BioDetection Systems

Quantitative high throughput screening methodologies to assess biological activity of chemicals and water samples; their use in the context of REACH and the Water Framework Directive

Bart van der Burg

Chemscreen

DEMEAU
Demonstration of promising technologies to address emerging pollutants in water and waste water

BE-Basic Foundation

BioDetection Systems

Focus:

develops, markets and applies bio-based detection methods for safety assessment and quality control chemicals and pharmaceuticals, bio-based materials, food, water and the environment

Activities:

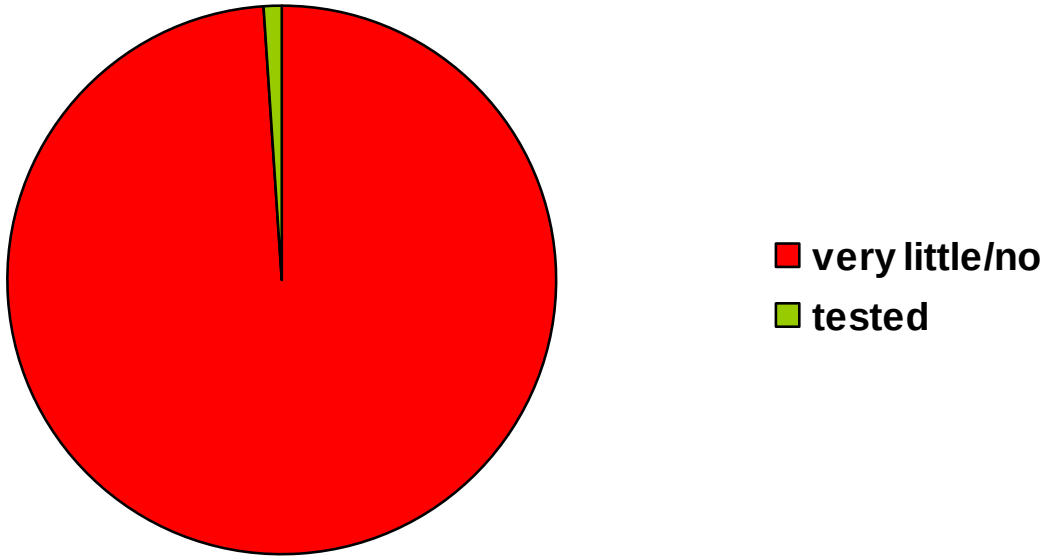
- **Analytical services**
- **Licenses**
- **Training**
- **Research and Development**
- **Consultancy**

RvA TESTING RvA L 401

ISO 17025

International marketing network

In vivo bioassays in animals: problem speed and capacity



- *100,106 industrial chemicals on the market in 1981 (“existing substances”):*
- *1% tested on toxicity!*
- *This lead to new legislation: REACH*

Chemscreen

Chemical substance in vitro/in silico screening system to predict human- and ecotoxicological effects

- **Generation of a simple, rapid screening system for reprotoxic effects of chemicals**

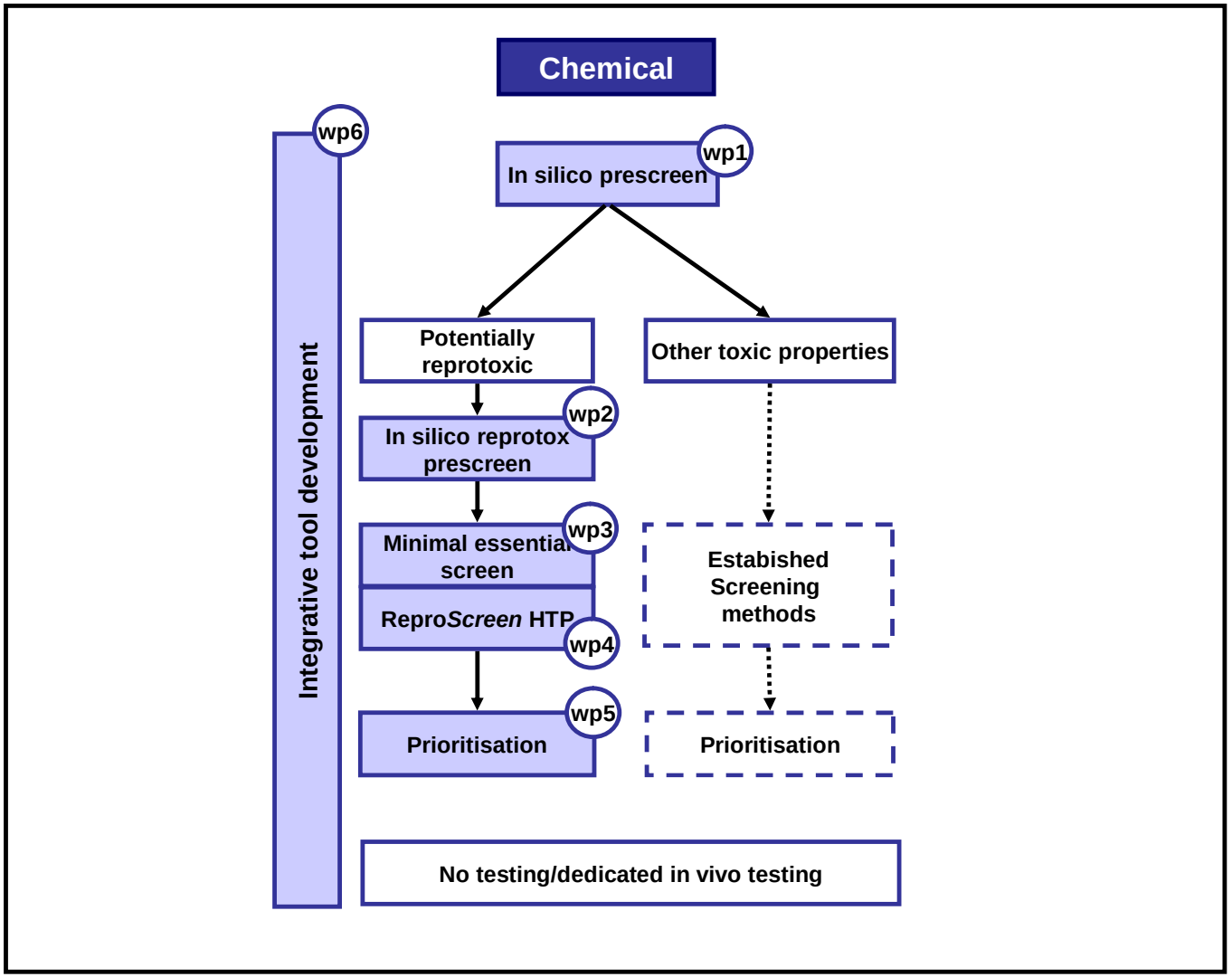
- **widespread implementation**
  suitable for regulatory purposes
  within the tight time schedule of the REACH program
  mechanistic base
  OECD/ECVAM validated methods as anchors/quality control
  cost effective and transferable

SEVENTH FRAMEWORK PROGRAMME

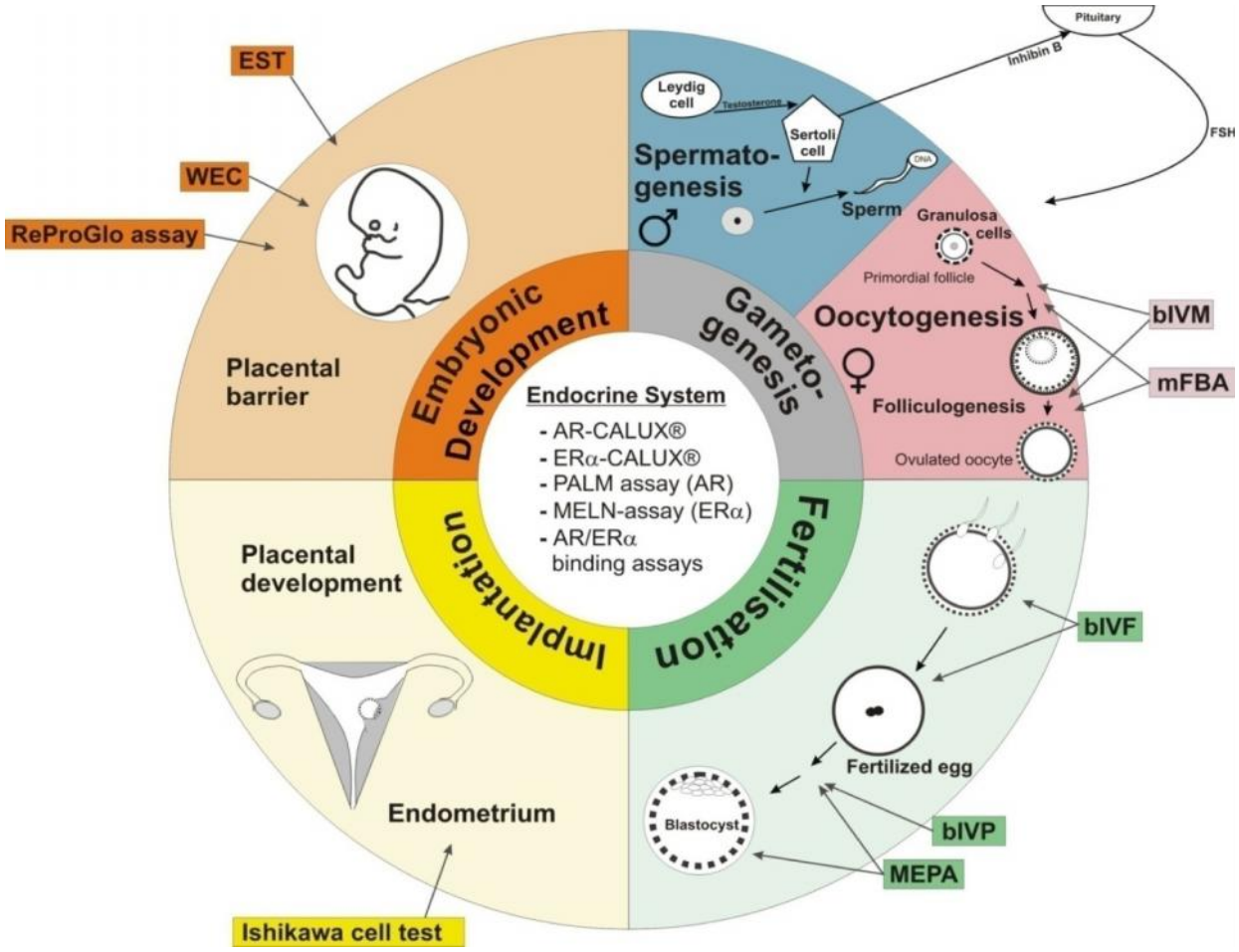
Why reprotox?

- **Prioritised in REACH**
- **Reproductive toxicity is important to assess both human and environmental toxicity**
- **Uses the most animals in toxicity testing**
- **Very little alternative methods**

ITS-like work package structure



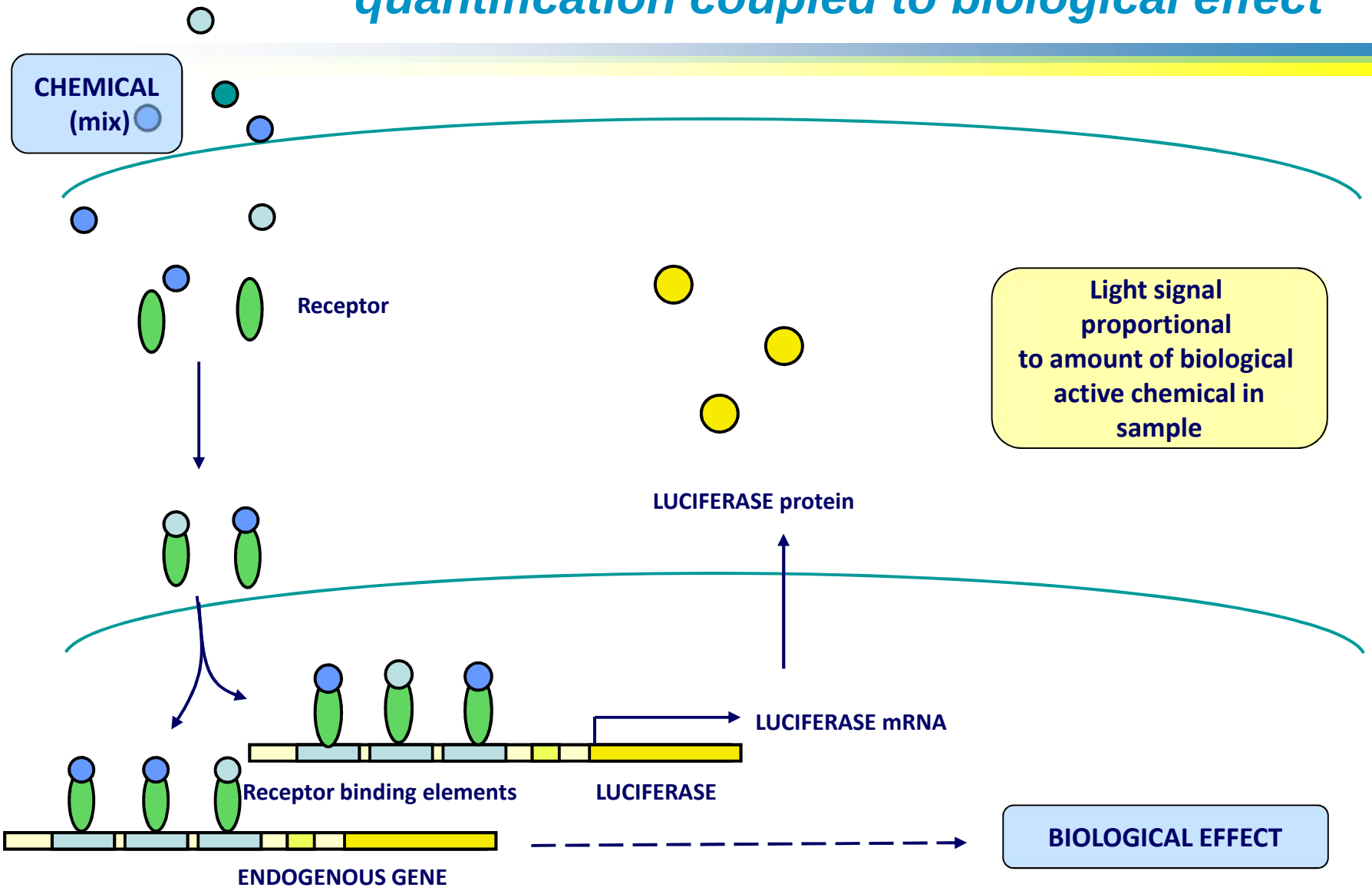
ChemScreen; in vitro screening



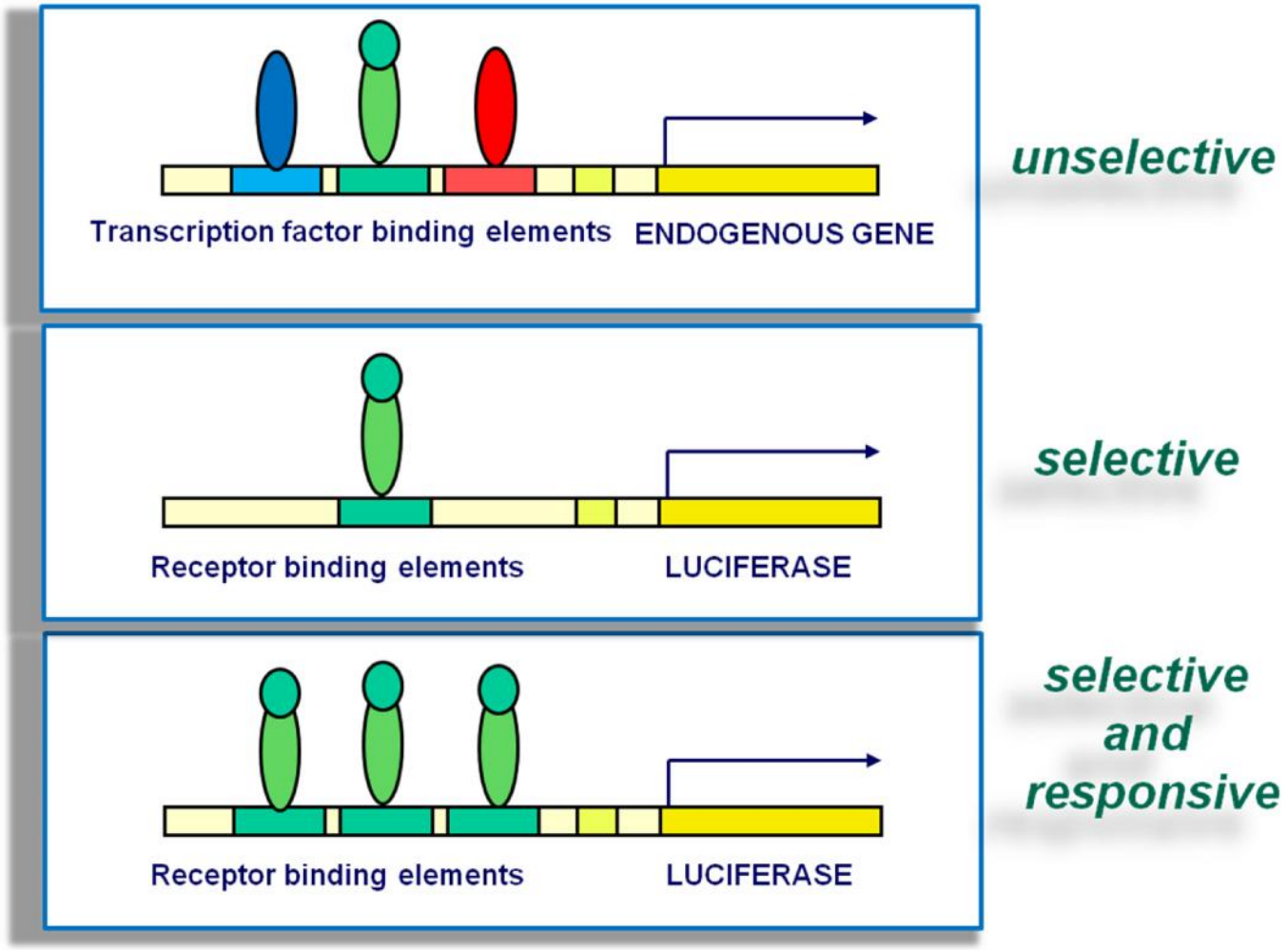
- ✓ ***ReProTect:***
  Complexity reproductive cycle be captured with a limited amount of apical tests
- ✓ ***ChemScreen:***
  Is further simplification/higher throughput possible & can we use these methods in a regulatory context?

Schenk et al. 2010 Reproductive Toxicology 30, 200-218

Rapid in vitro bioassays: CALUX® chemical quantification coupled to biological effect



CALUX human pathway selective and responsive reporter gene assays



Legler et al (1999) Toxicological Sciences 48, 55-66.

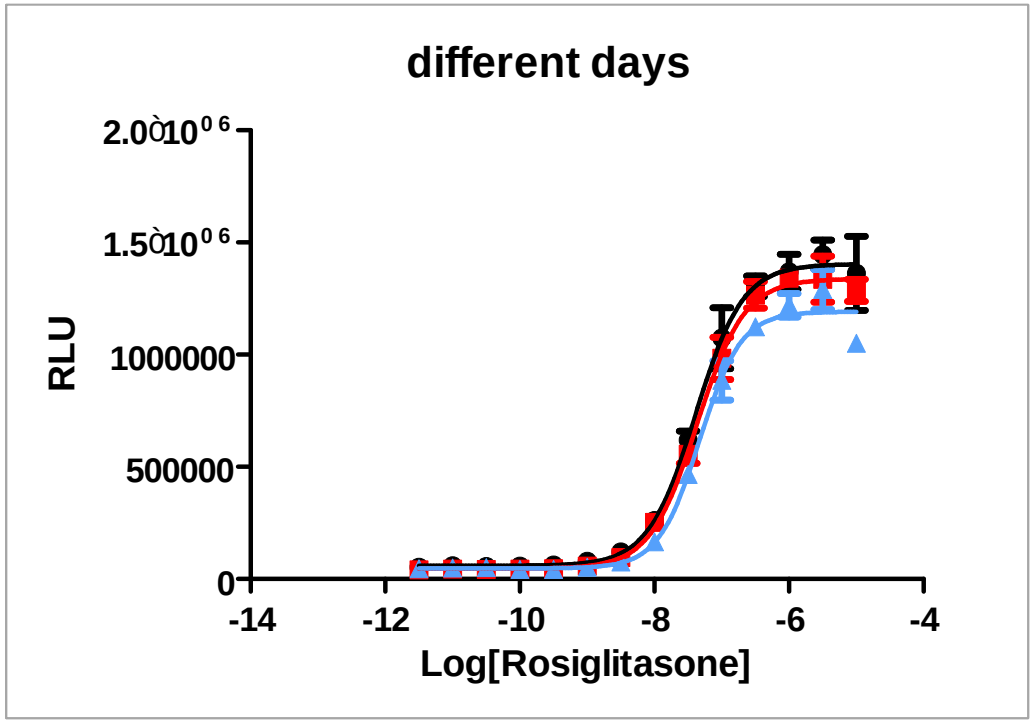
CALUX® panel approach

Advantages low background, high selectivity and inducibility:

- **High sensitivity**
- **Better 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**

Chemscreen

Automation

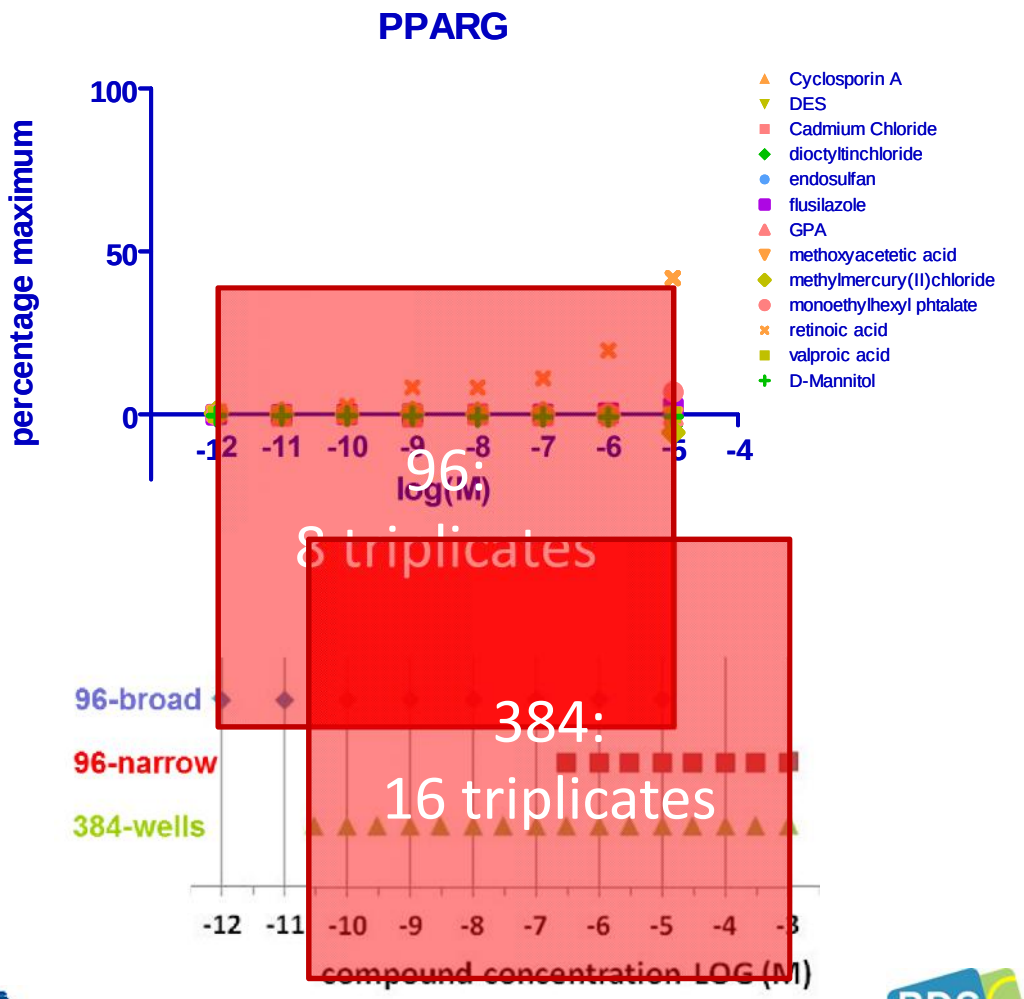


BDS
BioDetection Systems

Quantitative HTS in 384 wells:
hundreds of dose-response curves per day

SEVENTH FRAMEWORK PROGRAMME

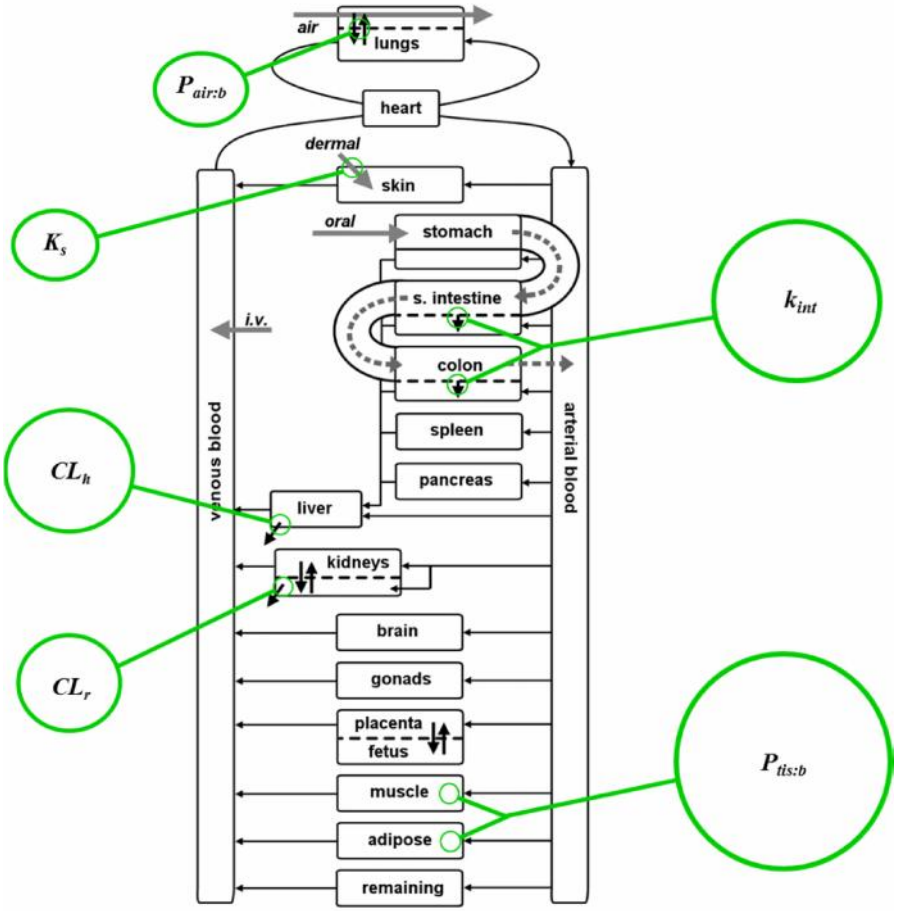
Example screening result



- ***Format 96 > 384 wells***
- ***Use frozen cells***
- ***Expansion-dose response: automated potency determination***

BioDetection Systems

ChemScreen HTS PBPK model



- **Many compartments, processes included, but…**
- **Minimal input required:**
  - **High Throughput PK:**
    - **fraction unbound (fu)**
    - **hepatic clearance ($CL_{h,int}$)**
    - **intestinal permeability (P_{app})**
  - ***in silico* (QPPR):**
    - **logP, ionization**
  - **Default assumptions: CL_r**

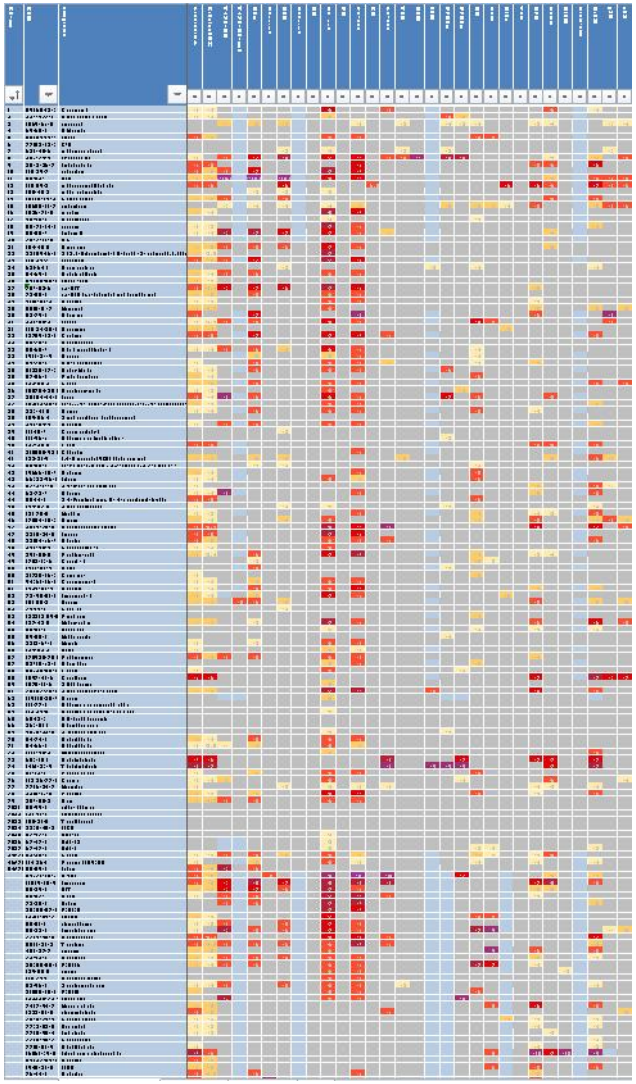
Battery performance :Feasibility study 1

compound	Toxicity in vivo	EST	ZET	ReProGlo	Cyp17	Cyp19	CALUX	CALUX PBPK	battery
Cyclosporin A									
Monoethylhexyl phthalate									
Sodium valproate									
D-mannitol									
Flusilazole									
Glufosinate ammonium									
Methoxy acetic acid									
Retinoic acid									
Dioctyltin chloride									
Endosulfan									
Diethylstilbestrol									
Methylmercury chloride									

- ✓ **Correct prediction 11/12 compounds**
- ✓ **Like in ReProTect Glufosinate missed: mechanism bypassed in culture**
- ✓ **PBPK modeling improves predictions (e.g. in CALUX)**
- ✓ **Simple HTS model same prediction as EST/ZET**

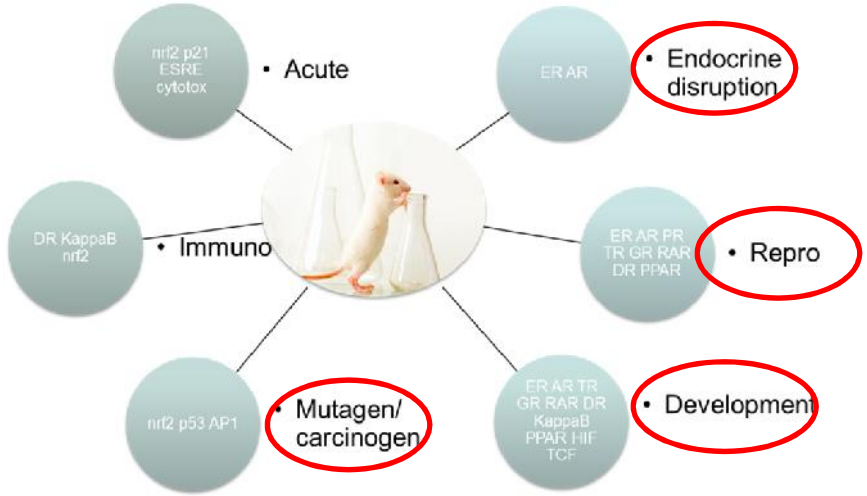
Piersma et al. 2013 Reproductive Toxicology

In vivo toxicity prediction possible?



Identify subsets of assays to predict specific types of toxicity

?



Dominant responses: antiandrogenic, antiprogestagenic estrogenic

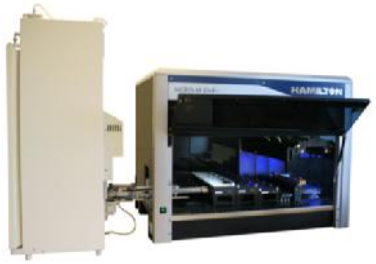
Grouping & read across: feasibility study 2

FeDTex
278
chemicals

ERalpha/AR
CALUX
HTS screen
220
chemicals

89 chemical categories with OECD (Q)SAR Toolbox

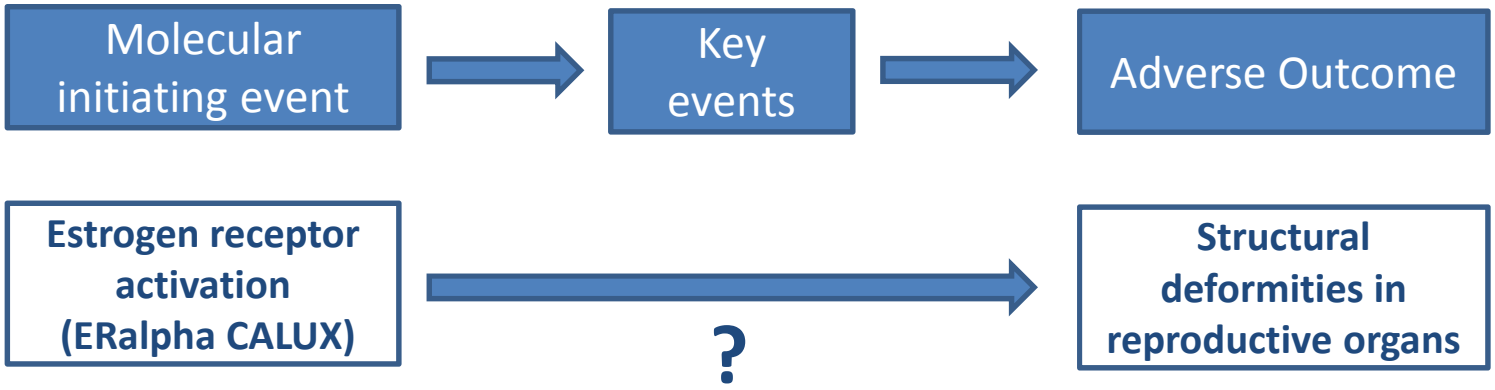
LOEL any effect on reproductive targets males and females



1. No chemical category associated with reproductive effects
2. Hormonal compounds in almost any chemical category
3. Effect concentrations in vitro (i.e. biological category) correlated well with LOELs in vivo (R^2=0.71; without PBPK)

Lewin et al., Reproductive Toxicology, submitted

AOP application: feasibility study 3



Compounds	overall accuracy (%)	correct/total
all (NTD+RO, controls)	66	25/38
Neural tube defects	47	9/19
Reproductive organ deformities	84	16/19

- ✓ **Estrogen receptor activation in ERalpha CALUX clearly links to structural deformities in reproductive organs**

Van der Burg et al., Reproductive Toxicology, submitted

Example read across

CAS	compound	CALUX Cytotox10%	CALUX Cytotox50%	ERalpha	ERalpha-anti	Erbeta	ERbeta-anti	AR	AR-anti	PR	PR-anti	GR	GR-anti	TRb	RAR	LXR	PPARa	PPARg	DR	PAH	Hif1a	AP1	ESRE	Nrf2	p21	p53	CALUX prediction
1118-46-3	Monobutyltinchloride								-4,5															-5			
683-18-1	Dibutyltindichloride	-6,5	-6,3										-7,5					-6,5				-6,7	-6,8	-6,8			
1461-22-9	Tributyltinchloride	-7,5	-7,2										-8,5			-8,7	-8,5	-8,5					-6,9	-7,2			

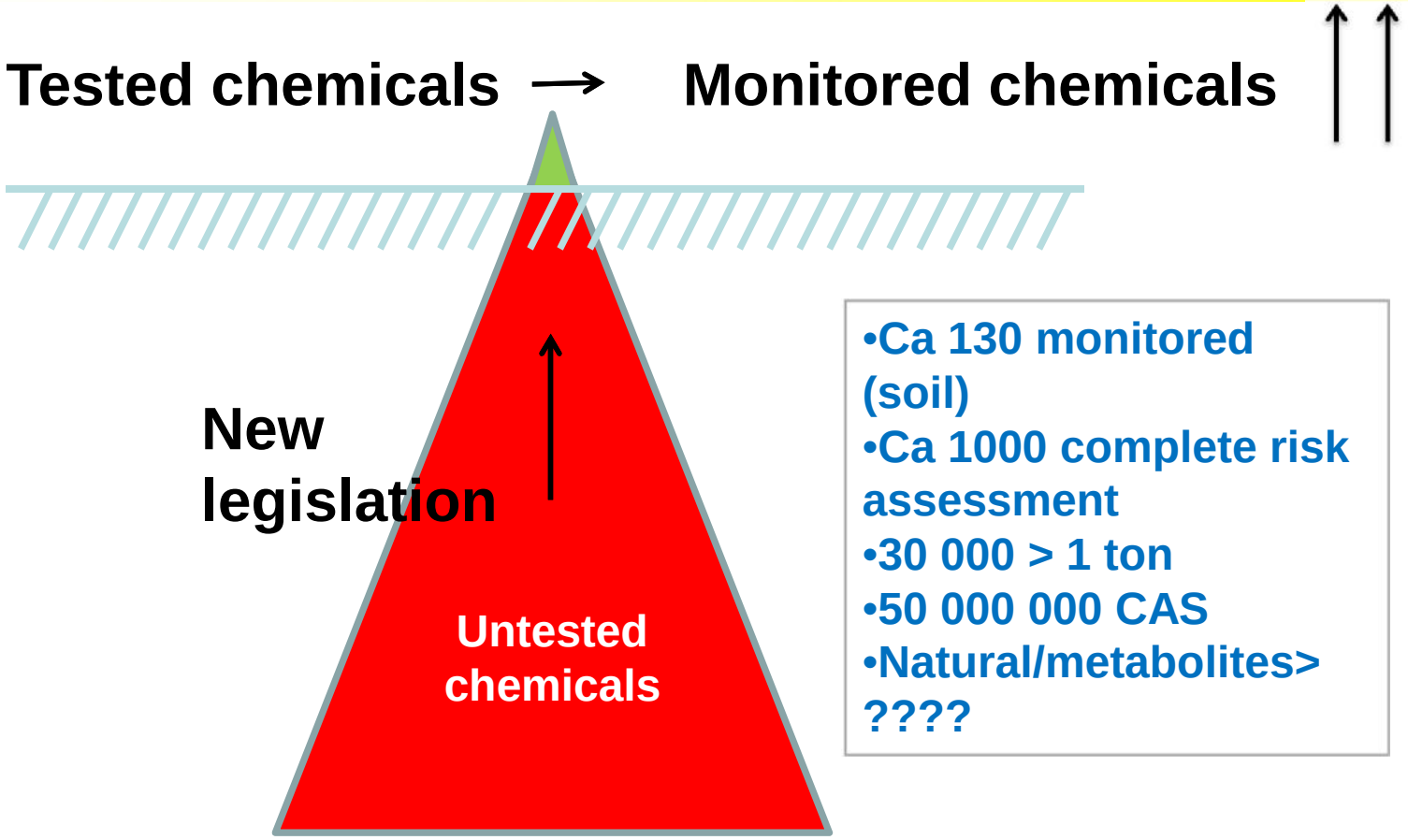
- ✓ **Examples with 3 chemical classes (Alkyl alkanoic acids, phthalates, organotin chlorides); all three successful**
- ✓ **Read-across used in approx. 30% reproductive tox dossiers (100-1000TPA) in REACH (ECHA 2014)**
  (new testing proposal only few %)

Kroese et al., Reproductive Toxicology, submitted

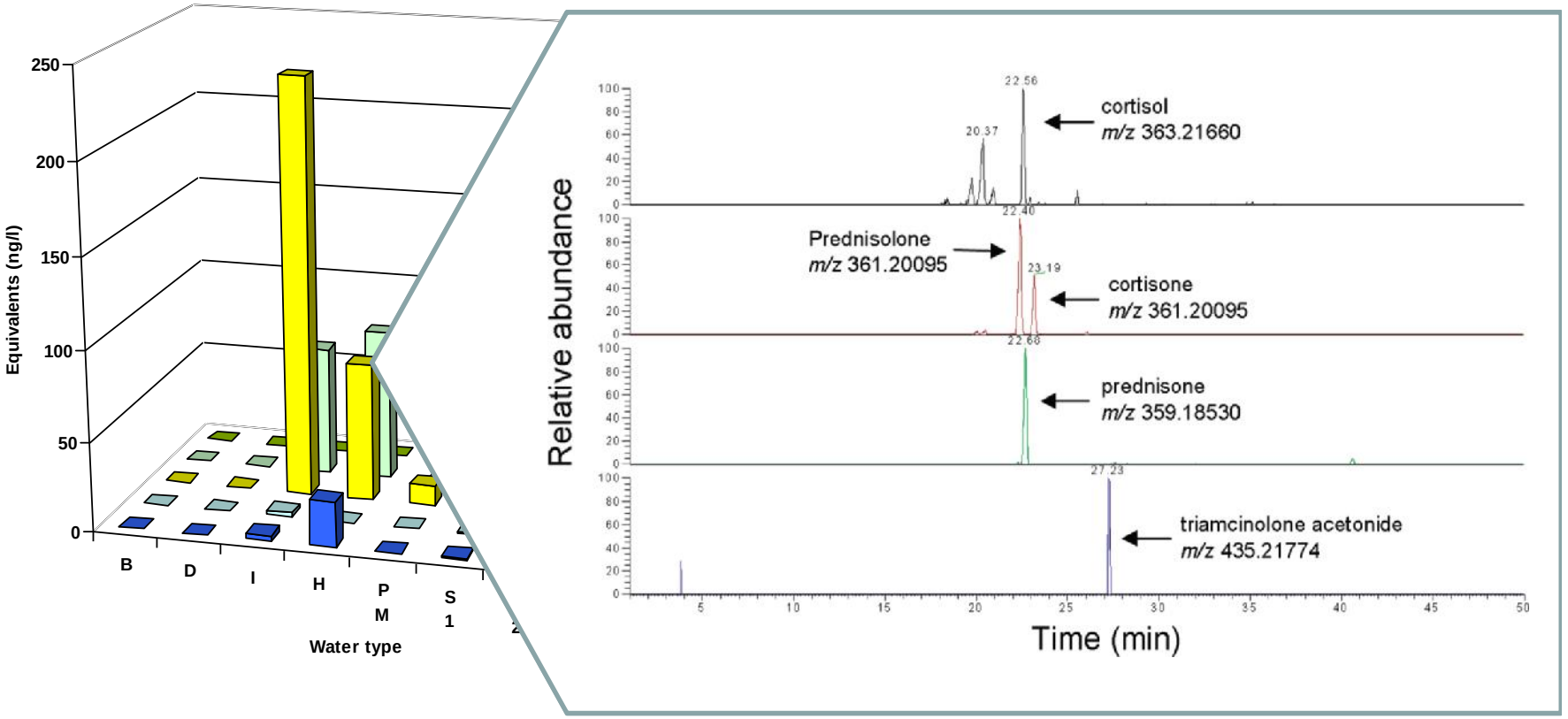
Fields of application CALUX® battery

Major challenge: risk assessment of complex mixtures

Chemical monitoring alone insufficient



Effect profile water samples with CALUX® cells

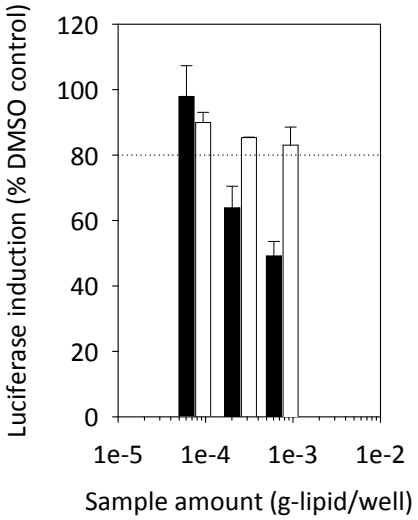


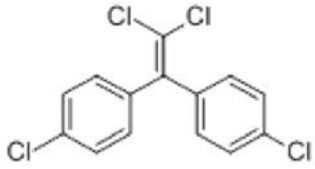
Selection assays:

- ***Priority effects & compound groups***
- ***Overlapping chemical domains of assays: hot spots of activity?***

Van der Linden et al. 2008, Environ. Sci. Technol. 42: 5814;
Schriks et al. 2010, Environ. Sci. Technol. 44: 4766-74.

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





p,p'-DDE
(persistent DDT metabolite)

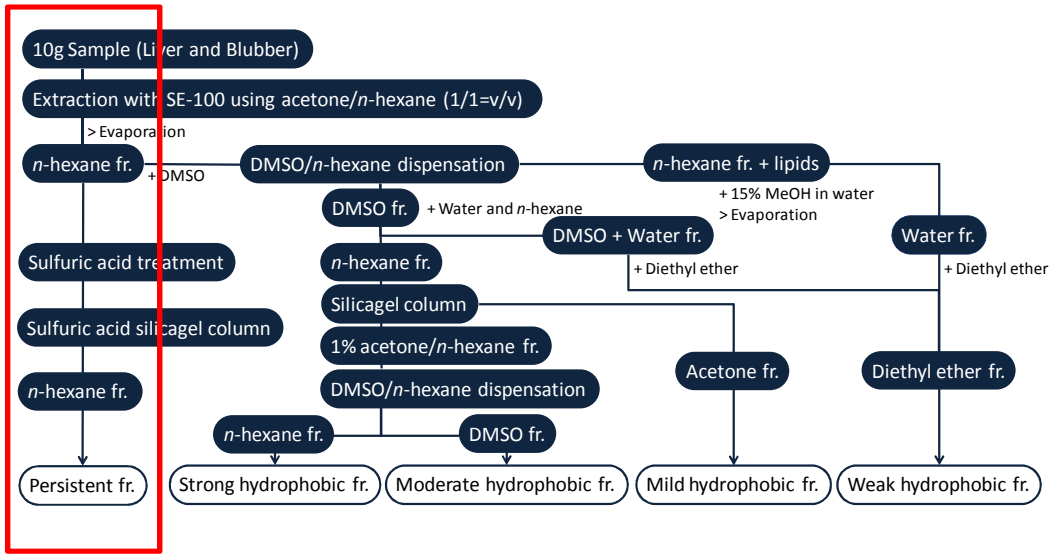
EHIME UNIVERSITY

Suzuki et al. 2011 Environ. Sci. Technol. ePub 18 Oct

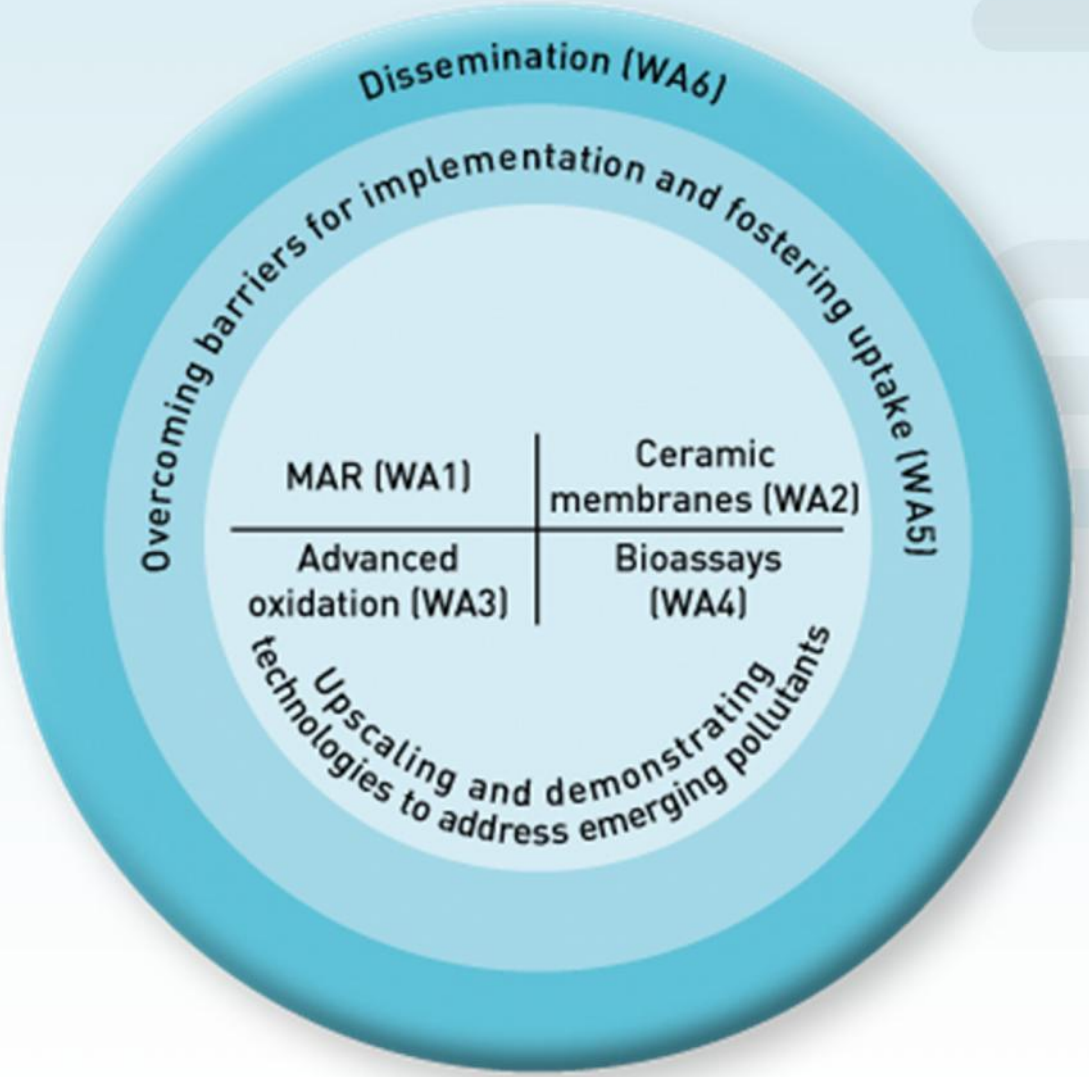
Bioactivities in persistent hydrophobic fractions

	Androgens		Estrogens		Glucocorticoids		Progestins		Dioxins	
	ago	antago	ago	antago	ago	antago	ago	antago	ago	antago
Baikal seal blubber 2005										
Baikal seal blubber 1992										
Baikal seal liver 2005										
Baikal seal liver 1992										
Common cormorant liver										
Raccoon dog liver										
Finless porpoise liver										

10 mg/well
1.0 mg/well
0.1mg/well
0.01 mg/well



Suzuki et al. 2011 Environ. Sci. Technol. ePub 18 Oct


Dissemination (WA6)
Overcoming barriers for implementation and fostering uptake (WA5)
MAR (WA1)
Ceramic membranes (WA2)
Advanced oxidation (WA3)
Bioassays (WA4)
Upscaling and demonstrating technologies to address emerging pollutants

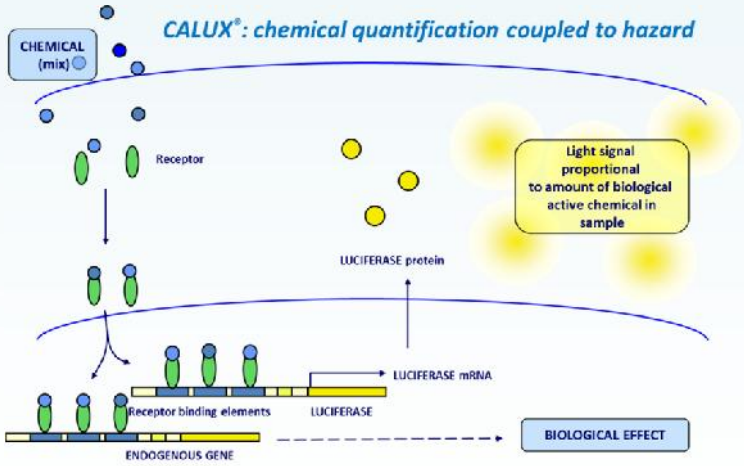
WA 4 Implementation of bioassays

4.1 Selection and validation

- Selection criteria
- Bioassay selection
- Automation
- Trigger values
- Validation

4.2 Implementation for monitoring

- Regulatory acceptance
- Testing framework
- Introduction to water utilities
- Demonstration

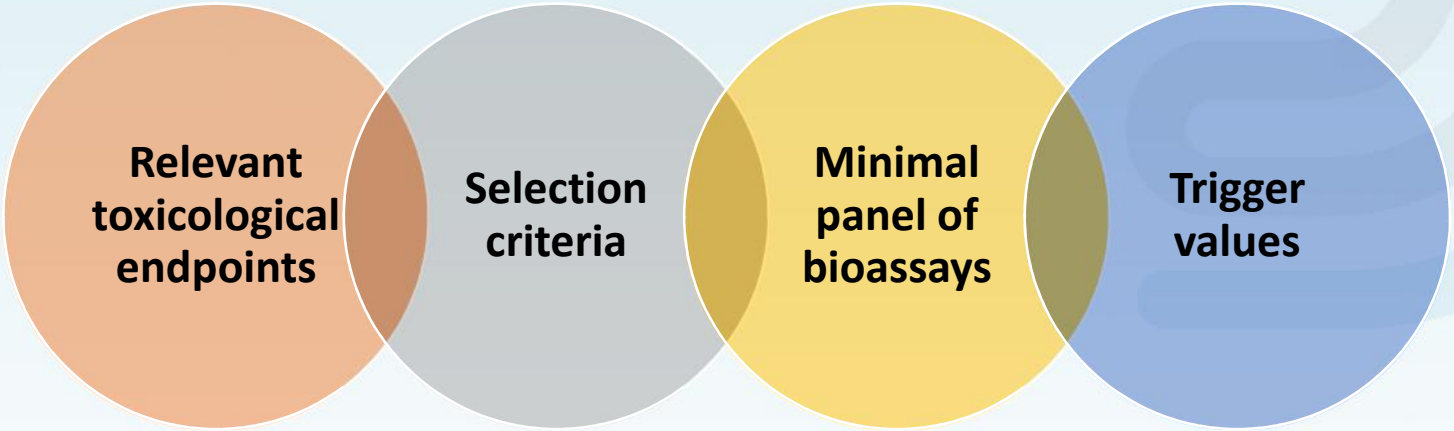


Market **application**

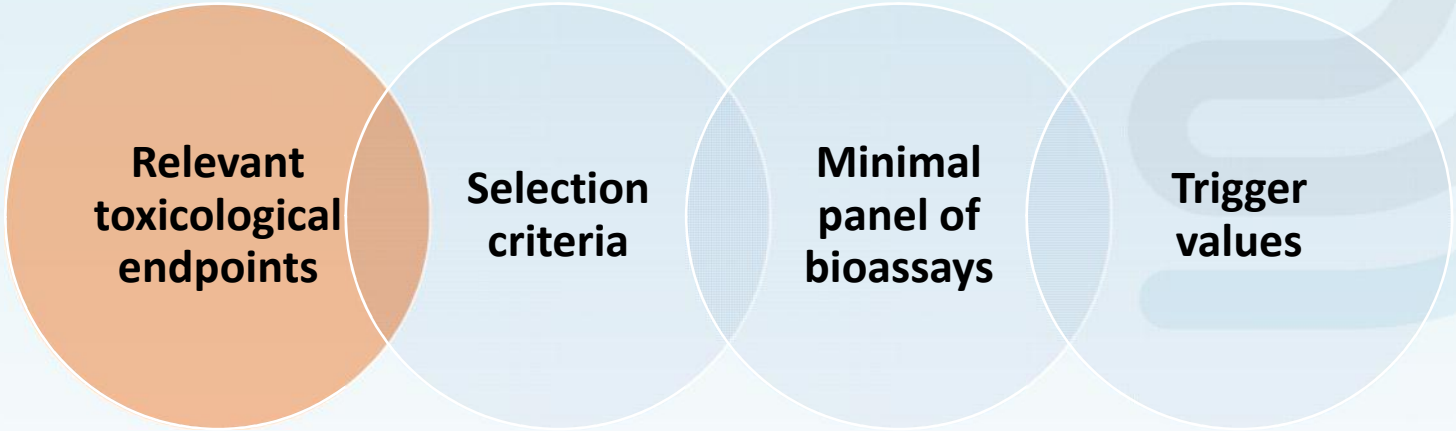
DEMEAU

Demonstration of promising technologies to address emerging pollutants in water and waste water

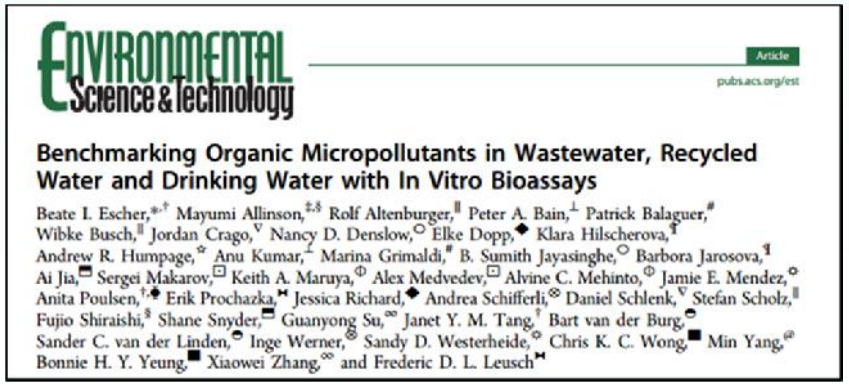
WP41 SELECTION AND VALIDATION OF BIOASSAYS QUALITY ASSESSMENT



WP41 SELECTION AND VALIDATION OF BIOASSAYS QUALITY ASSESSMENT



- Xenobiotic metabolism
- Hormone-mediated MoA
- Reactive MoA
- Developmental toxicity
- Adaptive stress response


Environmental Science & Technology

Article

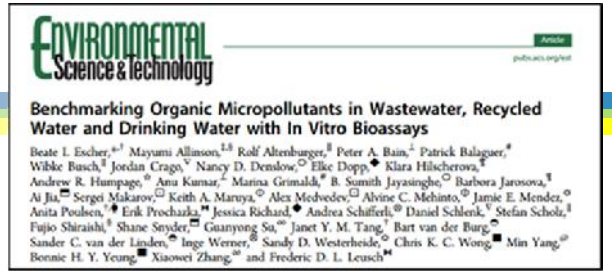
pubs.acs.org/est

Benchmarking Organic Micropollutants in Wastewater, Recycled Water and Drinking Water with In Vitro Bioassays

Beate I. Escher, Mayumi Allinson, Rolf Altenburger, Peter A. Bain, Patrick Balaguer, Wibke Busch, Jordan Crago, Nancy D. Denslow, Elke Dopp, Klara Hilscherova, Andrew R. Humpage, Anu Kumar, Marina Grimaldi, B. Sumith Jayasinghe, Barbora Jarosova, Ai Jia, Sergei Makarov, Keith A. Maruya, Alex Medvedev, Alvine C. Mehinto, Jamie E. Mendez, Anita Poulsen, Erik Prochazka, Jessica Richard, Andrea Schifferli, Daniel Schlenk, Stefan Scholz, Fujio Shiraishi, Shane Snyder, Guanyong Su, Janet Y. M. Tang, Bart van der Burg, Sander C. van der Linden, Inge Werner, Sandy D. Westerheide, Chris K. C. Wong, Min Yang, Bonnie H. Y. Yeung, Xiaowei Zhang, and Frederic D. L. Leusch

Test panels emerging from case studies

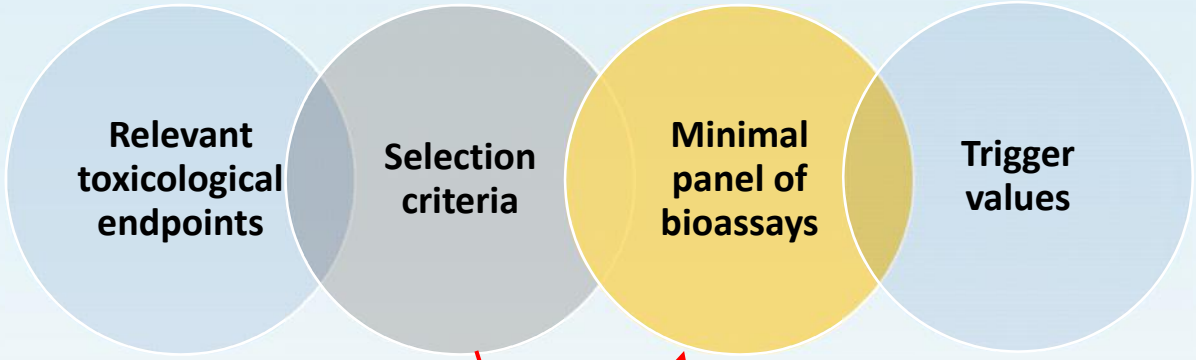
(GWRC, Australia, Dutch)



Screening toolbox

Relevant endpoints	B. Escher et. al	BE-Basic case study
Xenobiotic metabolism	PXR activation AHR activation CAR	DR/PAH-CALUX
Hormone-mediated MoA	Estrogenicity Anti-androgenicity Glucocorticoid activity Progestagenic activity Thyroid activity	Erα-CALUX AR-CALUX PR-CALUX GR-CALUX TRβ-CALUX RAR-CALUX
Reactive MoA	Mutations (AMES, SOS) DNA repair (umuC) DNA damage response (Micronucleus)	P53-CALUX P53 S9+ CALUX (?)
Adaptive stress response	Oxidative stress pathway	Nrf2-CALUX
Developmental toxicity	Preimplantation toxicity Embryonic development Placenta	ZFET
Lipid metabolism	PPARα, PPARγ	PPARα, PPARγ, PPARδ
Photosynthesis	Photosynthesis	-
General response	Cytotoxicity Viability Vibrio fischeri (Microtox) Algae growth	Cytotoxicity Cytotoxicity S9+

WP41 SELECTION AND VALIDATION OF BIOASSAYS FOR WATER QUALITY ASSESSMENT

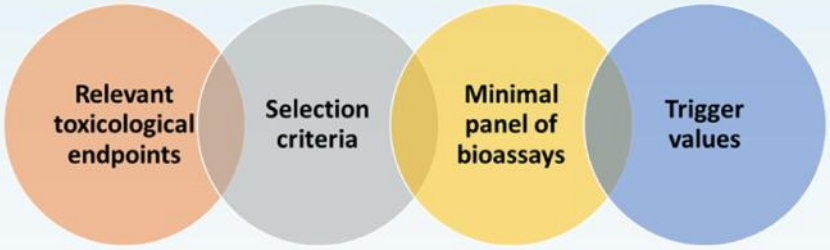


- Performance
- Validation status
- Cost
- Service
- etc

1	poor
2	good
3	excellent
NA	Not Available

		Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
Assay applicability	Applied to environmental samples	1	3	2	3	2
	Validated to water samples	1	3	3	2	1
	Generic sample handling and/or preparation is adequate	1	3	2	3	2
	Standardized protocol available/maturity	2	3	2	3	2
	Service and support available	1	3	2	3	1
	Ease of use	1	3	2	2	2
	Costs	3	3	1	3	1
Score		10	21	14	19	11
Assay performance	Selectivity	1	3	1	2	3
	Accuracy	1	2	1	2	3
	Reproducibility	1	3	2	3	3
	Robustness	1	3	2	3	2
	Sensitivity	1	3	3	3	3
	Specificity	1	2	2	3	3
	LOD	1	3	2	2	2
	Cytotoxicity control	3	3	3	3	3
	Quick	3	2	2	3	1
	Clear/Straightforward read-out	1	3	2	2	2
	High-throughput capacity	3	3	2	3	2
Score		17	30	22	29	27
Total score		27	51	36	48	38

Toxicity endpoints	DEMEAU bioassay(s)
Xenobiotic metabolism	DR/PAH-CALUX PXR-CALUX
Hormone-mediated MoA	ER-CALUX antiAR-CALUX GR-CALUX
Reactive MoA	P53-CALUX
Developmental tox	ER-CALUX antiAR-CALUX
Adaptive stress response	Nrf2-CALUX



TEQ approach


Environment International 55 (2013) 109–118
Contents lists available at SciVerse ScienceDirect
Environment International
journal homepage: www.elsevier.com/locate/envint

Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays

Walter Brand [a,*,1], Cindy M. de Jongh [a,1], Sander C. van der Linden [b], Wim Mennes [c], Leo M. Puijker [a], Cornelis J. van Leeuwen [a], Annemarie P. van Wezel [a], Merijn Schriks [a,**], Minne B. Heringa [a,2]

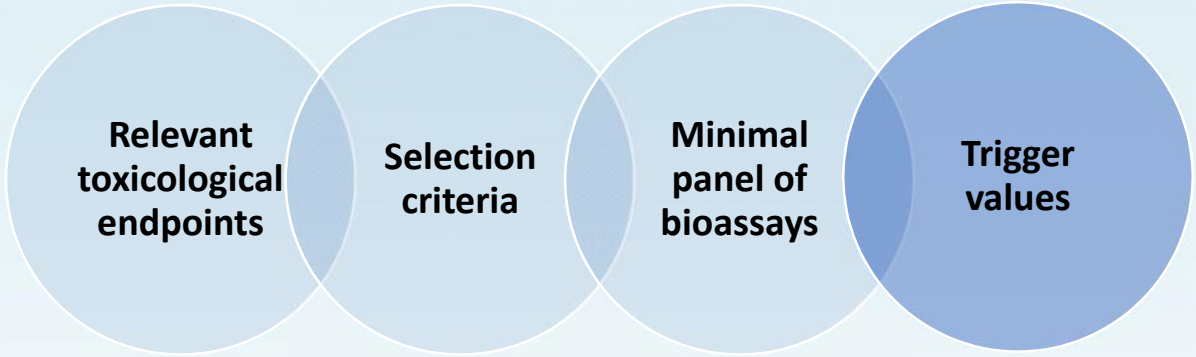
[a] KWR Watercycle Research Institute, Groningenhaven 7, 3433 PE Nieuwegein, The Netherlands
[b] BioDetection Systems B.V., Amsterdam, Science Park 406, 1098 XH Amsterdam, The Netherlands
[c] National Institute for Public Health and the Environment (RIVM), Antonie van Leeuwenhoeklaan 9, PO Box 1, 3720 BA Bilthoven, The Netherlands

Assay	Trigger value
ER-CALUX	3.8 ng E2-eq / L
AR-CALUX	11 ng DHT-eq / L
GR-CALUX	3.8 ng DEX-EQ / L
PR-CALUX	3.8 ng Org2058-eq / L

Trigger values in practice

Demonstration of promising technologies to address emerging pollutants in water and waste water

WP41 SELECTION AND VALIDATION OF BIOASSAYS FOR WATER QUALITY ASSESSMENT



Large volume grab samples	ZFET	Erα	AR	PR	GR	TRβ	RAR	PPARα	PPARγ	PPARδ	DR total	PAH	Nrf2	p53	p53 S9+	Cyto	Cyto S9+
Bottled water																	
Drinking water																	
Surface water Lekkannaal (Rhine)																	
Surface water Keizersveer (Maas)																	
Waste water Cuijk																	
Waste water Hapert																	
Waste water Grouw																	

WA 4 Implementation of bioassays

4.1 Selection and validation

- ✓ Selection criteria
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- ✓ Automation
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4.2 Implementation for monitoring

- ✓ Regulatory acceptance
- Testing framework
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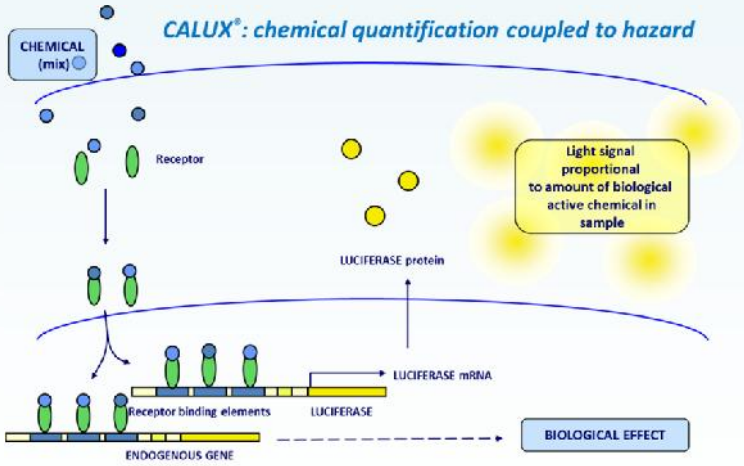


Market **application**

Conclusions

- **Mechanistic bioassays available that can predict various important types of toxicity in animals (and humans)**
- **Quantitative**
- **Validated and accredited**
- **Pharmacokinetic (PBPK) modeling improves predictions**
- **Even without complete coverage of toxicity mechanisms applicable for prioritization and read-across of pure chemicals**
- **Particularly suitable for complex mixtures: e.g. prioritized effects (endocrine disrupters, genotoxicity, etc), and to identify pollution hotspots vs clean samples, efficiency of purification processes.**

WA4 partners

KWR Watercycle Research Institute

eawag aquatic research

VEOLIA ENVIRONNEMENT Recherche & Innovation

Griffith UNIVERSITY

waternet

BDS BioDetection Systems

Chemscreen

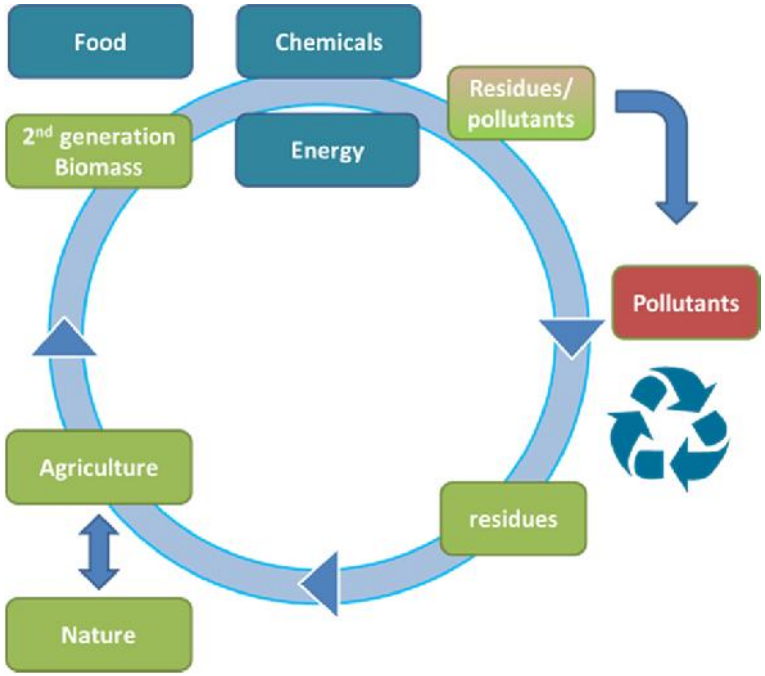
partners

Partner	
BioDetection Systems (BDS)	Bart van der Burg
Fraunhofer Institute for Toxicology and Experimental Medicine (FhG)	Inge Mangelsdorf
Netherlands Organization for Applied Scientific Research (TNO)	Dinant Kroese
Simpple (SIM)	Eduard Pauné
National Institute for Public Health and the Environment (RIVM)	Aldert Piersma
Danish Technical University Food Institute (DTU)	Jay Niemalä
Procter & Gamble Eurocor (P&GEN)	Joanna Jaworska
Eberhard Karls University of Tübingen (EKUT)	Michael Schwarz
University of Konstanz (UKON)	Daniel Dietrich

SEVENTH FRAMEWORK PROGRAMME

Towards a bio-based economy

- **Increased (re)use of biological materials: safety issues related to complex mixtures rather than single compounds**



www.be-basic.org