Search
- SIDS contacts
- Sponsored chemicals
- Category chemicals
- Login
- Help

Reports

- Overall Status
- All Sponsored Substances
- Publications

Open all / Toggle all

Acid Chloride Category (4)

Alkyl chlorosilanes (4)

Alkyl phenate sulfides (9)

Alkyl Sulfates, Alkane Sulfonates and αOlefin Sulfonates (61)

Alkyl-substituted Peroxy Esters (5)

Alkylamidopropyl betaines (3)

Alpha-Olefins (5)

Amine oxides (15)

Ammonia (4)

Amorphous silica silicates (5)

Anthracene oils (5)

Aryl-substituted Peroxy Esters (2)

Critical Reviews in Toxicology, 36:695–726, 2006
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A Category Approach for Reproductive Effects of Phthalates

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In regulatory toxicology, the experimental assessment of reproductive toxicity is one of the most costly endpoints to perform. Categorizing chemicals is an approach that can be used to reduce animal tests in risk assessments of chemicals, for example, via REACH (Registration, Evaluation, and Authorization of Chemicals). The category approach was tested for reproductive toxicity with a group of 10 *ortho*-phthalate esters, with different side chain lengths. Three *ortho*-phthalates were used for testing the category. Phthalates with side-chain lengths C4 to C6 that are commonly known to cause reproductive effects were included, as well as the recently discovered mechanism that indicates antiandrogenic effects. The differences in physicochemical properties, absorption rates, and metabolism between the phthalates



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based on adverse

mada^a, J. Yamada^a

ution, Tokyo, Japan;

Adoption of the data-gap filling method for complex endpoints such as repeated dose toxicity (RDT) and reproductive/developmental toxicity is one of the most important issues affecting international chemical management at present. A categorization method based on adverse outcome pathways (AOPs) has recently been investigated for such complex endpoints. In this paper, we report results of the categorization of nitrobenzenes for RDT based on the AOPs obtained by

Develop MoA-based chemical category

Integrative Chemical–Biological Read-Across Approach for Chemical Hazard Classification

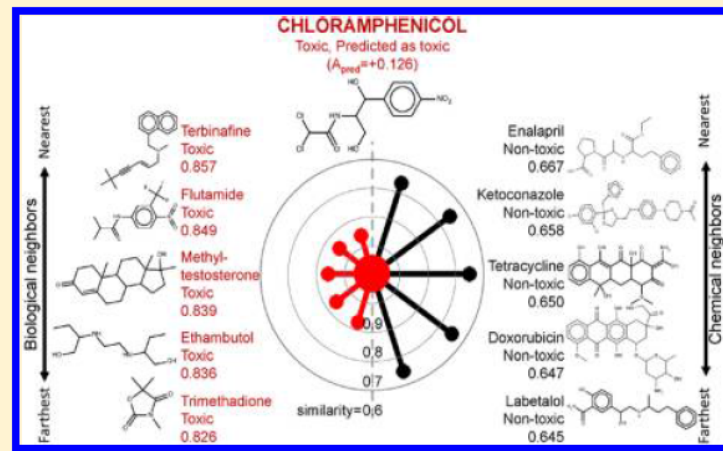
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Supporting Information

ABSTRACT: Traditional read-across approaches typically rely on the chemical similarity principle to predict chemical toxicity; however, the accuracy of such predictions is often inadequate due to the underlying complex mechanisms of toxicity. Here, we report on the development of a hazard classification and visualization method that draws upon both chemical structural similarity and comparisons of biological responses to chemicals measured in multiple short-term assays (“biological” similarity). The Chemical–Biological Read-Across (CBRA) approach infers each compound’s toxicity from both chemical and biological analogues whose similarities are determined by the Tanimoto coefficient. Classification accuracy of CBRA was compared to that of classical

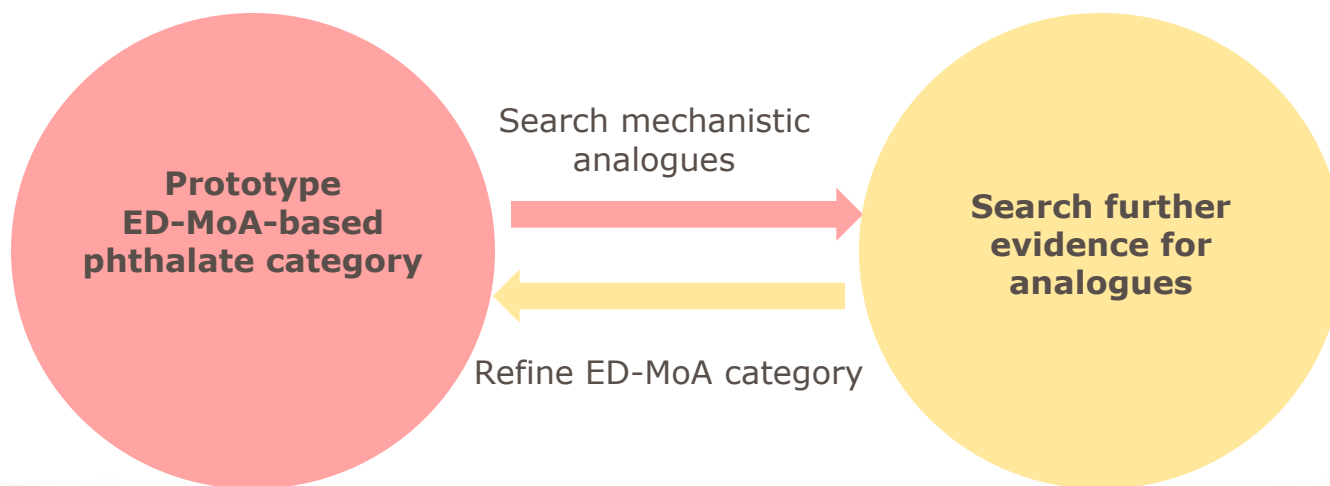


Phthalate case study: Background

- Endocrine Disrupting chemicals display little structural commonality and may cause toxicity through a variety of mechanisms  nearly impossible to define single chemical descriptors to encompass all EDs.
- Knowledge of ED MoAs supports the development of EDs chemical categories and computational profilers able to identify potential EDs.
- Some phthalates are considered to be EDs, they cause reproductive and developmental toxicity (mainly in males) and their toxicological modes of action are relatively well known (data-rich).

Phthalate case study: Aim

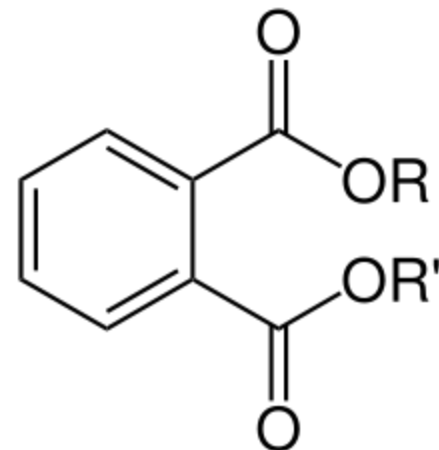
- Use the ED-MoA knowledge of the phthalates group to identify mechanistic analogues and search for further evidence in literature to support the development of ED-MoA-based chemical category.
- The analysis is done profiling different data-sources to identify phthalates-analogues. At this stage, identification of analogues is only done by using existing data without using testing methods.



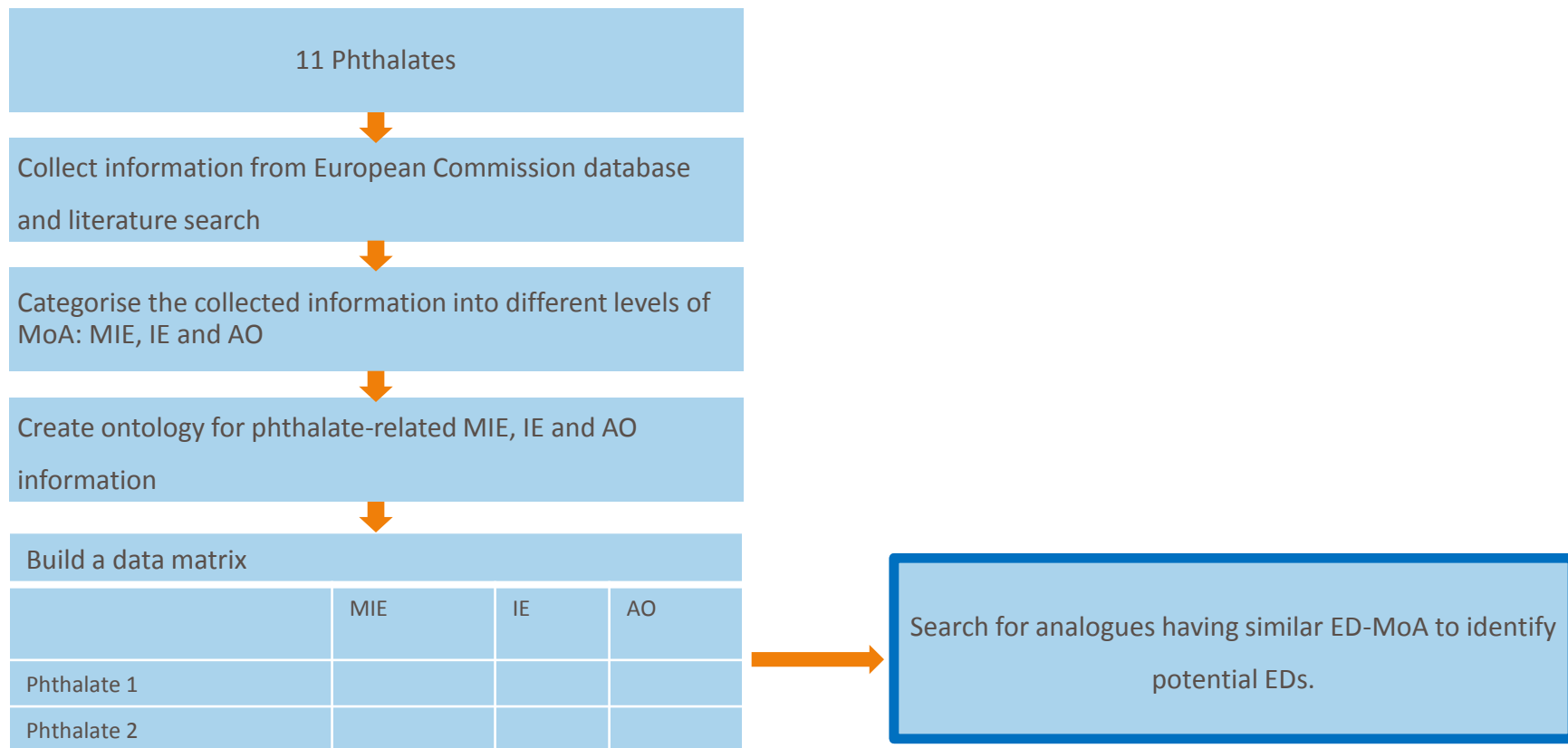
Phthalate case study: category formation

Members of phthalate class present in a database, developed by European Commission, containing potential ED chemicals.

11 Phthalate ortho-diester (side chain length C2-C10)	
Chemical Name	Side Chain Length (number carbon atoms)
Di ethyl phthalate	C2 / linear
Di propyl phthalate	C3 / linear
Di isobutyl phthalate	C4 / branched
Di pentyl phthalate	C5 / linear
Di hexyl phthalate	C6 / linear
Di cyclohexyl phthalate	C6 / branched (ring)
Butyl benzyl phthalate	C7 / branched and C4 / linear
Di octyl phthalate	C8 / linear
Di ethylhexyl phthalate	C8 / branched
Di isononyl phthalate	C9 / branched
Di isodecyl phthalate	C10 / branched



Phthalate case study: data collection



Data matrix displaying information at MIE, IE and AO levels

	Estrogenic activity	Activation/binding glucocorticoid receptor	Activation/binding PPARalpha	Activation/binding PPARbeta/delta	Activation/binding PPARgamma	Activation/binding AR	Activation/binding AhR	Activation/binding thyroid (T3/T4 agonist antagonist)	Activation/binding PR	Activation/binding PXR	antagonists of CB(1) receptors
Diethyl phthalate (DEP)	3					1					
Diisobutyl phthalate	1										
MEHP				1	2	3				1	
Di-n-pentylphthalate (DPP)	2										
BBP	7		1	1	1	3	3	1			1
Dicyclohexyl phthalate (DCHP)		1			1						
Dihexyl phthalate (DHP)	3					1					
DEHP	2		3	1	3	2	1	2		1	1
DnOP	1										1
Diisononyl phthalate (DINP)	3					1	1				
Diisodecyl phthalate (DIDP)	3					1	1				

	Sertoli cells	spermatocytes maturation process	Alteration of Leydig cells	Decreased testosterone	Decreased testicular zinc concentration	Degeneration of seminiferous tubules	Increased serum androgen binding protein	Down-regulation genes steroidogenesis	Histological alterations of reproductive organs
Diethyl phthalate (DEP)			1					1	1
DnOP									
Diisobutyl phthalate	1	2	2	4+1		1		3	
MEHP	8			7		2			1
Di-n-pentylphthalate (DPP)	1	2		3	1	2	1	3	
RRP		1	1	4		1			1
Dicyclohexyl phthalate (DCHP)	1	2	1	1		1		1	
Dihexyl phthalate (DHP)	3	2	2	2		1		1	
DEHP		2		10	1	2			1
DnOP					1				2
Diisononyl phthalate (DINP)	1	4	1			2		1	
Diisodecyl phthalate (DIDP)				2		2		1	

Adversity/Phthalate name	general toxicity/Maternal toxicity	Reproductive performance (refers to parental generation)	Offspring viability	Male reproductive tract abnormalities	Abnormalities of sperm parameters	External, visceral, skeletal malformation	Genitalia malformations	Change ano-genital distance (decreased/increased in male/female)	Nipple retention	Abnormalities of liver
Diethyl phthalate (DEP)		2				3		2		
DnOP	2	1	3	3	2		1	1		3
Diisobutyl phthalate	4			4		1	3	3	1	
MEHP	3		1	3	2	1	1	1		4
Di-n-pentylphthalate (DPP)	2	2	4		2			2	1	
Rutylbenzylphthalate (RRP)	10	2	17	3	3	11	4	6	2	5
Dicyclohexyl phthalate (DCHP)	1	1			1	2	1	3	1	4
Dihexyl phthalate (DHP)[6]			4		2	1	2	2	1	3
Di-(2-ethylhexyl)phthalate (DEHP)[6]		1	3	7	4	1	2	4	1	5
Di-n-propylphthalate(DnOP) [8]	1	1		3	3	2				5
Diisononyl phthalate (DINP) [8]		2			2	2			1	3
Diisodecyl phthalate (DIDP) [9]	2	1	2		1	2+1		1	1	8

Collapse all mechanistic data (MIE, IE, AO) in one data matrix

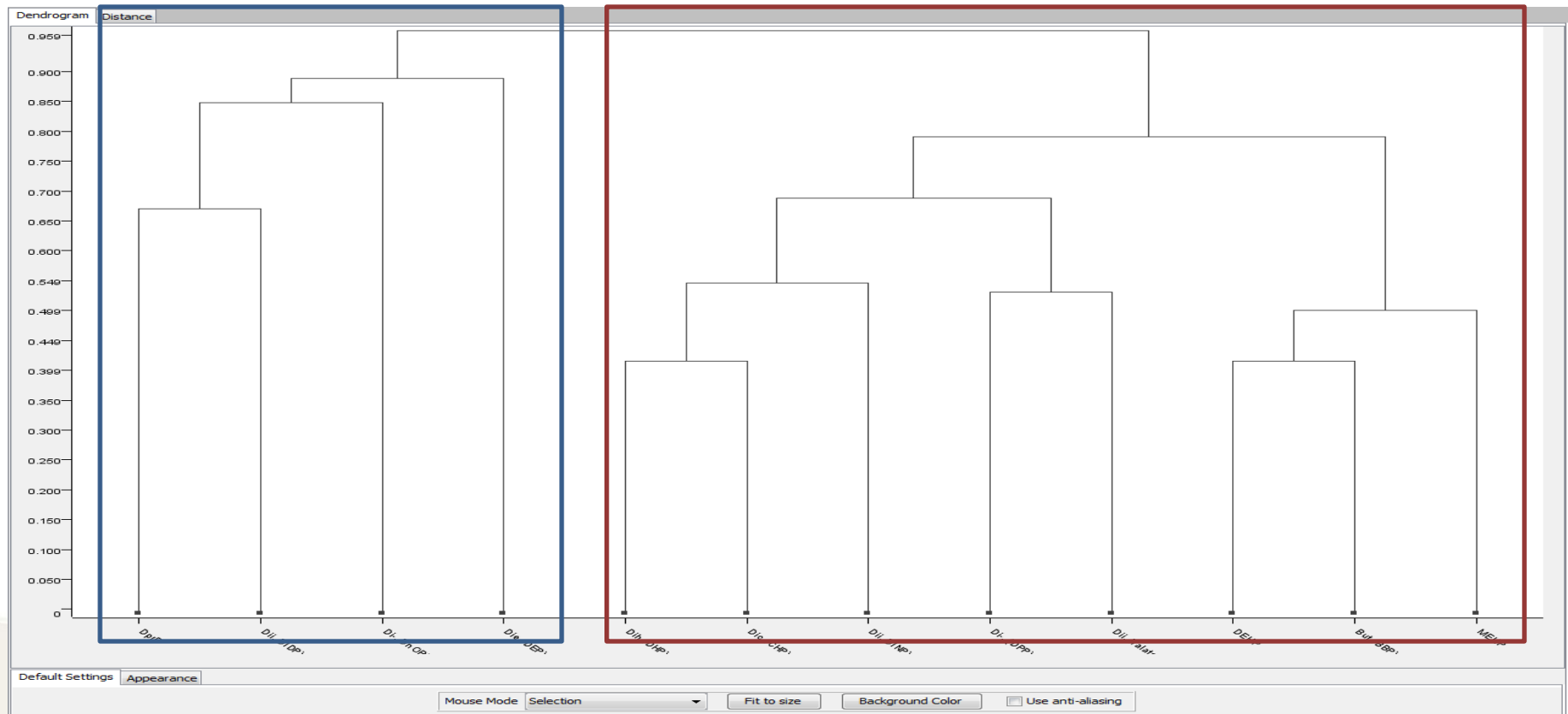
	Molecular Initiating Event							Intermediate Events									Adversity										
	ER	GR	PPARalpha	PPARbeta/delta	PPARgamma	AR	AhR	Sertoli	spermatocyte maturation	Leydig	change testosterone	Testicular zinc concentration	Seminiferous tubules	Androgen binding protein	steroidogenesis	reproductive organs	general toxicity/Maternal toxicity	Reproductive performance	Offspring viability	Male reproductive tract abnormalities	Abnormalities of sperm parameters	External, visceral, skeletal malformations	Genitalia malformations	Change anogenital distance	Nipple retention	Abnormalities of liver	Abnormalities of kidney
Diethyl phthalate (DEP)	0									1	1				0	0	1	0	0	0	/	0		0		1	0
Diisobutyl phthalate	1							1	1	1	1		1		1		0		/	1		1	1	1	1		
Di-n-pentylphthalate (DPP)	0							1	1		1	1	1	1	1		0	1	1	1	1			1	1	/	0
Dicyclohexyl phthalate (DCHP)	0	1			0			1	1	1	1		1		1		1	0	0	1	1	0	1	1	1	1	1
Dihexyl phthalate (DHP)	0					0		1	1	1	1		1		1		/		1	1	1	1	1	1	1	1	1
Diisononyl phthalate (DINP)	0					0	0	1	1	1	1		0		1		1	0	0	0	1	1		/	1	1	1
Diisodecyl phthalate (DIDP)	0					0	1				0		0		0		1	0	0	/	1	1		0	0	1	1
Di-n-propylphthalate (DnOP)	0											/				1	0	0	0	0	1	1				1	1
Bis(4-phenyl)phthalate (BBP)	1		1	1	1	1	1		1	1	1		1			1	1	1	1	1	1	1	1	1	1	1	1
DpP																	1	1	1	1	1	/	1	1		1	1
MEHP			/	1	1	1		1	1	1	1		1			1	1		1	1	1	1	1	1		1	
DEHP	/		1	1	1	/	1	1	1	1	1	1	1			1	1	1	1	1	1	1	1	1	1	1	1

- For each phthalate, a bit string (0,1 outcome) is constructed displaying all the data at MIE, IE and AO levels.
- By chemo-informatics, it is possible to search for phthalates being similar for their MoA: identify subgroups as more or less toxic.
- Furthermore it is possible to compute which key events are the best descriptors to predict the apical endpoints

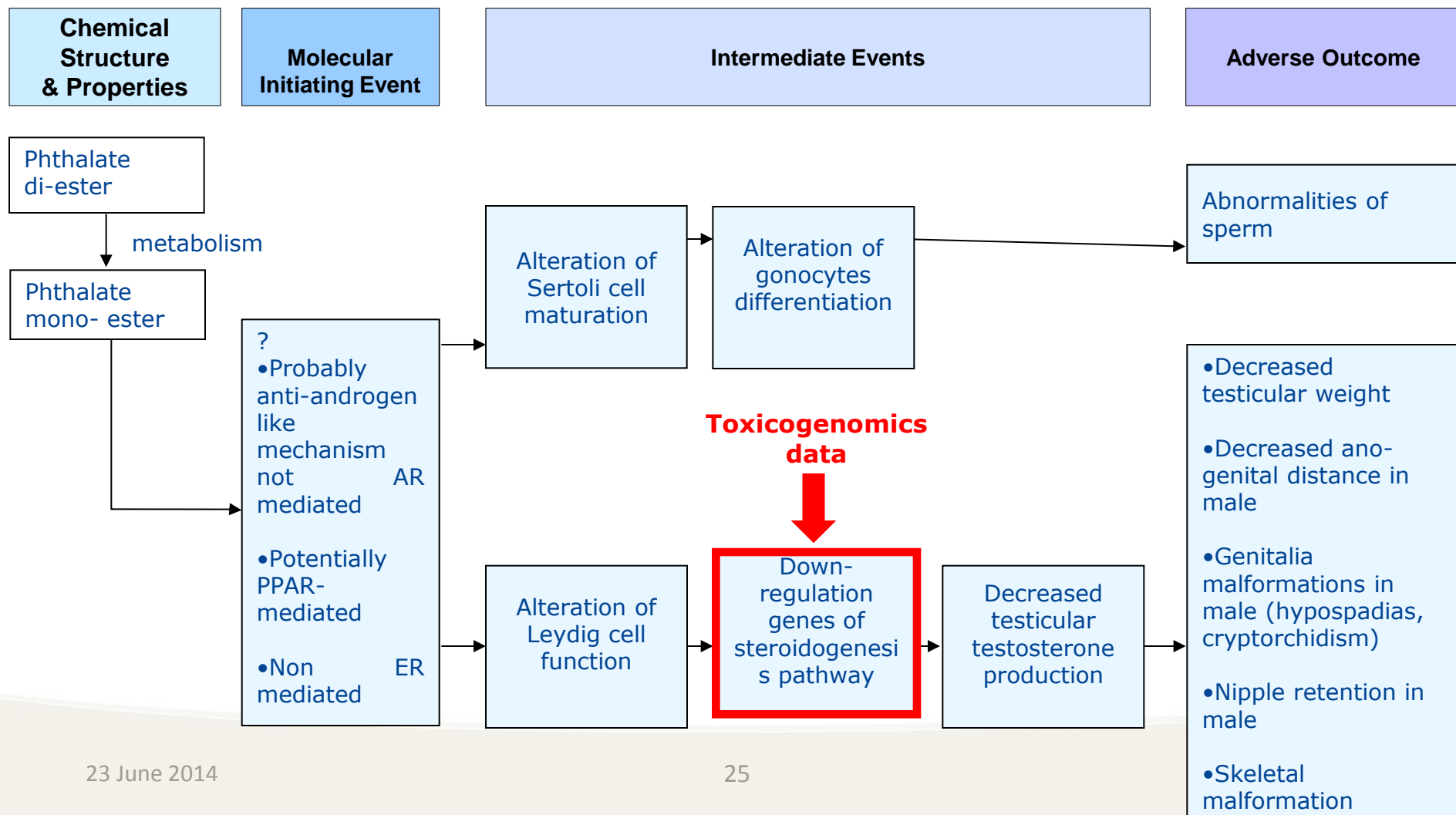
Phthalates sub-groups

Less toxic

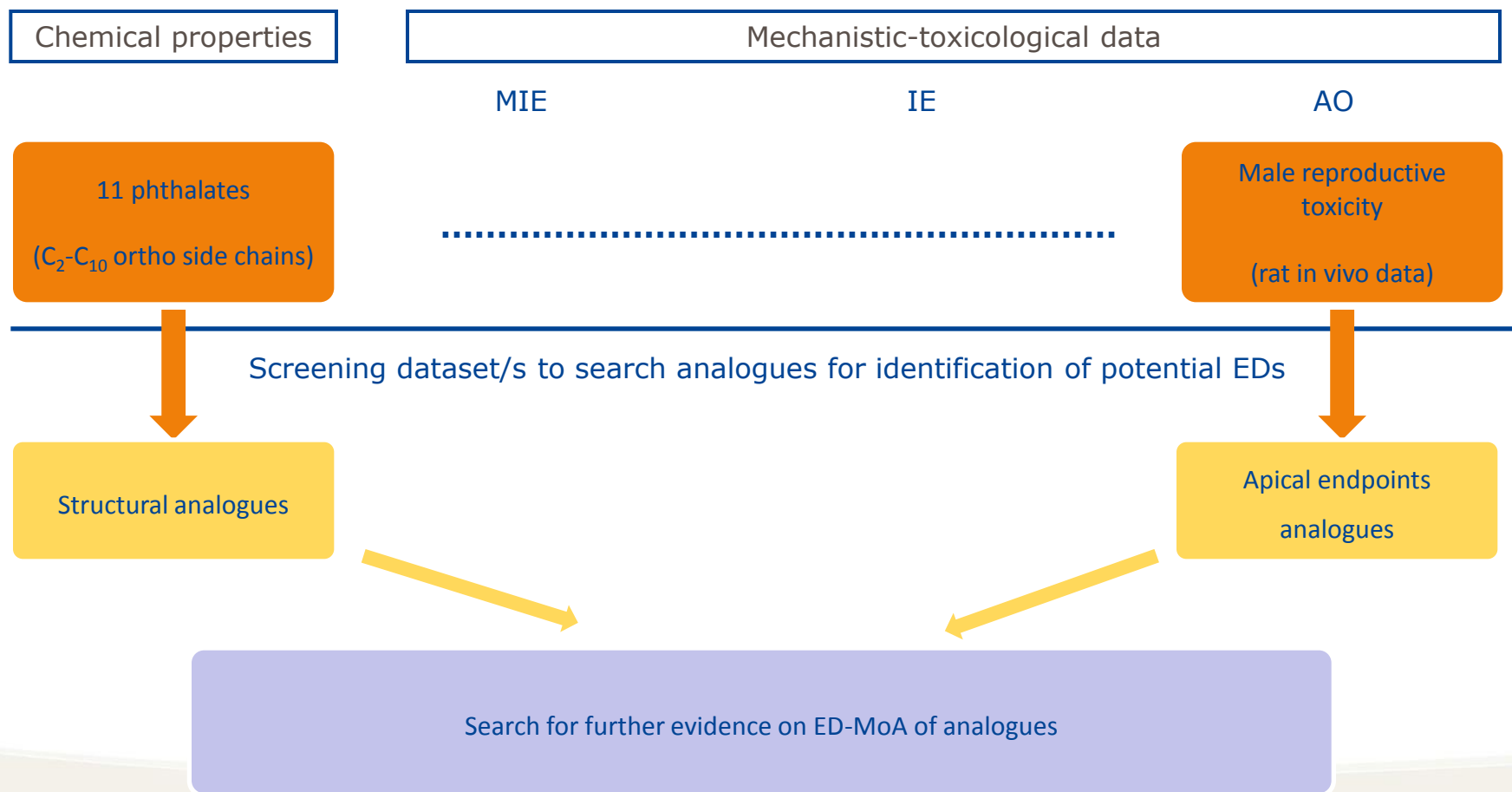
More toxic



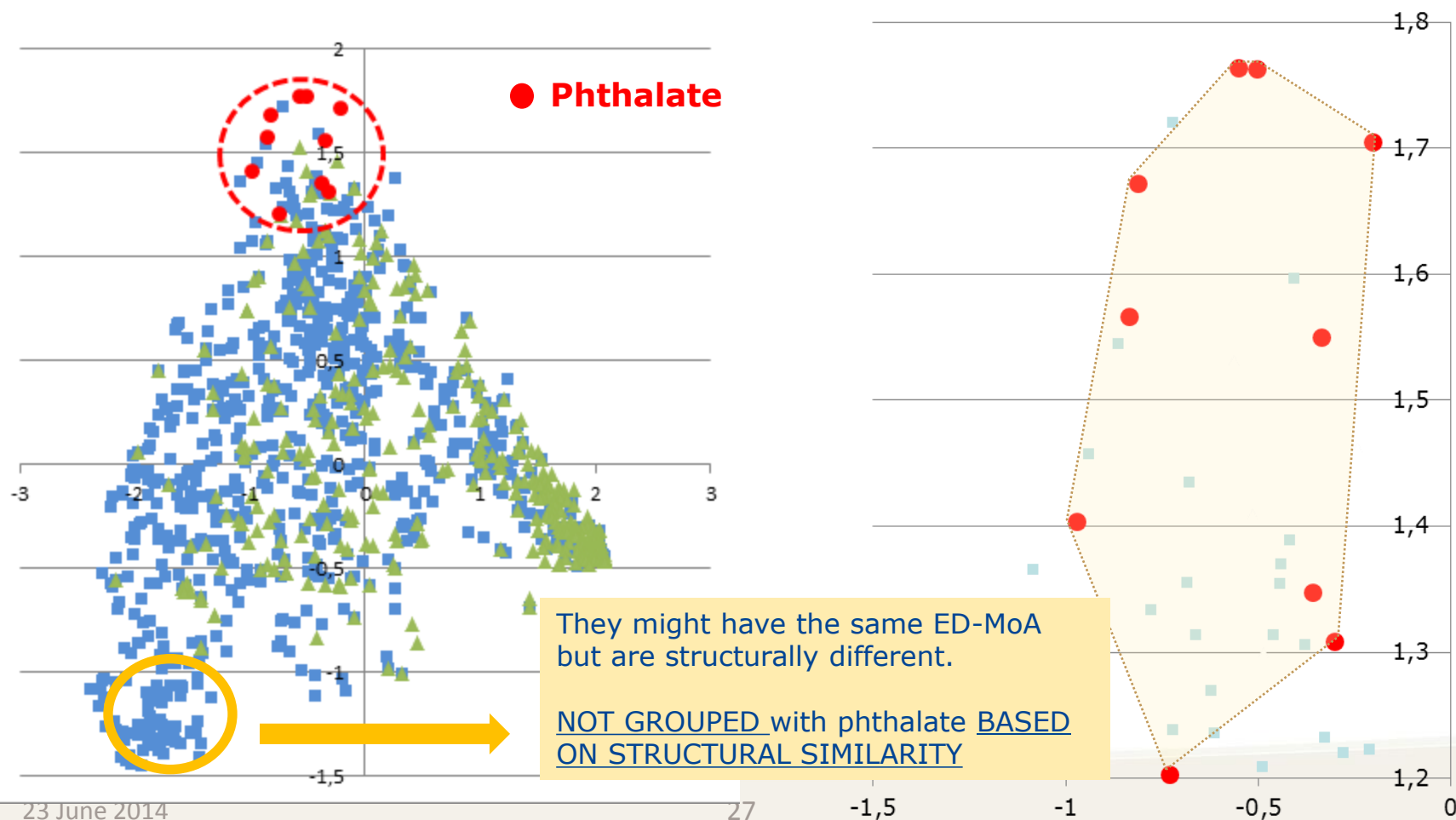
Prototype MoA for phthalate related toxicity



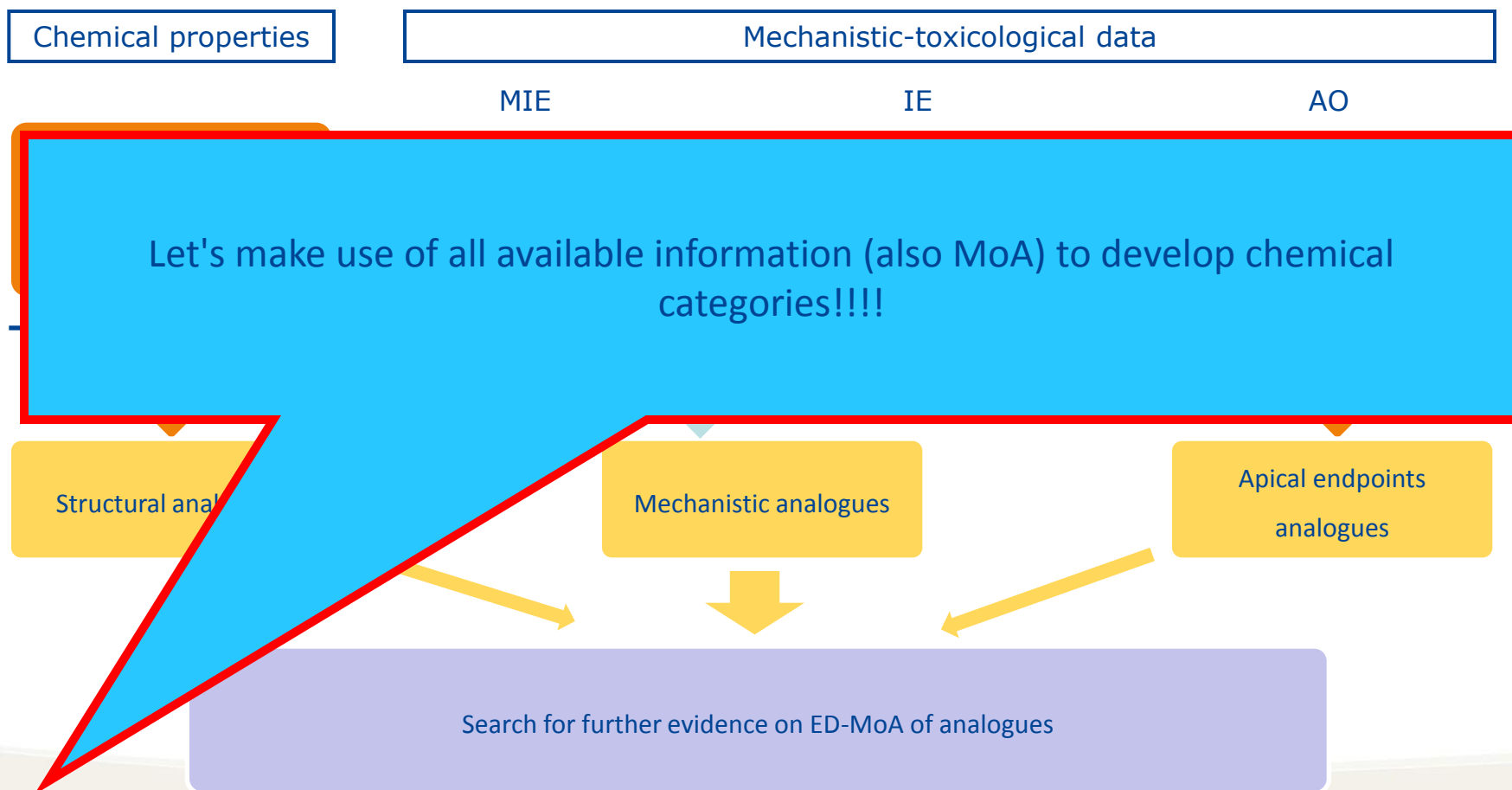
Searching for analogues: structural-based



Searching structural analogues



Searching for analogues: MoA-based



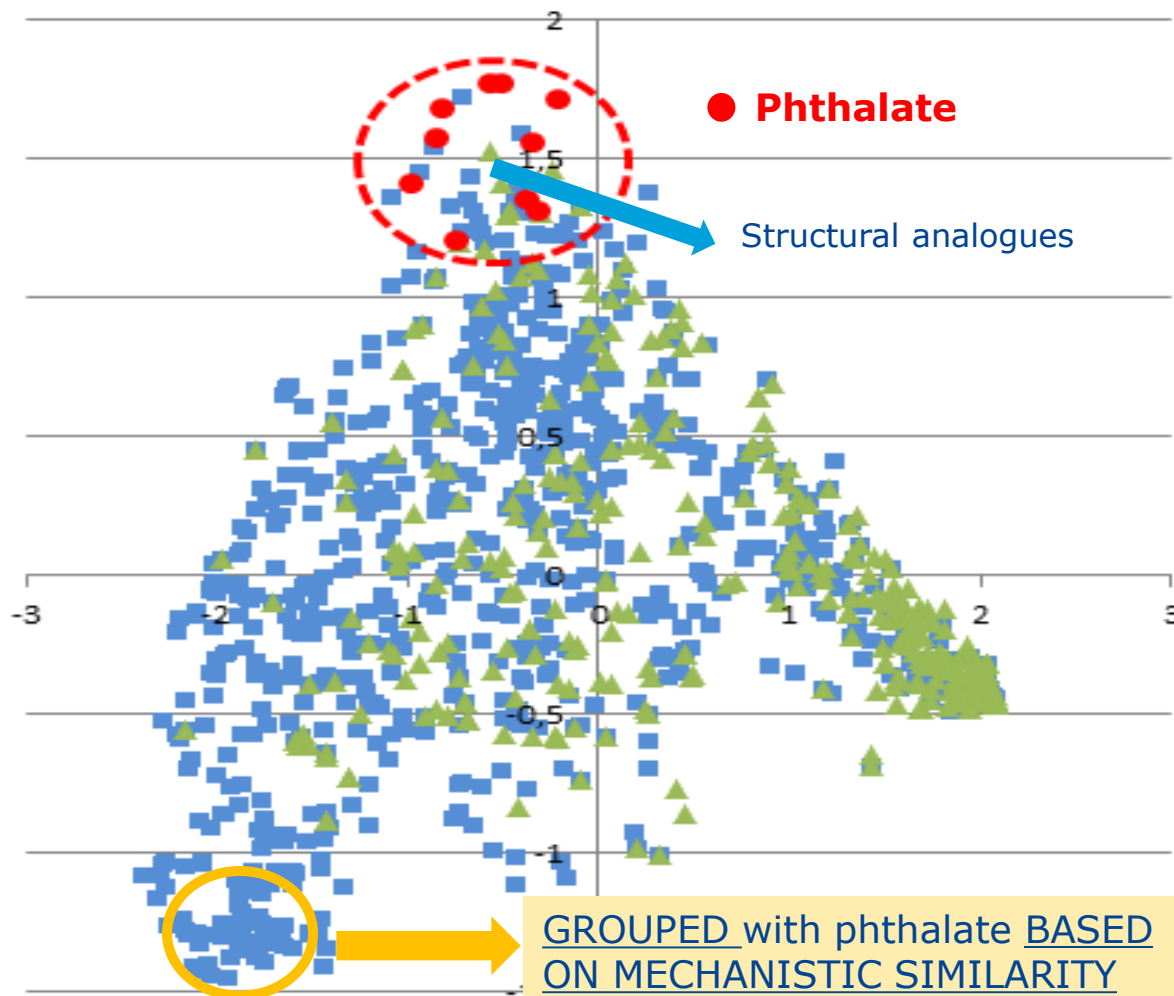
Searching mechanistic analogues

Intermediate
Events of phthalate
MoA

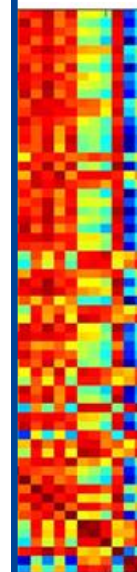
Toxicogenomics (TG)
profile datasets for
analogues.

There are available d

23 June 2014



profile



Conclusion

- Grouping and read-across is already widely used to fill data gaps
- Trend from structure-based to MoA-based chemical categories
- Use all available information (MoA) to develop alternative methods

Practical example 2

The System Toxicology Unit case study for the SEURAT-1 project:

"Mode of Action-based classification model for repeated dose liver toxicity"

Prediction Goal

Develop a classification model to correctly discriminate between hepatotoxic and non-hepatotoxic chemicals.

Hepatotoxicity mainly related to: Cholestasis, Fibrosis and Steatosis (MoAs available within SEURAT-1)

CRITERIA for SUCCESS

Main goal:

Distinguish between hepatotoxicants and non-hepatotoxicants (binar outcome: yes/no toxic)

Added value:

Predict also if a chemical causes hepatotoxicity through cholestasis/fibrosis /steatosis.

Application:

support the SEURAT-1 3rd level proof of concept

1. Screening and Prioritisation:

- Hazard profiling of large datasets and priority setting

2. Preliminary Risk Assessment:

- Define Thresholds of Toxicological Concern (TTC) of chemicals predicted as positive and negative

Mechanistic basis

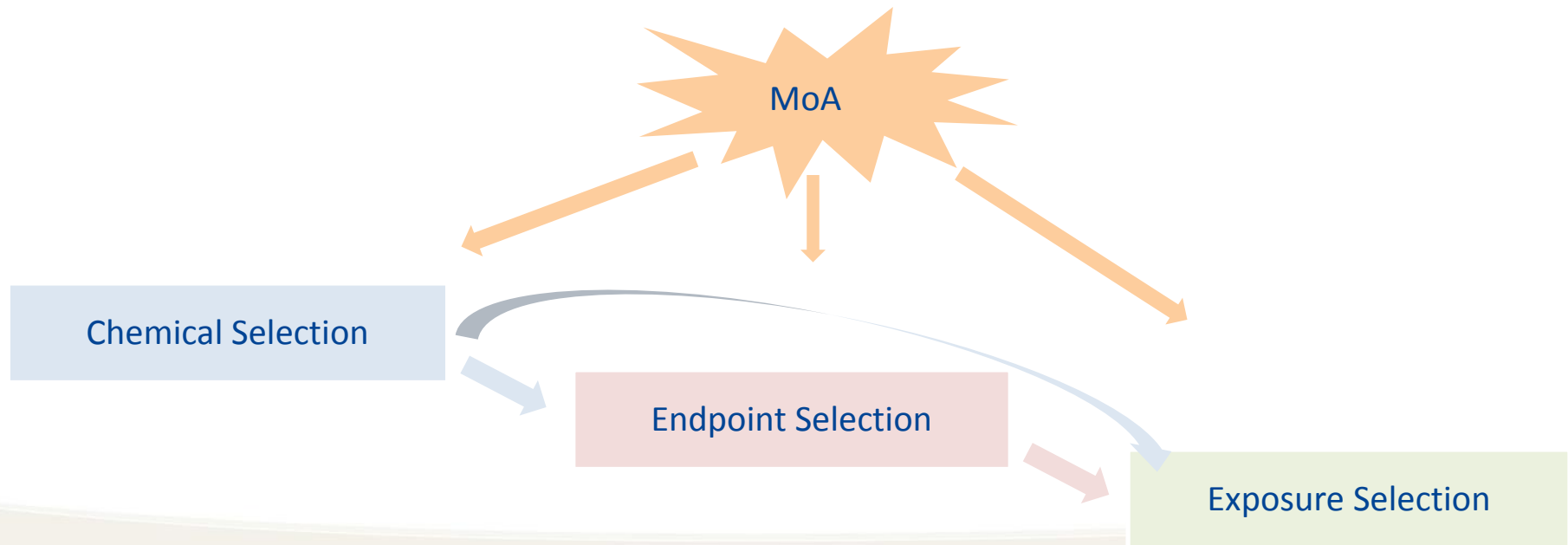
We focused on hepatotoxicity prediction because:

- Good mechanistic understanding of MoAs for cholestasis, fibrosis and steatosis
- Previous experiments performed in JRC using HepaRG

} Selection of endpoints
and
Define experimental setup

Study design

- 92 chemicals (75% hepatotoxic: 25% non-hepatotoxic) incubated with HepaRG cells under repeated dose exposure
- High Throughput Screening platform used to test reference chemicals at 16 concentrations each in triplicate
- Read-out of *in vitro* endpoints by High Content Screening (automated imaging)



Challenges of the project

- Test 92 chemicals using knowledge of 3 MoAs to design a test system using 1 *in vitro* model: many parameters to be considered
- Define repeated dose exposure scenarios relevant to the selected MoAs (and not just based on optimal conditions for cell growth)
- Consider always how to practically translate the project proposal into the testing phase (put the project in the context of our laboratory facility)

Project Status

Chemical Selection

COMPLETED (maybe minor changes to replace some chemicals because solubility, better quality in vivo data, etc.)

Endpoint Selection

COMPLETED (the list of selected endpoints can be extended and improved)

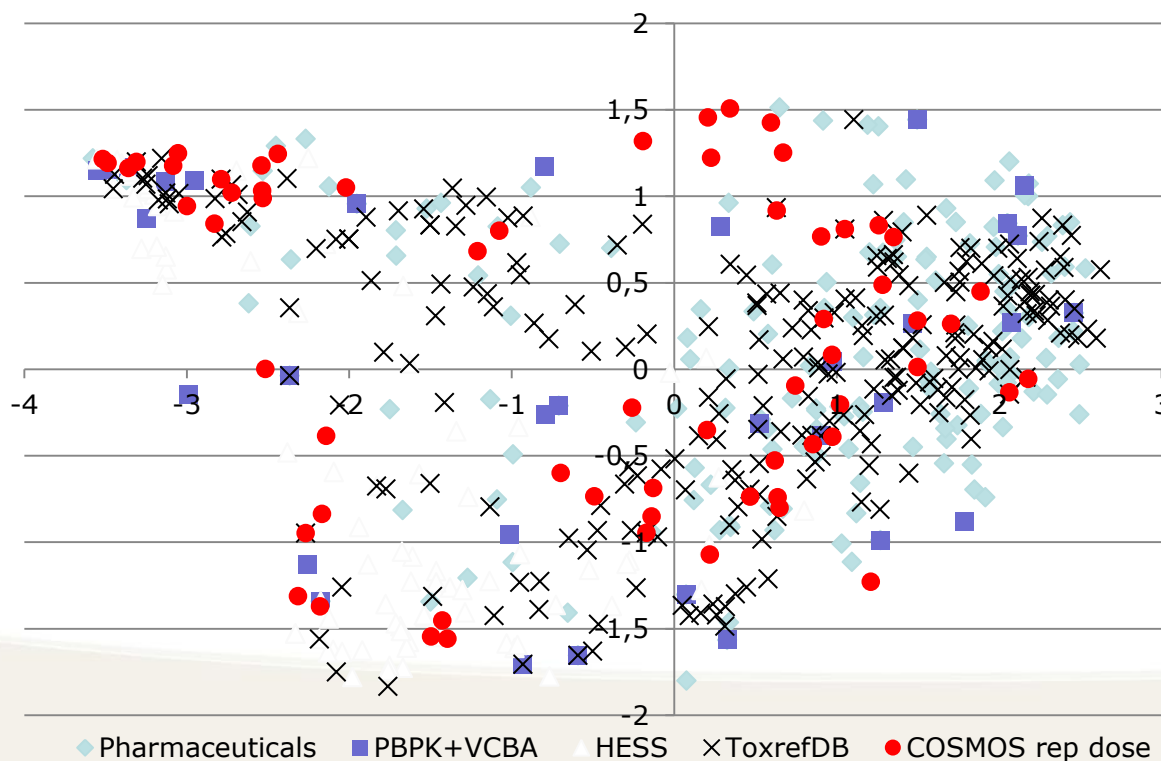
Exposure Selection

COMPLETED

Chemical Selection

Sources used:

1. Literature search (pharmaceuticals)
2. COSMOS database
3. Hazard Evaluation Support System (HESS) database
4. ToxRef database
5. JRC-IHCP's data warehouse
6. SEURAT-1 Gold Compounds



Criteria used for selection:

- Select ± 100 chemicals
- Cover cosmetic structural space
- High reliability on hepatotoxicity
- High structural diversity
- Overlap with other SEURAT-1 projects
- Include toxicogenomics data

Endpoint Selection

Biological feature/effect	Parameter/marker
Cell loss / DNA degradation / Nuclear size	Cell number
	Cell morphology
Oxidative stress	Reactive oxygen species (ROS)
Mitochondrial damage	Mitochondrial membrane potential
Genotoxicity	Phosphorylation of Histone H2A.X
Apoptosis (in-/extrinsic pathway)	Caspase 3 activation
Accumulation of neutral lipids	Steatosis

Reasoning process

Balance between MoA-relevance and technical considerations

Priority given:

- MoA-based
- Observable in HepaRG (limitation for fibrosis)
- Already implemented in the in house HTS platform and compatible with high content screening (imaging)

Exposure Selection

Rational

Define exposure protocol where chemical concentration, to which HepaRGs are exposed, is kept relatively constant over time within a certain range (continuous exposure)

Why?

- Continuous exposure ensures that the observed changes of *in vitro* effects are due specifically to pharmacodynamics and not to pharmacokinetics
(e.g. rate of metabolism of a parent compound, which by defining the compound bioavailability/concentration influences triggering *in vitro* effects).
- To capture both toxic chemicals that induce toxicity through a maximum concentration- (C_{\max}) or Area under the Curve- (AUC) effect or through a combination of both.

Protocol

Expose HepaRGs every 6 h to chemicals (frequency based on metabolic clearance knowledge)

Results

Tested Chemical	<i>In vivo</i> liver toxicity	<i>In vitro</i> endpoint		
		E_1	E_2	E_n
Chemical 1	Yes
Chemical 2	No
Chemical 3	Yes
Chemical n	Yes

Classification model (minimise false-negative rate):

- Built using the *in vitro* endpoint read-outs
- Include molecular descriptors (COSMOS chemotypes)
- Performance = *in vitro* prediction vs *in vivo* data

Acknowledgments

All System Toxicology Unit

Particularlry:

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Elisabeth Joossens

Novak Jaroslav

Nepelska Malgorzata

Horvat Tomislav

Julien Burton

Towards the Replacement of in vivo Repeated Dose Systemic Toxicity Testing

Thank you for your attention