Quantitative high throughput screening methodologies to assess biological activity of chemicals and water samples; their use in the context of REACH and the Water Framework Directive

Bart van der Burg
Focus:
develops, markets and applies bio-based detection methods for safety assessment and quality control chemicals and pharmaceuticals, bio-based materials, food, water and the environment

Activities:
• Analytical services
• Licenses
• Training
• Research and Development
• Consultancy
International marketing network
In vivo bioassays in animals: problem speed and capacity

- 100,106 industrial chemicals on the market in 1981 (“existing substances”): 1% tested on toxicity!
- This lead to new legislation: REACH
Chemical substance in vitro/in silico screening system to predict human- and ecotoxicological effects

- Generation of a simple, rapid screening system for reprotoxic effects of chemicals

- Widespread implementation suitable for regulatory purposes within the tight time schedule of the REACH program mechanistic base.
  OECD/ECVAM validated methods as anchors/quality control.
  Cost effective and transferable.
Why reprotox?

- Prioritised in REACH
- Reproductive toxicity is important to assess both human and environmental toxicity
- Uses the most animals in toxicity testing
- Very little alternative methods
In silico prescreen

Chemical

Potentially reprotoxic

Other toxic properties

Minimal essential screen

ReproScreen HTP

Prioritisation

Established Screening methods

No testing/dedicated in vivo testing
**ReProTect:**
Complexity reproductive cycle be captured with a limited amount of apical tests

**ChemScreen:**
Is further simplification/higher throughput possible & can we use these methods in a regulatory context?

Schenk et al. 2010 Reproductive Toxicology 30, 200-218
Rapid in vitro bioassays: CALUX® chemical quantification coupled to biological effect

CHEMICAL (mix)

Light signal proportional to amount of biological active chemical in sample

Receptor binding elements

LUCIFERASE mRNA

LUCIFERASE protein

LUCIFERASE

ENDOGENOUS GENE

BIOLOGICAL EFFECT
CALUX human pathway selective and responsive reporter gene assays

Advantages low background, high selectivity and inducibility:

- High sensitivity
- Better quantification
  - Single mechanism, avoid cross-talk and artifacts
  - Straight-forward interpretation and risk assessment
  - Better extrapolation to other species
  - Suitable to measure bioactivity in complex mixtures
Quantitative HTS in 384 wells: hundreds of dose-response curves per day
Example screening result

PPARG

- Format 96 > 384 wells
- Use frozen cells
- Expansion-dose response: automated potency determination
• Many compartments, processes included, but...

• Minimal input required:
  • High Throughput PK:
    • fraction unbound (fu)
    • hepatic clearance ($CL_{h,\text{int}}$)
    • intestinal permeability ($P_{\text{app}}$)
  • in silico (QPPR):
    • logP, ionization
  • Default assumptions: $CL_r$
Correct prediction 11/12 compounds
Like in ReProTect Glufosinate missed: mechanism bypassed in culture
PBPK modeling improves predictions (e.g. in CALUX)
Simple HTS model same prediction as EST/ZET

**Piersma et al. 2013 Reproductive Toxicology**
Identify subsets of assays to predict specific types of toxicity

**Dominant responses:** antiandrogenic, antiprogestagenic estrogenic
1. No chemical category associated with reproductive effects

2. Hormonal compounds in almost any chemical category

3. Effect concentrations in vitro (i.e. biological category) correlated well with LOELs in vivo ($R^2=0.71$; without PBPK)

Lewin et al., Reproductive Toxicology, submitted
Estrogen receptor activation in ERalpha CALUX clearly links to structural deformities in reproductive organs

Van der Burg et al., Reproductive Toxicology, submitted
Examples with 3 chemical classes (Alkyl alkanoic acids, phthalates, organotin chlorides); all three successful

Read-across used in approx. 30% reproductive tox dossiers (100-1000TPA) in REACH (ECHA 2014) (new testing proposal only few %)
Fields of application CALUX® battery
Major challenge: risk assessment of complex mixtures

Toxic waste  Chemicals  Pharmaceuticals  Toxins

Complex mixtures

Environmental health, Food, Human health
Chemical monitoring alone insufficient

- Tested chemicals → Monitored chemicals

- New legislation

- Untested chemicals

- Ca 130 monitored (soil)
- Ca 1000 complete risk assessment
- 30 000 > 1 ton
- 50 000 000 CAS
- Natural/metabolites> ????

NIES 100614
Effect profile water samples with CALUX® cells

Selection assays:
• Priority effects & compound groups
• Overlapping chemical domains of assays: hot spots of activity?

Occurrence and identification of androgen receptor antagonists in high trophic-level animals

# Bioactivities in persistent hydrophobic fractions

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<tr>
<th></th>
<th>Androgens</th>
<th>Estrogens</th>
<th>Glucocorticoids</th>
<th>Progestins</th>
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<tr>
<td>Common cormorant liver</td>
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<td>Raccoon dog liver</td>
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<td>Finless porpoise liver</td>
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</table>

10 mg/well
1.0 mg/well
0.1 mg/well
0.01 mg/well

**Suzuki et al. 2011 Environ. Sci. Technol.** ePub 18 Oct
4.1 Selection and validation
- Selection criteria
- Bioassay selection
- Automation
- Trigger values
- Validation

4.2 Implementation for monitoring
- Regulatory acceptance
- Testing framework
- Introduction to water utilities
- Demonstration

Market application
WP41 SELECTION AND VALIDATION OF BIOASSAYS QUALITY ASSESSMENT

- Relevant toxicological endpoints
- Selection criteria
- Minimal panel of bioassays
- Trigger values
WP41 SELECTION AND VALIDATION OF BIOASSAYS QUALITY ASSESSMENT

- Relevant toxicological endpoints
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- Xenobiotic metabolism
- Hormone-mediated MoA
- Reactive MoA
- Developmental toxicity
- Adaptive stress response
### Test panels emerging from case studies

*(GWRC, Australia, Dutch)*

<table>
<thead>
<tr>
<th>Relevant endpoints</th>
<th>B. Escher et. al</th>
<th>BE-Basic case study</th>
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<tbody>
<tr>
<td>Xenobiotic metabolism</td>
<td>PXR activation, AHR activation, CAR</td>
<td>DR/PAH-CALUX</td>
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<tr>
<td>Hormone-mediated MoA</td>
<td>Estrogenicity, Anti-androgenicity, Glucocorticoid activity, Progestagenic activity, Thyroid activity</td>
<td>Era-CALUX, AR-CALUX, PR-CALUX, GR-CALUX, TRβ-CALUX, RAR-CALUX</td>
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<tr>
<td>Reactive MoA</td>
<td>Mutations (AMES, SOS), DNA repair (umuC), DNA damage response (Micronucleus)</td>
<td>P53-CALUX, P53 S9+ CALUX (?)</td>
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<tr>
<td>Adaptive stress response</td>
<td>Oxidative stress pathway</td>
<td>Nrf2-CALUX</td>
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<td>Developmental toxicity</td>
<td>Preimplantation toxicity, Embryonic development, Placenta</td>
<td>ZFET</td>
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<td>Lipid metabolism</td>
<td>PPARα, PPARγ</td>
<td>PPARα, PPARγ, PPARδ</td>
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<td>Photosynthesis</td>
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<tr>
<td>General response</td>
<td>Cytotoxicity, Viability, Vibrio Fischeri (Microtox), Algae growth</td>
<td>Cytotoxicity, Cytotoxicity S9+</td>
</tr>
</tbody>
</table>
**WP41 SELECTION AND VALIDATION OF BIOASSAYS FOR WATER QUALITY ASSESSMENT**

### Relevant toxicological endpoints
- Relevant toxicological endpoints

### Selection criteria
- Minimal panel of bioassays
- Trigger values

#### Assay applicability
- Applied to environmental samples
- Validated to water samples
- Generic sample handling and/or preparation is adequate
- Standardised protocol available/maturity
- Service and support available
- Ease of use
- Costs

### Assay performance
- Selectivity
- Accuracy
- Reproducibility
- Robustness
- Sensitivity
- Specificity
- LOQ
- Cytotoxicity control
- Quick
- Clear/straightforward read-out
- High-throughput capacity

<table>
<thead>
<tr>
<th>Toxicity endpoints</th>
<th>DEMEAU bioassay(s)</th>
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<tr>
<td>Xenobiotic metabolism</td>
<td>DR/PAH-CALUX, PXR-CALUX</td>
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<td>Hormone-mediated MoA</td>
<td>ER-CALUX, antiAR-CALUX, GR-CALUX</td>
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<td><strong>Score</strong></td>
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<td><strong>Score</strong></td>
<td>17</td>
<td>30</td>
<td>22</td>
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</table>

Total score:
- Assay 1: 27
- Assay 2: 31
- Assay 3: 36
- Assay 4: 41
- Assay 5: 36

**Relevant toxicological endpoints**
- Performance
- Validation status
- Cost
- Service
- etc
TEQ approach

<table>
<thead>
<tr>
<th>Assay</th>
<th>Trigger value</th>
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<tbody>
<tr>
<td>ER-CALUX</td>
<td>3.8 ng E2-eq / L</td>
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<tr>
<td>AR-CALUX</td>
<td>11 ng DHT-eq / L</td>
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<td>GR-CALUX</td>
<td>3.8 ng DEX-EQ / L</td>
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<tr>
<td>PR-CALUX</td>
<td>3.8 ng Org2058-eq / L</td>
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</table>
WP41 SELECTION AND VALIDATION OF BIOASSAYS FOR WATER QUALITY ASSESSMENT

Relevant toxicological endpoints

Selection criteria

Minimal panel of bioassays

Trigger values

Trigger values in practice

Demonstration of promising technologies to address emerging pollutants in water and waste water

Large volume grab samples

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<thead>
<tr>
<th></th>
<th>ZFET</th>
<th>Ere</th>
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<th>PR</th>
<th>GR</th>
<th>TRB</th>
<th>RAR</th>
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<th>PPARγ</th>
<th>PPARδ</th>
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<th>PAH</th>
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<th>p53 SO +</th>
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- Selection criteria
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4.2 Implementation for monitoring

- Regulatory acceptance
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  - Introduction to water utilities
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Market application
Conclusions

- Mechanistic bioassays available that can predict various important types of toxicity in animals (and humans)
- Quantitative
- Validated and accredited
- Pharmacokinetic (PBPK) modeling improves predictions
- Even without complete coverage of toxicity mechanisms applicable for prioritization and read-across of pure chemicals
- Particularly suitable for complex mixtures: e.g. prioritized effects (endocrine disrupters, genotoxicity, etc), and to identify pollution hotspots vs clean samples, efficiency of purification processes.
<table>
<thead>
<tr>
<th>Partner</th>
<th>Partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioDetection Systems (BDS)</td>
<td>Bart van der Burg</td>
</tr>
<tr>
<td>Fraunhofer Institute for Toxicology and Experimental Medicine (FhG)</td>
<td>Inge Mangelsdorf</td>
</tr>
<tr>
<td>Netherlands Organization for Applied Scientific Research (TNO)</td>
<td>Dinant Kroese</td>
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<tr>
<td>Simpple (SIM)</td>
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<td>National Institute for Public Health and the Environment (RIVM)</td>
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<td>Danish Technical University Food Institute (DTU)</td>
<td>Jay Niemalä</td>
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<td>Procter &amp; Gamble Eurocor (P&amp;GEN)</td>
<td>Joanna Jaworska</td>
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<tr>
<td>Eberhard Karls University of Tübingen (EKUT)</td>
<td>Michael Schwarz</td>
</tr>
<tr>
<td>University of Konstanz (UKON)</td>
<td>Daniel Dietrich</td>
</tr>
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</table>
Towards a bio-based economy

- Increased (re)use of biological materials: safety issues related to complex mixtures rather than single compounds

www.be-basic.org