State of the Art Biodetectors
Special emphasis on health monitoring

Abraham Brouwer,
CEO of BDS & MLS, Amsterdam
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: Food, Feed, Water, Health, Environment, Chemicals, Consumer products

Novel bioanalytical solutions
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

<table>
<thead>
<tr>
<th>Nuclear receptors</th>
<th>Signaling pathways</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>name</td>
<td>status</td>
<td>cell</td>
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<tr>
<td>DR CALUX</td>
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<td>PAH CALUX</td>
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<td>ER CALUX</td>
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<td>ERAlpha CALUX</td>
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<td>ERbeta CALUX</td>
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<td>ERAlpha CALUX</td>
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<td>ERbeta CALUX</td>
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<td>AR CALUX</td>
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<td>PR CALUX</td>
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<td>GR CALUX</td>
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<td>TR CALUX</td>
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<td>RAR CALUX</td>
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<td>PPARγ1 CALUX</td>
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<td>PPARγ2 CALUX</td>
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<td>PPARα CALUX</td>
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<td>PPARδ CALUX</td>
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<td>LXR CALUX</td>
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<td>PXR CALUX</td>
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<td>VDR CALUX</td>
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<tr>
<td>MR CALUX</td>
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</tbody>
</table>

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

**CALUX: n=28**

**Agonist/antagonist: 25x2=56 assays**
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

\[ y = 0.9565x \]
\[ R^2 = 0.9889 \]
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**
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.
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>TOXICITY</th>
<th>EST diff</th>
<th>zebrafish</th>
<th>CALUX panel</th>
<th>CALUX with PBPK</th>
<th>cyp19</th>
<th>PREDICTIO N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Cyclosporin A (CSA)</td>
<td>developmental (immuno) toxicant</td>
<td>differentialed</td>
<td>no effect</td>
<td>represses anti AR, weak</td>
<td>no effect</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>2 Monoethylhexylphthalate (MEHP)</td>
<td>male reproductive organ</td>
<td>differentialed</td>
<td>developmen tal toxicant</td>
<td>weakly positive in many assays, consistent with HDAC inhibition</td>
<td>no effect</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>3 Sodium valproate (VPA)</td>
<td>neurodevelopmental toxicant</td>
<td>differentialed</td>
<td>developmen tal toxicant</td>
<td>no effect</td>
<td>positive</td>
<td></td>
<td></td>
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<tr>
<td>4 D-mannitol (DML)</td>
<td>negative control</td>
<td>no effect</td>
<td>no effect</td>
<td>represses anti AR, weak</td>
<td>no effect</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>5 Flusilazole (FLU)</td>
<td>craniofacial and axial skeletal malformations</td>
<td>differentialed</td>
<td>developmen tal toxicant</td>
<td>cytoxic,antiPR/antiGR/antiGR weak DR/PAH</td>
<td>inhibitor at high conc in H295R</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>6 Glufosinate ammonium (GPA)</td>
<td>neurodevelopmental toxicant</td>
<td>no effect</td>
<td>no effect</td>
<td>represses anti AR, weak</td>
<td>no effect</td>
<td>negative</td>
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</tr>
<tr>
<td>7 Methoxyacetic acid (MAA)</td>
<td>growth and developmental retardation</td>
<td>differentialed</td>
<td>developmen tal toxicant</td>
<td>negative</td>
<td>no effect</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>8 Retinoic acid (RA)</td>
<td>neural crest cell migration affected</td>
<td>differentialed</td>
<td>developmen tal toxicant</td>
<td>strong RAR/RXR activity</td>
<td>inhibitor in H295R</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>9 Dioctyltin dichloride/</td>
<td>developmental (immuno)toxicant</td>
<td>cytotoxic</td>
<td>no effect</td>
<td>represses toxic,antiproges tin, stress-related pathways</td>
<td>inhibitor in H295R</td>
<td>positive</td>
<td></td>
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<tr>
<td>dichlorodioctylstannane (DO TC)</td>
<td>Endosulfan (ESF)</td>
<td>neurotoxicant</td>
<td>cytotoxic</td>
<td>represses toxic,antiproges tin, stress-related pathways</td>
<td>inhibitor in H295R</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>1 Diethylstilbestrol (DES)</td>
<td>transplacental carcinogen</td>
<td>cytotoxic</td>
<td>developmen tal toxicant</td>
<td>strong estrogen: antiAR, antiPR, stress- and genotoxicity</td>
<td>no effect</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>2 Methylmercury chloride (MMC)</td>
<td>neurodevelopmental toxicant</td>
<td>cytotoxic</td>
<td>developmen tal toxicant</td>
<td>stress-related pathways affected, estrogen, GR agonist</td>
<td>Inducer in H295R, inhibitor in HPMs</td>
<td>positive</td>
<td></td>
</tr>
</tbody>
</table>

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
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":

AOP Example 3: p53-Calux and carcinogens
Typical p53 CALUX responses by chemical carcinogens
Performance characteristics of the p53 CALUX

<table>
<thead>
<tr>
<th></th>
<th>p53 CALUX (+/−S9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>82</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>90</td>
</tr>
</tbody>
</table>

Validated using the ECVAM recommended list of 61 compounds (Kirkland et al 2008. Mutat. Res. 653, 99–108)
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 diabetes-type 2.
**CALUX panel can measure AOP-based signatures**

*Option: identify 21st century priority chemicals*

<table>
<thead>
<tr>
<th>Substance</th>
<th>Endocrine</th>
<th>Dioxin receptor</th>
<th>Stress Pathways</th>
<th>Acute Toxicity</th>
<th>Non-Related</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dirty Dozen POPs</strong></td>
<td></td>
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<tr>
<td>Chlorodane</td>
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<tr>
<td>DDT</td>
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<tr>
<td>Dieldrin</td>
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<tr>
<td>Endrin</td>
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<tr>
<td>Heptachlor</td>
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<tr>
<td>Hexachlorobenzene</td>
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<tr>
<td>Mirex</td>
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<tr>
<td>Toxaphene</td>
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<td>PCB118</td>
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<td>PCB126</td>
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<td>PCB128</td>
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<td>PCB156</td>
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<tr>
<td>TCDD</td>
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<tr>
<td>Furan</td>
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<tr>
<td><strong>Additional POPs</strong></td>
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<tr>
<td>dibenzo[a,h]fluoranthene</td>
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<tr>
<td>dibenzo[a,h]pyrene</td>
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<tr>
<td>benzo[a]pyrene</td>
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<tr>
<td>tributyltinacetate</td>
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<tr>
<td>methyl mercury iodide chloride</td>
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<tr>
<td><strong>Heavy metals</strong></td>
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<tr>
<td>Lead chloride</td>
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<tr>
<td>Mercuric chloride</td>
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<tr>
<td>Cadmium chloride</td>
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<tr>
<td>Cobaltous chloride</td>
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<tr>
<td>Copper chloride</td>
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<tr>
<td>copper sulphate</td>
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<tr>
<td>Zinc sulphate</td>
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<tr>
<td>Sodium arsenite</td>
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<tr>
<td>Nickel(Ill) chloride</td>
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<tr>
<td>chromium(vi) oxide</td>
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</tbody>
</table>

**Dirty Dozen POPs:**
- endocrine
- dioxin receptor

**Additional POPs:**
- dioxin receptor
- stress pathways

**Heavy metals:**
- acute toxicity
- stress pathways

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

BDS is supported by grants from BE Basic

10th Biodetectors Sorrento 2017
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)
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 insulin 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-Akhurst¹, E.M. Norton¹, N. Schultz¹, R. Geor², J. Mickelson¹, M.E. McCue¹

¹University of Minnesota College of Veterinary Medicine, St Paul, MN
²University of Massey, Turitea, NZ

10th Biodetectors Sorrento 2017
Equine Metabolic Syndrome

- Increased adipose deposition
  - Regionally
  - Generalized obesity

- Insulin dysregulation

- Predisposition to laminitis
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
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
**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
## Results

- signifies $p > 0.05$

<table>
<thead>
<tr>
<th>EMS phenotype</th>
<th>AHR-EDC</th>
<th>ER-EDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting insulin</td>
<td>-</td>
<td>$p = 0.003$</td>
</tr>
<tr>
<td>INS-OST</td>
<td>-</td>
<td>$p = 0.002$</td>
</tr>
<tr>
<td>Resting glucose</td>
<td>$p = 0.042$</td>
<td>$p = 0.002$</td>
</tr>
<tr>
<td>GLU-OST</td>
<td>-</td>
<td>$p = 0.012$</td>
</tr>
<tr>
<td>NEFA</td>
<td>$p = 0.047$</td>
<td>-</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>$p = 0.011$</td>
<td>-</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leptin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ACTH</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

10th Biodetectors Sorrento 2017
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
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 Stigum, 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)
Newgeneris Project summary

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

DR-teq

**Box Plot of DR CALUX pgTeq/g lipid**

- DR CALUX pgTeq/g lipid for CB Denmark, Maternal Denmark, CB Spain, Maternal Spain, CB Crete, and Maternal Crete.
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
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 Tornqvist7, 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: Possible relation between dioxin exposure and 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!