Coming to grips with unfamiliar uncertainties of a new predictive toxicology paradigm

Maurice Whelan

European Commission Joint Research Centre

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European Union Reference Laboratory for Alternatives to Animal Testing

Shaping the future of food safety together"

EFSA@EXPO, Milano 2015

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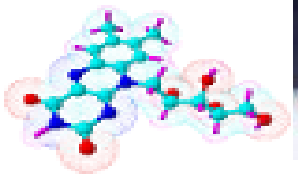
Systems Toxicology Unit – Support to policy focus

Regulation of Chemicals and the Protection of Animals*

- Animal-free methodology to assess the hazard posed to consumers, workers and the environment
- Advancing safety assessment science to support regulatory decision making
- Facilitating industry to comply with regulation while striving for innovation and competiveness

*Industrial chemicals, cosmetic ingredients, food constituents, pesticides, biocides, drugs



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Established under the Directive 2010/63/EU on the protection of animals used for scientific purposes

The European Union Reference Laboratory for Alternatives to Animal Testing

Responsibilities

- Guide research
- Coordinate validation
- Disseminate information
- Facilitate stakeholder dialogue
- Promote international acceptance

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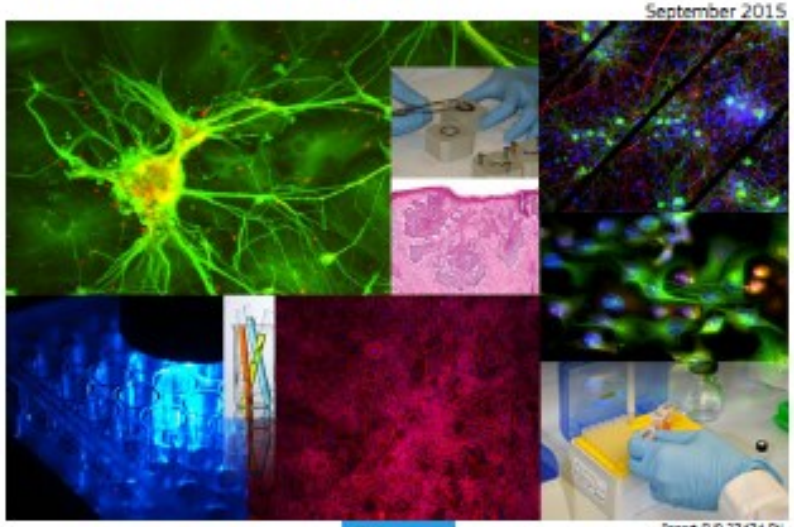
JRC SCIENCE AND POLICY REPORT

EURL ECVAM Status Report on the Development, Validation and Regulatory Acceptance of Alternative Methods and Approaches (2015)

Valérie Zuang, Bertrand Desprez, João Barroso, Susanne Belz, Elisabet Berggren, Camilla Bernasconi, Jos Bessems, Stephanie Bopp, Silvia Casati, Sandra Coecke, Raffaella Corvi, Coralie Dumont, Varvara Gouliarmou, Claudius Griesinger, Marlies Halder, Annett Janusch-Roi, Aude Kienzler, Brigitte Landesmann, Federica Madia, Anne Milcamps, Sharon Munn, Anna Price, Pilar Prieto, Michael Schäffer, Jutta Triebe, Clemens Wittwehr, Andrew Worth and Maurice Whelan

September 2015



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http://publications.jrc.ec.europa.eu/repository/handle/JRC97811

Extrapolating from early to late effect

Extrapolating across dosing duration

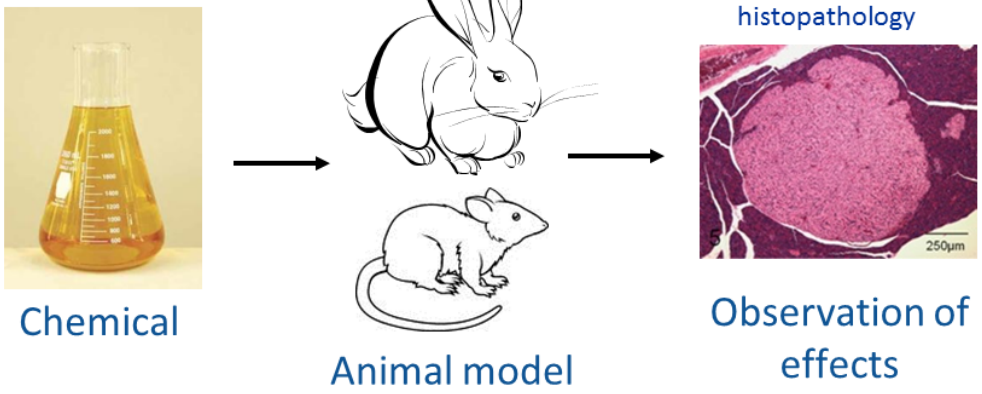
Extrapolating across dosing patterns

Conventional Toxicology

Determination of a PoD

Extrapolating to low-effect levels

Estimating Intra-species variability



Extrapolating across exposure metrics

Extrapolating across agents

Sources of 'familiar' uncertainty

Inter-species extrapolation

Estimating the impact of missing studies

Extrapolating from *in vitro* or *in chemico* to *in vivo* data

WHO-IPCS (2014) Guidance document on evaluating and expressing uncertainty in hazard characterization.

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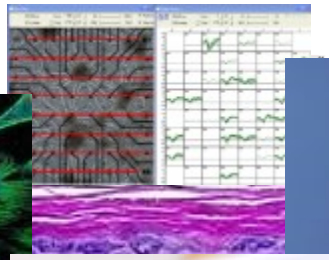
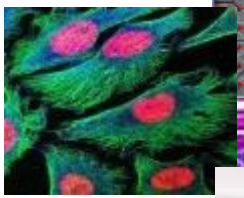
Predictive Toxicology

Organ-on-a-chip

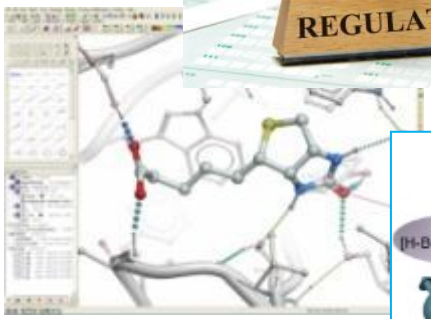


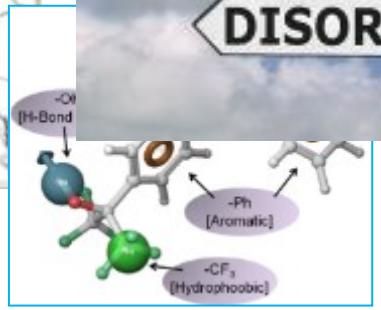
Cell cultures



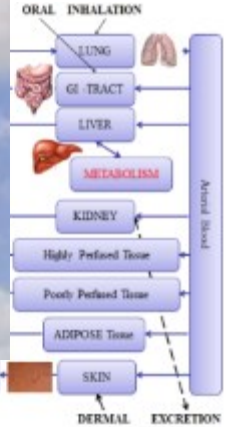
'OMICS

MoA knowledge





Cheminformatics & Comp. chemistry



Exposure modeling

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Integrated Approaches to Testing and Assessment (IATA): Proposed Working Definition

A hypothesis-driven framework for hazard identification, hazard characterisation and/or safety assessment of a chemical or group of chemicals, which strategically integrates and weights all relevant existing data and guides the targeted generation of new data where required to inform regulatory decision-making.

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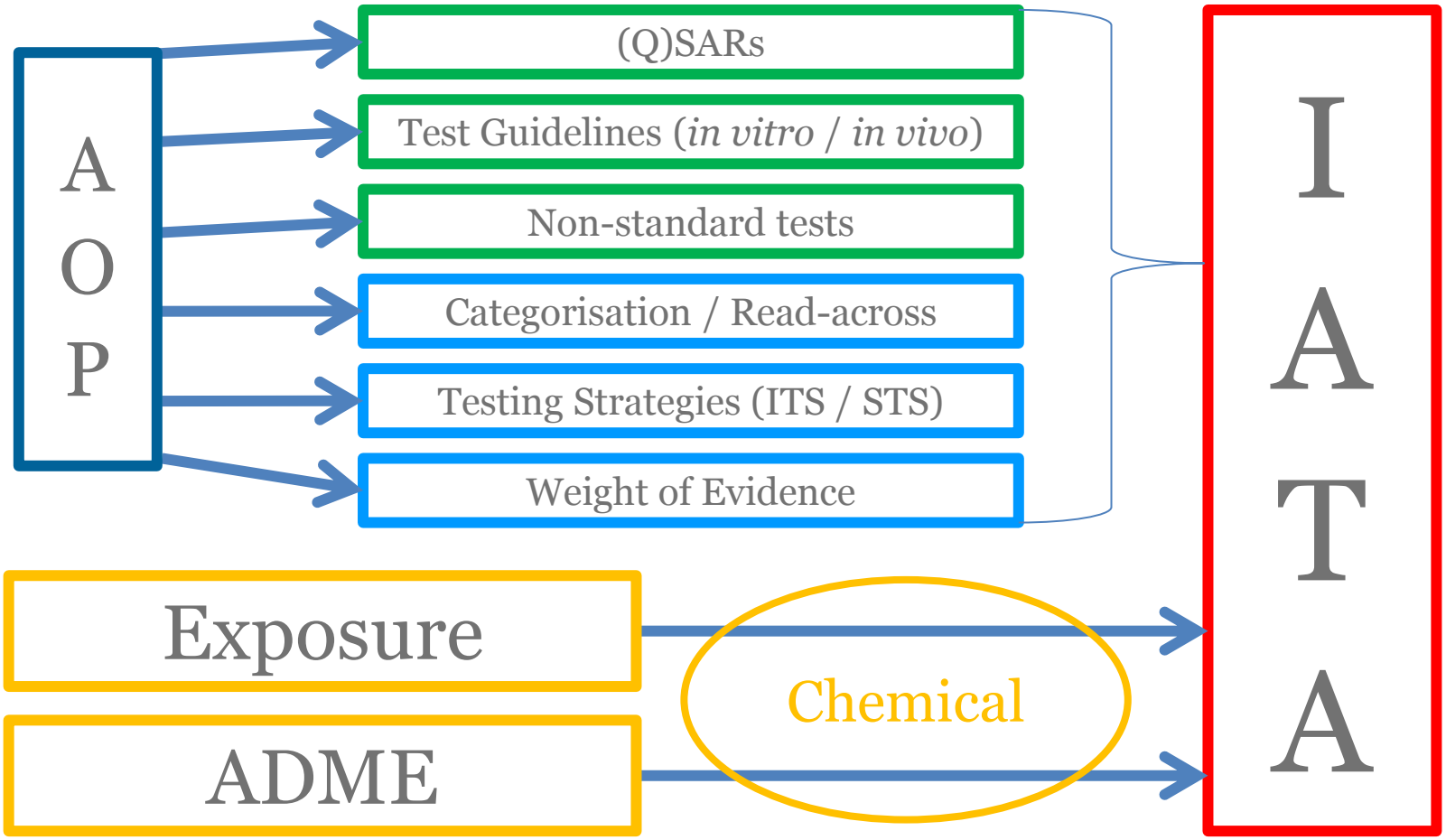
OECD

Elements within IATA



Modified from OECD STA No. 215

Six Principles: Essential Information for Regulatory Application of an IATA

1. A defined endpoint of regulatory concern
2. A defined purpose/application
3. A description of the rationale, including the mechanistic basis, underlying the construction of the IATA
4. A description of the individual information sources constituting the IATA
5. A description of how the individual information sources are integrated to derive the final prediction/assessment
6. A description of the known uncertainties

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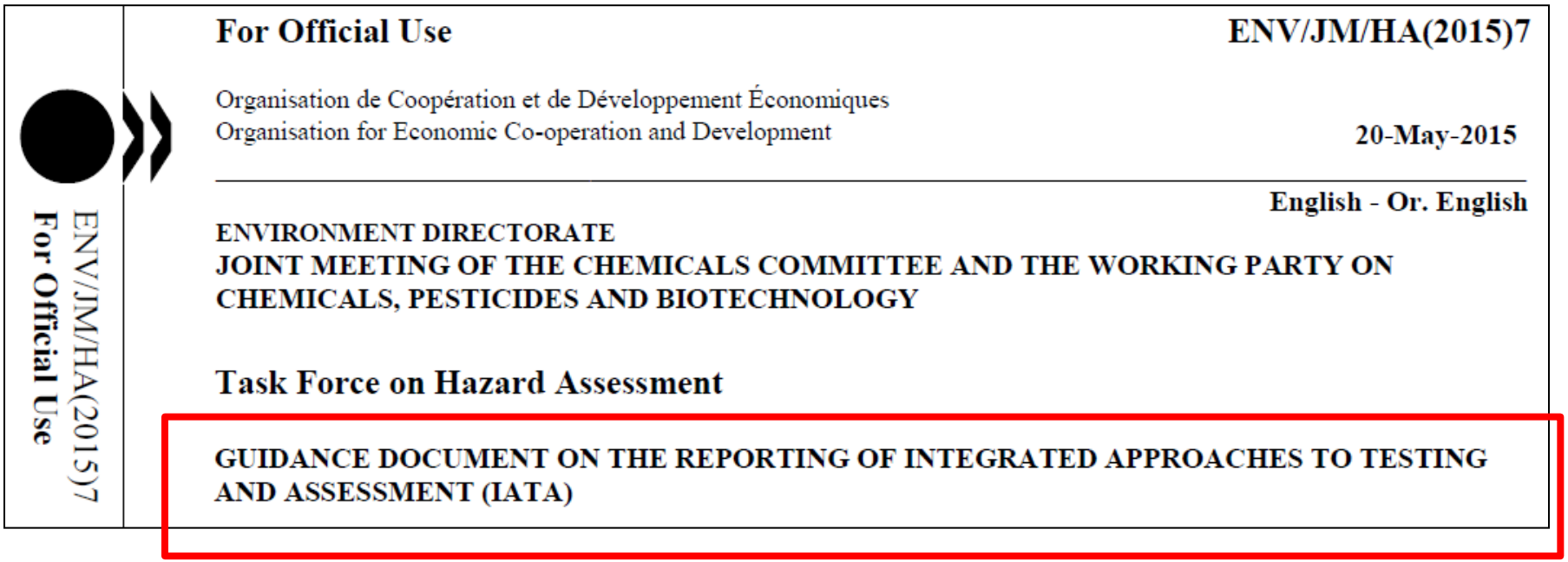
Guidance Document on the Reporting of IATA



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Organisation de Coopération et de Développement Économiques
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20-May-2015

English - Or. English

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

Task Force on Hazard Assessment

GUIDANCE DOCUMENT ON THE REPORTING OF INTEGRATED APPROACHES TO TESTING AND ASSESSMENT (IATA)

Scope: To promote consistent evaluation and application of IATA within OECD member countries by providing guidance towards a harmonised approach for the reporting of IATA

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IATA reporting template

1	**Summary**	*concise overview of the approach*
2	**General information**	*identifier, date, authors, updates, references, proprietary aspects*
3	**Endpoint addressed**	*e.g. skin sensitisation*
4	**Purpose**	*e.g. screening, hazard assessment, potency prediction*
5	**Rationale underlying its construction**	*including reason for the choice of information sources and their linkage to known biological mechanisms (e.g. key events)*
6	**Brief description of the individual information sources used**	*including response(s) measured and respective measure(s), detailed descriptions in the dedicated template*
7	**Process applied to the derive the prediction assessment**	*e.g. sequential testing strategies, regression models, 2 out of 3 WoE, scoring systems, machine learning approaches, Bayesian networks, etc…*
8	**Chemicals used to develop and test the approach**	*approach used for selection of training and test sets, relevant information on both sets: chemical names, composition, reference data (e.g. in vivo data), readouts, predictions*
9	**Limitations (and strengths) in the application of the approach**	*with regard to technical constrains or wrong predictions*
10	**Predictive capacity**	*misclassifications and unreliable predictions rationalised to the extent possible*
11	**Known uncertainties associated with the application**	*how key assumptions related to model structure and information sources translate to prediction uncertainty described to the extent possible*

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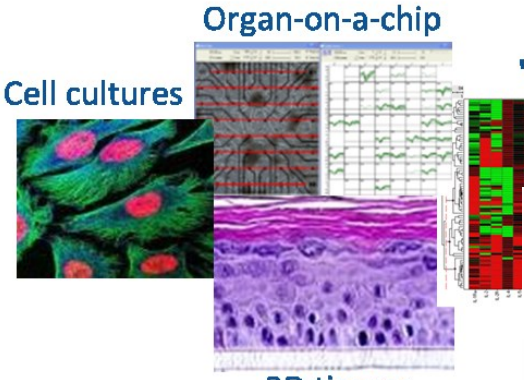
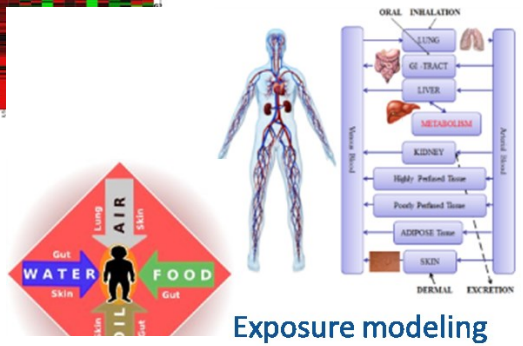
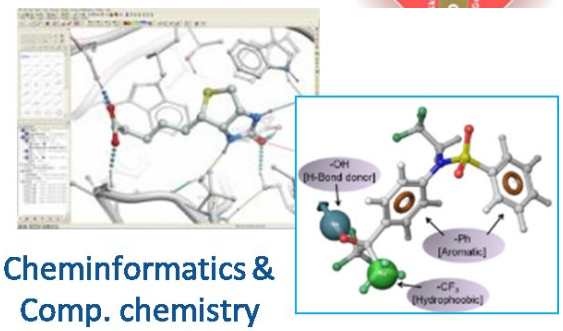
Predictive Toxicology

Inputs

- Ambiguity
- Measurement uncertainty
- Sampling uncertainty
- Assumptions incl. default values
- Extrapolation uncertainty
- Distribution uncertainty
- Other uncertainties







Types of uncertainty

SCIENTIFIC OPINION

Guidance on Uncertainty in EFSA Scientific Assessment

EFSA Scientific Committee[1, 2]

European Food Safety Authority (EFSA), Parma, Italy

DRAFT

EFSA Journal

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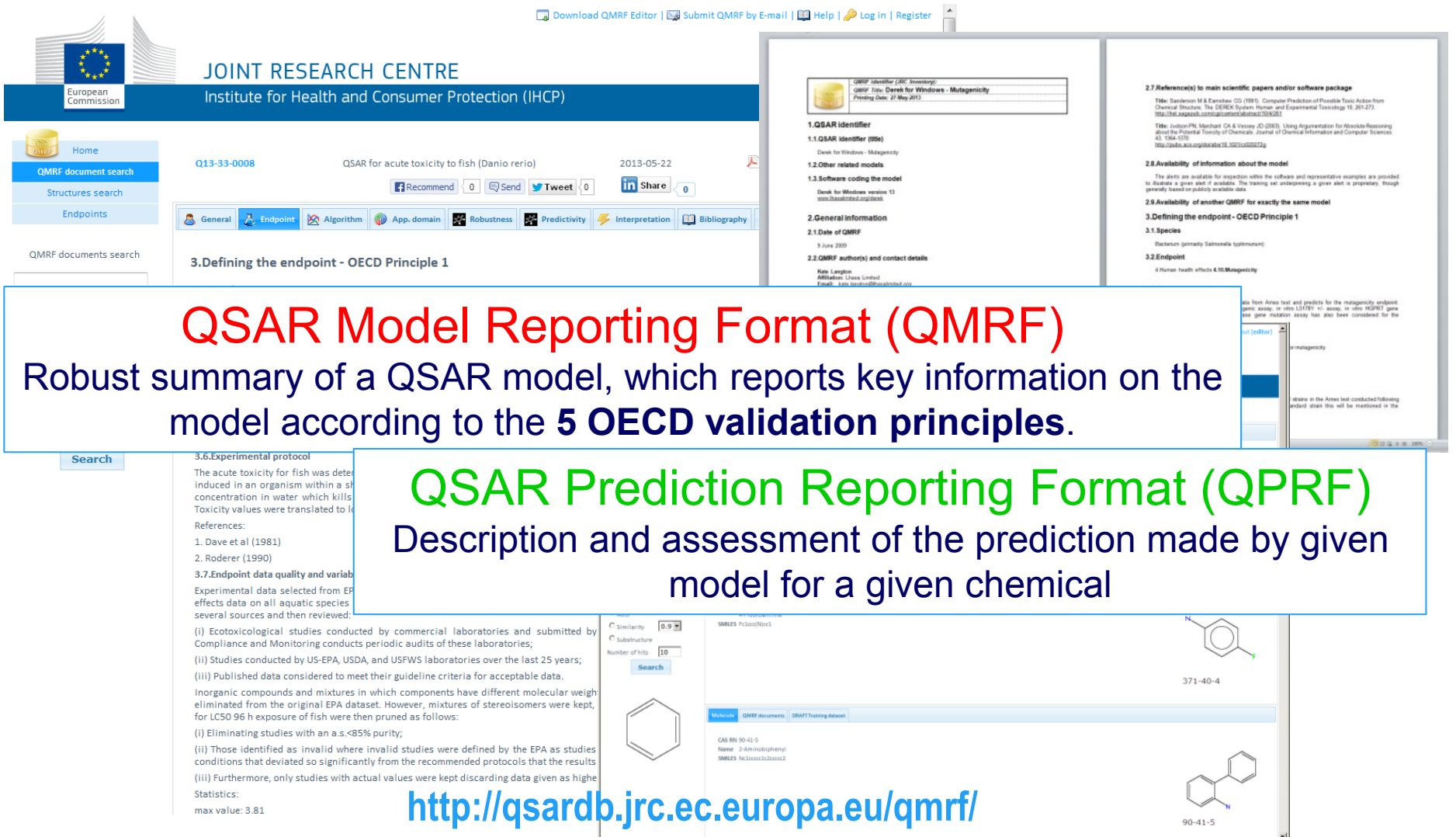
JRC QSAR Model Database



QSAR Model Reporting Format (QMRF)

Robust summary of a QSAR model, which reports key information on the model according to the **5 OECD validation principles**.

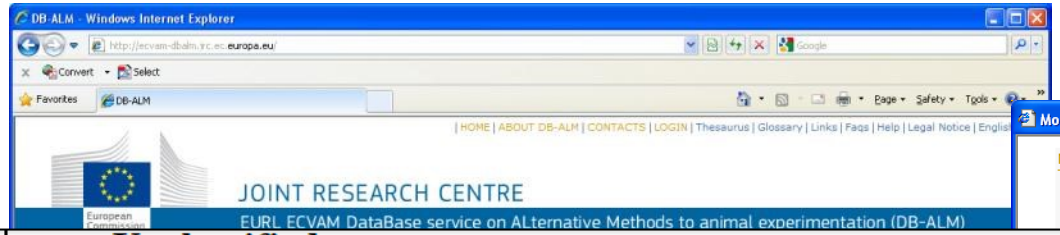
QSAR Prediction Reporting Format (QPRF)

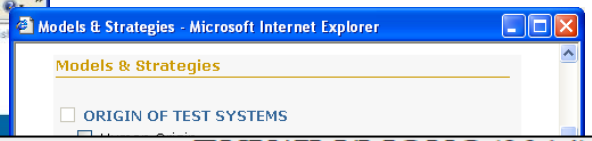
Description and assessment of the prediction made by given model for a given chemical

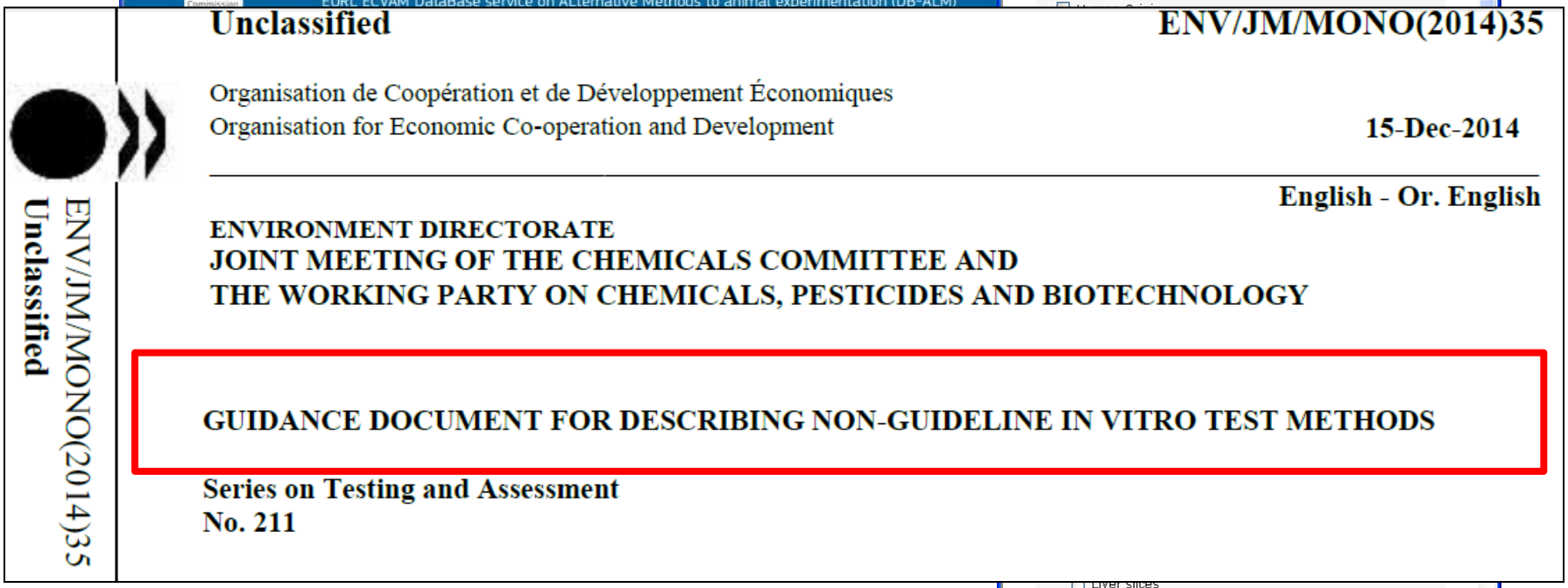
http://qsardb.jrc.ec.europa.eu/qmrf/

ECVAM Data Base service on Alternative Methods

DB-ALM







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Organisation de Coopération et de Développement Économiques
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15-Dec-2014

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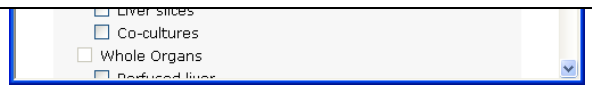
GUIDANCE DOCUMENT FOR DESCRIBING NON-GUIDELINE IN VITRO TEST METHODS

Series on Testing and Assessment
No. 211

Unclassified

ENV/JM/MONO(2014)35



http://ecvam-dbalm.jrc.ec.europa.eu

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Validation of alternative methods in a regulatory context *...the 3Ps*

*While the **principles** of validation are scientifically grounded and remain relatively constant, the **purpose** and **process** of validation need to evolve in order to keep pace with scientific progress and address the needs of decision makers.*

Characterise reliability, relevance, and uncertainty!

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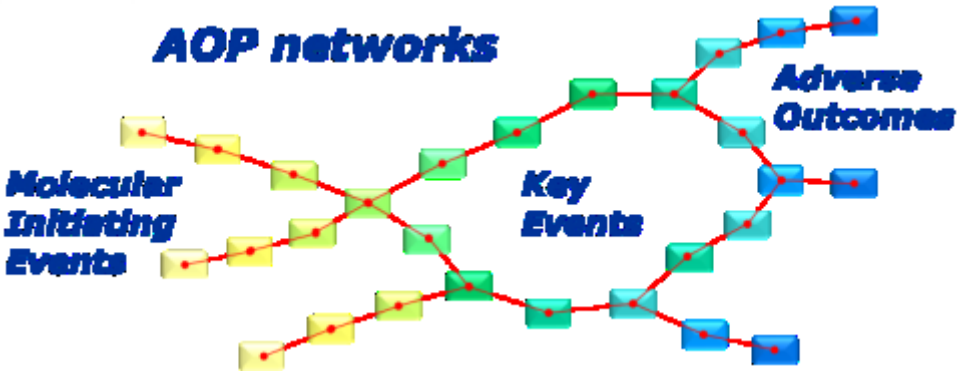
Predictive Toxicology

Combining inputs

- Ambiguity
- Excluded factors
- Relationship between components
- Distribution uncertainty
- Structure of the assessment
- Comparisons with independent data
- Dependency between uncertainties
- Other …



$$f(x|n) = \binom{n}{x} p^x q^{n-x},\ 0 \le x \le n$$

$$f(x,n) = \binom{n}{x} p^x q^{n-x} a_n,\ 0 \le x \le n$$

$$m(x) = \sum_{n=x}^{\infty} \binom{n}{x} p^x q^{n-x} a_n = p^x \sum_{n=x}^{\infty} \binom{n}{x} q^{n-x} a_n = p^x \sum_{k=0}^{\infty} \binom{x+k}{x} q^k a_{k+x}$$

$$f(n|x) = \frac{f(x,n)}{m(x)} = \frac{\binom{n}{x} p^x q^{n-x} a_n}{p^x \sum_{k=0}^{\infty} \binom{x+k}{x} q^k a_{k+x}} = \frac{\binom{n}{x} q^{n-x} a_n}{\sum_{k=0}^{\infty} \binom{x+k}{x} q^k a_{k+x}}$$

Testing Strategies (ITS / STS)

Weight of Evidence

Types of uncertainty

SCIENTIFIC OPINION

Guidance on Uncertainty in EFSA Scientific Assessment

EFSA Scientific Committee[1, 2]

European Food Safety Authority (EFSA), Parma, Italy

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EFSA Journal

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IPCS
INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY
IPCS Harmonization Project
World Health Organization

Guidance Document on Evaluating and Expressing Uncertainty in Hazard Characterization

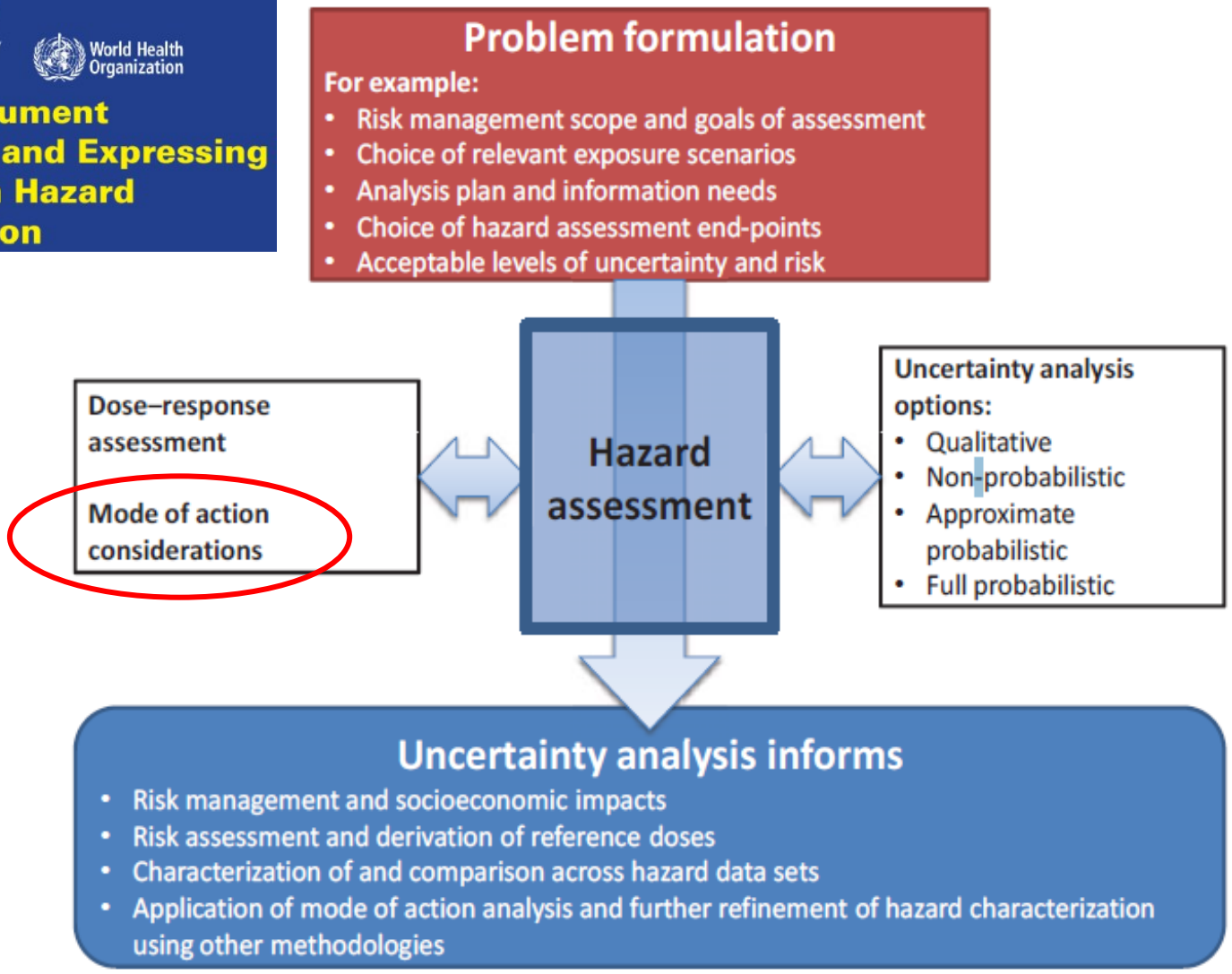

Fig. 2.1: Uncertainty analysis in the context of problem formulation.

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HTS "Toxicity" pathway assay

7. Validation of assay performance using a limited chemical set

6. Implement and optimise for running on a HTS platform

5. Select optimum in vitro assay operational parameters

4. Characterise and describe pathway response dynamics

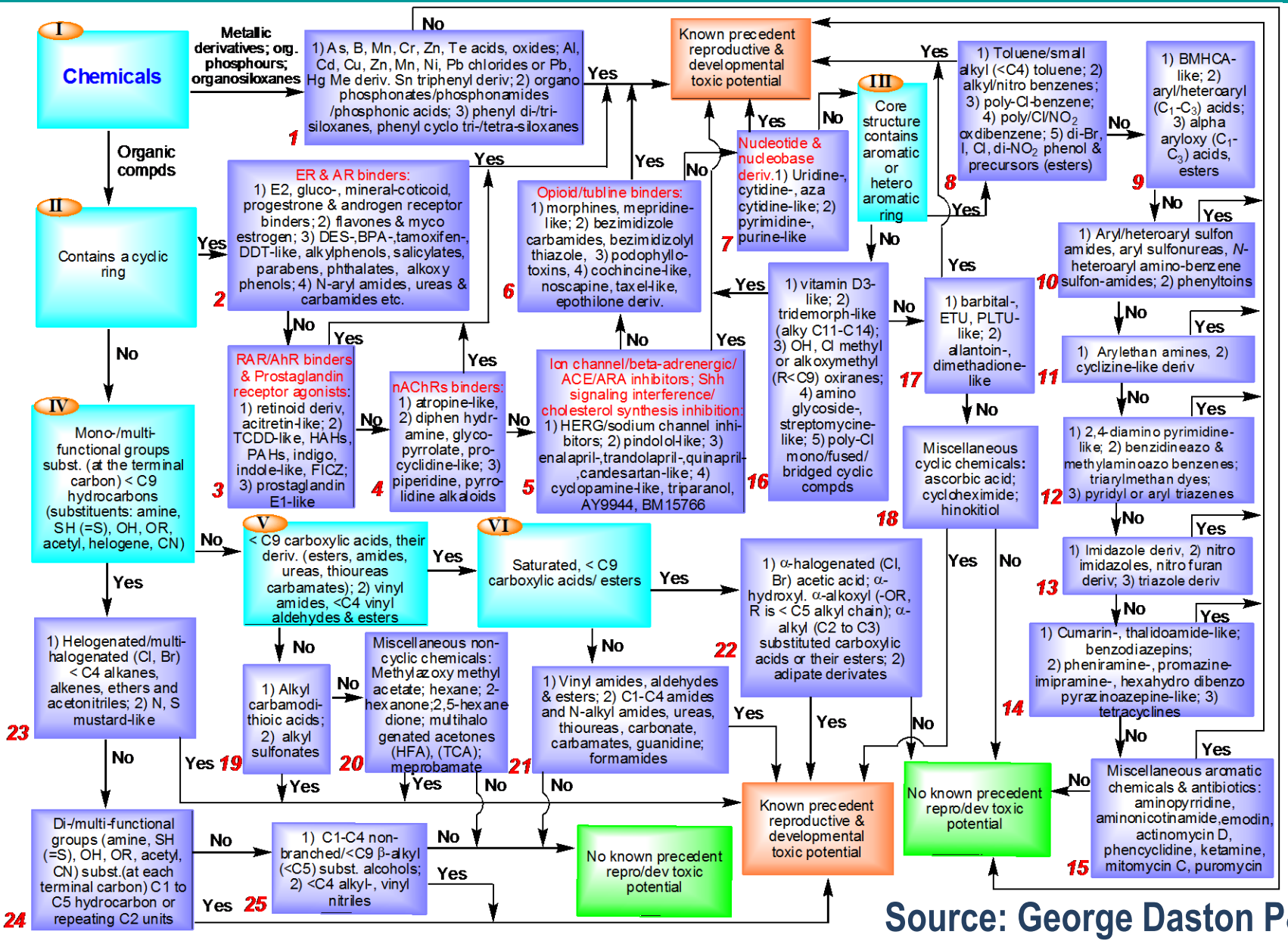
3. Select sensitive/specific biomarkers and probe-chemicals to measure pathway response

2. Selection and characterisation of cellular model expressing the physiological pathway

1. Selection of physiological pathway that has toxicological relevance

Physiological pathway

Expert system decision tree for repro/dev toxicity



Source: George Daston P&G

OECD AOP Development Programme

Responsibility of the Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST)

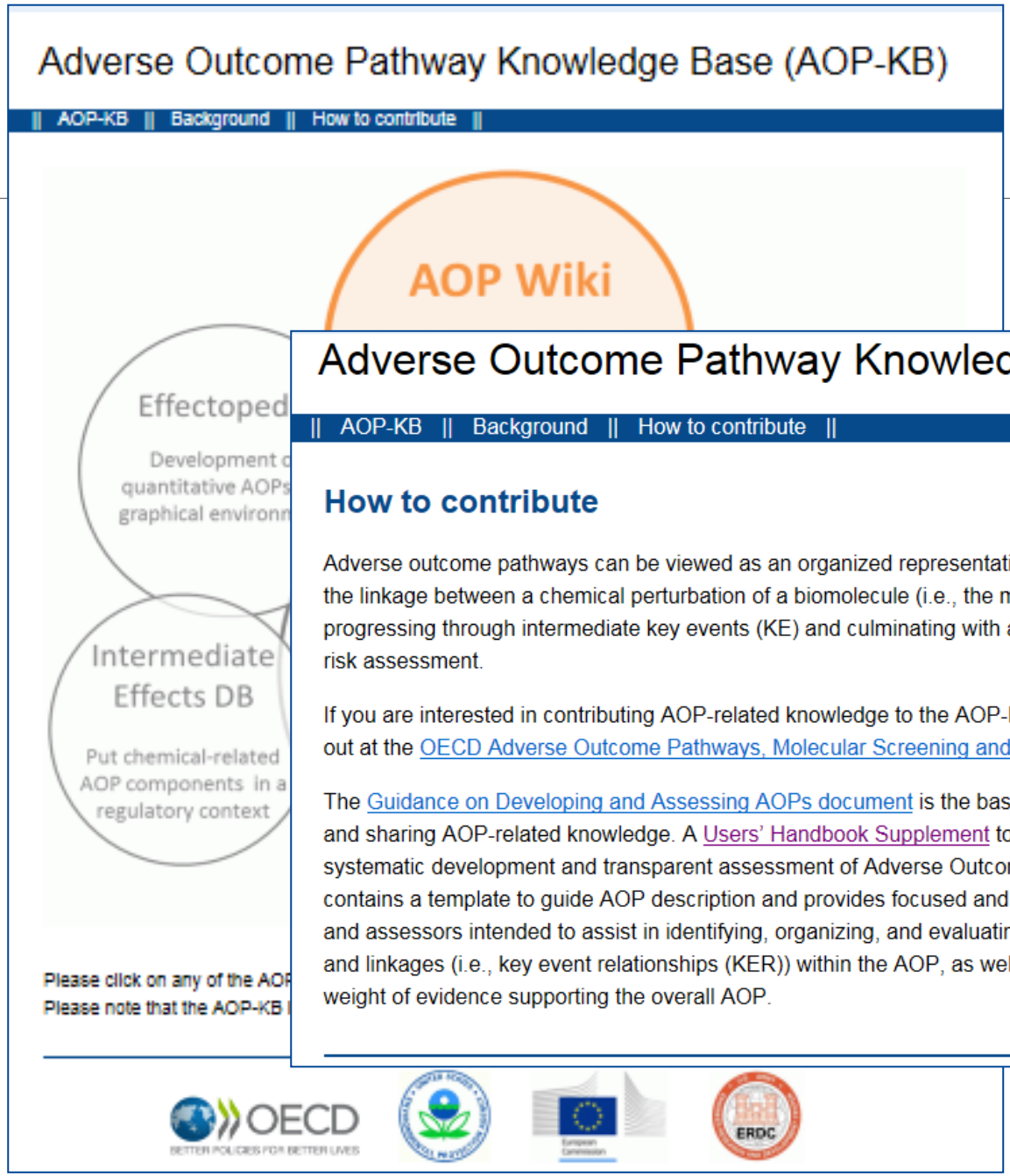
Co-chairs: Robert Kavlock (US EPA) & Maurice Whelan (EC JRC)

Villeneuve et. al., (2014) 'Adverse Outcome Pathway (AOP) Development I: Strategies and Principles', *Toxicol. Sci.,* 142 (2), 312-320.

Villeneuve et. al. (2014), 'Adverse Outcome Pathway (AOP) Development II: Best Practices', *Toxicol. Sci.,* 142 (2), 321-330.

- *OECD Template and Guidance on developing and assessing the completeness of Adverse Outcome Pathways (2013)*
- *Supplementary 'User Handbook' (Sept 2014).*

www.aopkb.org



Adverse Outcome Pathway Knowledge Base (AOP-KB)
|| AOP-KB || Background || How to contribute ||

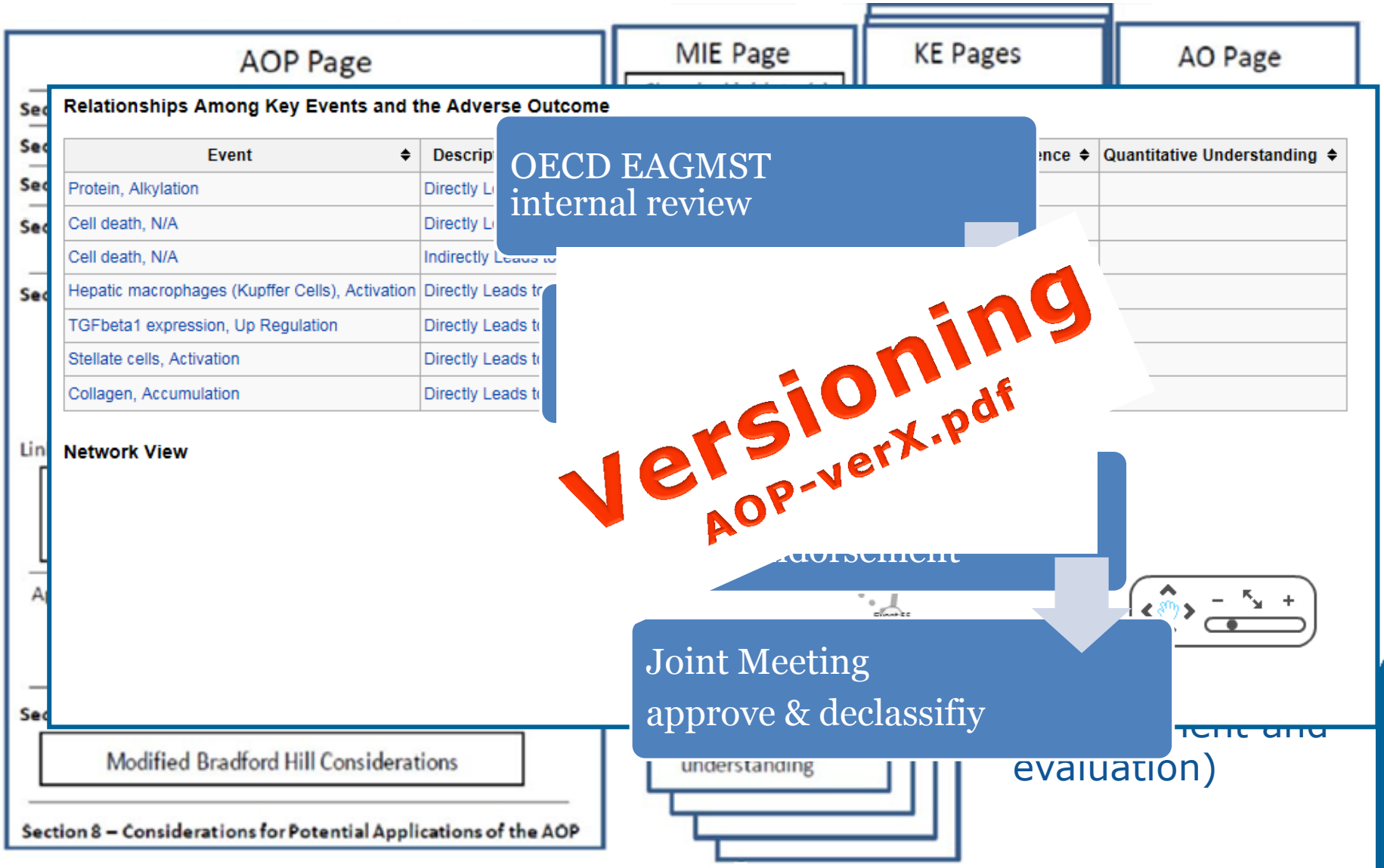
How to contribute

Adverse outcome pathways can be viewed as an organized representation of existing knowledge concerning the linkage between a chemical perturbation of a biomolecule (i.e., the molecular initiating event (MIE)), progressing through intermediate key events (KE) and culminating with an adverse outcome (AO) relevant for risk assessment.

If you are interested in contributing AOP-related knowledge to the AOP-KB, please follow the instructions laid out at the OECD Adverse Outcome Pathways, Molecular Screening and Toxicogenomics page.

The Guidance on Developing and Assessing AOPs document is the basis for all work related to contributing and sharing AOP-related knowledge. A Users' Handbook Supplement to this Guidance has been written to aid systematic development and transparent assessment of Adverse Outcome Pathways (AOPs). The handbook contains a template to guide AOP description and provides focused and practical instructions for developers and assessors intended to assist in identifying, organizing, and evaluating critical information on key events and linkages (i.e., key event relationships (KER)) within the AOP, as well as guidance on how to assess the weight of evidence supporting the overall AOP.

AOP Wiki – Document (Article) structure

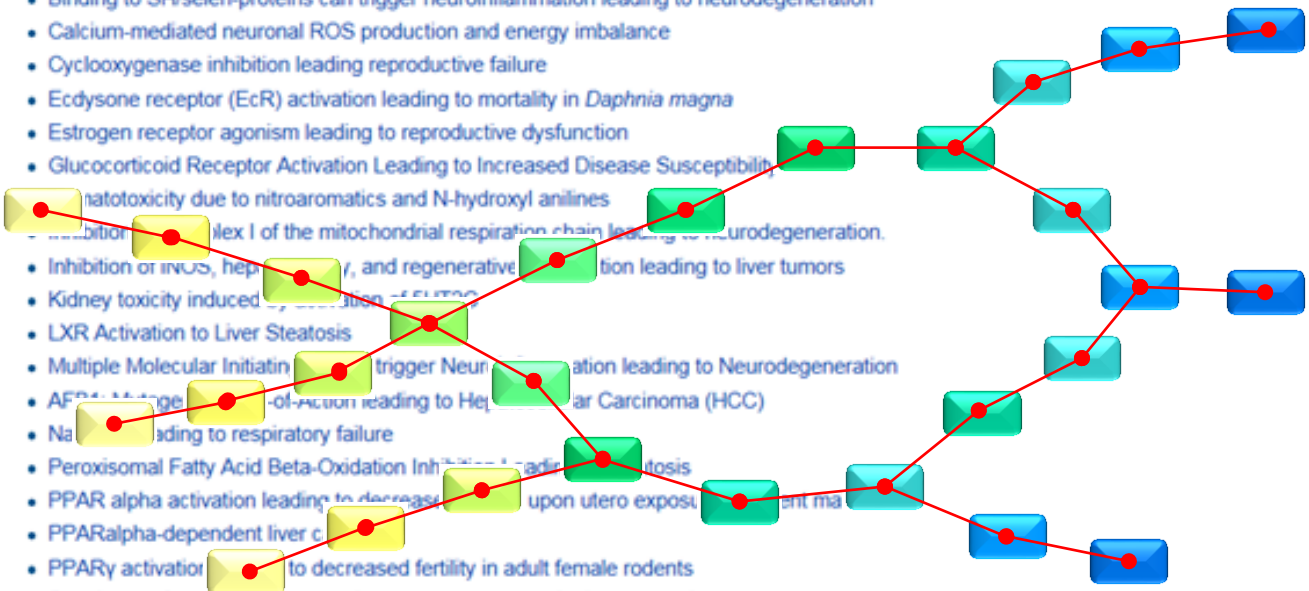


AOP Knowledge Base

Adverse Outcome Pathway WIKI

AOPs Under Development

- AhR activation leading to embryo toxicity in fish
- Androgen receptor antagonism leading to adverse effects in the male foetus (mammals)
- Androgen receptor antagonism leading to reproductive dysfunction
- Binding to glutamatergic ionotropic receptors can trigger neuroinflammation leading to neurodegeneration
- Binding of antagonist to N-methyl-D-aspartate (NMDA) receptors during brain development (synaptogenesis) induces impairment of learning and memory abilities
- Binding of agonists to N-methyl-D-aspartate receptor (NMDAR) in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to reduction (or loss) of cognitive function
- Binding of antagonists to NMDAR can trigger neuroinflammation leading to neurodegeneration
- Binding to electron chain transfer complexes in the mitochondria can trigger neuroinflammation and lead to neurodegeneration
- Binding to SH/selen-proteins can trigger neuroinflammation leading to neurodegeneration
- Calcium-mediated neuronal ROS production and energy imbalance
- Cyclooxygenase inhibition leading reproductive failure
- Ecdysone receptor (EcR) activation leading to mortality in *Daphnia magna*
- Estrogen receptor agonism leading to reproductive dysfunction
- Glucocorticoid Receptor Activation Leading to Increased Disease Susceptibility
- natotoxicity due to nitroaromatics and N-hydroxyl anilines
- lex I of the mitochondrial respiration chain leading to neurodegeneration.
- Inhibition of iNOS, hep , and regenerative tion leading to liver tumors
- Kidney toxicity induced
- LXR Activation to Liver Steatosis
- Multiple Molecular Initiatin trigger Neur ation leading to Neurodegeneration
- AF ar Carcinoma (HCC)
- Na ding to respiratory failure
- Peroxisomal Fatty Acid Beta-Oxidation Inh tosis
- PPAR alpha activation leading upon utero exposu
- PPARalpha-dependent liver c
- PPARγ activatio to decreased fertility in adult female rodents
- Respiratory Sensitisation/Allergy induced by covalent binding to proteins
- Skin Sensitisation Initiated by Covalent Binding to Proteins
- Sustained AhR Activation leading to Rodent Liver Tumours
- Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals
- VEGF Signaling and Vascular Disruption Leading to Adverse Developmental Outcomes
- PPARα activation leading to impaired fertility in adult male rodents
- Inhibition of Na+/I- symporter (NIS) decreases TH synthesis leading to learning and memory deficits in children



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To conclude …

- IATA … an international standard for reporting hazard and risk assessments (approaches) based on the integration of predictive toxicology methods.
- We shouldn't 'reinvent the wheel' when it comes to assessing and describing uncertainty within/of IATA but instead adapt existing (excellent) guidance.
- The need/use of mode-of-action knowledge is the major difference between hazard/risk assessment based on conventional and predictive toxicology.
- The 'unfamiliar' uncertainties will become familiar when we start identifying and characterising them!

  Validation can help!

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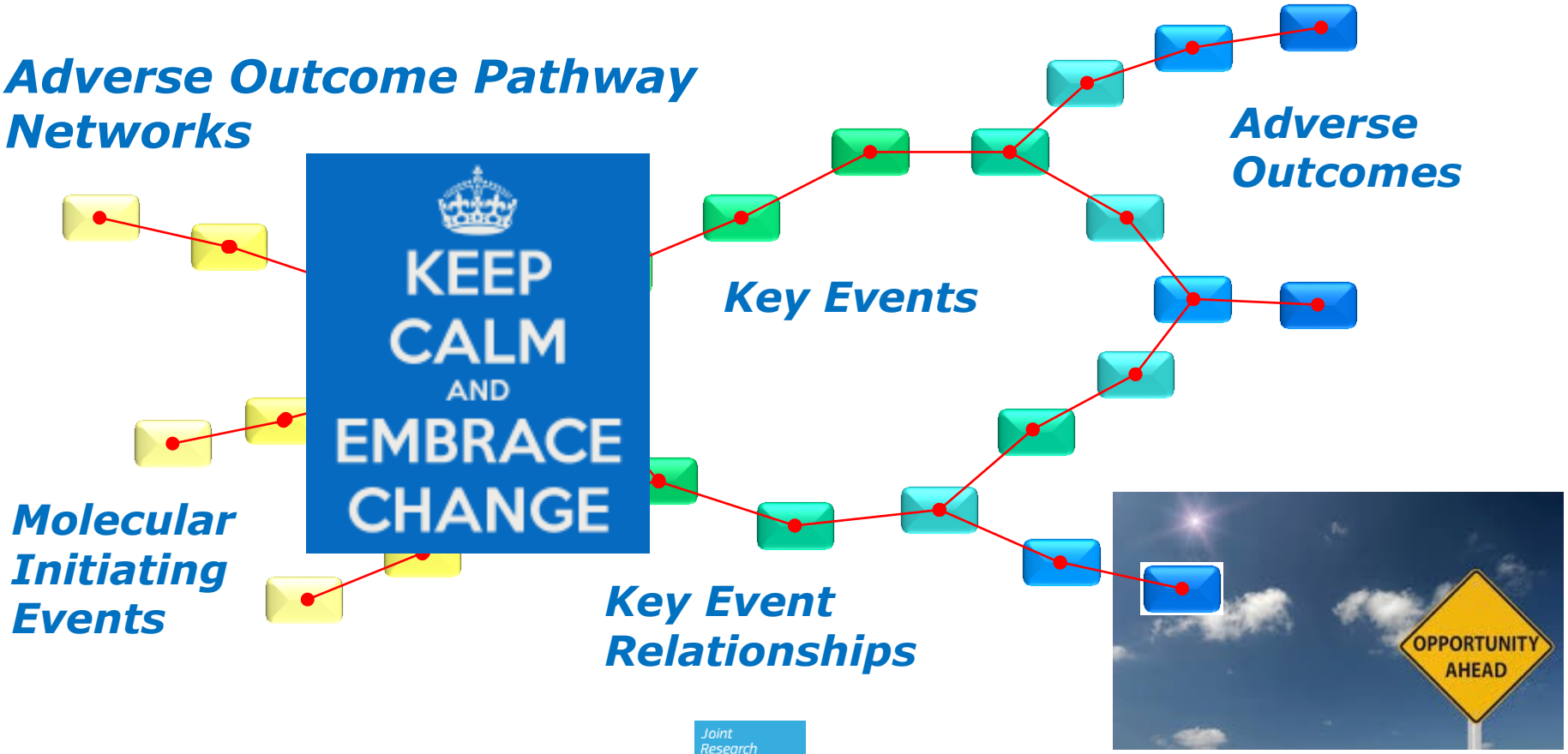
Transitioning to a new way of describing toxicological hazard …?



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