WaterTOP COST Action (CA18225)
for Water Taste and Odor Problems

Risk Assessment approaches for water T&O 16-18 October 2023

Istituto Superiore di Sanità (ISS), Rome, Italy

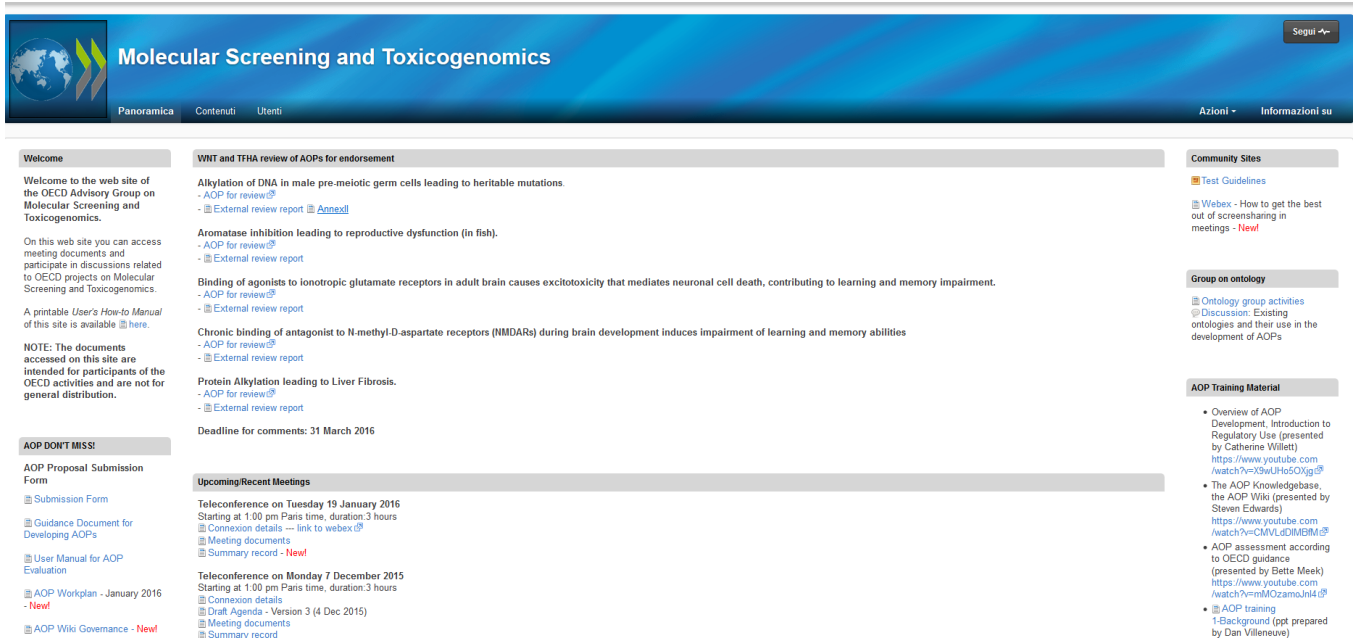
Presenter:
Olga Tcheremenskaia
ISS, Department of Environment and Health

Adverse Outcome Pathways (AOPs):
from research to regulation

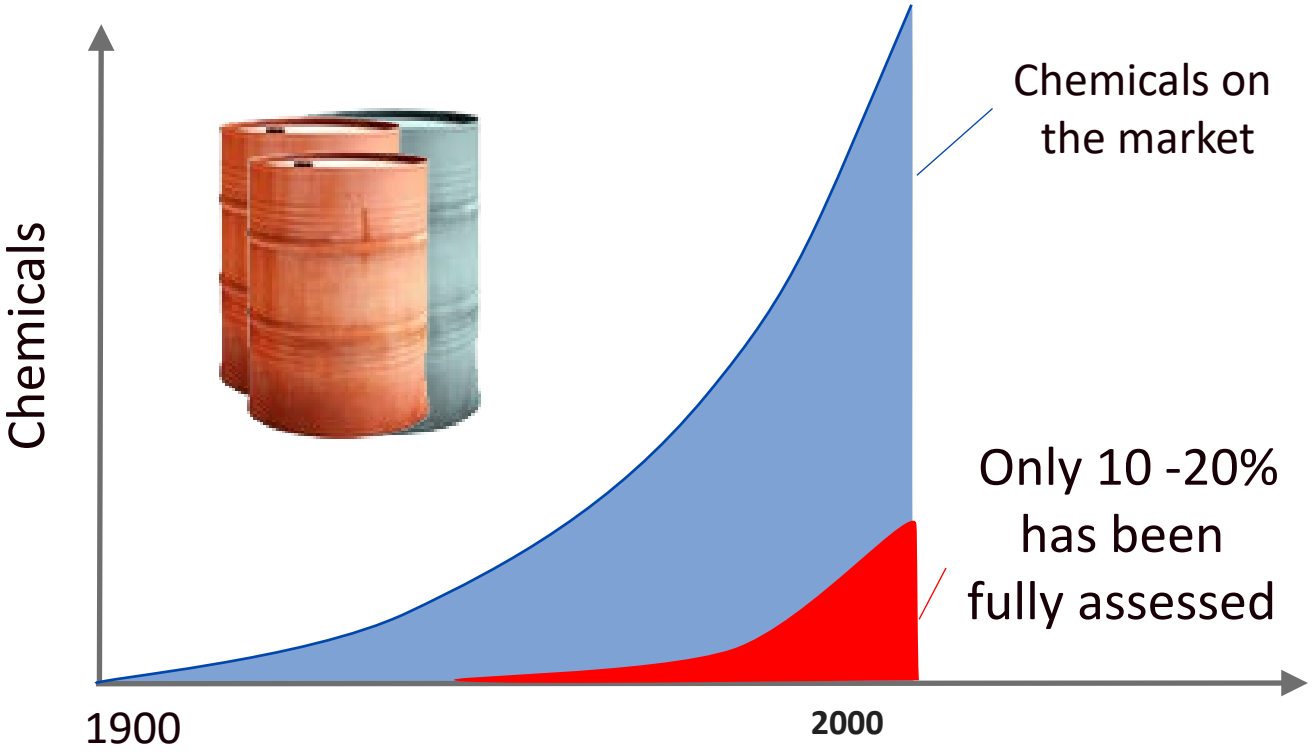
Adverse Outcome Pathways Development Programme

was launched by OECD in 2012, under the responsibility of the EAGMST group (The Extended Advisory Group of Molecular Screening and Toxicogenomic)

AOPs are a central concept in future work at OECD on predictive toxicology, enabling improving uses and applications of mechanistic information



Growing concern over lack of toxicological data

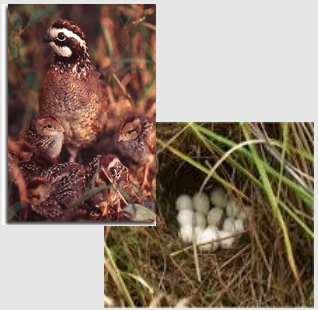


Standard toxicity testing is costly, time consuming and requires many animals

5000 animals / chemical

Test duration
30 – 720 days

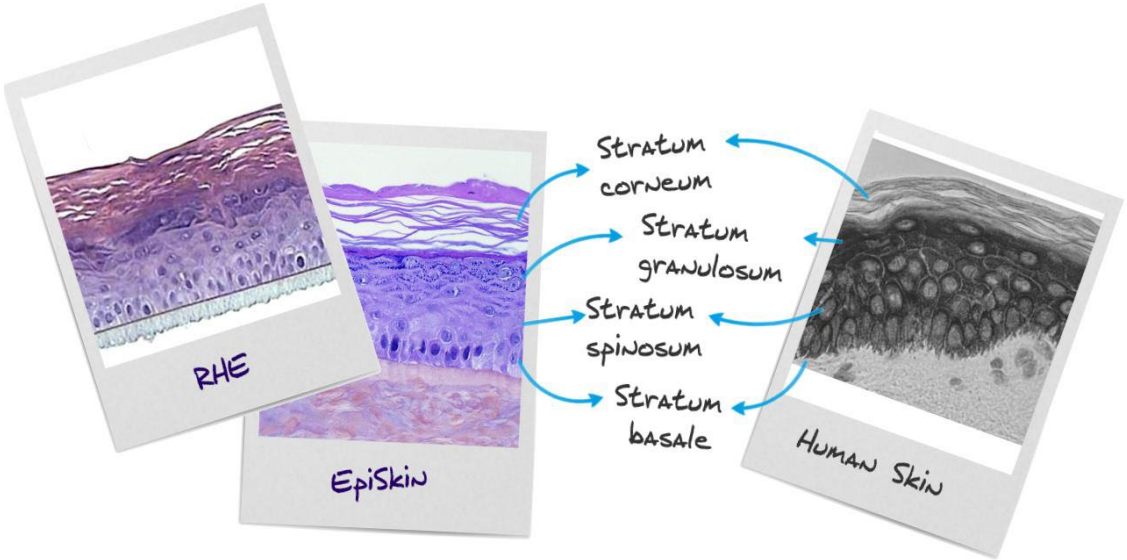
Costs
€2,000 - €2,000,000



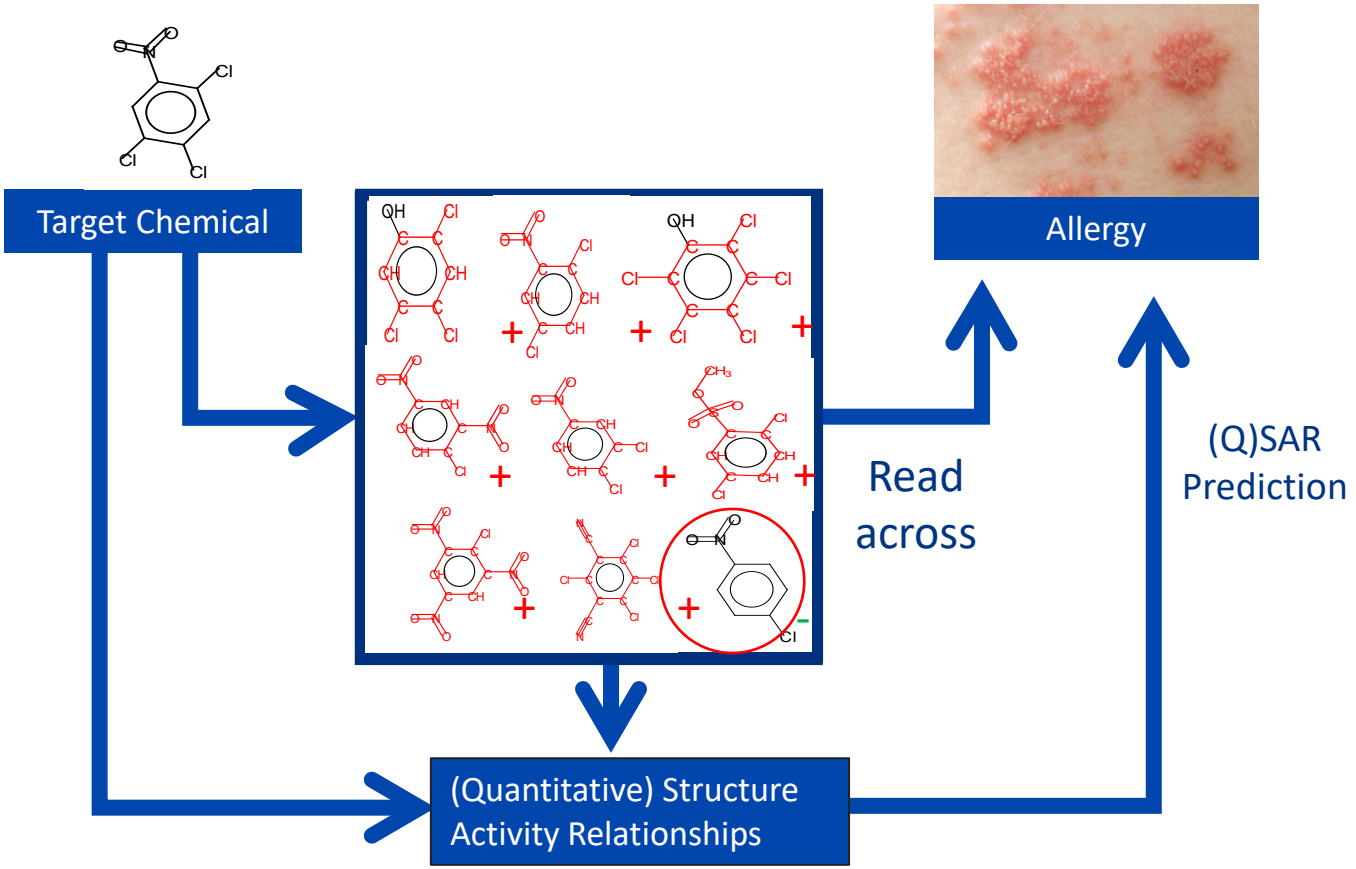
Avian reproduction study (OECD TG 206)
Animals: > 200
Test duration: > 30 weeks
Cost: > $250,000

Promoting the use of non-animal methods

OECD Test Guidelines based on non-animal methods, for example, for skin and eye corrosion / irritation, phototoxicity, skin absorption, genotoxicity, skin sensitisation



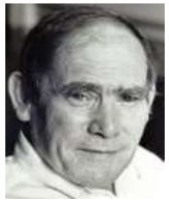
Developing models to predict toxicity



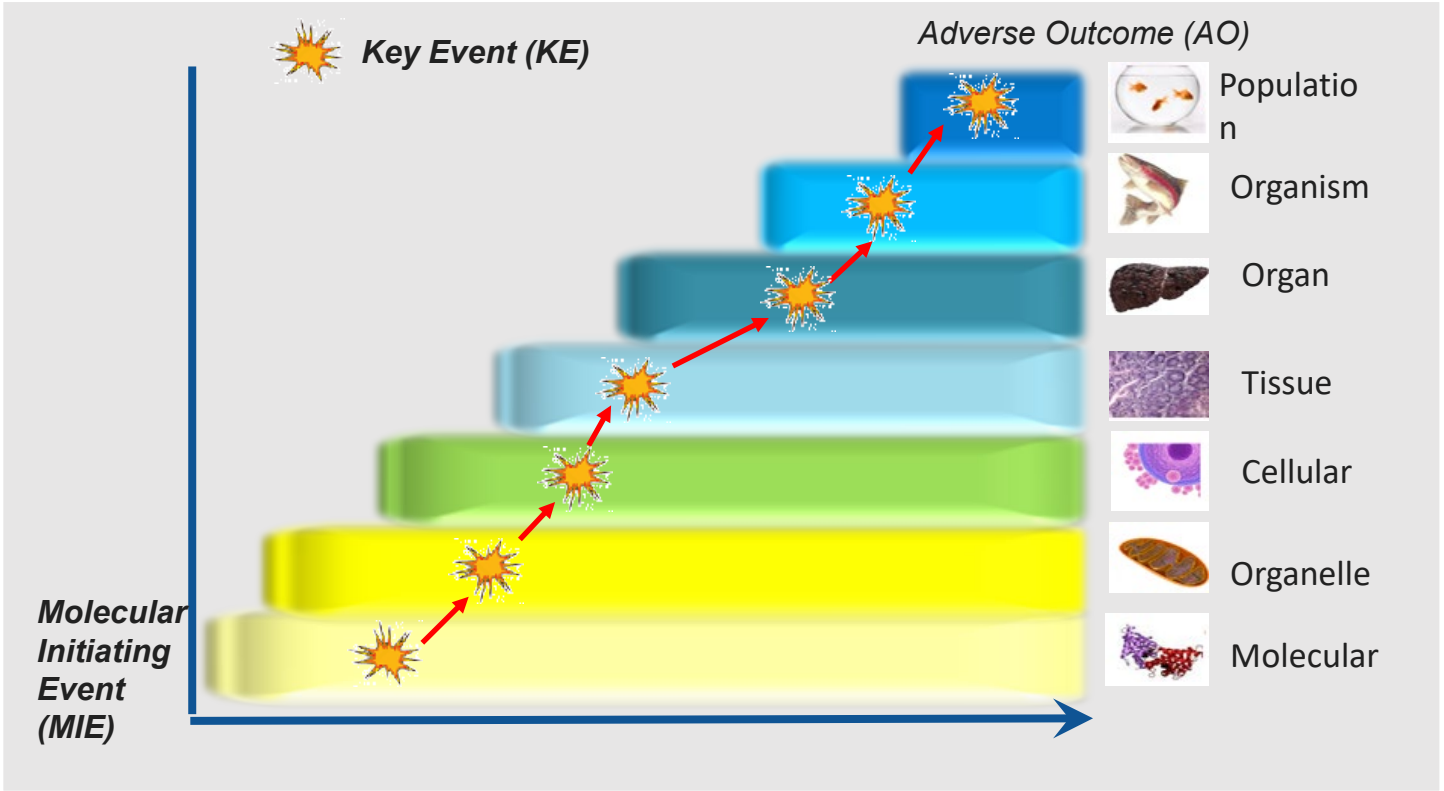
Need of framework for knowledge and data management

"We now have unprecedented ability to collect data about nature but there is now a crisis developing in biology, in that completely unstructured information does not enhance understanding. We need a framework to put all of this knowledge and data into - that is going to be the problem in biology. We've reached the stage where we can't talk to each other - we've all become highly specialized. … driving toward that framework is really the big challenge."

- Sydney Brenner. Molecular Biologist and Nobel Laureate, NIH-BISTI Symposium 2003

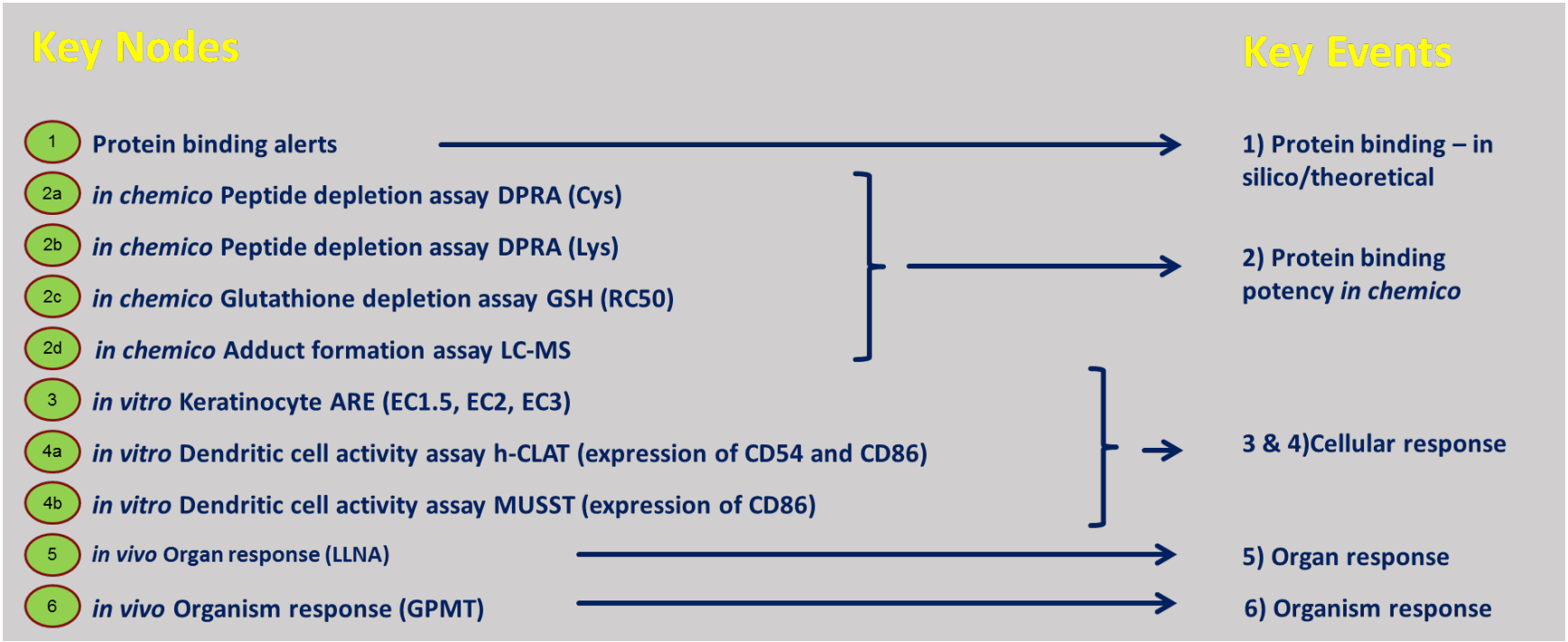


AOP structure



Covalent Protein binding leading to Skin Sensitisation

https://aopwiki.org/aops/40



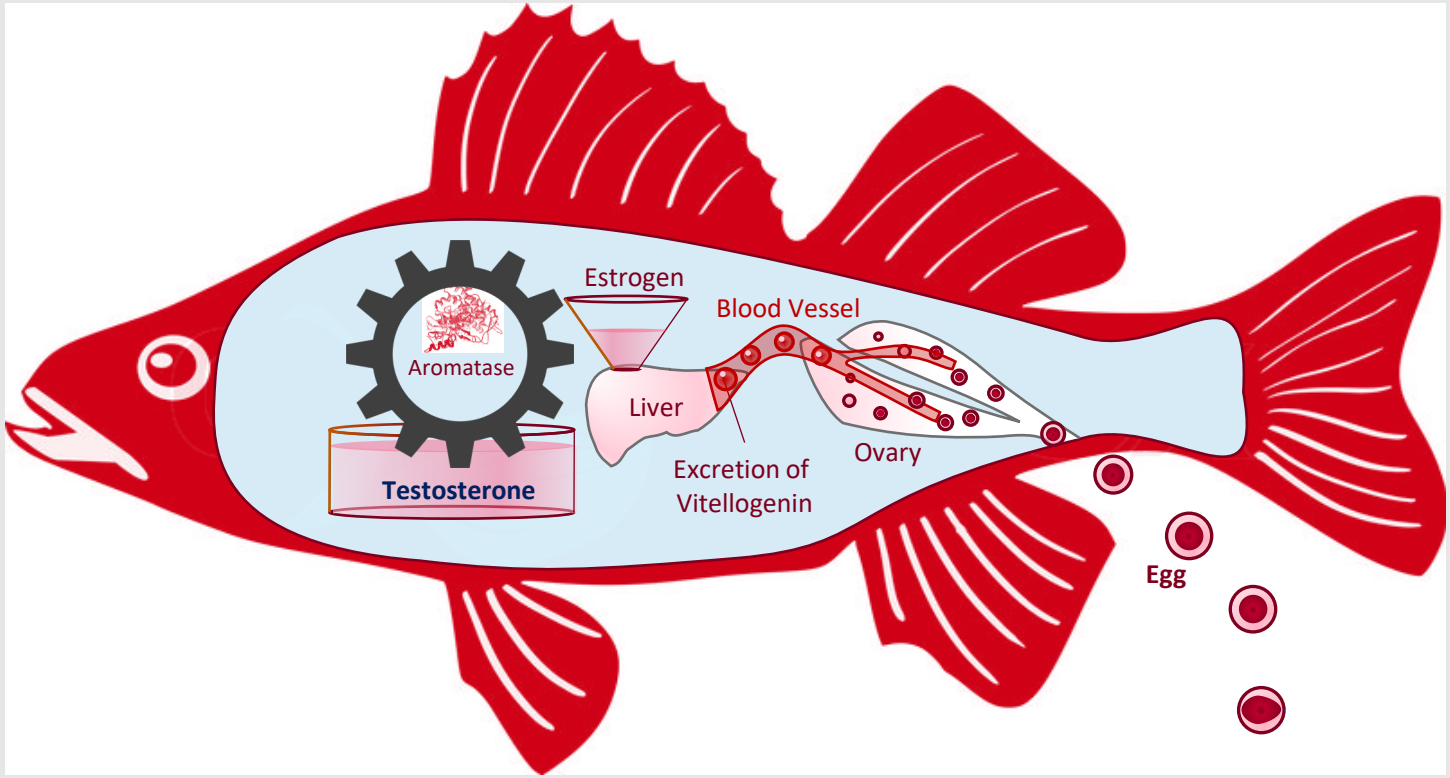
Adapted from The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence OECD ENV/JM/MONO(2012) 10 PART 1

Aromatase inhibition leading to reproductive dysfunction (in fish)
https://aopwiki.org/wiki/index.php/Aop:25

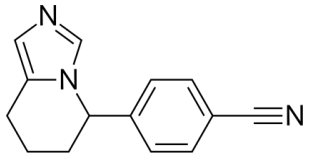
Cumulative fecundity is the most apical endpoint considered in the OECD 229 Fish Short Term Reproduction Assay. The OECD 229 assay serves as screening assay for endocrine disruption and associated reproductive impairment

Event	Description	Triggers	Weight of Evidence	Quantitative Understanding
Aromatase, Inhibition	Directly Leads to	17beta-estradiol synthesis by ovarian granulosa cells, Reduction	Strong	Moderate
17beta-estradiol synthesis by ovarian granulosa cells, Reduction	Directly Leads to	Plasma 17beta-estradiol concentrations, Reduction	Strong	Moderate
Plasma 17beta-estradiol concentrations, Reduction	Directly Leads to	Vitellogenin synthesis in liver, Reduction	Strong	Moderate
Vitellogenin synthesis in liver, Reduction	Directly Leads to	Plasma vitellogenin concentrations, Reduction	Strong	Moderate
Plasma vitellogenin concentrations, Reduction	Directly Leads to	Vitellogenin accumulation into oocytes and oocyte growth/development, Reduction	Moderate	Weak
Vitellogenin accumulation into oocytes and oocyte growth/development, Reduction	Directly Leads to	Cumulative fecundity and spawning, Reduction	Moderate	Moderate
Cumulative fecundity and spawning, Reduction	Directly Leads to	Population trajectory, Decrease	Moderate	Moderate

AOP example: Aromatase and normal egg production

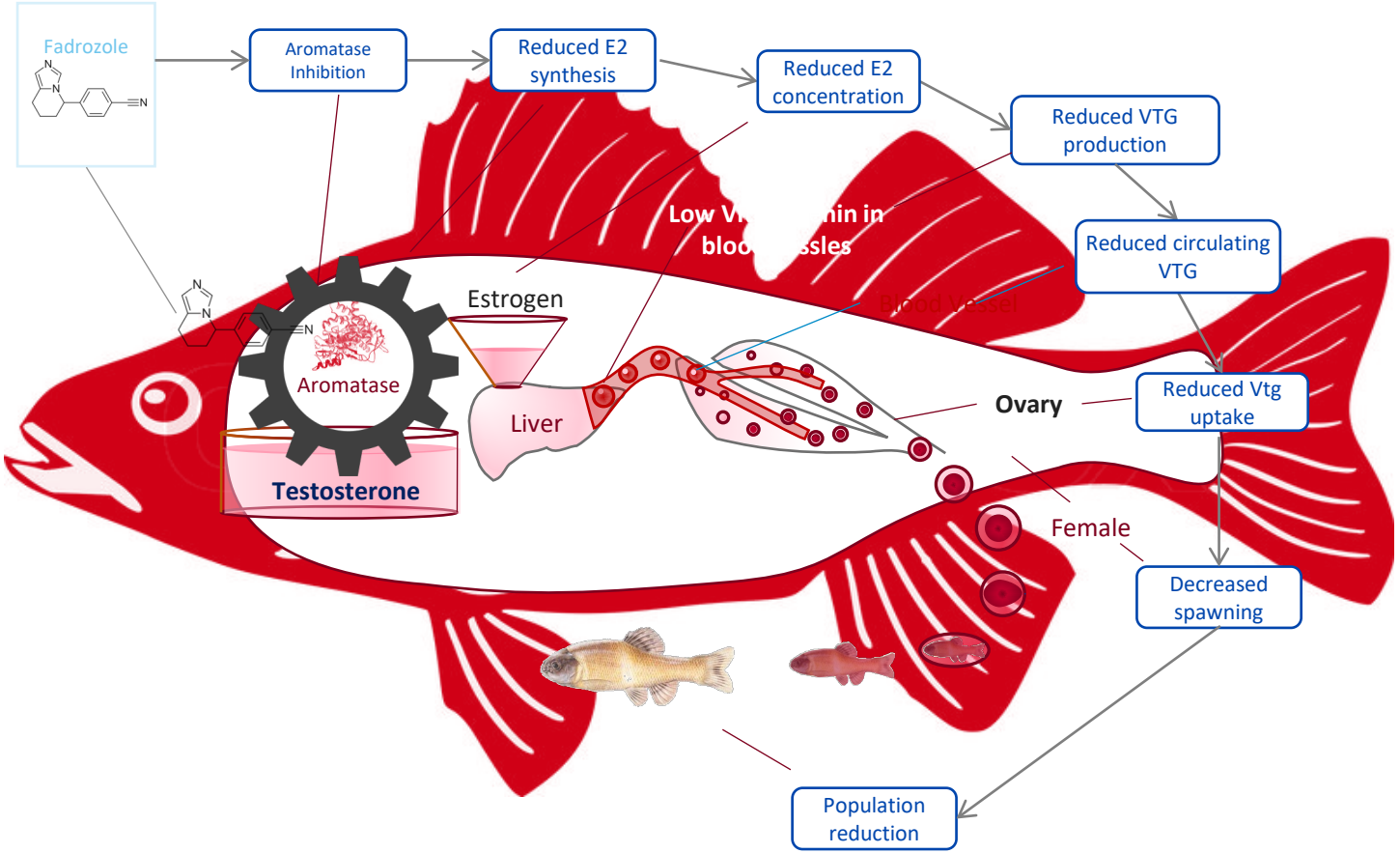


An Endocrine Disruptor In Action

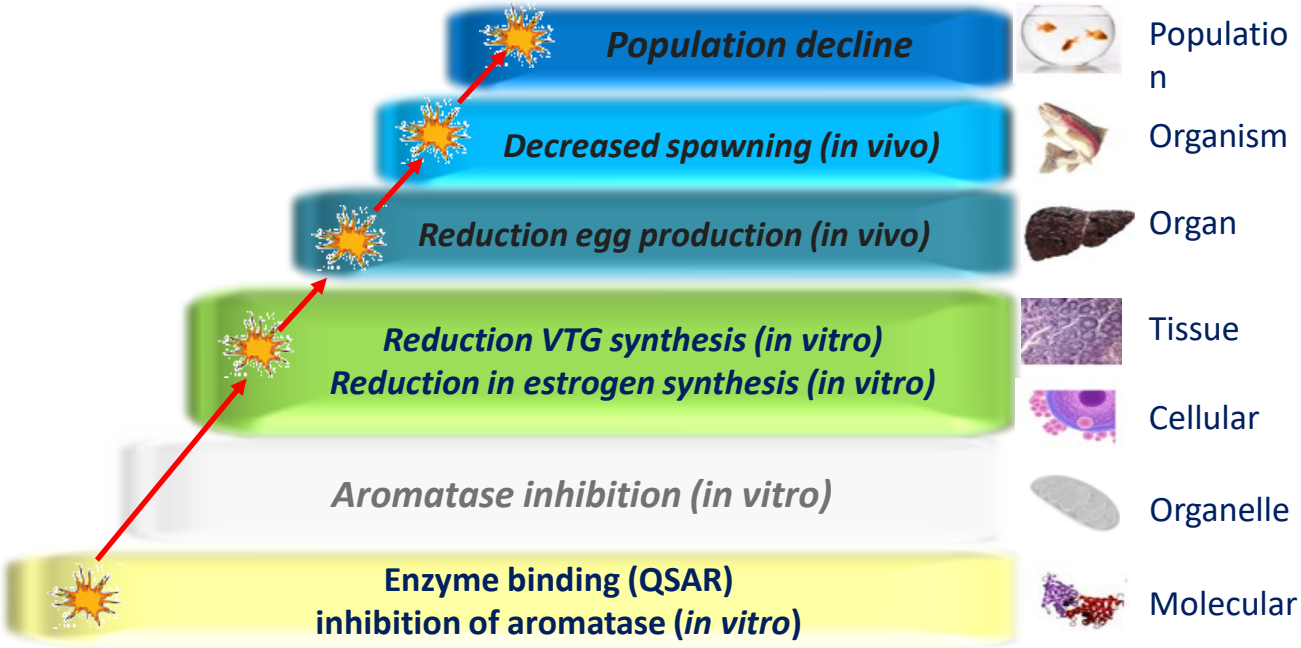


Fadrozole

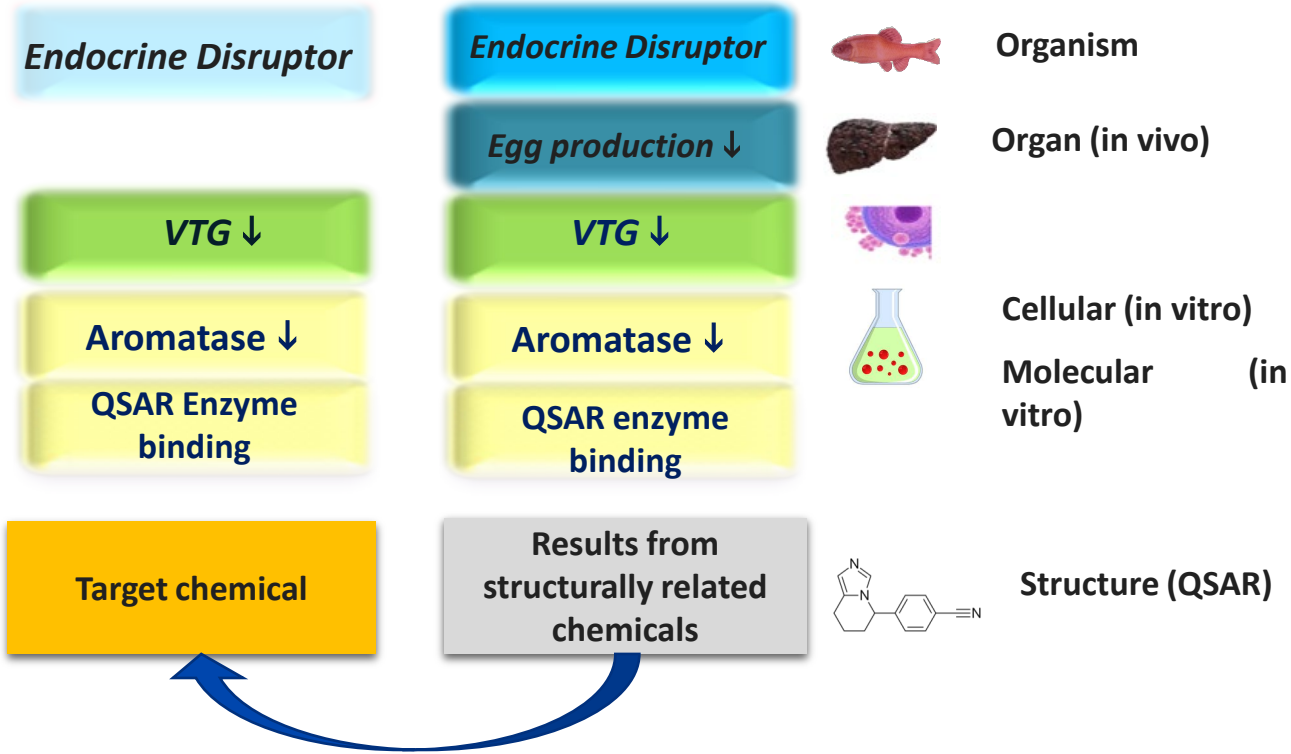
AOP describes the key events caused by aromatase inhibition that lead to population reduction



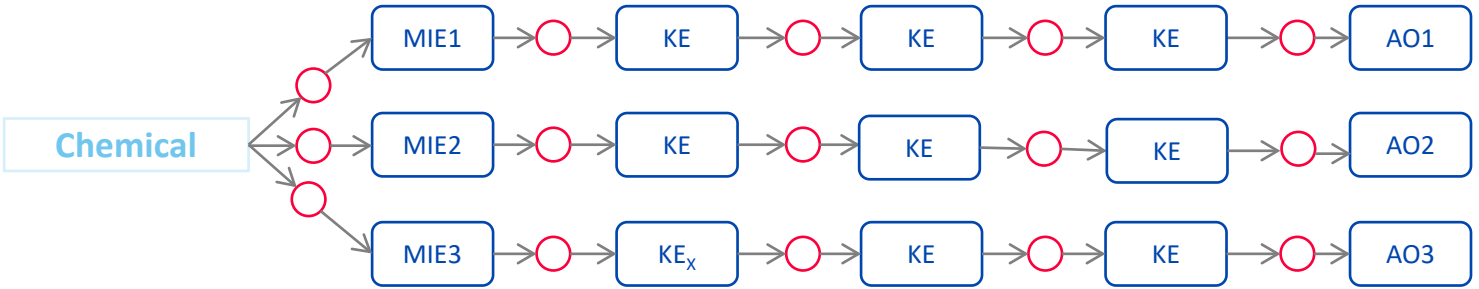
Early key events can be measured with non-animal tests, which can be used to predict the adverse outcome

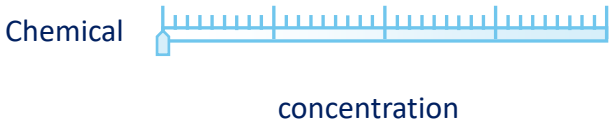


Development of Integrated approaches to testing and assessment (IATA). Read-across based on mechanistic understanding

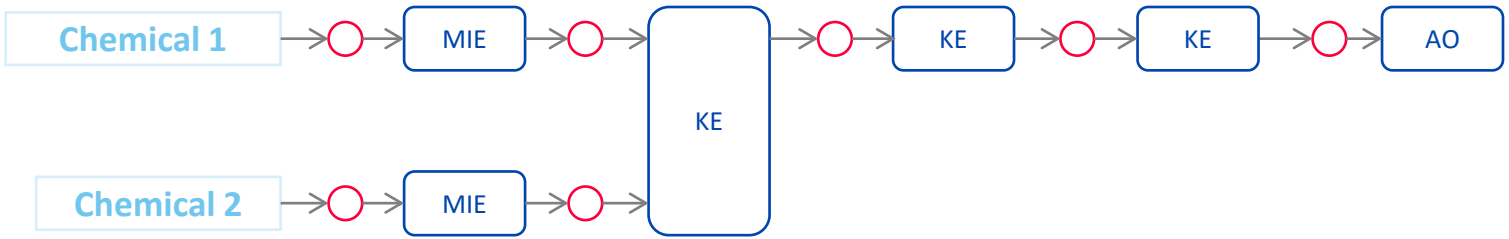


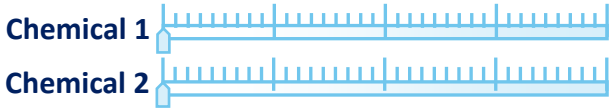
A chemical can activate different MIEs leading to different adverse effects





Use of AOP Networks to assess toxicity of mixtures

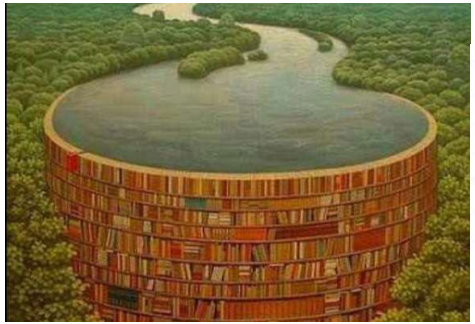




concentration

AOPs are a way of organizing information

Sea of existing information



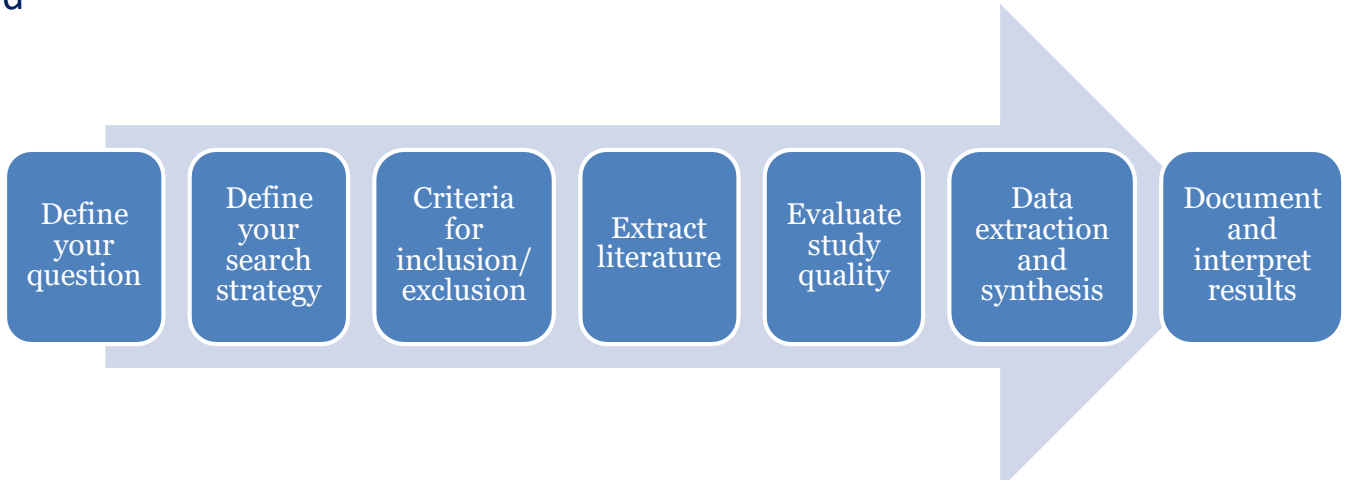
AOP developer

AOP consumers

Start with quality ingredients

AOP development will typically involve literature review

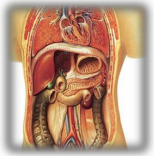
- Documenting how the literature search/reviews were done may be helpful to developers and users.
- Transparency
- Efficient – easy to understand how to update or expand



AOP development often requires a team of collaborators

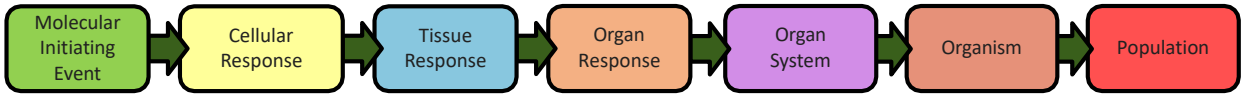
Rare for a “single person” to produce a full AOP

Molecular biologists



Physiologists

Risk Assessors



Cellular biologists

Ecologists

AOP Wiki: a community resource for AOP development

A-O-P-Wiki AOPs Key Events KE Relationships Prototypical Stressors Developers' Handbook

Welcome to the Collaborative Adverse Outcome Pathway Wiki (AOP-Wiki)

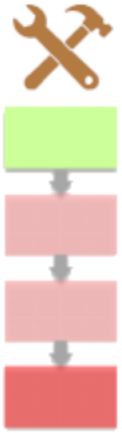
Version 2.6 was released on April 29, 2023. More details regarding the new release are available here: Release 2.6.

Interested in helping plan for Version 3.0? Please submit your ideas on the AOP Forum here.

View Content

AOPs | Key Events | KE Relationships | Prototypical Stressors

Get access to the main elements of an Adverse Outcome Pathway managed in the AOP-Wiki



Contribute

Register

Start a new AOP

Developers' Handbook

OECD AOP Development Programme

AOP Wiki Access: three levels

1. Anyone can access the wiki, search and read entries
2. To leave comments, you will need an account

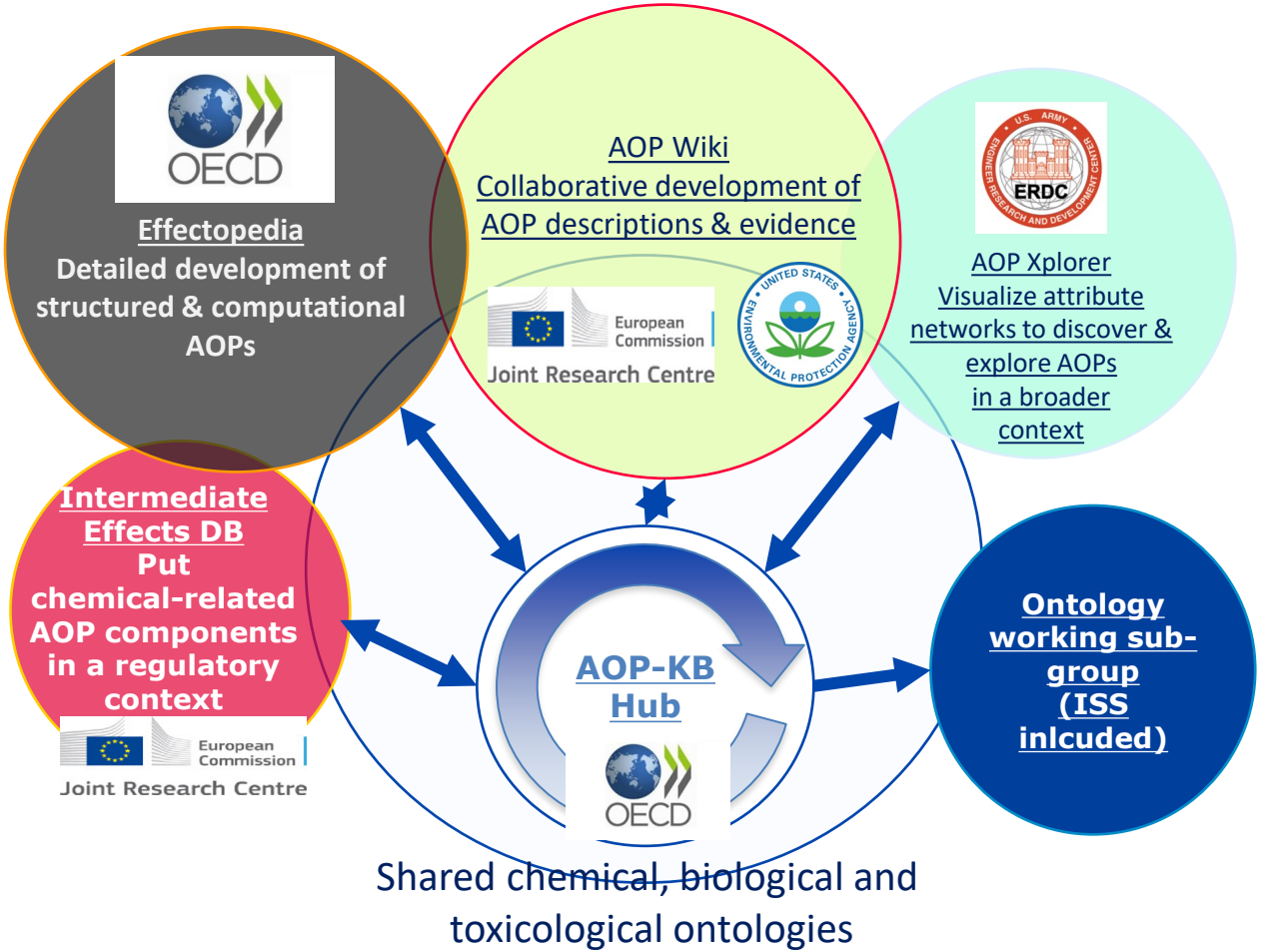
   Request an account through

   www.aopwiki.org or www.saaop.org
3. To gain write access

   Request write access the same way

   You should have a familiarity with the wiki and desire to build an AOP

OECD AOP knowledge base (AOP-KB)



The AOP Knowledge Base

https://aopkb.oecd.org/

- Main entry point for the AOP-KB
- Search engine for all AOPs
  Houses the status of all AOPs & links to the official copies
- Allows browsing of review reports

Adverse Outcome Pathway Wiki

https://aopwiki.org/

- Entry level module for evaluating an AOP's scientific evidence
- Supports OECD review of AOPs
- Default go-to module for all qualitative AOPs

AOP XML

Effectopedia

https://www.effectopedia.org/

- Captures quantitative information and models
- Provides standard visual representation of AOPs and associated test methods

AOP XML

Third Party Tools

AOPXplorer

http://apps.cytoscape.org/apps/aopxplorer

AOP-DB

On the Web this Summer 2020!

Intermediate Effects Database (IEDB)

New AOP proposals: acceptance by OECD case by case

AOP Development Programme is evolving fast with participation of multiple groups of experts in various areas of toxicology

The public can make project proposals to develop AOPs

AOP can be developed in parallel with scientific publications

OECD ADVERSE OUTCOME PATHWAY

Project Submission Form

(Revised 29 September 2015 only to update Secretariat contact details)

If you require further information please contact the OECD Secretariat
Return completed forms to Fiona Macfarlane (Fiona.MACFARLANE@oecd.org) and Christina Quaglio (Christina.QUAGLIO@oecd.org)

PROJECT TITLE

AhR / beta-catenin signalling leading to placental vascular disruption

SUBMITTED BY (Country / European Commission / Secretariat)

Italy

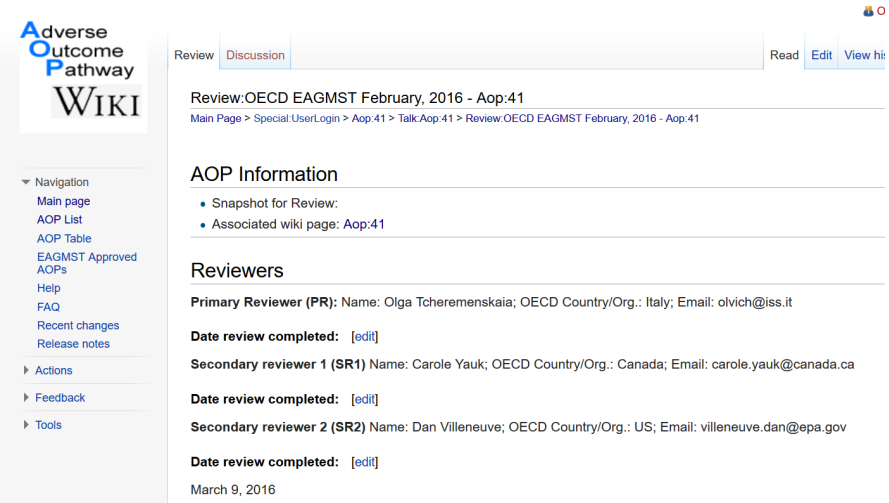
DATE OF SUBMISSION TO THE SECRETARIAT

18/11/2015

DETAILS OF LEAD COUNTRY/CONSORTIUM

Country/Organisation:	Italy
Agency/ministry/Other:	Istituto Superiore di Sanità
Contact person(s):	Sabrina Tait Cinzia La Rocca
Mail Address:	Istituto Superiore di Sanità Dept. Food Safety and Veterinary Public Health Viale Regina Elena 299 00161 – Rome Italy
Phone/fax:	+39 6 4990 2839 (Tait) +39 6 4990 2992 (La Rocca)
Email:	sabrina.tait@iss.it cinzia.larocca@iss.it

Internal review process: EAGMS group



Adverse Outcome Pathway WIKI

Review | Discussion | Read | Edit | View his

Review:OECD EAGMST February, 2016 - Aop:41

Main Page > Special:UserLogin > Aop:41 > Talk:Aop:41 > Review:OECD EAGMST February, 2016 - Aop:41

Navigation
- Main page
- AOP List
- AOP Table
- EAGMST Approved AOPs
- Help
- FAQ
- Recent changes
- Release notes

Actions

Feedback

Tools

AOP Information

- Snapshot for Review:
- Associated wiki page: Aop:41

Reviewers

Primary Reviewer (PR): Name: Olga Tcheremenskaia; OECD Country/Org.: Italy; Email: olvich@iss.it

Date review completed: [edit]

Secondary reviewer 1 (SR1) Name: Carole Yauk; OECD Country/Org.: Canada; Email: carole.yauk@canada.ca

Date review completed: [edit]

Secondary reviewer 2 (SR2) Name: Dan Villeneuve; OECD Country/Org.: US; Email: villeneuve.dan@epa.gov

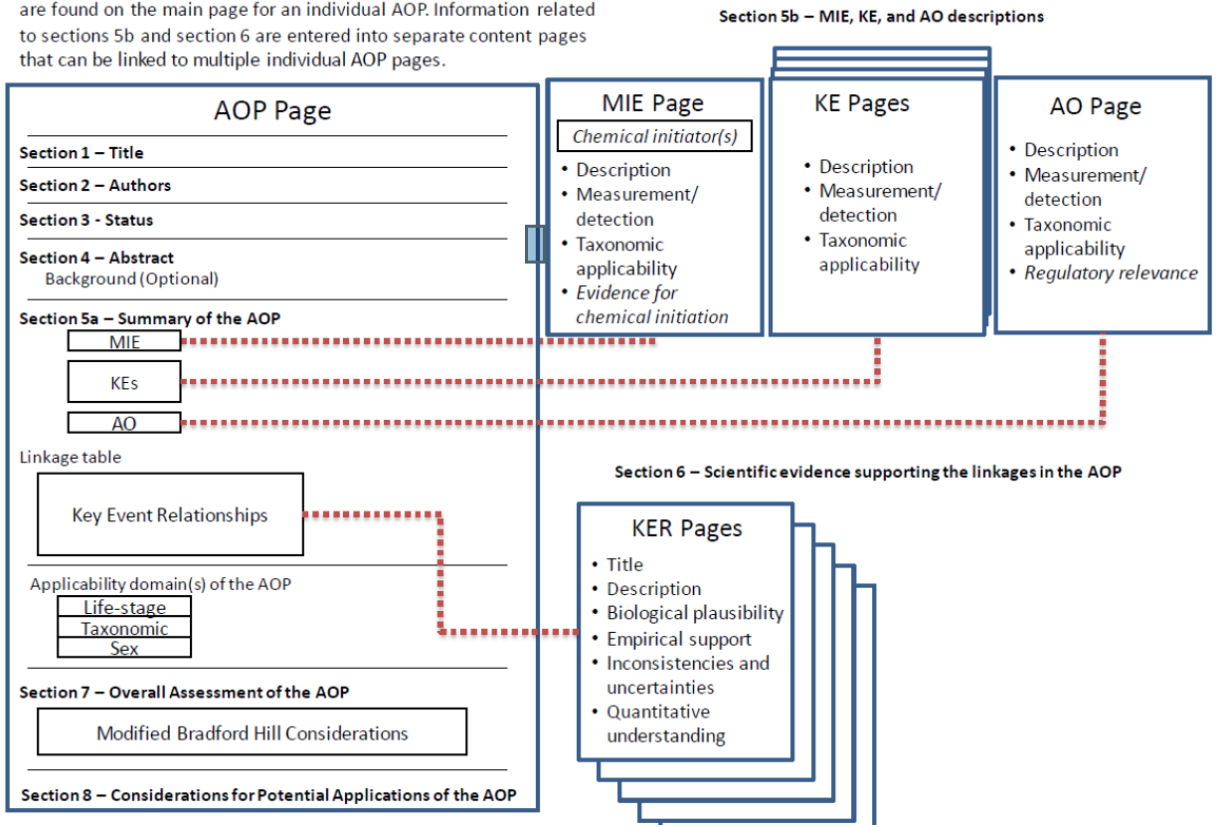
Date review completed: [edit]

March 9, 2016

Assignment of a coach to each AOP included in the OECD plan: from 2019

AOP documentation required

Figure 2. Overview of the organization of content pages in the AOP-wiki relative to sections of the AOP template. Sections 1, 4, 5a, and 7 are found on the main page for an individual AOP. Information related to sections 5b and section 6 are entered into separate content pages that can be linked to multiple individual AOP pages.



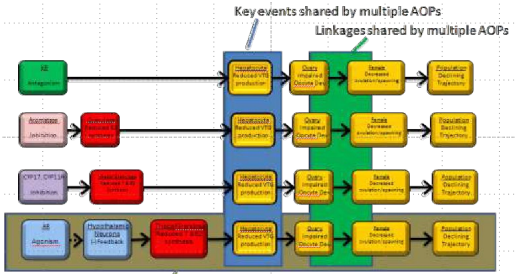
Bradford-Hill criteria

for establishing causation:

- strength of association,
- consistency of the evidence,
- specificity of the relationship, association, consistency of the evidence,
- specificity consistent temporal relationships,
- dose-response relationships,
- biological plausibility,
- coherence of the evidence,
- and consideration of alternative explanations

Bradford-Hill, A. (1965). The environment and disease: association or causation? Proc R Soc Med 58, 295-300.

AOP-KB supports principles of collaborative AOP development



AOPs are modular

- KEs and KERs are shared by multiple AOPs
- No need to re-write the same descriptions over and over
- Reusability (best practices)

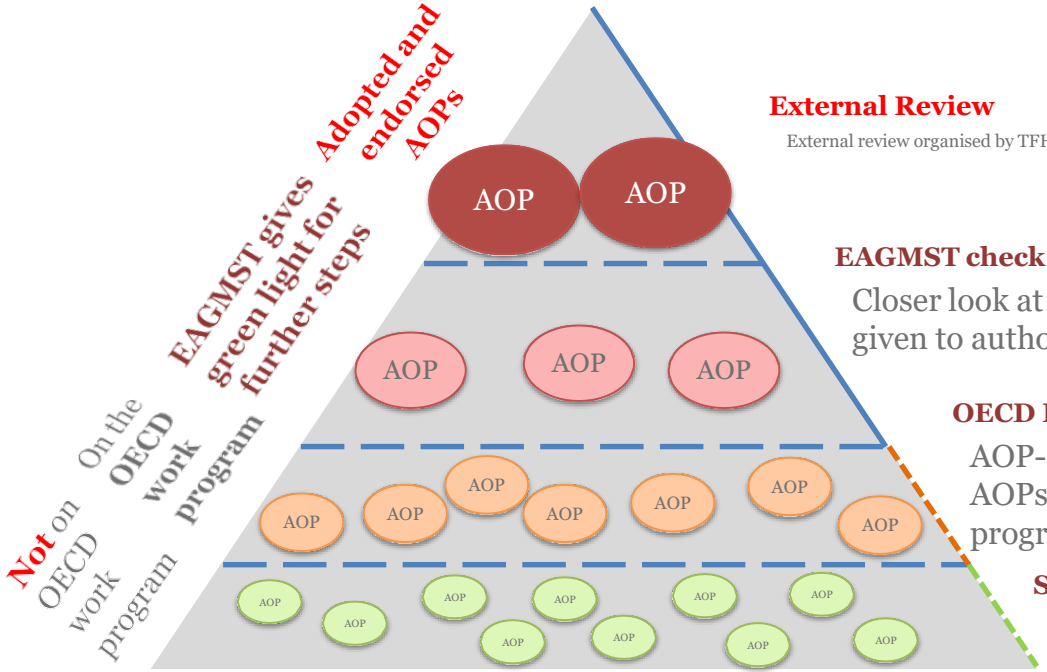
AOPs are living documents

- KE and KER descriptions can be expected to evolve over time
- As descriptions are updated and expanded – all AOP descriptions they link to update automatically

AOP networks for prediction

- Entry of structured information in KB allows for de-facto assembly of AOP networks.

AOPs' lifecycle



External Review

External review organised by TFHA/WNT

EAGMST check

Closer look at selected AOPs Support given to authors First reality check

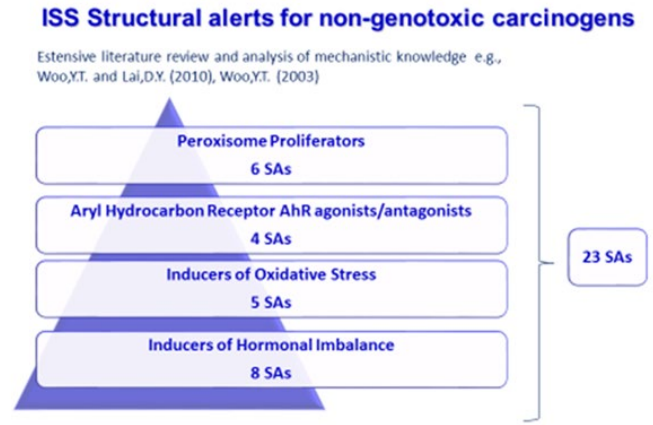
OECD Entrance Gate

AOP-KB write access via SAAOP
AOPs get directly on the OECD work program

SAAOP Entrance Gate

AOP-KB write access via SAAOP
AOPs from the scientific community

EAGMST 2014: ISS presentation



Zoom automatico

iskaia (Italy) highlighted the potential benefits of using an AOP-based approach genicity assessment, which is difficult to detect and currently requires rodent ural alerts for non-genotoxic carcinogens, implemented in QSAR Toolbox and Toxtree, can be used as triggers of different MIEs which in turn can follow multiple mechanisms. The need using standardised terminology based on pathway ontologies was also highlighted.

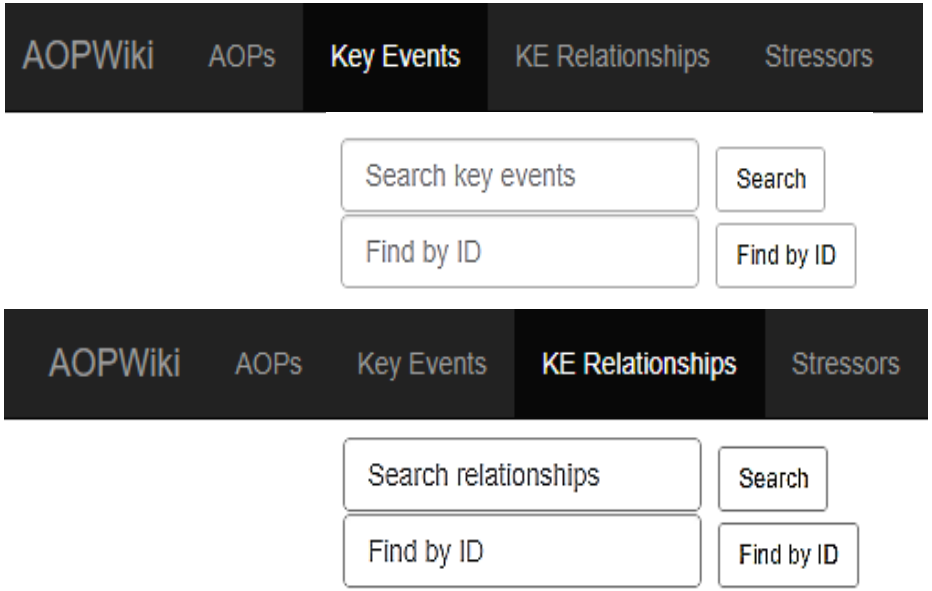
23. Steve Edwards (US) presented activities of the Toxicogenomics Interoperatability Interest Group at Research Data Alliance project. The group focus is on alleviating the barriers of data availability and interoperability. The exchange TG-GATES and DrugMatrix datasets between diXa and CEBS using ISATAB was chosen as a case study for illustrating the problem and potential solution.

24. Rick Becker (BIAC) presented the challenges and opportunities in developing scientific confidence in HTS-derived prediction models. The presentation brought the question of how much accuracy is needed for a given application and how to build such models using analytical validation.

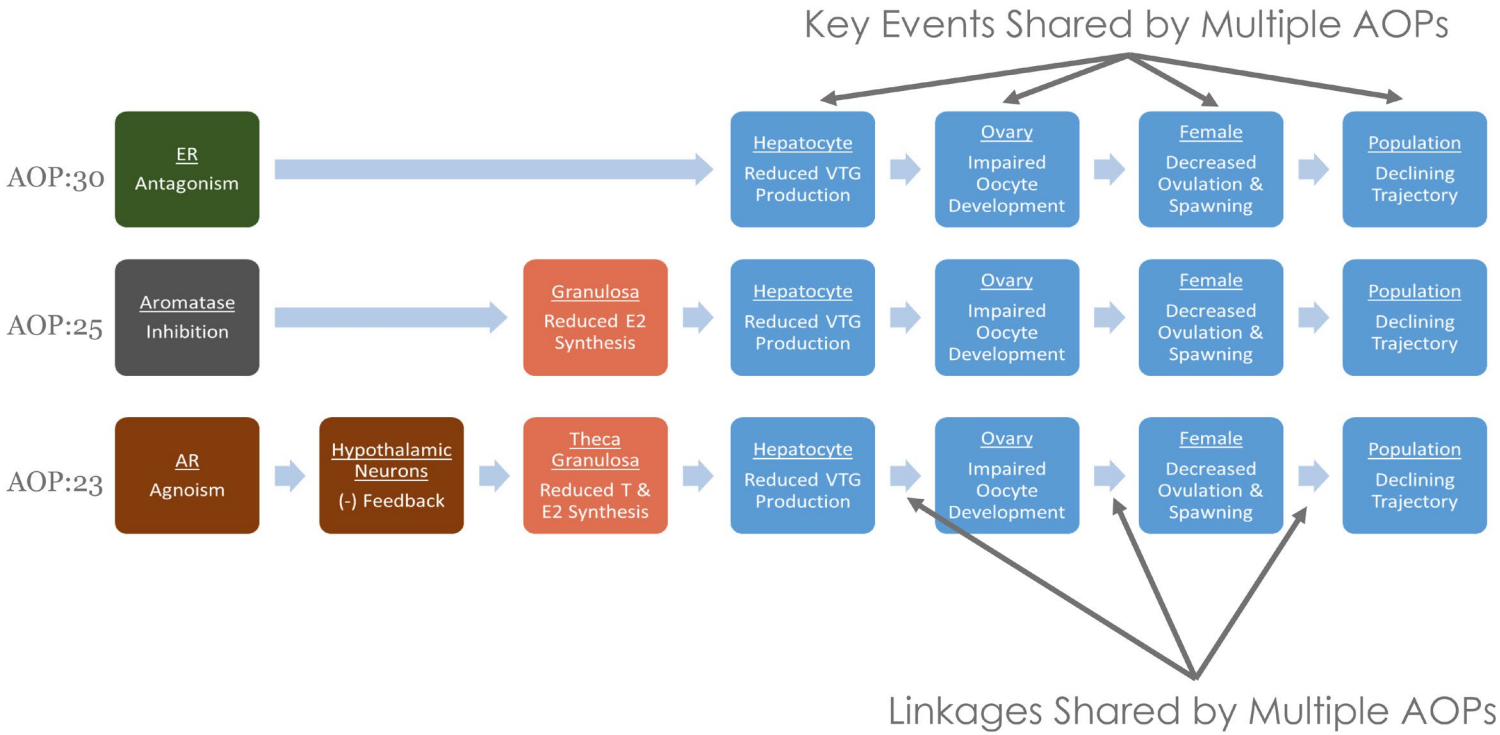
Fusion and collaboration

No need to start from scratch

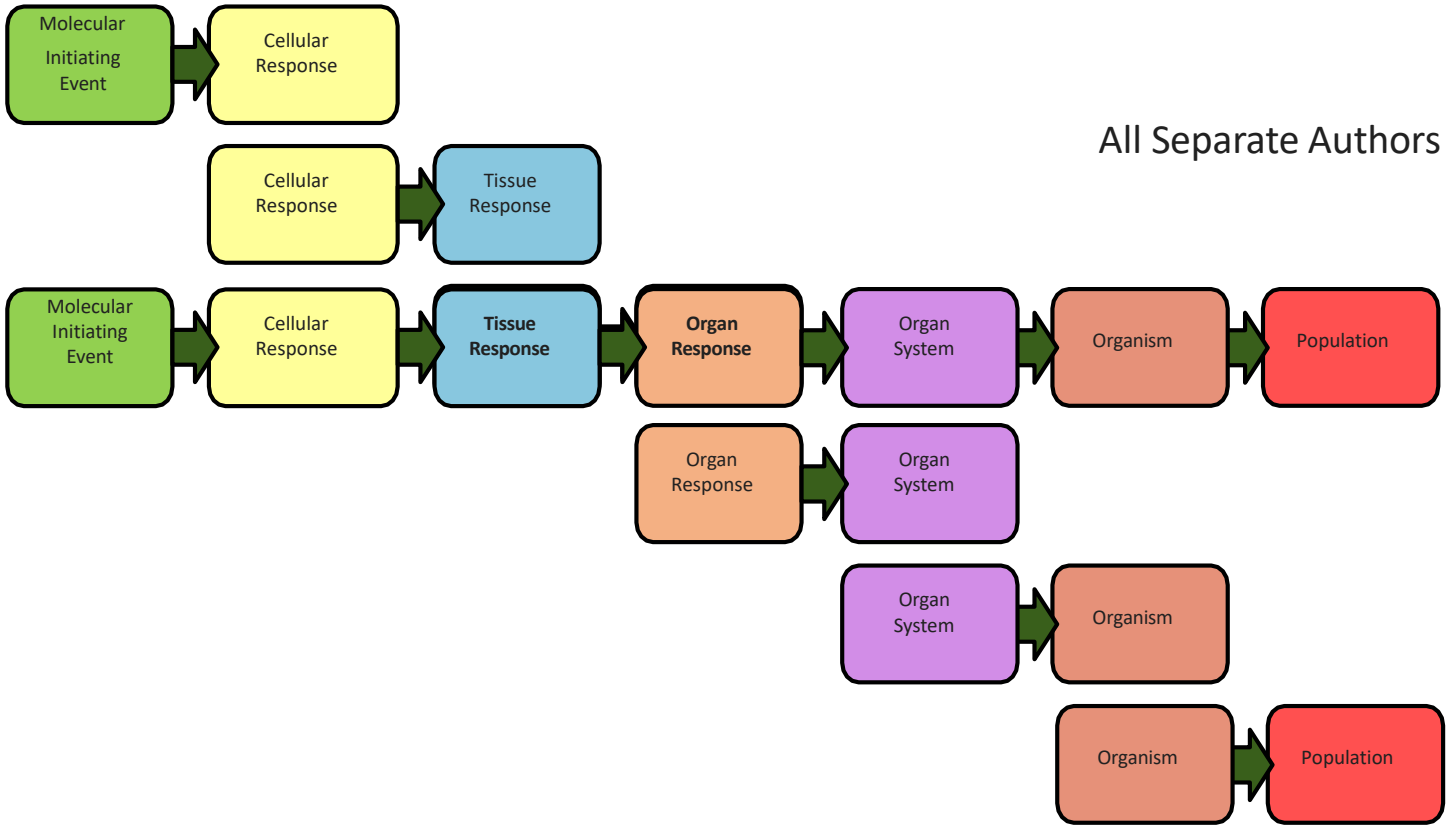
Build from existing ingredients whenever possible



Construction of AOPs networks

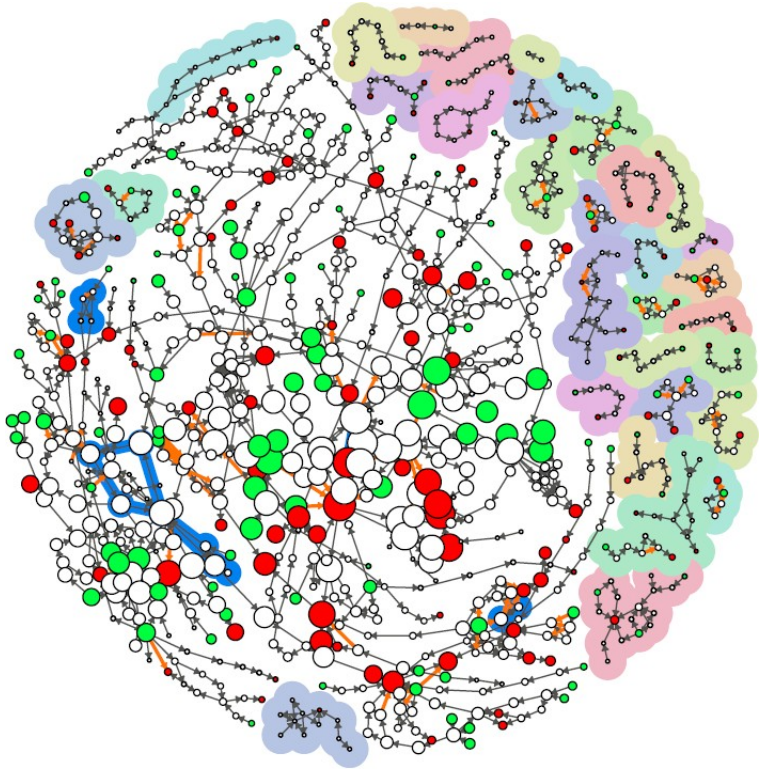


Collaborate: Single Elements



All Separate Authors

All contributions help generate NEW knowledge

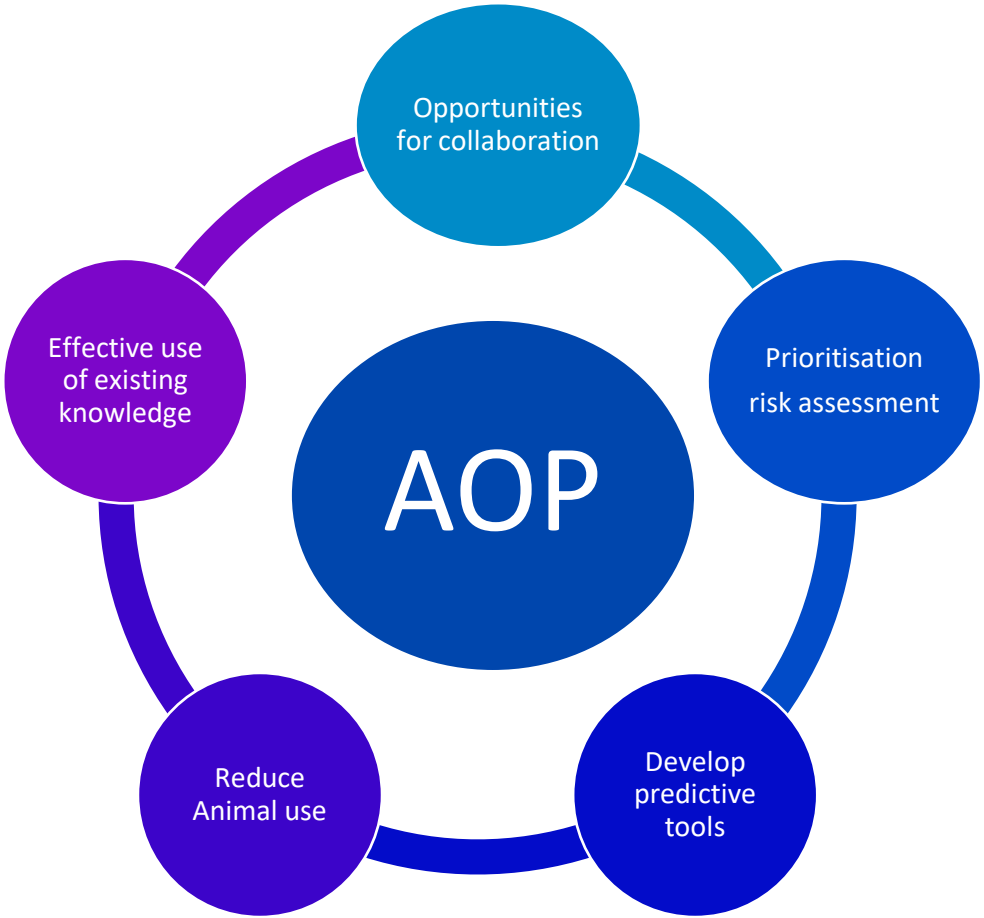


NETWORK

>9000 unique paths
(from MIE to AO)

NEW knowledge

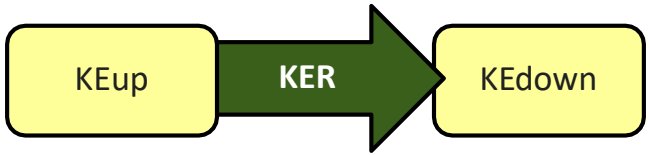
Benefits of AOPs



Single Elements: Key Event Relationship

How Upstream
Event is Measured

How Downstream
Event is Measured

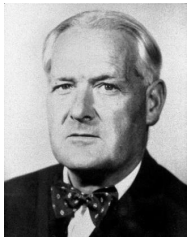


Experimental Evidence Linking KEup and KEdown

- Causal Evidence
- Weight of Evidence Evaluation
- Principal Unit of Extrapolation

Background – WOE Analysis for AOPs

- Based on modified Bradford Hill (B/H)considerations
  - Initially introduced to assess causality of associations in epidemiological studies



The Environment and Disease: Association or Causation?

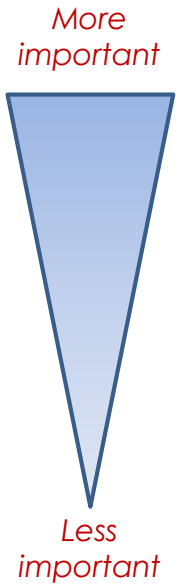
by Sir Austin Bradford Hill CBE DSC FRCP(hon) FRS (*Professor Emeritus of Medical Statistics, University of London*)

Amongst the objects of this newly-founded Section of Occupational Medicine are firstly 'to provide a means, not readily afforded elsewhere, whereby physicians and surgeons with a special knowledge of the relationship between sickness and injury and conditions of work may discuss their problems, not only with each other, but also with colleagues in other fields, by holding joint meetings with other Sections of the Society'; and, secondly, 'to make available information about the physical, chemical and psychological hazards of occupation, and in particular about those that are rare or not easily recognized'.

  - Subsequently adopted by a wide range of communities
  - Subset of B/H considerations modified for AOP assessment
    - based on regulatory experience in assessing chemical specific mechanistic data (mode of action analysis)
  - Continue to evolve, with additional experience in assessment and application

Weight/Extent of the Evidence - AOPs

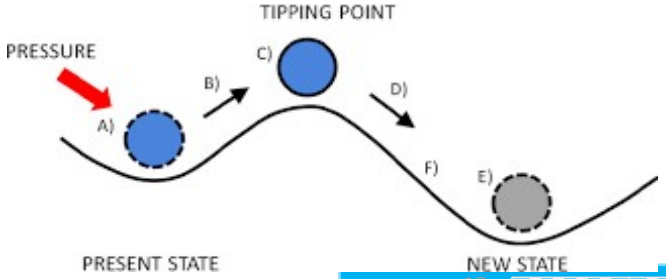
- Biological Plausibility – *KERs* H M L
  - Extent of knowledge of the biology of the pathway
  - Knowledge of the structural-functional relationships
- Essentiality – *KEs within AOP* H M L
  - Necessity of Key Events
  - Experimental support normally from specialized studies to block or modify key events, stop/recovery studies
- Empirical Support – *KERs* H M L
  - Pattern of Quantitative Associations among Key Events often considered through application of stressors

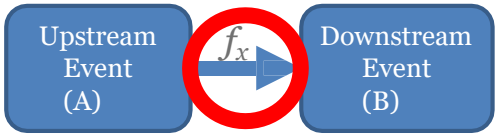


Quantitative adverse outcome pathway (qAOP)

- An AOP for which the quantitative understanding of relationships that underlie transitions from one KE to the next, as well as critical factors that modulate those relationships, are sufficiently well defined to allow quantitative prediction of the probability or severity of the AO for a given level of activation/perturbation of the MIE.

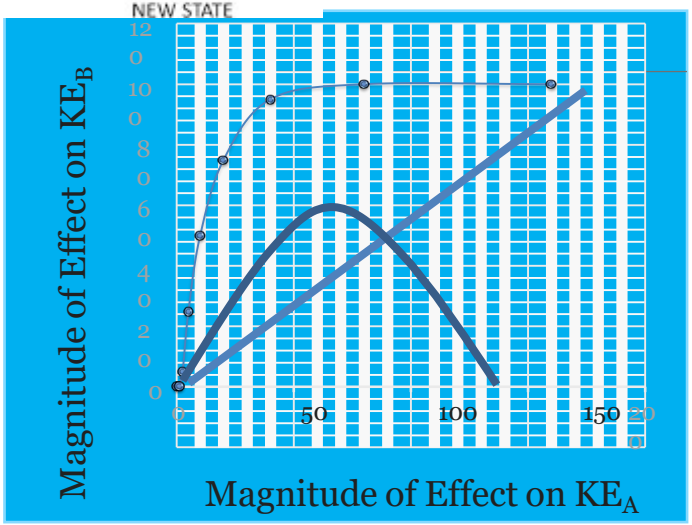
i.e., - no longer need to assume tipping points, we can evaluate whether the exposure is likely to surpass the tipping points along the pathway.



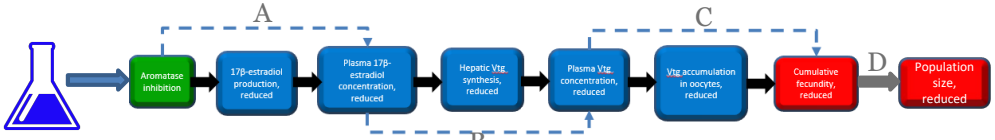


Quantitative Understanding of KERs

- Response - Response Relationship
- Time-scale of the transition
- Modulating factors that can shift or alter the R-R relationship

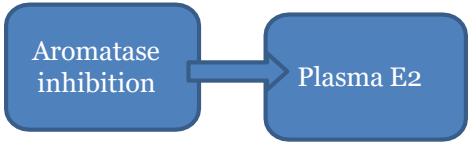


Quantitative adverse outcome pathway (qAOP): AOP 25 Aromatase inhibition leading to reproductive dysfunction



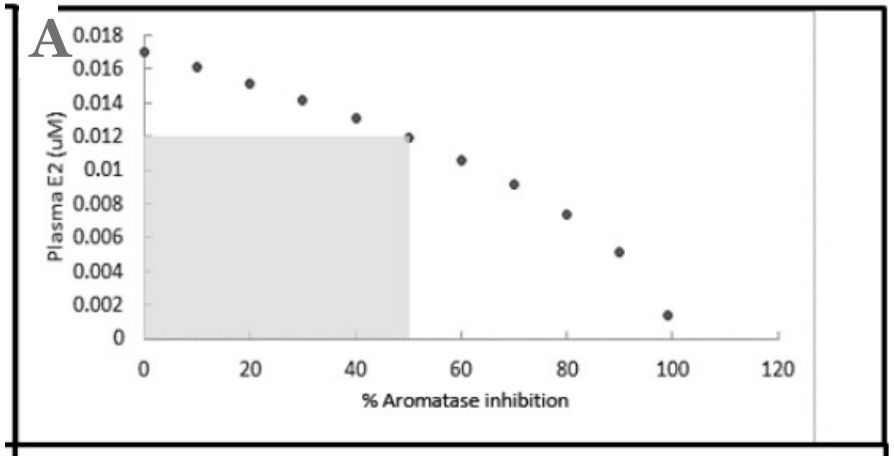
https://aopwiki.org/aops/25

Response-Response Relationship



In vitro, HTS

In vivo

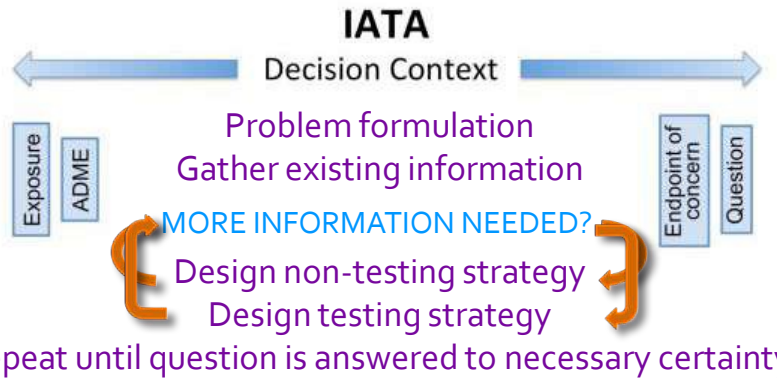


$$y = -8e^{-7}x^2 - 7e^{-5}x + 0.016$$

Conolly RB, Ankley GT, Cheng W, Mayo ML, Miller DH, Perkins EJ, Villeneuve DL, Watanabe KH. Quantitative Adverse Outcome Pathways and Their Application to Predictive Toxicology . Environ Sci Technol. 2017 Apr 18;51(8):4661-4672. doi: 10.1021/acs.est.6b06230.

AOP: a knowledge bridge

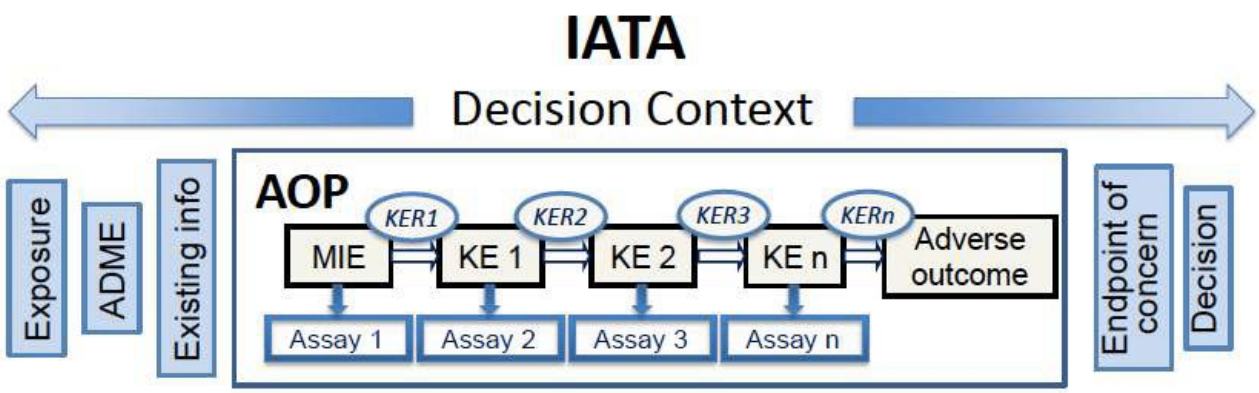
Integrated Approach to Testing and Assessment (IATA): OECD working definition



"a structured approach that strategically integrates and weights all relevant data to inform regulatory decisions regarding potential hazard and/or risk and/or the need for further targeted testing and therefore optimising and potentially reducing the number of tests that need to be conducted."

Report of the Workshop on a Framework for the Development and Use of Integrated Approaches to Testing and Assessment. 2015. OECD Series on Testing and Assessment No. 215

Using an AOP within the context of an IATA



AOP provides biological rationale

- For weight-of-evidence interpretation
- For design of integrated, iterative testing strategy

Transparent communication of certainty

Quantitative information allows prediction

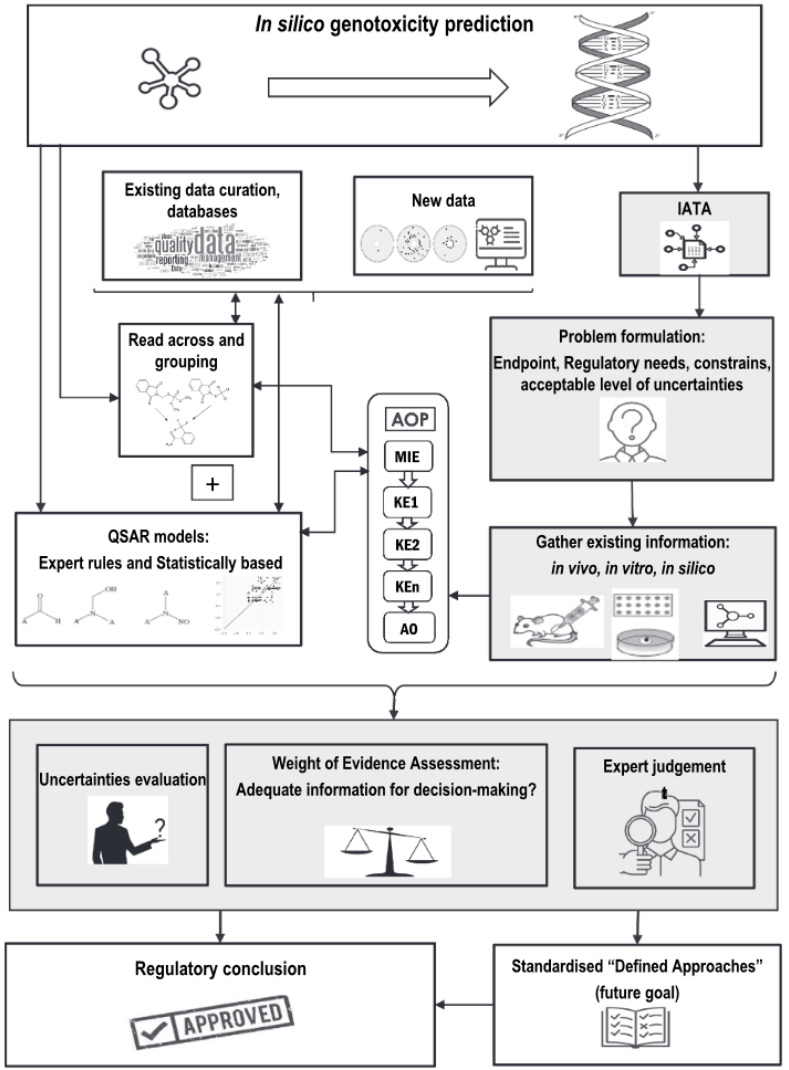
Current state of art of in silico tools for genotoxicity prediction (applicable also for other endpoints)

AOPs

- informs chemical grouping and subsequent data gap filling by read-across or trend analysis
- provides an opportunity to group chemicals based on their intrinsic chemical properties as well as their biological activity at different levels of biological organization.

AOP-informed IATA

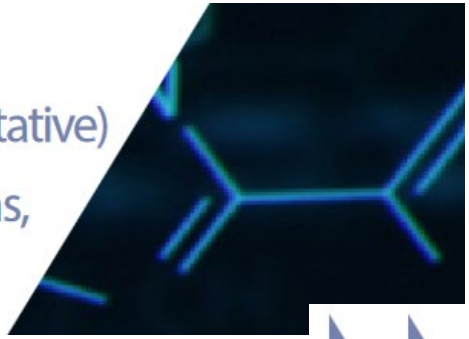
- provides a more robust support framework for assessing toxicological potential for new and untested chemicals,
- improves the predictive performance of in silico method by bringing more transparent mechanistically based data integration.



https://www.tandfonline.com/doi/epdf/10.1080/17425255.2021.1938540?needAccess=true

(Q)SAR Assessment Framework:

Guidance for the regulatory assessment of (Quantitative) Structure Activity Relationship models, predictions, and results based on multiple predictions



Series on Testing and Assessment
No. 386

(Q)SAR model: a model that predicts the property of a substance using as input information on the structure

1.5 Mechanistic interpretation

According to Principle 5 (OECD, 2007), a (Q)SAR "should be associated with a mechanistic interpretation, if possible". Statistical methods used to describe relationships between chemical structure and activity are not intended to replace other knowledge from chemistry and toxicology when such knowledge exists. Assessors may require that the model documentation includes considerations on how the rationale behind a (Q)SAR model is consistent with or accounts for the knowledge related to the predicted property (such as known Adverse Outcome Pathways, AOPs, relevant for the predicted property), namely a mechanistic interpretation. Toxicokinetic considerations are also part of the mechanistic interpretation, if relevant for the property of interest.

The Model Checklist includes the following AE related to mechanistic interpretation:

- Plausibility of the mechanistic interpretation

https://www.oecd.org/chemicalsafety/risk-assessment/qsar-assessment-framework.pdf

Regulatory acceptance of IATA: specific case Defined Approaches

OECD Guidance document 255: Guidance Document on the **Reporting of Defined Approaches to be Used Within Integrated Approaches to Testing and Assessment (2016)**

Six Principles: Essential Information for Regulatory Application of an IATA

1. A defined endpoint
2. A defined purpose
3. A description of the rationale 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 associated with the IATA application

Guideline No. 497: Defined Approaches on Skin Sensitisation

A Defined Approach (DA) consists of a selection of information sources (e.g in silico predictions, in chemico, in vitro data) used in a specific combination, and resulting data are interpreted using a fixed data interpretation procedure (DIP) (e.g. a mathematical, rule-based model). DAs use method… More

04 Jul 2023 | 53 pages | English | Also available in: French

https://doi.org/10.1787/b92879a4-en | 9789264903005 (PDF)

Author(s): OECD

A Defined Approach (DA) consists of a selection of information sources (e.g in silico predictions, in chemico, in vitro data) used in a specific combination, and resulting data are interpreted using a fixed data interpretation procedure (DIP) (e.g. a mathematical, rule-based model).

The DAs included in this Guideline have shown to either provide the same level of information or be more informative than the murine Local Lymph Node Assay (LLNA; OECD TG 429) for hazard identification (i.e. sensitiser versus non-sensitiser).
In addition, two of the DAs provide information for sensitisation potency categorisation that is equivalent to the potency categorisation information provided by the LLNA.

AOP implementation in the OECD QSAR Toolbox

SKIN SENSITIZATION

HISTORICAL ANIMAL TESTS

Regulatory adopted animal-based tests, which are part of Council Regulation No 440/2008, include:

- 1965 - **Buehler** occluded patch test in the guinea pig (OECD TG 406)
- 1969 - **GPMT**, by Magnusson & Kligman (OECD TG 406)
- 1999 - **LLNA** (OECD TG 429), and its non-radioactive modifications, LLNA-DA (OECD TG 442A) and LLNA-BrdU Elisa (OECD TG 442B)

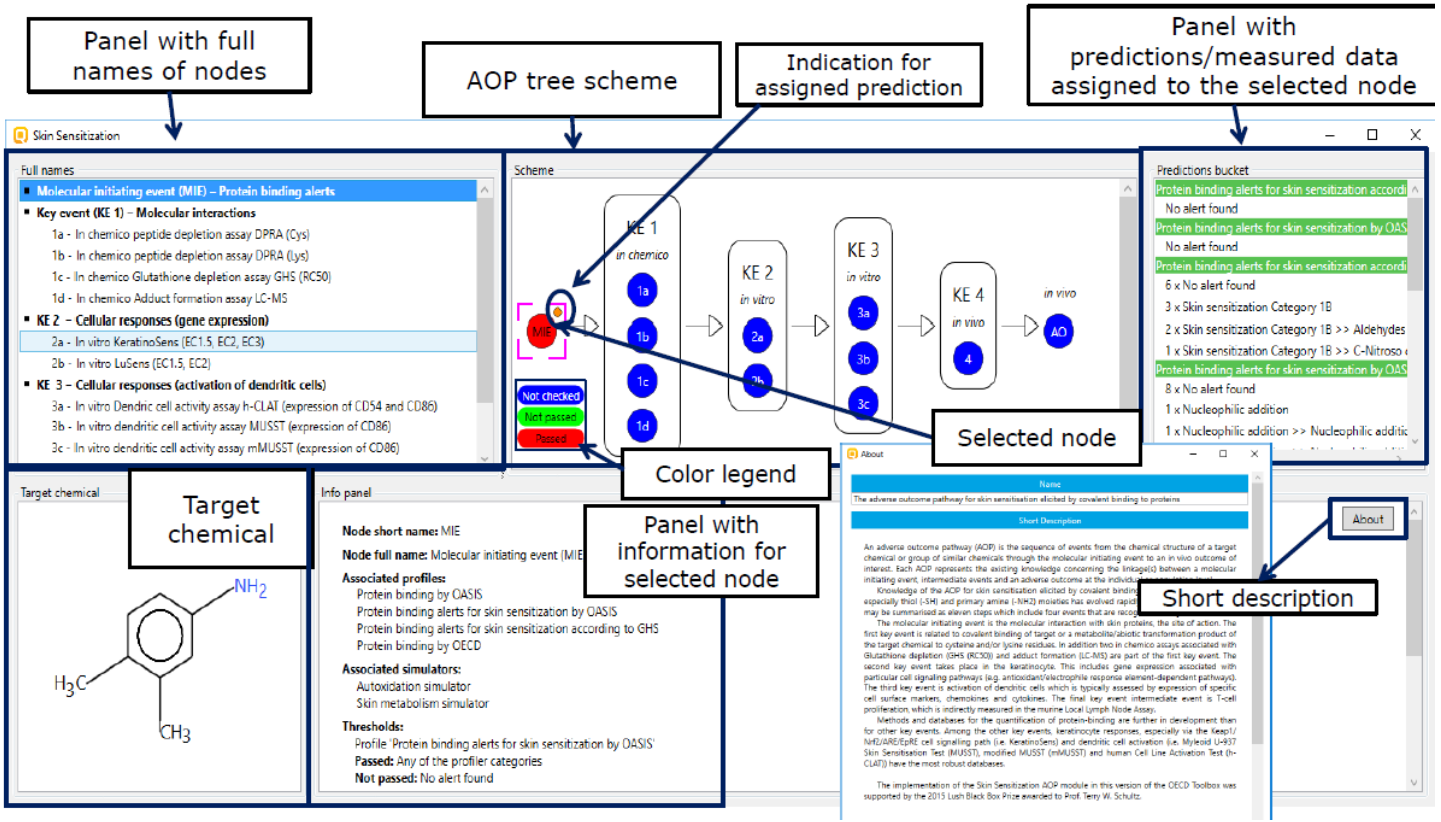
VALIDATED ALTERNATIVE METHODS

OECD tests guidelines and define approaches (DA)

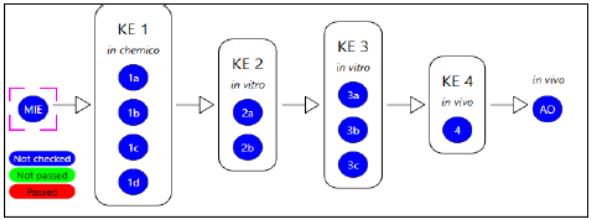
OECD guideline	Methods	AOP
OECD TG 442C	Direct Peptide Reactivity Assay (**DPRA**)	KE1: protein binding
OECD TG 442D	ARE-Nrf2 Luciferase Test Method: **KeratinoSens**	KE2: keratinocyte activation
OECD TG 442E	1) human Cell Line Activation Test (**h-CLAT**) 2) U937 cell line activation test (**U-SENS**) 3) Interleukin-8 Reporter Gene Assay (**IL-8 Luc assay**)	KE3: dendritic cell activation
OECD Project 4.106:	New TG: Genomic Allergen Rapid Detection test for skin (**GARD™skin**) test: An in vitro method for identification of skin sensitizers based on a genomic interpretation of the impact of chemicals on human dendritic cell-like cells (AOP key event 3).	KE3: dendritic cell activation
OECD Project 4.107:	New TG: Toxicogenomic analysis on 3D reconstituted epidermis for measuring skin sensitization potency – **the SENS-IS assay.**	KE1: protein binding KE2: keratinocyte activation

Overview of the AOP scheme as implemented in Toolbox

Details of AOP window



AOP workflow for skin sensitization



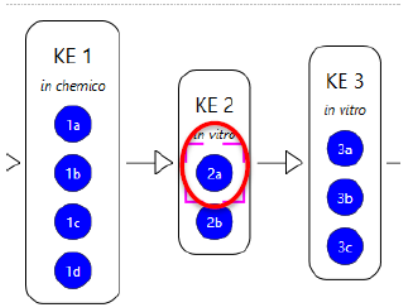
	Key node		Key event
	MIE	Protein binding alerts	Protein binding – in silico/theoretical
In chemico	KE1	1a Peptide depletion assay DPRA (Cys)	Protein binding potency in chemico
		1b Peptide depletion assay DPRA (Lys)	
		1c Glutathione depletion assay GSH (RC50)	
		1d Adduct formation assay LC-MS	
In vitro	KE2	2a KeratinoSens assay(EC1.5, EC2, EC3)	Cellular response (gene expression)
		2b LuSens (EC1.5, EC2, EC3)	
	KE3	3a Dendritic cell activity assay h-CLAT (expression of CD54 and CD86)	Cellular response (activation of dendritic cells)
		3b Dendritic cell activity assay MUSST (expression of CD86)	
		3c Dendritic cell activity assay mMUSST (expression of CD86)	
In vivo	KE4	4 Organ response (EC3 LLNA)	Organ response
		AO Organism response (GPMT)	Organism response

Overview of the AOP scheme as implemented in Toolbox

Implemented thresholds for the AOP nodes

- Thresholds are implemented for each AOP node
- Each threshold is available in the description panel of the AOP node
- Threshold are identified based on assay data related to the corresponding node
- The status of the each node (passed/not passed) depends on the implemented thresholds
- Thresholds of the AOP nodes determined by expert group are provided on the next slide:

Thresholds:
Scale name 'Gene expression EC (ordinal)'
Scale type 'Ordinal'
Passed: High | Low | Moderate | Very High
Not passed: Negative

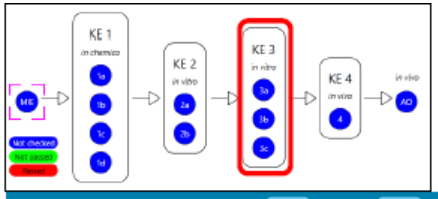


Implemented thresholds for the AOP nodes

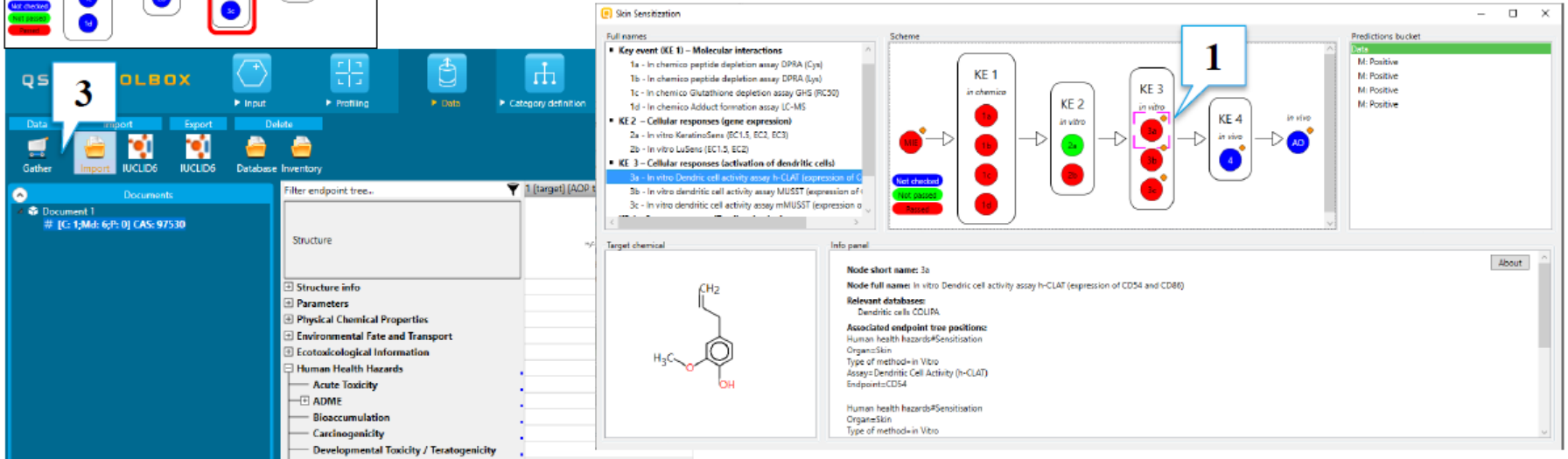
Node name	Data thresholds	Node status: Pass	Node status: Not pass
MIE - Protein binding alerts		presence of alert	absence of alert
1a and 1b *in chemico* DPRA Cys and Lys	Peptide depletion, PD (%): PD > 9 - Passed PD <=9% - Not passed	> 9 % - Passed	<=9 % - Not passed
1c - *in chemico* Glutathione depletion assay GSH (RC50)	RC50 (mmol/L) ≤ 0.099 – Extremely reactive 0.1 ≥ RC50 ≤ 0.99 – Highly reactive 1 ≥ RC50 ≤ 15 – Moderately reactive 16 ≥ RC50 ≤ 70 – Slightly reactive 70.1 ≥ RC50 ≤ 135 – Suspect RC50 > 135 – Not reactive	Extremely Reactive| Highly Reactive | Moderately Reactive | Slightly Reactive	Suspect | Not Reactive | Not reactive at saturation
1d - *in chemico* Adduct formation assay LC-MS	Adduct formation (%) ≥ 30% - Positive Adduct formation (%) < 30% - Negative	Positive	Negative
2a - in vitro Keratinocyte (EC1.5, EC2, EC3) AND 2b – *in vitro* LuSens (EC1.5, EC2)	EC3 (%) ≤ 20 – Very High 20 > EC3 ≤ 50 – High 50 > EC3 ≤ 100 – Moderate 100 > EC3 ≤ 2000 – Low EC3 > 2000 - Negative	Very High |High| Moderate | Low	Negative
3a;3b and 3c *in vitro* Dendritic cell activity assay h-CLAT; MUSST and mMUSST (expression of CD54 and CD86)	expression of CD54 and CD86 Positive Negative	Positive	Negative
4 - in vivo Organ response (LLNA)	0 ≥ EC3 (%) <50 – Positive EC3 ≥ 50 – Negative Or	Positive	Negative
AO - in vivo Organism response (GPMT)	Data provided: Strong sensitizer; Moderate sensitizer; Weak sensitizer; Non sensitizer	Strong sensitizer |Moderate sensitizer	Weak sensitizer |Non sensitizer

https://www.oecd.org/env/ehs/risk-assessment/Tutorial_11_TB%204.1_Implementation%20of%20AOP%20workflow%20in%20Toolbox%20SS.pdf

Implementation AOP workflow in Toolbox: Skin Sensitization



KE3, node 3a: Check if there are any data for the target chemical for the *in vitro Dendric cell activity assay*



1. Select **node 3a**
2. Select database related to node 3a
3. **Gather** data and click **OK** in the appeared message
4. The experimental data appears on Data matrix. Based on data found for the target chemicals status of nodes 3a, 3b and 3c was changed to **Passed.**

Thank you very much!