In vitro and in silico toxicology specialty section:

In²TOX SS

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Helena KANDAROVA
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Marc PRINCIVALLE
Mathieu VINKEN
MOTIVATION

- **Ethics**
  - Refinement
  - Reduction
  - Replacement

- **Legislation**
  - Directive 2010/63/EU
  - Regulation (EC) No 1223/2009
  - Regulation (EC) No 1907/2006
  - Regulation (EC) No 178/2002

- **Research**
  - Predictive
  - Mechanistic
  - Interdisciplinary
GOALS

☑ To serve as a focal point for interaction of members of EUROTOX interested in the field of *in vitro* and *in silico* toxicology

☑ To propose scientific programs, such as workshops and symposia, to be held at EUROTOX annual conferences, and to promote educational activities that emphasize developments in fundamental and applied *in vitro* and *in silico* toxicology

☑ To relate those developments to the activities of EUROTOX in order to stimulate growth in the science of *in vitro* methods, *in silico* modelling and new approach methodologies in general
ORGANIZATION

✔ Membership

- Members of EUROTOX and/or national member societies
- Application through website or via secretary

✔ Operational guidelines

- Drafted by Jean-Lou Dorne and Mathieu Vinken
- Revised and informally approved by some members of the Executive Committee
- Should be formally approved by the entire Executive Committee

✔ Officers

- Chair, secretary, chair-elected, 2 officers
- 3-years term renewable once, except for the chair and chair-elected
- Voted by members through e-mail voting
OFFICERS

☑ Chair: Mathieu Vinken (Belgium/academia)

☑ Secretary: Jean-Lou Dorne (France-Italy/regulatory)

☑ Special officers
  - Communication: Helena Kandárová (Slovakia/consultancy&academia)
  - Relations societies and specialty sections: Nynke Kramer (The Netherlands/academia)
  - Relations industry: Marc Princivalle (Switzerland-UK/industry)
Events

- Proposal and organization of sessions in EUROTOX conferences
- Organization of symposia and workshops
- Organization of webinars
- Organization of mentoring and career activities

Establishing links and setting up collaborations

- EUROTOX specialty sections
- SOT specialty sections
- EUROTOX affiliated societies (ESTIV)
- Journals
TOOLS

☐ Website

- Team, objectives, operational guidelines, presentations, reports
- Activities, documents, pictures, links

☐ Social media

- LinkedIn/Facebook
- Activities and events

☐ Networks

- Members/officers
- Specialty sections/affiliated societies/industry/regulatory agencies
CASE STUDY 1

✔ Development and testing of a repeated dose toxicity ontology model for chemical risk assessment purposes: liver effects as a case study

✔ Cosmetics Europe’s Long Range Science Strategy (LRSS)
  - Established in 2016
  - Goal: develop and strategically combine non-animal approaches for safety assessment purposes and support regulatory acceptance

✔ Partners
  - Mathieu Vinken: Vrije Universiteit Brussel-Belgium (VUB)
  - Anne Kienhuis: National Institute for Public Health and the Environment-The Netherlands (RIVM)
  - Bob van de Water: Leiden University-The Netherlands (LACDR)
  - Mark Cronin: Liverpool John Moores University-United Kingdom (LJMU)
  - Nynke Kramer: Utrecht University-The Netherlands (UU)
  - Dinant Kroese: The Netherlands Organization for applied scientific research-The Netherlands (TNO)
  - George Daston: Procter&Gamble-USA (P&G)
  - Chihae Yang: Molecular Networks GmbH-Germany (MN)
CASE STUDY 1

☑ Ontology

A framework centered around mode-of-action to organize and structure key aspects critical for the prediction of repeat dose toxicity induced by chemicals in human

Key aspect 1: kinetics
Key aspect 2: chemistry
Key aspect 3: mechanism
Key aspect 4: toxicology
CASE STUDY 1

✅ Work packages (WP)
- WP1: chemistry data collection
- WP2: kinetics data collection
- WP3: mechanistic data collection
- WP4: toxicology data collection
- WP5: liver pathology ontology model integration and testing

✅ PERT chart

Liver pathology ontology model development

Liver pathology ontology model testing

WP5
Proof-of-concept for integrating AOP-based *in vitro* assays and QIVIVE modelling into a tiered testing strategy for repeat-dose nephrotoxicity testing.
CASE STUDY 2

- **Clinical Note**: Lysosome per cell area
- **Lysosome**: Mean intensity
- **Cathespin release (IF)**: Mean/Max intensity
- **Number of nuclei (PerkinElmer’s Opera™ System)**
- **CellTiter Glo® Cytotoxicity assay**

**Graphs and Data**

- **Graph 1**: Log concentration vs. response [%]
  - Axes: Log concentration (µM) vs. response [%]
  - Data points: 1h, 2h, 3h, 4h

- **Graph 2**: Intracellular concentration vs. Exposure time [h]
  - Axes: Intracellular concentration [nmol/well] vs. Exposure time [h]
  - Data points: 0h, 6h, 12h, 24h

- **Graph 3**: Lysosome per cell area
  - Axes: Concentration in µM vs. Lysosome per cell area
  - Data points: LOD = 50 nM, LOQ = 15 nM, 134 µM
CASE STUDY 2

Clinical authorized dose:
15,000 – 25,000 units/kg BW/day, IV
10,000 units = 1 mg polymyxin B

Dose = 1.5–2.5 mg/kg/day
Given in 2 doses, so 0.75 – 1.25 mg/kg per dose.

In vitro kinetics

Intracellular concentrations

AOP outcomes

concentration-KE biomarker response
CASE STUDY 3

☑ IN²TOX for food safety: open source tools and case studies

- New data requirements for pesticides (283/284 2013): comparison in vitro metabolism rat/human

- Collaborative case studies with national/international (2016-2020):
  - Use of human in vitro metabolism data and QIVIVE models in risk assessment
  - Prediction of human kinetics for compounds relevant to EFSA panels, such as pesticides, contaminants, food additives and botanicals

*Guidance on use of human in vitro metabolism pesticide panel (November 2019)*
CASE STUDY 3

✓ IN²TOX for food safety: open source tools and case studies

- Generic TK, IVIVE Models and TK platform (2018-2020)
- Human, fish and farm animal models: open source data and model codes

TKPlate: all models for description and prediction of TK (2021)
CASE STUDY 3

IN²TOX for food safety: open source tools and case studies

- **OpenFoodTox**: Linking Open Data and QSARs: published *versus* predicted

**Properties**
- Phys-chem, TK data, Tox, bioaccumulation, exposure, mechanistic

- **New and updated OECD harmonised templates (JRC EFSA OECD)**
  - Weight of evidence, biological relevance and uncertainty
  - Update mechanistic (OHT 201) and TK template
CASE STUDY 4

Endocrine disruptor (ED) chemicals: an integrated approach

- **Current regulations**: EU and EPA (hazard versus risk approaches)
- **Mammalian safety**: EATS-Tiered approach
- **Complexity**: new versus existing chemicals/cost/timelines

**Point of entry for existing chemicals**
- Review of Tox package
- Detailed EATS assessment
- Read across

**Assessment of existing Data**
- **In silico**
  - ToxCast Models and HTS (bioactivity)
  - QSAR Models (prediction)
- **In vitro**
  - EAS OECD Assays
  - Thyroid homeostasis platform (non-OECD, MOA)
  - Liver Metabolism of T
- **In vivo**
  - Specific Mechanistic EATS endpoints
  - Thyroid MOA
  - Liver Metabolism of T
  - 3Rs

**ED assessment**
- Annex E compilation
- Opinion
- Submission to ECHA/EPA

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Current QSAR and future ToxCast models

<table>
<thead>
<tr>
<th>EDSP Tier 1 screening assays</th>
<th>US EPA HTP screening and in silico model alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
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<tr>
<td>ER Transactivation</td>
<td>OCSPP 890.1300 ToxCast ER model</td>
</tr>
<tr>
<td>ER Binding</td>
<td>OCSPP 890.1250 ToxCast ER model</td>
</tr>
<tr>
<td>AR Binding</td>
<td>TG OPPTS 890.1150 AR model (future)</td>
</tr>
<tr>
<td>Steroidogenesis</td>
<td>OCSPP 890.1550 STR model (future)</td>
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<tr>
<td>Aromatase</td>
<td>TG OPPTS 890.1200 STR model (future)</td>
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<tr>
<td><strong>In vivo mammalian</strong></td>
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<tr>
<td>Uterotrophic</td>
<td>OCSPP 890.1600 ToxCast ER model</td>
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<tr>
<td>Hershberger</td>
<td>OCSPP 890.1400 AR model (future)</td>
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<tr>
<td>Pubertal male</td>
<td>OCSPP 890.1500 AR, STR and THY model (future)</td>
</tr>
<tr>
<td>Pubertal female</td>
<td>OCSPP 890.1450 ER, STR and THY model (future)</td>
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<table>
<thead>
<tr>
<th>QSAR Model System</th>
<th>Insight</th>
<th>Modality</th>
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<tbody>
<tr>
<td>DEREK Nexus</td>
<td>Based on expert rules</td>
<td>E A T S</td>
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<tr>
<td>VEGA NIC</td>
<td>Statistic-based model</td>
<td>X</td>
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<tr>
<td>OECD QSAR Toolbox</td>
<td>Some limitations</td>
<td>X</td>
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<tr>
<td>Endocrine Disruptome</td>
<td>Focuses on the receptors</td>
<td>X X X X</td>
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<tr>
<td>Danish QSAR Database</td>
<td>Only useful if the chemical is already included</td>
<td>X X X X</td>
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Building a weight-of-evidence argument for next steps

Establishment:
- The data are sufficient to show that a substance does not meet the ED criteria
- Additional information is needed (data gaps)
- A MOA analysis is required as a next step to conclude on the substances’ ED properties.
EAS in vitro platform

Thyroid homeostasis in vitro platform

<table>
<thead>
<tr>
<th>Assay type</th>
<th>E</th>
<th>A</th>
<th>T</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG 493: In Vitro Oestrogen Receptor Binding Assay (890.1250)</td>
<td></td>
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<tr>
<td>TG 455: In Vitro Oestrogen Receptor Transactivation Assay (890.1300)</td>
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<tr>
<td>TG 458: In Vitro Androgen Receptor Transactivation Assay (880.1150)</td>
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<tr>
<td>TG 456: H295R Steroidogenesis Assay (890.1200)</td>
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<td>X</td>
<td></td>
<td>X</td>
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</tbody>
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Thyroid homeostasis assessment: cross-species

- Inhibition of rat and human deiodinase 1, 2 or 3
- Sodium/iodide symporter inhibition (NIS) (rat)
- TPO inhibition (multiple species)
- In vivo and in vitro hepatic thyroid hormone metabolism (multiple species)

Conclusion

1. Difference US EPA versus EU ECHA
2. Complex processes
   1. Existing molecules
   2. New molecules
3. EAS in vitro -> in silico models (EPA)
4. T assays: new platform for MOA
5. Gap assessments and bridging strategy
6. ED classification

IC_{50} values obtained are consistent with those reported in the literature.