Current acceptance of *in vitro* toxicology tools and future prospects

Chantra Eskes, PhD Eng.
ESTIV president
Member of OECD expert group eye and skin hazard
ACTIP executive secretary
SeCAM, Switzerland

Lausanne, 14 April 2016

Henry Spira
New York Times,
15 April 1980 and
7 October 1980

1959: The 3Rs Concept

Refinement alternatives alleviate or minimise potential pain, suffering and distress

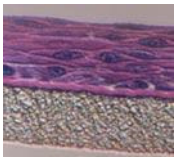
Reduction alternatives obtain a comparable level of information from the use of fewer animals, or more information from the same number of animals

Replacement alternatives permit a given purpose to be achieved without using animals

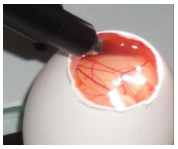
Russell, W.M.S. & Burch, R.L. (1959). *The Principles of Humane Experimental Technique*. Methuen, London.

1986 (updated in 2010): EU Directive on the Protection of Animals used for Scientific Purposes

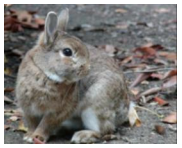
Directive 2010/63 (updated Directive 86/609)



Article 4.1: **Member States shall ensure that,** wherever possible, **a *scientifically satisfactory method or testing strategy*, not entailing the use of a live animal, shall be used instead of a procedure** (~ use of animal for experimental or other scientific purposes)



Article 13.1: **Member States shall ensure that a procedure is not carried out if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognised under the legislation of the Union**.



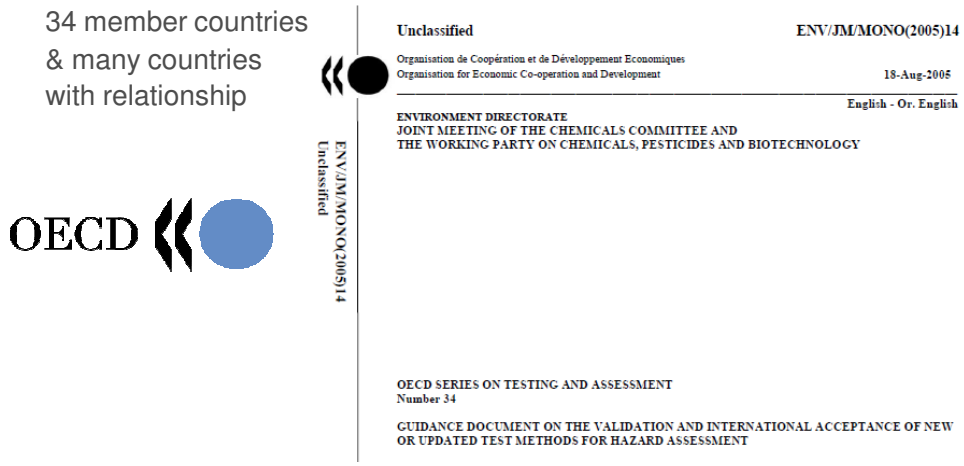
Article 47: **The Commission and the Member States shall contribute to the development and validation of alternative approaches** which could provide the same or higher levels of information as those obtained in procedures using animals, but which do not involve the use of animals or use fewer animals or which entail less painful procedures, and they shall take such other steps as they consider appropriate to encourage research in this field.

Article 48: **European Union Reference Laboratory**

2005: OECD Guidance Documents 34 on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment

34 member countries
& many countries
with relationship

OECD



Unclassified ENV/JM/MONO(2005)14

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development 18-Aug-2005

English - Or. English

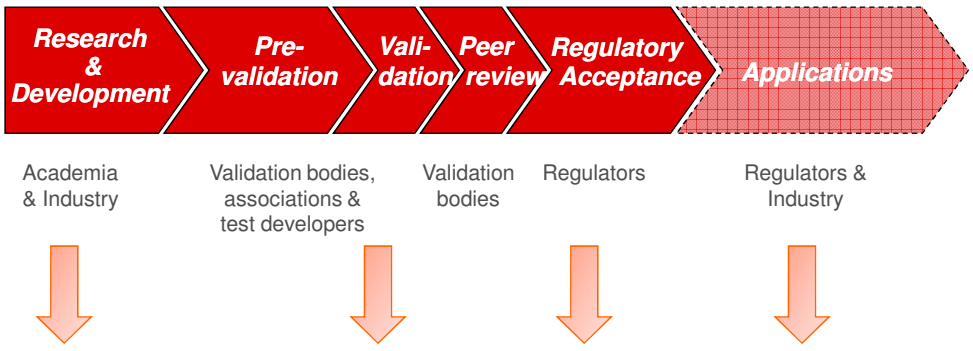
ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

ENV/JM/MONO(2005)14
Unclassified

OECD SERIES ON TESTING AND ASSESSMENT
Number 34

GUIDANCE DOCUMENT ON THE VALIDATION AND INTERNATIONAL ACCEPTANCE OF NEW OR UPDATED TEST METHODS FOR HAZARD ASSESSMENT

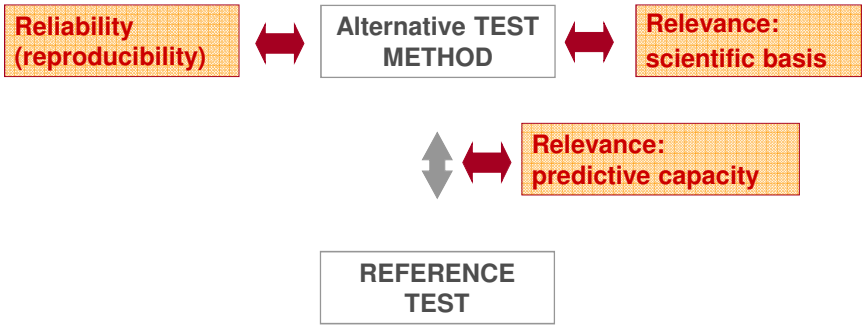
The Validation Process



Collaboration, communication & joint progress

Definition of Validation

"…to establish the reliability and relevance of the method for a particular purpose"



Regulatory drivers

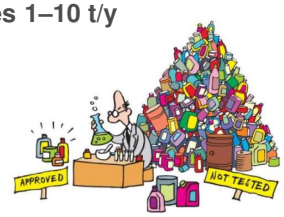
Regulatory drivers (EU)

➢ **Cosmetics: EU Directive 2003/15/EC (Regulation 1223/2009)**

- **Ban on animal testing for finished products (2004)**
- **Ban on animal testing for cosmetic ingredients (2009)**
- **Marketing ban on cosmetics tested on animals (2013)**

➢ **New & existing chemicals (≥ 1 t/y): REACH Reg. 1907/2006**

- **Skin & eye irritation: *in vitro* testing only for substances 1–10 t/y**
- **General rules for adaptation: *in vitro* tests** (annex XI)
  - Validated assays: non- and hazardous effects
  - Suitable assays: only hazardous effects
  - Other assays: mechanistic insights



Example of Integrated Testing Strategy: Skin and Eye Irritation

1. Existing data on physico-chemical properties
2. Existing human data
3. Existing animal data from skin/eye irritation/corrosion studies
4. Existing data from acute dermal toxicity (skin: also sensitization studies)
5. Existing (Q)SAR data and read-across
6. Existing *in vitro* data
7. Weight of evidence analysis
8 & 9. New *in vitro/ex vivo* test skin and eye corrosion and irritation
~~9 or 10. New *in vivo* test for skin/eye irritation (annex VIII)~~

If *in vitro* validated full replacement(s) available

United Nations Globally Harmonized System

- **From 2013 (5th revision): incorporates weigh-of-evidence evaluation**

  1. existing human or animal skin corrosion/irritation data,
  2. other existing skin data in animals
  3. existing *ex vivo* / *in vitro* data (REACH: position 6 after SAR)
  4. pH-based assessment (and acid/alkaline reserve of the substance) (REACH: position 1)
  5. validated SAR methods
  6. weight of evidence

  Although information can be gained from the single parameters within a tier, ***the totality of existing information shall be considered*** *to make an overall weight of evidence determination, especially when there is conflict in information available on some parameters*
  .

Mixtures: EU CLP Regulation 1272/2008

- Introduction of GHS classification in the EU 2010: substances, 2015: mixtures
- Use of **tiered weight-of-evidence strategy encouraged**
- Validated *in vitro* methods required to confirm classification of extreme pH formulations with alkaline or acid reserve indicating non-corrosion

Biocides: Regulation 528/2012

- **Testing on vertebrates only as a last resort**
- Toxicological information required: **testing strategy, existing information, reduction and refinement assays**
- **General rules for adaptation: *in vitro* tests** (annex VI)
  - Validated assays: hazardous effects
  - Suitable assays: mechanistic insights

  Non-hazardous effects: confirmation may be requested on a case- by-case basis

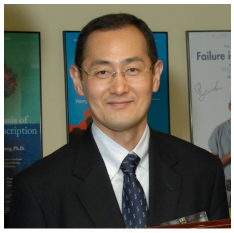
Scientific drivers

Drug development

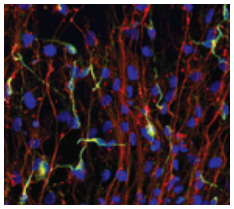
~ 95% of drugs that enter clinical trials do not make it to the market

- **20 - 40% poor prediction of side effects by toxicology & safety tests**
  - Rats and mice together 43% prediction only - Olson et al., 2000, RTP 32, 56-67
  - Lethal dose of endotoxin in humans to produce shock is a million-fold less than in mice - McGonigle & Ruggeri, 2013, Bioch Pharm *in press*
  - No correlation between murine and human genetic responses to inflammation - Leist & Hartung, 2013, Arch Tox 87, 563-567
- **Lack of efficacy: 51 % of failure in phase II clinical studies**
  - Animal studies only 37 to 55% reproducible in humans - Hartung 2013, ALTEX 30, 275-291
  - Over 500 neuroprotective treatments for cerebral ischemia that worked in animals failed in man - van der Workp, 2010, Plos Med 7
  - Inadequacies of animal studies in the area of mood disorders (depression, anxiety), contributed to the withdrawal of research in this area - McGonigle & Ruggeri, 2013, Bioch Pharm *in press*

Induced Pluripotent Stem Cells (iPSC)



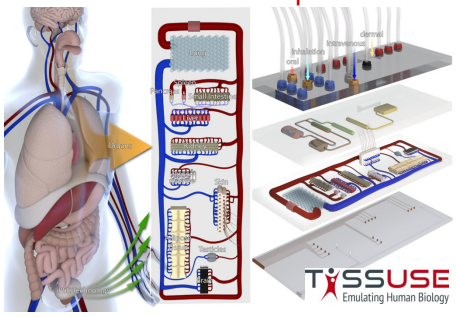
Shinya Yamanaka



2012 Nobel Prize in Physiology or Medicine together with John Gurdon
for the discovery that “mature cells can be reprogrammed to become pluripotent."

Inoue & Yamanaka, 2011, Cli Pharm Therap 89, 655-661

Human on a chip



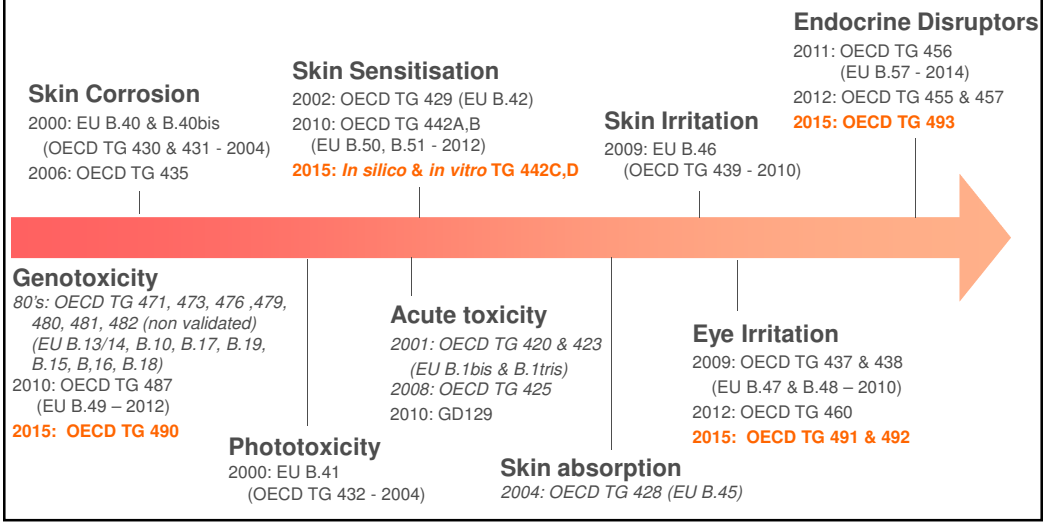
Marx et al., ATLA 2012

High throughput screening

Current status

International Acceptance of Alternative Methods

OECD Chemicals



Full replacement
&
1st Integrated Approaches for Testing and Assessment (IATA)

Skin irritation & corrosion: full replacement in EU

1 *in vivo* TG → 4 *in vitro* TG → 10 Test Methods

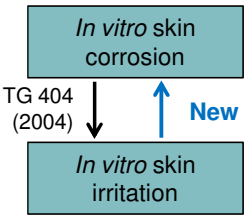
- Reconstructed human Epidermis (RhE) models
  - EPISKIN™ SIT (2007)
  - EpiDerm™ EPI-200-SIT (2009)
  - SkinEthic™ SIT42bis (2009)
  - Labcyte EPI-MODEL24 SIT (2012)
- Transepidermal Resistance test
- RhE models
  - TER (1998)
  - EPISKIN™ (1998)
  - EpiDerm™ (1998)
  - SkinEthic™ RHE (2006)
  - epiCS® (EST1000, 2009)
- Membrane barrier test*
  - Corrositex® (1999)

* Under revision

OECD Guidance Document on Integrated Approaches to Testing and Assessment (IATA) of skin corrosion & irritation

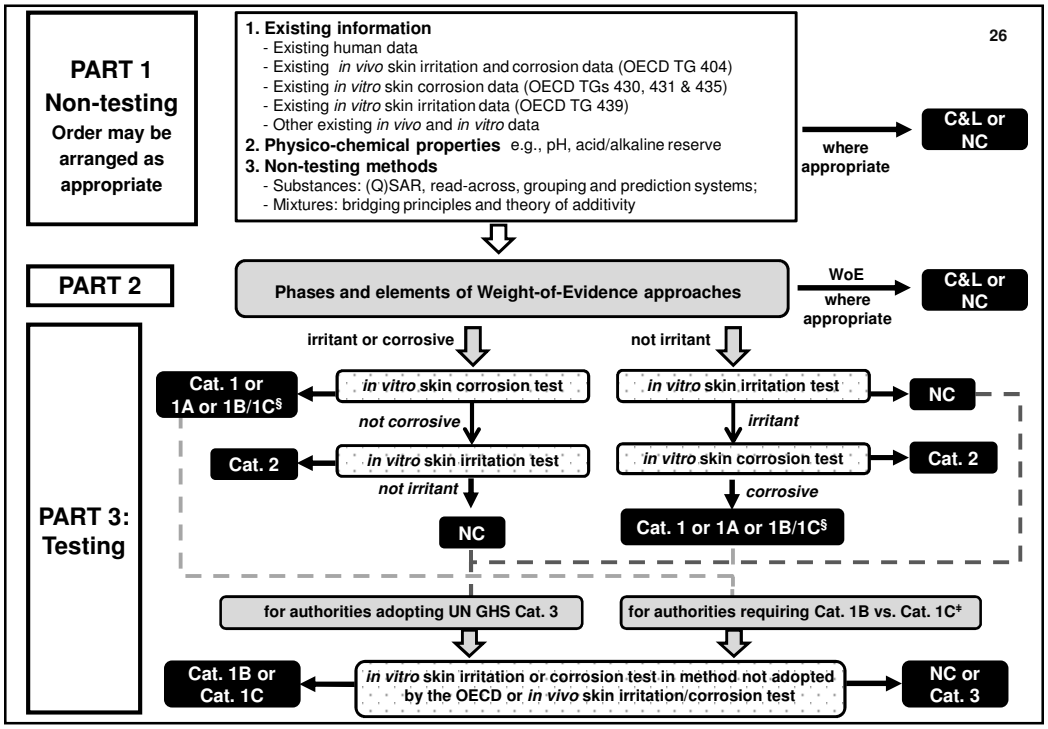
➢ Aim: combining OECD TG 404, 430, 431, 435 and 439 with the aim to minimize the use of animal testing to the extent possible, while ensuring human safety

- Modules tackled:
  - Human data
  - Animal data
  - *In vitro* data on skin corrosion and irritation
  - Non-testing data (physico-chemical properties, QSARs, etc)
  - Guidance on weigh-of-evidence analyses



- ***Possibility to identify non-hazard effects*** *if sufficient and appropriate evidence*
- Applicability, benefits and limitations of the existing TGs and modules
- Suitability of the OECD TGs for mixtures and preparations

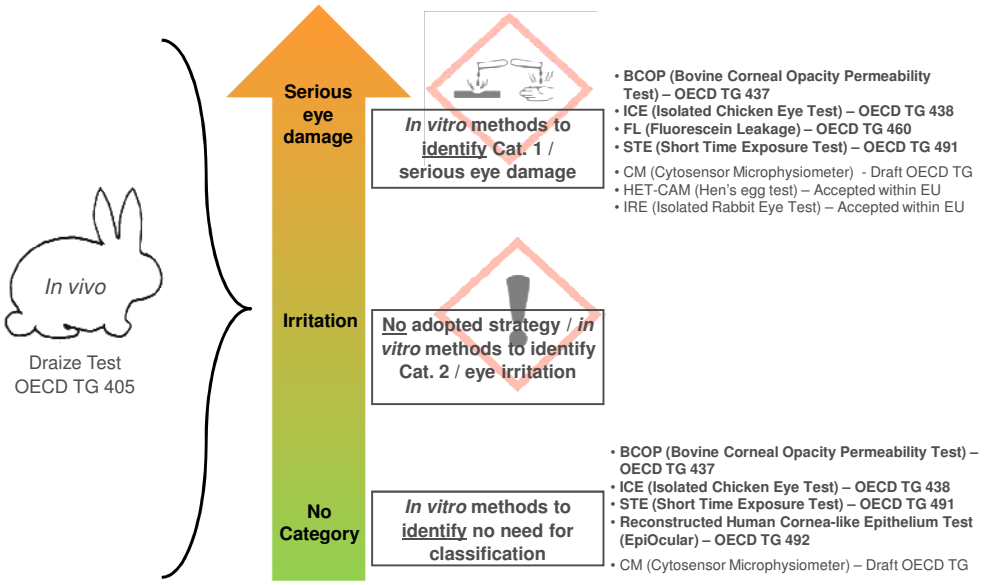
⇨ **Guidance Document 203 adopted at OECD WNT meeting in April 2014**
TG 404, 430, 431, 435 & 439 updated accordingly



SeCAM

Partial replacement

In vitro test methods for eye hazard identification



Evaluation of irreversible versus reversible effects

- **The reversibility of tissue lesions are not evaluated *per se* in the current OECD TG methods accepted to identify serious eye damage / Cat. 1**
  - ICE & surfactants: 67% (6/9) false negatives, where 4/9 surfactants, that were classified based on persistent effects only, were all under-predicted by the ICE standard test
- **Persistence of tissue effects (at day 21) count for a significant proportion of serious eye damage / Cat. 1 classification**
  - Cos EU / EURL-ECVAM joint activity: 55.5% (106/191) of Cat. 1 chemicals from public and new databases are classified based on persistence of effects only - Adriaens *et al.* 2014, Arch Tox 88. 701-723
  - Surfactants: 67% (18/27) from public databases classified due to persistence of effects only
- **Histopathology as an adjunct to the ICE test method**
  - Histopathology criteria developed by A.I.S.E. - Cazelle et al. 2014 (TIV 28, 657-666), 2015 (TIV 29, 609-616)
  - Allowed a better prediction of EU CLP / UN GHS Cat. 1: Identification of Cat. 1 classified *in vivo* based on persistence of effects, and avoid misclassification of Cat. 1 classified *in vivo* based on severity of effects
  - Inter-laboratory reproducibility study ongoing

⇨ **Under discussions at the OECD level** (Draft revised TG 438 from 23.09.2015)

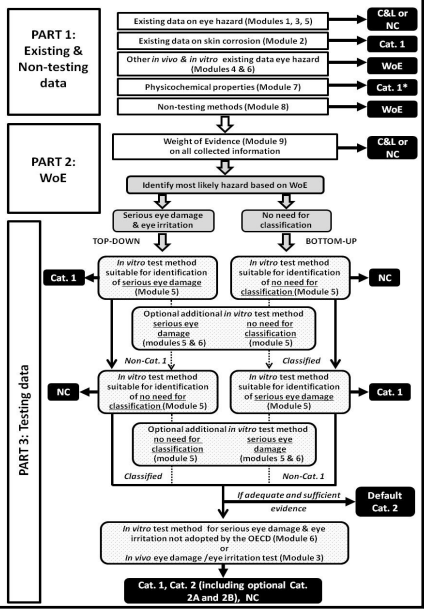
Integrated Approaches for Testing and Assessment (IATA) on serious eye damage / eye irritation

OECD activity (lead: US & EU)

- Decision making on UN GHS classification and labeling (Cat. 1, Cat. 2, No Cat.)

  Minimize the use of animals to the extent possible, whilst ensuring human safety

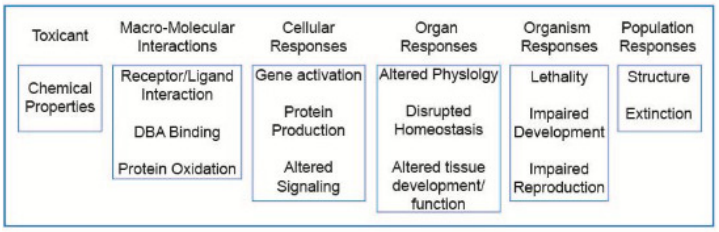
- Addresses
  - Key characteristics of each information source comprising IATA
  - Guidance on how and when to integrate the information sources
  - Bottom-Up & Top-Down testing approaches
  - **Discuss statistical modeling of testing strategies → EU CLP Cat. 2**
  - **Considerations on intrinsic characteristics of Draize rabbit eye test method**



SeCAM

Adverse Outcome Pathways & Defined Approaches to Testing and Assessment

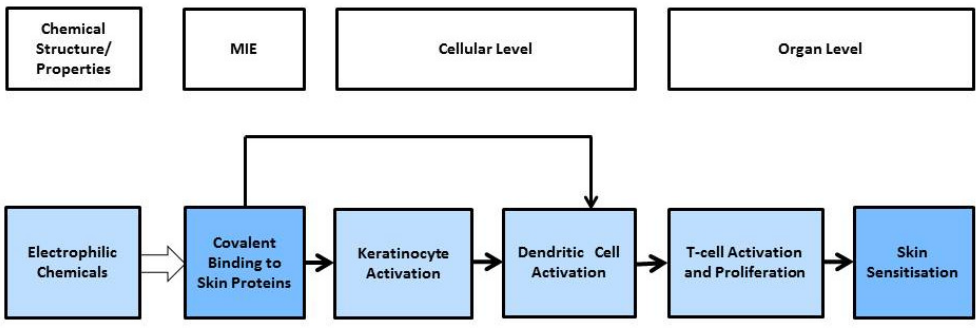
OECD & Adverse Outcome Pathways



Sequential chain of causally linked events leading to an adverse effect

- **AOP knowledge base**
  - Interactive web-based platform for AOP development
  - AOP Wiki: https://aopkb.org/aopwiki/index.php/Main_Page
- **Possibility to make an AOP project proposal to the OECD**
  http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

Skin Sensitisation: Adverse Outcome Pathways



AOP Wiki, March 2016

Skin Sensitization

- **Defined Approaches to Testing and Assessment (DATA)**
  - AOP-based IATA: different information sources target different key events & toxicity pathways
  - DATA: integration of read-outs of a suite of *in silico*, *in chemico* and *in vitro* methods
  - Examples: ITS, STS, ITS+STS, integrated decision strategy, consensus decision tree model, artificial neural network models
  - Based on: *fixed set of information sources* & *fixed data interpretation procedure* (DIP) to convert inputs from information sources into a prediction
  - Fixed weighting of the different information sources (≠ WoE approaches)
  - Outcome may be used on its own or as a component of IATA
  - Chemical space for the DATA usually > IATA (customised to chemical (class) evaluated)

⇨ In preparation: OECD Draft Guidance Document on reporting of defined approaches and individual information sources to be used within IATA for skin sensitization (including 12 case-studies)

Reporting of IATA

- AOP represents a solid scientific framework for development of an IATA
- Different IATA solutions possible depending on chemical, regulatory need and specific geographical requirements
- Harmonized approach for reporting of IATA to promote consistent application and evaluation of IATA
- General principles:
  - Defined endpoint
  - Defined purpose
  - Rationale underlying IATA construction
  - Description of individual information sources of IATA
  - Description of how information sources are integrated to derive final prediction/assessment
  - Known uncertainties associated with IATA
- Reporting template for DATA
- Reporting template for individual information sources

⇨ In preparation: OECD Draft Guidance Document on reporting of IATA to facilitate consistent evaluation and application

Challenges & prospects

Challenges

- **Pathway-based approaches**
  - Major hurdle: moving from qualitative to quantitative AOP
  - Flexibility needed to validate based on MoA, rather than on apical endpoint in high dose animal studies
  - Require: targeted cellular assays, new cell biology-based extrapolation modes, and MoA based approaches based on human biology
  - Evidence-based approaches, systemic reviews and evidence quality assessment can help (e.g. reference standard)
- **Transcriptomics-based *in vitro* methods**
  - Can support MoA approach
  - Challenges on data interpretation, level of confidence and threshold of adversity
- **Stem cells**
  - Standardization, identity, sources of variability, characterization of pluripotency, functional potential, quality & microbiological controls, characterization of differentiation, stability & window of use
  - Bioreactors: validation based on process characterization (versus final 'product')
- **Human-on-a-chip / microphysiological systems**
  - Qualification: reproducibility, cellular systems, combination of different growing cells, stability in time, organ functionality, standardized testing protocol, etc
  - Adherence to existing guidelines & fit for purpose validation with representative groups of substances in coordination with regulators

OECD GD 211 for describing non-guideline *in vitro* methods (Dec 2014)

Information to be ideally provided for describing non-guideline *in vitro* methods

- Harmonize method description & facilitate assessment
- Not prescriptive, allows flexible structure, completeness of information may depend on level of development of *in vitro* assay
- Novel *in vitro* assays e.g., high throughput screening, complex models

1. **General information:** Name, developer, status, references…
2. **Test method definition:** Purpose, principle, exposure, quality/acceptance criteria, known limitations & strengths
3. **Prediction model:** Assay responses, data analyses and interpretation
4. **Performances:** Reproducibility, predictive capacity, scope & limitations
5. **Potential regulatory applications**
   Support read-across / Priority setting / Screening purpose / Component of IATA

Pharmaceuticals

EMA/CHMP/CVMP/JEG-3Rs/450091/2012

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

⇨ **Applicable to t*esting approaches*** subject to regulatory guidance ***for human and veterinary medicinal products*** used to support regulatory applications: clinical trial applications, marketing authorisation applications

(early screening: no regulatory involvement (in-house validation))

1 3 October 2014
2 EMA/CHMP/CVMP/JEG-3Rs/450091/2012
3 Committee for Medicinal Products for Human Use (CHMP)
4 Committee for Medicinal Products for Veterinary Use (CVMP)

5 Guideline on regulatory acceptance of 3R (replacement,
6 reduction, refinement) testing approaches
7 Draft

Draft Agreed by JEG 3Rs	March 2014
Draft agreed by SWP, SWP-V, BWP, IWP and EWP-V	By July 2014
Adoption by CVMP for release for consultation	11 September 2014
Adoption by CHMP for release for consultation	24 September 2014
Start of consultation	3 October 2014
End of consultation (deadline for comments)	31 December 2014

8
9 This guideline replaces the Position on Replacement of Animal Studies by *in vitro* Models
10 (CPMP/SWP/728/95).

Criteria for regulatory acceptance

1. Formal **method validation** (OECD, ECVAM, ICCVAM)

2. Demonstration that the new or substitute method or testing strategy provides either new data that **fill a recognised gap** or **data that are at least as useful as, and preferably better than those obtained using existing methods**
   → *If no formal validation, evaluation on a case-by-case basis by National Control Authorities and/or relevant Working Parties or Expert Working Groups*

3. Demonstration of **adequate testing of medicinal products under real-life conditions** (human and veterinary) which can be generated through the "*Safe Harbour Process*":
   Period of voluntary submission of data obtained using a new 3R testing approach in parallel with data generated using existing methods. Data generated with 3R approach will be solely used for the purpose of evaluation of novel 3R testing approaches for possible future regulatory acceptance

Conclusions

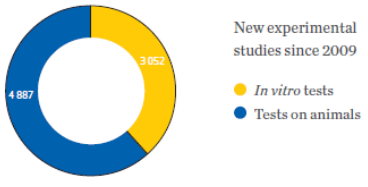
- **Ethical, regulatory and scientific drivers**

  Cosmetics, chemicals, mixtures, biocides, pharmaceuticals

- **Validation succeeded to facilitate regulatory acceptance**
  - Full replacement: skin corrosion & irritation, phototoxicity, skin absorption
  - Partial replacement: eye hazard & skin sensitization
  - Reduction and refinement: acute toxicity, genotoxicity, endocrine disruptors

The Use of Alternatives to Testing on Animals for the REACH Regulation

- **ca. 8000 new experiments (tests) in October 2013 (since 2009)**
  - ~ **40% (3052) *in vitro* tests**
  - ~ **60% (4887) tests in animals**



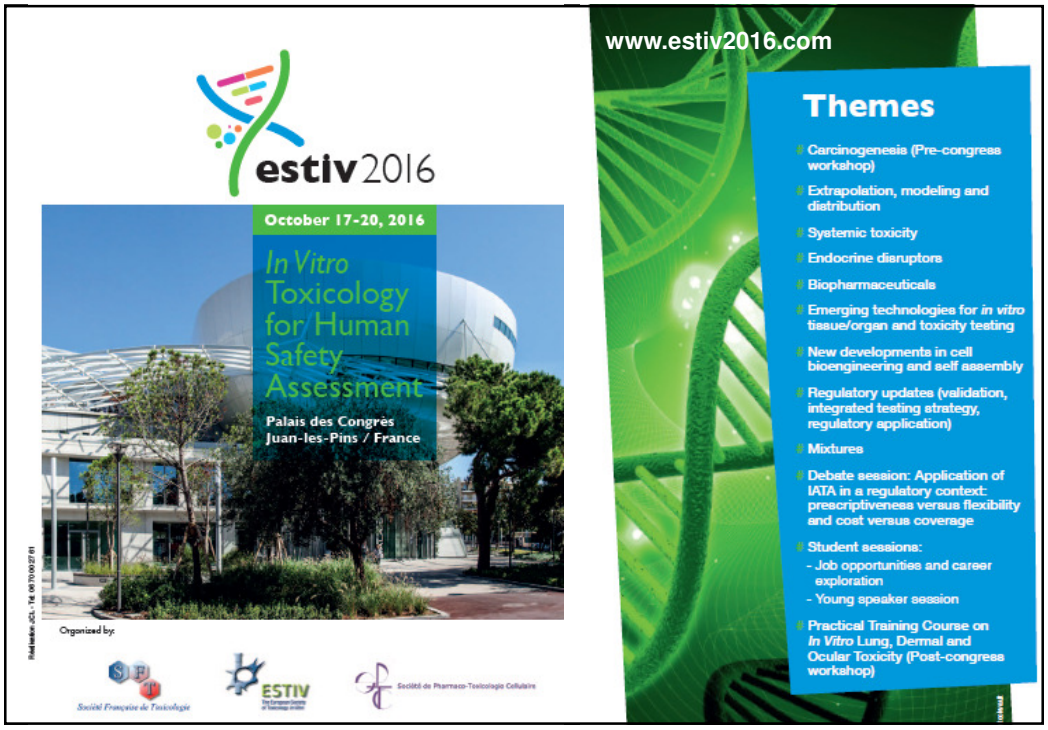
- **ca. 9000 registrations for 2998 substances ≥ 100 tones/year**
  - **75% make use of Read-Across → most commonly used alternative**

    Especially for higher tier endpoints, where alternative tests or strategies not yet available (e.g., sub-chronic toxicity, pre-natal developmental toxicity or toxicity to reproduction)
  - **20% of dossiers contained *in vitro* studies**

    Either alone or combined with other information
  - ***In vitro* tests for skin and eye hazard: x3 from 2011 to 2013** (from 442 to 1410)

- **Current challenges**
  - Need to keep pace with scientific progress
  - Acceptance criteria more stringent than for animal testing
  - Tendency to consider animal as a 'gold standard' over human effects

- **Opportunities**
  - Fit-for-purpose / flexible validation approaches
  - IATA: validation to add value rather than hindering progress
  - Performance standards to classes of methods/integrated approaches that provide similar information
  - Collaborative efforts between developers & user communities




Thank you!
chantra.eskes@secam-ce.eu