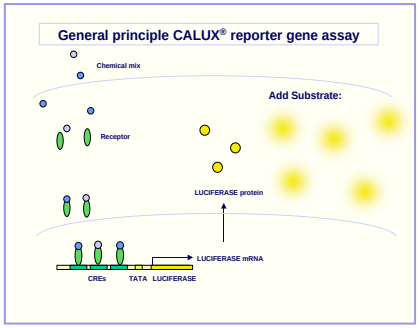
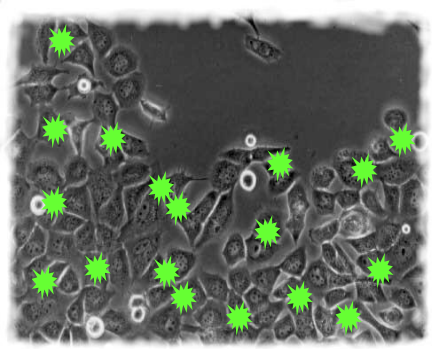
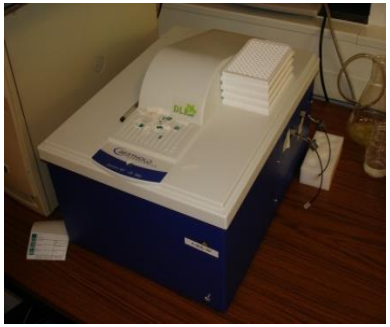
BioDetection Systems

State-of-the-art Biodetectors







Prof. Dr. Bram Brouwer
CEO of BioDetection Systems B.V

Professor of Environmental Toxicology VU University, Amsterdam

Turkey, Istanbul,
07-08 November 2013

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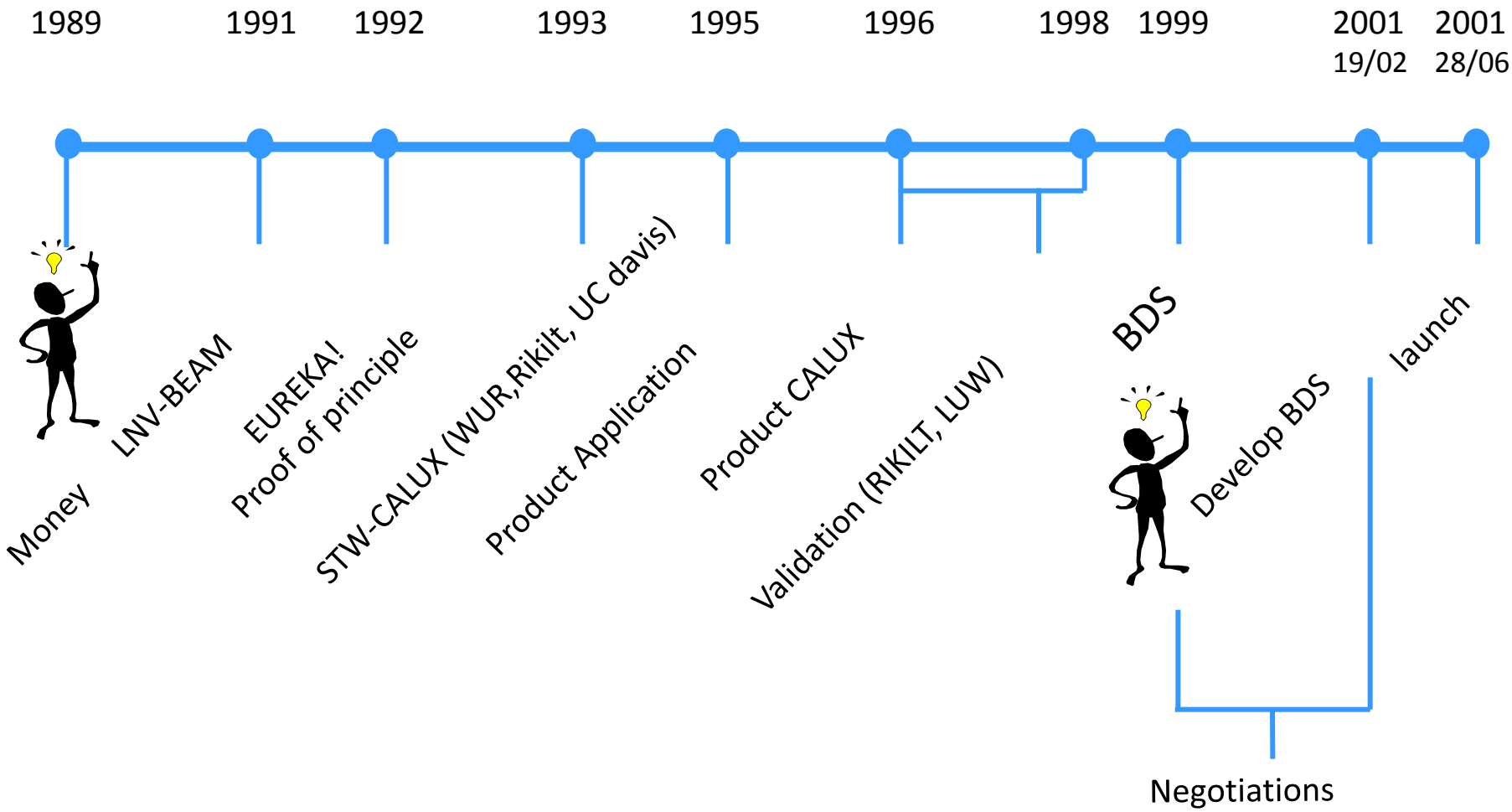
History: Idea for CALUX bioassay development started 25 years ago



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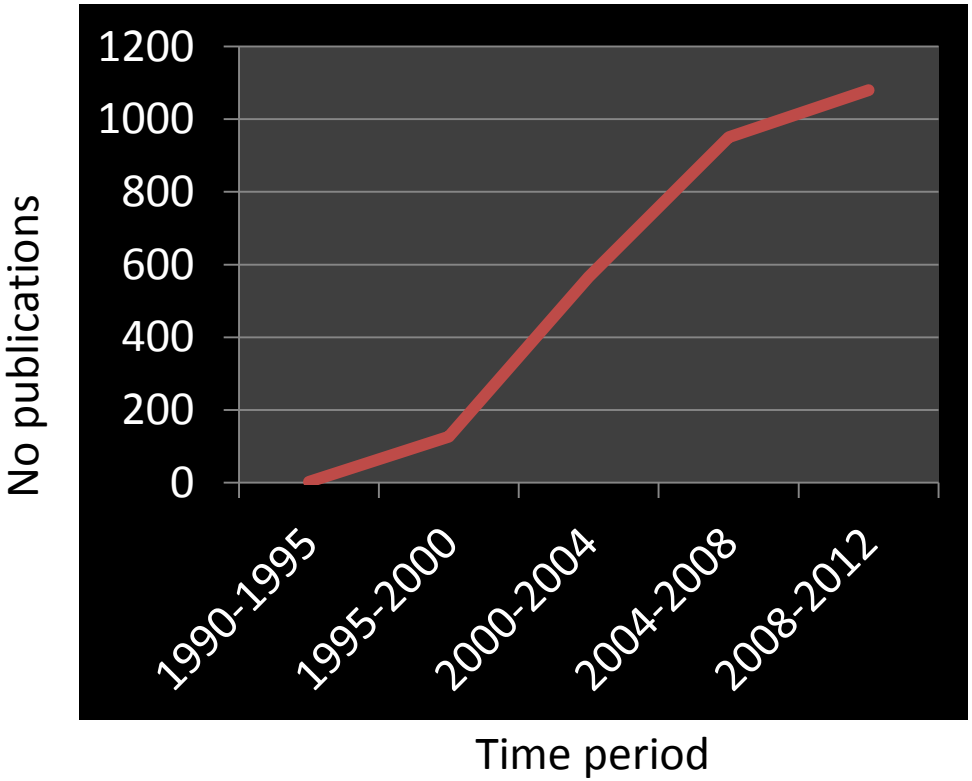
Increase in publications using CALUX bioassay(s)



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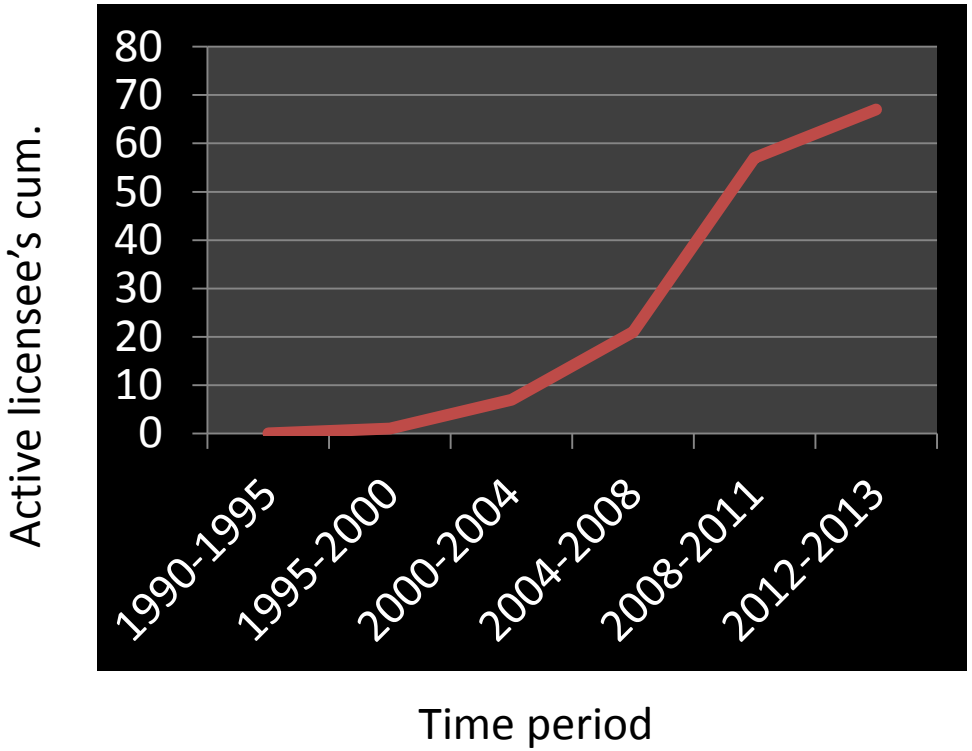
Increase no of active licensee's CALUX technologies



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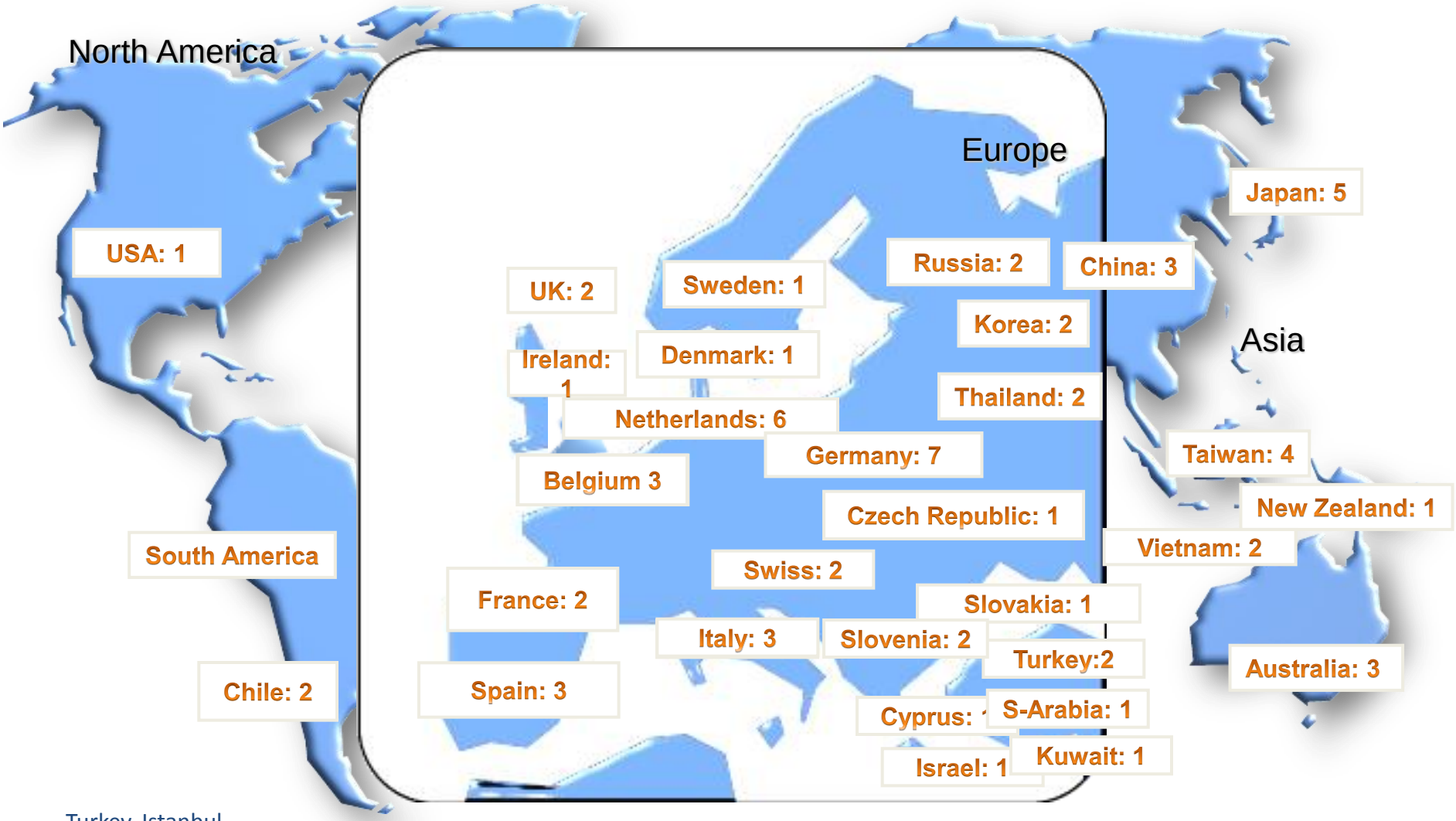
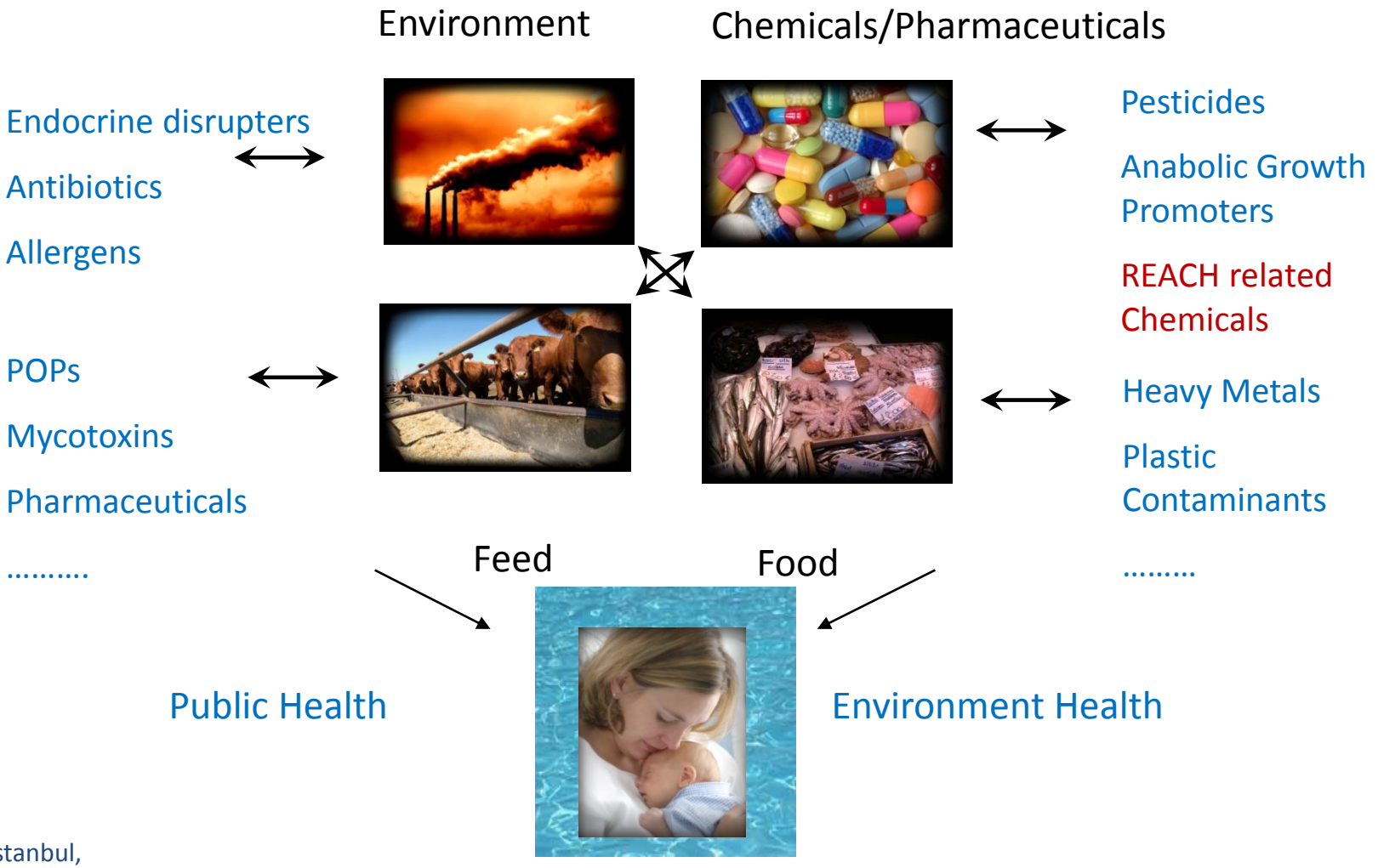
BDS global network of Licensee's

> 70



Why use Bioassays?

We are exposed to chemical cocktails rather than to single compounds



Why use Bioassays?

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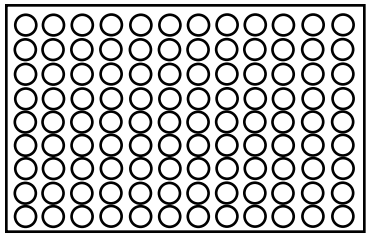
Dilemma: more and more compounds to be tested, how to manage the risks of mixtures?

Solution: Paradigm shift from chemical to effect oriented analysis:



Chemical oriented,
Compound by compound

Effect oriented,
complex mixtures

To know if our soup is safe, rather than what exactly is in it

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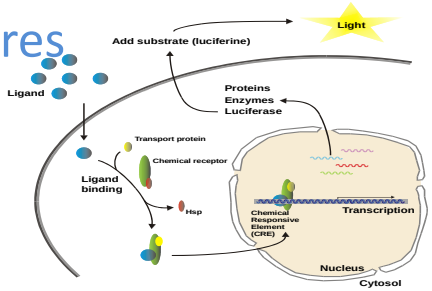
Why use Bioassays?

Modern in vitro bioassays should be:

- Designed in biologically relevant host
- Provide an integrated measure for complex mixtures
- Mechanism-based
- Predictive for hazard (risk) identification



Bioassays can be operated in an automated mode and are extremely useful for screening purposes

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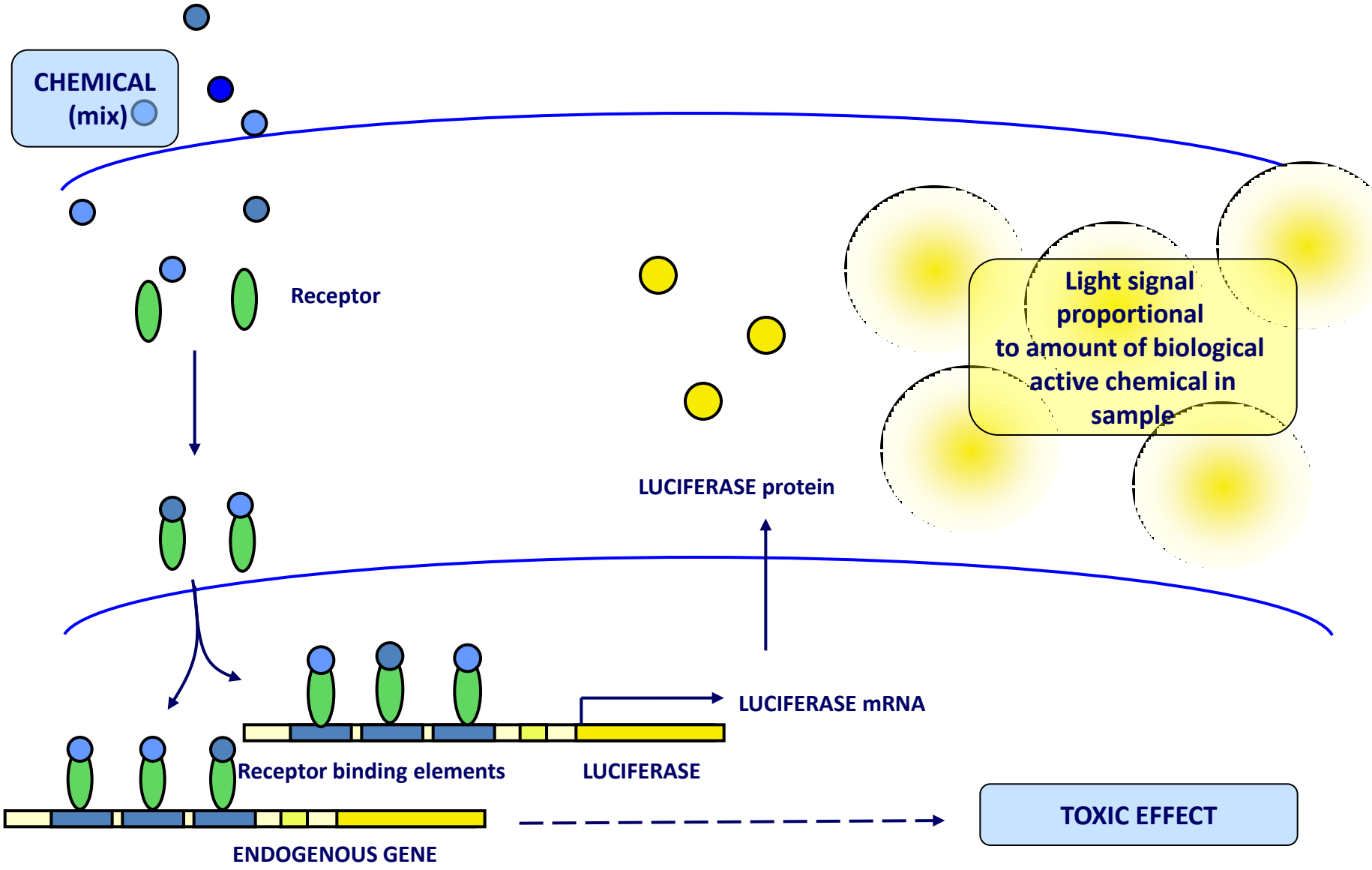
CALUX®: effect-based compound quantification



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Available CALUX® assays: list of important "mode of actions"

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	✓	U2OS	STAT CALUX	✓	U2OS			
PPARδ CALUX	✓	U2OS	Myc CALUX	✓	U2OS			
LXR CALUX	✓	U2OS	TGFbeta CALUX	✓	U2OS			
PXR CALUX	✓	U2OS	Metal CALUX	✓	T47D			
VDR CALUX	✓	U2OS						
MR CALUX	✓	U2OS						

CALUX: n=28

Agonist/antagonist: 25x2=56 assays

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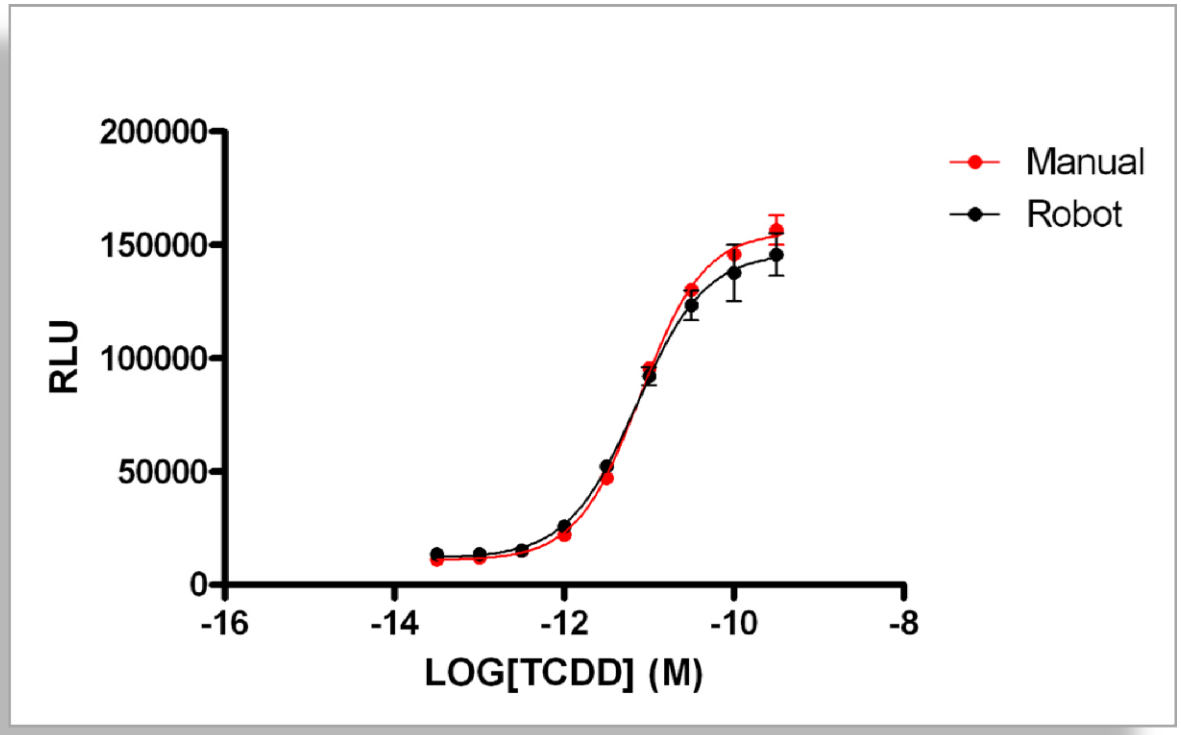
Bioassay automation



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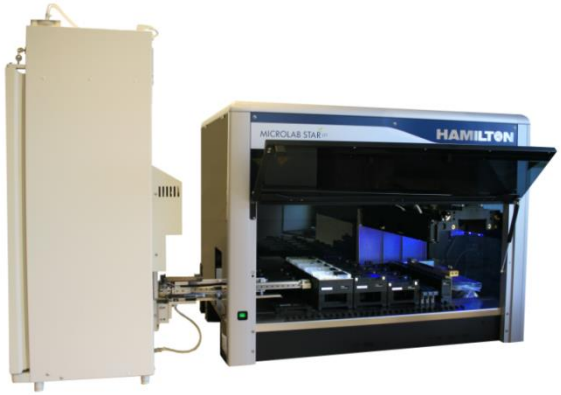

Quantitative HTS: hundreds of samples per week

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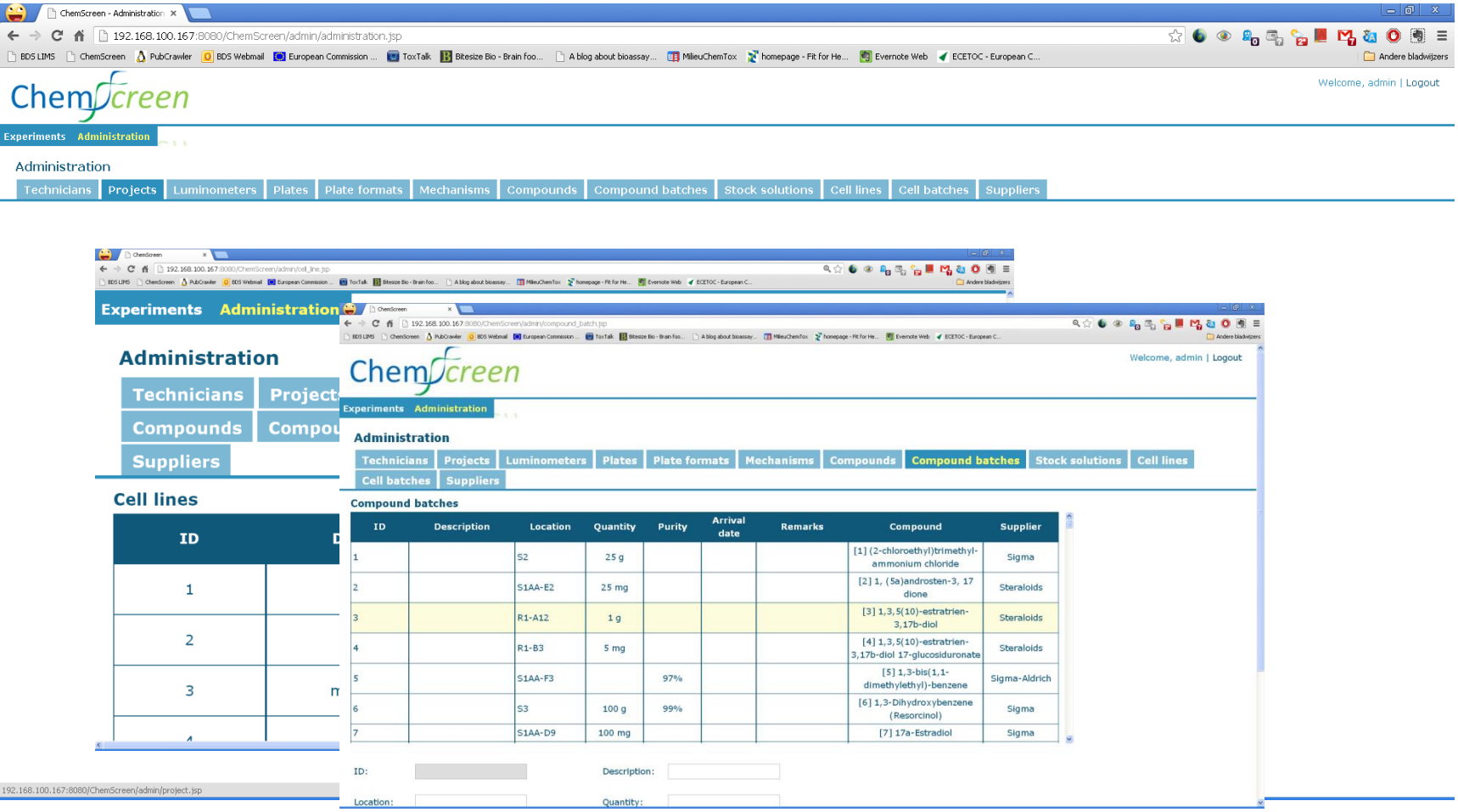
Data storage and calculation tool



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simple efficient solutions

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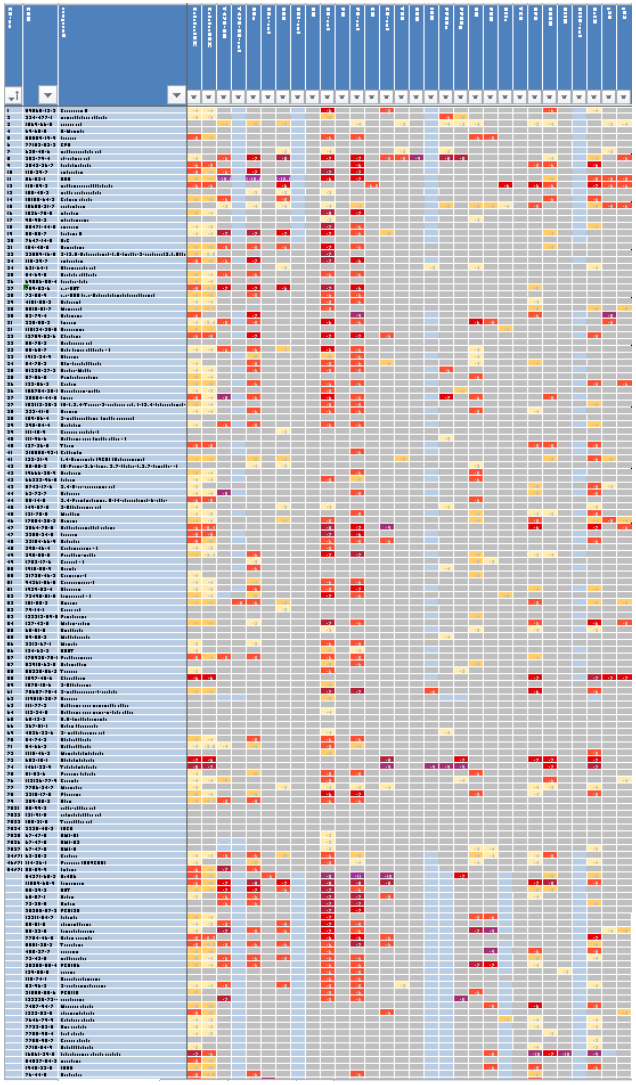
HTS profiling of pure compounds



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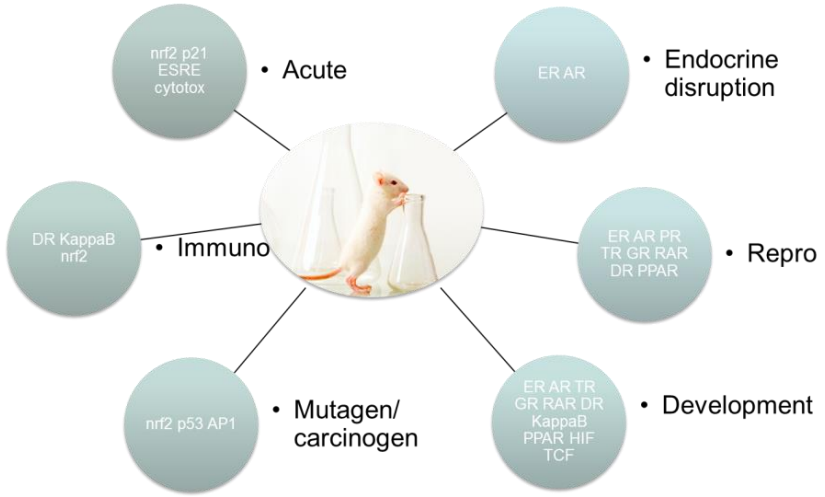

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Predictability of CALUX for Reproductive hazard (risk) identification

	COMPOUND	TOXICITY	EST diff	zebrafish	CALUX panel	CALUX with PBPK		cyp19	PREDICTION
1	Cyclosporin A (CSA)	developmental (immuno) toxicant	differentiation effect	no effect	anti AR, weak antiPR and GR and ESRE		ect	no effect	positive
2	Monoethylhexylphthalate (MEHP)	male reproductive organ malformations	differentiation effect	developmental toxicant	PPARg and PPARalpha agonist		ect	no effect	positive
3	Sodium valproate (VPA)	neurodevelopmental toxicant	differentiation effect	developmental toxicant	weakly positive in many assays, consistent with HDAC inhibition		ect	no effect	positive
4	D-mannitol (DML)	negative control	no effect	no effect	negative		ect	no effect	negative
5	Flusilazole (FLU)	craniofacial and axial skeletal malformations	differentiation effect	developmental toxicant	cytotoxic antiPR/antiGR weak DR/PAH		or	inhibitor at high conc in H295R	positive
6	Glufosinate ammonium (GPA)	neurodevelopmental toxicant	no effect	no effect	negative		ect	no effect	negative
7	Methoxyacetic acid (MAA)	growth and developmental retardation	differentiation effect	developmental toxicant	negative		ect	no effect	positive
8	Retinoic acid (RA)	neural crest cell migration affected	differentiation effect	developmental toxicant	strong RAR/RXR activity		ect	inhibitor in H295R	positive
9	Dioctyltin dichloride/ dichlorodioctylstannane(DOTC)	developmental (immuno)toxicant	cytotoxic	no effect	cytotoxic,antiprogestin, stress-related pathways		or 5R	inhibitor in H295R	positive
10	Endosulfan (ESF)	neurotoxicant	cytotoxic	developmental toxicant	cytotoxic, ER, antiAR, antiGR		ect	inhibitor in H295R	positive
11	Diethylstilbestrol (DES)	transplacental carcinogen	cytotoxic	developmental toxicant	strong estrogen: antiAR, antiPR, stress- and genotoxicity pathways		or an	no effect	positive
12	Methylmercury chloride (MMC)	neurodevelopmental toxicant	cytotoxic	developmental toxicant	stress-related pathways affected, estrogen, GR agonist		or an	Inducer in H295R, inhibitor in HPMs	positive

Piersma et al. 2013
Reprod Toxicol. 38:53-64.

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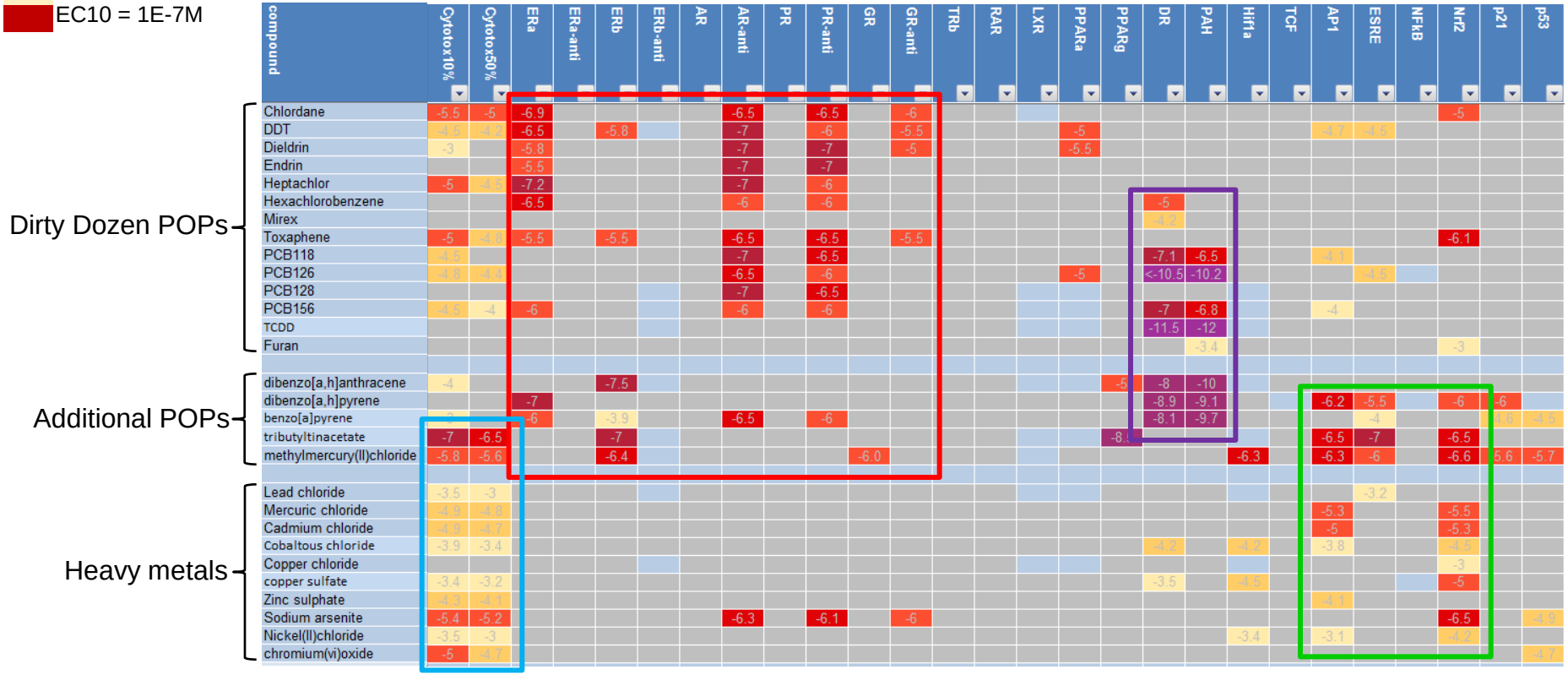
Toxicity pathways activated by environmental chemicals

no activity
EC10 = 1E-3M
EC10 = 1E-7M



Dirty Dozen POPs: endocrine | dioxin receptor

Additional POPs: dioxin receptor | stress pathways

Heavy metals: acute toxicity | stress pathways

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Identification of Green Chemicals

compound	Cytotox10%	Cytotox50%	ERa	ERa-anti	ERb	ERb-anti	AR	AR-anti	PR	PR-anti	GR	GR-anti	TRb	RAR	PPARa	PPARg	DR	PAH	Hif1a	TCF	AP1	ESRE	NFkB	Nrf2	p21	p53
bisphenol A	-4	-3.7	-7.3		-6.8			-6.8		-5.5		-4.5										-4.3				
Butyl benzyl phthalate	-3.9	-3.5	-5.7		-4.4			-6.1		-5.7							-3.7									
Di(n-hexyl)phthalate	-3.5		-5					-5		-5.5		-4.5					-4				-4.2					
Dibutylphthalate	-4.5	-4	-5.2					-5.5		-5.5																
Diethylphthalate	-3.5	-3.0	-4.3					-5		-4.3																
Diisobutyl phthalate	-4.5	-4	-5.7					-6		-6																
Nonylphenol	-4.9	-4.7	-5.1		-5.6			-6.5		-5.5												-4.6				
FDCA																										

Case study: CALUX panel identifies FDCA as a potentially non-toxic alternative to current plastic ingredients/building blocks

bird engineering

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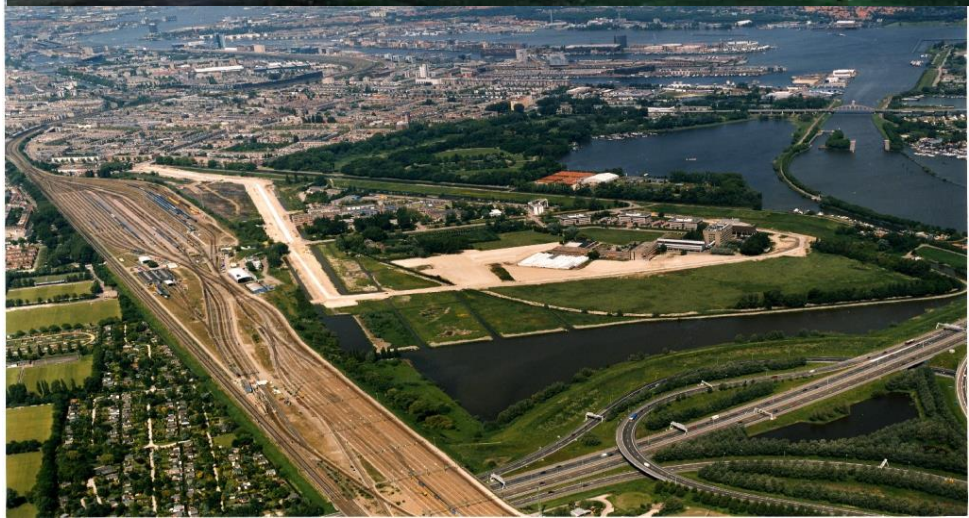
BDS Amsterdam: Thank you for your attention!



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