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Alternative methods for ecotoxicology/bioaccumulation: Route to validation and regulatory application

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ECETOC/Cefic LRI Environment Review & Scoping Meeting 2019, 28-29 January, Brussels
Outline

- **Introduction to validation**
  - Definition, validation bodies, routes

- **Regulatory acceptance / application**
  - Fish embryo test - OECD TG236
  - Rtgill-W1 cytotoxicity assay
  - Fish intrinsic clearance – OECD TGs 319A & B
Validation - Definition

OECD GD 34 §11

- Test method validation is a **process** based on **scientifically sound principles** by which the **reliability** and **relevance** of a particular test, approach, method, or process are established for a specific purpose.

- New and updated test methods need to be both **reliable** and **relevant**, i.e., validated.
Validation - Reliability & Relevance

- **Reliability**
  - defined as the extent of reproducibility of results from a test within and among laboratories over time, when performed using the same standardised protocol

- **Relevance**
  - describes the relationship between the test and the effect in the target species; i.e. is the test meaningful and useful for a defined purpose, with the limitations identified; to which extent does it correctly measure or predict the (biological) effect of interest, as appropriate.
  - Regulatory need, usefulness and limitations of the test method are aspects of its relevance.
OECD – Test Guidelines Programme
  • Solna Workshop in 1996; GD34 in 2005
  • Validation studies initiated by member countries

The European Union Reference Laboratory for Alternatives to Animal Testing
  • Since 1991; formerly became EURL ECVAM with Directive 2010/63 in 2011
  • collaborates with the International Cooperation on Alternative Test Methods (ICATM) and its members in US, Japan, Canada, South Korea, Brazil, China
Routes to Validation – EURL ECVAM

1. Test method submission (pre- and full)

2. Assessment of submission

3. Conduct of validation study, if appropriate, involvement of ICATM partners & EU-NETVAL labs

4. Independent peer review by EURL ECVAM Scientific Advisory Committee

5. EURL ECVAM recommendation: validity status of test method; regulatory use; further steps

EURL ECVAM - TSAR

TSAR = Tracking System for Alternative Methods towards Regulatory acceptance

• Methods from ICATM partners
  • EU, USA, Japan, Canada, South Korea, and Brazil

• Access to method descriptions, key records, status, comments

https://tsar.jrc.ec.europa.eu/
Routes to Validation - OECD

Test Guidelines Programme (TGP)

1. Submission of a project to be included in TGP workplan
   • Standard Project Submission Form by National Coordinator/EC/OECD secretariat
   • Describes test method, status of validation, regulatory need, contribution to 3Rs, workplan

2. Project on TGP – decision by OECD WNT
   • Lead country/ies coordinate validation study
   • Validation should follow OECD GD34
   • Project updates to be provided on yearly basis
   • Discussion with VMG-eco

3. Draft TG & validation reports go through several WNT commenting rounds

4. WNT approval -> OECD adoption -> publication of TG
Points to be considered

1. Test method: well defined & developed; SOP(s) available
2. Number of laboratories (at least 3)
3. Selection of test chemicals (phys-chem properties, area of use, range of toxicity, etc)
4. Number of test chemicals
5. Advice of statistician on design of validation study
6. Quality of reference data (e.g. high variability)
7. Use of historical data (e.g. how similar are the protocols)
8. Consultation with stakeholders, regulators, end users
Adoption as OECD TG ≠ Regulatory acceptance

However, regulators prefer standardised tests

Regulatory acceptance

- A method / TG is mentioned for a given endpoint in a regulatory document (regulation, guidance, etc)
- Decision in the EU by member state committees, expert groups of EU agencies
Alternatives for ecotoxicity/bioaccumulation

**Acute fish toxicity**

- Fish embryo test (TG236) since 2013
- RTgill-W1 cytotoxicity assay

**Bioaccumulation**

- Determination of in vitro intrinsic clearance using:
  - cryopreserved rainbow trout hepatocytes (RT-HEP); OECD 319A
  - rainbow trout liver S9 sub-cellular fraction (RT-S9); OECD 319B
Fish embryo test (TG236)

- OECD validation (lead country Germany, coordinated by EURL ECVAM)
- Predictive capacity demonstrated by comparison of fish / FET data (Belanger et al 2013)
- ECHA performs comparison, allows the use of FET in WOE approach
- Data sets used for comparison differ in many aspects
- Fish data used by ECHA not published
- FET not put in context with Daphnia / algae data
Fish embryo test – what next?

- Rawlings et al (2019) show that fish embryo tests and acute fish toxicity tests are interchangeable in a threshold approach context

- OECD project 2.54 Guidance on IATA for fish acute toxicity testing
  - Combine QSARs, fish embryo test, RTgill-W1 assay in a threshold context

- Time to update REACH guidance on aquatic toxicity testing (written in 2006)?
RTgill-W1 cytotoxicity assay

- SPSF submitted to OECD with Switzerland and Norway as lead countries
  - Decision to be included in TGP in April 2019 by WNT
- Submitted to EURL ECVAM as pre-submission in 2014; full submission in September 2018
  - Assessment ongoing
- Should be part of IATA for fish acute toxicity, OECD 2.54
TG 319 A & B

• OECD project finalised in 2018 (US/HESI & EC co-lead)

• Use of measured metabolism data improve the reliability of BCF prediction models

• Lot of discussions on IVIVE model proposed in the guidance accompanying the TGs (GD280)

• Start open dialogue with regulators on how to use the methods in a weight-of-evidence approach for bioconcentration / bioaccumulation testing
• Include regulators at early stages of validation

• Up-front discussion re: limitations and domain of applicability

• Access to data not only on the new method but the existing studies is critical – databases are key for analysis and to increase ‘comfort’ with the method

• Open dialogue / collaboration between multi-stakeholder groups and regulators
Thank you

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