

New CALUX technologies



Analytical Challenges

Chemical contaminants in food/environment: sensitive, cost-effective methods needed to analyse and determine hazards of complex mixtures with similar effects (EDCs, dioxins, PAHs, etc)

Need to rapidly screen hazards of chemicals with reduced use of experimental animals (REACH, development of new chemicals)

Exposure to chemical cocktails

Ingredients



Toxic waste



Chemicals



Pharmaceuticals



Toxins



Environment



Food



Human

Complex mixtures

How to assess hazard?

Toxicity of most chemicals unknown; e.g. industrial chemicals



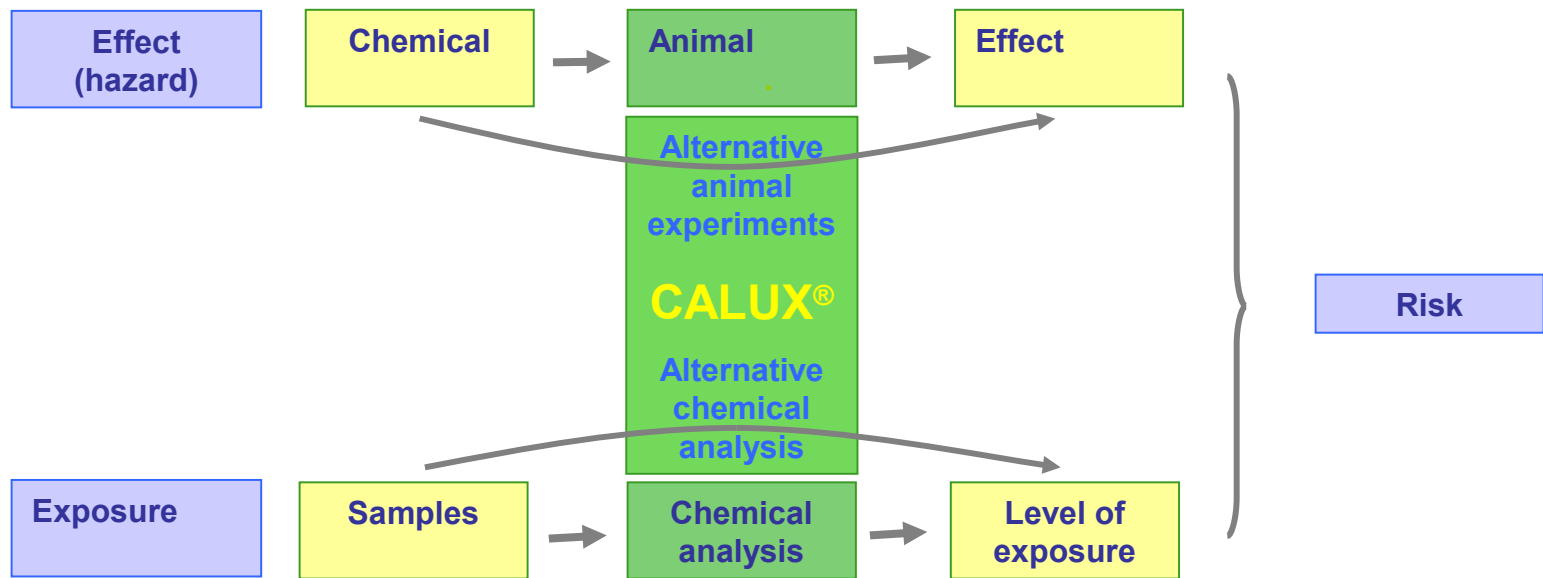
- EU white paper on chemicals: over 100,000 chemicals on market;
1% tested on toxicity



-
- *How to establish hazards without prior knowledge on the chemicals in a sample?*
 - *How to establish complex mixture effects?*



CALUX[®] assays in risk assessment





- *At start of BDS (2001): DR CALUX and ER CALUX available*
- *We want more!*

- **Introduction biodetection systems and biomonitoring**
- **Projects on new biodetection systems and applications**
 - Dutch Projects Ecogenomics and EcoLinc – healthy soil, DNA barcoding
 - Technological collaboration project Economic affairs – genomics-based biodetection
 - EU Project DIFFERENCE – dioxin/PCB screening in food/feed
 - EU Project HORIZONTAL – dioxin/PCB screening in soil, sludge/biowaste
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 - EU project METAEXPLORE- metagenomics
 - Dutch project Genes for Water- water safety
 - Netherlands Toxicogenomics Centre- genomics and non animal testing
 - Top Institute Pharma project – tests for adverse drug reactions
 - STW project – tests for genotoxic compounds



Major field of activity BDS

- **Food and Feed (safety/functional foods)**
- **Water**
- **Environment**
- **Chemicals and biologicals (safety/discovery)**
- **Human health (clinical/epidemiology/doping)**
- **Pharmaceuticals (safety/discovery)**



BDS' projects are in different fields

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- **Technological collaboration project Economic affairs**

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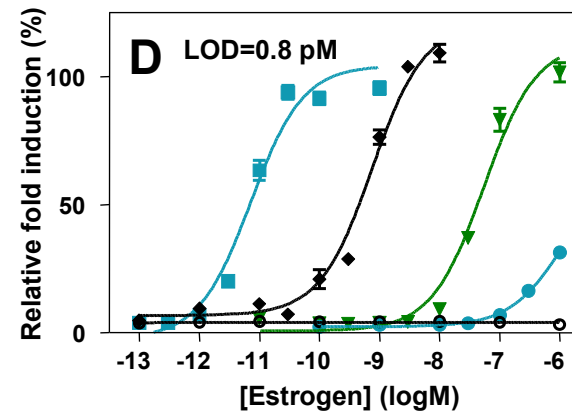
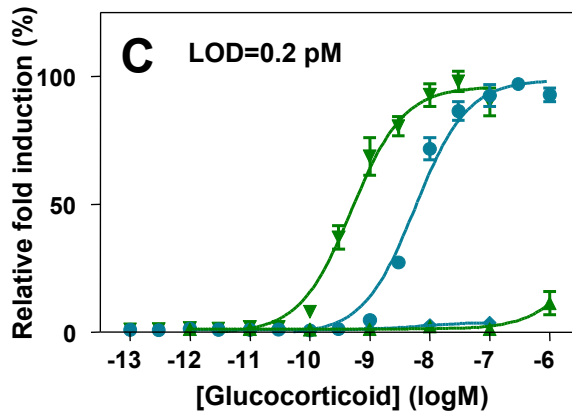
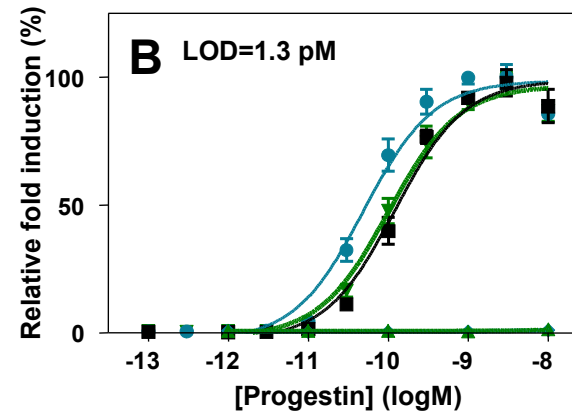
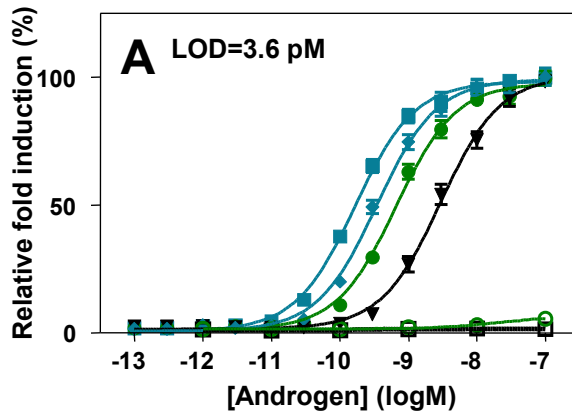
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More endpoints: expanding array of CALUX® in vitro assays



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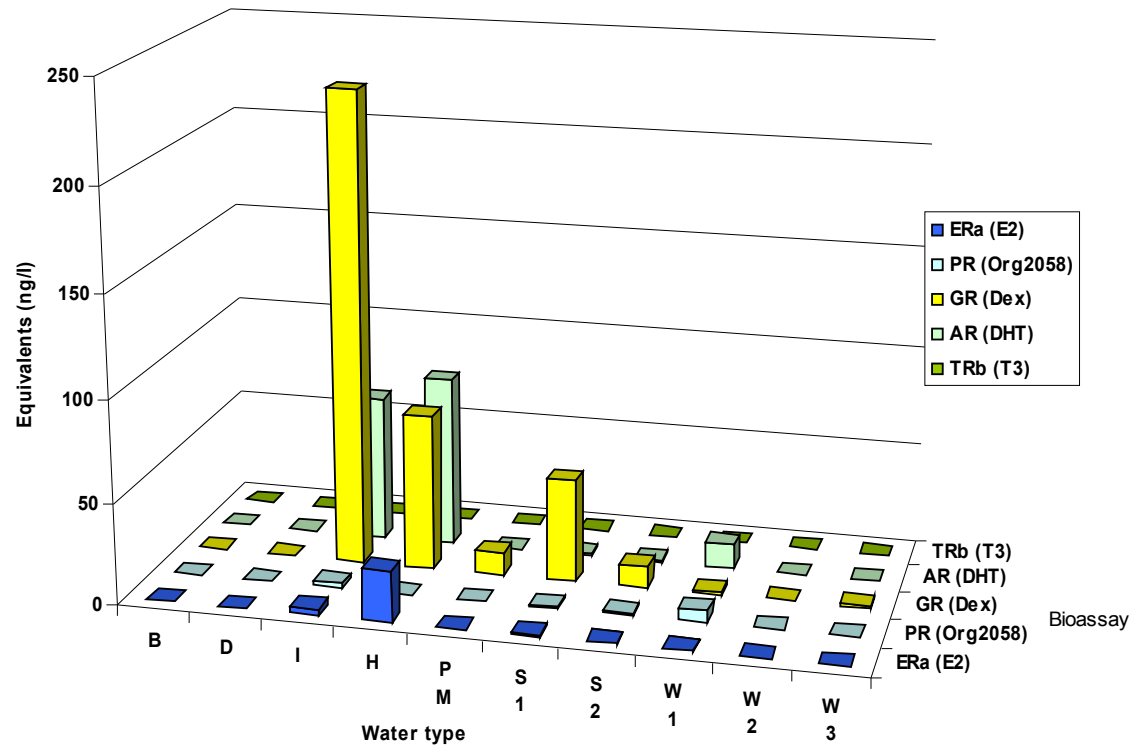
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Example effect profile with CALUX® cells

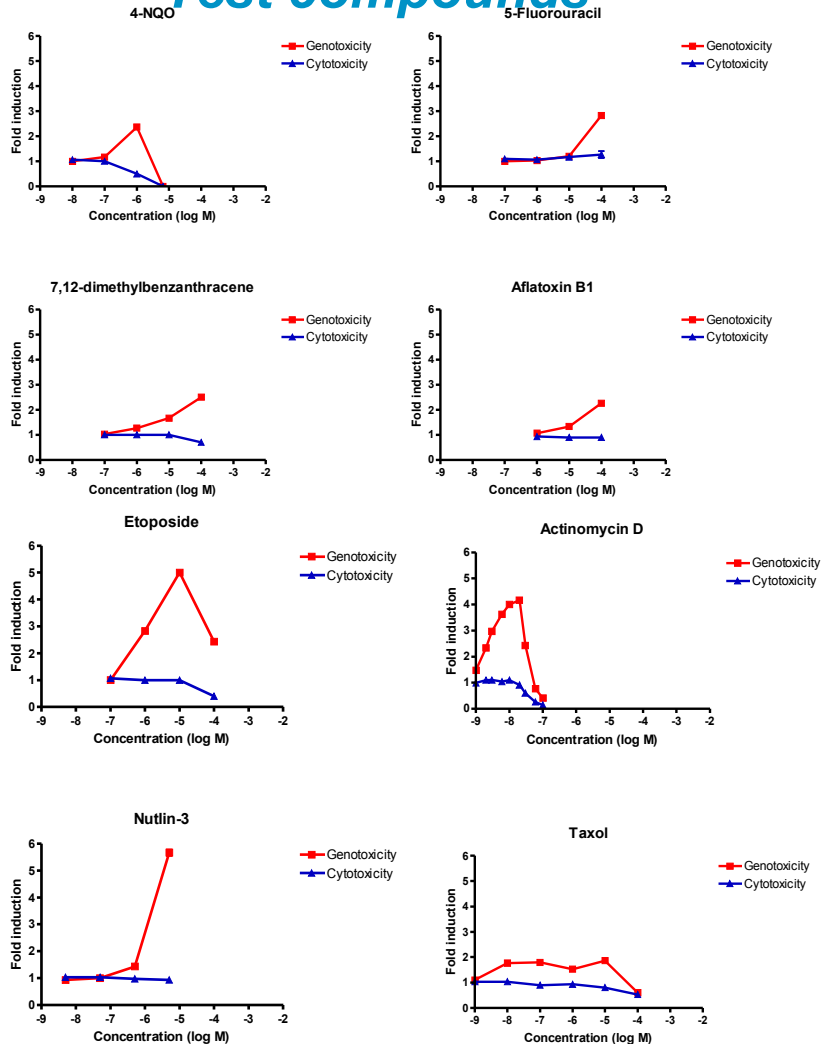


- Identification of specific effects/compound groups; glucocorticoids
- Identification of hot spots of pollution

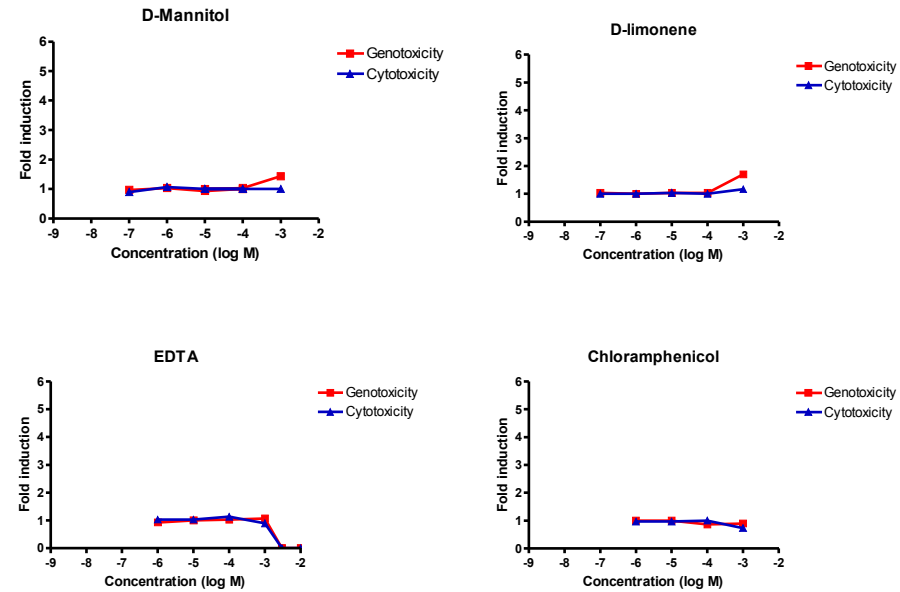


Genotoxic/carcinogenic compounds: p21 CALUX

Test compounds



Controls





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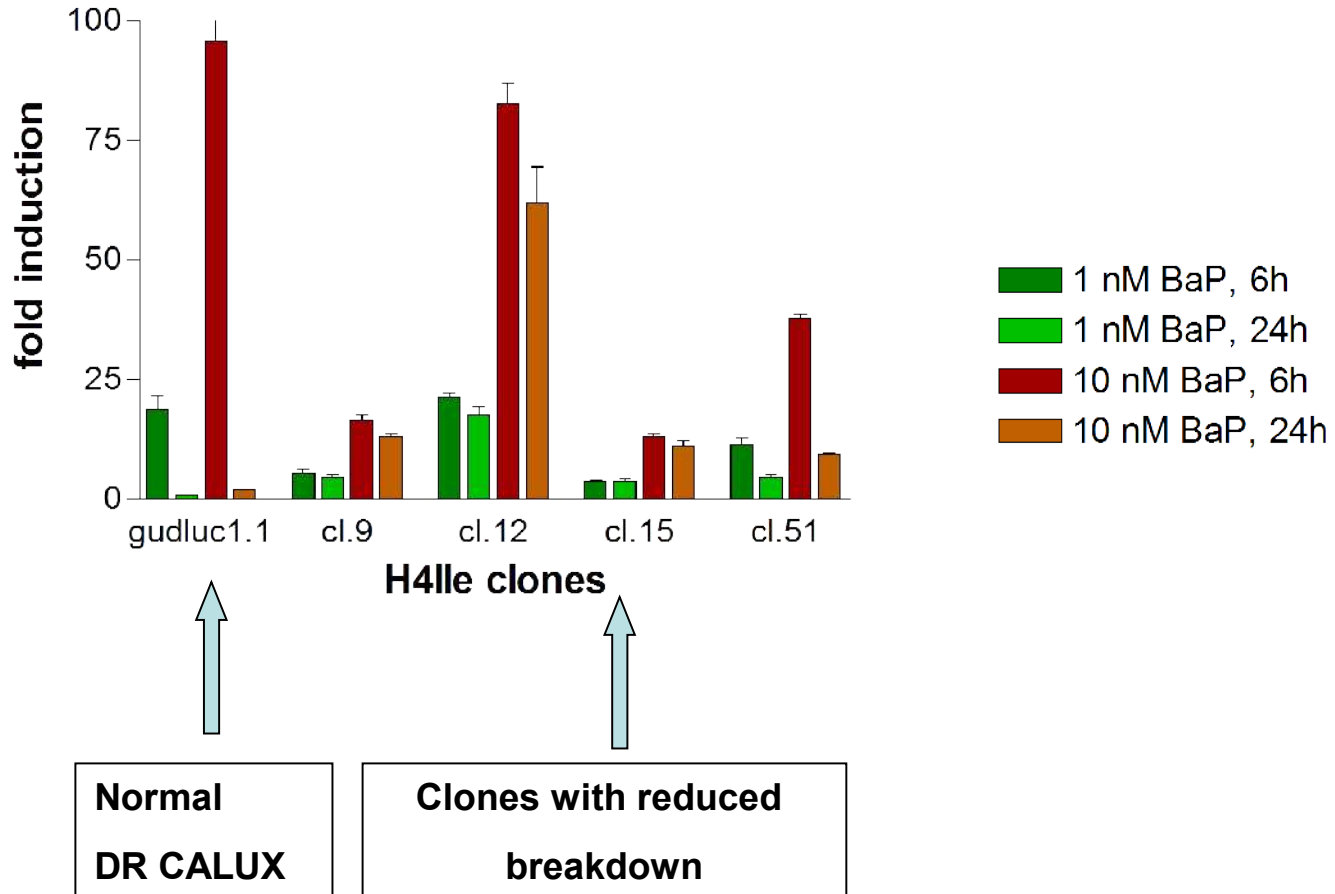
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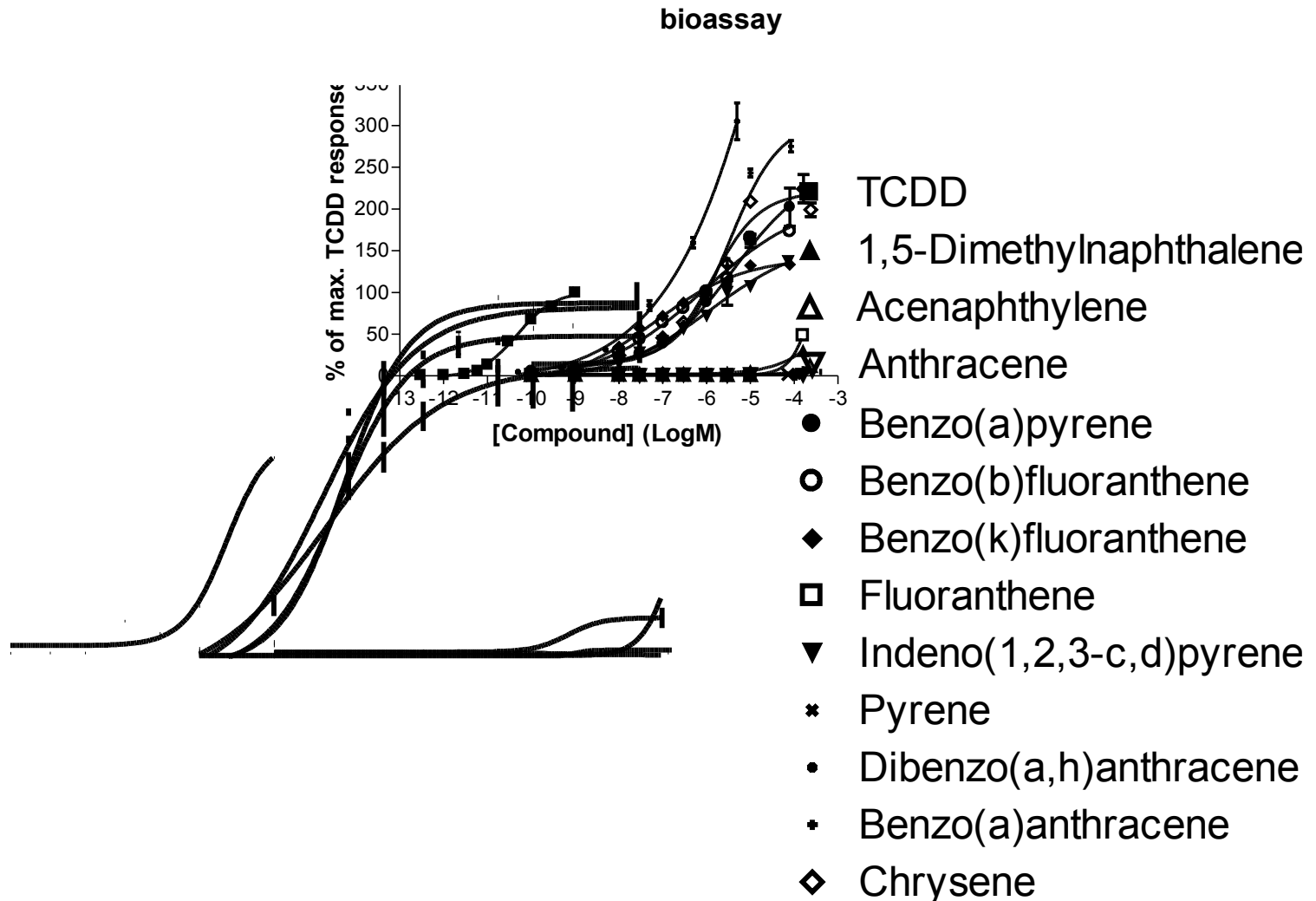
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PAH CALUX assay: less stringent work-up and short incubation/metabolically less active variant



Results PAH CALUX assay; pure compounds





Results PAH CALUX assay

	Genotoxicity	WHO	CALUX
Benzo(k)fluoranthene	0.04-0.1	pos	0.7
Chrysene	0.001-0.3	pos	0.03
Indeno (1,2,3-c,d)pyrene	0-0.2	pos	0.59
Benzo(a)pyrene	1	pos	1
Dibenz(a,h)anthracene	0.7-1.1	pos	0.6
Benzo(b)fluoranthene	0.06-0.14	pos	1.1
Benz(a)anthracene	0-0.14	pos	0.3
Benzo (g,h,i)perylene	0.01-0.03	neg	>0.005
Anthracene	0.0005-0.01	neg	>0.005
Pyrene	0-0.001	uncertain	>0.005
Fluoranthene	0-0.05	uncertain	>0.005
Phenanthrene	0.0005	uncertain	>0.005
Acenaphthene			>0.005
Acenaphthylene			>0.005
Fluorene			>0.005
Naphthalene			>0.005
1,5-Dimethylnaphthalene			>0.005



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- **EU projects ReProTect/ ChemScreen- non animal testing for REACH**

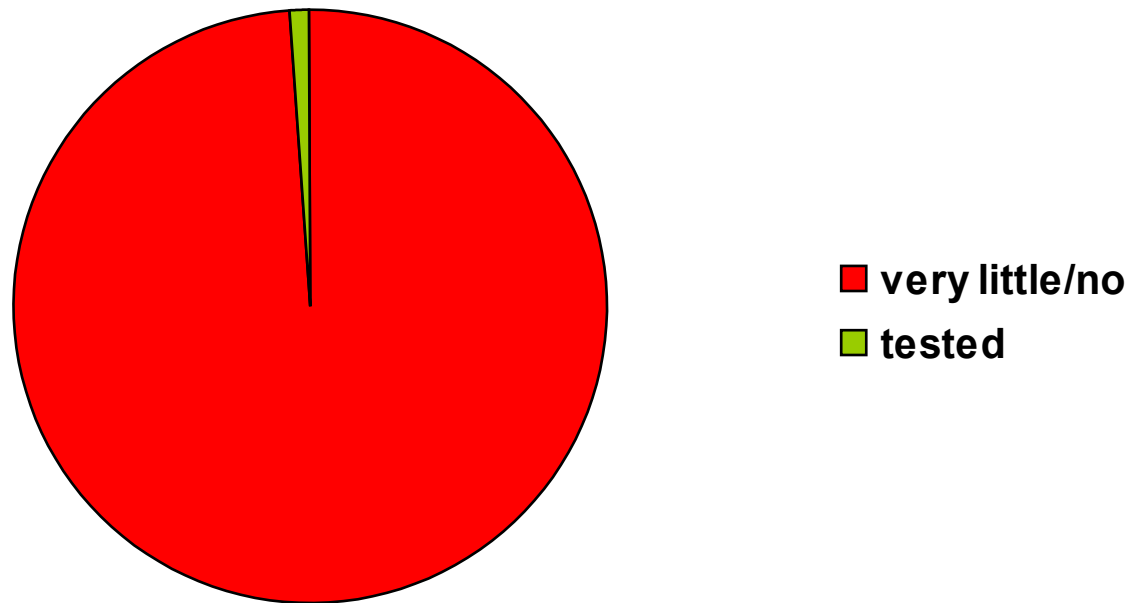
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*100,106 chemicals on market in 1981 (“existing substances”);
1% tested on hazardous properties*



REACH & alternative testing

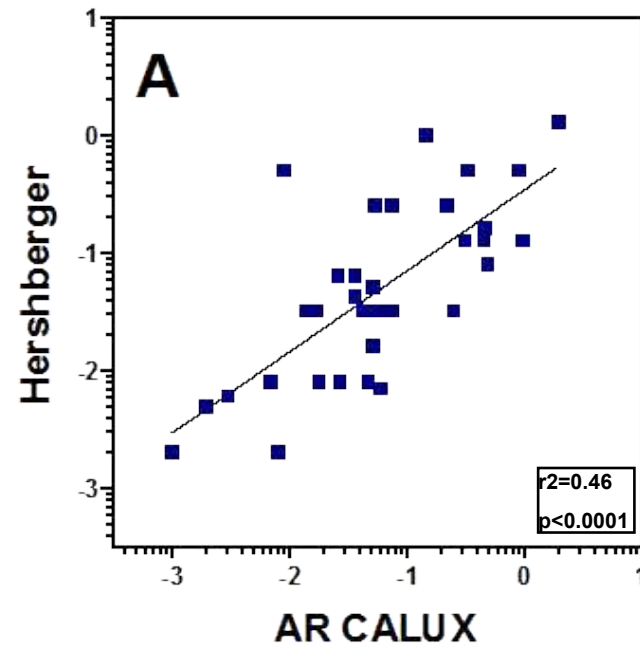
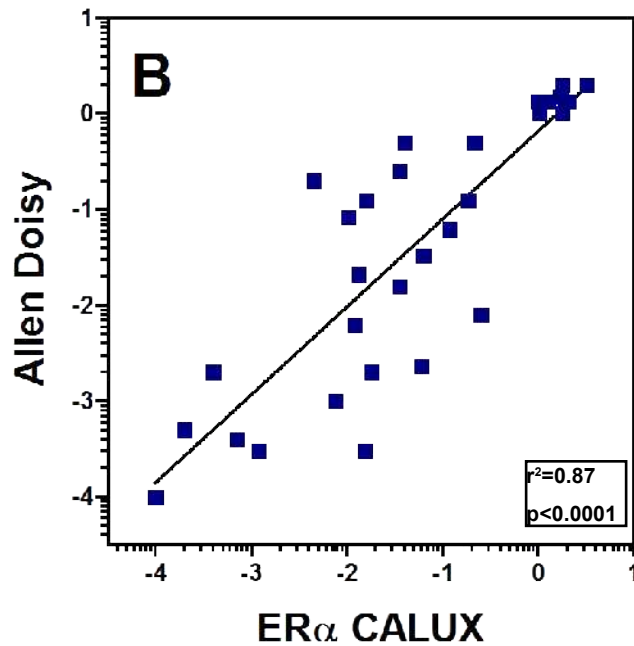
When traditional animal tests are used progress of REACH will be seriously hampered by:

1. **Ethics**: resistance to the excessive use of animals.
2. **Costs**: particular those linked to labour intensive animal testing
3. **Capacity**: lack of capacity to carry out these tests.
4. **Speed**: the use of the same traditional methods will not allow major advances in speed of the process to be made

>In order to be successful cost-effective, rapid in vitro tests need to be adopted

Example of effect (endpoint) module

- In vivo -



- In vitro -



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

ChemScreen

- **Panels of cost effective bioassays needed for rapid screening, coupled to efficient data storage, retrieval and interpretation:**
- **Integrated Testing Strategies**
 - **EU FP7, Environment program**
 - **4.6 million Euro (3.5 EU contribution)**



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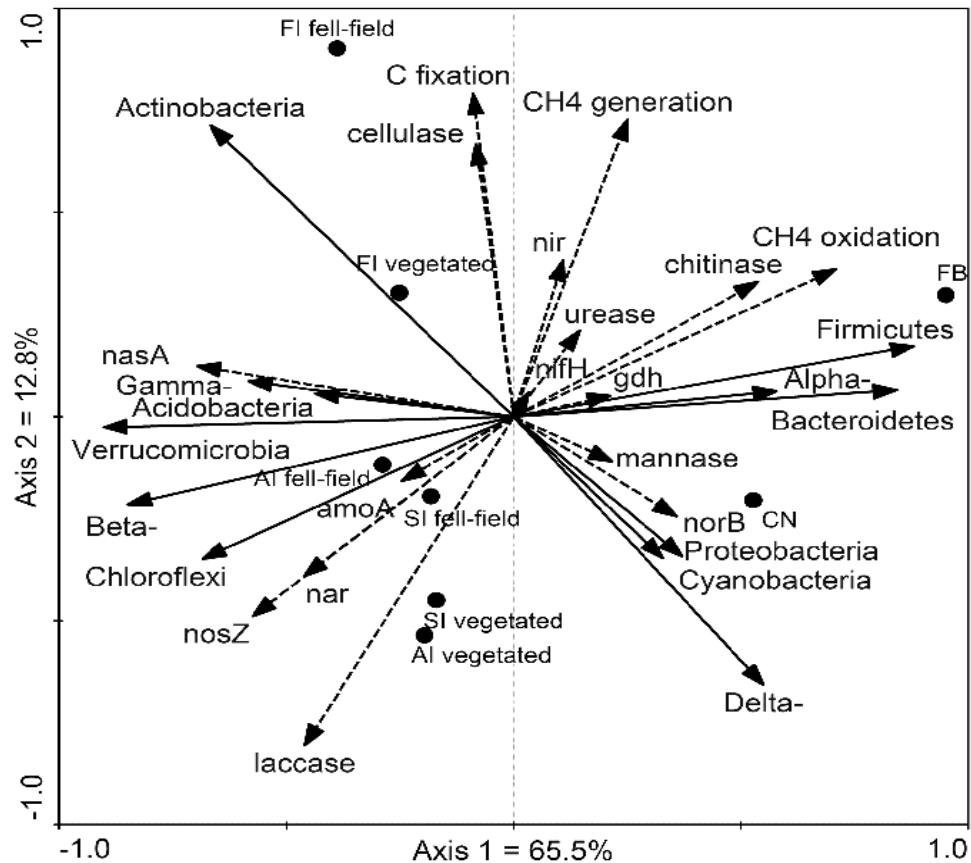
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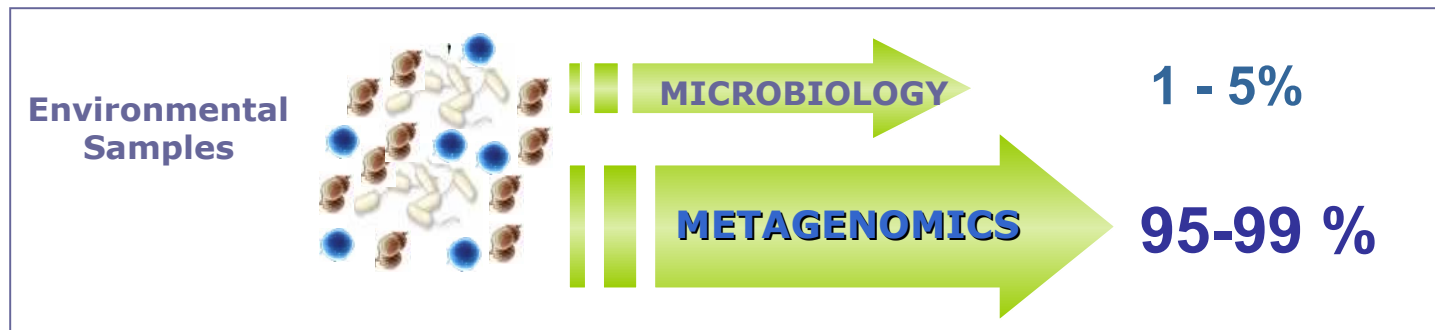
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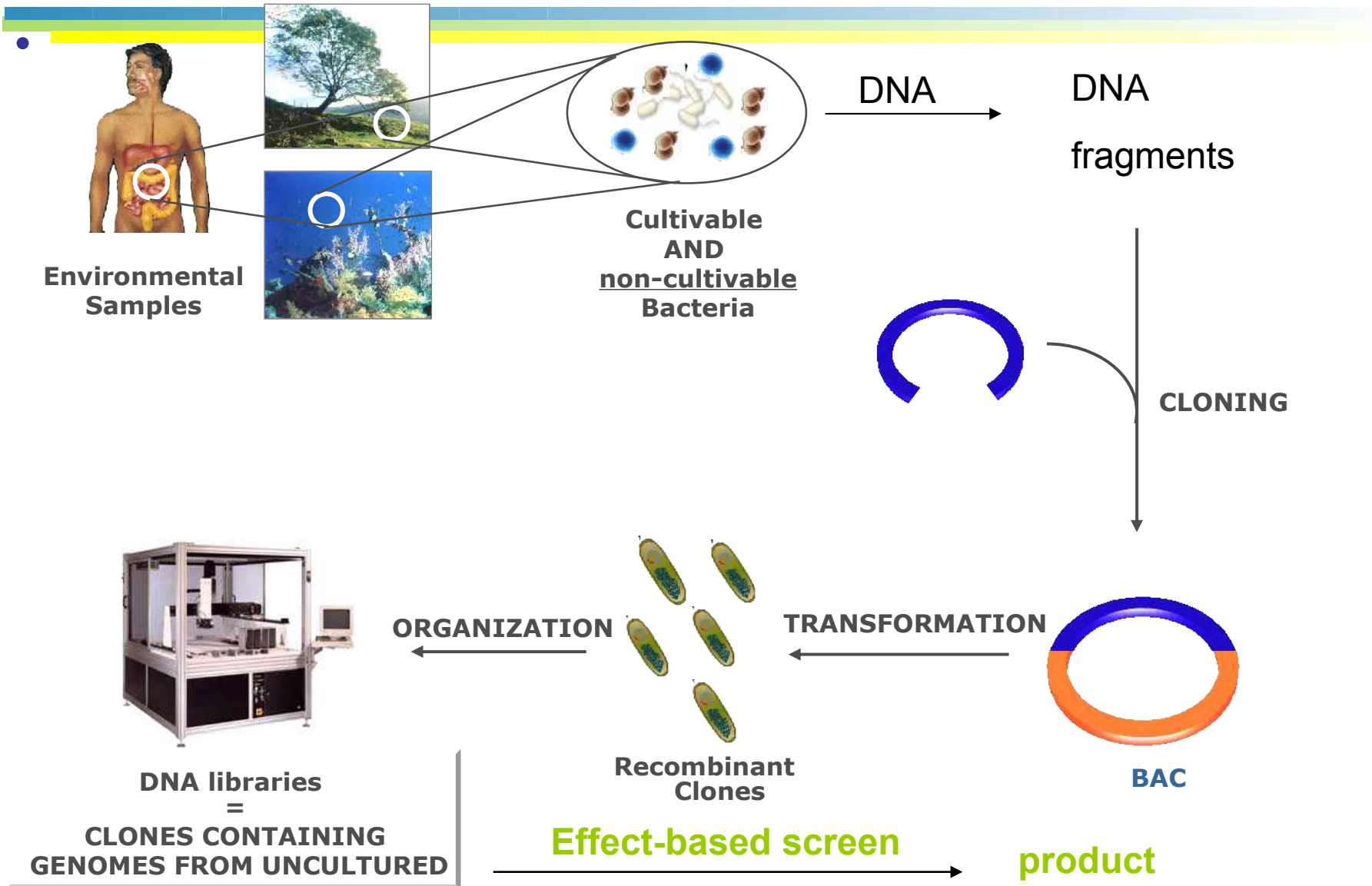
- **Dutch Project EcoLinc – metagenomics approaches**

- Soil ecosystems: Monitoring presence of species and/or classes of genes ('barcoding')





ACCESS TO THE REMAINING 95%-99% : METAGENOMICS





Results BDS projects

- **New cell lines and protocols**
- **New applications**
- **Data storage and bioinformatics**
- **Methods to modulate metabolism**
- **New (molecular) methods for novel applications**



Automation CALUX® battery

Microsoft Access

File Edit View Insert Format Records Tools Window Help

Type a question for help

MS Sans Serif 8 B I U

Data

BDS no: 1
O.q code: O.q 2317
Source: Scme
compound: Estra-1,3,5(10)-triene-3,17b-diol
Trivial name: 17b-estradiol (E2)
Structure:

	CALUX	log EC50	RAA	RTA	log IC50	RIA	RTI	MW	Formula
AR	-6.281	0.000000	32	-8.007	0.960000	50	272.3664	C18H24O2	
ER	-11.107	1.000000	100	>-5.0	0	<5			
ERa	-11.80	1.000000	100	>-5.0	0.000000	<5			
ERb	-9.631	1.000000	100						
PR	>-5.0	0.000000	<5	-7.328	0.003359	84			
GR	>-6.0	0.000000	<5						
DR									

ERa RAA: 1.000000 EF
AR RAA: <0.001 AI
PR RAA: 0.001 PI
ERb RAA: 1.000000 EI
GR RAA: 0.003 G

CHO

Binding: AR: 0.038333 ERa: 1.000000
PR: 0.003 GR: 0.001

In Vivo Hersberger SC Hersberger P0 Alen Doisy

Record: 11 of 262

Form View

Start

Luciferase induction (% of max. E2 activity)

E2	
BOTTOM	1.187
TOP	98.80
LOG EC50	-10.80
EC50	1.570e-011





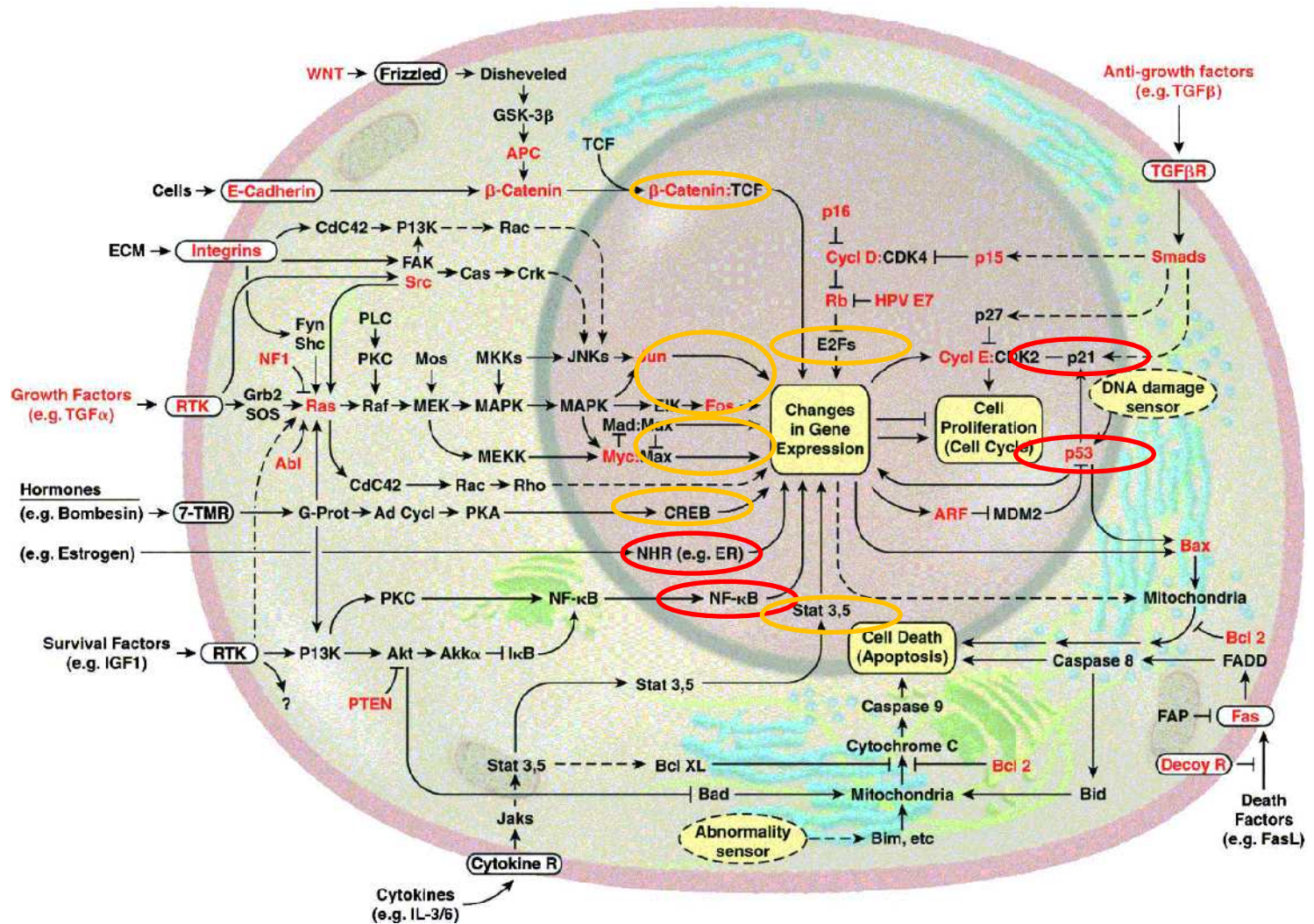
Different areas, same problems:

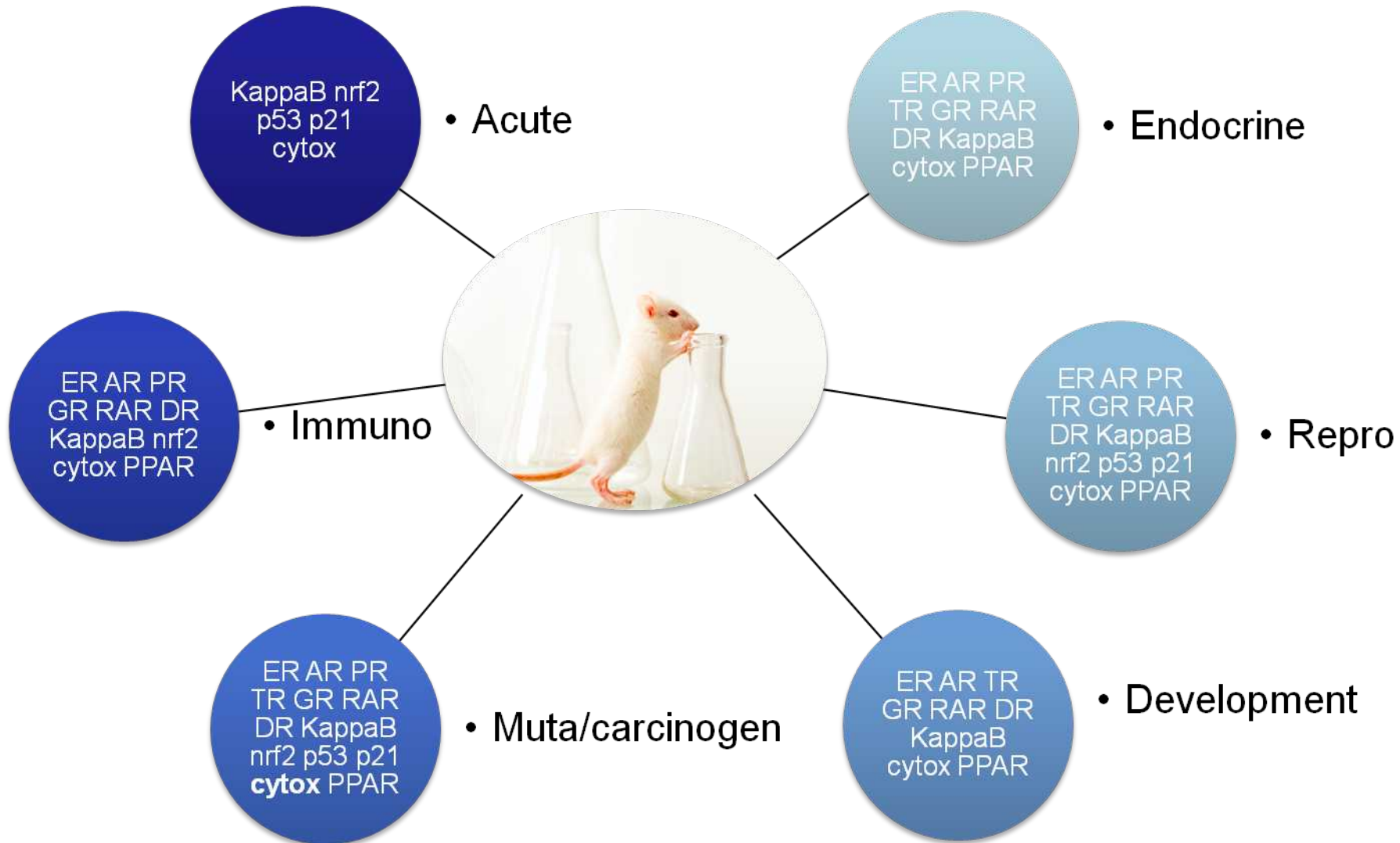
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and solutions:

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Toxicity pathways: carcinogens







CALUX battery

Name	Examples applications	Ligands
DR CALUX	Clinical, food, environment, reproduction, cancer	Dioxins and dioxin-like chemicals
PAH CALUX	Clinical, food, environment, reproduction, cancer	Carcinogenic PAHs
ER CALUX	Clinical, food, pharma, environment, reproduction, cancer	Estrogens, EDCs
ERalpha CALUX	Clinical, food, pharma, environment, reproduction, cancer	Estrogens, EDCs
ERbeta CALUX	Clinical, food, pharma, environment, reproduction, cancer	(Phyto)Estrogens, EDCs
AR CALUX	Clinical, food, pharma, environment, reproduction, cancer, doping	Androgens, EDCs
PR CALUX	Clinical, food, pharma, environment, reproduction, cancer	Progestins, EDCs
GR CALUX	Clinical, food, pharma, environment, doping, inflammation	Glucocorticoids, EDCs
TR CALUX	Clinical, food, pharma, environment, energy metabolism	Thyroid hormones, EDCs
RAR CALUX	Clinical, food, pharma, reproduction, cancer, teratogenicity, cosmetics	Retinoids
PPAR CALUX	Clinical, food, pharma, environment, cancer, metabolic syndrome	Wide range
kappaB CALUX	Clinical, food, pharma, environment, inflammation, stress	Pro-inflammatory cytokines
P21 CALUX	Clinical, food, pharma, environment, cell/DNA damage	Genotoxic agents
Cytox CALUX	Environment, food, pharma, cytotoxicity, specificity control	Cytotoxic agents
Nrf2 CALUX	Clinical, food, pharma, environment, cancer, cell protection	Electrophiles, ox stress
P53 CALUX	Clinical, food, pharma, environment, cell/DNA damage	Cytotoxic agents
AP1 CALUX	Clinical, food, pharma, environment, reproduction, cancer	Carcinogens, UV
Etc..		

