Revolution in developmental toxicity testing

Aldert H. Piersma

Center for Health Protection
RIVM Bilthoven-NL

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Reductionistic models for human chemical hazard assessment
Adverse Outcome Pathways

Development of AOP

MIE1 → KE1 → KE2 → KE3 → AO1

Evidence in the literature?
Homeostasis through feedback mechanisms: the Hypothalamus-Pituitary-Gonadal axis
Adverse Outcome Pathways

- **MIE1** → **KE1** → **KE2** → **KE3** → **AO1**
- **MIE2** → **KE5** → **KE6** → **AO2**
- **MIE3** → **KE5** → **KE6** → **AO3**
- **MIE4** → **KE7** → **KE8** → **KE9** → **AO4**
- **MIE5** → **KE7** → **KE8** → **KE9** → **AO5**
Toxicological Ontology

molecule-cell-tissue-organ-organism physiology

chemical → outcome
chemical → outcome
chemical → outcome
chemical → outcome
chemical → outcome
Animal-free risk assessment

molecule-cell-tissue-organ-organism physiology

chemical
chemical
chemical
chemical
chemical

outcome
outcome
outcome
outcome
outcome

test 1


test 2


test 3


test 4


test 5


test 6


toxicological ontology model

toxicity profile
Toxicological Ontology Model

- biology (fundam.res.)
- chemistry (SAR/phys/..)
- toxicology (tests/MOA/..)

Substance -> toxicological ontology model -> Toxicity
An RA neural tube - axial patterning AOP Framework

Tonk, Pennings & Piersma 2015
Retinoic acid and neural differentiation

retinoic acid exposure regulation and malformations

Rhinn 2012
EST Neural Differentiation

Theunissen et al., 2011
MeHg effects on tissue gene sets over time

- Embryonic time gene sets decrease
- Early gene sets decrease
- Neuroectodermal gene sets increase
- Alternative germ layers decrease
- Agrees with studies showing enhanced neural differentiation causing brain malformations

Theunissen et al., 2011
Zebrafish selected gene set regulation - Flusilazole

Hermsen et al., 2011
Chemical Structure and Developmental Toxicity

Daston et al., Chem. Res. Toxicol. 2013, 26, 1840–1861
Toxicological Ontology Model

- Biology (fundam.res.)
- Chemistry (SAR/phys/..)
- Substance
- Toxicology (tests/MOA/..)
- Toxicity
A Computational Model Predicting Disruption of Blood Vessel Development

Nicole Kleinstreuer¹, David Dix¹, Michael Rountree¹, Nancy Baker², Nisha Sipes¹, David Reif¹, Richard Spencer², Thomas Knudsen¹*

¹ National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, United States of America, ²Lockheed-Martin, Research Triangle Park, North Carolina, United States of America
Animal-free risk assessment

molecule-cell-tissue-organ-organism physiology

test 1
chemical
outcome

test 2
chemical
outcome

test 3
chemical
outcome

test 4
chemical
outcome

test 5
chemical
outcome

test 6
chemical
outcome

toxicological ontology model
toxicity profile

Graph showing chemical interactions and outcomes from animal-free tests.
Animal-free Human Risk Assessment

- ADME
- Ontology Model
- external exposure
- target concentration
- key events
- AOPs
- toxicity profile
- risk assessment

organism

molecule
To do list

• Map human physiology from the molecular to the organism level
  – Aim at level of detail fit for the purpose of toxicity testing

• Integrate existing chemistry and toxicity knowledge
  – Identify the major Modes of Action of human toxicity
  – Map the integrated AOP landscape fit for the purpose of toxicity testing
  – Identify rate-limiting Key Events and related biomarkers
  – Design biomarker-related test systems

• Build ontology-based computational tools for toxicity prediction
  – Integrate quantitative test output into ontology model
  – Define thresholds of adversity at the integrated ontology model level

• Embed toxicodynamic ontology model within overall risk assessment
  – Consider use patterns and expected exposure scenarios
  – ADME – model external to internal exposure – target organ concentration modelling
  – Consider timing, duration and life cycle segment(s) of exposure
  – Design flexible compound-dependent case-by-case fit-for-purpose testing strategy
Revolution in hazard and risk assessment

- Based on integrated knowledge of the biology of the system
- Fit for purpose - Comprehensive as to toxicity pathways
- Employing all existing knowledge in chemistry and toxicology (incl *man* and *animal* and *in vitro* and *in silico*)
- Targeting the human
- Avoiding the detour of the animal (time, cost, ethics)
Animal-free risk assessment
Reference:

An adverse outcome pathway framework for neural tube and axial defects mediated by modulation of retinoic acid homeostasis.

Tonk EC, Pennings JL, Piersma AH.

Modular validation approach (ECVAM)

- **Test definition**
- **Within-lab variability**
- **Transferability**
- **Between-lab variability**
- **Predictive capacity**
- **Applicability domain**
- **Performance standards**

**Reliability**
- **Technical performance**
- **Sensitivity specificity**

**Relevance**
- **Biological domain**
- **Chemical domain**

**Review by Validation Management Group**

**Independent Peer Review**

Hartung et al., 2004
Test system requirements

• Biological domain
  – Describes the biology of the system in terms of MoA, AOP, key event(s) covered and end point measured

• Technical performance
  – Standardization, variability, transferability

• Chemical domain
  – Solubility, volatility, ...

• Sensitivity / specificity
  – Validate each individual test with known positives and known negatives against its biological domain only, “mechanistic validation”
  – Validate test battery as a whole against in vivo toxicity, based on sufficient mechanistic coverage of biology/toxicology
Battery prediction example

- 11 dev. toxicants and one negative control in a 25 assay battery
- The battery predicted better than individual assays
- One ‘false’ (!) negative, its MoA was absent from the battery

Piersma et al., 2013