ReSolve
SAFE AND EFFECTIVE BIO-BASED SOLVENTS

Applying the CALUX bioassay panel: GREEN toxicology

BioDetectors Conference 2018

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BIO-BASED INDUSTRIES Public-Private Partnership

Bio·based Industries Consortium

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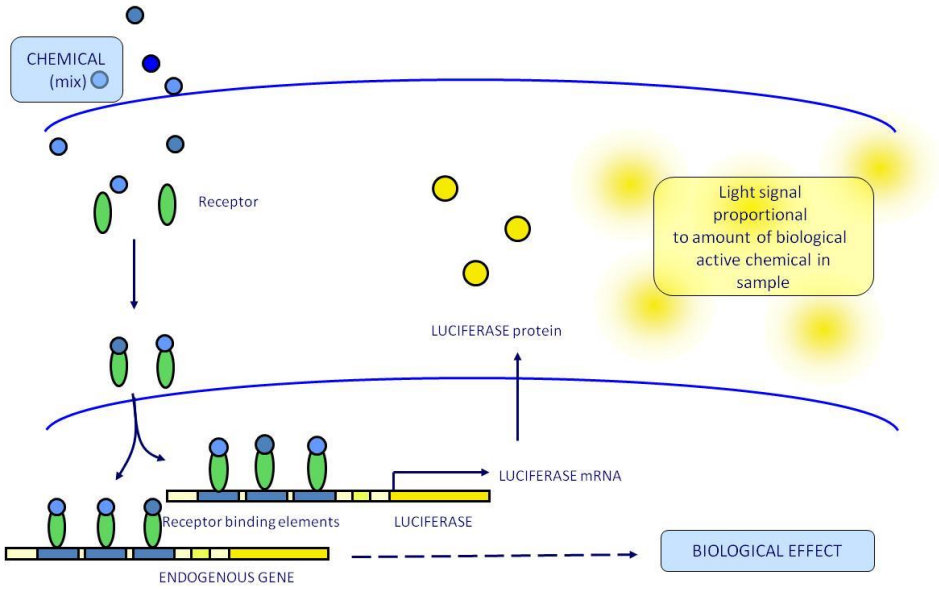
CALUX assay principle

- Effect-based risk assessment, focusing on molecular initiation events
  - chemical – receptor interaction (agonism/antagonism)
  - chemical – cell signaling pathway interaction (activation/inhibition)
- Suitable for single compounds or complex mixtures
- >30 CALUX assays available



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

CALUX profiles

- CALUX panel analysis results in a hazard profile:

Assays ->

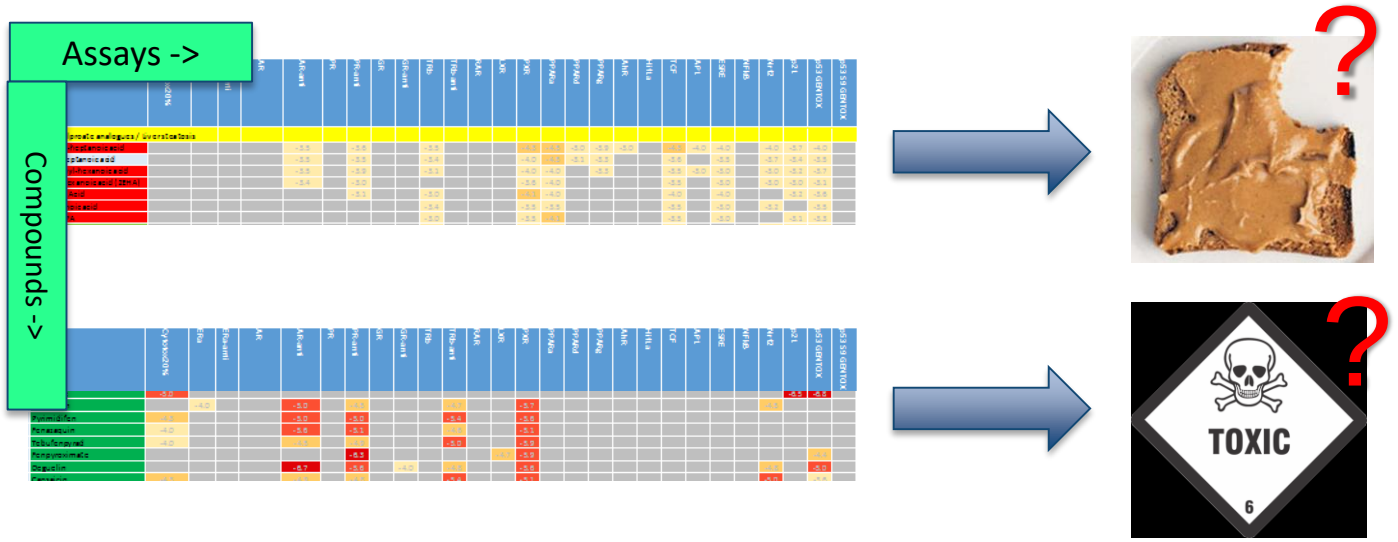
<- Compounds

compound	Cytotox20%	ERa	ERa-anti	AR	AR-anti	PR	PR-anti	GR	GR-anti	TRb	TRb-anti	RAR	LXR	PXR	PPARa	PPARd	PPARg	AhR	Hif1a	TCF	AP1	ESRE	NFkB	Nrf2	p21	p53 GENTOX
Compound 1			-4.6		-4.0				-5.0													-4.8		-5.5		
Compound 2	-4.9	-7.0			-6.0		-5.7							-6.0										-4.5		

activity - +

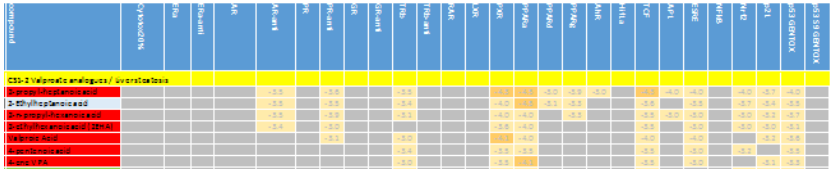
CALUX interpretation

- BUT: how to interpret the results??



Profile interpretation: approach

- Screen a ‘training set’: compounds of known (lack of) toxicity
- Link *in vitro* profile to *in vivo* toxicity



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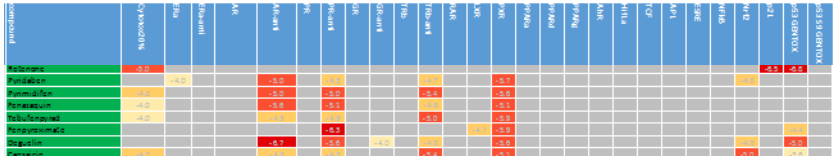

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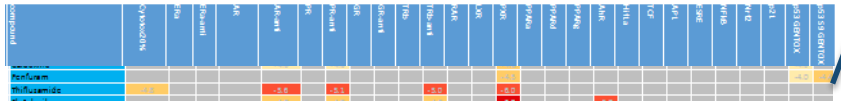
Predict *in vivo* activity of unknown compounds based on their *in vitro* profile:



➢ **This strategy requires the analysis of many compounds.**

Increasing the throughput: automation

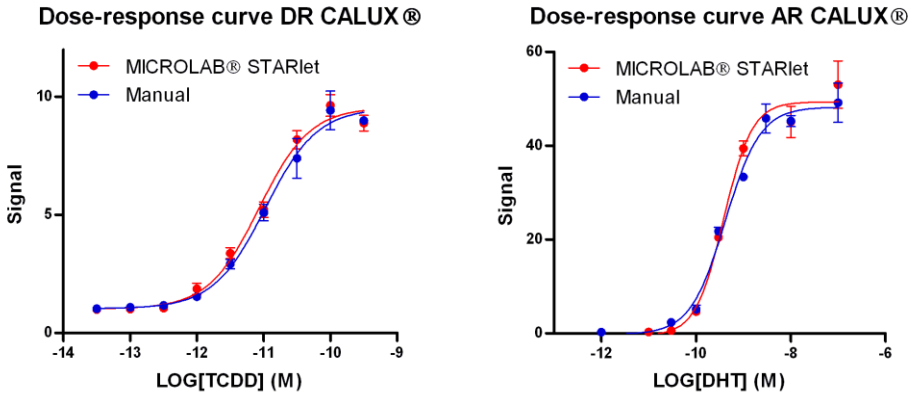
To increase throughput AND improve quality: automate the most time consuming steps

- cell seeding (cell dispenser)
- compound dilution (liquid handling robot)
- compound exposure (liquid handling robot)
- luminescence detection (luminometer + stacker)

Validation of robot performance

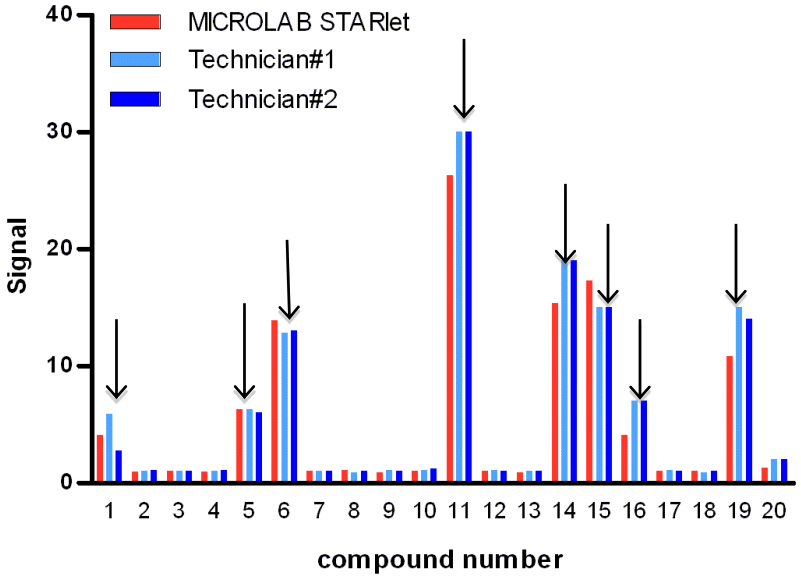
Manual vs robot

Identical EC50-, SD- and RLU values:



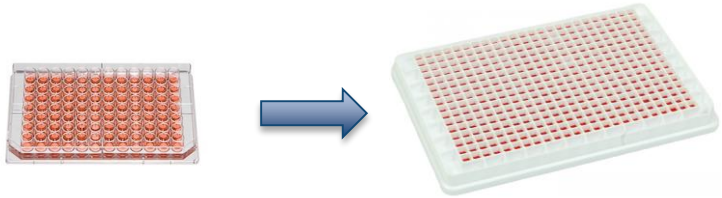
- Labour time
- Interindividual variation
- Human errors
- Reproducibility
- Data quality

Same ‘hits’ are found by robot as by two experienced technicians:

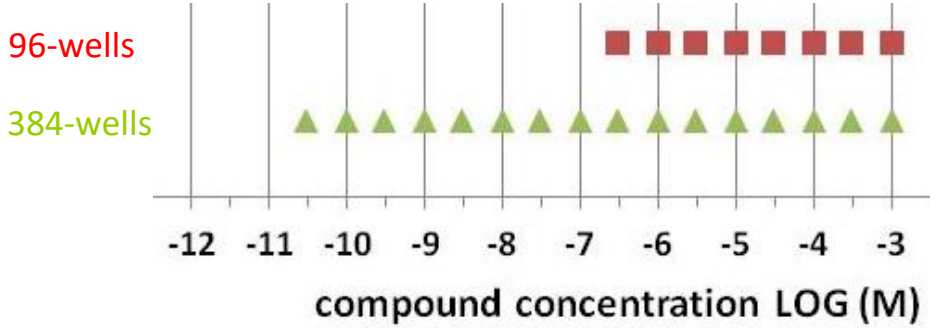


Increasing the throughput: miniaturization

96-wells -> 384-wells plates resulted in:

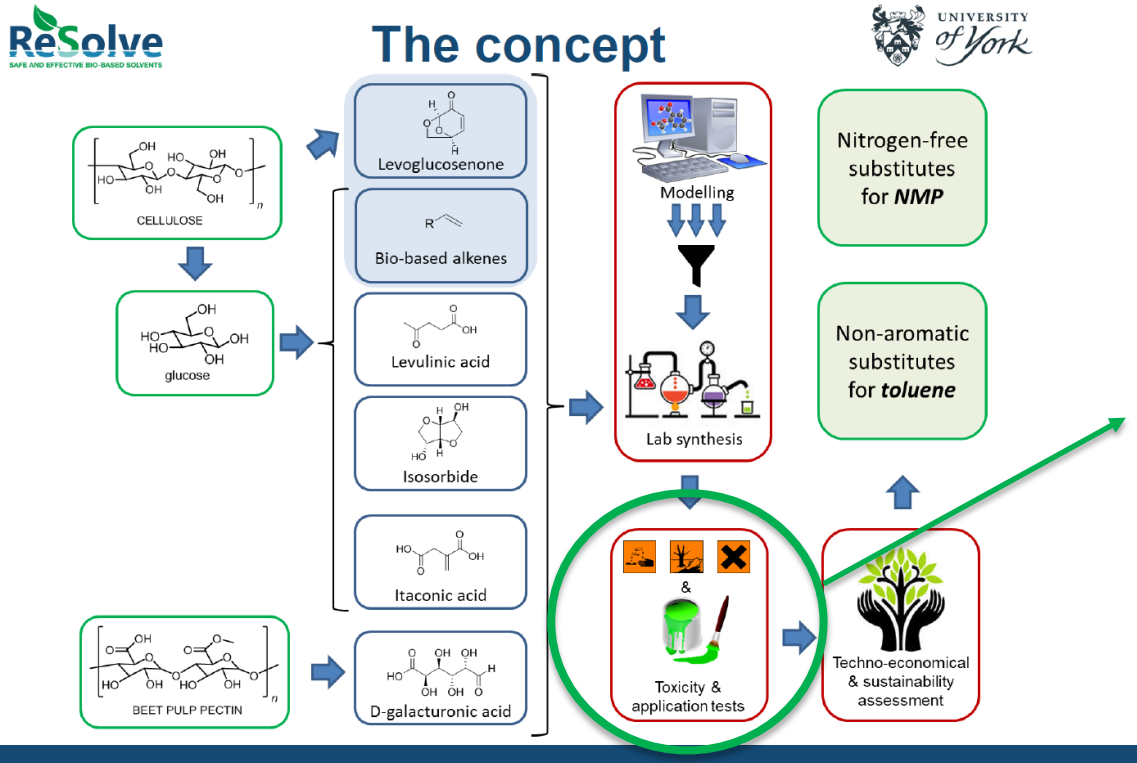


- 2 -> 6 compounds per plate
- 8 -> 16 concentrations per compound
- 1 control -> full dose-response curve per plate
- 100 -> 200 dose-response curves per day
  - broader concentration range
  - more controls
  - more reliable and accurate data
  - automated analysis possible
  - lower sample volumes



The ReSolve project

- Toluene and N-methyl-2-pyrrolidone (NMP) are two of the most toxic solvents currently in use.
- The primary aim of the ReSolve project is to replace these solvents with safe, biobased alternatives derived from non-food carbohydrates.



Establishing an integrated testing strategy to evaluate safety issues

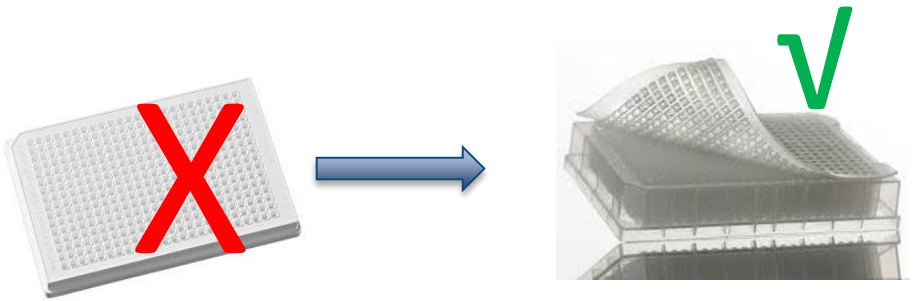
- *in vitro*: human CALUX cell-based reporter gene assay panel
  - major types of toxicity
  - rapid

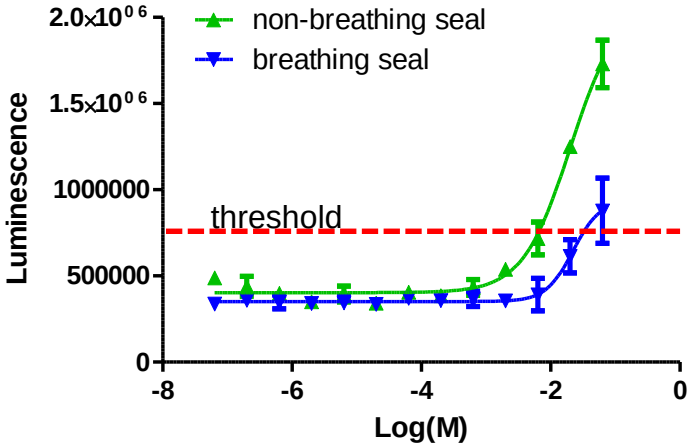
- *in silico*: DIAMONDS, CoMSAS, TTC
  - combining existing data: read-across
  - structure/activity evaluation

- Analyse solvents currently in use. Their profile can be seen as a point of departure: toxicity profiles of novel candidates should be more favourable.

Modify CALUX protocols to enable screening of volatile solvents

- Expose solvents at 1% v/v: exposure concentration ~0.1M instead of 1E^{-4}M (standard procedure)
- Cover the CALUX assay plates with non-breathing seals after exposure to prevent solvent evaporation
- Use CO_2-independent cell culture medium to allow cell survival in a closed environment (due to the seal)





CALUX panel results for reference solvents

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BDS
BioDetection Systems

toxicity | xenobiotics sensor | stress pathways

compound	Cytotox20%	ERa	ERa-anti	AR	AR-anti	PR	PR-anti	GR	GR-anti	TRb	TRb-anti	RAR	LXR	PXR	PPARa	PPARd	PPARg	AhR	Hif1a	TCF	AP1	ESRE	NFkB	Nrf2	p21	p53 GENTOX +/-S9
NMP	-1.4						-1.7		-1.5							-2.2		-2.0		-2.5	-1.5				-2.0	
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		-2.5			-1.5				-2.0	
1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone	-1.1									-2.5						-2.6		-2.6		-2.6					-2.0	
DMF																									-1.3	
DMAC																-2.2		-2.2		-2.5	-1.7				-2.0	
benzene														-1.7												

activity - +

CALUX panel results for solvent candidates

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BDS
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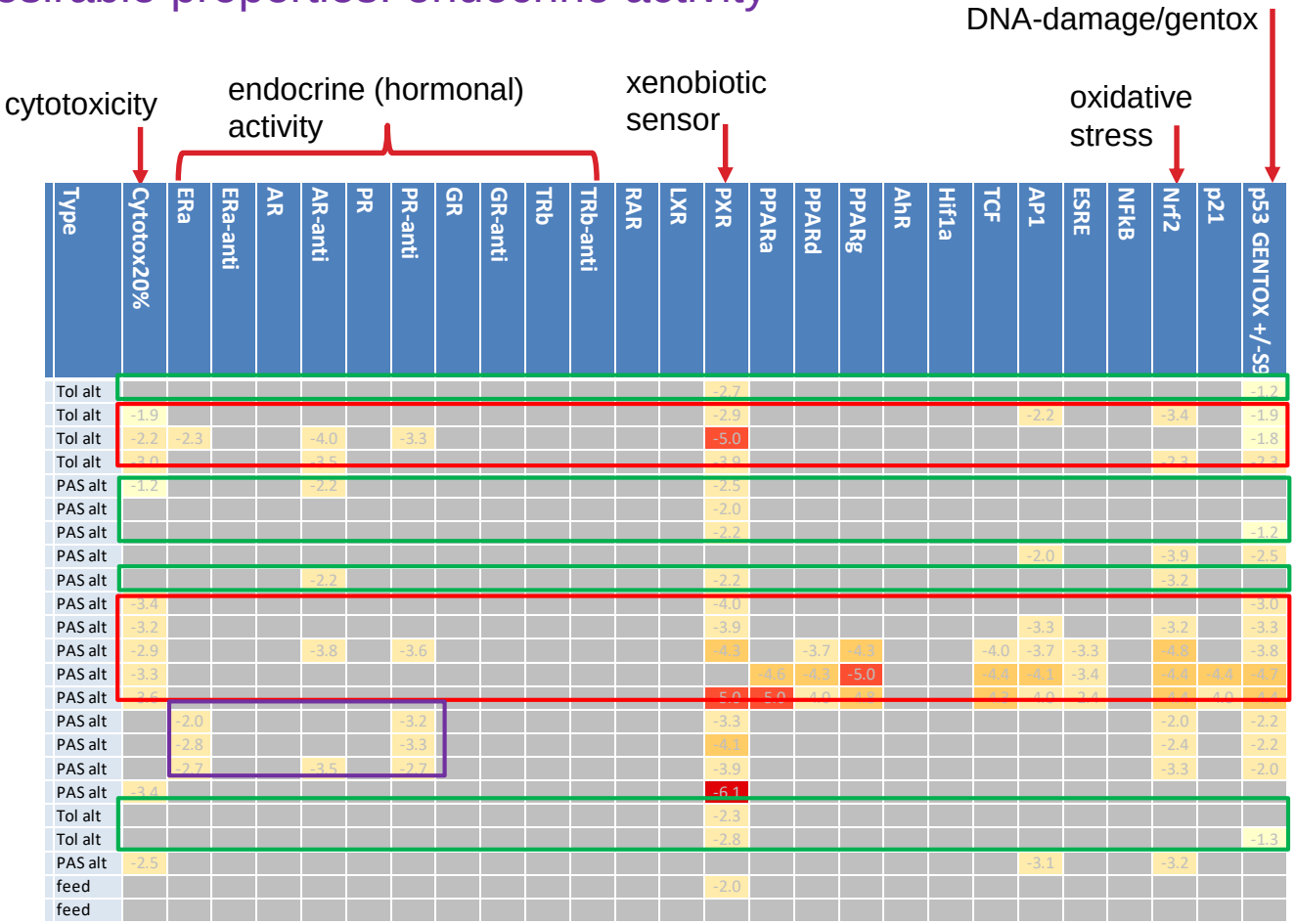
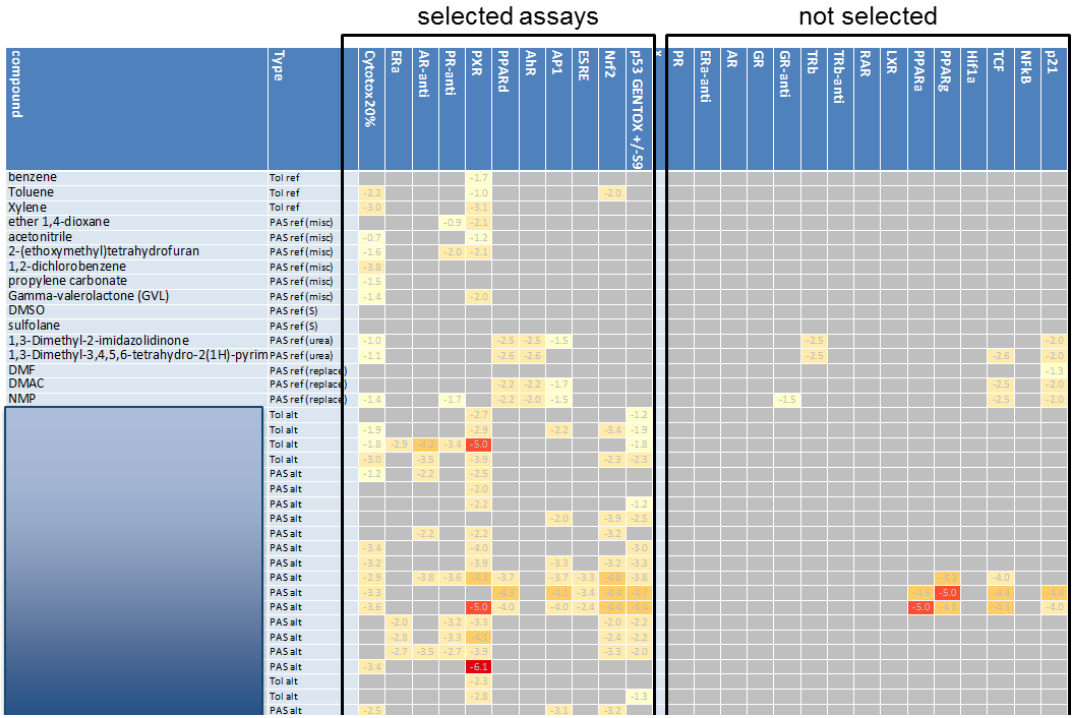
- Some candidates look promising
- Some are (cyto)toxic and/or active on too many assays
- Some possess undesirable properties: endocrine activity



Based on the CALUX panel profiles:

- several novel solvent candidate groups (‘platforms’) were discontinued due to unfavourable safety profiles
- for platforms with a favourable profile, efforts were intensified and more structural variants were synthesized
- a ‘sub-panel’ of relevant CALUX assays could be selected, to allow screening of more compounds in shorter time:



Conclusions

- The CALUX panel was successfully optimized for this specific class of compounds (volatile solvents)
- The CALUX results were used to identify candidates with a favourable safety profile, for additional (tox) studies and structural optimization
- Some of the results obtained so far had not been predicted based on structural information and physicochemical properties of the candidates
- The ReSolve project is therefore a good example of the added value of effect-based bioassays
- Currently, the CALUX *in vitro* data is being combined with *in vivo / in silico* data to identify missing endpoints in the current panel

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