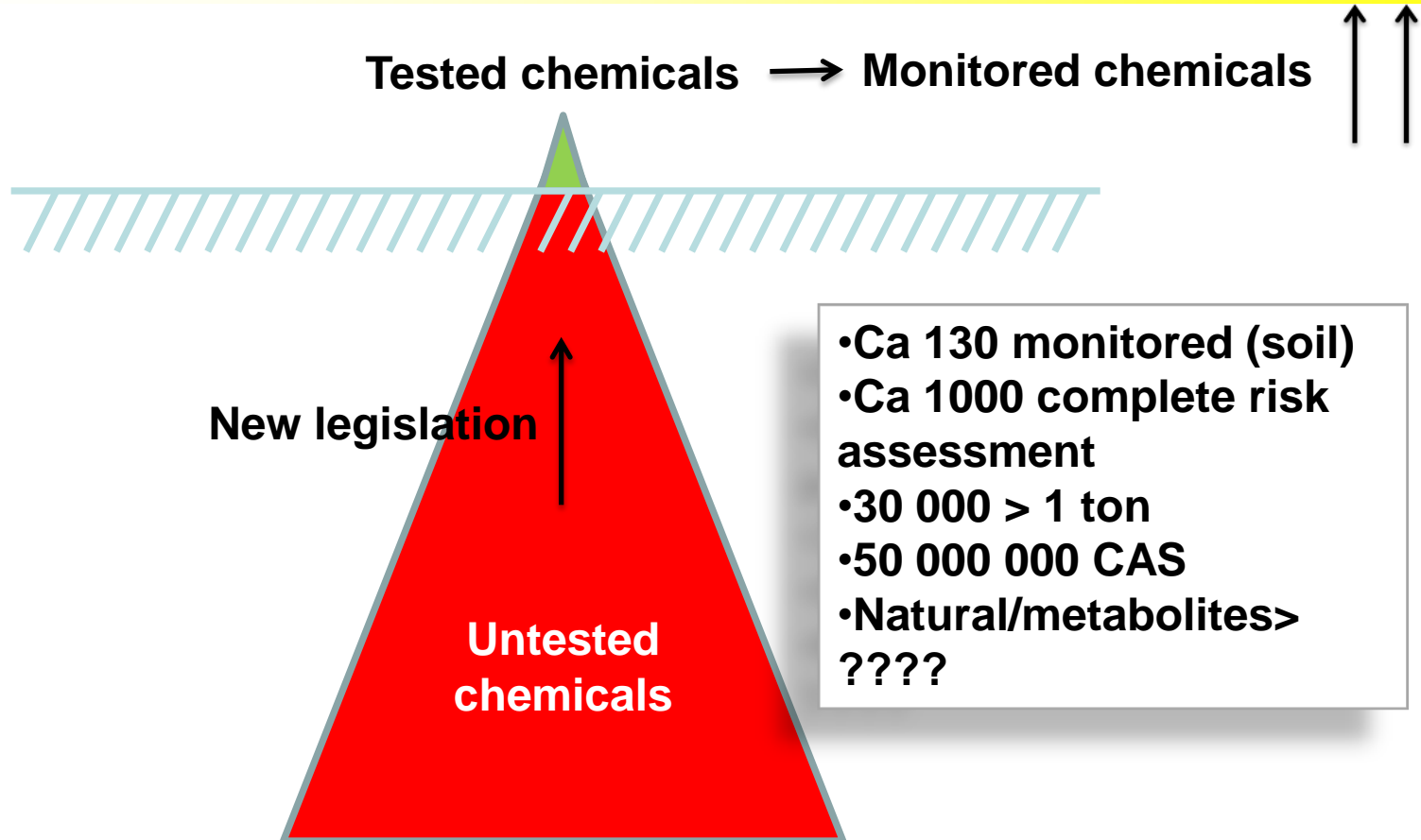


50 CALUX tests – latest R&D

Bart van der Burg

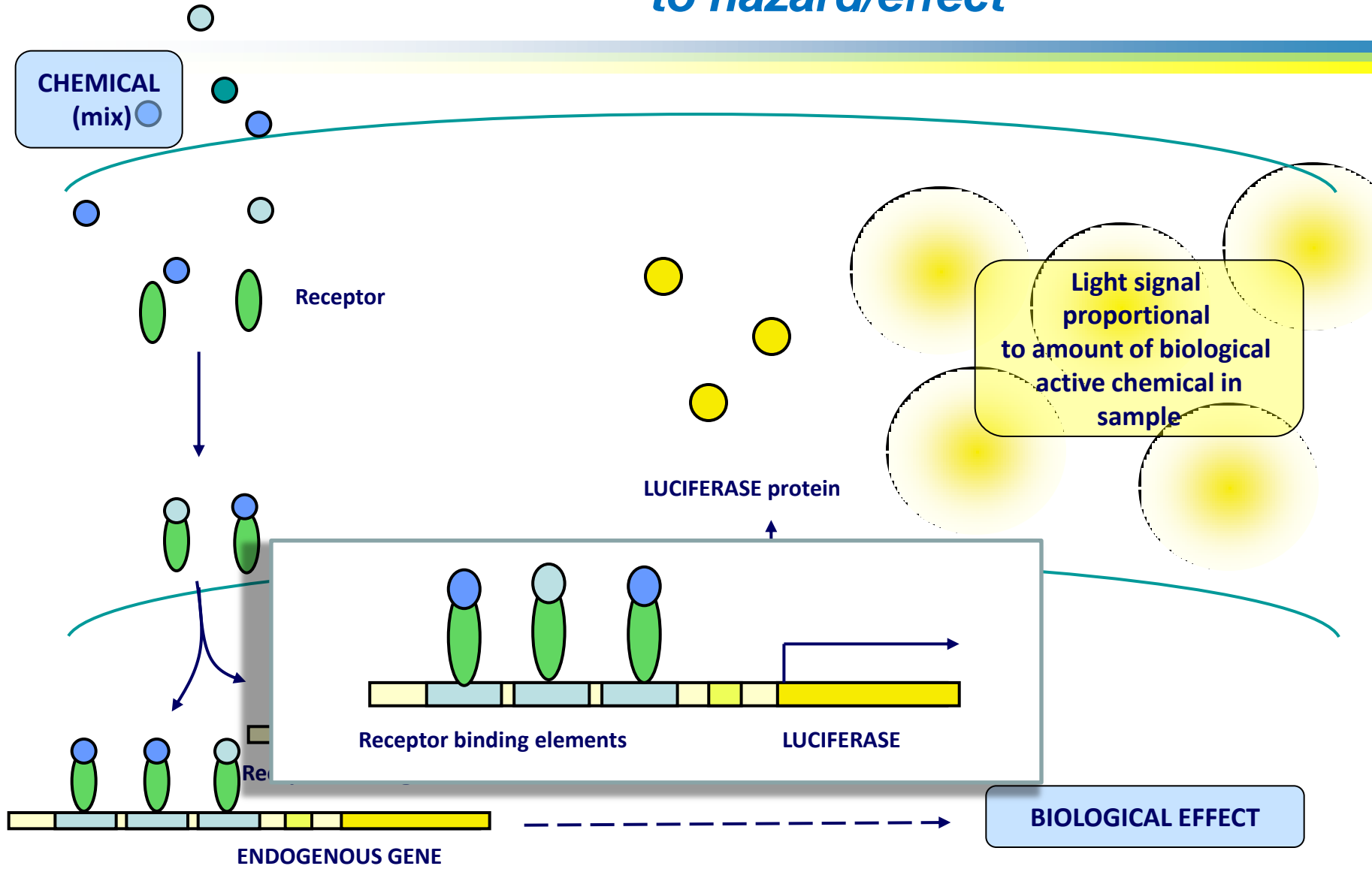


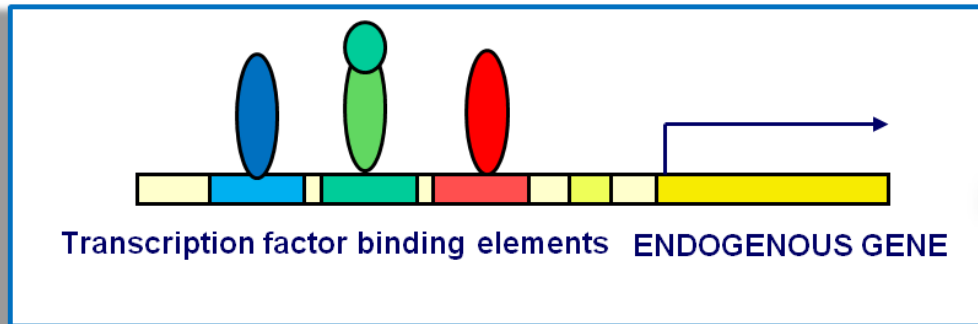
Environmental monitoring: integrative (biological) methods needed



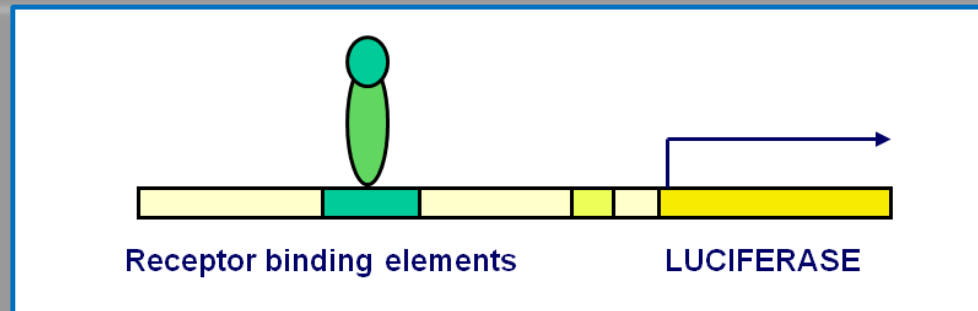


CALUX[®] : chemical quantification coupled to hazard/effect

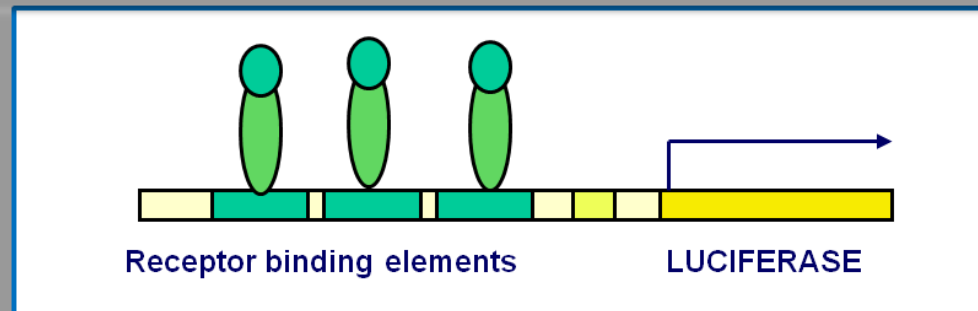




unselective



selective



*selective
and
responsive*

- **Low background, high selectivity and inducibility**
- **High sensitivity**
- **Excellent quantification**
 - **Single mechanism, avoid cross-talk and artifacts**
 - **Straight-forward interpretation and risk assessment**
 - **Better extrapolation to other species**
 - **Suitable to measure bioactivity in complex mixtures**
 - **Low rate of false positives greatly reduces error in panel of assays**



Available CALUX[®] lines

Nuclear receptors			Signaling pathways			Controls		
name	status	cell	name	status	cell	name	status	cell
DR CALUX	✓	H4IIE	kappaB CALUX	✓	U2OS	Cytox CALUX	✓	U2OS
PAH CALUX	✓	H4IIE	P21 CALUX	✓	U2OS	MTT	✓	all
ER CALUX	✓	T47D	Nrf2 CALUX	✓	U2OS	LDH leakage	✓	all
ERalpha CALUX	✓	U2OS	P53 CALUX	✓	U2OS	Visual	✓	all
ERbeta CALUX	✓	U2OS	P53 CALUX	✓	HepG2			
ERalpha CALUX	✓	HEK293	TCF CALUX	✓	U2OS			
ERbeta CALUX	✓	HEK293	AP1 CALUX	✓	U2OS			
AR CALUX	✓	U2OS	HIF1alpha CALUX	✓	U2OS			
PR CALUX	✓	U2OS	ER stress CALUX	✓	U2OS			
GR CALUX	✓	U2OS	CRE CALUX	✓	U2OS			
TR CALUX	✓	U2OS	ETS CALUX	✓	U2OS			
RAR CALUX	✓	U2OS	GLI CALUX	✓	U2OS			
PPARγ1 CALUX	✓	U2OS	NOTCH CALUX	✓	U2OS			
PPARγ2 CALUX	✓	U2OS	E2F CALUX	✓	U2OS			
PPARα CALUX								
PPARδ CALUX								
PXR CALUX								
LXR CALUX								
VDR CALUX								
MR CALUX	✓	U2OS						

Latest count
Stable CALUX: n=25
Agonist/antagonist: 25x2=50assays

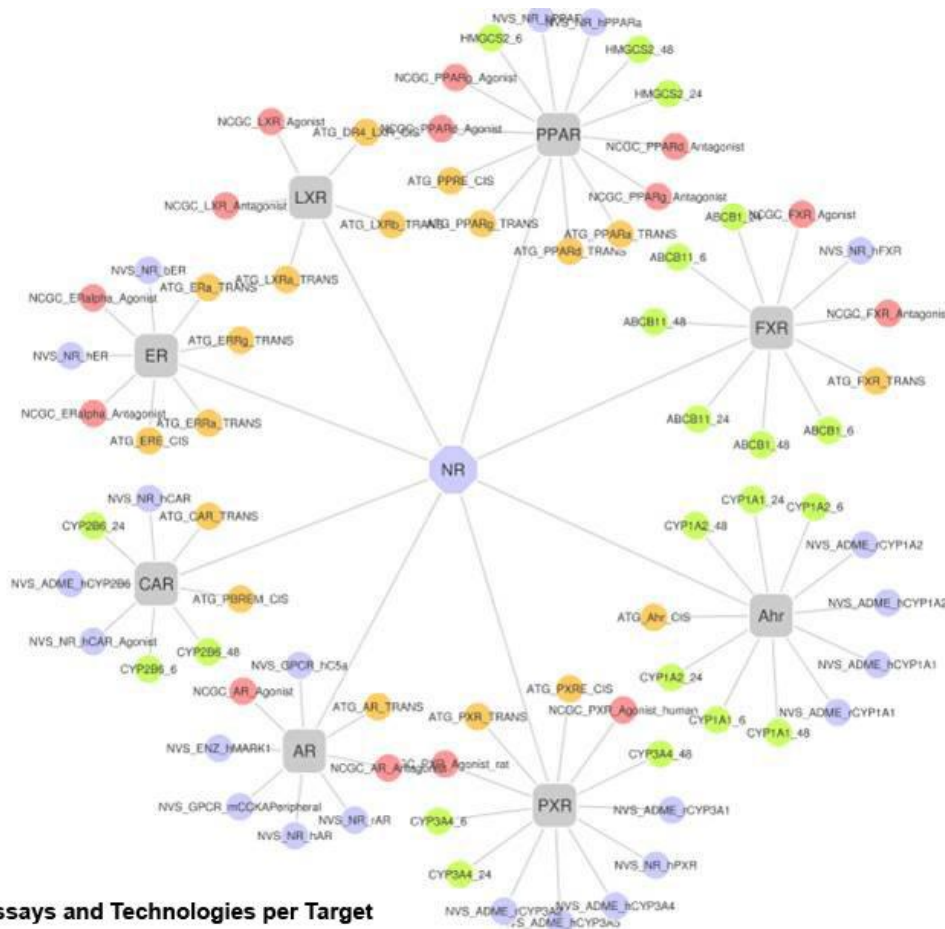


Additional systems

Under evaluation:

- **Differentiated cells; mouse embryonal stem reporter cells:**
 - ReProGlo: TCF reporter
 - ToxTracker
 - other genomics-based systems
- **Bacterial reporter cells**
- **Offline/online systems**

Stringent selection of most informative assays



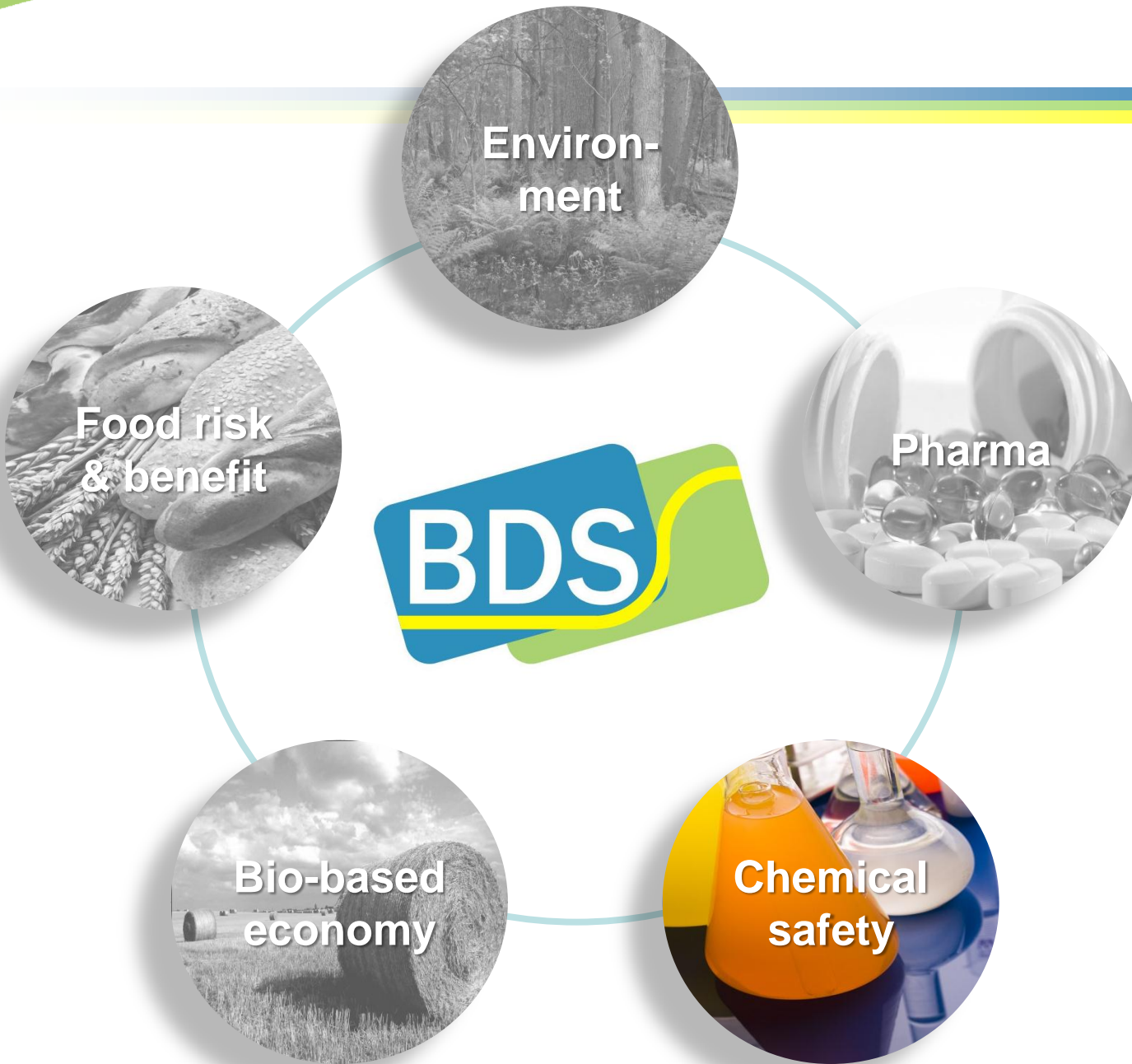
Multiple Assays and Technologies per Target

- **ToxCast: many assays/endpoints per effect class**
- **Our approach: One assay with minimal false positives**

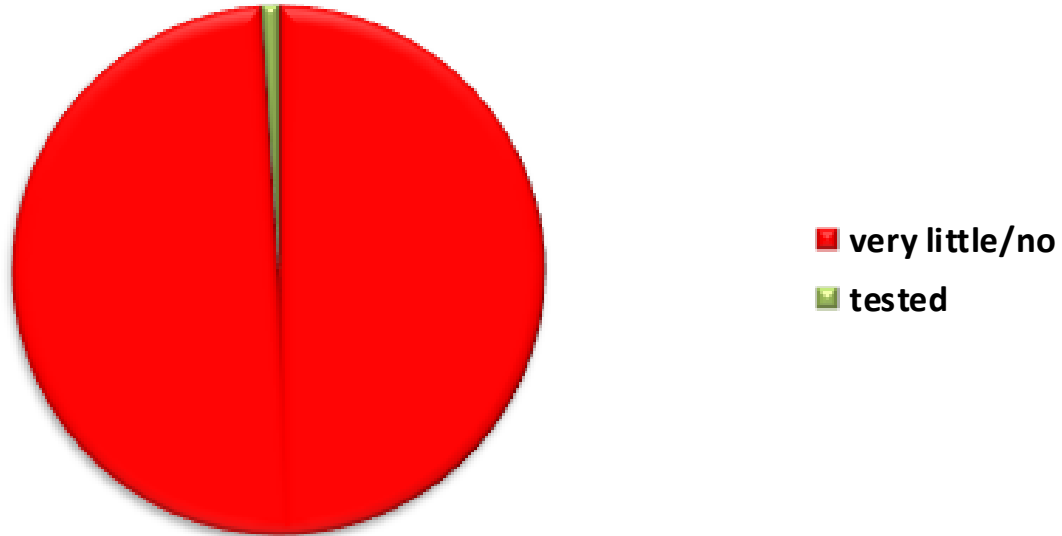


Many fields of application CALUX panel





Market: rapid screening methods are needed



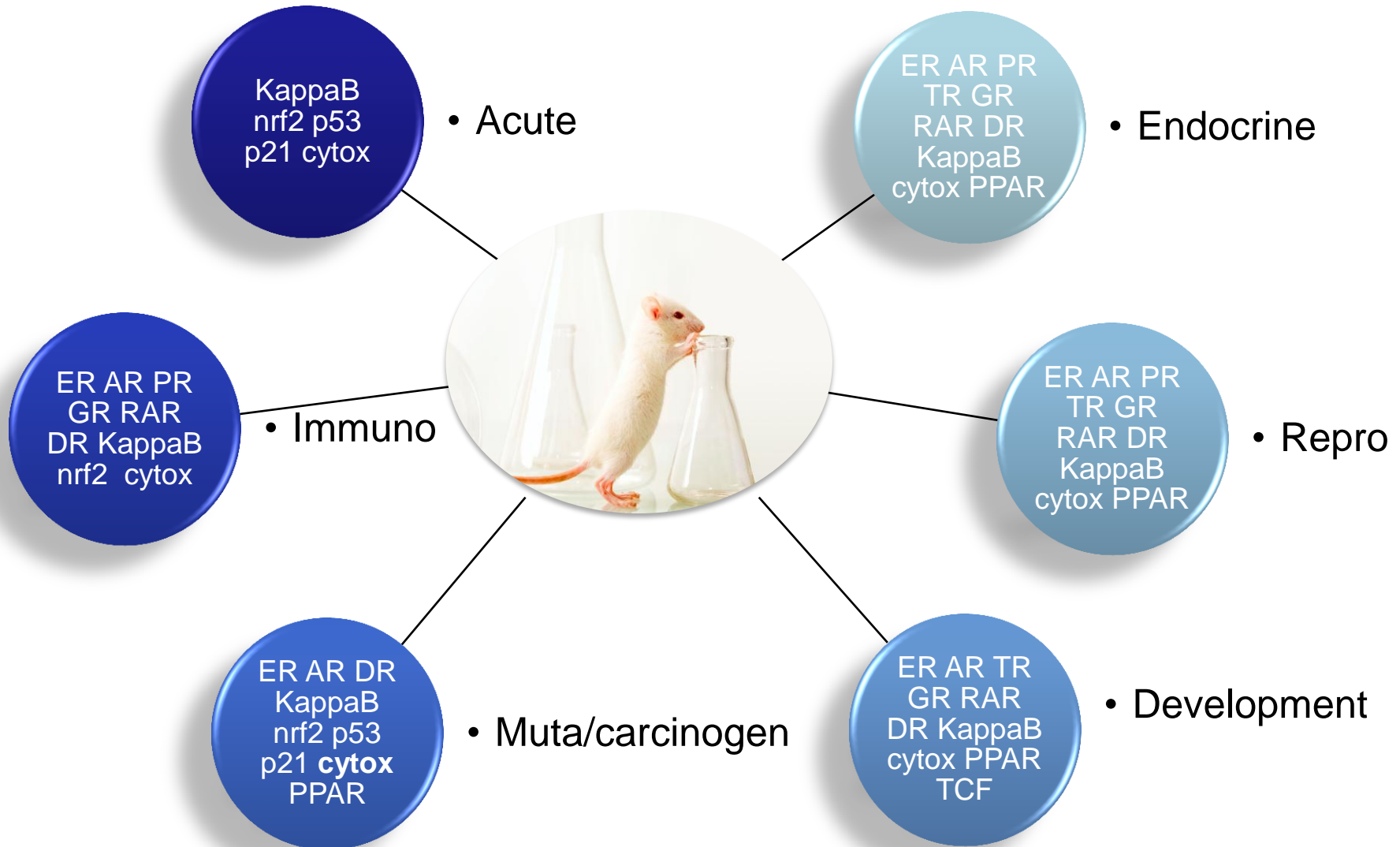
***100,106 chemicals on market in 1981 (“existing substances”);
1% tested on hazardous properties: REACH***

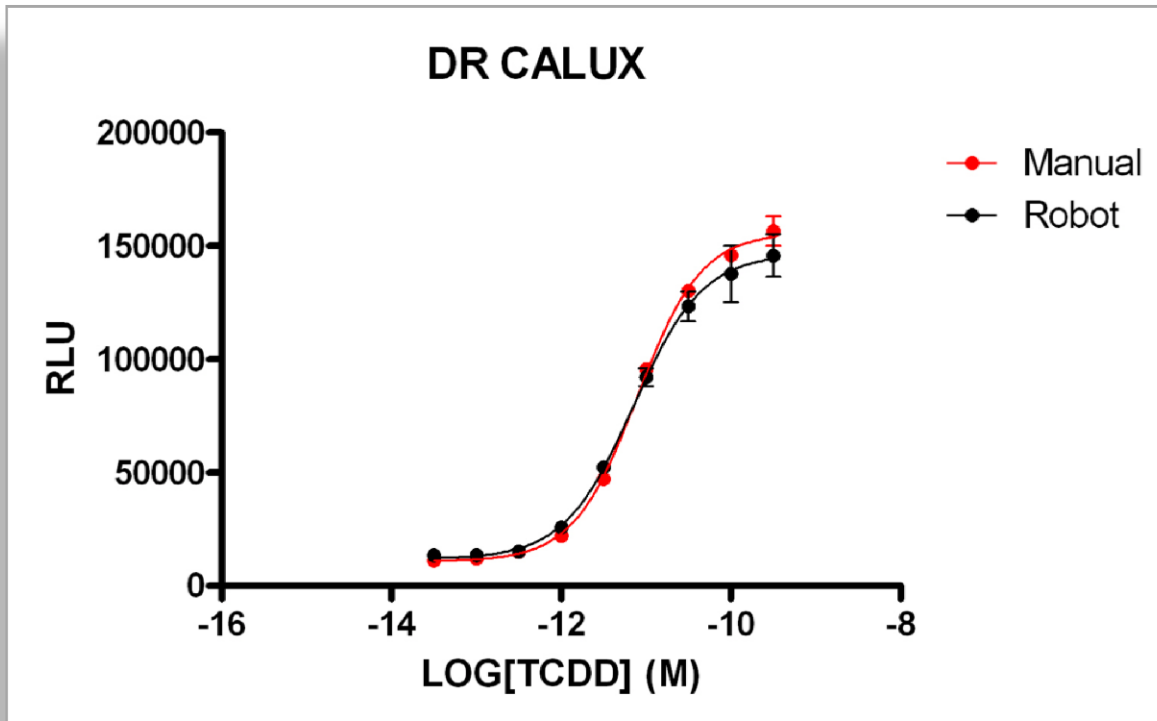
Problems with animal testing in context of REACH:

1. **Speed**
2. **Costs**
3. **Capacity**
4. **Ethics**

>Cost-effective, rapid in vitro tests need to be adopted

Effect profiling with CALUX[®] battery?





CALUX Quantitative HTS:
200 dose-response curves (16 triplicates) per day



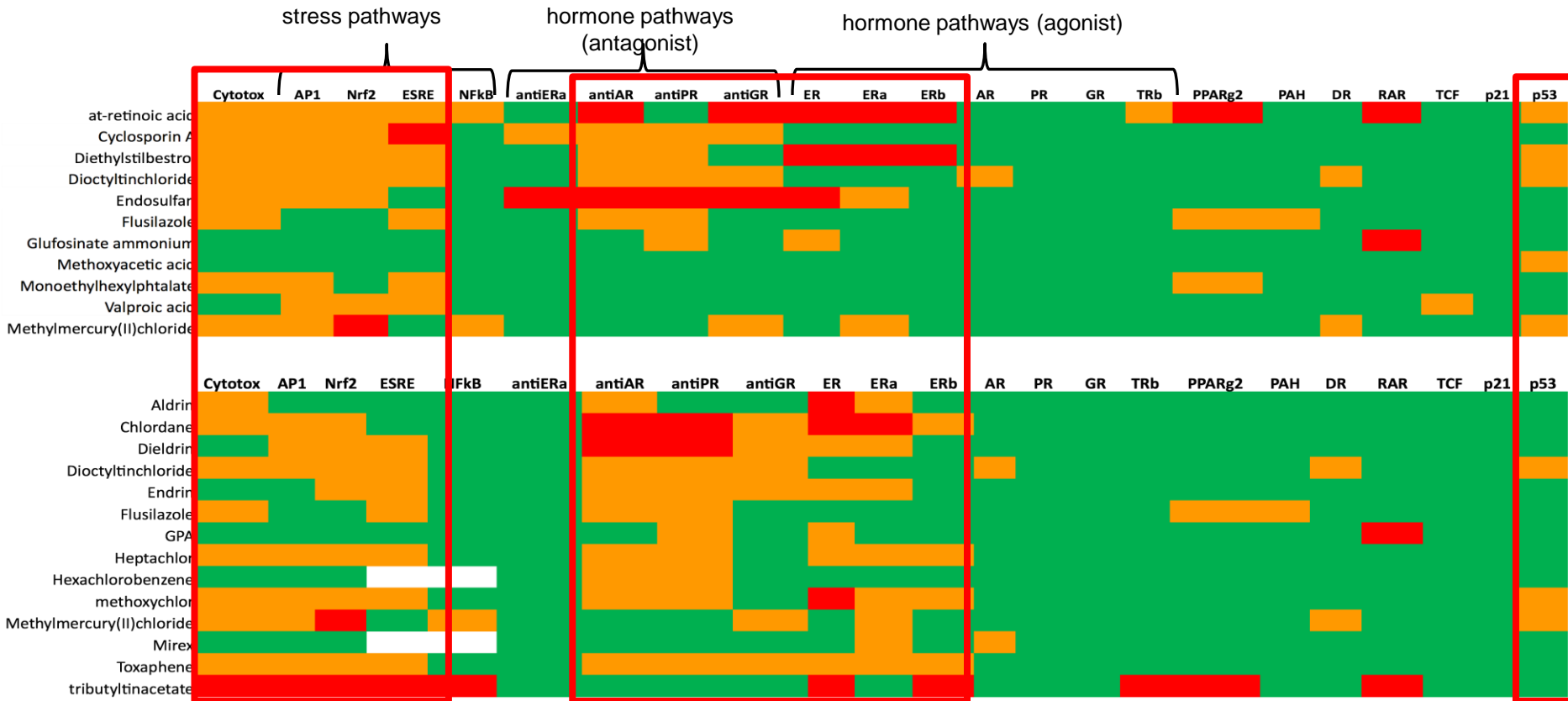


Hot spots of activity in CALUX panel

stress pathways

steroid receptors

genotoxicity



Top: 11 reprotoxicants

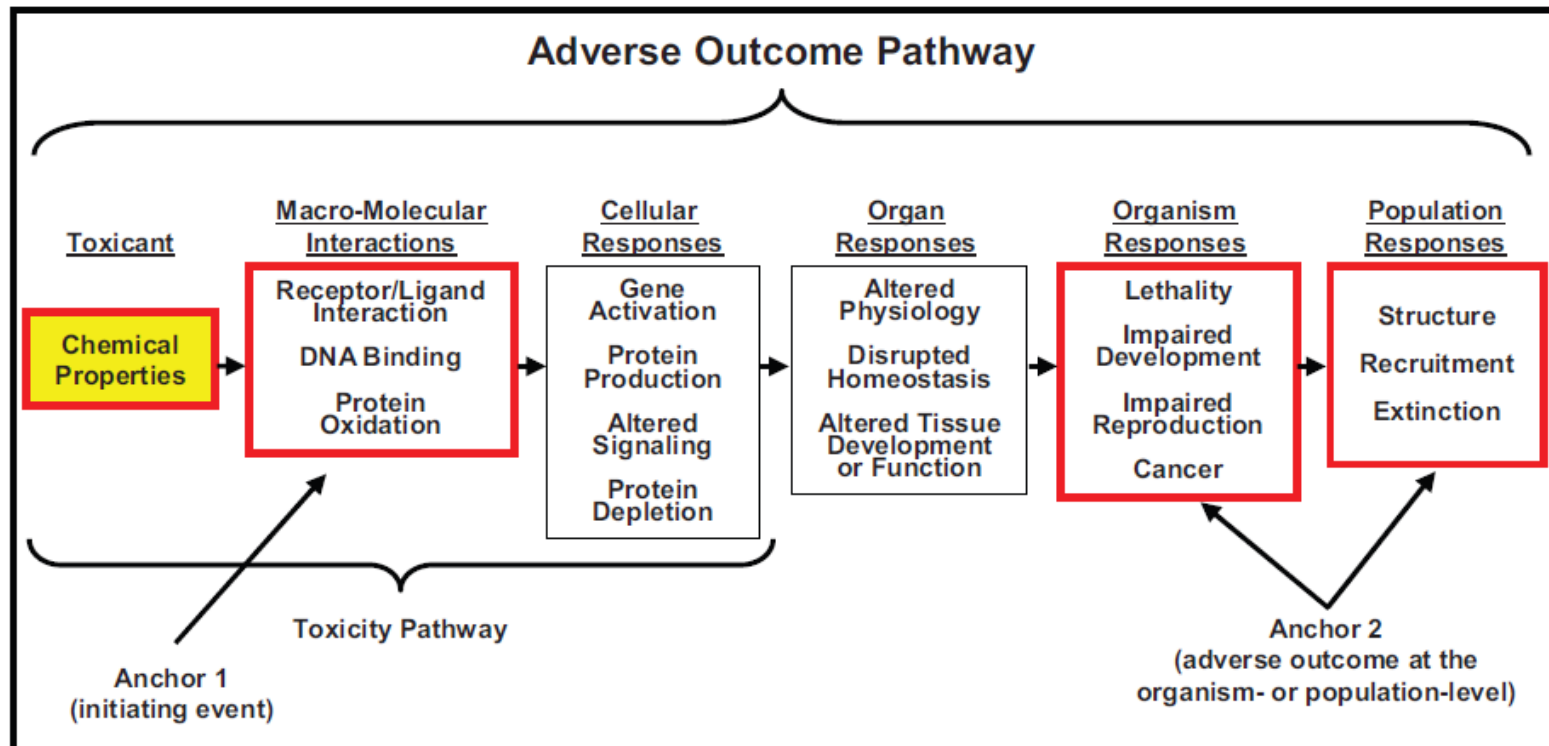
Bottom: 14 pesticides

Prediction reproductive toxicants

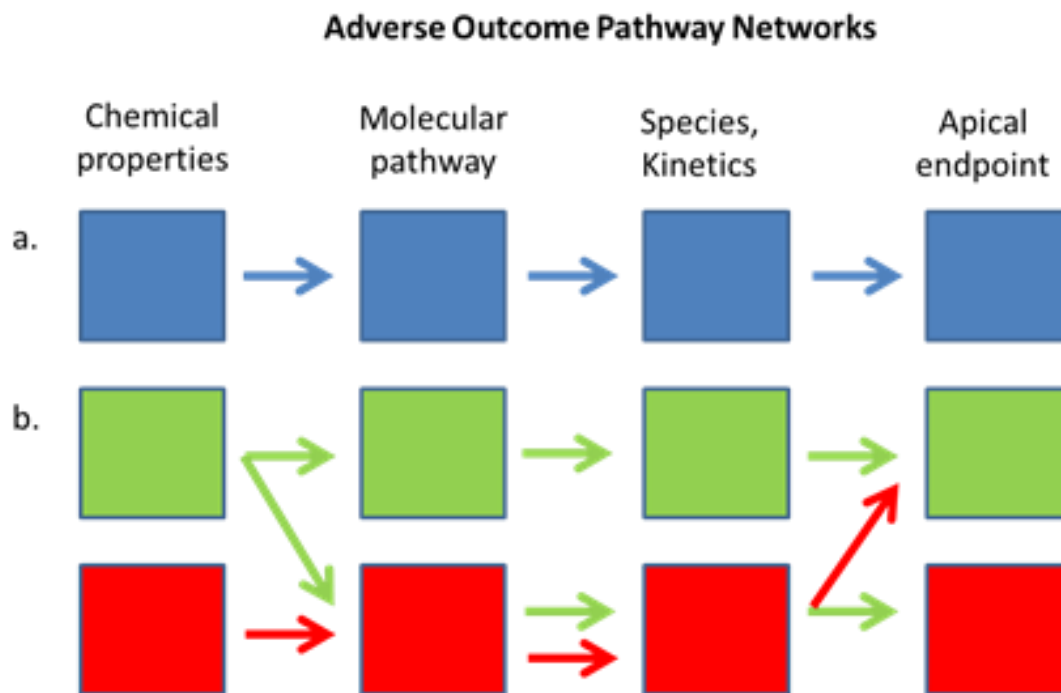



No	Compound	In vivo phenotype	In vivo class	EST	zebrafish	ProGlo	CALUX panel	cyp17	cyp19	bIVM	QSAR	PREDICTION
1	Cyclosporin A (CSA)	immunosuppressant	immune system parameters	specific effect on differentiation	No effect	repressor	weak antiandrogen/glucocorticoid	no effect	no effect	not done	positive	
2	Monoethylhexylphthalate (MEHP)	male reproductive organ malformations	repro (male)	specific effect on differentiation	developmental toxicant	no effect	negative, slight ppar activation : PPAR alpha activator	no effect	no effect	positive		
3	Sodium valproate (VPA)	neurodevelopmental toxicant	development	differentiation effect	low potency developmental toxicant	inducer	negative	no effect	no effect	negative	positive	
4	D-mannitol (DML)	negative control	negative control	no effect	negative	no effect	negative	no effect	no effect	not done		
5	Flusilazole (FLU)	craniofacial and axial skeletal malformations	development	differentiation effect	developmental toxicant	repressor	weak antiandrogen/progestin	inhibitor	inhibitor	not done		
6	Glufosinate ammonium (GPA)	neurodevelopmental toxicant	development, repro (female)	no effect	negative	no effect	antiprogesterin	not done	not done	not done		
7	Methoxyacetic acid (MAA)	growth and developmental retardation	development	no effect	low potency developmental toxicant	inducer	negative	no effect	no effect	not done		
8	Retinoic acid (RA)	neural crest cell migration affected	development	differentiation effect	developmental toxicant	no effect	retinoid; strong developmental and reproductive toxicant	no effect	inhibitor	positive	positive	
9	Diocetyl tin dichloride/dichlorodicylstannane(DOTC)	developmental (immuno)toxicant	immune system	toxic	negative	no effect	weak antiandrogen/progestin, stress-related pathways	inhibitor	inhibitor	not done	not done	
10	Endosulfan (ESF)	neurotoxicant	development (secondary)	toxic	negative	no effect	steroid receptor antagonist. Weak estrogen: reproductive effects	no effect	inhibitor	not done	positive	
11	Diethylstilbestrol (DES)	transplacental carcinogen	reproductive (development)	toxic	negative	inducer	strong estrogen: reproductive effects		no effect	positive	positive	
12	Methylmercury chloride (MMC)	neurodevelopmental toxicant	development	toxic	developmental toxicant	no effect	weak activation of stress related/carcinogenic pathways. Weak steroid receptor interactions	inhibitor	inhibitor	not done	not done	

Interpretation of screening data



Interpretation of screening data



**Molecular screening assays predict toxicity,
but phenotype of change is species-, dose-, and life-stage dependent**



Environ-
ment



Food risk
& benefit



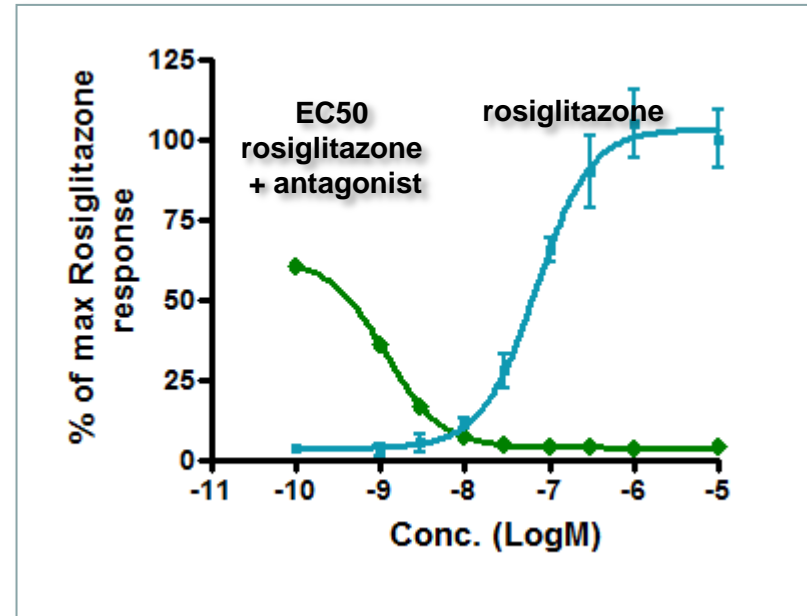
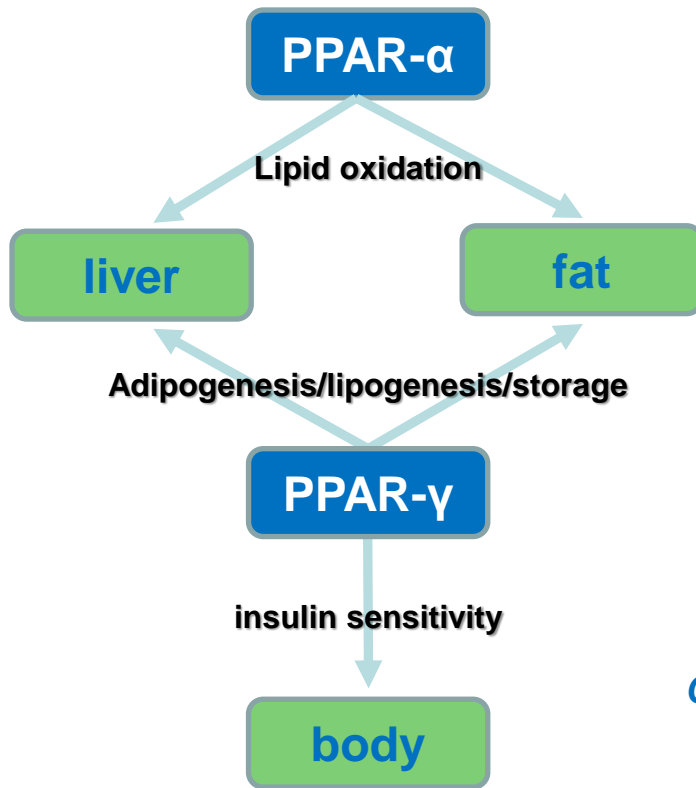
Pharma



Bio-based
economy

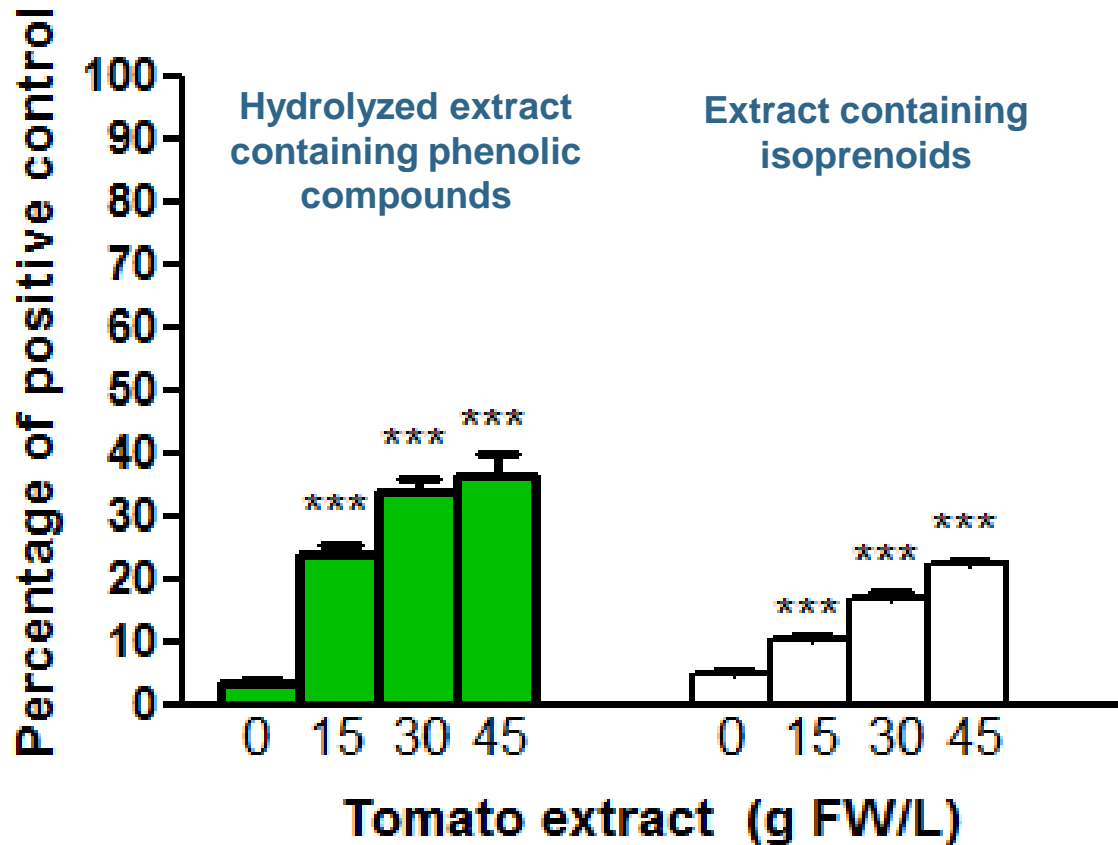


Chemical
safety

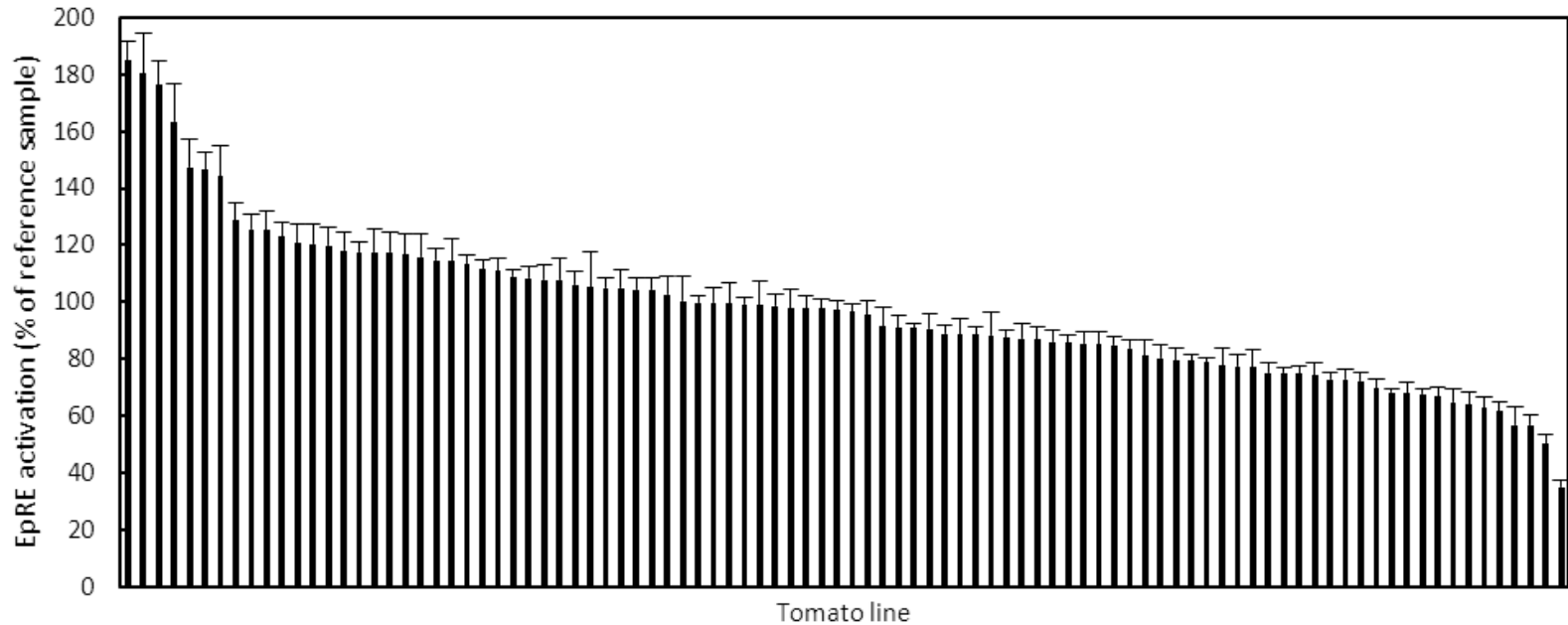


Gijsbers et al., 2011 Analytical Biochemistry 414:77-83

PPAR γ activation by tomato extracts



Reporter gene assay based optimisation of breeding strategies



Different tomato varieties vary in their potential for Nrf2 activation



Environment; complex mixtures

Ingredients



Toxic waste



Chemicals



Pharmaceuticals

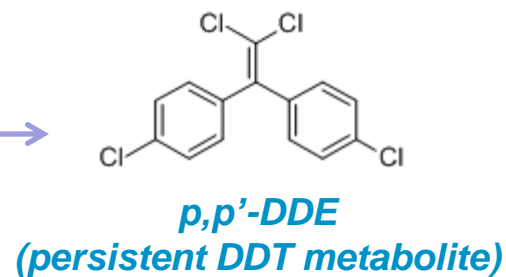
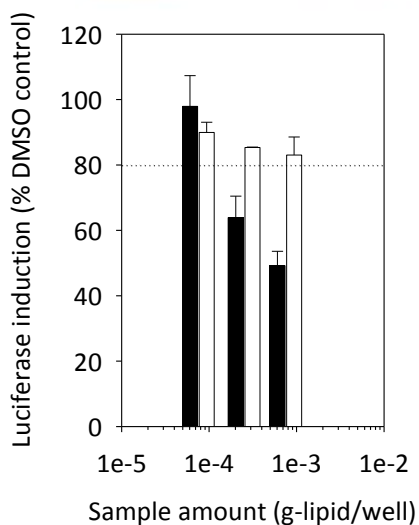


Toxins



How to assess hazard?

Occurrence and identification of androgen receptor antagonists in high trophic-level animals



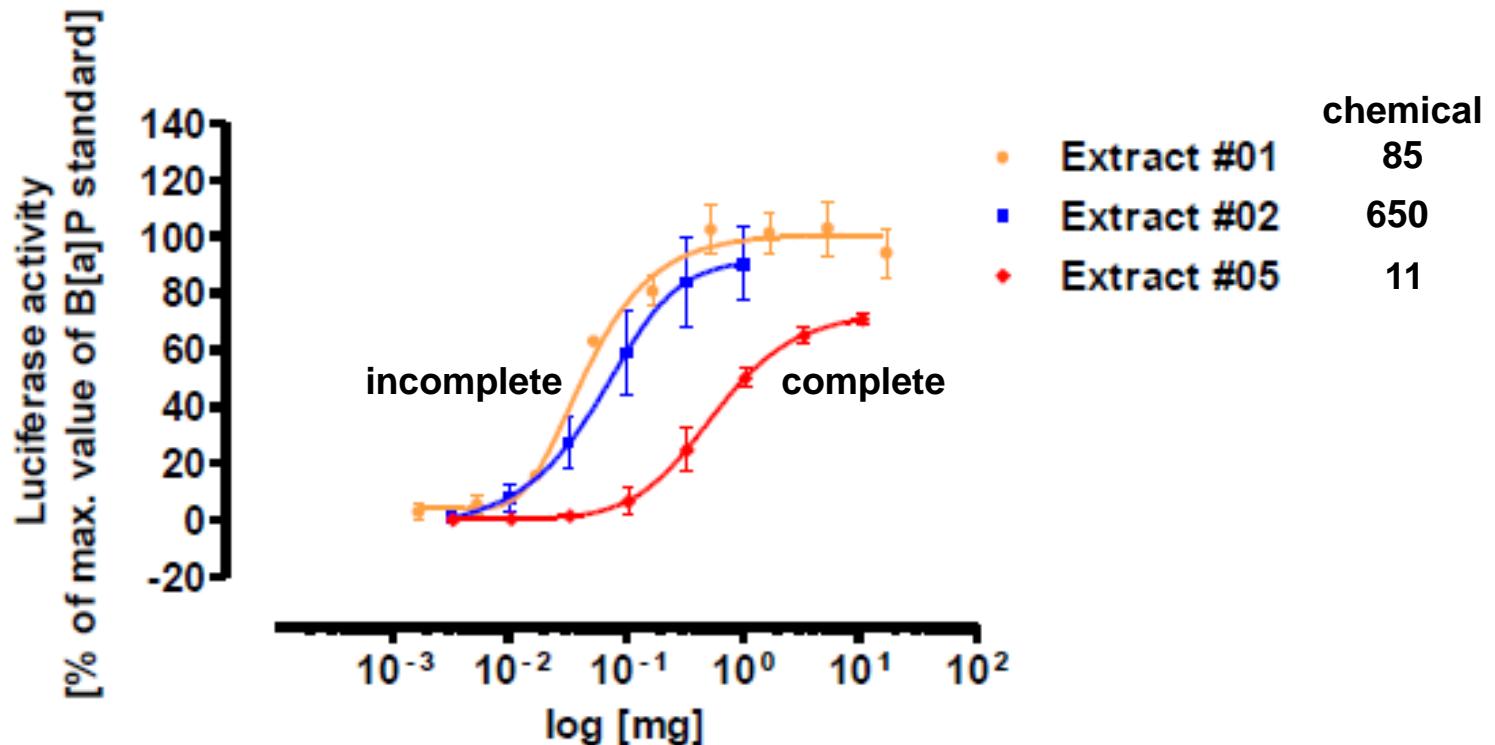
Characterization of biologically available wood combustion particles in cell culture medium

Susanne Gauggel, Cassandra Derreza-Greven, Julia Wimmer, Mark Wingfield, Bart van der Burg and Daniel R Dietrich



*Human and Environmental
Toxicology, University of
Konstanz, Konstanz, Germany*

Distinction complete- and incomplete wood combustion in PAH CALUX



Chemical analysis poorly predicts dioxin-receptor-mediated toxicity of PAHs





Bio-based Ecologically Balanced Sustainable Industrial Chemistry

5/29/2012



Carbon-based compounds

(Dr. Hein Stam, DSM; Prof. dr. Gerrit Eggink, Wageningen UR)

Nitrogen-based specialties

(Prof. dr. Arnold Driessen, University of Groningen; Prof. dr. Isabel Arends, Delft University of Technology)

Bioconstructions

(Dr. Wouter van der Star, Deltares)

Recycling of rare resources

(under development)

Synthetic Biology

(Prof. dr. Bert Poolman, University of Groningen; Dr. Ton van Maris, Delft University of Technology)

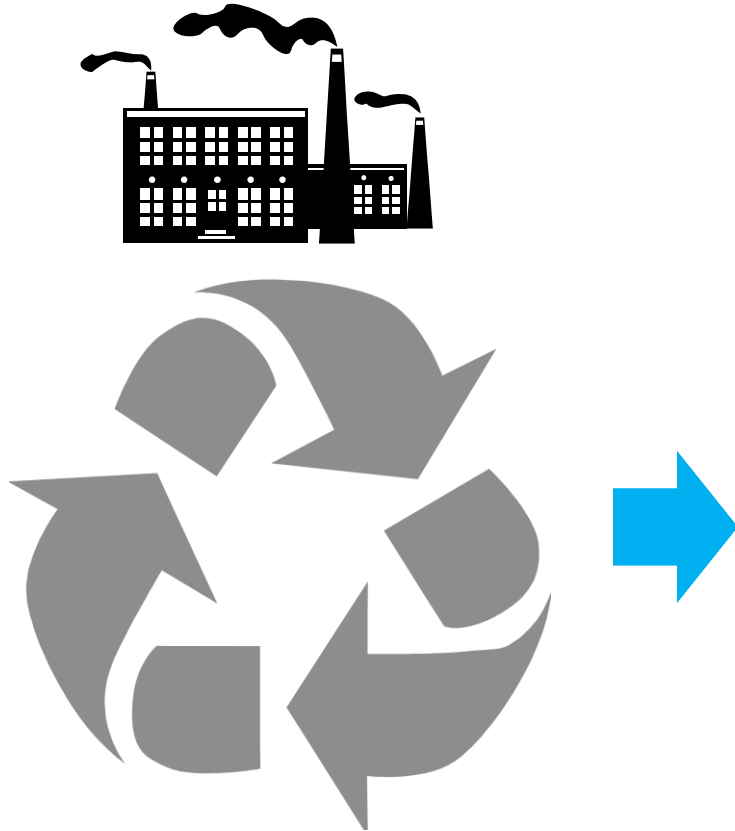
High-throughput experimentation and (meta)genomic mining

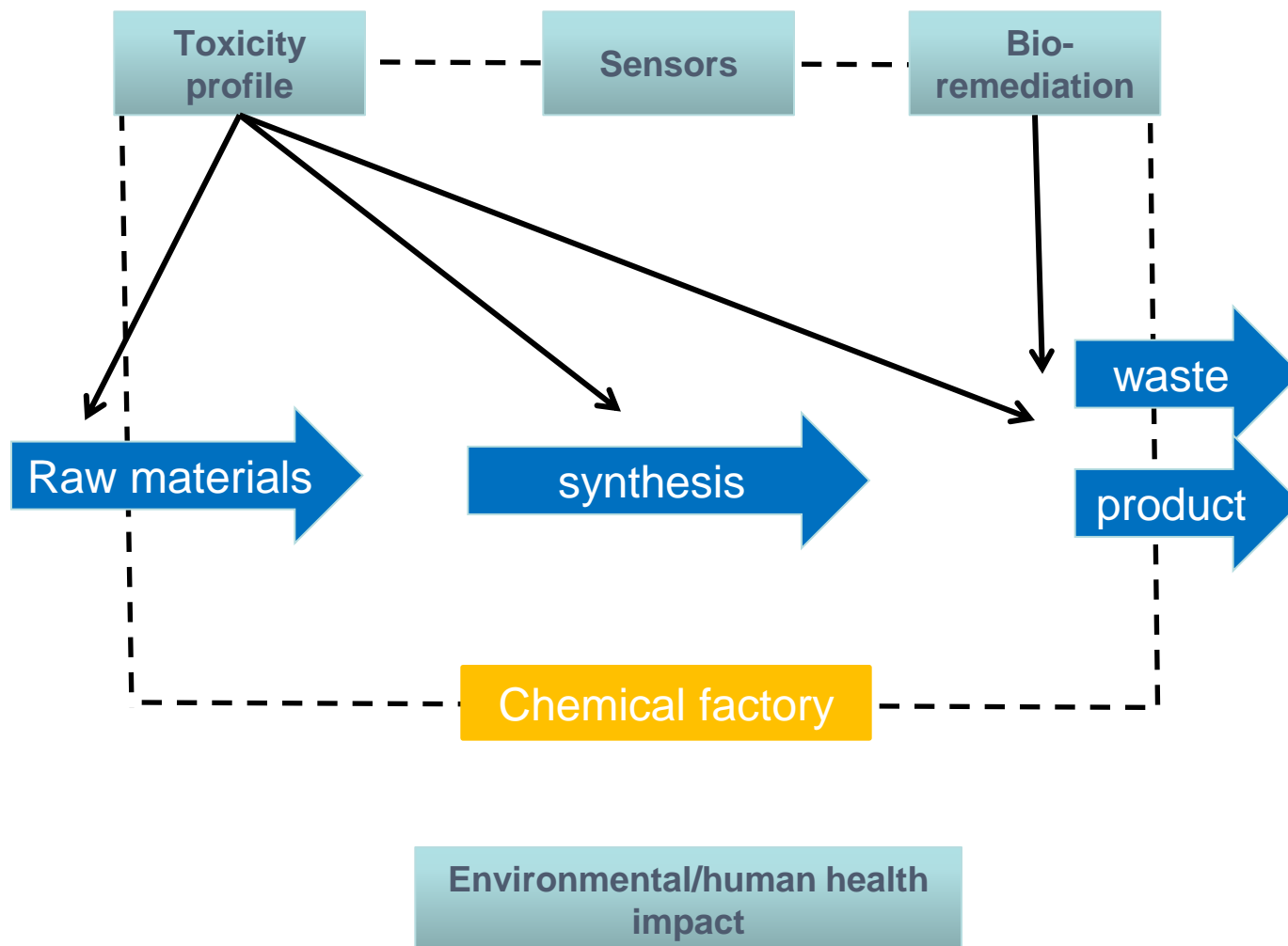
Environmental impact of chemicals, bio-based molecules and processes

(Dr. Bart van der Burg, BioDetection Systems; Prof. dr. Hauke Smidt, Wageningen UR)

Societal Embedding of a Biobased Economy

(Prof. dr. Patricia Osseweijer, Delft University of Technology)





- **Bioassays highly suited addition to current single chemical oriented monitoring programs**
- **Many mechanism-based molecular screening tools available**
 - **Screening models still to be developed further for several endpoints**
 - **Simplification/selection of panels needed and feasible**
 - **Extraction and workup important to distinguish between classes of compounds**
 - **Data analysis and interpretation key area of development**
- **Poor link to regulatory framework**



Acknowledgements

BDS R&D

Hai-Yen Man

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Lydia Jonker

Eszter Simon

Emiel Felzel

Harrie Besselink

Bart Pieterse

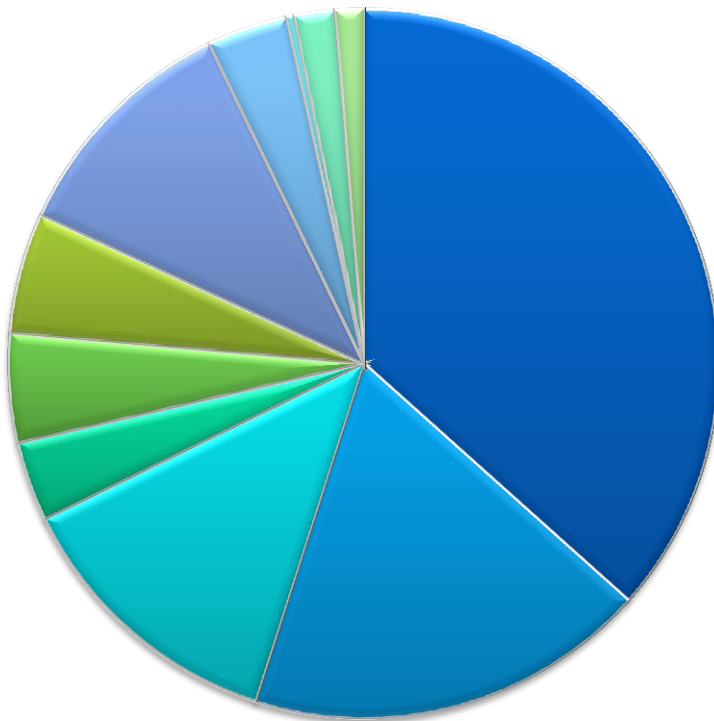
Thierry Janssen

Sander van der Linden

Barbara Lussenburg

Bart van der Burg

Bram Brouwer



- be-basic
- chemscreen
- ecolinc
- fnd
- metaexplore
- ntc
- plantlibra
- raak
- TI-pharma

