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Existing efforts and roadmaps: North American perspective

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Precedents for pathway-based toxicology

Dose-response modeling

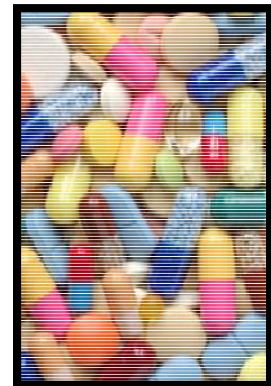
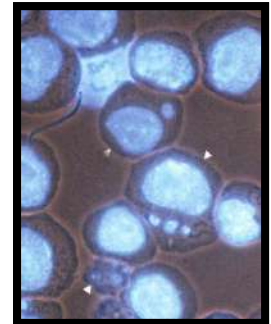
- Using pharmacokinetic and mechanistic information

IPCS/WHO mode of action frameworks

- Human relevance of rodent cancer findings
- Extrapolated to non-cancer endpoints

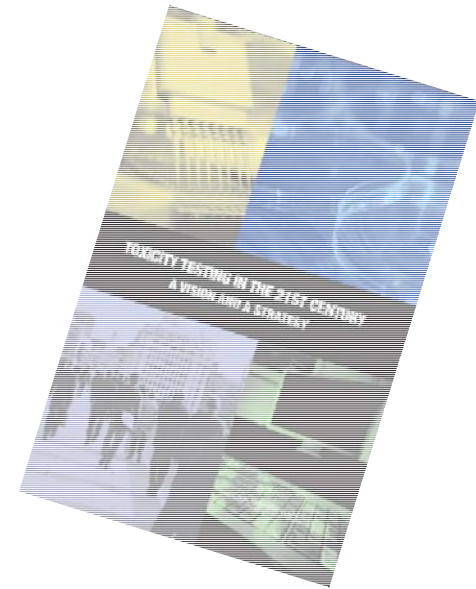
Mode of action pathways in drug and product development

- Drug and target-specific



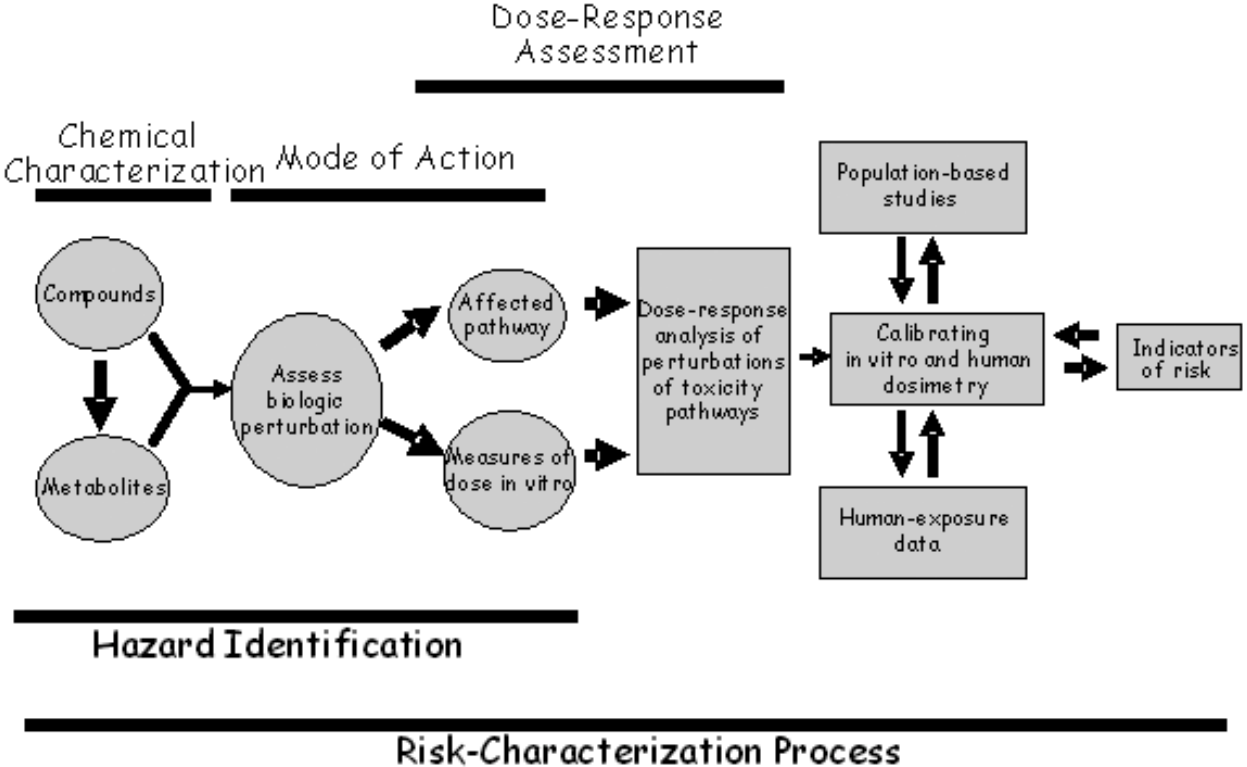
National Research Council in 2007 Report, Toxicity testing in the 21st century: A vision and a strategy

*“envisions a new toxicity-testing system that evaluates **biologically significant perturbations in key toxicity pathways** by using new methods in computational biology and a comprehensive array of in vitro tests **based on human biology**”*



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NRC 2007 Report



NRC 2007 Report recommendations

- The realization of the vision will entail considerable research over many years and require **substantial funding**—hundreds of millions of dollars
- Much of the research will be **interdisciplinary** and consequently, to be most effective, should not be dispersed among discipline-specific laboratories
- The research will need **high-level coordination** to tackle the challenges presented in the vision efficiently
- The research should **be informed by the needs of the regulatory agencies** that would adapt and use the emerging testing procedures, but the research program **should be insulated from the short-term orientation** and varied mandates of the agencies



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Related US Roadmaps

EPA Strategic Plan for Evaluating the Toxicity of Chemicals, 2009



	Pathway-based screening	Pathway-based risk assessment	Institutional transition
Issue	Need to assess 10,000's of chemicals for all toxicities	Current approach is slow, expensive, limited Lack of biological understanding needs addressing	Requires significant institution investment, organizational transition, public outreach
Drivers	Lower costs, improve speed, decrease uncertainty	New scientific understanding and tools: molecular, computation, informatics	EPA lacks appropriate resources, knowledge and training.
New Approach	Elucidation of toxicity pathways, combine all types of in silico, in vitro information linked to existing animal data	Reliance on increased understanding of pathways, perturbations at concentrations relevant to exposure	Proof-of-concept, verification studies. Staff training.
Impact	Focus limited resources on chemicals with greatest potential risk, reduce cost and testing.	Provide scientifically relevant data for risk decisions.	Well-informed public will have greater confidence in hazard and risk decisions.



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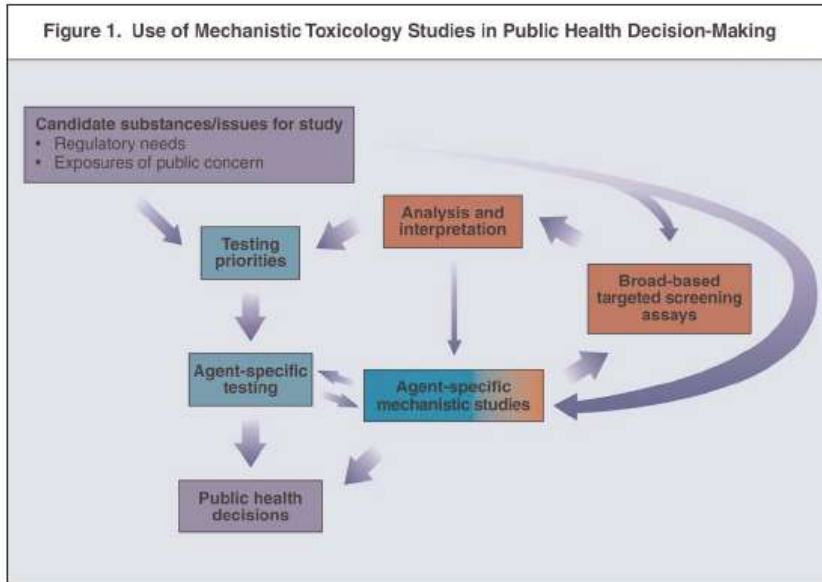
Related US Roadmaps

National Toxicology Program

A National Toxicology Program for the 21st Century, 2004



Figure 1. Use of Mechanistic Toxicology Studies in Public Health Decision-Making



Roadmap Activities: High-Throughput Screening (HTS)

Short-term Activities

- Catalogue available assays
- Convene working groups to provide advice on selection of assays
- Develop assays
- Identify initial set of chemicals for testing

Mid-term Activities

- Continue assay development
- Validate individual assays
- Develop methods for analysis of data
- Develop HTS database
- Review effectiveness

Long-term Activities

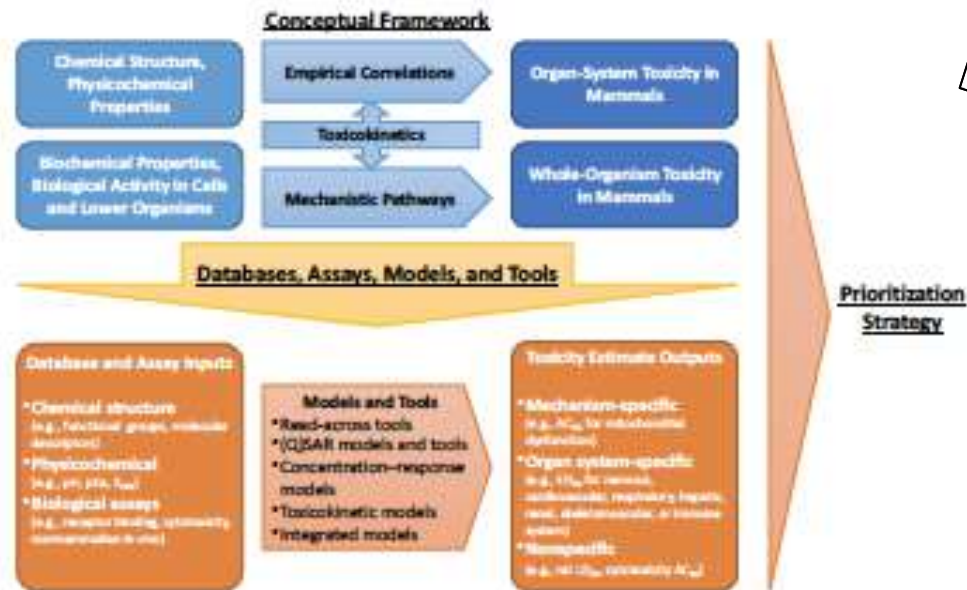
- Develop mechanisms to make chemical sets and tissue banks available for external researchers
- Evaluate HTS data for predictability of toxicity
- Develop a communication plan
- Review effectiveness



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Related US Roadmaps

Department of Defense: Application of Modern Toxicology Approaches for Predicting Acute for Military Assessments of Acute Exposures



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Related US projects

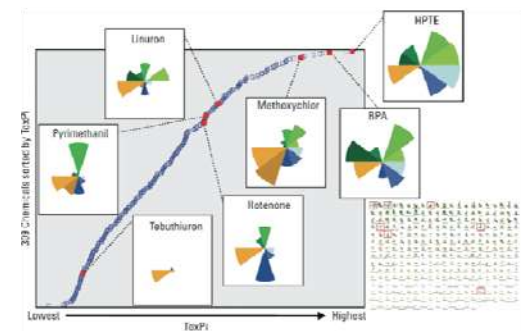
US Government

EPA: ToxCast

- High-throughput data generation
- With industry partners
- ~ 800 in vitro assays, thousands of endpoints
- ~300 pathways
- ~3000 chemicals at ~10 concentrations
- All data publically available
- Application to US EDSP: E, A and T pathways

Tox21: NIH/EPA/FDA

- Screening 10,000 chemicals, including drugs
- At the NIH Center for Advancing Translational Science using innovative robotic technology



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Pathway-related projects

US Government, cont.

“Human on a Chip”: DARPA/NIH/FDA

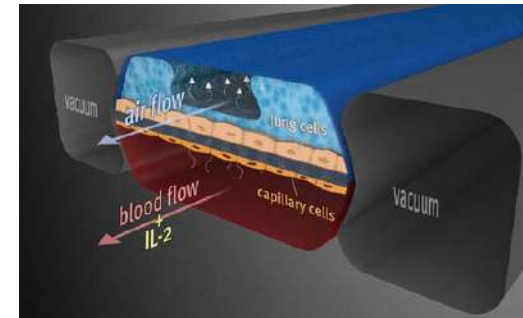
- \$132 million over 5 years to universities
- lung, liver, intestine, heart, brain
- Goal is 10 organs in 5 years

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

- Endocrine and developmental pathway development
- Assay development and evaluation

EPA: Mid-Atlantic division

- QSARs, AOPs for aquatic toxicity
- Estrogen receptor-mediated reproductive impairment
- Aromatase inhibition-mediated reproductive impairment



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Pathway-related projects

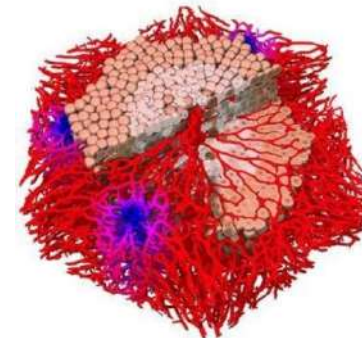
US Government, cont.

EPA Office of Research and Development

- Virtual liver
- Virtual embryo

US Army Corps of Engineers

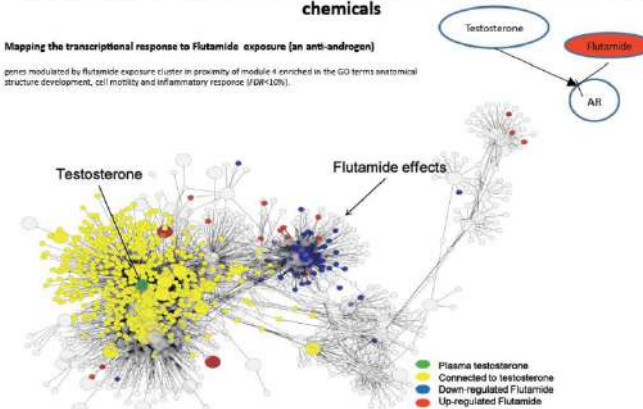
- AOPs for ecotoxicology
- Aromatase inhibition
- androgen agonism
- HTG axis
- Chemical-specific case studies



A fish ovary molecular network tool to assess reproductive mode of toxicity of chemicals

Mapping the transcriptional response to Flutamide exposure (an anti-androgen)

genes modulated by flutamide exposure cluster in proximity of module 4 enriched in the GO terms anatomical structure development, cell motility and inflammatory response (3/6/10/10).

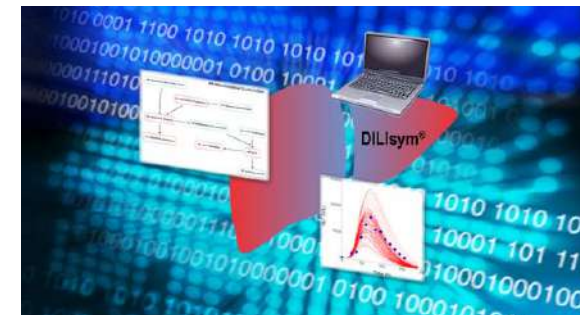
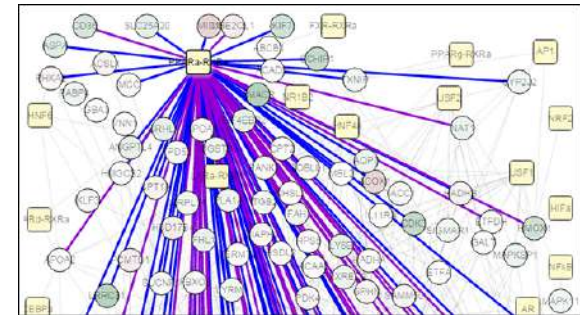


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Pathway-related projects

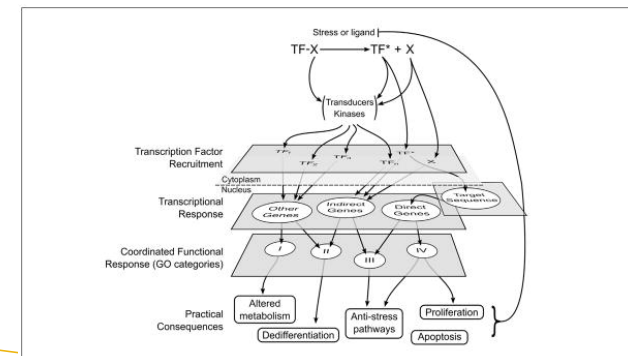
The Hamner Institutes

- “Tier 1 and done”
 - Complete estrogen receptor pathways
- PPAR α network
 - Systems biology approach to complex network interactions
- DiliSym:
 - computer model for drug-induced liver injury



Johns Hopkins School for Public Health Center for Alternatives to Animal Testing

- Pathways of Toxicity
 - “omics” approaches to mapping all pathways
 - Goal of establishing the “Human Toxome”
- Evidence-based toxicology



Pathway-related projects

Organization for Economic Cooperation and Development

Advisory Group on Molecular Screening and Toxicogenomics

- Template for building AOPs, organizing information
- Guidance document on developing and assessing AOP (2013), No. 184 Series Testing and Assessment

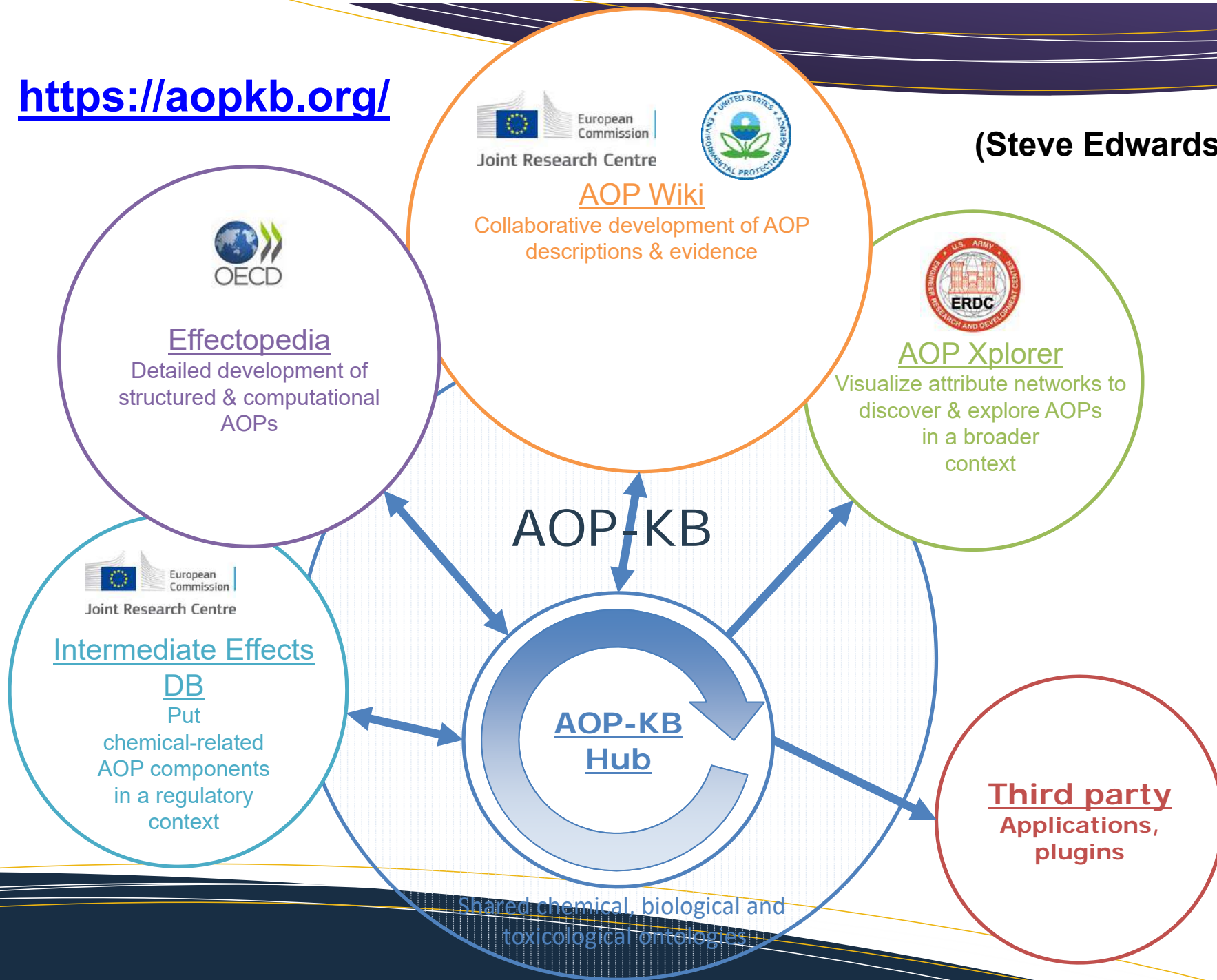


OECD QSAR toolbox

- Large collection of QSAR and SAR models
- databases
- Guidance

<https://aopkb.org/>

(Steve Edwards)





AOP Wiki

Collaborative development of AOP descriptions & evidence

- Qualitative, **text-based descriptions** of an AOP in a structured environment
- Focus is on documenting the weight of **evidence** in support of the AOP
- **Synchronized** with the **OECD** guidance and **handbook** documents
- Online only access to encourage **crowd-sourcing** of AOP development
- Interfaces with the **AOP Xplorer** to provide AOP information in a **network** context

Effect
Detailed &
structured

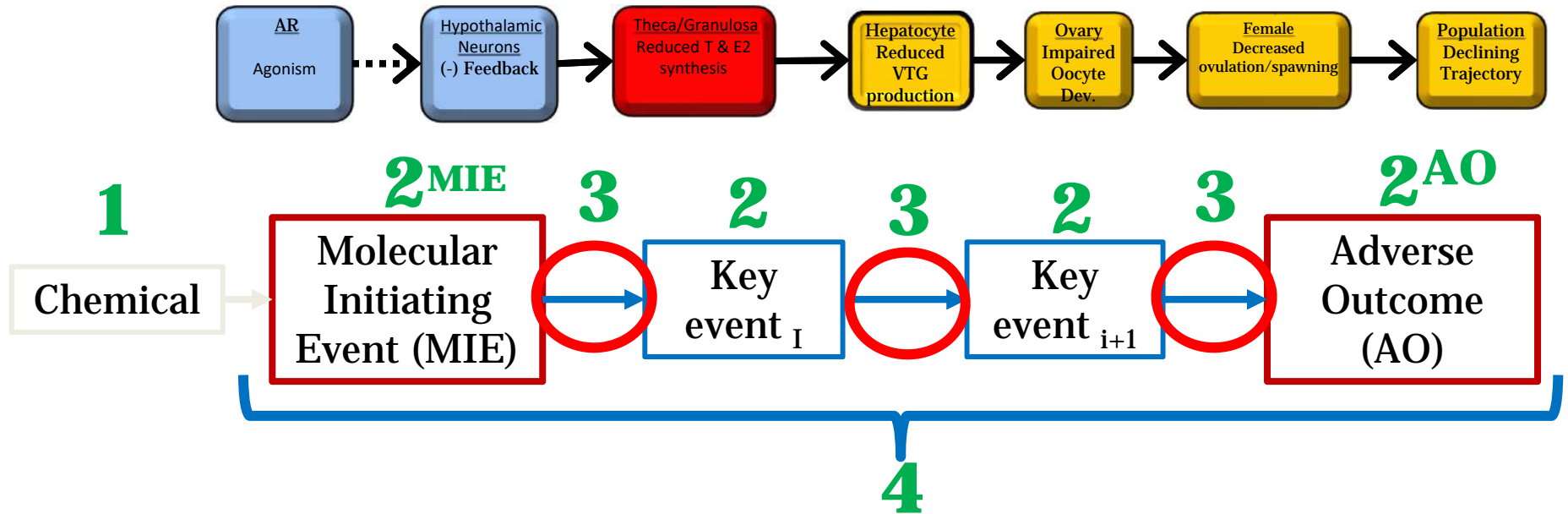
Joint Research Centre
Economic
Commission
Intermed
Effects D
Put
chemical-related
AOP components
in a regulatory
context

Third party
Applications,
plugins

System
Size

Shared chemical, biological and
toxicological ontologies

Structuring and Storing AOP Information



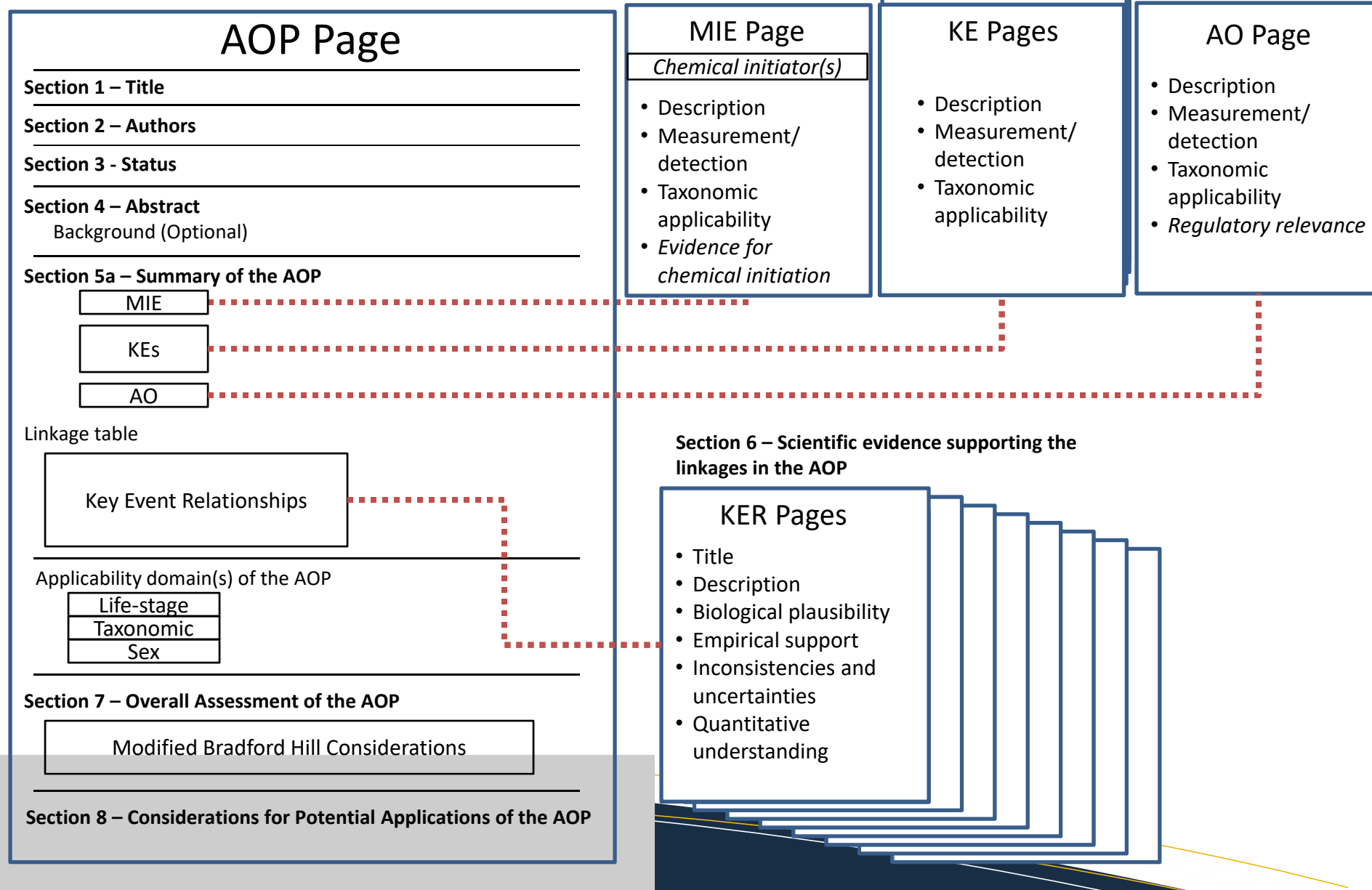
AOP Components are mapped to specific entities in the KB

1. Chemical initiator
2. Key event (nodes)
MIE & AO are special cases of KEs
3. KE Relationship (linkage; edge)
4. AOP

Wiki Matches OECD Guidance & Handbook

https://aopkb.org/common/AOP_Handbook.pdf

Section 5b – MIE, KE, and AO descriptions



AOP Page in Wiki

Adverse Outcome Pathway Wiki

Estrogen receptor agonism leading to reproductive dysfunction

Authors [edit]

Status [edit]

Free Text

Abstract [edit]

The AOP describes the linkage between agonism of the estrogen receptor (ER) and population-relevant impacts in a range of invertebrate vertebrates including amphibians, birds and fish. The information in this AOP for ER agonism does not apply to mammalian species and was not re-evaluated.

Organisms are sensitive to ER agonists during the transformation from zygote to juvenile frog as these include critical periods of metamorphic development and sex differentiation that may be particularly sensitive to endocrine disruption. Larvae exposed to ER agonists during mid-metamorphosis show developmental effects, a subsequent strong hormone-based sex ratio which suggests that transient early life stage exposure to ER agonists can produce effects on the reproductive organs that persist into the beginning of adult life stages. Birds are also known to be sensitive to ER agonists causing disruption of estrogen-regulated functions such as sexual differentiation and sexual behaviour. Model species such as the Japanese quail have been widely used as a model for studying various organ system effects after embryonic exposure to ER agonists. In terms of breast fat, exposure to ER agonists leads to a suite of adverse outcomes depending upon whether exposures occur during or beyond the larval, juvenile and adult stages. For example, aquatic exposure to potent ER agonists during the larval and juvenile life stages may lead to gonadal and liver pathology and testicular atrophy in adult fish (generally 100% females). Larval juvenile and adult male fish exposed to the same ER agonist display abnormal plasma or whole body levels of androgen (VTG), Cumulative Mortality in adult populations are also adversely affected by ER agonists and this is an important endpoint in the OECD Test Guideline 228 Fish Short-Term Reproduction Assay. In summary, this AOP has utility in supporting the application of tiered methods for detecting ER agonists, or in other predictions of the biological effects of ER agonists and in the broader context of endocrine-disrupting chemicals.

Structured Content

Summary of the AOP

Molecular Initiating Event

Add Molecular Initiating Event to Table

Molecular Initiating Event	Support for Essentiality
Estrogen receptor agonism	Strong

Key Events

Add Event to Table

Event	Support for Essentiality
Cumulative mortality and spawning reduction	Strong
Estrogen receptor agonism	Strong
Plasma vitellogenin concentrations increase	Strong
Vitellogenin synthesis in liver increase	Strong
Renal pathology due to VTG deposition increase	Strong

Adverse Outcome

Add Adverse Outcome to Table

Adverse Outcome	Support for Essentiality
Population mortality decrease	Strong
Reproductive behaviour altered	Strong
Larval development altered	Strong
Reproductive organs impaired development of	Strong

Key Events

Add Event to Table

Event	Support for Essentiality
Cumulative mortality and spawning reduction	Strong
Estrogen receptor agonism	Strong
Plasma vitellogenin concentrations increase	Strong
Vitellogenin synthesis in liver increase	Strong
Renal pathology due to VTG deposition increase	Strong

Adverse Outcome

Add Adverse Outcome to Table

Adverse Outcome	Support for Essentiality
Population mortality decrease	Strong
Reproductive behaviour altered	Strong
Larval development altered	Strong
Reproductive organs impaired development of	Strong

Relationships among Key Events and the Adverse Outcome

Add record to table

Step	Event	Description	Triggers	Weight of Evidence	Quantitative Understanding
1	Estrogen receptor agonism	Directly Leads to	Reproductive organs, impaired development of	Strong	Strong
4	Renal pathology due to VTG deposition increase	Directly Leads to	Larval development, Altered	Strong	Strong
1	Estrogen receptor agonism	Directly Leads to	Vitellogenin synthesis in liver, increase	Strong	Strong
3	Plasma vitellogenin concentrations increase	Directly Leads to	Renal pathology due to VTG deposition, increase	Strong	Strong
4	Estrogen receptor agonism	Directly Leads to	Reproductive behaviour, Altered	Strong	Strong
2	Vitellogenin synthesis in liver increase	Directly Leads to	Plasma vitellogenin concentrations, increase	Strong	Strong

Structured Content

Click nodes or edges.

Life Stage Applicability

Add Life Stage to table

Life Stage	Evidence	Links
Juvenile	Strong	<input checked="" type="checkbox"/> <input type="checkbox"/>
Embryo	Strong	<input checked="" type="checkbox"/> <input type="checkbox"/>

Taxonomic Applicability

Add Species from list

Name	Scientific Name	Evidence	Links
Fathead minnow	<i>Pimephales promelas</i>	Strong	<input checked="" type="checkbox"/> <input type="checkbox"/>
Japanese quail	<i>Coturnix coturnix</i>	Strong	<input checked="" type="checkbox"/> <input type="checkbox"/>
Northern leopard frog	<i>Rana pipiens</i>	Strong	<input checked="" type="checkbox"/> <input type="checkbox"/>
Medaka	<i>Oryzias latipes</i>	Strong	<input checked="" type="checkbox"/> <input type="checkbox"/>
Zebrafish	<i>Danio rerio</i>	Strong	<input checked="" type="checkbox"/> <input type="checkbox"/>

Sex Applicability

Add a Sex to table

Sex	Evidence	Links
Male	Strong	<input checked="" type="checkbox"/> <input type="checkbox"/>

Graphical Representation

Click to download template for graphical representation

Free Text

Overall Assessment of the AOP [edit]

In terms of the criteria associated with Key Events in the AOP, the following observations have been made as shown in parentheses:

1. concordance of dose-response relationships? [There is a strong dose-response relationship concordance over a wide range of experimental studies using ER agonists in well-defined animal models, including amphibians, birds and fish].
2. temporal concordance among the key events and adverse effect? [There is strong temporal concordance from partial and full life-cycle studies using ER agonists in well-defined animal models].
3. strength, consistency, and specificity of association of adverse effect and initiating event? [In fish, there is a strong and consistent association between ER agonist exposure, disruption of sexual development and reproductive dysfunction. The same is true for amphibians and birds although the published studies are less numerous].
4. biological plausibility, coherence, and consistency of the experimental evidence? [For the vertebrate species frequently studied to date, there is a high level of biological plausibility, coherence, and consistency across the published experimental evidence].
5. alternative mechanisms that logically pre-empt themselves and the extent to which they may distract from the postulated AOP? [Other mechanisms of relevance to estrogen-mediated sexual development include the disruption of the neuroendocrine pathways (eg see the AOP for aromatase inhibition in fish) and this alternative AOP should be considered alongside ER agonism in the context of elevated plasma VTG levels, disrupted sexual development of reproductive dysfunction. The possibility of other AOPs arising should be kept in mind through critical analysis of the updated pre-reviewed literature].
6. uncertainties, inconsistencies and data gaps? [An important aspect of uncertainty is quantifying the degree to which disrupted sexual development leads to a population-relevant impact via reproductive dysfunction. Experimental and validated population modeling is a key need to address the data gap and uncertainty. In the authors' view, there are no major scientific inconsistencies with regard to the ER agonist AOP and associated Key Events].

Weight of Evidence Summary [edit]

Summary Table

Provide an overall summary of the weight of evidence based on the evaluations of the individual linkages from the Key Event Relationship pages

Essentiality of the Key Events [edit]

Molecular Initiating Event Summary, Key Event Summary

Provide an overall assessment of the essentiality for the key events in the AOP. Support calls for individual key events can be included in the molecular initiating event, key event, and adverse outcome tables above.

Quantitative Considerations [edit]

Summary Table

Provide an overall discussion of the quantitative information available for this AOP. Support calls for the individual relationships can be included in the Key Event Relationship table above.

Applicability of the AOP [edit]

Life Stage Applicability, Taxonomic Applicability, Sex Applicability

In terms of the taxonomic domain of applicability, exposure to ER agonists is capable of disrupting sexual development and causing reproductive dysfunction in vertebrate species such as amphibians, birds and fish (see examples of peer-reviewed literature cited below).

References [edit]

Dang, Z., Traas, T., Vemere, T. (2011) Evaluation of the fish short-term reproduction assay for detecting endocrine disruptors. *Chemosphere* 85: 1692-1693

Haldon, K., Axelsson, J., Brunstrom, B. (2005) Effects of endocrine modulators on sexual differentiation and reproductive function in male Japanese quail. *Brain Research Bulletin* 65: 211-218

Hogan, N.S., Duarte, P., Wade M.G., Lean D.R.S., Trudeau, V.L. (2000) Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (*Rana pipiens*): identifying critically vulnerable periods of development. *General and Comparative Endocrinology* 156: S15-S23

Hutchinson T.H. (2002) Impacts of endocrine disruptors on fish development: opportunities for adopting OECD Test Guideline 210. *Environmental Sciences* 9: 439-450

Länge R., Hutchinson T.H., Crundace C.P., Segmund P., Schwemfurn H., Hampe P., Paster G.H., Sunzter J.P. (2001) Effects of the synthetic oestrogen 17- α -ethynylestradiol over the life-cycle of the fathead minnow. *Environmental Toxicology and Chemistry* 20: 1216-1227

Lane, N.L., Jensen K.M., Kinley, G.T. (2005) Gonadal histology and characteristic hepatopathy associated with endocrine disruption in the adult fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Pharmacology* 19: 65-80

Ottinger, M.H., Carr, T., Bahannon, M., Bates, L., Maroek, A.M., Mookman, M., Dean, K.H., Laviec, E., Abbenashi, M. (2013) Assessing effects of environmental chemicals on endocrine systems: Potential mechanisms and functional outcomes. *General and Comparative Endocrinology* 195: 194-202

Categories: Adverse Outcome Pathway | Pages with broken file links

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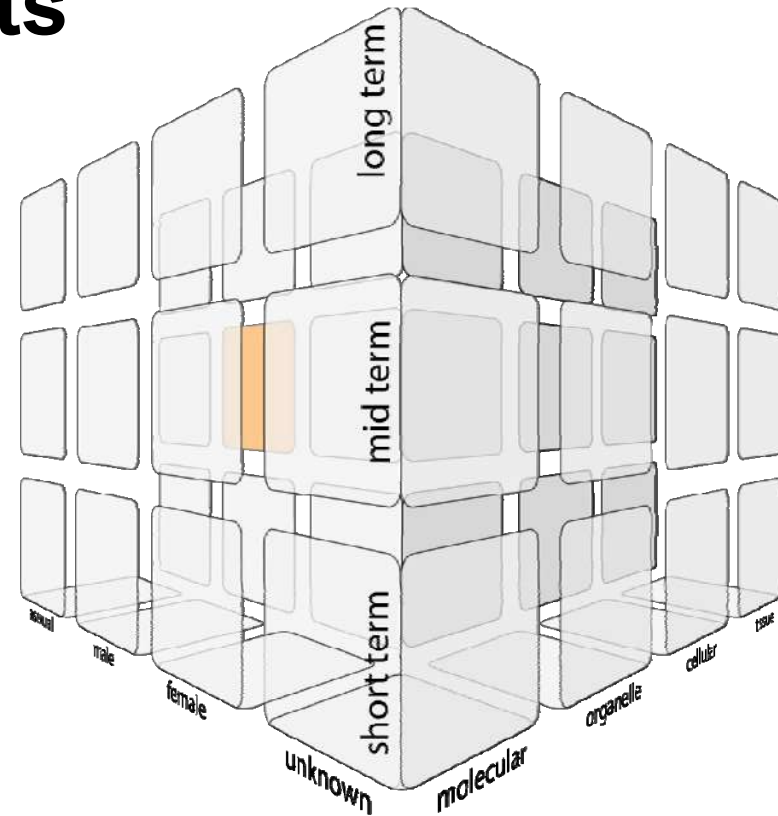
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Pathway-related projects

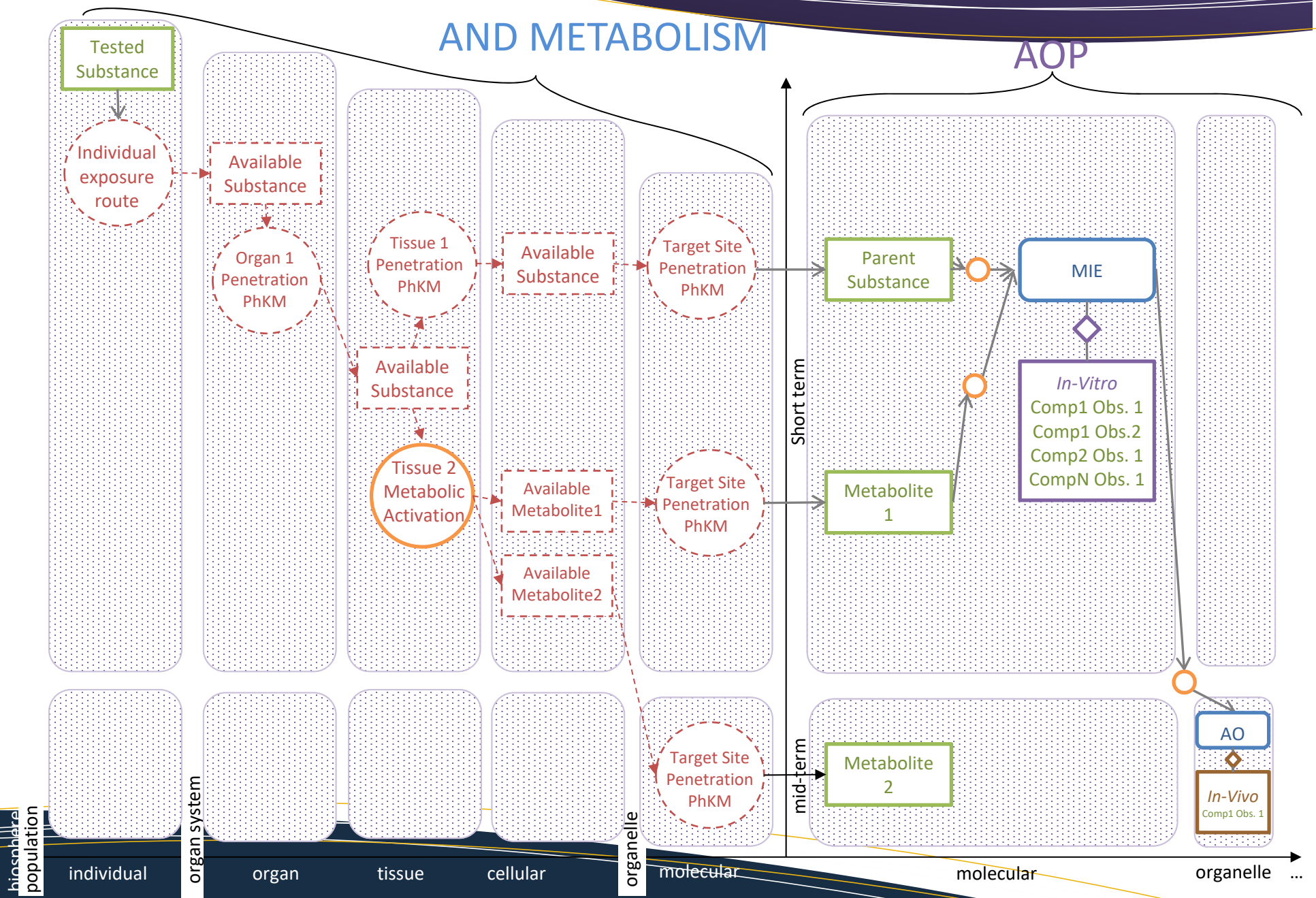
Effectopedia

- Life stage
- Taxonomy
- Gender
- Generation
- Time to effect
- Level of biological organization
- ...



User expandable set of biological context dimensions
Interface allows easy switching between pathway space 2D
projections

FUTURE PENETRATION MODELS AND METABOLISM



US Funding

NIH: worlds largest medical research institution

“mission is to seek fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to enhance health, lengthen life, and reduce illness and disability.”

- 30.1 billion USD annually
 - 27 institutes and centers
 - 80% to fund 50,000 competitive grants
- NCATS: \$635,710,000 FY 15
- NIEHS: \$665,080,000 FY 15
 - Small number of competitive grants to fund non-animal method development and validation (new)

US Funding

US EPA: 7.89 billion USD (FY2015)

- Science and Technology: 7.64 billion
- Chemical safety: \$672,918,000 FY 15
- Chemical Safety and Sustainability, Human Health Risk Assessment, and Homeland Security Research programs: \$162,600,000 FY 15
 - ToxCast
 - Research into non-animal methods, mechanisms of action, QSAR, modeling

HSI/HSUS Efforts through the Humane Society Legislative Fund

Focus has been on shifting appropriations within existing programs to prioritize funding for non-animal approaches to toxicological assessment:

- Senate and House FY15 Labor HHS Appropriations Report Language:
 - To Office of the Director - Supporting High Throughput Screening, Toxicity Pathway Profiling, and Biological Interpretation of Findings National Institutes of Health
- House FY15 Department of the Interior, Environment and Related Agencies Report Language
 - Research: Chemical Safety and Sustainability — rejecting decreases in funding new methods
- Endocrine Disruptor Research — supporting implementing the toxicity testing agenda that the 2007 National Academy of Sciences (NAS) report on Toxicity Testing in the 21st Century puts forth

Conclusions

The US has aligned strategic plans within:

- NIH, EPA, DARPA
- Based on 2007 NRC report

International collaboration is essential:

- To tackle the magnitude of the project
- For sharing/harmonized use of information

Potential funding is only partially tapped for the transition

Potential funding for a similar approach to disease and medicine is available through NIH's 17 institutes

Public/private partnerships, so successful in the EU, are underutilized in the US