State of the Art Biodetectors
Special emphasis on health monitoring

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Professor of Environmental Toxicology & Ecogenomics,
VU University Amsterdam
Managing Director of BE-Basic Consortium on biobased economy

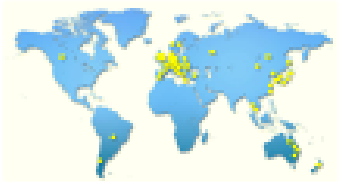
Core activity: Develop and apply novel bioassays for health & safety assessment

Safety assessment Authenticity testing Screening for bioactive compounds

Prototype (Bio)-analytical tools → Products, application → Automation → Bio-analytical services → Consultan-cy

Projects, partnerships

Demonstration studies



Novel bioanalytical solutions

Safety assessment:

- **Food,**
- **Feed,**
- **Water,**
- **Health**
- **Environment**
- **Chemicals**
- **Consumer products**

CALUX bioassays make use of cellular signal transduction pathways i.e., AOP-based bioassays

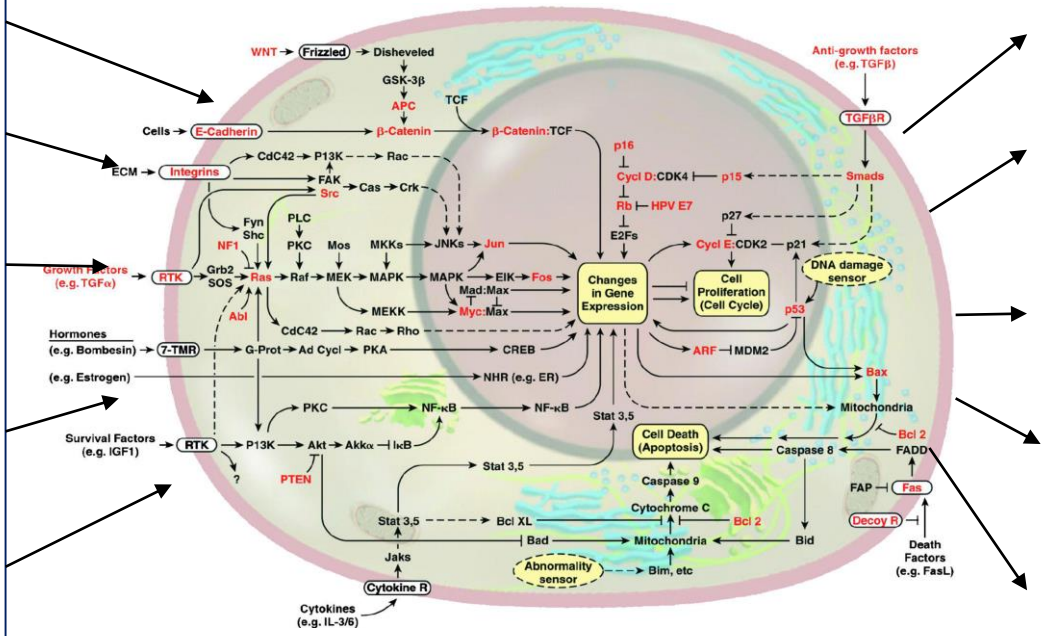
Dioxins, PAH

Toxic metals

PPAR activators

Endocrine Disruptors

Genotoxic compounds



Dioxin receptor

ROS, Nrf pathway

PPAR receptors

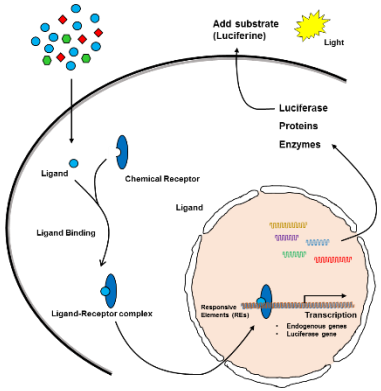
Hormone receptors

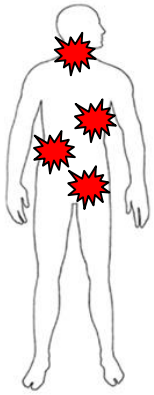
P53 pathway

Key benefits of using AOP-based Bioassays in health & safety assessment

Key Benefits:

- High predictivity of health related-effects
- Good estimate of total effect from mixtures
- Can predict unknown effects of chemicals
- Can discover unknown chemicals in matrices
- Level of precision similar to instrumental methods
- Low cost, high capacity, easy to operate





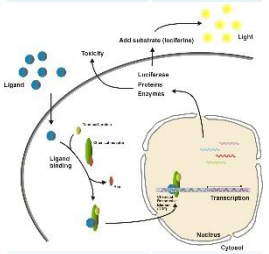
Best Useage:

- Most valuable tool for (human) biomonitoring
- Powerful screening tool for safety assessment e.g food, water
- Good *in vitro* alternative for chemical safety assessment

Many CALUX® assays available with different AOP

Nuclear receptors			Signaling pathways			Controls		
name	status	cell	name	status	cell	name	status	cell
DR CALUX	✓.	U2OS					✓.	U2OS
PAH CALUX	✓.	all					✓.	all
ER CALUX	✓.	all					✓.	all
ERalpha CALUX	✓.	all						
ERbeta CALUX								
ERalpha CALUX								
ERbeta CALUX								
AR CALUX								
PR CALUX								
GR CALUX								
TR CALUX								
RAR CALUX								
PPARγ1 CALUX								
PPARγ2 CALUX								
PPARα CALUX	✓.	U2OS	STAT CALUX	✓.	U2OS			
PPARδ CALUX	✓.	U2OS						
LXR CALUX	✓.	U2OS						
PXR CALUX	✓.	U2OS						
VDR CALUX	✓.	U2OS						
MR CALUX	✓.	U2OS						

- **Acute toxicity**
- **Oxidative stress**
- **AhR pathway**
- **Endocrine effects/EDCs**
- **Obesogens**
- **Reproductive effects**
- **Genotoxicity/carcinogenicity**
- **Metabolism**
- **etc**

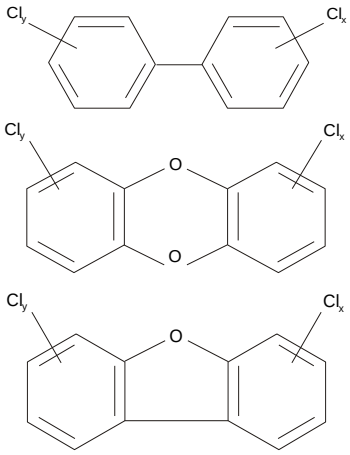


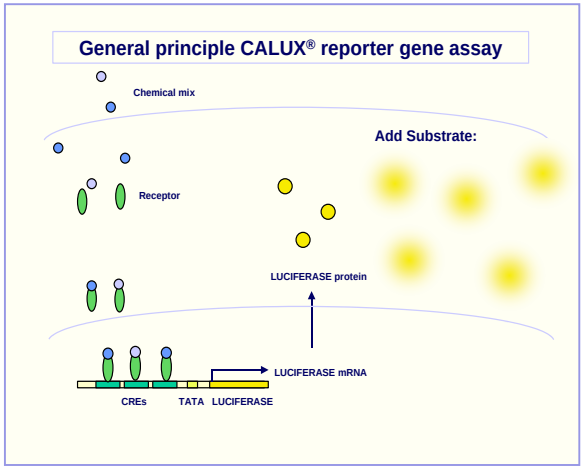
CALUX: n=28

Agonist/antagonist: 25x2=56 assays

AOP Example 1: Ah-Receptor and dioxins

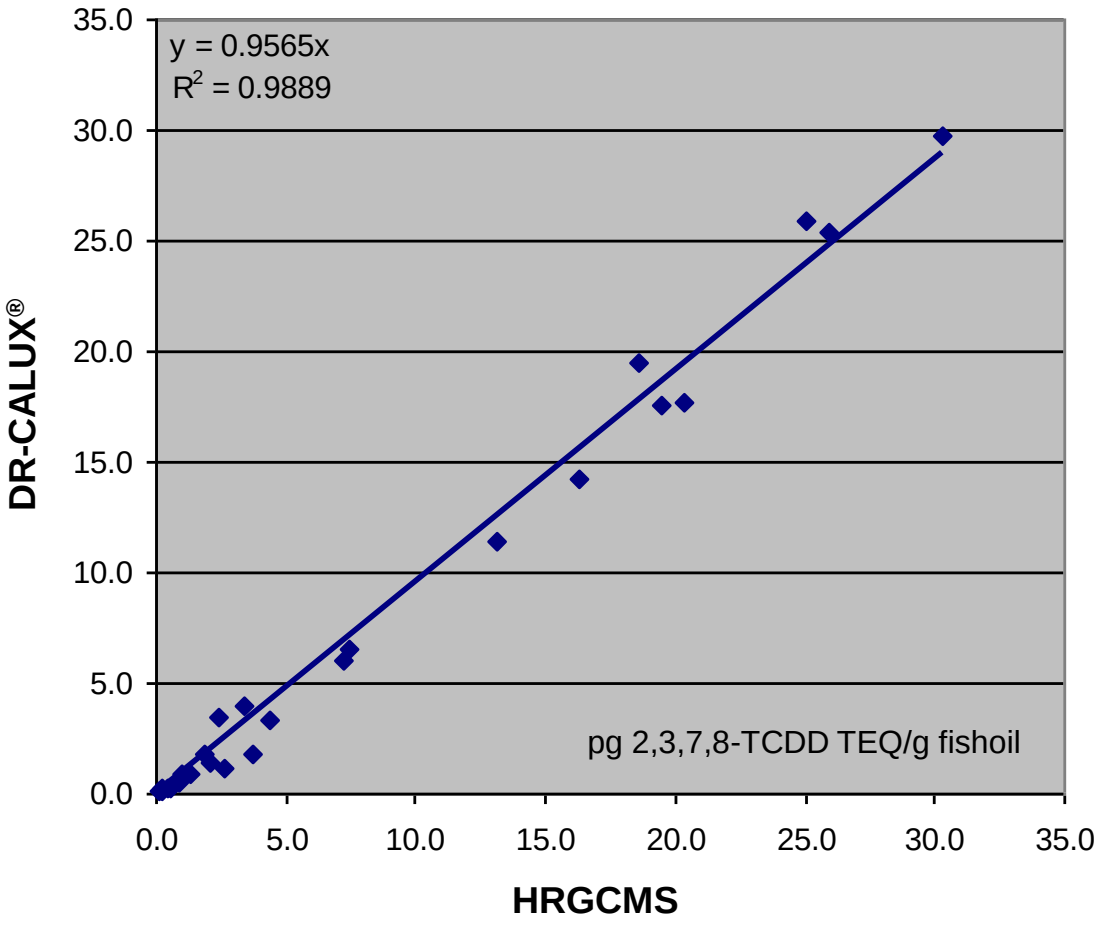
Using the Ah-Receptor as AOP in DR-CALUX for monitoring dioxin toxicity by mixtures of dioxin-like compounds





Comparison of Dioxin analysis in e.g food DR CALUX® vs HR-GC/MS

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



BDS develops bio-based detection methods and applies those in a wide range of sectors

Environment technology

Human epidemiology

Medicin

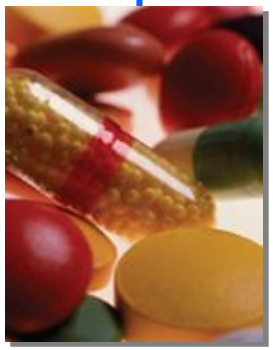
Technique and processes

Quality Management

Feed

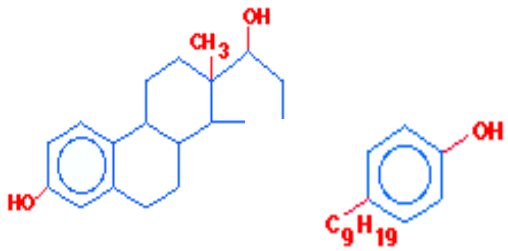
Food

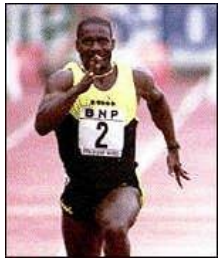
Pharma



AOP Example 2: Hormone Receptors and endocrine disruptors

Using adverse endocrine/reproductive pathways to develop a set of ER,AR,PR,GR,TR, etc-CALUX systems in a human cell line for monitoring endocrine disrupters and reprotoxic chemicals





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

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

Application domains for Hormone CALUX panel

Surface water quality

Waste water treatment

Human monitoring

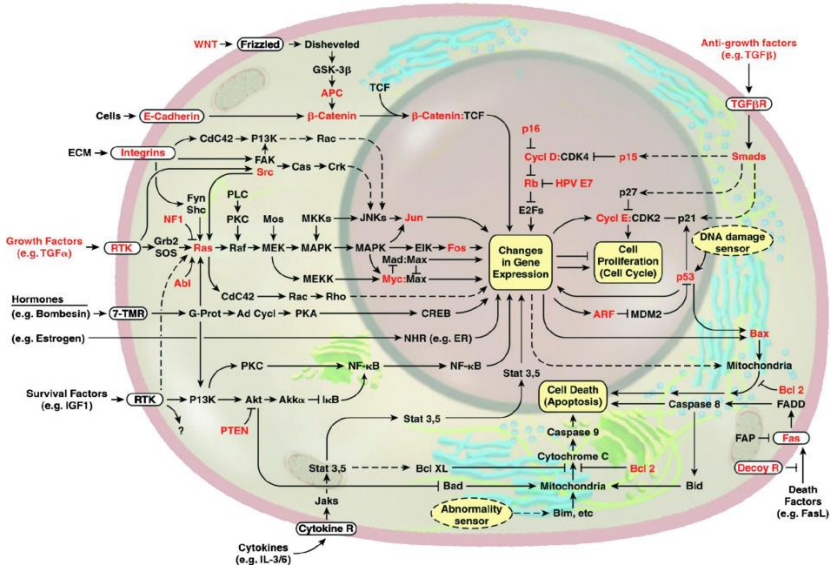
Safety & quality of Food packaging materials

Anabolic steroid abuse

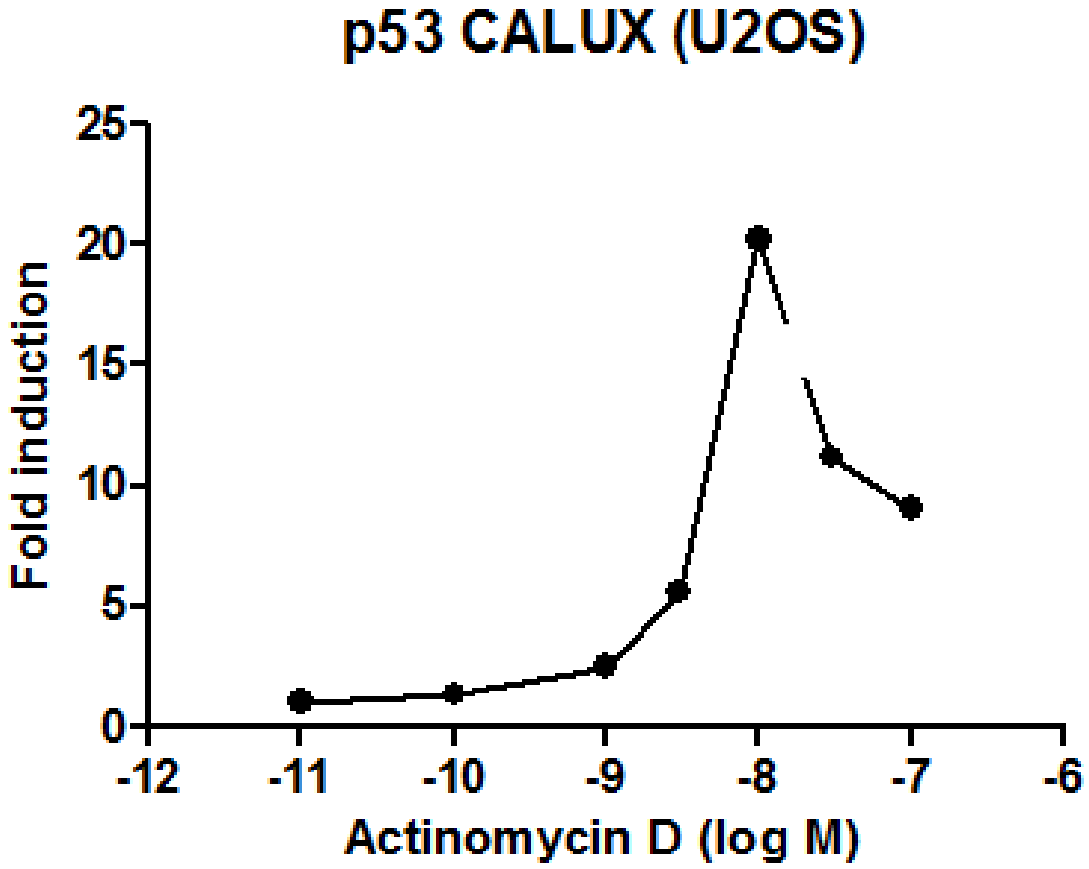
AOP Example 3: p53-Calux and carcinogens

Using a cancer-based adverse outcome pathway to develop a p53-CALUX in a human cell line for chemical carcinogens and mutagens testing

P53 "the guardian of the genome":



Typical p53 CALUX responses by chemical carcinogens



Performance characteristics of the p53 CALUX

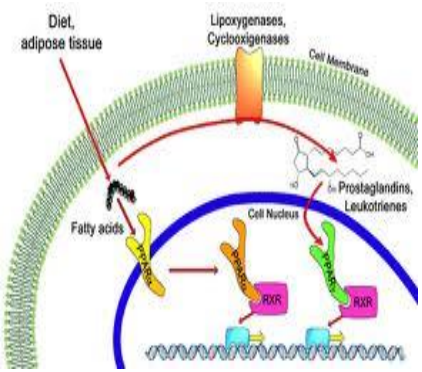
	p53 CALUX (+/−S9)
Sensitivity (%)	82
Specificity (%)	90

Validated using the ECVAM recommended list of 61 compounds (Kirkland et al 2008. Mutat. Res. 653, 99–108)

Van der Linden et al, 2014. Mutat Res 760:23-32

AOP Example 4: PPAR-Calux and obesity & diabetes-type 2

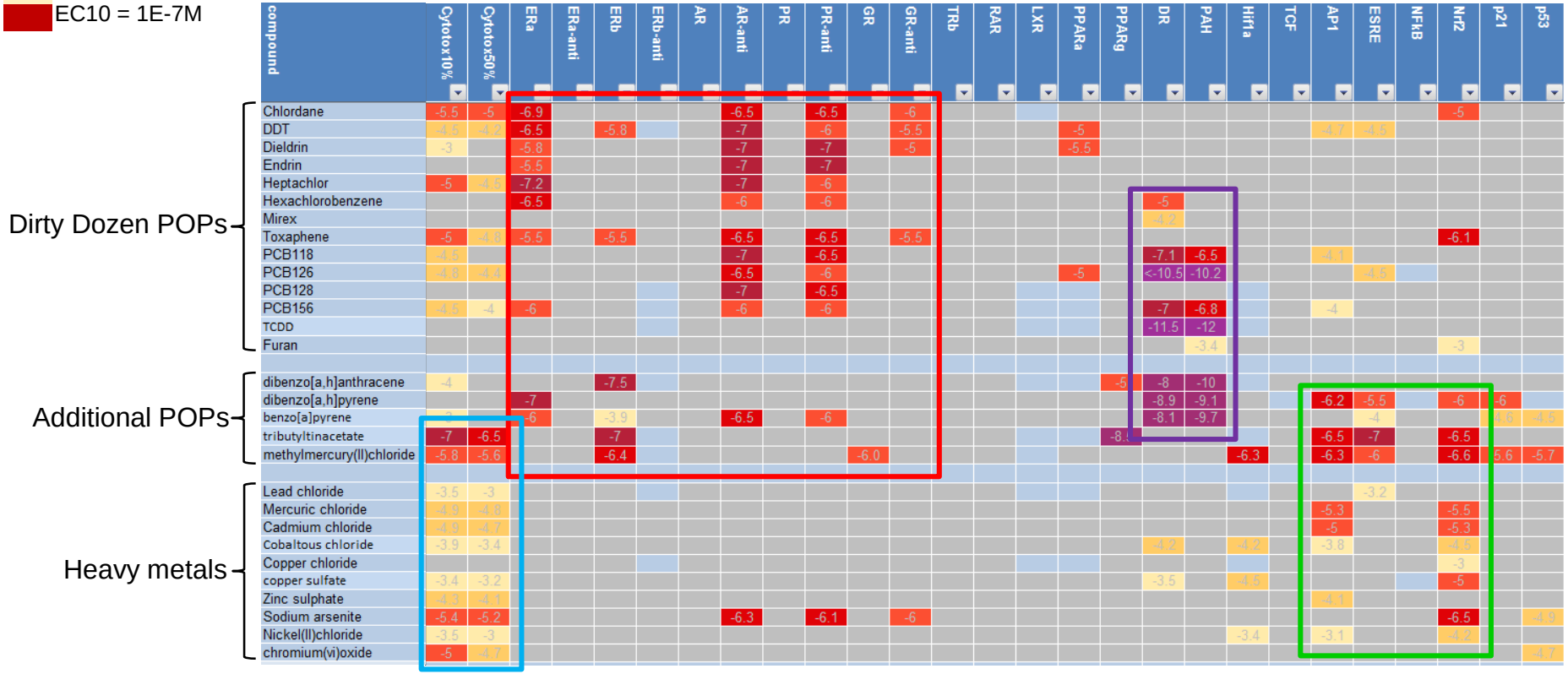
Using an metabolic syndrome/obesogen-based adverse outcome pathway to develop a set of PPAR-CALUX systems in a human cell lines for testing of chemicals that can induce obesity and diabets-type 2



CALUX panel can measure AOP-based signatures

Option: identify 21st century priority chemicals

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

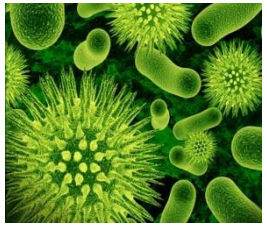


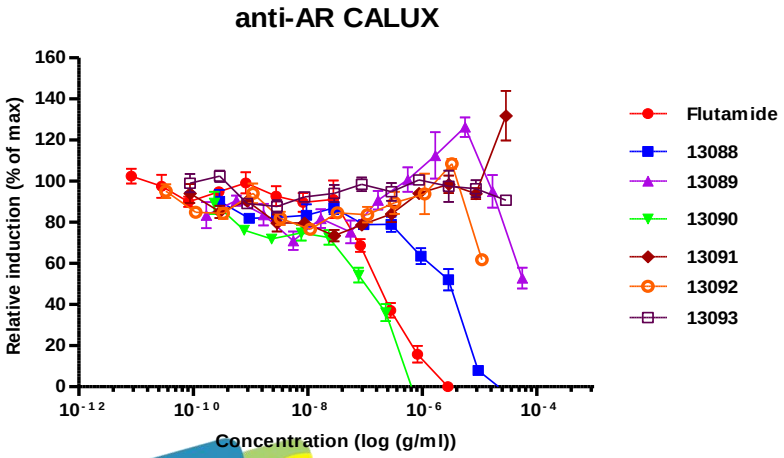
Dirty Dozen POPs: endocrine | dioxin receptor

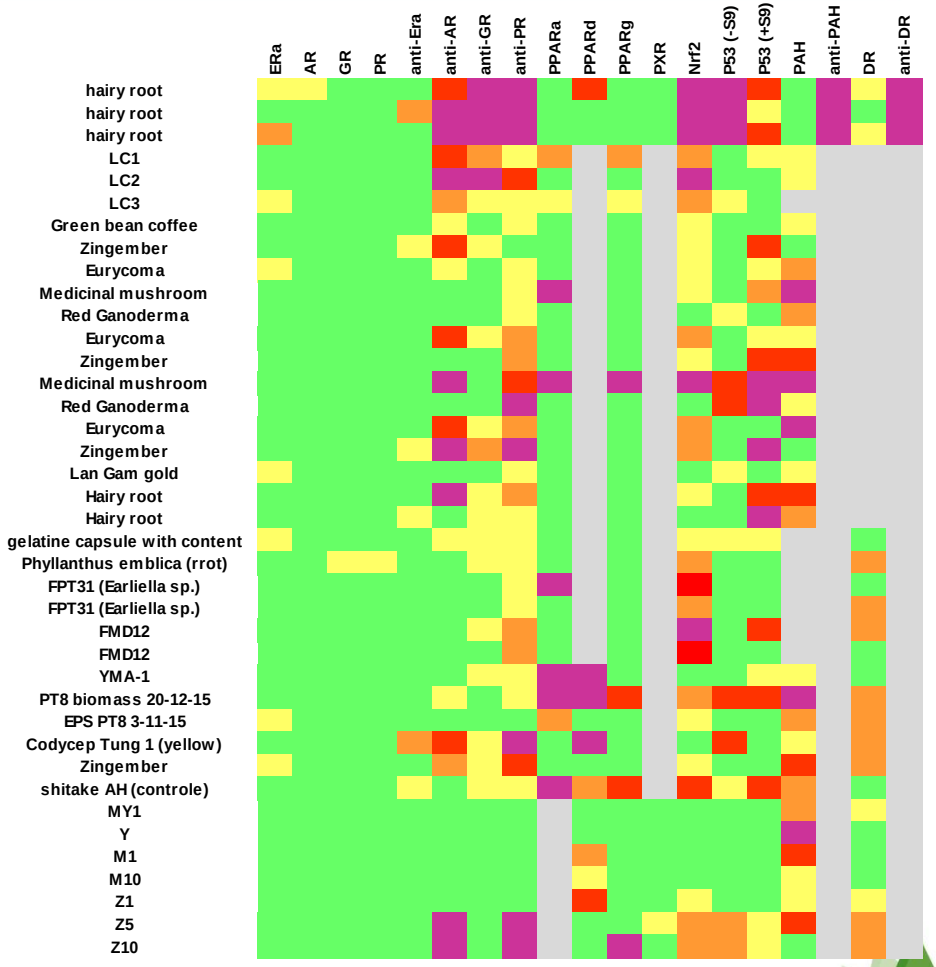
Additional POPs: dioxin receptor | stress pathways

Heavy metals: acute toxicity | stress pathways

BDS: Bioactivity: discovery of highly potent anti-tumor, Antibiotic, anti-oxidant and anti-obesity activities in plants & biomass







BDS
BioDetection Systems

BDS is supported by grants from BE Basic

BE-Basic

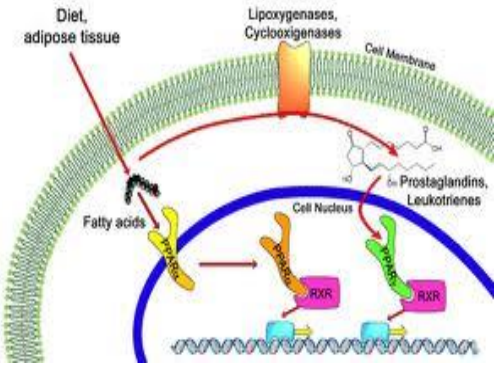
Applications of CALUX bioassays in health monitoring (focus on metabolic syndrome)

Some examples of BDS involvement in health monitoring

- Association between EDCs and equine metabolic syndrome
- (collaboration with Dr Mickelson and Dr. McCue of University of Minnesota College of Veterinary Medicine)
- Newborns and Genotoxic exposure risks in EU-Newgeneris
- (collaboration with many parties within the EU-Newgeneris consortium)

Health monitoring: Endocrine disruptors and Metabolic syndrome development

- **Dioxins cause wasting syndrome, *AhR* maybe involved in metabolic syndrome development**
- **Endocrine *ER, TR* and *PPAR* pathways are involved in regulation of fat and energy metabolism and insuline sensitivity**
- **Dioxins interfere in *ER* and *TR* pathways;**
- **Dioxins and endocrine disruptors suspected of supporting Diabetes type II development**



The association between endocrine disrupting chemicals and equine metabolic syndrome

S.A. Durward-Akhurst[1], E.M. Norton[1], N. Schultz[1], R. Geor[2], J. Mickelson[1], M.E. McCue[1]

[1]University of Minnesota College of Veterinary Medicine, St Paul, MN

[2]University of Massey, Turitea, NZ

10th Biodetectors Sorrento 2017

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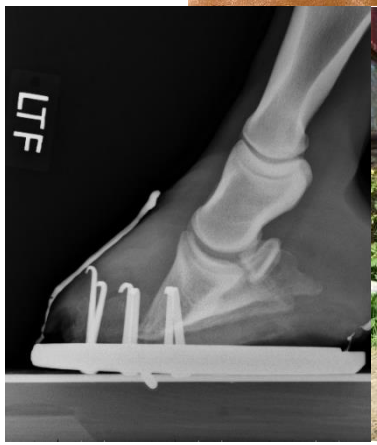
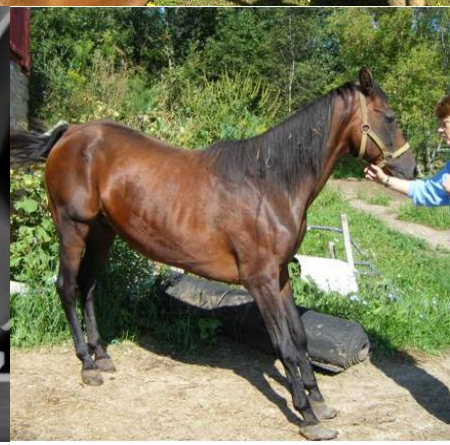
Equine Metabolic Syndrome

- Increased adipose deposition
  - Regionally
  - Generalized obesity
- Insulin dysregulation
- Predisposition to laminitis





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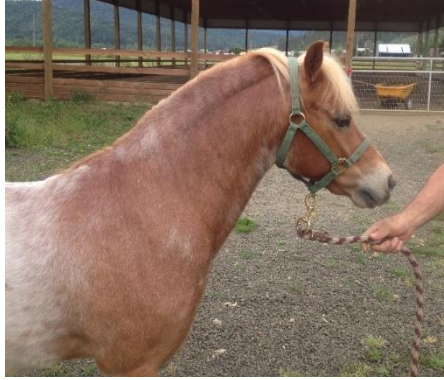
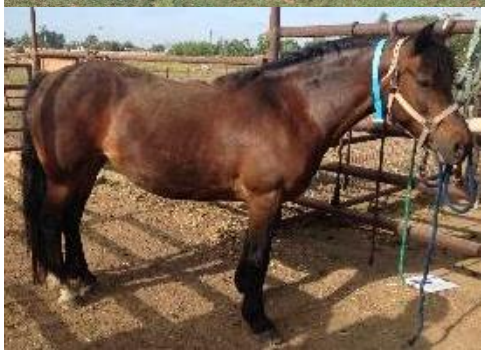


Hypothesis

1. Endocrine disrupting chemicals are associated with the EMS phenotype

2. The *AHR* and/or *ER* genotype of an individual modulates the metabolic response secondary to exposure to EDCs

Study design



- 161 Morgan horses
  - 18 farms
- 140 Welsh ponies
  - 14 farms

- Phenotypic measurements:

- Fasting glucose and insulin
- Post OST glucose and insulin
- Triglycerides
- ACTH
- Leptin
- Adiponectin
- NEFAs

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Statistics

- Non-normally distributed data was transformed

- Univariate linear model

- Response variables:

- Fasting glucose and insulin
- Post OST glucose and insulin
- Triglycerides
- ACTH
- Leptin
- Adiponectin
- NEFAs

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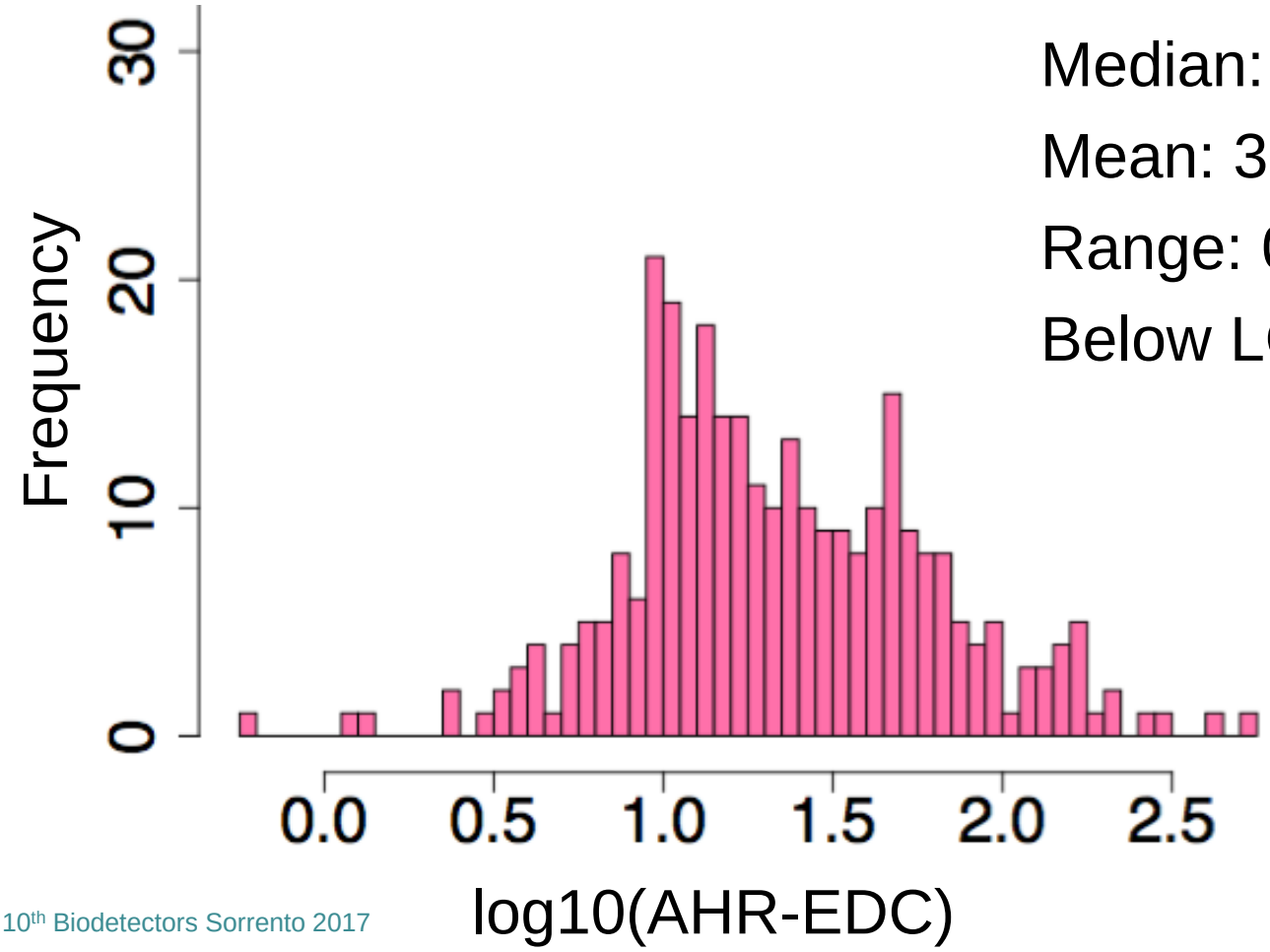
AHR-EDC results



Median: 19.29 pg/g fat

Mean: 38.51 pg/g fat

Range: 0.59-536.36 pg/g fat

Below LOD: 131 samples

n = 301

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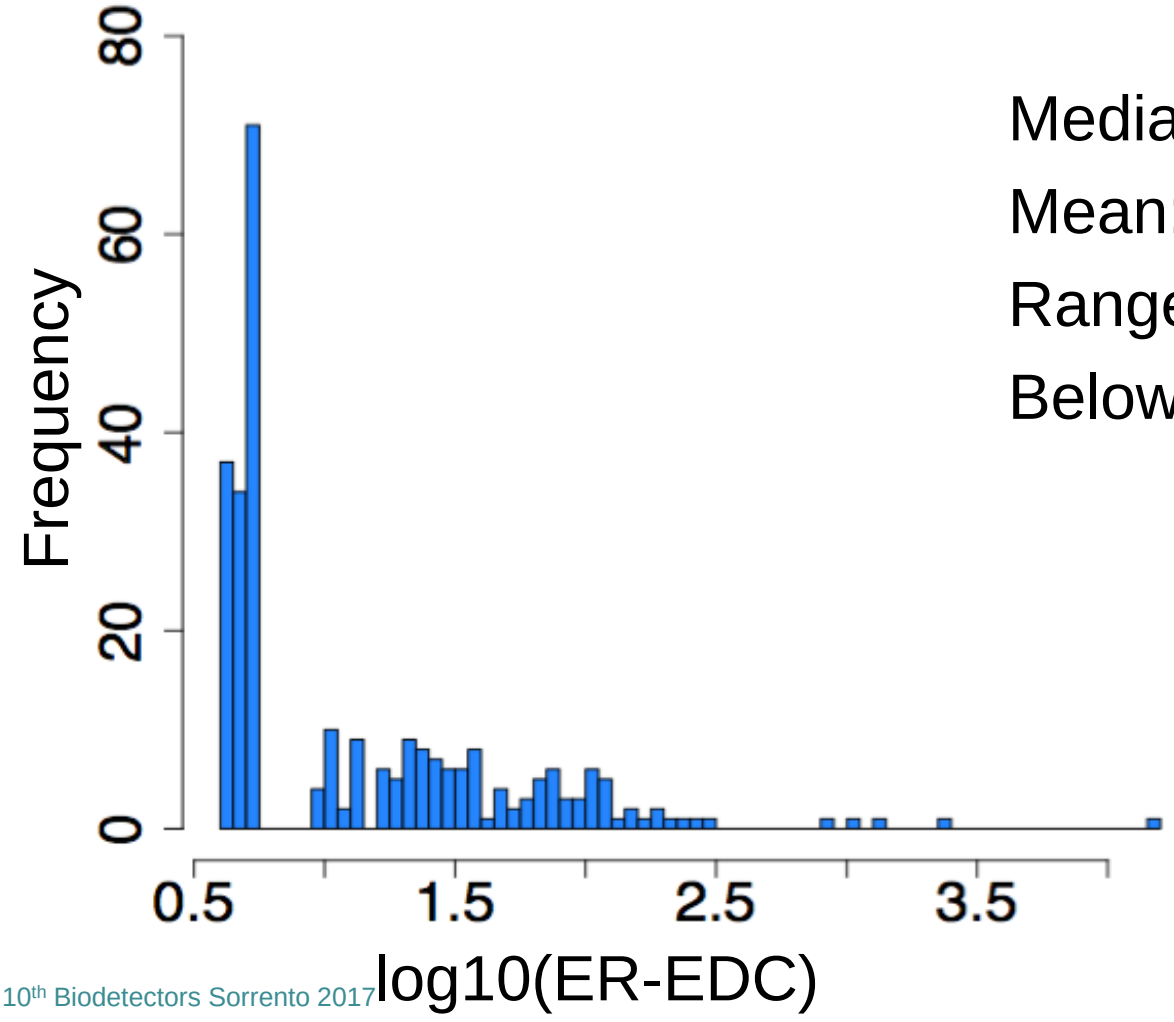
ER-EDC results



Median: 5.50 pg/ml

Mean: 101.91 pg/ml

Range: 4.35-15000.00 pg/ml

Below LOD: 119 samples

n = 276

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Results

- signifies p > 0.05

EMS phenotype	*AHR*-EDC	*ER*-EDC
Resting insulin	-	p = 0.003
INS-OST	-	p = 0.002
Resting glucose	p = 0.042	p = 0.002
GLU-OST	-	p = 0.012
NEFA	p = 0.047	-
Triglycerides	p = 0.011	-
Adiponectin	-	-
Leptin	-	-
ACTH	-	-

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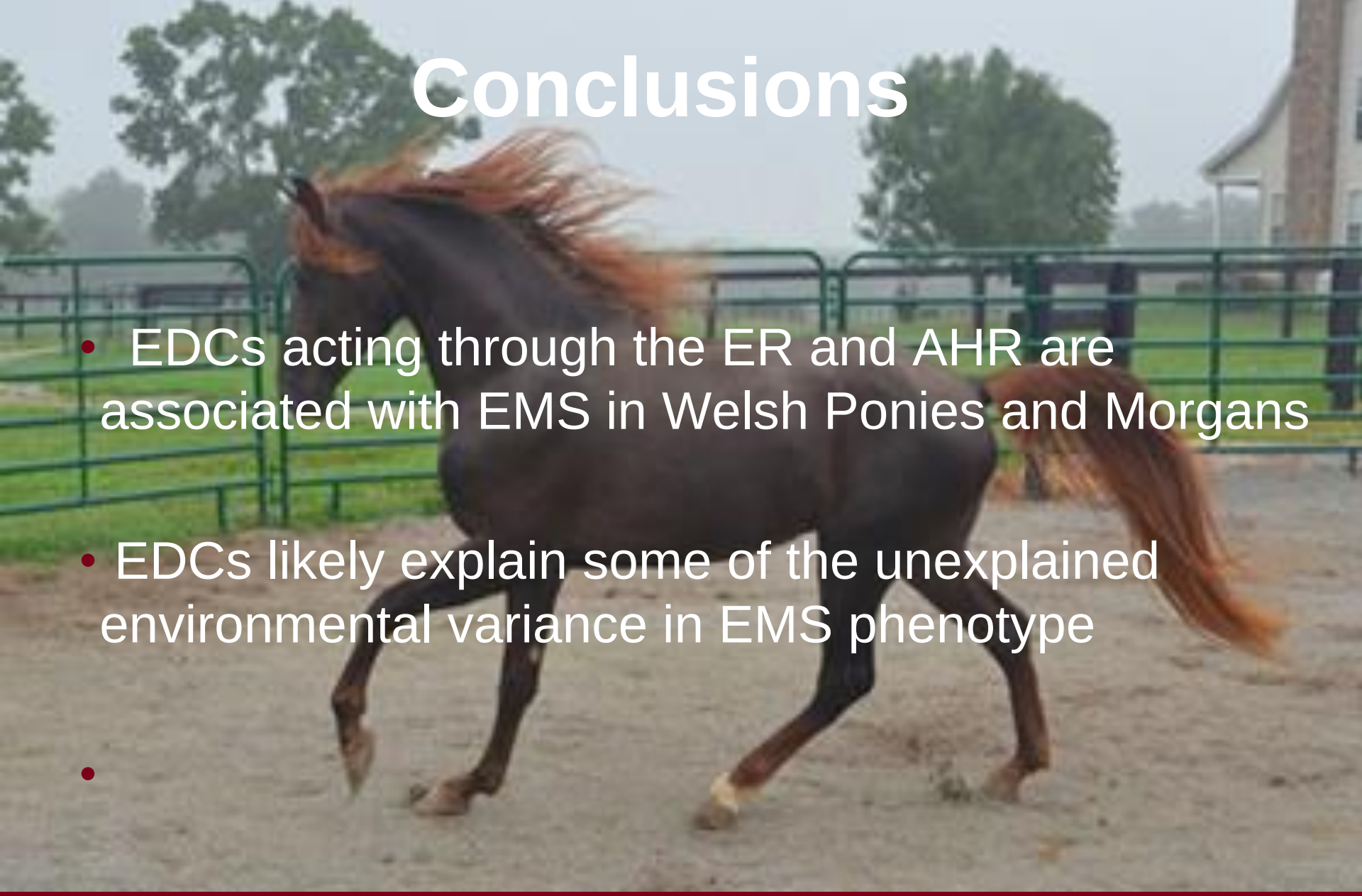
Conclusions



- EDCs acting through the ER and AHR are associated with EMS in Welsh Ponies and Morgans
- EDCs likely explain some of the unexplained environmental variance in EMS phenotype
-

A recent study has also found evidence for a possible association between Dioxins and Human Metabolic Syndrome

Perinatal exposure to dioxins and dioxin-like compounds and infant growth and body mass index at seven years: A pooled analysis of three European birth cohorts

Nina Iszatt a, Hein Stiguma, EvaGovarts b, Lubica PalkovicovaMurinova c, Greet Schoeters b,d,e, Tomas Trnovec c,Juliette Legler f,1, Cathrine Thomsen g, Gudrun Koppen b, Merete Eggesbø a,

RESULTS

At 7 years, dioxins exposure was associated with a statistically significant increase in BMI in girls (adjusted estimate for BMI units β=0.49, 95% CI: 0.07, 0.91) but not in boys (β=−0.03, 95% CI:−0.55, 0.49) (p-interaction=0.044). Furthermore, girls had a 54% (−6%, 151%) increased risk of overweight at 7 years (p-interaction = 0.023).

Conclusions

Perinatal exposure to dioxin and dioxin-like compounds was associated with increased early infant growth, and increased BMI in school age girls. Studies in larger sample sizes are required to confirm these sexspecific effects.

Please cite this article as: Iszatt, N., et al., Perinatal exposure to dioxins and dioxin-like compounds and infant growth and body mass index at seven years: A pooled analysis of..., Environ Int (2016), http://dx.doi.org/10.1016/j.envint.2016.04.040

Applications of CALUX bioassays in health monitoring (early life stage exposure & effects)

Some examples of BDS involvement in health monitoring

- **Association between EDCs and equine metabolic syndrome**
- **(collaboration with Dr Mickelson and Dr. McCue of University of Minnesota College of Veterinary Medicine)**
- **Newborns and Genotoxic exposure risks in EU-Newgeneris**
- **(collaboration with many parties within the EU-Newgeneris consortium)**

BioDetection Systems

Newgeneris Project summary

NewGeneris
Newborns and Genotoxic exposure risks

Hypothesis to be tested:

Maternal exposure to dietary compounds with carcinogenic and immunotoxic properties results in *in utero* exposure and molecular events in the unborn child leading to increased risk of cancer and immune disorders later in childhood.

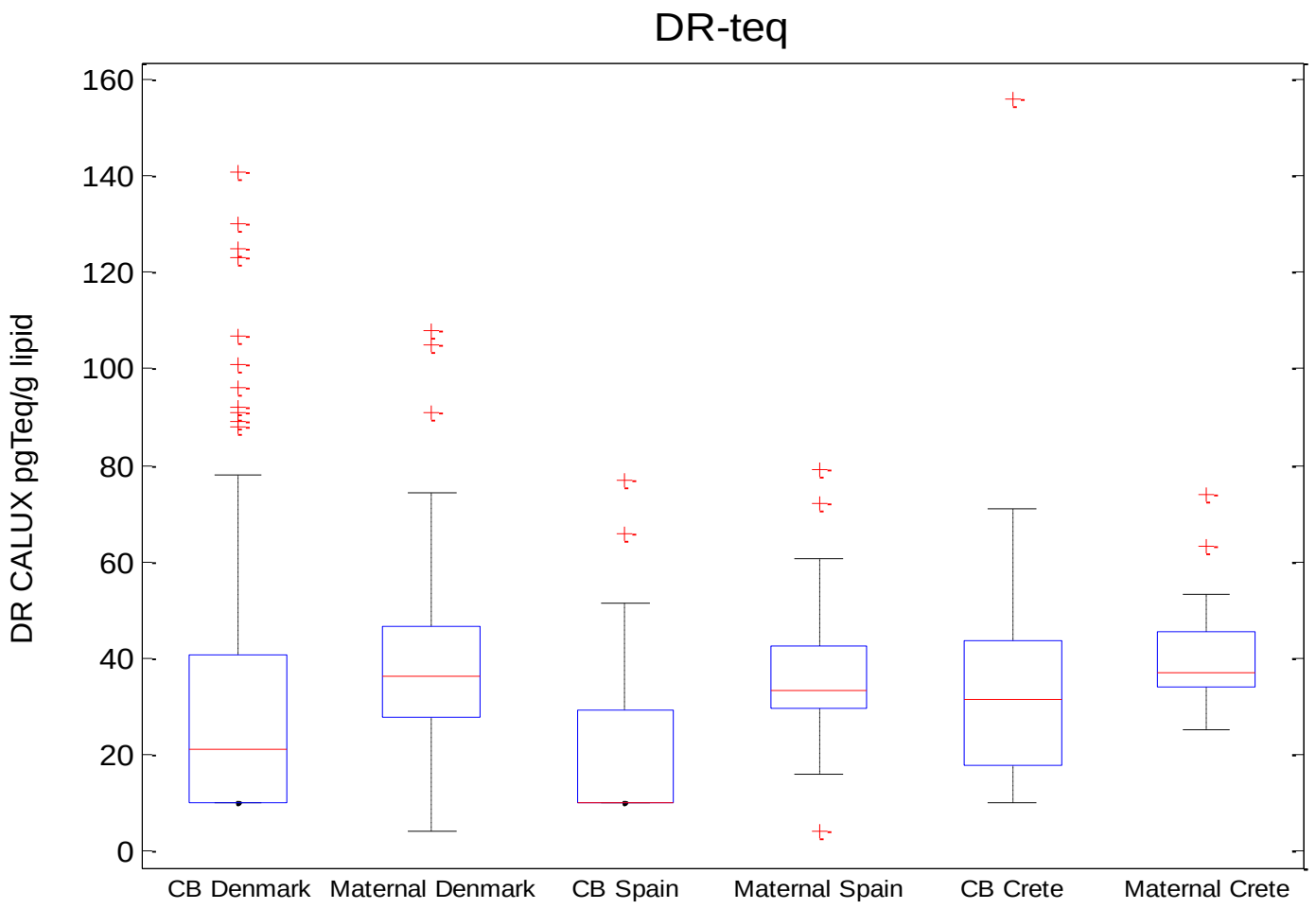
Existing mother-child cohorts will be used while new bio banks will be set-up

Overall goal: Development and application of two categories of biomarkers in relation to dietary exposure and childhood disease.

1 - biomarkers of exposure to chemicals with carcinogenic and immunotoxic properties

2 - biomarkers of pre-carcinogenic and immunotoxic effects

Box Plot of DR CALUX results on blood plasma from Newgeneris Cohorts



Outcome of Newgeneris Study

- Pathway specific bioassays are valuable for human monitoring
- Small volume sample analysis of human plasma is feasible with CALUX bioassays
- 11 papers published:
  The NewGeneris human early lifestage epidemiology studies show associations between exposure to dioxins and/or EDCs (especially with cord serum) and adverse Health outcome in children,
  in particular:
  - Associations between DR-CALUX responses and childhood leukemia
  - Associations between DR-CALUX responses and low birth weight; and shorter gestational age
  - Associations between DR-CALUX responses and changes in AGD in young boys
  - Prenatal exposure to DR-CALUX responses via food is associated with effects on the immune system functions at 1 and 3 year old children

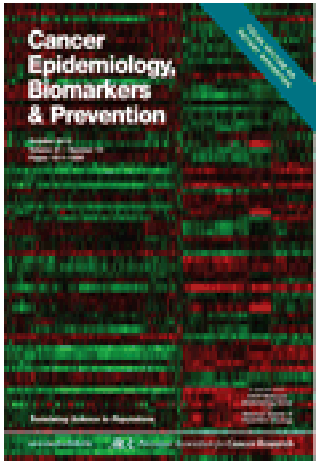
NewGeneris results:

Possible relation between dioxin exposure and incidence of childhood leukemia

Global Gene Expression Analysis in Cord Blood Reveals Gender-Specific Differences in Response to Carcinogenic Exposure In Utero

Kevin Hochstenbach1, Danitsja M. van Leeuwen1, Hans Gmuender4, Ralf W. Gottschalk1, Martinus Løvik5,Berit Granum5, Unni Nygaard5, Ellen Namork5, Micheline Kirsch-Volders6, Ilse Decordier6, Kim Vande Loock6,Harrie Besselink2, Margareta T€ornqvist7, Hans von Stedingk7, Per Rydberg7, Jos C.S. Kleinjans1,Henk van Loveren1,3, and Joost H.M. van Delft1 *Cancer Epidemiol Biomarkers Prev* 2012;21:1756-1767. Published OnlineFirst August 9, 2012.

Methods: Global gene expression was applied in umbilical cord blood samples, **the CALUX-assay** was used for measuring dioxin(-like), androgen(-like), and estrogen(-like) internal exposure, and acrylamide–hemoglobinadduct levels were determined by mass spectrometry adduct-FIRE-procedureTM. To link gene expression to an established phenotypic biomarker of cancer risk, micronuclei frequencies were investigated



Conclusions/Impact: This study reveals different transcriptomic responses to environmental carcinogens between the sexes. In particular, male-specific TNF-alpha-NF-kB signaling upon dioxin exposure and activation of the Wnt-pathway in boys upon acrylamide exposure might represent possible mechanistic explanations for gender specificity in the incidence of childhood leukemia

NewGeneris results:

Env. carcinogens, endocrine disrupters may mechanistically contribute to carcinogen-induced childhood leukemia

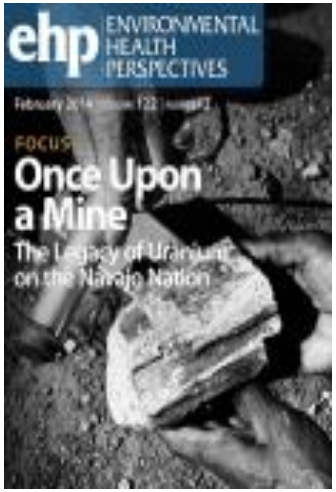
Micronuclei in Cord Blood Lymphocytes and Associations with Biomarkers of Exposure to Carcinogens and Hormonally Active Factors, Gene Polymorphisms, and Gene Expression: The NewGeneris Cohort

Domenico Franco Merlo,1 Silvia Agramunt,2 Lívia Anna,3 Harrie Besselink,4 Maria Botsivali,5 et al.,

Environ Health Perspect 122:193–200, february 2014; ; http://dx.doi.org/10.1289/ehp.1206324

CALUX-relevant part of Results

Gene expression levels were significantly lower for 11 genes in association with the highest versus lowest category of plasma AR CALUX® (chemically activated luciferase expression for androgens) (8 genes), ERα CALUX® (for estrogens) (2 genes), and DR CALUX® (for dioxins).



Conclusion: We measured *in utero* exposure to selected environmental carcinogens and circulating hormonally acting factors and detected associations with Micronuclei frequency in newborns circulating T lymphocytes. The results highlight mechanisms that may contribute to carcinogen-induced leukemia and require further research.

We are happy to discuss any options for future collaboration

Thank *you* for your attention!