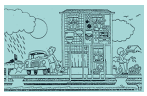
BioDetection Systems

Water safety assessment by Bioassays

Cell based toxicity screening by a panel of CALUX bioassays



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Who we are

BioDetection Systems B.V. ("BDS") is a Dutch company and ISO 17025 accredited service laboratory providing biological detection systems, such as the innovative CALUX® bioassays for the determination of ultra low levels of a variety of highly potent materials.

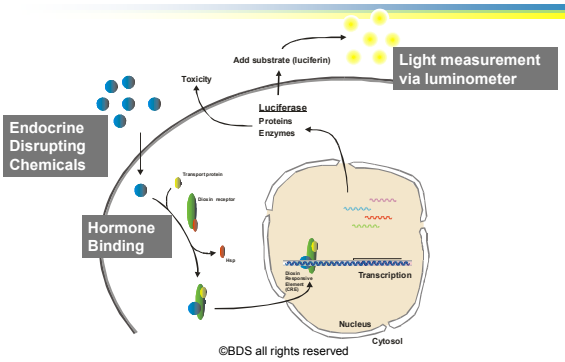
Mission

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

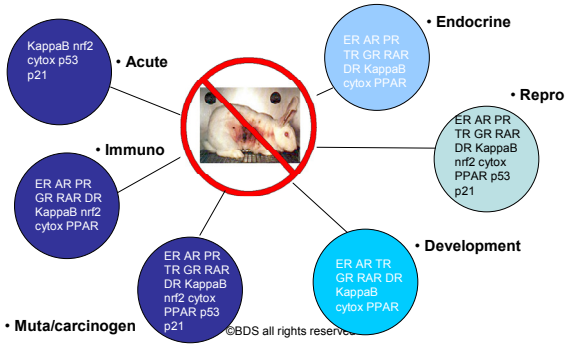
Partner in many international projects related to water:

- EC FP6 "TECHNEAU"
- Rhine Monitoring Project (RIWA)
- EC FP7 ChemScreen for REACH/3Rs
- Dutch Projects, z. Bsp. LEOS, ZORG, Genes4Water

CALUX® Principles



Panel of CALUX® biotests



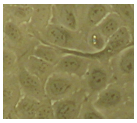
BDS's CALUX and typical applications (1)

- DR-CALUX (dioxins, furans, PCBs, POPs, PAKs, Ah receptor ligands)
- ERα-CALUX (natural, synthetic and plant-like estrogens, pseudo-estrogens, DES, endocrine disrupters, tamoxifen and other anti-estrogens)
- ERβ-CALUX (like ERα-CALUX, but more for plant-like estrogens)
- AR-CALUX (androgens, anabolic steroids, pseudo-androgens, antibiotic growth promoters, nandrolone, flutamide e.a. steroidal drugs)
- PR-CALUX (progesterone, MPA and other progestagene-activ compounds, steroid-based antidiuretics, aldosterone antagonists, aromatase inhibitors)

BDS's CALUX and typical applications (2)

- GR-CALUX (cortisol, corticosteroids)
- TRβ-CALUX (thyroid hormons, anti-thyroid agents, goitrogenic compounds, contrasting agents, POPs, environmental pollutants, nitrofen)
- RAR-CALUX (retinol, vitamin A, retinoids, retinoic acid)
- NF-κB-CALUX (endotoxins, TNFalfa-modulators, cytokines)
- PPARα and PPARγ-CALUX (fatty acid derivatives, peroxisome proliferators, alkyl tin, phthalates)
- P53 CALUX (the Guardian of DNA, genotoxic compounds)

Water Hormone - Bioanalysis by CALUX



Recommendation: STOWA, the Netherlands (2010)

Endocrine disrupting chemical analyzed by ER-CALUX – Drinking- and surface water

a) Drinking Water (RIVM) 'trigger-value: 7 ng EEQ/l.

b) Surface Water (RIVM) 'trigger-value: 1 ng EEQ/l.

In case of higher values it is recommended to find the responsible compound and to evaluate the destruction by water treatment plants.

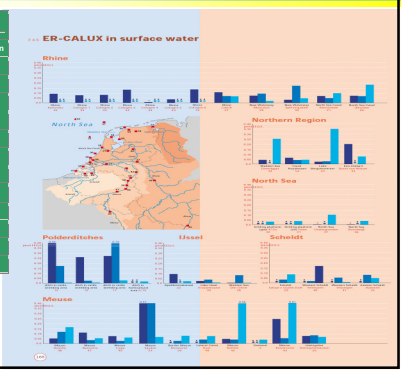
Source: Mennes, W. (2004). Assessment of human health risks for estrogenic activity detected in water samples, using the ER-CALUX assay. RIVM-notation, RIVM, Bilthoven; see also at http://themas.stowa.nl/Themas/Informatie.aspx?mID=7216&rID=1115&aID=1984

Dutch National Water Research (LOES 2002): Industrial and Municipal Wastewater

Estradiol equivalents (pmol EEQ/l)

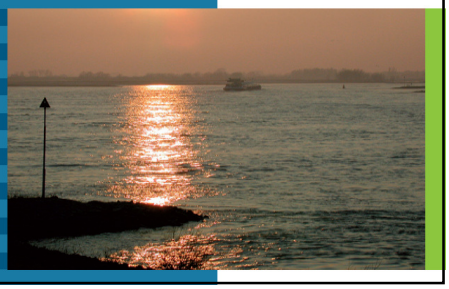
Compartment	*n*	Range (*n* > l.o.d.)	Median
Industrial wastewater:			
Effluent	3	0.2–9.5 (3)	0.9
Influent	5	5.8–560.4 (4[a])	317
Municipal wastewater:			
Effluent	10	<l.o.d.–2.2 (9)	0.3
Untreated influent	13	2.4–275.1 (13)	27.4
Surface water:			
Surface water	90	<l.o.d.–0.61 (85)	0.07
Polder ditches	11	0.003–0.74 (11)	0.03
Rainwater	3	0.01–0.22 (3)	0.13

Dutch River water: Dommel highest ER CALUX and intersex in bream

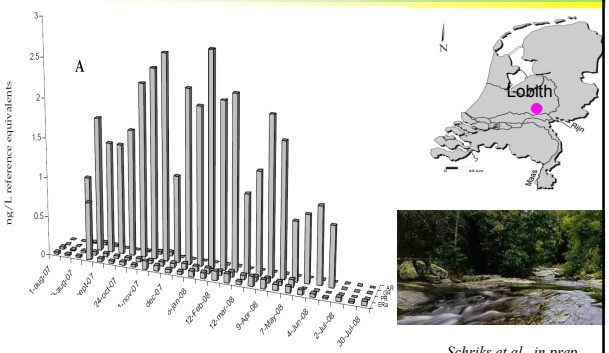


Temporal variation in multiple hormonal activities of surface waters located in the Dutch part of the Rhine basin

RIWA Rhine Water Works The Netherlands



BDS CALUX panel monitoring from Rhine surface water

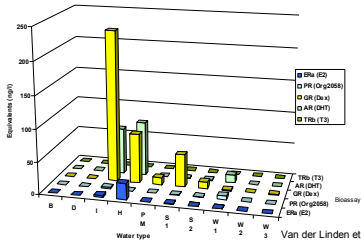


Schriks et al., in prep

Appendix 2. Raw data of the present study (location Lobith), as equivalents of the given reference compound.

CALUX	ERa	PR	GR	AR	TRB
LOBITH	E2 (ng/L)	Org2058 (ng/L)	Dex (ng/L)	DHT (ng/L)	T3 (ng/L)
1-aug-07	0.029	0.031	<LOD	<LOD	<LOD
14-aug-07	0.027	0.020	<LOD	<LOD	<LOD
29-aug-07	0.026	0.027	0.92	0.017	<LOD
12-sept-07	0.73	0.039	1.7	0.034	<LOD
26-sept-07	0.04	0.038	1.4	0.025	<LOD
10-oct-07	0.032	0.028	1.4	<LOD	<LOD
24-oct-07	0.041	0.032	1.6	<LOD	<LOD
7-nov-07	0.031	0.042	2.2	0.017	<LOD
21-nov-07	0.1	0.046	2.4	0.031	<LOD
5-dec-07	0.068	0.049	2.6	0.051	<LOD
19-dec-07	0.044	0.055	1.1	<0.05	<LOD
2-jan-08	0.042	0.078	2.2	<0.05	<LOD
16-jan-08	0.075	0.068	2	<0.05	<LOD

High levels of glucocorticoids found in hospital waste water by GR CALUX®



Van der Linden et al. Env. Sci. Techn. 2008, 42, 5814–5820

- Several hormonal activities found in waste and surface water
- Glucocorticoids are new problems, especially in hospital waste water?
- Profile shows hotspots of compound classes to focus further on

Comparison GR CALUX vs. chemical analysis for different water samples (Schriks et al., EST 2010)

Water sample	GR-CALUX (ng dex EQs/L) *(vd Linden et al., 2008)*	Detected glucocorticoids (LC-MS/MS)	Conc. LC-MS/MS [ng/L]	REP
Industry wastewater	243	•Prednisolone •Dexamethasone •Cortisone •Cortisol •*Fluocortin/fluprednidene* •*Hydrocortisone aceponate*	180 80 20 10 *Not confirmed* *Not confirmed*	41 80 0.07 0.7 --- --- ∑ *122*
Hospital wastewater	96	•Cortisone •Prednisolone •Cortisol •Prednisone •Triamcinoloneacetonide •*Fluocortin/fluprednidene* •*Hydrocortisone aceponate*	290 230 210 90 30 *Not confirmed* *Not confirmed*	0.2 52.7 15.5 0.2 67.8 --- --- ∑ *136*
Paper mill treated WW	11	No compounds detected	---	---
STP effluent	38	•Triamcinoloneacetonide •*Hydrocortisone aceponate*	10 *Not confirmed*	23 --- ∑ *23*

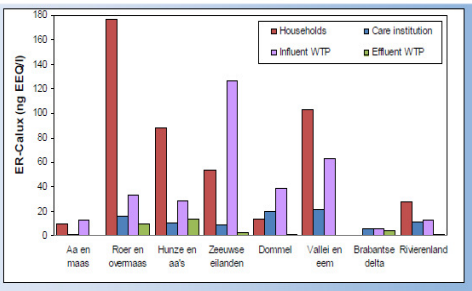
Emission of pharmaceuticals from Dutch care institutions into wastewater: chemical en effect monitoring

Barry Pieters[1], Lideke Vergouwen and Stefan Kools

[1]Grontmij, Amsterdam, The Netherlands, barry.pieters@grontmij.nl

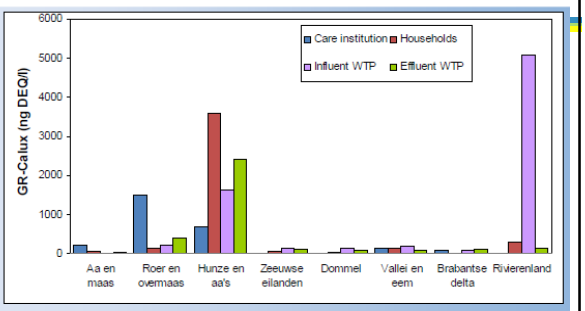
Grontmij stowa planning connecting respecting the future

ER-Calux: estrogen activity



- high removal rates in WTP
- care institutions: except ZE, lower activity than households
- households: RO, HA and VE high activity

GR-Calux: glucocorticoid activity



- differing removal rates in WTP
- care institutions: RO an HA high activity
- households: HA high activity

Dutch National Quality criteria's for ER CALUX (since 2002)

- Reference sample : 3 pM E2/well via Shewhart chart: 3s
- Induction of sample between LOD and EC_{50}
- Induction factor > 6
- Estradiol Standard curve linearity R^2 > 0.98

Rijkswaterstaat

Directoraat -Generaal

Rijksinstituut voor Kust en Zee/RIKZ

Standaardvoorschrift RIKZ/ER-CALUX® (Estrogen Receptor mediated - Chemical Activated LUciferase gene eXpression) bioassay.

Trefwoorden: estrogen receptor, reportergen, ER-CALUX®, estradiol

General EU guideline for Screening Methods e. g. EC/1883/2006 and SANCO 10376/2011

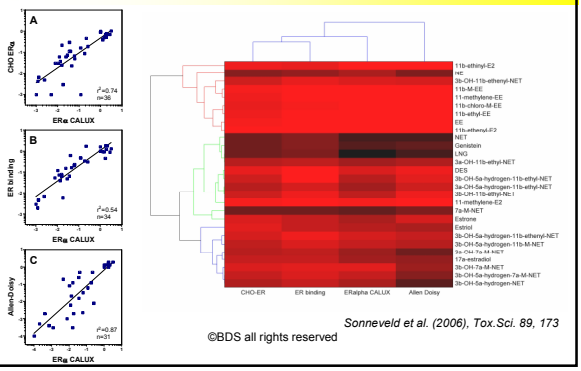
Quantitative Analysis:

a) LOD 1/5th of the relevant concentration (e.g. in Holland for surface water 1 ng EEQ/l x 1/5tel = 0,2 ng EEQ/l water
b) Standard deviations at ½x, 1x and 2x of the regulated levels in an acceptable range
c) Participation in proficiency testing
d) ISO 17025 accredited laboratory
e) False Negative Rate below 5%
f) Standard dilution series: linearity of standard R^2>0.95
g) Triple measurement of each dilution with standard deviation below15%; precision of 3 experiments below 30%

OECD Standardization via ICCVAM/ECCVAM: REACH/ToxCast & Alternatives

…..ongoing intensive efforts of EC-ECVAM, US- ICCVAM/ToxCAST and OECD: Developments of alternative non-animals testing

Evaluation for EC-CVAM: Comparison ERα CALUX® vs. other *in vivo* assays



Sonneveld et al. (2006), Tox.Sci. 89, 173

EC Project „Techneau“ for Water Safety (2006-2011)

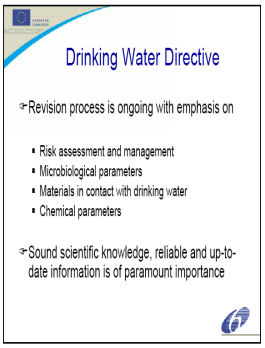


Table 1. Indicative evaluation of different effect assays for hormonal disruption (Mons, 2008).

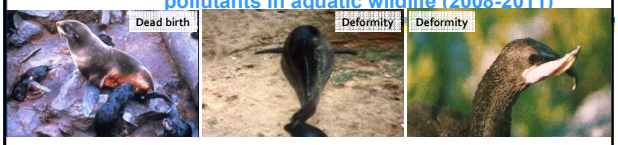
	Sensitivity		Robustness		Time to result	Operational specifications	
	Sources	Drinking water	Operational robustness	Selectivity		Ease-of-use	Maintenance requirements
Bioassays for estrogenic activity							
ER CALUX®	4	4	5	3	3	4	3
MVLN and MELN	2	2	2	2–3	2		
T47D-KBluc	3	3			3		
YES	1	1	4	3	2–3	3	3
E-screen	4	4	4	2	1	3	3
Bioassays for androgenic activity							
AR CALUX®	3	3	3	5	3	4	3
MDA-kb2	3	3		1	3		
PALM	3	3	3	3	3		
YAS	2	1	3	3	3		
A-screen	3	3		2	1		
Bioassays for progestagenic activity							
PR CALUX®	3	3	3	5	3	4	3
TM-Luc	3	3		1	3		
Yeast based assays	2	1		4	3		
Bioassays for glucocorticoid activity							
GR CALUX®	3	3	3	5	3	4	3
TGRM-Luc	3	3		1	3		
MDA-kb2	3	3		1	3		
Bioassays for thyroid activity							
TRβ CALUX®			3		3	4	3
T-Screen					1		
PC-DR-Luc							
xL58-TRE-Luc							

1 = very poor, 2 = poor, 3 = average, 4 = good, 5 = very good; when insufficient information was available, the box was left empty.

International projects and applications

- **USA**
- **Germany**
- **Japan**
- **European Union**

Japan:
Decline of aquatic biodiversity/increase diseases – pollutants in aquatic wildlife (2008-2011)



Relation with contaminants?

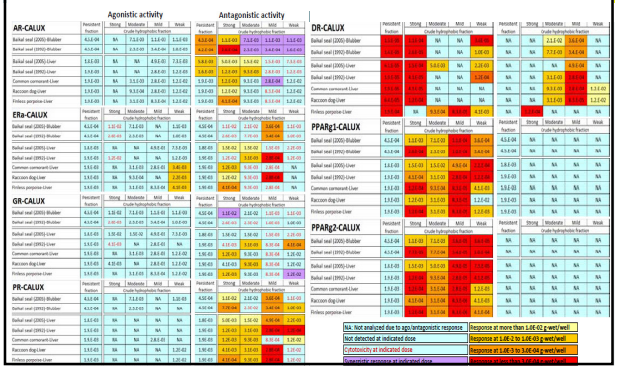
Dr. Go SUZUKI

Center for Marine Environmental Studies, Ehime University, Japan

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]

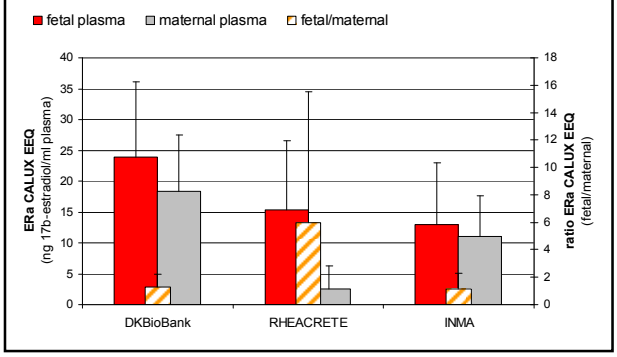
[1]*Center for Marine Environmental Studies, Ehime University, Matsuyama 790-8577, Japan,* [2]*BioDetection Systems b.v., 1098 XH Amsterdam, the Netherlands,* [3]*Institute for Environmental Studies, VU University, 1081 HV Amsterdam, the Netherlands,* [4]*Faculty of Earth and Life Sciences, VU University, 1081 HV Amsterdam, the Netherlands,* [5]*National Museum of Nature and Science, Tokyo 110-8718, Japan*



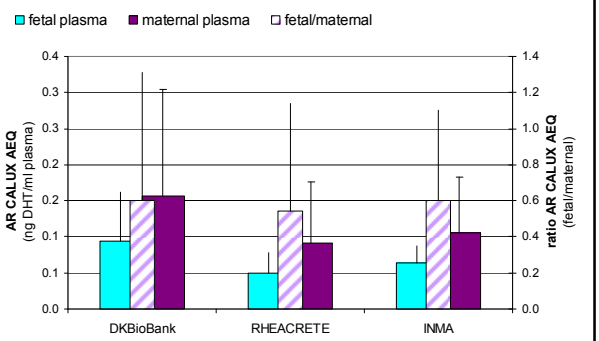
European Union: CALUX Hormone biomarkers for mother- newborn baby cohortes

- NEW GENERIS project from 2006 to 2009
- ca. 1200 mother- newborn baby cohorts from
- 6 different countries (Denmark, Greek, Spain, Norway, England)
- Cord blood was taken at day of birth

Estrogen-like compounds by ER CALUX



Androgen-like compounds by AR CALUX



Take home message

- Multiple biodetectors or Effect based analysis tools have been evaluated in many international projects for many environmental applications – they are currently also in several countries parallel in the last evaluation phase (EU, China, Japan, Australia)
- Endocrine disrupters are not only female hormones – male and other hormones (PR, GR, PPAR, RXR, TR) need more attention!
- Complex mixture cocktails and multi-pollutants effects are relevant and can be now evaluated by a panel of CALUX tests
- Search for R&D partners and policymakers to move Multiple biodetectors or Effect based analysis tools forward..interested ?

6th BioDetectors Workshop in Amsterdam in May 2012