Interpretation of bioassay results

A roller-coaster along new tools in the framework of smart monitoring, trigger values and regulatory acceptance

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29 January 2015

This project has received funding from the European Union’s Seventh Programme for Research, Technological Development and Demonstration under Grant Agreement no. 308339.
• Application of bioassays in a smart monitoring strategy;
• Trigger values for aquatic ecotoxicological bioassays;
• Trigger values for \textit{in vitro} bioassays;
• Regulatory acceptance of \textit{in vitro} bioassays
Regulatory acceptance of bioassays: Approach and goal of DEMEAU report

- Overview of *in vitro* bioassays for human health in international regulatory frameworks
- Focus on water quality assessment, with examples from other purposes such as food safety and chemical regulation
- Roadmap to regulatory acceptance
Validation

Validation assures reliability and reproducability

1. Formal validation
   • **Effort in terms of time, costs and motivation (Spielmann, 2000)**
   • **Based on accuracy, precision, robustness, selectivity and sensitivity**
   • **Performed by ECVAM, ESAC, ICCVAM, JaCVAM**

2. Harmonization
   • **ISO standards**
   • **OECD guidelines**
Regulatory acceptance in water quality

- Regulations: Drinking Water Directive (EU), Safe Drinking Water Act (USA), Drinking water protection Act (Canada), Australian Drinking Water Guidelines
- Currently no formal acceptance of *in vitro* bioassays
- Accepted *in vivo* bioassays in waste water quality (Power and Bumprey, 2004, Kienle *et al.*, 2011) and fresh/marine water quality (Nagpal *et al.*, 2013)
- Australian guidelines mention *in vitro* bioassays as useful tools for water quality of source water and treated recycled water
Regulatory acceptance in food safety

- Standards in food regulation in the EU (Hoogenboom et al., 2010) for groups of chemicals: dioxins (TEQ concept)

- Proposed (EFSA): tiered approach for genotoxicity
  1. *In vitro* bioassays (Ames test and micronucleus test)
  2. Advanced animal toxicity studies for individual compounds
Regulatory acceptance in chemical regulation

- Chemical safety assessment: Tiered approach for genotoxicity hazard in the EU including *in vitro* bioassays

<table>
<thead>
<tr>
<th>Regulation</th>
<th>Leads to <em>in vivo</em> testing?</th>
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<tbody>
<tr>
<td>1. REACH and CLP</td>
<td>positive outcome</td>
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<tr>
<td>2. Cosmetic products</td>
<td>prohibited</td>
</tr>
<tr>
<td>3. Biocides</td>
<td>positive outcome</td>
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<tr>
<td>4. Plant protection products</td>
<td>always</td>
</tr>
<tr>
<td>5. Pharmaceuticals</td>
<td>always</td>
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<td>6. Veterinary drugs</td>
<td>always</td>
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Requirements for regulatory acceptance

Three factors that play a role in acceptance of an *in vitro* bioassay:

- The necessity (e.g. in vivo testing is prohibited)
- The usefulness (what are the benefits?)
- The performance (reliability and reproducibility)
Factors for successful implementation

• Successful examples:

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<th>TEQ (food safety)</th>
<th>Ames test (chemical regulation)</th>
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<tbody>
<tr>
<td>The necessity</td>
<td>Standards for food safety (dioxins)</td>
<td>Regulatory requirements (cosmetics)</td>
</tr>
<tr>
<td>The usefulness</td>
<td>Summed effects compare well to exposure</td>
<td>Important endpoint, ethical aspects, time and costs</td>
</tr>
<tr>
<td>The performance</td>
<td>Same effect mechanism (trigger value)</td>
<td>Historical data, known performance, ISO and OECD</td>
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• The barriers:
  • Official validation is a costly and time consuming process
  • Battery of *in vitro* assays is to replace a single *in vivo* test → which battery?
  • The relevance
Further steps

• Derivation of health-based trigger values (Escher and Leusch, 2012):
  1. No observed effect (non-specific and reactive toxicity)
  2. Effect-based trigger values (specific toxicity), example by Brandt et al. (2013)
  3. Response leads to guideline

• *In vitro* bioassays can act as a filter mechanism -> further analysis effect above treshold
• Thank you!

Acknowledgements

Bas Blaauboer (IRAS, Utrecht University, The Netherlands), Toine Boivee (RIKILT, The Netherlands), Fred Leusch (Griffith University, Smart Research Centre, Australia) and Sander van der Linden (BioDetection Systems, The Netherlands), Rosa Sjerps (KWR, The Netherlands)
Thank you for your attention!

This project has received funding from the European Union’s Seventh Programme for Research, Technological Development and Demonstration under Grant Agreement no. 308339.