ReSolve
SAFE AND EFFECTIVE BIO-BASED SOLVENTS

Solvent modelling and selection

FERGAL BYRNE

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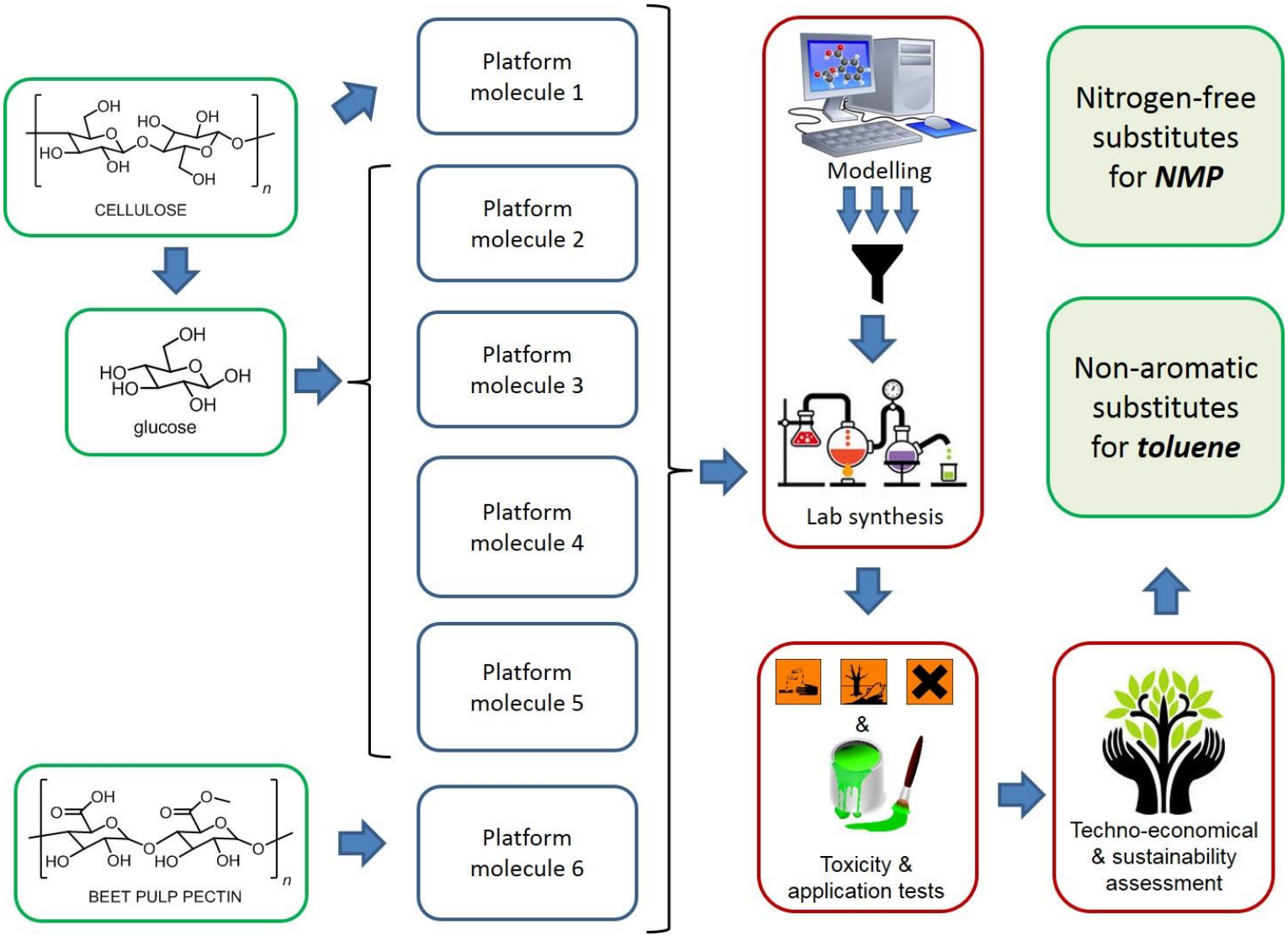
BBI JU

Bio-based Industries Consortium

Horizon 2020
European Union Funding
for Research & Innovation

This project has received funding from the Bio Based Industries Joint Undertaking under the European Union‘s Horizon2020 research and innovation programme under agreement No 745450

The concept


CELLULOSE
glucose
BEET PULP PECTIN
Platform molecule 1
Platform molecule 2
Platform molecule 3
Platform molecule 4
Platform molecule 5
Platform molecule 6
Modelling
Lab synthesis
&
Toxicity & application tests
Techno-economical & sustainability assessment
Nitrogen-free substitutes for NMP
Non-aromatic substitutes for toluene

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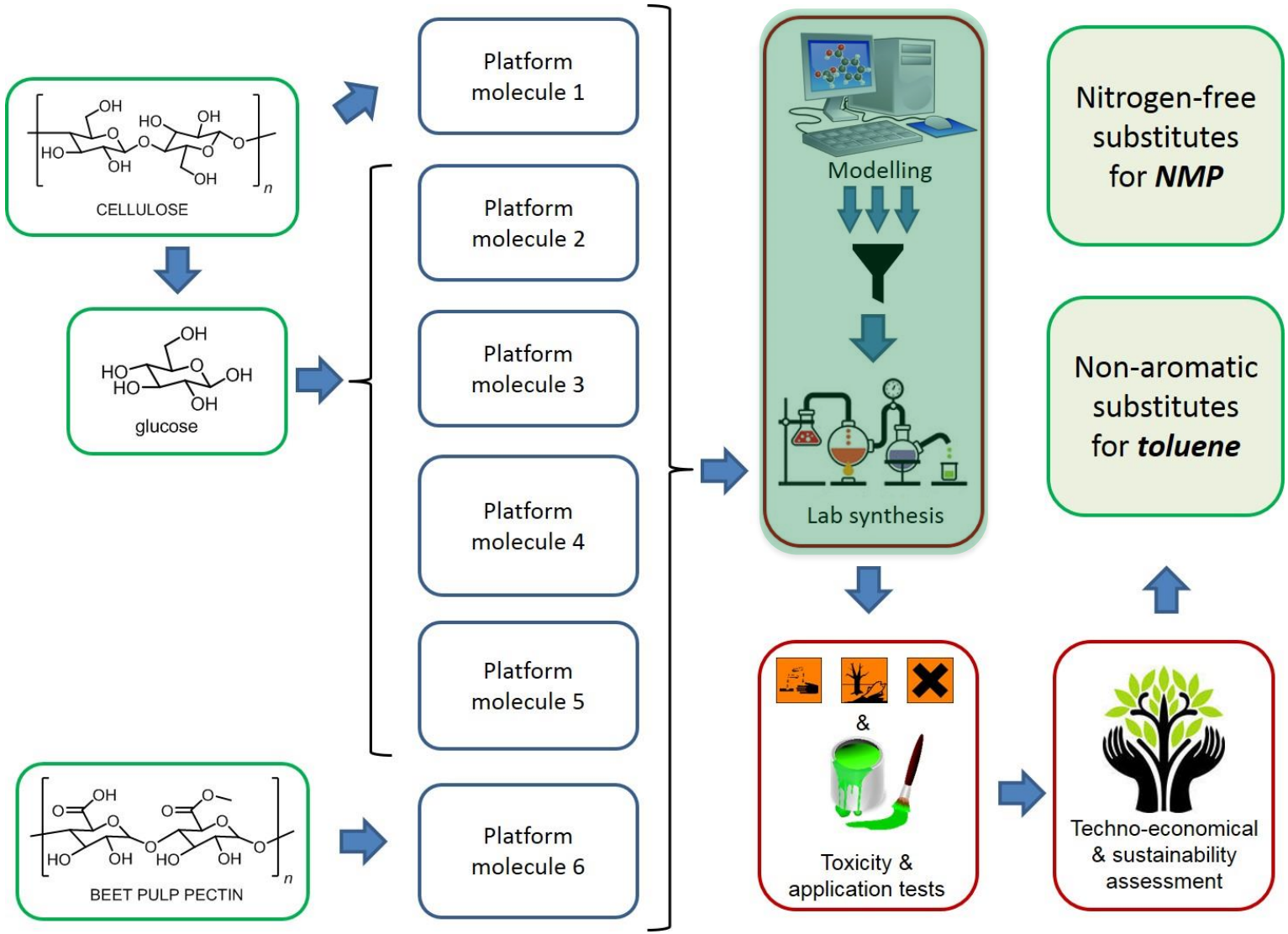
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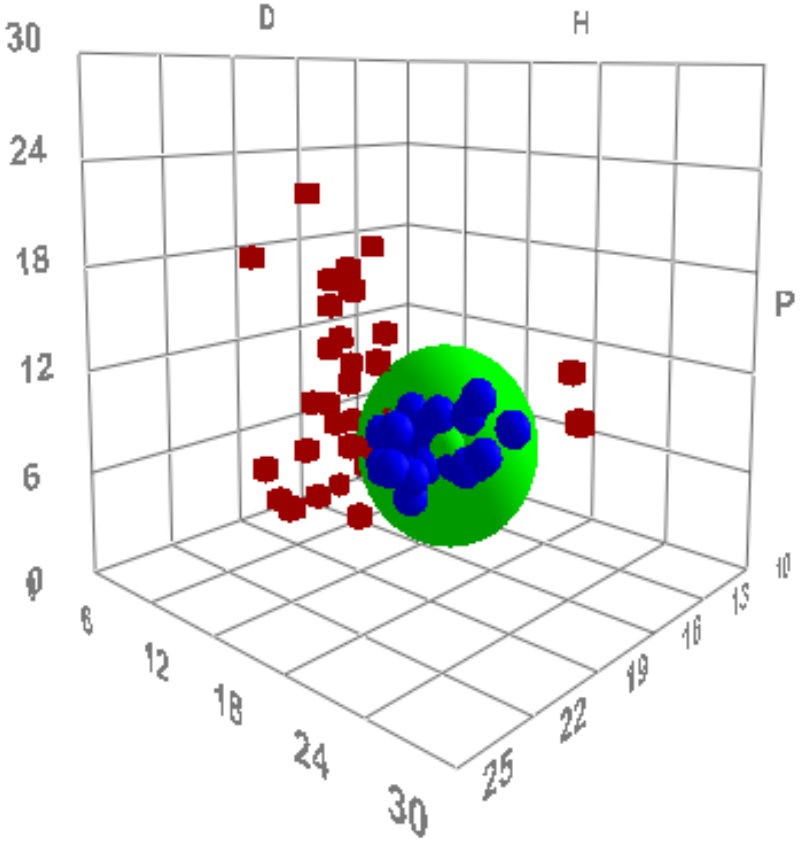
Designing New solvents

Solubility:



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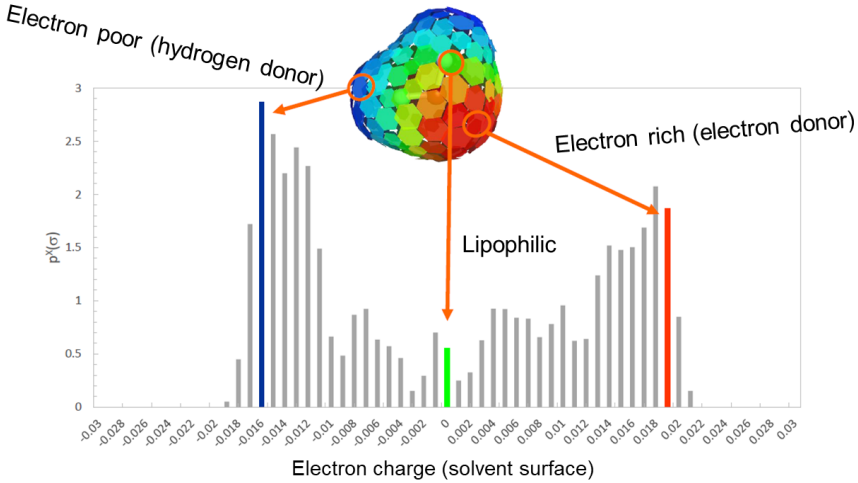
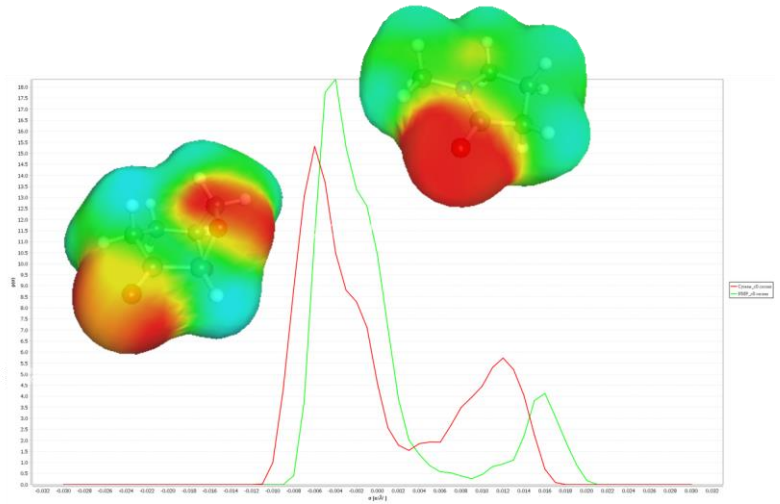
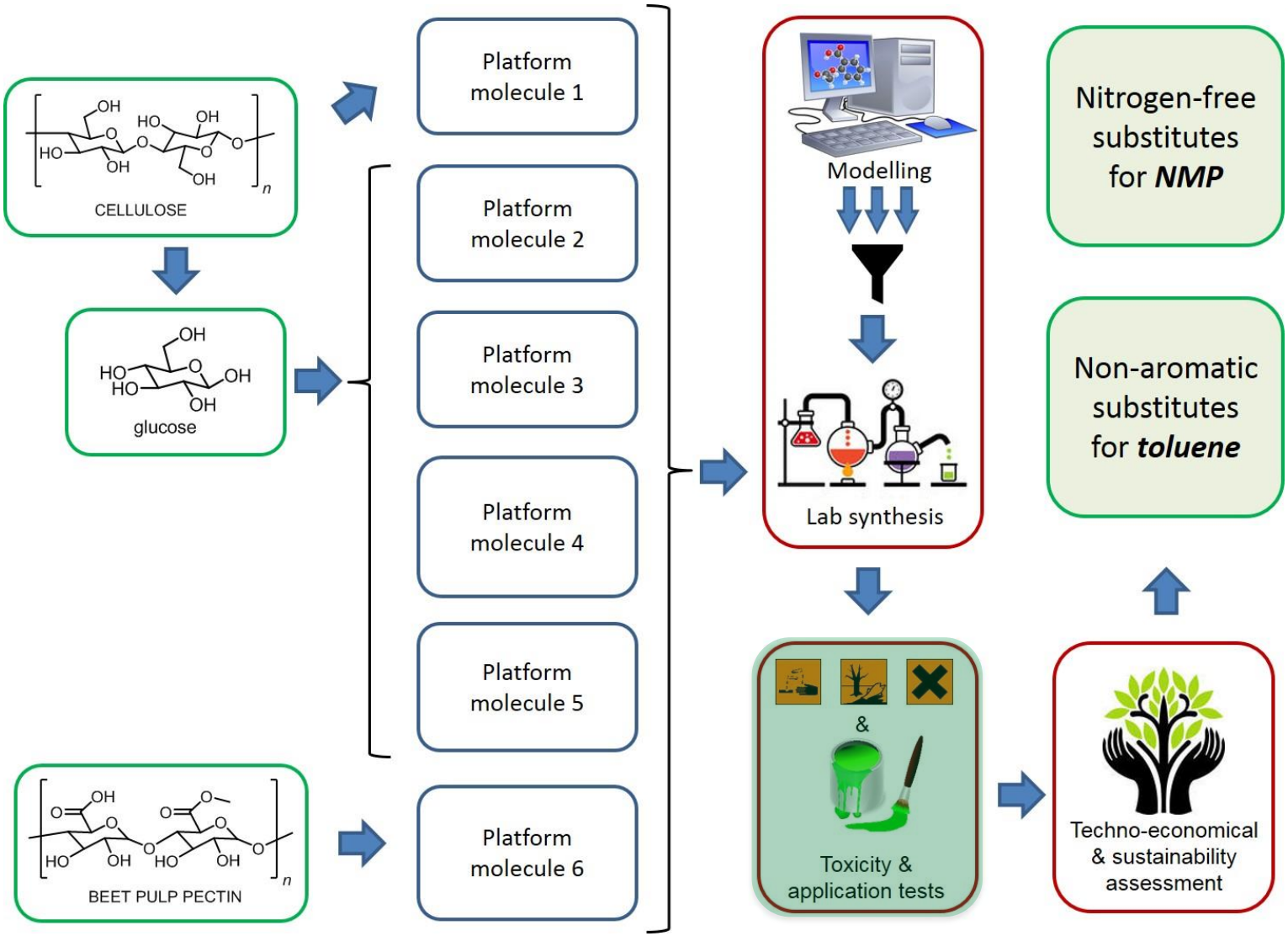




Courtesy of HSPiP
and COSMOlogic

The concept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ReSolve
SAFE AND EFFECTIVE BIO-BASED SOLVENTS

An integrated testing strategy to evaluate toxicological safety issues of candidate solvents

BARBARA VAN VUGT-LUSSENBURG

BioDetection Systems bv

BBI JU

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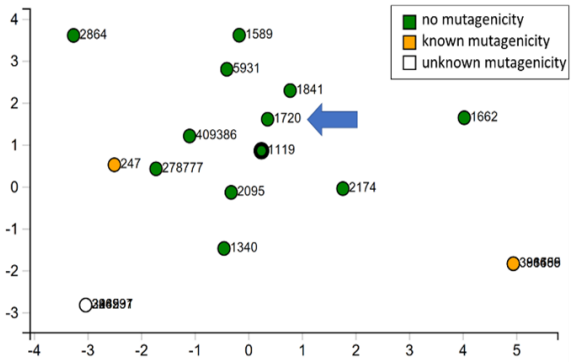
Advantages of early tox testing

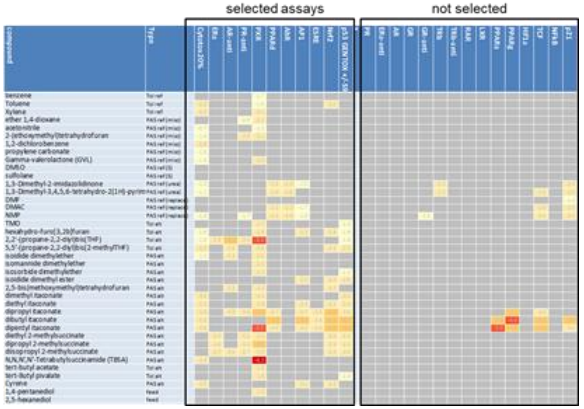
- It is vital for the project that final candidates have no/low toxicity
- ‘Green’ or ‘BioBased’ is no guarantee for low toxicity
- Early tox screening enables:
  - removal of toxic candidates from the workflow in an early stage
  - a feedback loop with structure-activity information to design / synthesis WP’s
- Overall: an integrated process combining the evaluation of:
  - ease of synthesis
  - functional performance
  - safety

Innovative testing strategy

in silico evaluation | reference data | *in vitro* evaluation



Integration & read-across



Compound information	Info obtained from BDS	Genotoxicity (mutagenicity)			Non-genotoxic carcinogenicity				Reproductive toxicity	
Substance name (ChemIDplus/Google/Chem	Alternative for	DTU oui	Toxtree	GHS	Rax	DTU oui	Toxtree	GHS	Rax	DTU terati	GHS
Toluene	NA		pos/neg/neg				neg				2
DOBO	Toluene		neg/neg/pos				neg				
Furan, 2,2'-(1-methylethylidene)bis(tetrahydrof	Toluene		neg/neg/pos				neg				
Furan, 2,2'-(1-methylethylidene)bis(5-methyltet	Toluene		neg/neg/pos		J - H341		neg		J - H351		
Furan, 2,2'-(ethylidene)bis(tetrahydrofuran)	Toluene		neg/neg/pos				neg				
Furan, 2,2'-(ethylidene)bis(5-methyltetrahydrof	Toluene		neg/neg/pos				neg				
Furan, 2,2'-(methylidene)bis(tetrahydrofuran)	Toluene		neg/neg/pos				neg				
Furan, 2,2'-(methylidene)bis(5-methyltetrahydr	Toluene		neg/neg/pos				neg				
2,2,3,5,- Tetramethylcyclopentanone	Toluene		neg/neg/neg				neg				
methyl n-butyrate	Toluene		neg/neg/neg				neg				
ethyl isobutyrate	Toluene		neg/neg/neg				neg				
methyl pivalate	Toluene		neg/neg/neg			?	neg				
pinacolone	Toluene		neg/neg/neg				neg				
2,5-dimethyl-2,4-hexadiene	Toluene; precursor TMO, contamination of AK1		neg/neg/neg		H - H341, J - H341		neg		H350, - J - H351, / - H350		
2,2,5,5-tetramethyloxolane (TMO)	Toluene		neg/pos/neg				neg				
tert-Butyl pivalate	Toluene		neg/neg/neg				neg				
Eucalyptol	Toluene alt; and TMO read across	?	neg/neg/neg				pos				
Terpineol	TMO read-across		neg/neg/neg				neg				

RAG status:

R = serious toxicity, discontinue

A = some toxicity, consider

G = limited toxicity, continue

Overview *in silico* assessment

- Three parts:
  - collection and evaluation of existing experimental toxicity data
  - toxicity predictions using QSAR models
  - read across (RAX) predictions

- prioritised human health endpoints:
  - carcinogenicity (C)
  - mutagenicity (M)
  - reproductive toxicity (R)
  - skin sensitisation (S)

} prioritised in regulation (REACH)

- Colour codes per endpoint:

Colour	Prediction
	Non toxic
	Low toxic
	Medium toxic
	Toxic
	Inconclusive

1. Existing experimental data

- Extract information from REACH registration dossiers
- Check for classification in the GHS system*

toxicity:	no	medium	high
based on experimental data	(EXP)	(EXP)	(EXP)
GHS classification exists		(GHS)	(GHS)

- Applicable for ‘existing’ chemicals only

*GHS: Globally Harmonized System of Classification and Labelling of Chemicals

2. QSAR-based predictions

In silico-tools to predict toxicity of compounds
Based on structure-activity relationships (SARs) or structural alerts

evaluated tools/models

Toxicity Endpoint	Tool/Model	Test data	Sens.	Spec.
Ames test	DTU/Case Ultra	Internal	0.86	0.86
Ames test	ToxTree	Ames data ISS	0.88	0.71
Ames test	VEGA/cons.	Ames data ISS	0.96	0.93
Carcinogenicity	VEGA/Antares*	Carc data ISS	0.85	0.92
Carcinogenicity	VEGA/ISSCAN-CGX*	Carc data ISS	0.99	0.96
Carcinogenicity	VEGA/CAESAR*	Carc data ISS	0.81	0.89
Carcinogenicity	VEGA/ISS*	Carc data ISS	1.00	1.00
Carcinogenicity	VEGA/TNOcons.*	Carc data ISS	1.00	0.99
Carcinogenicity	VEGA/TNOcons.	Carc data ISS	0.67	0.65
Carcinogenicity	DTU/CaseUltra-rodent	Internal	0.51	0.88
Carcinogenicity	DTU/Leadscope-rodent	Internal	0.70	0.76
TK *in vitro*	DTU/CaseUltra	Internal	0.69	0.92
TK *in vitro*	DTU/Leadscope	Internal	0.85	0.84
TK *in vitro*	DTU/SciQSAR	Internal	0.79	0.81
HGPRT *in vitro*	DTU/CaseUltra	Internal	0.75	0.85
HGPRT *in vitro*	DTU/Leadscope	Internal	0.82	0.78
HGPRT *in vitro*	DTU/SciQSAR	Internal	0.80	0.73
MN *in vivo*	ToxTree*	*In vivo* MN ISS	0.70	0.30
MN *in vivo*	DTU/CaseUltra	Internal	0.50	0.84
MN *in vivo*	DTU/Leadscope-rodent	Internal	0.64	0.78
MN *in vivo*	DTU/SciQSAR	Internal	0.60	0.90
CA *in vitro* / CHO	DTU/CaseUltra	Internal	0.40	0.95
CA *in vitro* / CHO	DTU/Leadscope	Internal	0.54	0.79
CA *in vitro* / CHO	DTU/SciQSAR	Internal	0.51	0.84
CA *in vitro* / CHL	DTU/CaseUltra	Internal	0.60	0.88
CA *in vitro* / CHL	DTU/Leadscope	Internal	0.75	0.75
CA *in vitro* / CHL	DTU/SciQSAR	Internal	0.73	0.73
DART	VEGA/Caesar	Wu, 2013	0.81	0.32
DART	VEGA/P&G	Wu, 2013	0.85	0.44
Teratogenicity	DTU/CaseUltra hum terato	Internal	0.65	0.85
Teratogenicity	DTU/Leadscope hum terato	Internal	0.72	0.86
Teratogenicity	DTU/SciQSAR hum terato	Internal	0.65	0.93
Skin sensitisation	ToxTree/Reaction Domain	Kleinstreuer,2018	0.76	0.70
Skin sensitisation	ToxTree/Protein Binding	Kleinstreuer,2018	0.90	0.24
Skin sensitisation	VEGA/CAESAR*	Kleinstreuer,2018	0.91	0.73
Skin sensitisation	DTU/CaseUltra	Internal	0.70	0.97
Skin sensitisation	DTU/Leadscope	Internal	0.75	0.96
Skin sensitisation	DTU/SciQSAR	Internal	0.62	0.97

selected tools/models

Health endpoint	Tool	Model
Carcinogenicity	DTU	Leadscope rodent
	VEGA	Carcinogenicity model (ISS) (version 1.0.2) Carcinogenicity model (IRFMN/ISSCAN-CGX) (version 1.0.0) Carcinogenicity model (IRFMN/Antares) (version 1.0.0) Carcinogenicity model (CAESAR) (version 2.1.9)
Mutagenicity	DTU	**Point mutations** Mutations in thymidine kinase (TK) locus in mouse lymphoma cells Mutations in HGPRT locus in Chinese hamster ovary (CHO) cells **Chromosome aberrations** Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Chromosome Aberrations in Chinese Hamster Lung (CHL) Cells
	VEGA	**Point mutations** Mutagenicity (Ames test) CONSENSUS model (version 1.0.2). Mutagenicity (Ames test) Consensus model, based on the predictions of the available VEGA mutagenicity models (Caesar, SarPy, ISS and KNN).
Reproductive toxicity	DTU	Teratogenic potential. Battery outcome i.e. combined CASE Ultra, Leadscope and SciQSAR
	VEGA	Developmental/Reproductive Toxicity library (PG) 1.0.0 Developmental Toxicity model (CAESAR) - v. 2.1.7
Skin sensitisation	DTU	Allergic contact dermatitis in guinee pig and human. Battery outcome i.e. combined CASE Ultra, Leadscope and SciQSAR.
	VEGA	Skin Sensitization model (CAESAR) 2.1.6
	Toxtree	Skin sensitization reactive domains

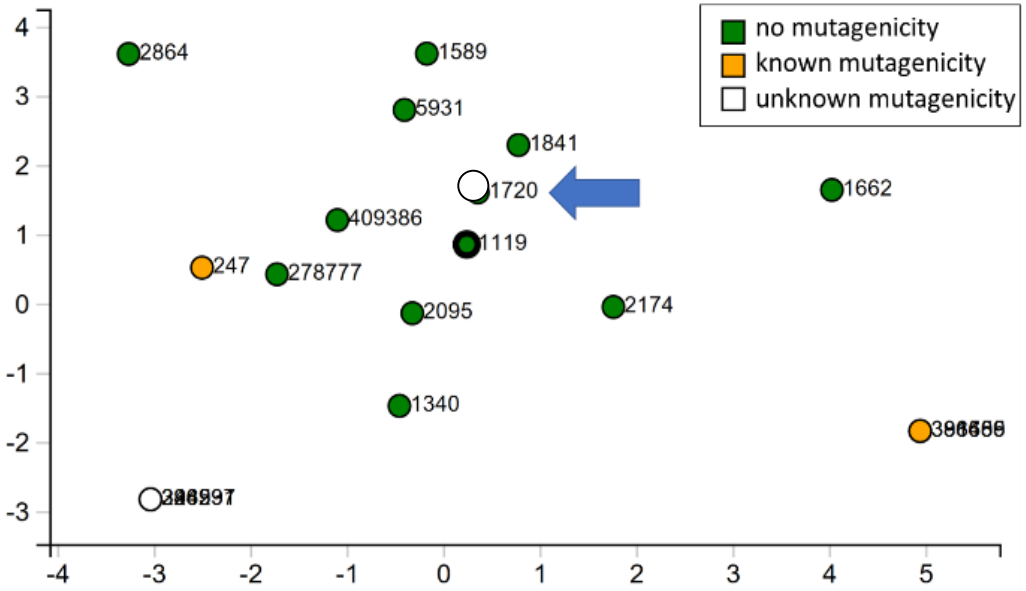
QSAR output

Colour	Prediction
	Non toxic
	Low toxic
	Medium toxic
	Toxic
	Inconclusive

3. Read-across (RAX)

DIAMONDS toolbox (TNO)

1. Identify structural similars of the candidate compound
2. Retrieve toxicity data of similars from various databases
3. Predict toxicity of candidate compound based on similars



ToxSpace shows toxicological properties of chemically most similar substances of target substance ID1720

Outcome *in silico* data

Integrate data (REACH, QSAR, RAX)

* If available, *in vivo* data (EXP) 'overrules' all other data
* Output: colour-coded 'barcode' with predictions on four endpoints
* Information on source data included (EXP, GHS, RAX)

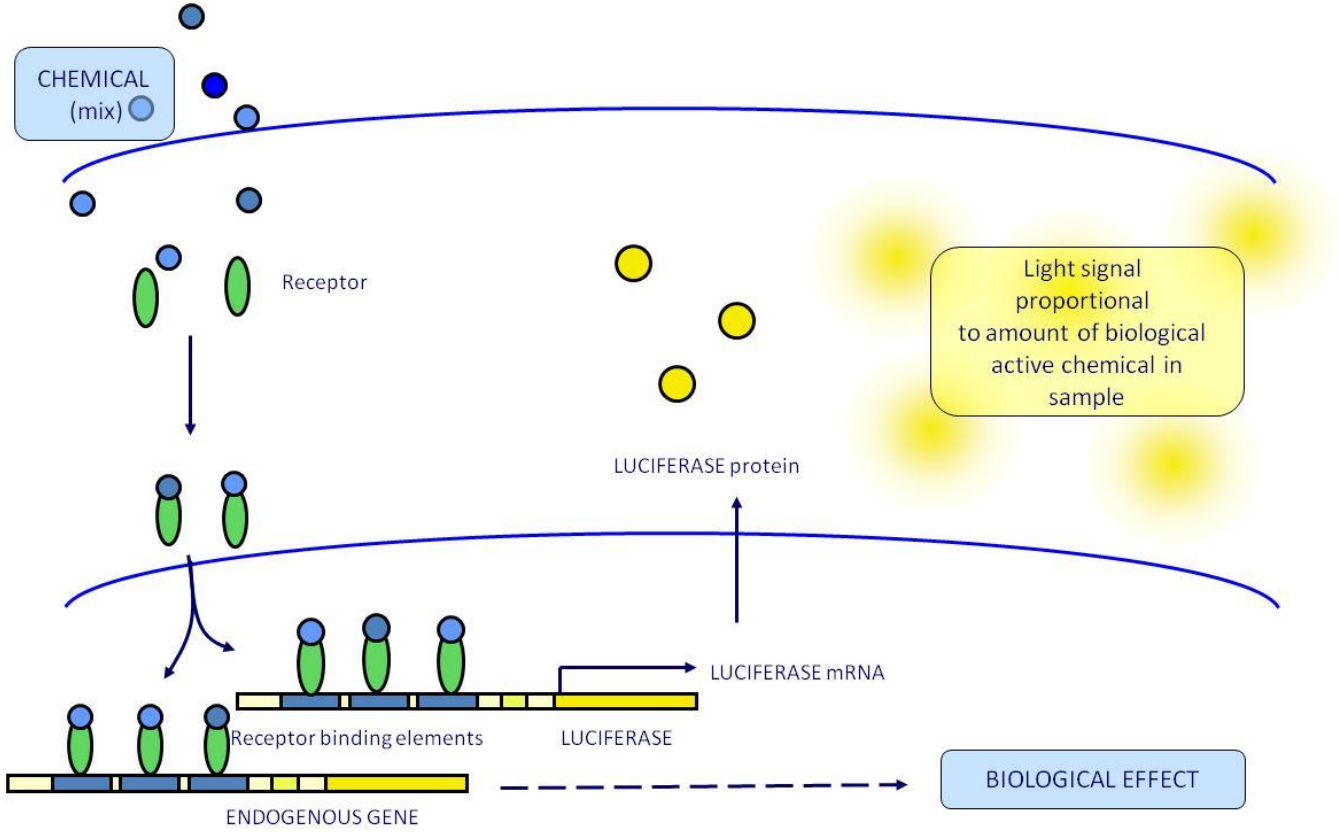
	Endpoint			
	C	M	R	S
comp. A			(EXP)	
comp. B				
comp. C				
comp. D	(GHS)		(RAX)	

- EXP: experimental data was used
- GHS: classification in GHS system used
- RAX: read-across was used

Colour	Prediction
	Non toxic
	Low toxic
	Medium toxic
	Toxic
	Inconclusive

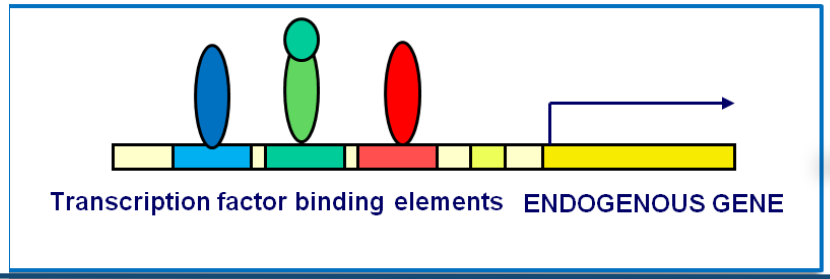
Overview *in vitro* assessment

- A panel of human cell based CALUX reporter gene assays
- Rapidly and selectively measures activation of one specific nuclear receptor or cell signaling pathway
- CALUX-profile gives clues on possible mode-of-action, can be linked to adverse outcome



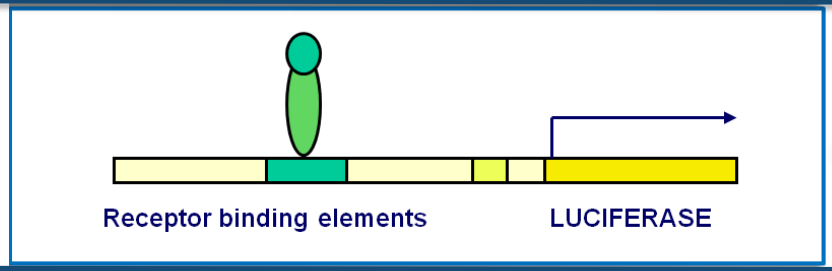
Advantages of the CALUX approach

Biological situation: endogenous gene under control of **multiple** RE's



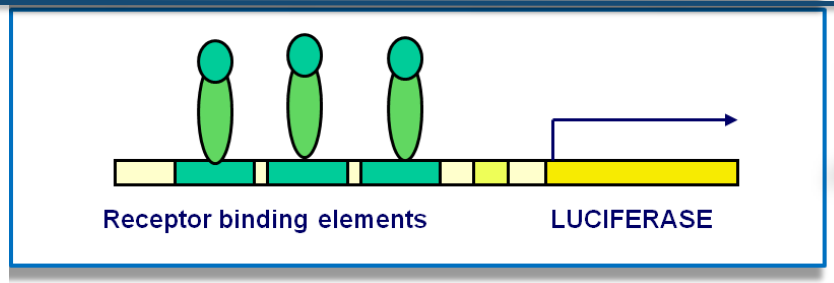
unselective

Reporter gene assay: reportergene under control of **one type** of RE



selective

CALUX assay: reportergene under control of **multiple copies** of one type of RE



selective and responsive

Available CALUX assays

Cell line	Endpoint
ERa CALUX (ago/anta)	Estrogen receptor (ant)agonists
AR CALUX (ago/anta)	Androgen receptor (ant)agonists
PR CALUX (ago/anta)	Progesterone receptor (ant)agonists
GR CALUX (ago/anta)	Glucocorticoid receptor (ant)agonists
TRb CALUX (ago/anta)	Thyroid receptor (ant)agonists
RAR CALUX	Retinoic acid receptor agonists
LXR CALUX	Liver X receptor agonists
PXR CALUX	Pregnane X receptor agonists
PPARa CALUX	Peroxisome proliferator activated receptor agonists
PPARg2 CALUX	Peroxisome proliferator activated receptor agonists
PPARd CALUX	Peroxisome proliferator activated receptor agonists
AhR CALUX	Aryl Hydrocarbon receptor agonists
Hif1a CALUX	Chemical hypoxia response
TCF CALUX	wnt/TCF pathway activation
AP-1 CALUX	AP1 pathway activation / cell cycle control
ESRE CALUX	Endoplasmic reticulum stress
NFkB CALUX	Activation of NF-kB pathway (immune response)
Nrf2 CALUX	Oxidative stress
p21 CALUX	Transcription of p21 inhibitor of cell cycle progression
p53 CALUX	p53-dependent pathway activation / genotoxicity
Cytotox CALUX	Cytoxicity

In vitro workflow

1. Determine appropriate exposure scenario
    * assess solubility, volatility, water-miscibility

2. Determine appropriate assays
    * screen representative reference compounds on broad panel
    * select sub-panel of assays representing the most relevant endpoints

3. Evaluate data
    * assign RAG status based on CALUX profile

4. Integrate with *in silico* outcome
    * assign overall RAG status based on integrated *in silico – in vivo* assessment

In vitro outcome: the CALUX profile

Lowest effect concentrations in Log(M)

activity - +

<-compounds	assays->	selected assays											not selected																
		Cytotox20%	ERa	AR-anti	PR-anti	PXR	PPARd	AhR	AP1	ESRE	Nrf2	p53 GENTOX +/-S9	PR	ERa-anti	AR	GR	GR-anti	TRb	TRb-anti	RAR	LXR	PPARa	PPARg	Hif1a	TCF	NFkB	p21
benzene						-1.7																						
Toluene		-2.2				-1.0					-2.0																	
Xylene		-3.0				-3.1																						
ether 1,4-dioxane					-0.9	-2.1																						
acetonitrile		-0.7				-1.2																						
2-(ethoxymethyl)tetrahydrofuran		-1.6			-2.0	-2.1																						
1,2-dichlorobenzene		-3.8																										
propylene carbonate		-1.5																										
Gamma-valerolactone (GVL)		-1.4				-2.0																						
DMSO																												
sulfolane																												
1,3-Dimethyl-2-imidazolidinone		-1.0					-2.5	-2.5	-1.5									-2.5									-2.0
1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrim		-1.1					-2.6	-2.6										-2.5							-2.6		-2.0
DMF																											-1.3
DMAC							-2.2	-2.2	-1.7																-2.5		-2.0
NMP		-1.4			-1.7		-2.2	-2.0	-1.5								-1.5								-2.5		-2.0

Data evaluation: RAG status

Rules have been established for CALUX RAG status

Number of hits	LEC (LogM)	RAG status
0-2	>-5	green
0-2	<-5	amber
3-5	>-5	amber
3-5	<-5	red
>5	any	red

RAG status:
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A = some toxicity, consider
G = limited toxicity, continue

Compound ID	Cytotox20%	ERa	AR-anti	PR-anti	PXR	PPARd	AhR	AP1	ESRE	Nrf2	p53 GENTOX
ID 1055											
ID 1116	-3.1				-5.0						
ID 1046					-2.6		-2.6			-1.1	-1.6
ID 1099	-4.9						-5.1	-5.1		-5.4	
ID 1100	-1.2		-2.7	-2.7	-3.7		-2.7	-1.7		-2.9	

In silico & in vivo integration

Work in progress!

	in vitro											bioavailability			*in silico*			
Compound Code	Cytotox20%	ERa	AR-anti	PR-anti	PXR	PPARd	AhR	AP1	ESRE	Nrf2	p53 GENTOX	[max tested]	max [medium]	Volatility	Carcinogenicity	Gentox	Reprotox	Skin sens
IS1	-1.2		-2.2		-2.5							-2.0	-2.0	N		RAX	RAX	RAX
IS2					-2.0							-2.0	-2.0	N		RAX	RAX	RAX
IS3					-2.2						-1.2	-1.2	-1.2	N		EXP	EXP	EXP
IS15					-2.2							-2.2		L				
AK1					-2.7					-1.7	-1.2	-1.2	-2.2	M				
AK4			-2.7		-1.5		-1.7				-1.1	-1.1	-1.1	M		EXP	RAX	
AK6					-1.1							-1.1	-1.1	M			RAX	
AK7		-1.6	-2.0		-3.1					-2.5	-1.8	-1.1	-1.1	H			RAX	
LG1	-3.0							-3.3		-3.5		-1.0	-1.0	N		EXP		EXP
LG6	-1.1				-1.6							-1.1	-1.1	L				
LG13	-4.9						-5.1	-5.1		-5.4		-1.1	-1.1	L				
LG23	-1.2		-2.7	-2.7	-3.7		-2.7	-1.7		-2.9		-1.2	x	L				
LG64	-1.5											-1.2	-1.2	M				
OT16			-2.2		-2.2					-3.2		-1.2	-1.2	N				
LV34			-2.8	-2.5	-3.0				-2.5	-2.2	-2.2	-1.2	-1.2	L		EXP		EXP

Conclusions

- An integrated strategy has been developed which combines:
  - available *in vivo* data (existing chemicals only)
  - *in silico* predictions based on QSARs and read-across
  - *in vitro* data assessing interaction with ~20 different nuclear receptors and cell signaling pathways

- Safety assessment early in the design process enables:
  - early go-no go decisions
  - feedback to design-team on chemical groups with unfavourable tox properties

Acknowledgements

In silico assessment:

TNO

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Marie Meima
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In vitro screening:

BioDetection Systems bv

Bart van der Burg
Hai-Yen Man
Faried Niamut

Thank you for your attention!