ENVIRONMENTAL COMPANY OF SAO PAULO STATE – CETESB

REGIONAL CENTRE OF STOCKHOLM CONVENTION ON POPs FOR LATIN AMERICA AND THE CARIBBEAN REGION

V INTERNATIONAL TRAINING PROGRAM ON ENVIRONMENTAL SOUND MANAGEMENT ON CHEMICALS AND WASTES, ESPECIALLY ON PERSISTENT ORGANIC POLLUTANTS (POPs) AND MERCURY (Hg)

Daniela Dayrell França

2016

Sao Paulo – SP – Brazil

JICA JBPP PROGRAMA DE PARCERIA BRASIL-JAPÃO ESCOLA SUPERIOR DA CETESB CETESB • Regional Centre for Stockholm Convention on POPs for Latin America and Caribbean Region CETESB AmBienteSP Sistema Ambiental Paulista GOVERNO DO ESTADO SÃO PAULO Secretaria do Meio Ambiente ABC AGÊNCIA BRASILEIRA DE COOPERAÇÃO MINISTÉRIO DAS RELAÇÕES EXTERIORES

CALUX test

Biodetectors for determination of dioxins, hormones and tributyltin

Daniela Dayrell França

Environmental assessment

Chemical analysis

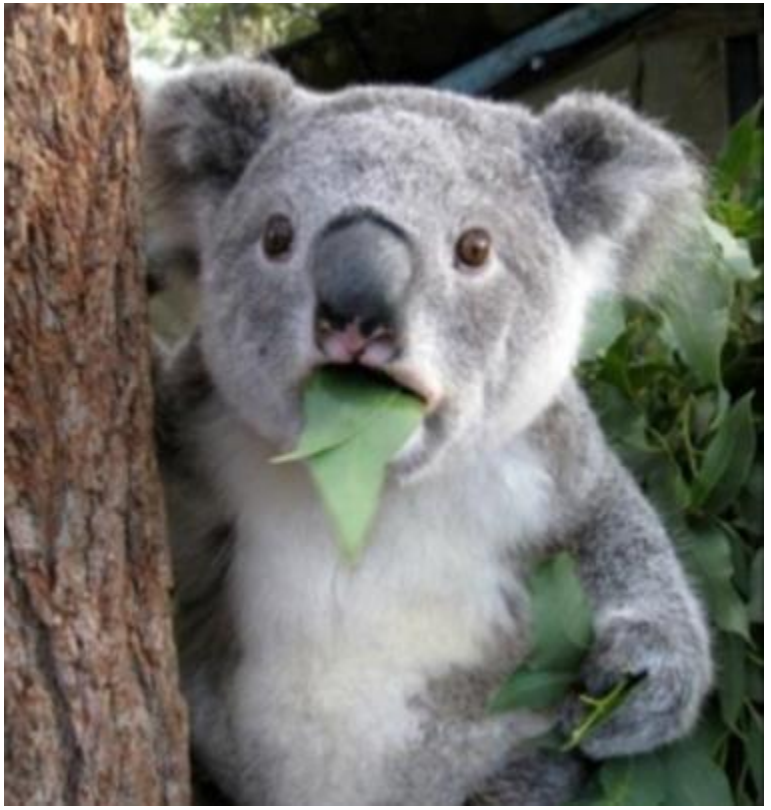
Biological assays

Physical properties

Chemical analysis

Requires knowledge on compounds of interest and the existence of standardized and sensitive methods for detection of trace levels of chemicals.

Why do we run chemical analysis?



Because chemicals have biological effects

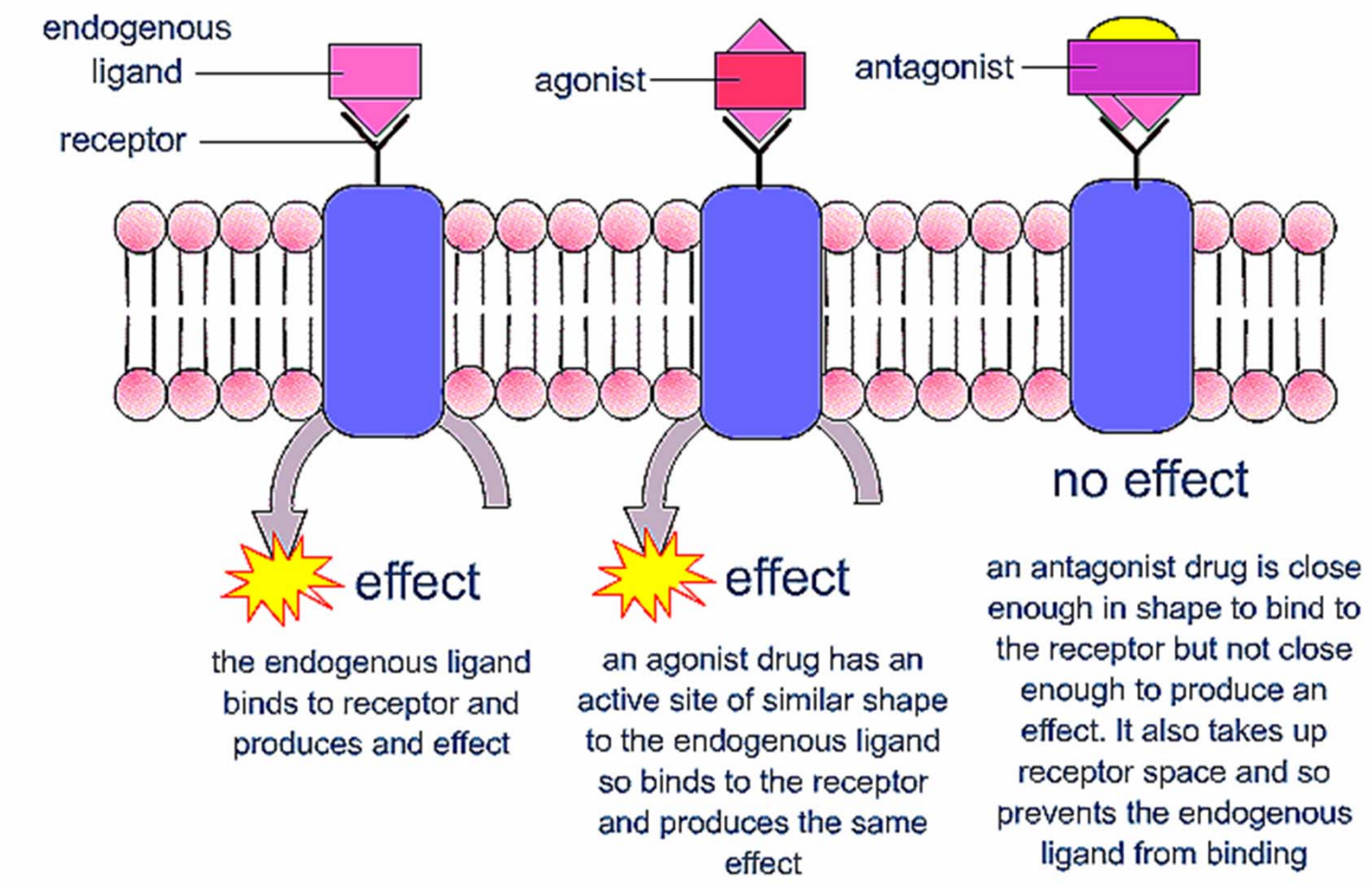
How do chemicals act on biological systems?

Receptors

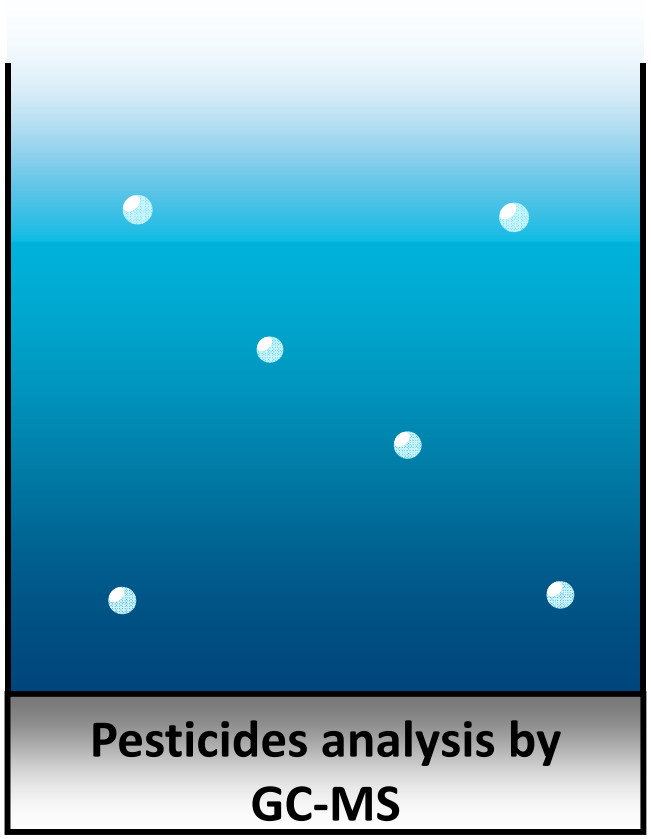
- A protein molecule that receives chemical signals from outside the cell

- When such chemical signals bind to a receptor, they cause some form of cellular/tissue response. In this sense, a receptor is a protein molecule that recognises and responds to endogenous chemical signals

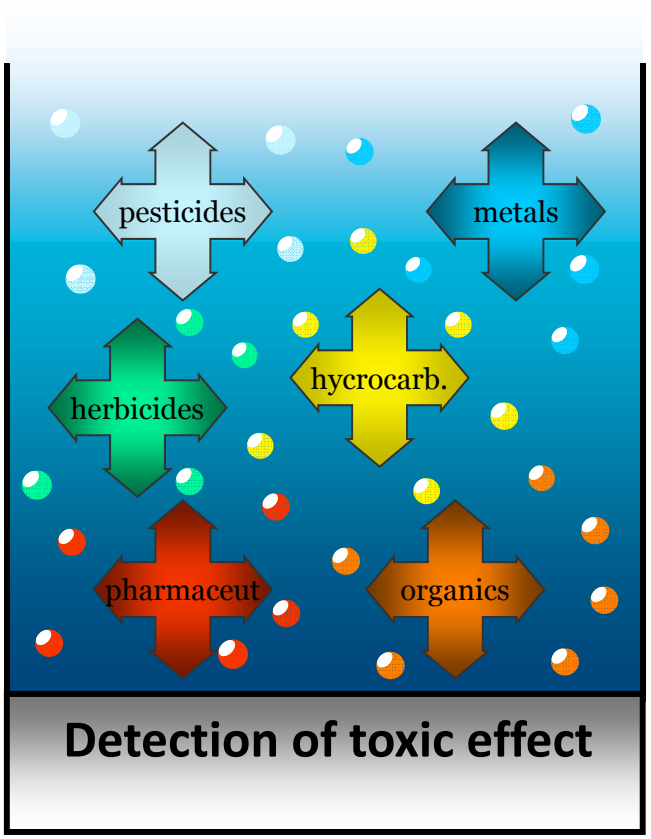
Environmental contaminants and receptors



Chemical analysis x bioassays

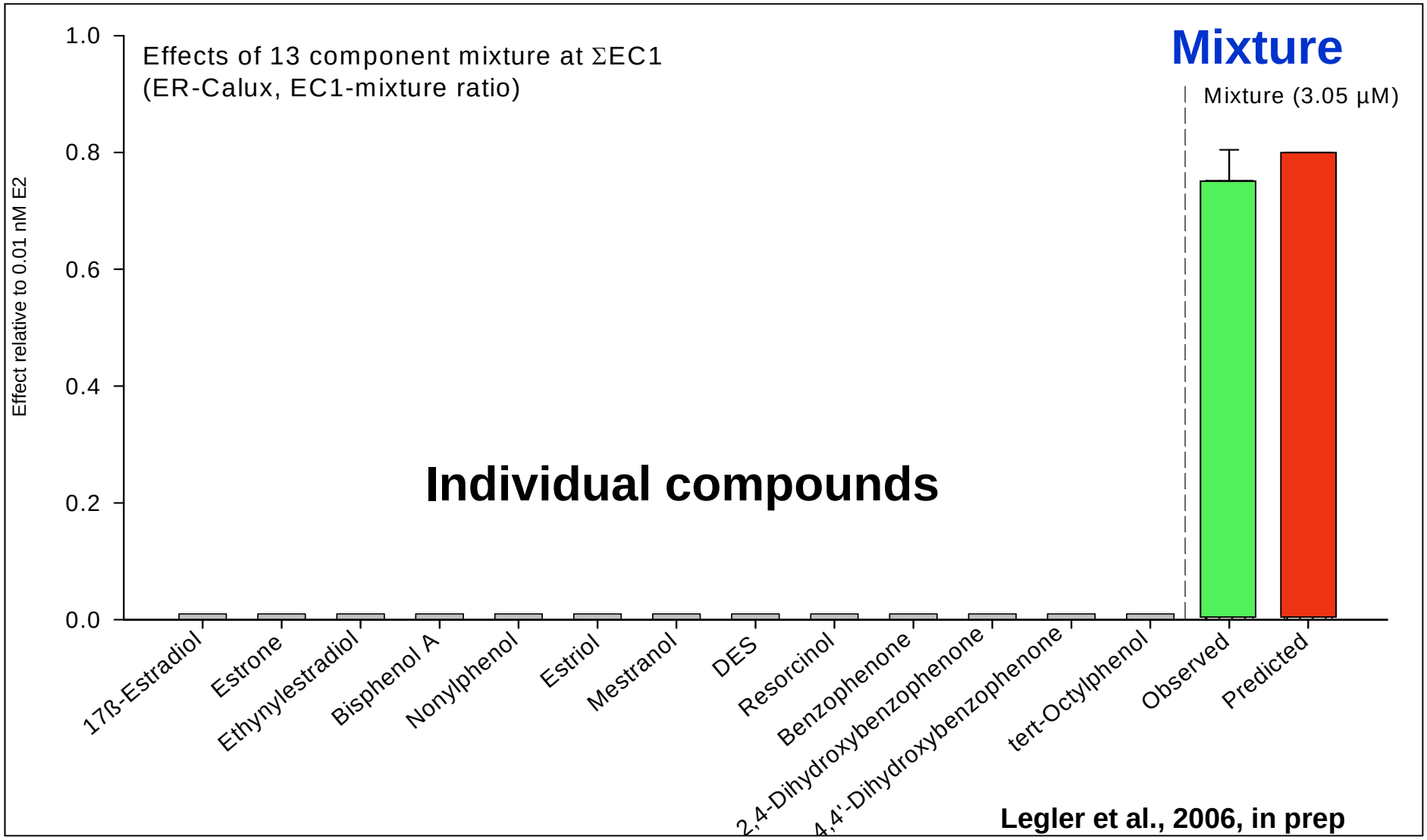


Pesticides analysis by GC-MS



Detection of toxic effect

Multi compound effects



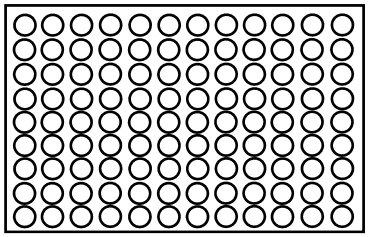
Bioanalytical assays

Genetically modified cells able to detect compounds based on their biological effect.

Why use Bioassays?

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

Determination of dioxins and dioxin-like PCBS
DR CALUX Technology

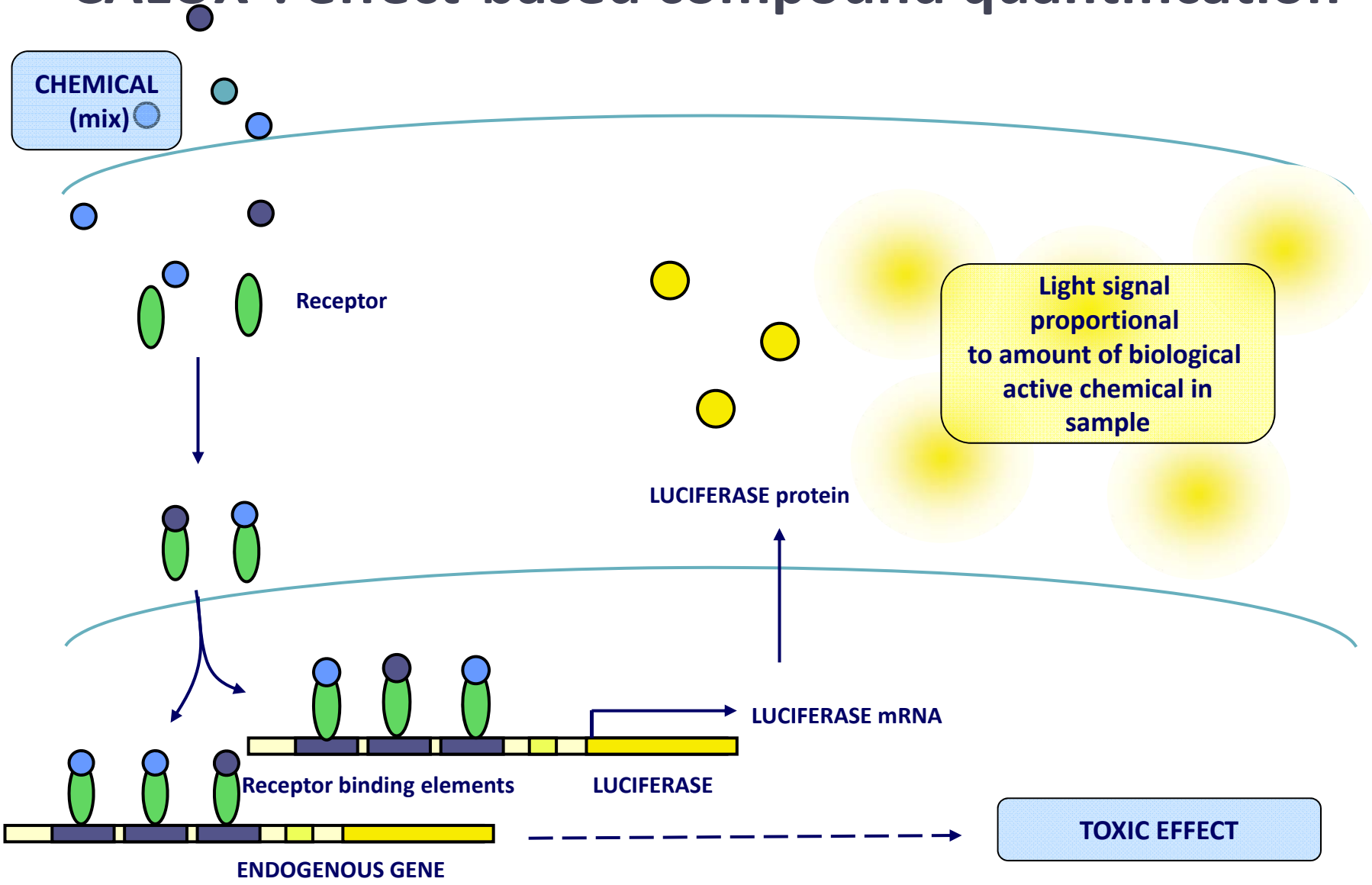
Developed by BioDetection Systems – BDS Netherlands

DR CALUX®
Dioxin **R**esponsive – **C**hemically **A**ctivated **LU**ciferase e**X**pression

• Correlates the response of a specific cell line to the presence of dioxins and dioxin-like PCBs, by its light emission.

• The amount of produced light is proportional to the amount of receptor AhR ligands, which correlates to the 2,3,7,8 TCDD toxic equivalents (TEQs).

CALUX®: effect-based compound quantification



Available CALUX® strains

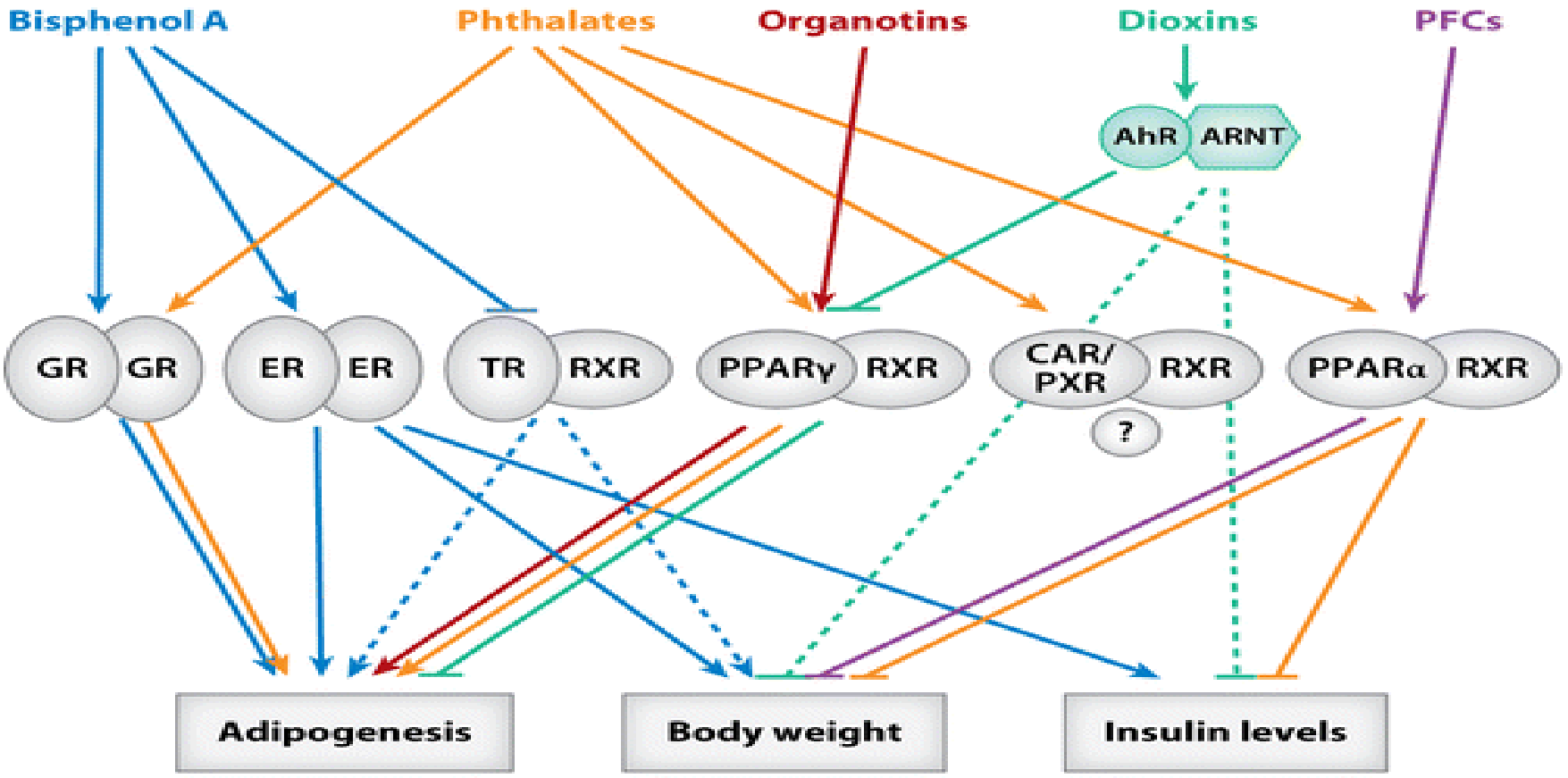
Nuclear receptors			Signaling pathways			Controls		
name	status	cell	name	status	cell	name	status	cell
DR CALUX	✓.	H4IIE	kappaB CALUX	✓.	U2OS	Cytox CALUX	✓.	U2OS
PAH CALUX	✓.	H4IIE	P21 CALUX	✓.	U2OS	MTT	✓.	all
ER CALUX	✓.	T47D	Nrf2 CALUX	✓.	U2OS	LDH leakage	✓.	all
ERalpha CALUX	✓.	U2OS	P53 CALUX	✓.	U2OS	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

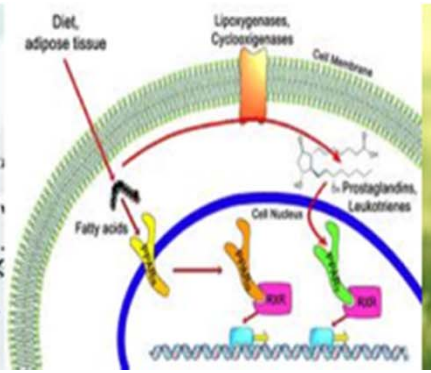
Why a panel of in vitro CALUX tests?

Link from important chemicals to important health risks



Casals-Calas C, Desvergne B. 2011 Annu. Rev. Physiol.73:135-62

Obesity testing by PPAR CALUX



PPAR Isoform	Organ specificity	Function
PPARα	Liver	Fatty acid metabolism
PPARγ	Adipose tissue	Lipid storage
PPARδ	ubiquitous	Energy homoeostasis

→ PFOA activates PPARα and PPARγ (but not PPARδ)
Vanden Heuvel et al. (2006) Toxicol Sci 92: 476-489

→ PFCAs activate PPARα
positive correlation between carbon chain length and the level of PPARα activation
Wolf et al. (2008) Toxicol Sci 106: 162-171

BioDetection Systems

BioDetection Systems B.V. ("BDS") is a Dutch company providing biological detection systems, such as the innovative CALUX® bioassays for the determination of ultra low levels of a variety of highly potent materials.

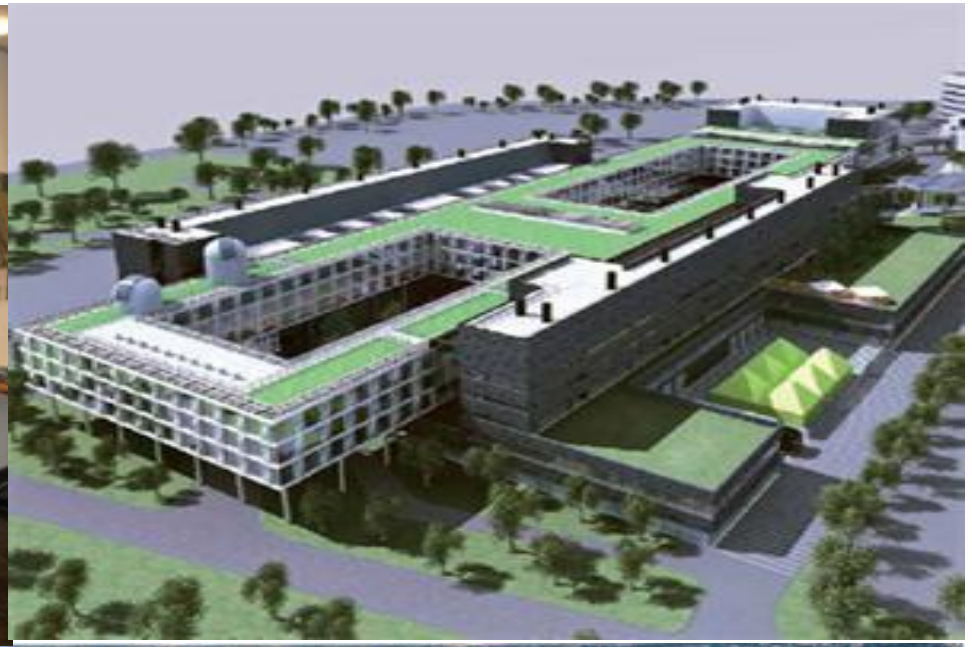
Mission

To provide innovative bioassays and implement their use to the highest international standards.

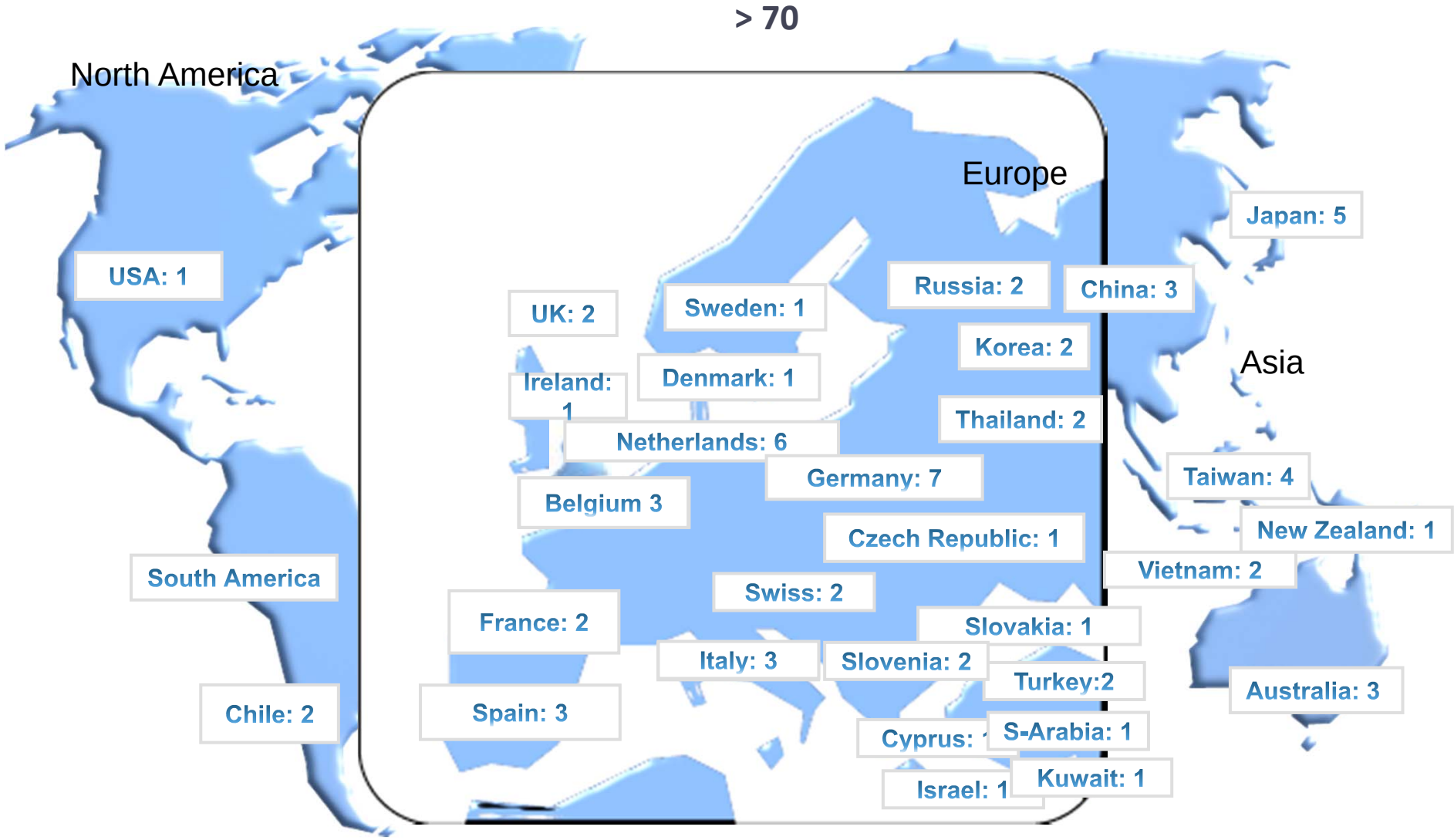
Activities:

- ISO 17025 accredited Laboratory – Contract analysis
- Licensing
- Training
- Research and Development
- Consultancy

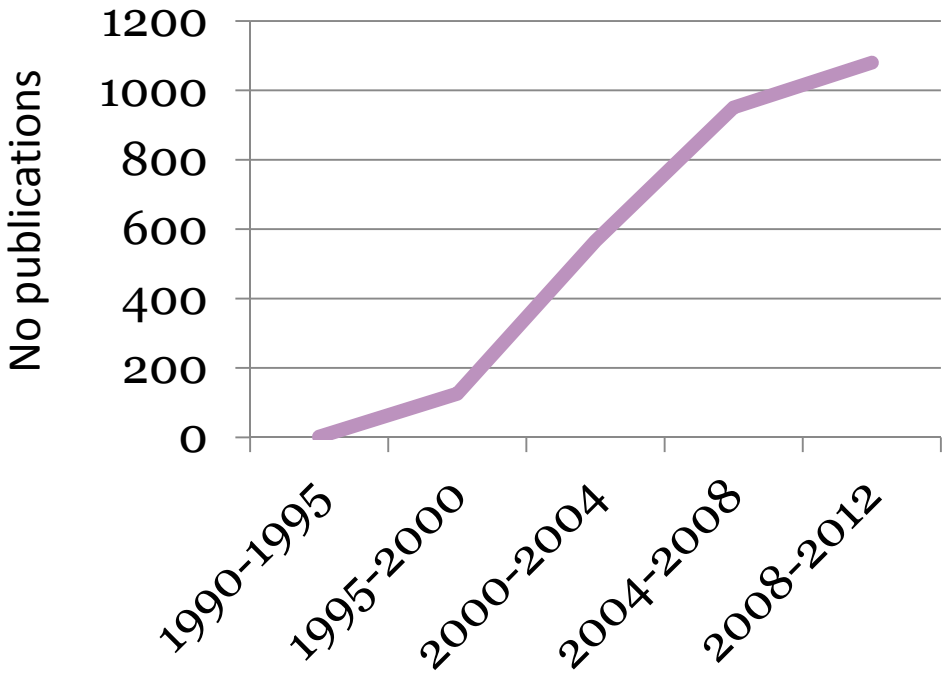
Amsterdam Sciencepark



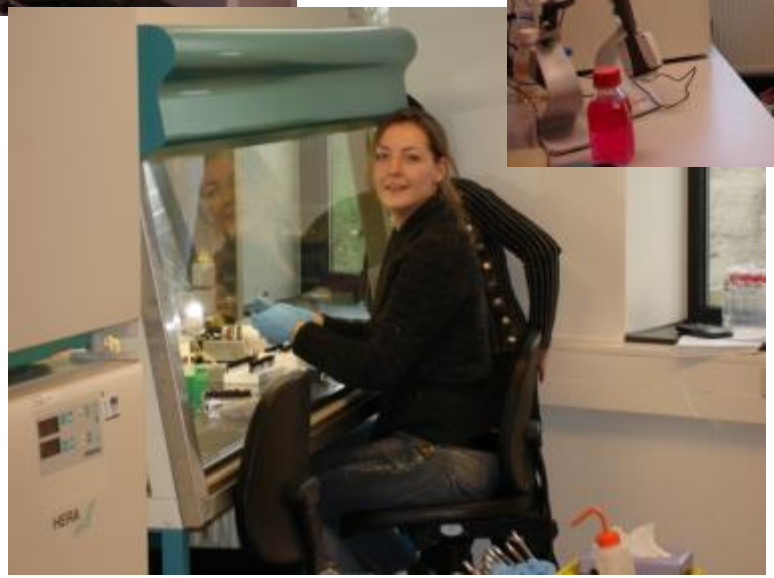
BDS global network of licensee's



Published articles on CALUX test



BDS lab



CALUX advantages

- **speed (possible in 28 hrs)**
- **Cost-efficient (ca. 70%)**
- **Sensitivity same as chemical HRGC/HRMS**
- **ISO 17025 accredited laboratory and training**
- **Weekly capacity of BDS more than 300 samples**

Equipment


Shaker


Autoclave

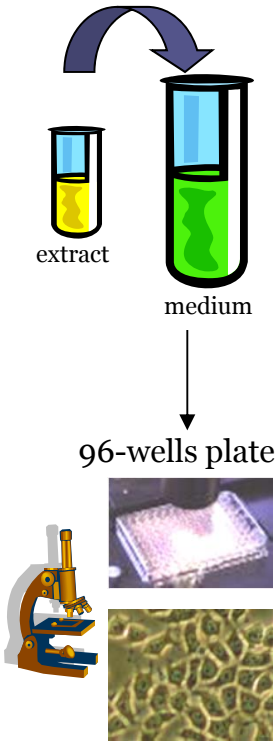

Microscope


Concentrator
PIERCE
Reacti-Therm III
HEATING MODULE

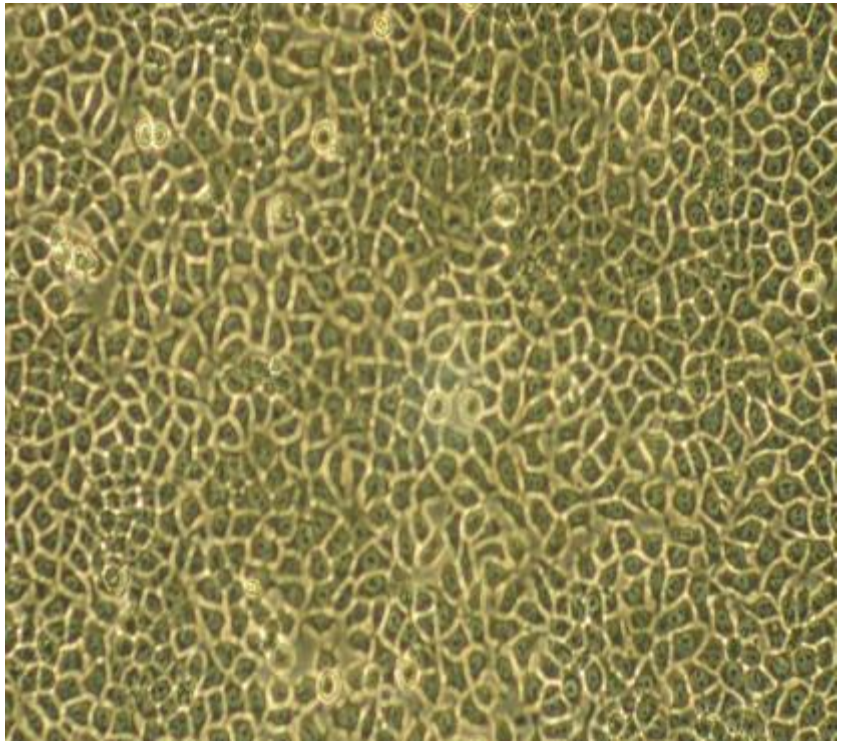

HERA cell
CO_2 incubator
Thermo

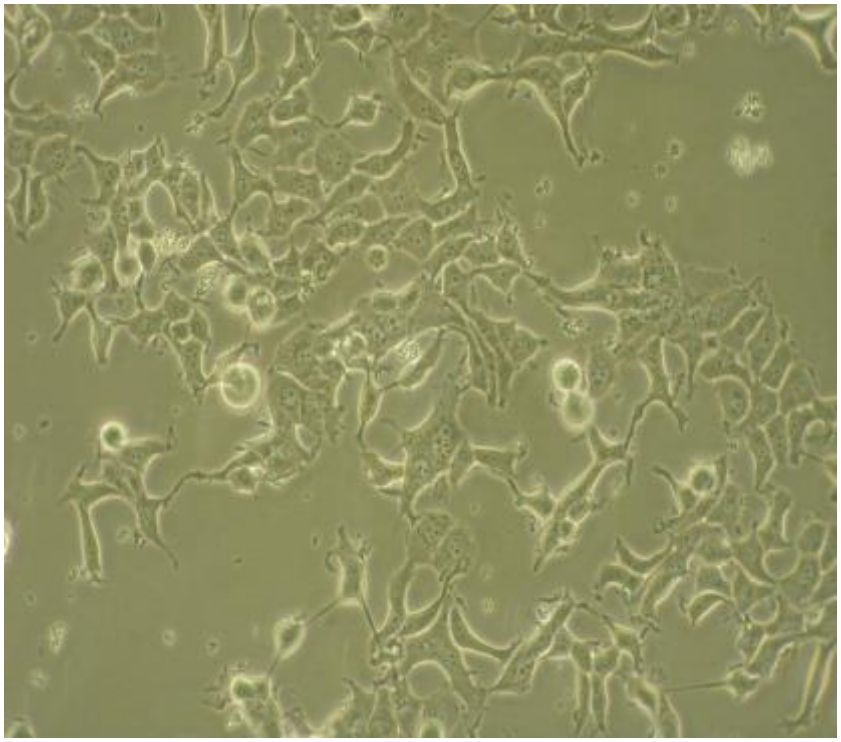

Luminometer

Cell culture



H4II rat cells under the microscope



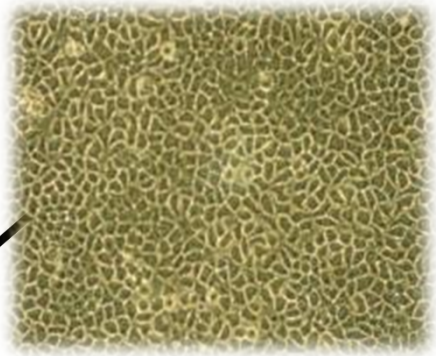


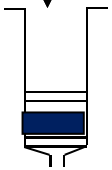
Environmental samples

Sample preparation

Extraction

Cell culture

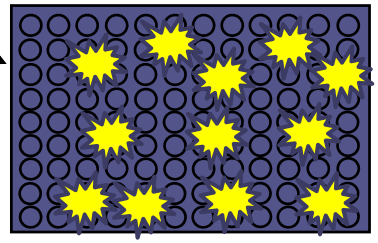




Silica Clean-Up

Methanol

DMSO

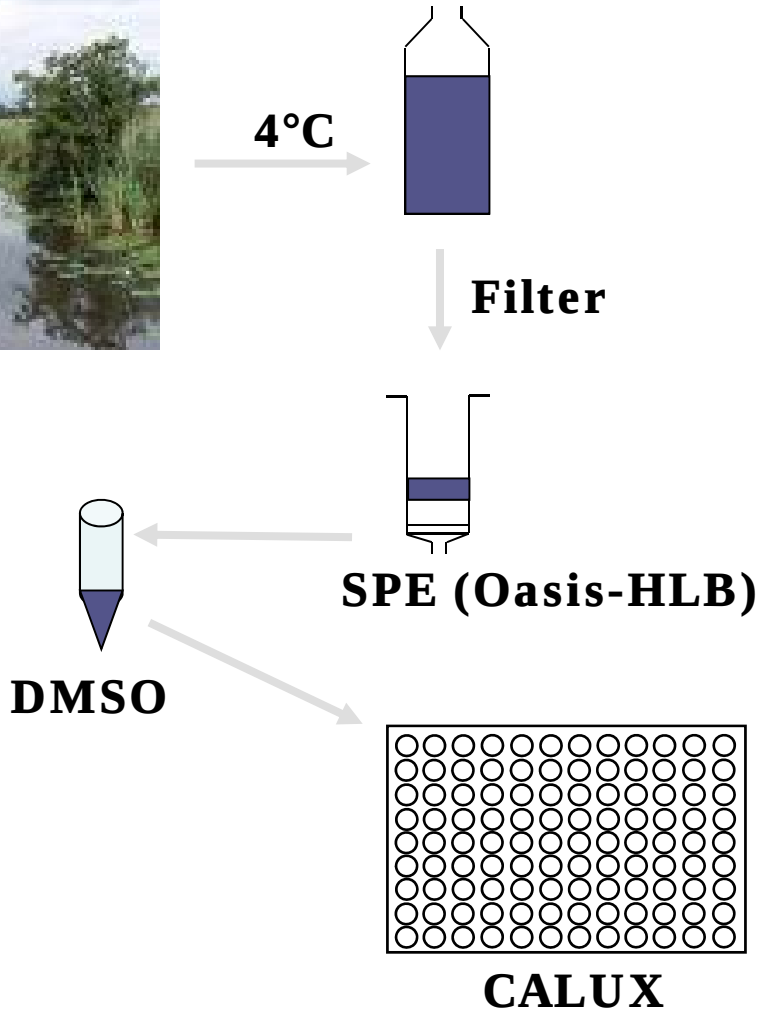


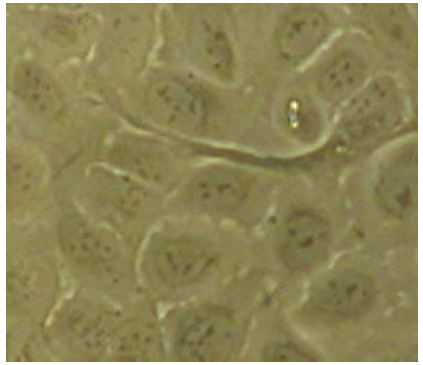
Microplates

Luminometer

Based on Dr. Peter A. Behnisch
BioDetection Systems , Amsterdam

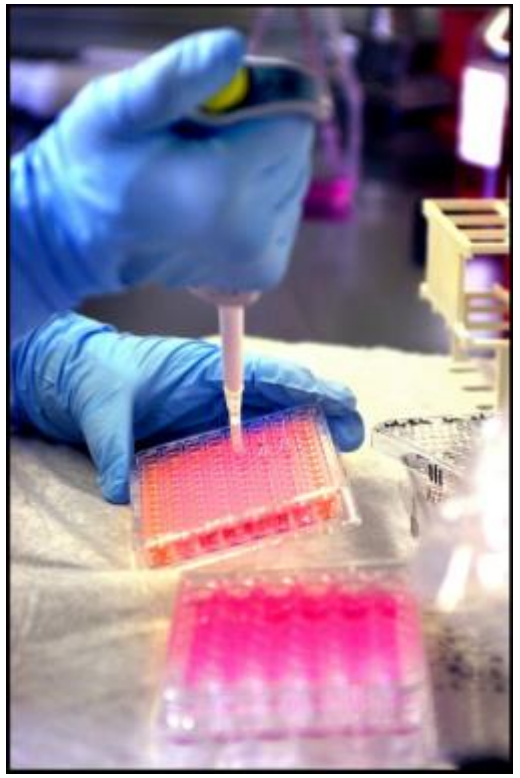
Sample preparation (water)





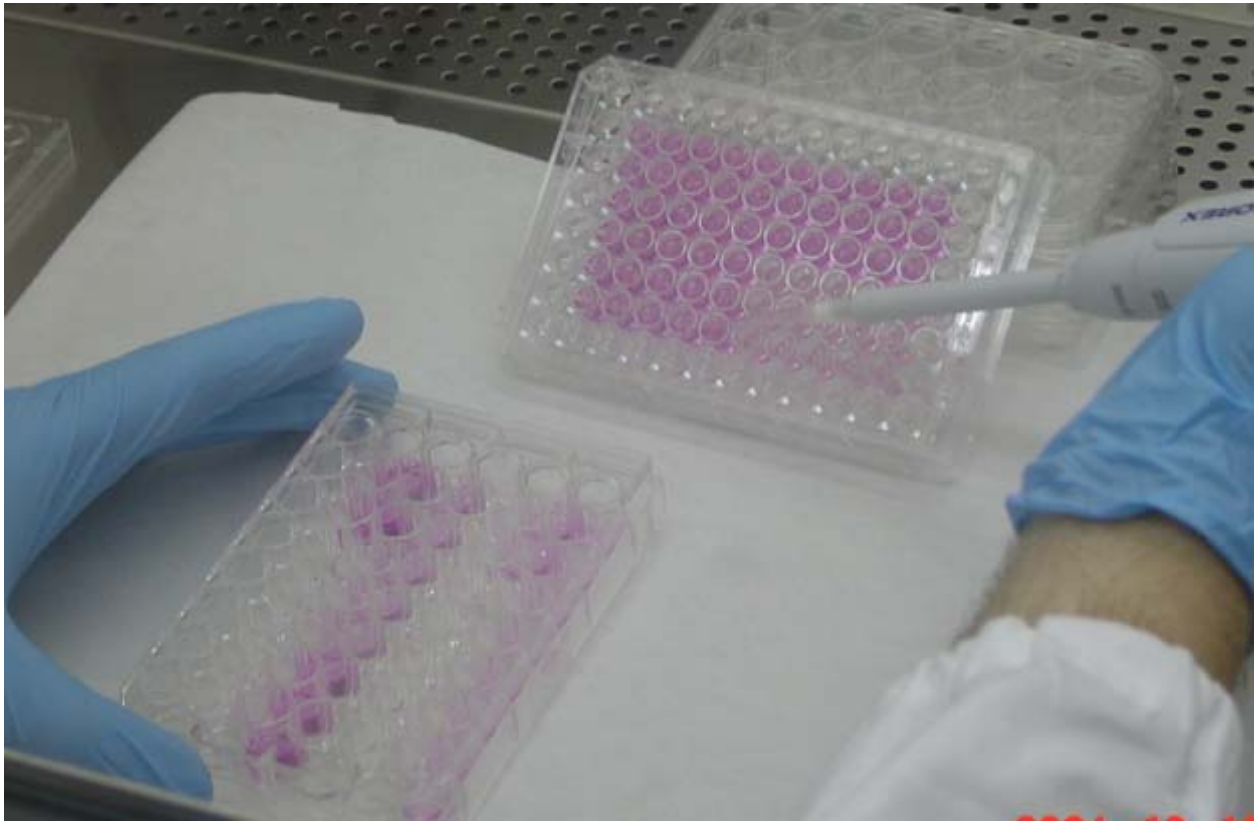
Seeding

Plate preparation : distribute cells on microplate and incubate for 24 hours



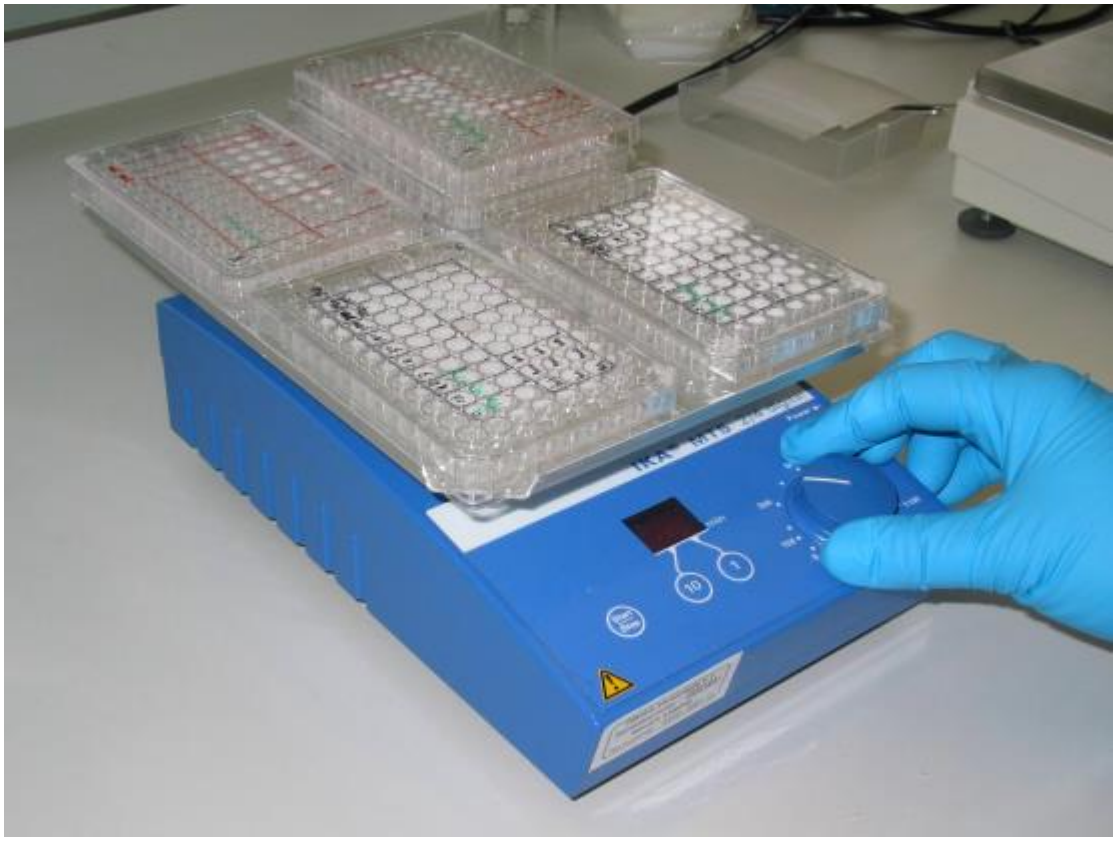
Exposure

Pipette samples and reference substance into the microplate



Exposure

Shake gently microplate and incubate for 24 hours



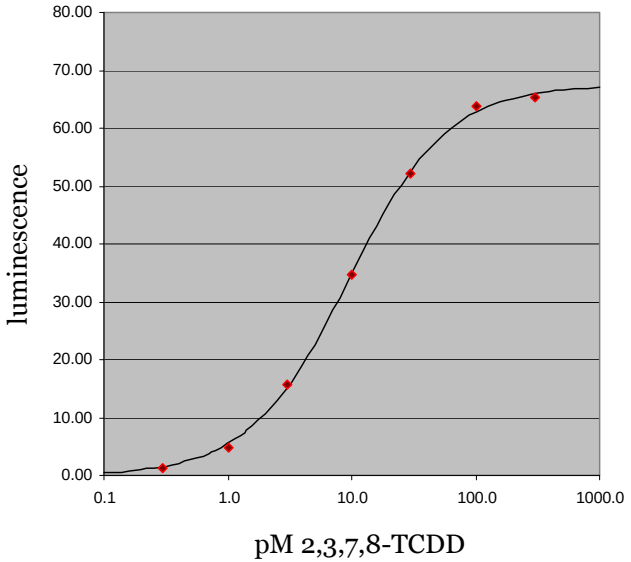
Reading

Lise cells, add luciferin and read luminescence

Data evaluation

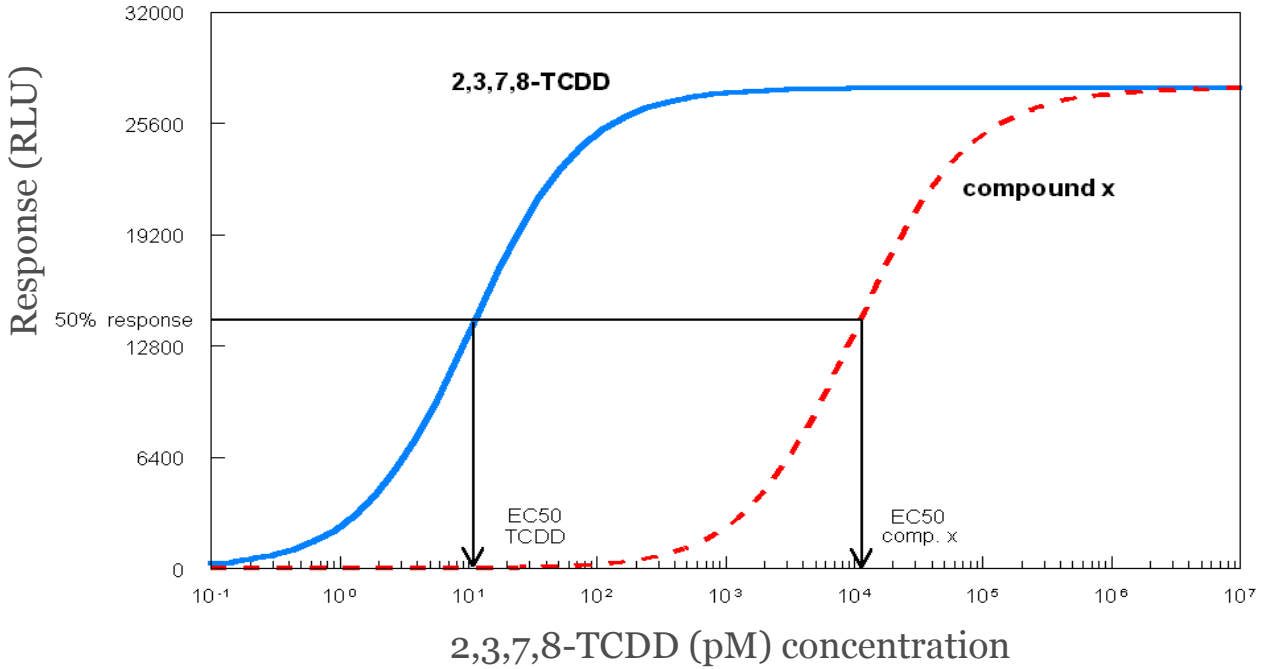
TCDD-0	TCDD-0.3	TCDD-1.0	TCDD-3.0	TCDD-10	TCDD-30	TCDD-100	TCDD-300	DMSO	5157
TCDD-0	TCDD-0.3	TCDD-1.0	TCDD-3.0	TCDD-10	TCDD-30	TCDD-100	TCDD-300	DMSO	5157
TCDD-0	TCDD-0.3	TCDD-1.0	TCDD-3.0	TCDD-10	TCDD-30	TCDD-100	TCDD-300	DMSO	5157
100xx	100xx	100xx	100xy	100xy	100xy	BW1	BW2	IRM1	IRM2
100xx	100xx	100xx	100xy	100xy	100xy	BW1	BW2	IRM1	IRM2
100xx	100xx	100xx	100xy	100xy	100xy	BW1	BW2	IRM1	IRM2

5.123	5.994	9.908	20.613	39.727	57.749	67.479	69.566	4.603	9.216
4.668	6.25	9.467	21.192	40.627	55.697	68.427	70.366	4.737	8.985
5.568	6.824	10.229	20.922	39.386	57.989	70.621	71.425	5.008	9.68
57.662	25.233	13.727	39.678	10.102	7.164	6.325	5.685	14.41	13.912
55.122	25.129	13.698	39.721	10.095	7.231	6.185	5.458	14.356	13.9225
56.335	24.956	13.721	39.796	10.147	7.211	6.421	5.777	14.421	13.856



Dilution	DR CALUX TEQ (pM) in well	TRUE/ FALSE	CHOICE (x)	Sample code	DR CALUX TEQ in sample	STDEV	Unit
1	30.0	**TRUE**		100xx	2.2E+01	1.3E+00	pg 2,3,7,8 TCDD TEQ/ g fat
10	5.2	**TRUE**		100xx	3.4E+00	1.4E-01	pg 2,3,7,8 TCDD TEQ/ g fat
30	1.7	**TRUE**	x	100xx	1.1E+00	1.5E-02	pg 2,3,7,8 TCDD TEQ/ g fat
1	10.9	**TRUE**		100xy	7.5E+00	5.8E-02	pg 2,3,7,8 TCDD TEQ/ g fat
10	1.1	**TRUE**	x	100xy	7.6E-01	2.9E+00	pg 2,3,7,8 TCDD TEQ/ g fat
30	0.4	**FALSE**		100xy	2.8E-01	1.0E+00	pg 2,3,7,8 TCDD TEQ/ g fat
1	0.2	**FALSE**	x	BW1	#NUM!	#NUM!	pg 2,3,7,8 TCDD TEQ/ g fat
1	0.1	**FALSE**	x	BW2	#NUM!	#NUM!	pg 2,3,7,8 TCDD TEQ/ g fat
10	1.5	**TRUE**	x	IRM1	1.0E+00	3.0E-02	pg 2,3,7,8 TCDD TEQ/ g fat
10	1.4	**TRUE**	x	IRM2	9.0E-01	4.0E-02	pg 2,3,7,8 TCDD TEQ/ g fat
1	0.84	**FALSE**	x	5157	#DIV/0!	#DIV/0!	pg 2,3,7,8 TCDD TEQ/ 0

Data evaluation



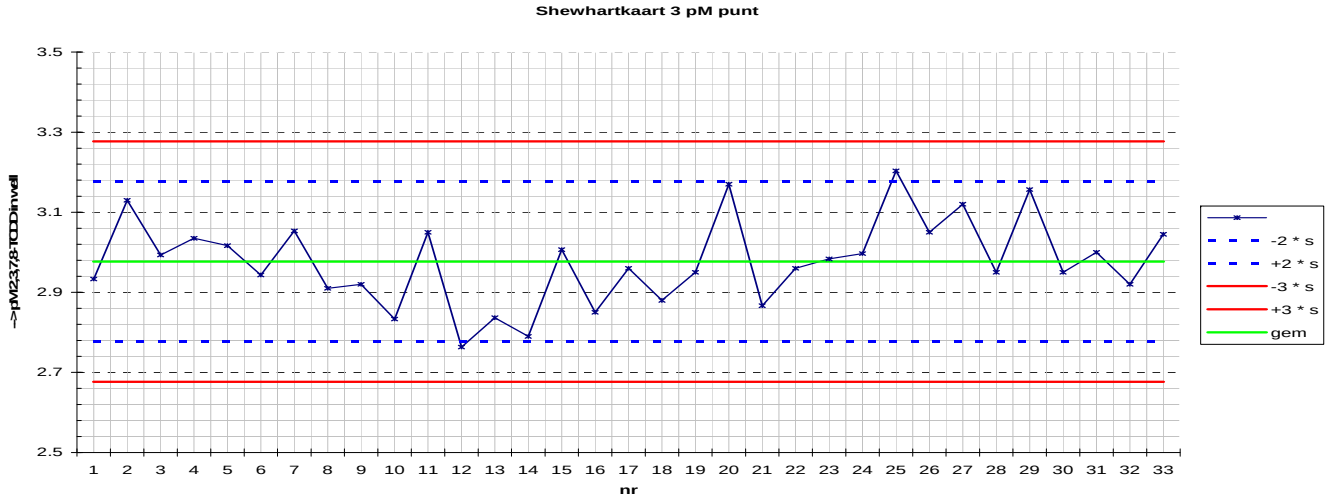
Formula: $TEF_x = ED50_{2,3,7,8\text{-}TCDD}/ED50_{,x}$

Performance criteria

- Every test run requires a series of reference concentrations of TCDD or a dioxin/furan/dl-PCB mixture (full dose-response curve with a $R^2 > 0.95$). However for screening purposes an expanded low level curve for analysing low level samples may be used

- A TCDD reference concentration (about 3 times the limit of quantification) on a quality control sheet shall be used for the outcome of the bioassay over a constant time period. An alternative may be the relative response of a reference sample in comparison to the TCDD calibration line since the response of the cells may depend on many factors

- A quality control (QC) chart for each type of reference material shall be recorded and checked to make sure the outcome is in accordance with the stated guidelines



TEQ calculation

HRGCMS TEQ calculation

Compound 1:	concentration 1	x TEF1 =	TEQ1	
Compound 2:	concentration 2	x TEF2 =	TEQ2	
Compound 3:	concentration 3	x TEF3 =	TEQ3	
Compound n:	concentration n	x TEFn =	TEQn	+
Total dioxin toxicity of mixture:			SumTEQ	

CALUX® TEQs – direct method

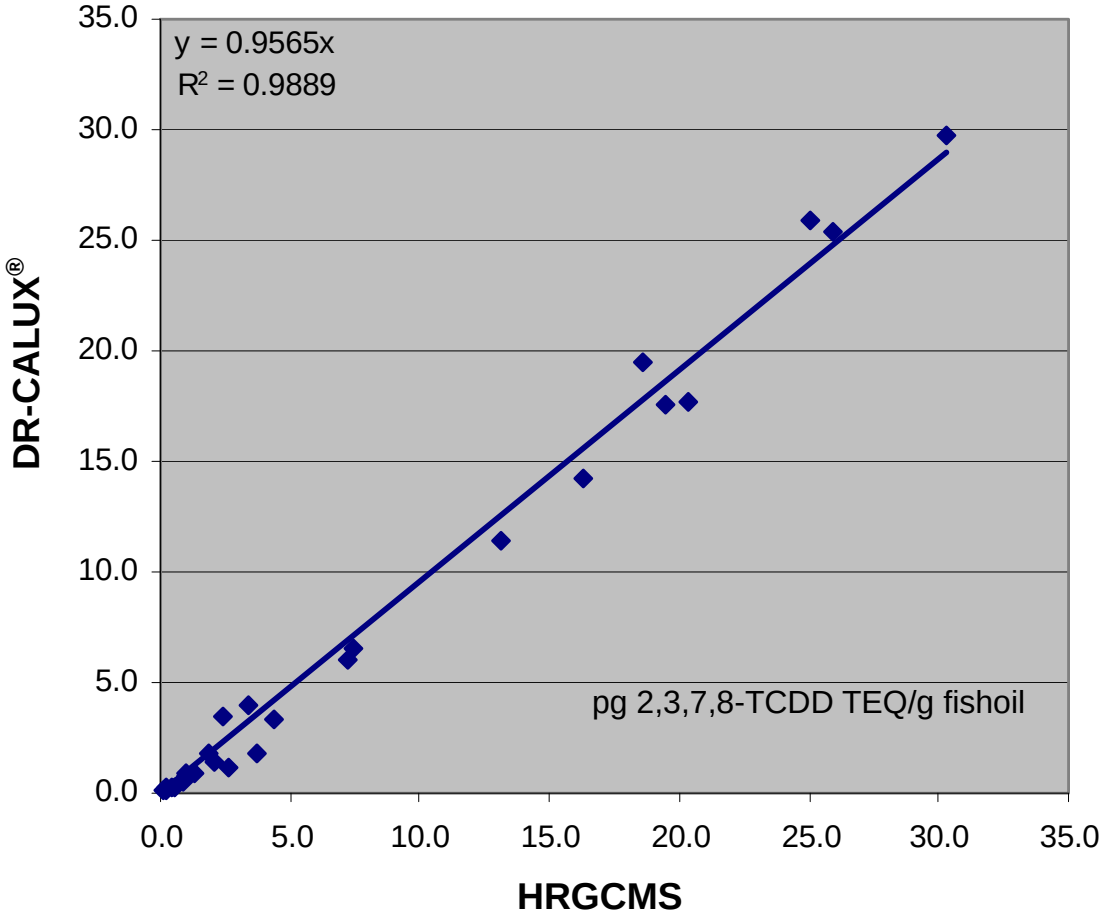
total TEQ value of sample (PCDDs/PCDFs/PCBs) → DR CALUX®

PCB specific TEQ value of sample (PCBs) → C-Split®

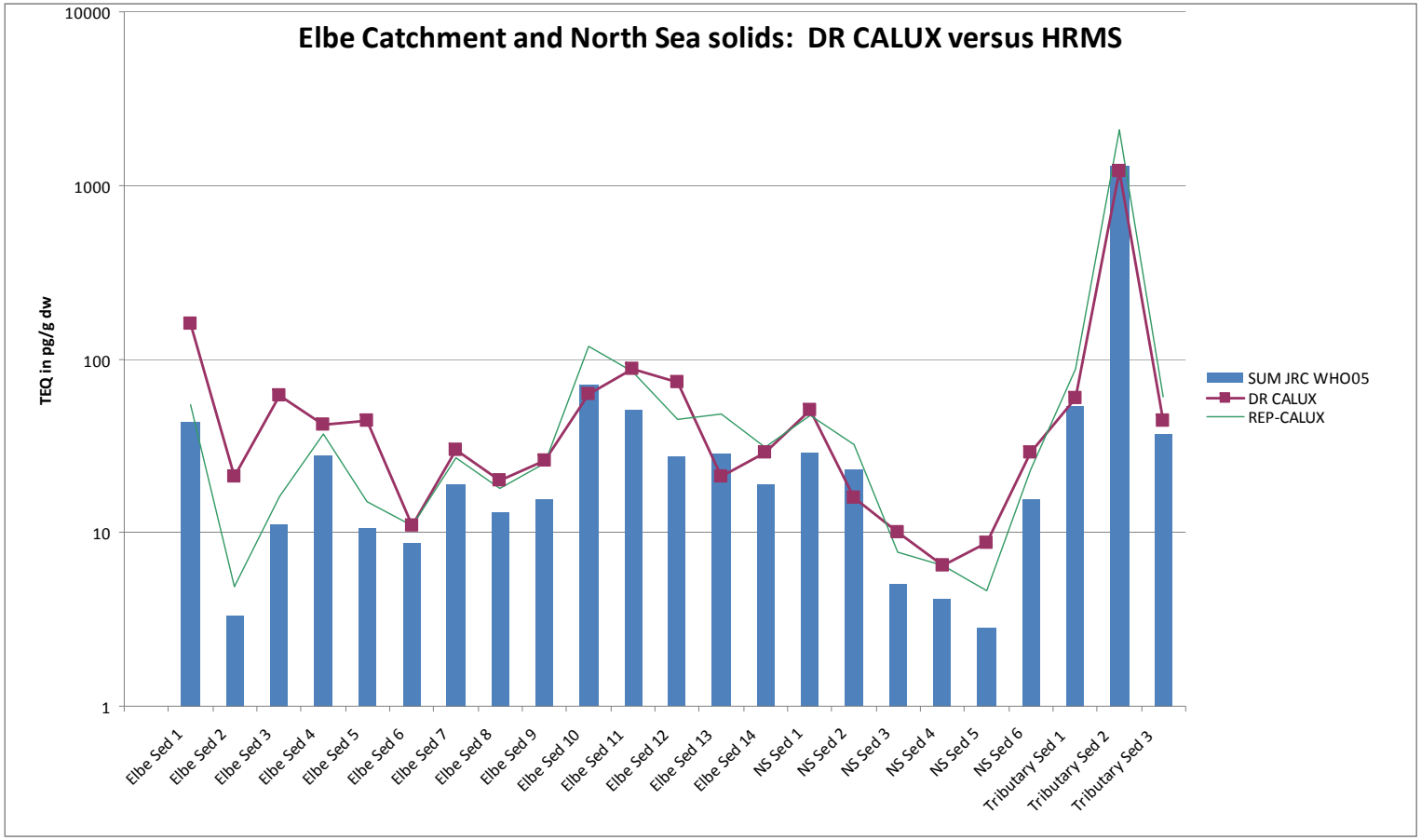
PCB specific TEQ value of sample (PCBs) → DR CALUX® - C-Split®

DR CALUX® vs HR-GC/MS

Total dioxin-levels (PCDDs, PCDFs and dioxin-like PCBs) in fishoil



DR CALUX vs. HR-GC/MS - sediment



Principle of the quantitative approach

- **Determine actual TEQ of test sample:**

  – TEQ response test sample lower than maximum EU limit - 25% value for respective matrix:

    sample declared **negative**

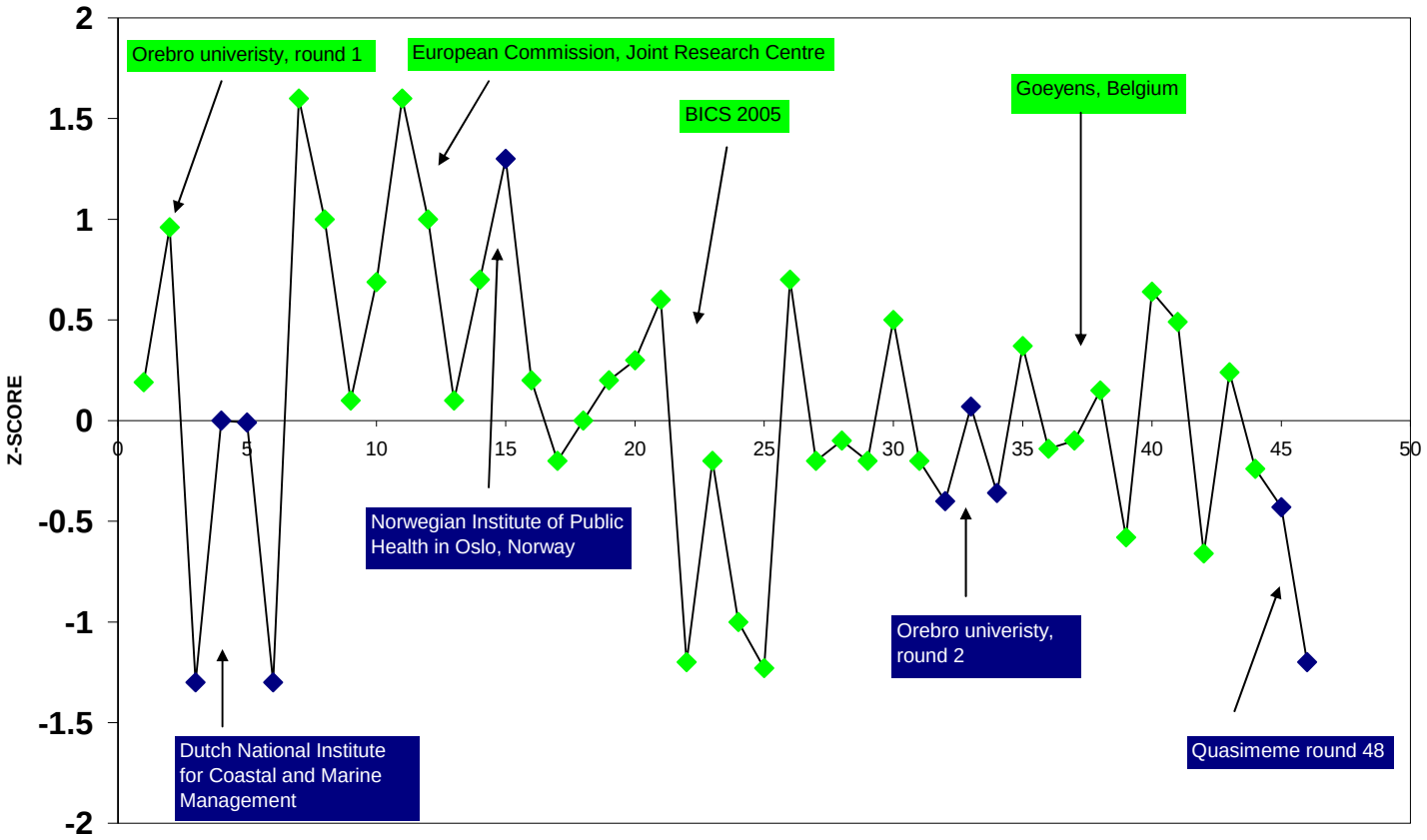
  – TEQ response test sample higher than maximum EU limit - 25% value for respective matrix:

    repeat DR CALUX analysis

  – TEQ response test sample higher than maximum EU limit value for respective matrix:

    sample declared suspected HR-GCMS confirmation required

Proficiency test performance BDS



EPA Method 4435

METHOD 4435

METHOD FOR TOXIC EQUIVALENTS (TEQS) DETERMINATIONS FOR DIOXIN-LIKE CHEMICAL ACTIVITY WITH THE CALUX® BIOASSAY

1.0 SCOPE AND APPLICATION

1.1 Method 4435 is a bio-analytical screening procedure for dioxin-like compounds in soils/sediments. This method is based on the ability of dioxin and related chemicals to activate the Ah receptor (AhR), a chemical-responsive DNA binding protein that is responsible for producing the toxic and biological effects of these chemicals. Measurement of the level of activation of AhR-dependent gene expression by a chemical or chemical extract provides a measure by which to estimate the relative potency and toxic potential of these chemicals and/or extracts with resulting values expressed as Toxic Equivalents (TEQs). Information on a commercially available genetically engineered cell line that contains the firefly luciferase gene under trans-activational control of the AhR (Ref. 41) can be found at the following website: http://www.dioxins.com/

Revision 0
February 2008

Some accreditations/approvals for DR CALUX®

Japan:

Bioassays are official screening methods for soil, sediments, *fly ash and emission gas*

Europe/Korea/Thailand/Taiwan/Australia:

Bioassays are official screening methods for *feed/food*

Norway:

CALUX testing used for evaluating toxicity of *sediments*

The Netherlands:

CALUX testing for evaluating dioxin-TEQ for *sediments*

USA:

Bioassay testing for screening of dioxin-TEQ for polluted sites and sediments (EPA 4435 for CALUX)

Evaluation of potential bioaccummulative compounds exerting endocrine-disrupting activities in wild animals using *in vitro* bioassays and chemical fractionation

Suzuki, G.[1,*], Tue, N.M.[1], van der Linden, S.[2], Someya, M.[1], Takahashi, S.[1], Brouwer, A.[2,4], van der Burg, B.[2], Lamoree, M.[3], van Velzen, M.[3], Isobe, T.[1], Tajima, Y.[5], Yamada, T.[5], Tanabe, S[1]

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Agonistic activity

AR-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.3.E-04	NA	7.1.E-03	1.1.E-01	1.1.E-03
Baikal seal (1992)-Blubber	4.3.E-04	NA	2.3.E-03	3.4.E-04	1.0.E-03
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	4.9.E-01	7.3.E-03
Baikal seal (1992)-Liver	1.9.E-03	NA	NA	2.8.E-01	1.2.E-03
Common cormorant-Liver	1.9.E-03	NA	3.1.E-03	2.8.E-01	1.2.E-02
Raccoon dog-Liver	1.9.E-03	NA	9.3.E-04	2.8.E-01	1.2.E-02
Finless porpoise-Liver	1.9.E-03	NA	3.1.E-03	8.3.E-04	1.2.E-02

ERa-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.3.E-04	1.1E-02	7.1.E-03	NA	1.1E-03
Baikal seal (1992)-Blubber	4.3.E-04	2.6E-03	2.3.E-03	NA	1.0E-03
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	4.9.E-01	7.3.E-03
Baikal seal (1992)-Liver	1.9.E-03	1.2E-02	NA	NA	1.2.E-03
Common cormorant-Liver	1.9.E-03	NA	3.1.E-03	2.8.E-01	3.4E-03
Raccoon dog-Liver	1.9.E-03	NA	9.3.E-04	NA	2.2E-05
Finless porpoise-Liver	1.9.E-03	NA	3.1.E-03	8.3.E-04	4.1E-03

GR-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.3.E-04	1.1E-02	7.1.E-03	1.1.E-01	1.1.E-03
Baikal seal (1992)-Blubber	4.3.E-04	2.6E-03	2.3.E-03	3.4.E-04	1.0.E-03
Baikal seal (2005)-Liver	1.8.E-03	1.5E-02	1.5E-02	4.9.E-01	7.3.E-03
Baikal seal (1992)-Liver	1.9.E-03	4.1E-03	NA	2.8.E-01	NA
Common cormorant-Liver	1.9.E-03	NA	3.1.E-03	2.8.E-01	1.2.E-02
Raccoon dog-Liver	1.9.E-03	4.1E-03	NA	2.8.E-01	1.2.E-02
Finless porpoise-Liver	1.9.E-03	NA	3.1.E-03	8.3.E-04	1.2.E-02

PR-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.3.E-04	NA	7.1.E-03	NA	1.1E-03
Baikal seal (1992)-Blubber	4.3.E-04	NA	2.3.E-03	NA	NA
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	NA	NA
Baikal seal (1992)-Liver	1.9.E-03	NA	NA	NA	NA
Common cormorant-Liver	1.9.E-03	NA	NA	2.8.E-01	NA
Raccoon dog-Liver	1.9.E-03	NA	NA	NA	1.2E-02
Finless porpoise-Liver	1.9.E-03	NA	NA	NA	1.2E-02

Antagonistic activity

AR-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.3.E-04	1.1.E-03	7.1.E-03	1.1.E-03	1.1.E-03
Baikal seal (1992)-Blubber	4.3.E-04	2.6.E-03	2.3.E-03	3.4.E-04	1.0.E-03
Baikal seal (2005)-Liver	1.8.E-03	5.0.E-03	1.5.E-02	1.5.E-03	7.3.E-03
Baikal seal (1992)-Liver	1.9.E-03	1.2.E-03	9.3.E-03	2.8.E-03	1.2.E-03
Common cormorant-Liver	1.9.E-03	1.2.E-03	9.3.E-03	2.8.E-04	1.2.E-02
Raccoon dog-Liver	1.9.E-03	1.2.E-02	9.3.E-03	8.3.E-04	1.2.E-02
Finless porpoise-Liver	1.9.E-03	4.1.E-04	9.3.E-03	8.3.E-04	1.2.E-02

ERa-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5E-04	1.1E-02	2.1E-02	3.6E-04	1.1E-03
Baikal seal (1992)-Blubber	4.5E-04	2.6E-03	7.7E-03	3.4E-04	1.0E-03
Baikal seal (2005)-Liver	1.8E-03	1.5E-02	1.5E-02	1.5E-03	2.2E-03
Baikal seal (1992)-Liver	1.9E-03	1.2E-02	3.1E-03	3.8E-04	1.2E-03
Common cormorant-Liver	1.9E-03	1.2E-03	9.3E-03	2.8E-04	NA
Raccoon dog-Liver	1.9E-03	1.2E-02	9.3E-03	2.8E-04	NA
Finless porpoise-Liver	1.9E-03	4.1E-04	9.3E-03	2.8E-04	NA

GR-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5E-04	1.1E-02	2.1E-02	1.1E-03	1.1E-03
Baikal seal (1992)-Blubber	4.5E-04	2.6E-03	2.3E-02	1.0E-03	1.0E-03
Baikal seal (2005)-Liver	1.8E-03	1.5E-02	1.5E-02	1.5E-03	2.2E-03
Baikal seal (1992)-Liver	1.9E-03	4.1E-03	3.1E-03	8.3E-04	4.1E-04
Common cormorant-Liver	1.9E-03	1.2E-03	9.3E-03	8.3E-04	1.2E-02
Raccoon dog-Liver	1.9E-03	4.1E-03	9.3E-03	8.3E-04	1.2E-02
Finless porpoise-Liver	1.9E-03	1.2E-03	9.3E-03	8.3E-04	1.2E-02

PR-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5E-04	1.1E-02	2.1E-02	3.6E-04	1.1E-03
Baikal seal (1992)-Blubber	4.5E-04	7.7E-04	2.3E-02	3.4E-04	1.0E-03
Baikal seal (2005)-Liver	1.8E-03	5.0E-03	1.5E-02	4.9E-04	2.2E-03
Baikal seal (1992)-Liver	1.9E-03	1.2E-03	3.1E-03	2.8E-04	1.2E-04
Common cormorant-Liver	1.9E-03	1.2E-03	9.3E-03	8.3E-04	1.2E-02
Raccoon dog-Liver	1.9E-03	4.1E-03	3.1E-03	2.8E-04	1.2E-02
Finless porpoise-Liver	1.9E-03	4.1E-04	9.3E-03	2.8E-04	1.2E-02

DR-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	1.1.E-05	1.1.E-04	NA	NA	3.6E-05
Baikal seal (1992)-Blubber	1.4.E-05	2.6.E-05	NA	NA	1.0E-03
Baikal seal (2005)-Liver	8.1.E-05	1.5.E-04	5.0.E-03	NA	2.2E-03
Baikal seal (1992)-Liver	1.2.E-05	4.1.E-05	NA	NA	1.2E-04
Common cormorant-Liver	7.9.E-06	4.1.E-05	NA	NA	NA
Raccoon dog-Liver	6.4.E-05	1.2.E-04	NA	NA	NA
Finless porpoise-Liver	1.9.E-04	NA	9.3.E-04	8.3.E-05	4.1E-03

PPARg1-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5.E-04	1.1.E-03	7.1.E-03	1.1.E-04	3.6.E-04
Baikal seal (1992)-Blubber	4.5.E-04	2.6.E-04	2.3.E-03	1.0.E-04	3.4.E-04
Baikal seal (2005)-Liver	1.8.E-03	1.5E-03	1.5.E-02	4.9.E-04	2.2.E-04
Baikal seal (1992)-Liver	1.9.E-03	4.1E-04	3.1.E-03	2.8.E-04	1.2.E-04
Common cormorant-Liver	1.9.E-03	1.2.E-04	9.3.E-04	8.3.E-05	4.1.E-03
Raccoon dog-Liver	1.9.E-03	1.2E-03	3.1.E-03	8.3.E-05	1.2.E-02
Finless porpoise-Liver	1.9.E-03	1.2.E-04	3.1.E-03	8.3.E-05	1.2.E-03

PPARg2-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5.E-04	1.1E-03	7.1.E-03	3.6.E-05	3.6.E-05
Baikal seal (1992)-Blubber	4.5.E-04	7.7E-04	7.7.E-04	3.4.E-05	1.0.E-04
Baikal seal (2005)-Liver	1.8.E-03	1.5E-03	5.0.E-03	4.9.E-05	7.3.E-05
Baikal seal (1992)-Liver	1.9.E-03	1.2E-04	9.3.E-04	2.8.E-05	4.1.E-05
Common cormorant-Liver	1.9.E-03	1.2.E-04	3.1.E-04	2.8.E-05	1.2.E-03
Raccoon dog-Liver	1.9.E-03	4.1.E-04	3.1.E-04	8.3.E-06	4.1.E-03
Finless porpoise-Liver	1.9.E-03	1.2.E-04	3.1.E-04	8.3.E-06	4.1.E-04

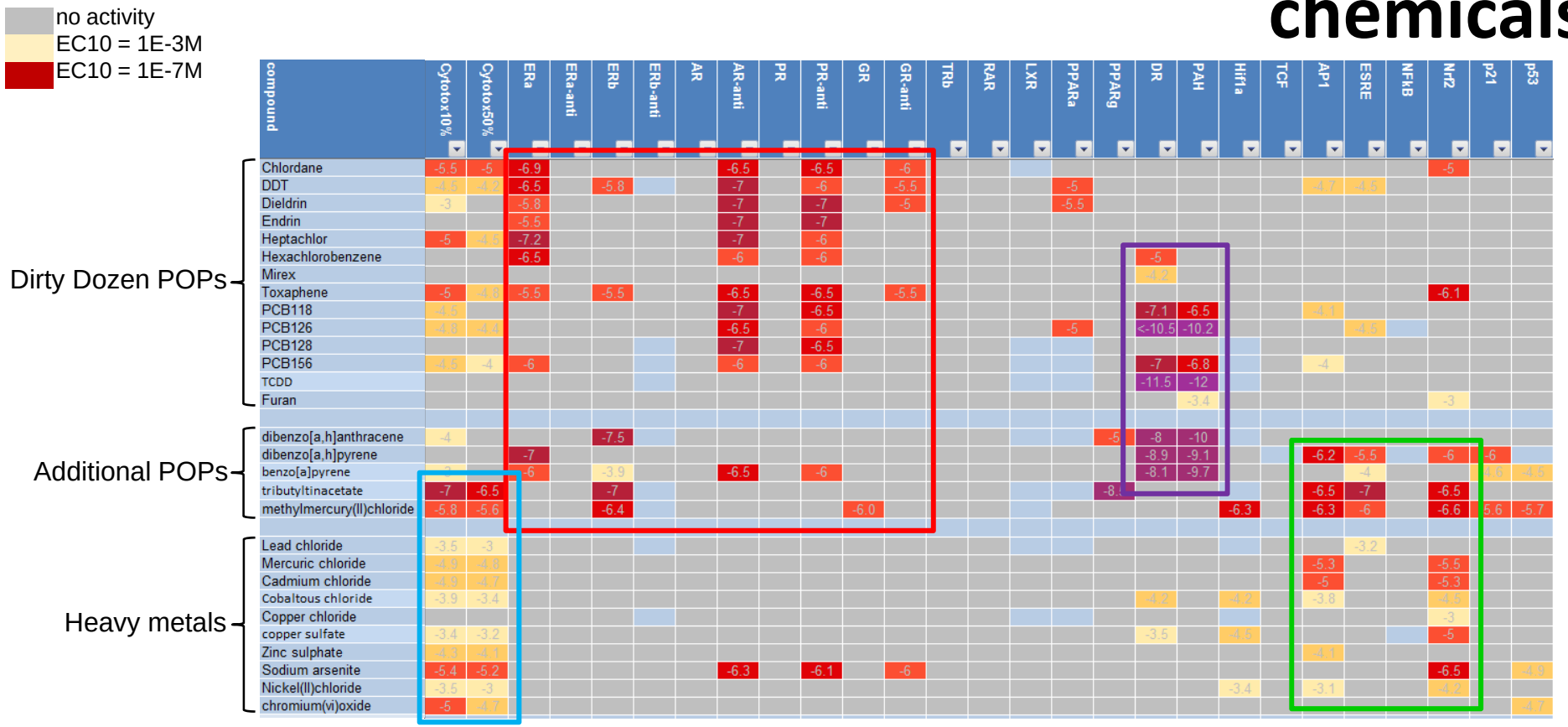
DR-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	NA	NA	2.1.E-02	3.6.E-04	NA
Baikal seal (1992)-Blubber	NA	NA	7.7.E-03	3.4.E-04	NA
Baikal seal (2005)-Liver	NA	NA	NA	4.9.E-04	NA
Baikal seal (1992)-Liver	NA	NA	3.1.E-03	3.8.E-04	NA
Common cormorant-Liver	NA	NA	9.3.E-03	3.8.E-04	1.2.E-02
Raccoon dog-Liver	NA	NA	3.1.E-03	8.3.E-05	1.2.E-02
Finless porpoise-Liver	NA	1.2.E-04	NA	NA	NA

PPARg1-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	4.5.E-04	NA	NA	NA	NA
Baikal seal (1992)-Blubber	4.5.E-04	NA	NA	NA	NA
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	NA	NA
Baikal seal (1992)-Liver	1.9.E-03	NA	NA	NA	NA
Common cormorant-Liver	1.9.E-03	NA	NA	NA	NA
Raccoon dog-Liver	1.9.E-03	NA	NA	NA	NA
Finless porpoise-Liver	1.9.E-03	NA	NA	NA	NA

PPARg2-CALUX	Persistent fraction	Crude hydrophobic fraction: Strong	Moderate	Mild	Weak
Baikal seal (2005)-Blubber	NA	NA	NA	NA	NA
Baikal seal (1992)-Blubber	NA	NA	NA	NA	NA
Baikal seal (2005)-Liver	NA	NA	NA	NA	NA
Baikal seal (1992)-Liver	NA	NA	NA	NA	NA
Common cormorant-Liver	NA	NA	NA	NA	NA
Raccoon dog-Liver	NA	NA	NA	NA	NA
Finless porpoise-Liver	NA	NA	NA	NA	NA

NA: Not analyzed due to ago/antagonistic response	Response at more than 1.0E-02 g-wet/well
Not detected at indicated dose	Response at 1.0E-2 to 1.0E-03 g-wet/well
Cytotoxicity at indicated dose	Response at 1.0E-3 to 3.0E-04 g-wet/well
Synergistic response at indicated dose	Response at less than 3.0E-04 g-wet/well

Toxicity pathways activated by environmental chemicals



Dirty Dozen POPs: endocrine | dioxin receptor

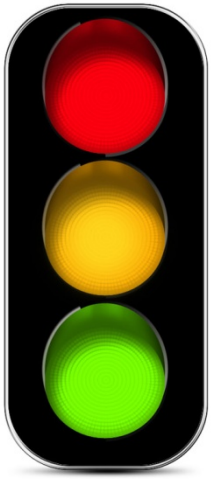
Additional POPs: dioxin receptor | stress pathways

Heavy metals: acute toxicity | stress pathways

CALUX based trigger values for drinking water

CALUX > trigger value → more detailed examination warrented

CALUX < trigger value → health risks can be waived



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

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Bioassay	Trigger value
ERα CALUX	3.8 ng E2-eq./L
AR CALUX	11 ng DHT-eq./L
GR CALUX	21 ng Dex-eq./L
PR CALUX	333 ng Org2058-eq./L
…. CALUX	….. eq./L

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