High Throughput Screening and Environmental Risk Assessment: Viewing the Forest and a Tree

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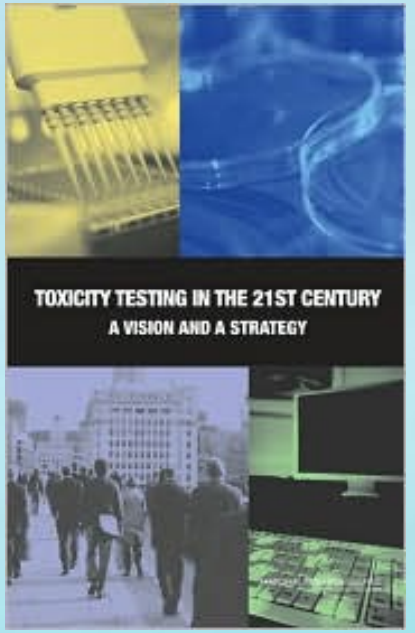
** The contents of this presentation neither constitute nor necessarily reflect US EPA policy*

Vision

"Transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin"

"The vision emphasizes the development of suites of predictive, high-throughput assays ….."

"The mix of tests in the vision include tests that assess critical mechanistic endpoints involved in the induction of overt toxic effects rather than the effects themselves."



Introduce participants to the HTS data sources, tools, and resources to aid their interpretation

Provide examples of current and emerging applications of HTS data in different regulatory/risk assessment contexts

Stimulate innovation that can further enhance the utility of HTS data for environmental risk assessment applications

High-Throughput Screening and Environmental Risk Assessment

STATE OF THE SCIENCE AND EMERGING APPLICATIONS

SETAC North America Focused Topic Meeting
16–18 APRIL 2018 | DURHAM, NC, USA

Outline

- Toxicity testing in the 21st century – six critical needs
- Perspectives on progress related to the critical needs
- Application of HTS, state of the science in 2018
- Opportunities for impact – moving forward

Outline

Macro perspective on how the field has evolved

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12
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Critical Perspective

High-Throughput Screening and Environmental Risk Assessment: State of the Science and Emerging Applications

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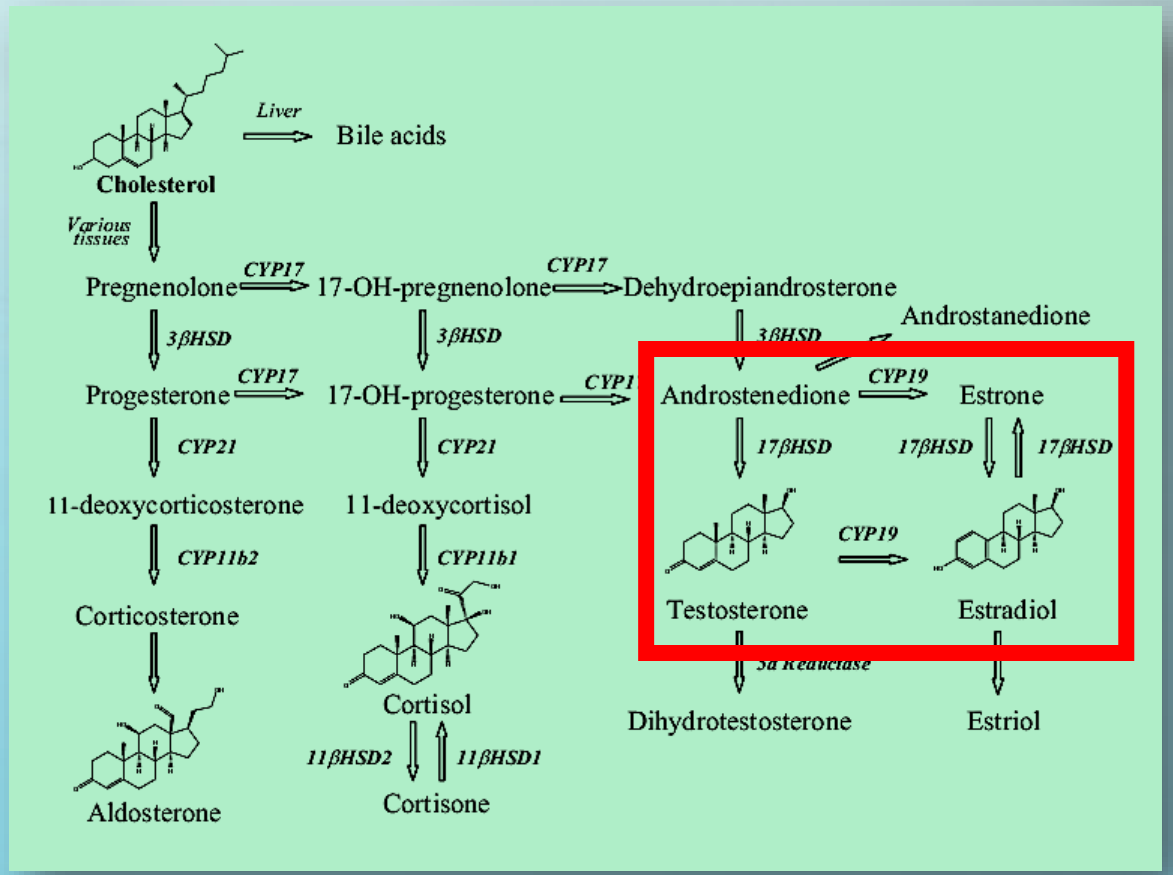
[f]Society of Environmental Toxicology and Chemistry, Durham, North Carolina, USA
[g]Environmental Laboratory, US Army Engineer Research and Development Center, Vicksburg, Mississippi

As informed by presentations, discussions, and follow up from 2018 FTM

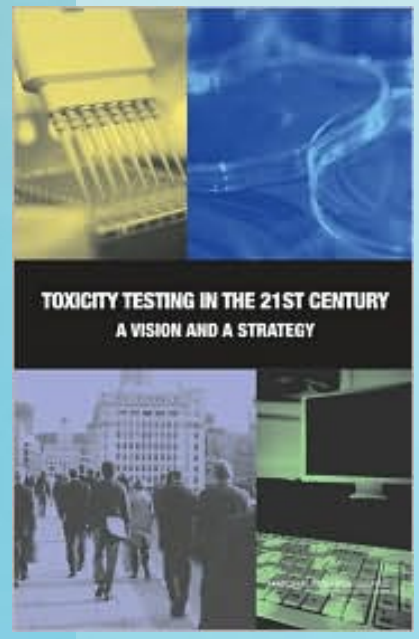
Outline

- Specific example reflecting progress over a decadal scale research effort.

- Inhibition of the enzyme aromatase (cyp19) – mode of endocrine disruption
- Use HTS assays/data & AOP
  - identify aromatase inhibitors,
  - understand their apical hazards
  - predict what exposures are likely to produce adverse effects
  - Identify assays/endpoints to confirm predicted effects



Six Critical Needs



1) development of appropriate suites of HTS assays

2) availability of targeted in vivo data to complement and provide an interpretive context for the HTS results

3) computational extrapolation models that could predict which exposures may result in adverse changes

4) infrastructure to support the basic and applied research to develop the assays and models

5) validation of the assays and data for incorporation into guidance regarding their interpretation and use

6) evidence that the new approaches are adequately predictive of adverse outcomes

1) Development of appropriate suites of HTS assays

ToxCast™

Tox21

- Over 1500 assay endpoints in the latest public release
- Commercial assays
- Targeted assay development to fill gaps
- Biological and chemical coverage continues to expand

1) Development of appropriate suites of HTS assays

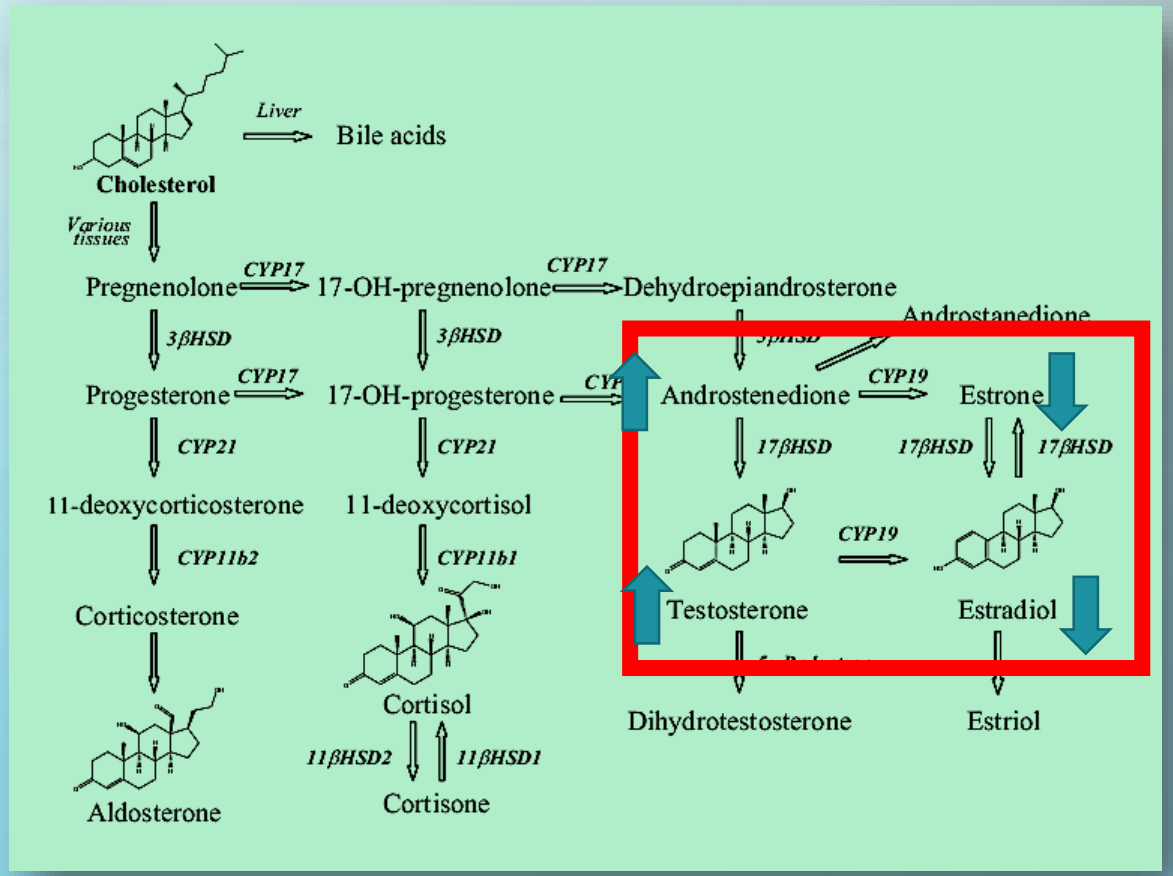
TOX21_Aromatase_Inhibition

- Luciferase reporter gene under control of ERE, loss of signal
- AI blocks endogenous E2 production

NVS_ADME_hCYP19A1

- Enzyme activity assay
- Aromatase inhibition = less production of fluorescent product

CEETOX_H295R_ESTRADIOL_dn
CEETOX_H295R_ESTRONE_dn
CEETOX_H295R_TESTOSTERONE_up
CEETOX_H295R_ANDR_up



2) Aggregation of in vivo data to complement and provide an interpretive context for the HTS results

EPA United States Environmental Protection Agency | Home | Advanced Search | Batch Search | Lists | Predictions | Downloads | Copy | Share | Submit Comment | Search all data

Phenol

108-95-2 | DTXSID5021124

Searched by DSSTox Substance Id.

- DETAILS
- EXECUTIVE SUMMARY
- PROPERTIES
- ENV. FATE/TRANSPORT
- HAZARD
- ADME
- EXPOSURE
- BIOACTIVITY
- SIMILAR COMPOUNDS
- GENRA (BETA)
- RELATED SUBSTANCES
- SYNONYMS
- LITERATURE
- LINKS
- COMMENTS

Executive Summary

Quantitative Risk Assessment Values

- IRIS values available
- No PPRTV values
- EPA RSL values available
- Minimum RfD: **0.20 mg/kg-day** (chronic, ACToR, inhalation, 4)
- Minimum RfC: **0.20 mg/m3** (chronic, RSL, inhalation, 7)
- IVIVE POD not calculated

Quantitative Hazard Values

- Minimum oral POD: **1.8 mg/kg-day** (chronic, EFSA, oral, 5)
- Minimum inhalation POD: **19 mg/m3** (chronic, Wignall, inhalation, 3)
- Lowest Observed Bioactivity Equivalent Level: ESR1

Cancer Information

- No cancer slope factor
- No inhalation unit risk value
- Carcinogenicity data available: IARC: undefinedEPA OPP cancer class: undefinedNLM ToxNet HSDB carcinogenicity warningUniversity of Maryland carcinogenicity warning;
- No genotoxicity findings reported

Reproductive Toxicology

- Reproductive toxicity PODs available

Chronic Toxicology

- Chronic toxicity PODs available

Subchronic Toxicology

- Subchronic toxicity PODs available

Developmental Toxicology

- Developmental toxicity PODs available

Acute Toxicology

- Acute toxicity PODs available

Subacute Toxicology

REGIONAL SCREENING

Class	THQ	Value
GIABS (unspecified)	THQ = 1	1
ABS (unspecified)	THQ = 1	0.1
RFDo (mg/kg-day)	THQ = 1	0.3
RFCi (mg/m3)	THQ = 1	0.2
screening level (residential Soil) (mg/kg)	THQ = 1	19000
screening level (industrial soil) (mg/kg)	THQ = 1	250000
screening level (residential air) (ug/m3)	THQ = 1	210
screening level (industrial air) (ug/m3)	THQ = 1	880
screening level (tap water) (ug/L)	THQ = 1	5800
risk-based SSL (mg/kg)	THQ = 1	3.3
GIABS (unspecified)	THQ = 0.1	1
ABS (unspecified)	THQ = 0.1	0.1
RFDo (mg/kg-day)	THQ = 0.1	0.3
RFCi (mg/m3)	THQ = 0.1	0.2
screening level (residential Soil) (mg/kg)	THQ = 0.1	1900
screening level (industrial soil) (mg/kg)	THQ = 0.1	25000
screening level (residential air) (ug/m3)	THQ = 0.1	21

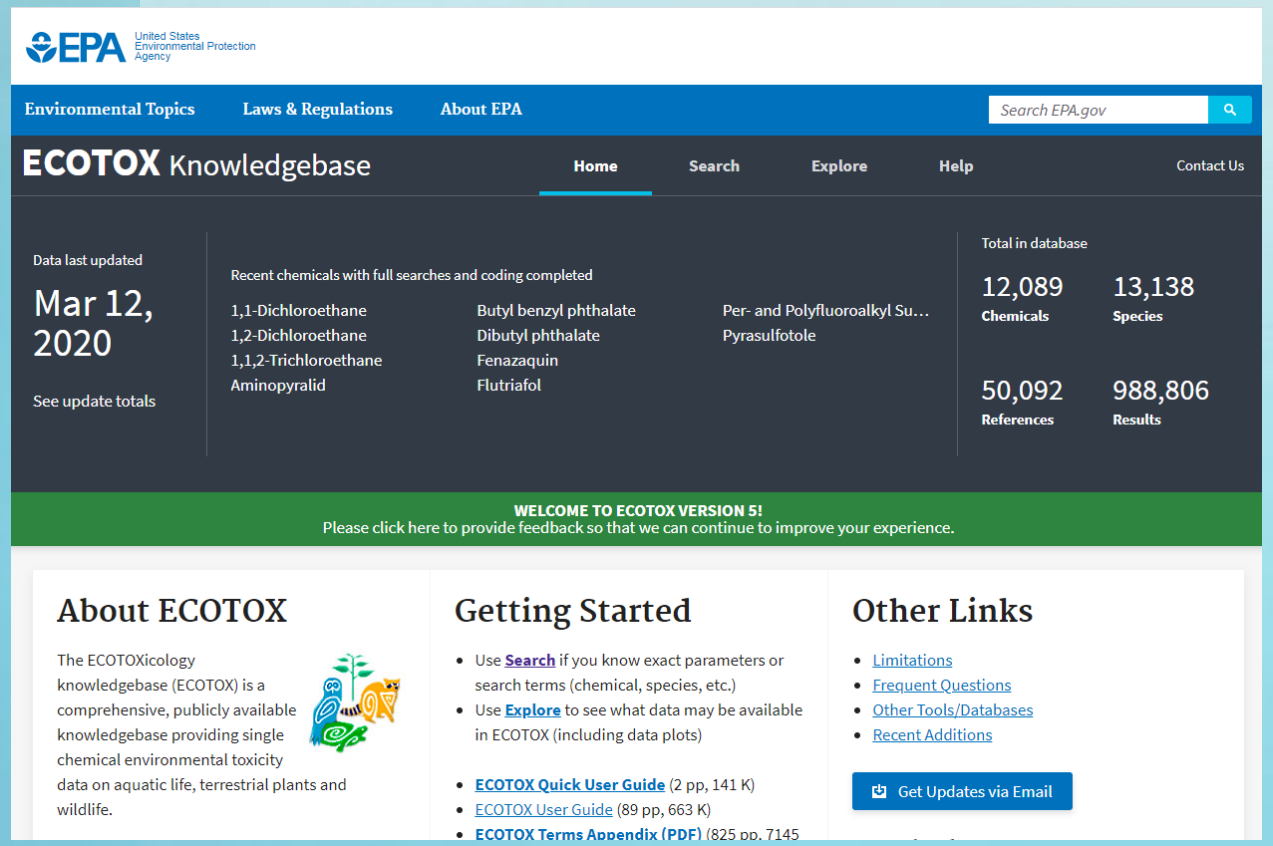
CompTox Chemicals Dashboard

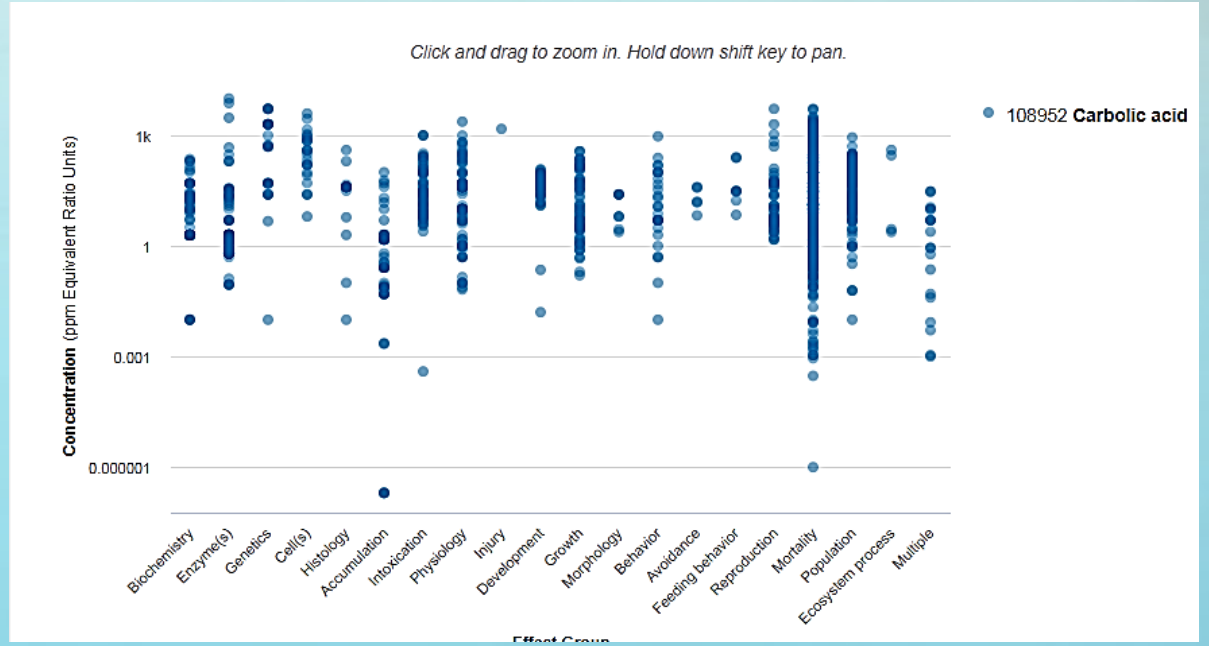
https://comptox.epa.gov/dashboard

Antony “Covid hair” Williams

2) Aggregation of in vivo data to complement and provide an interpretive context for the HTS results

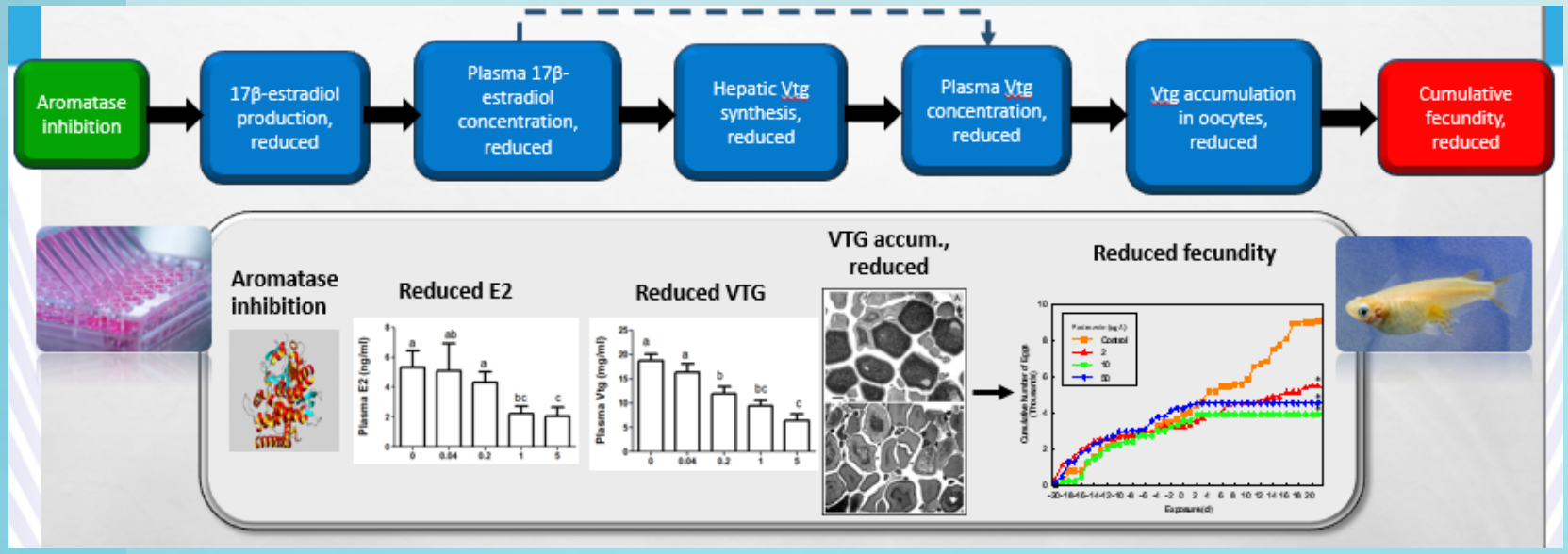
https://cfpub.epa.gov/ecotox/



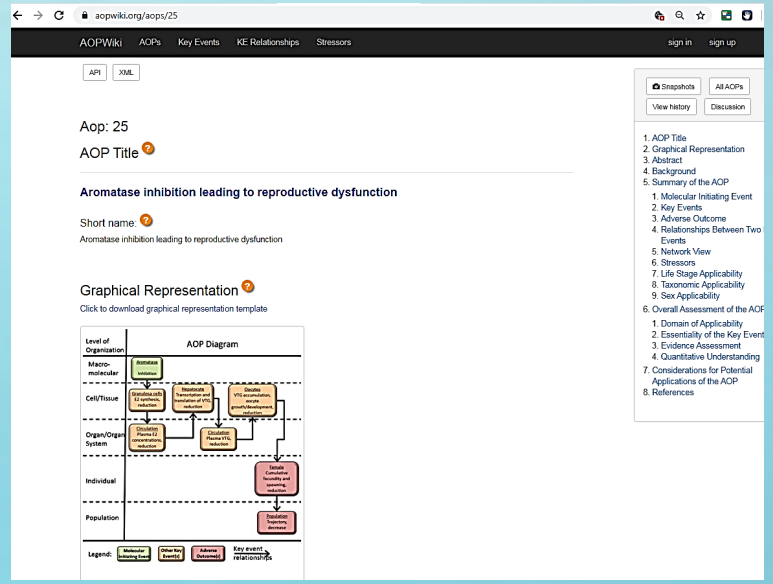


2) Aggregation of in vivo data to complement and provide an interpretive context for the HTS results

Adverse outcome pathway: Aromatase inhibition leading to reproductive impairment

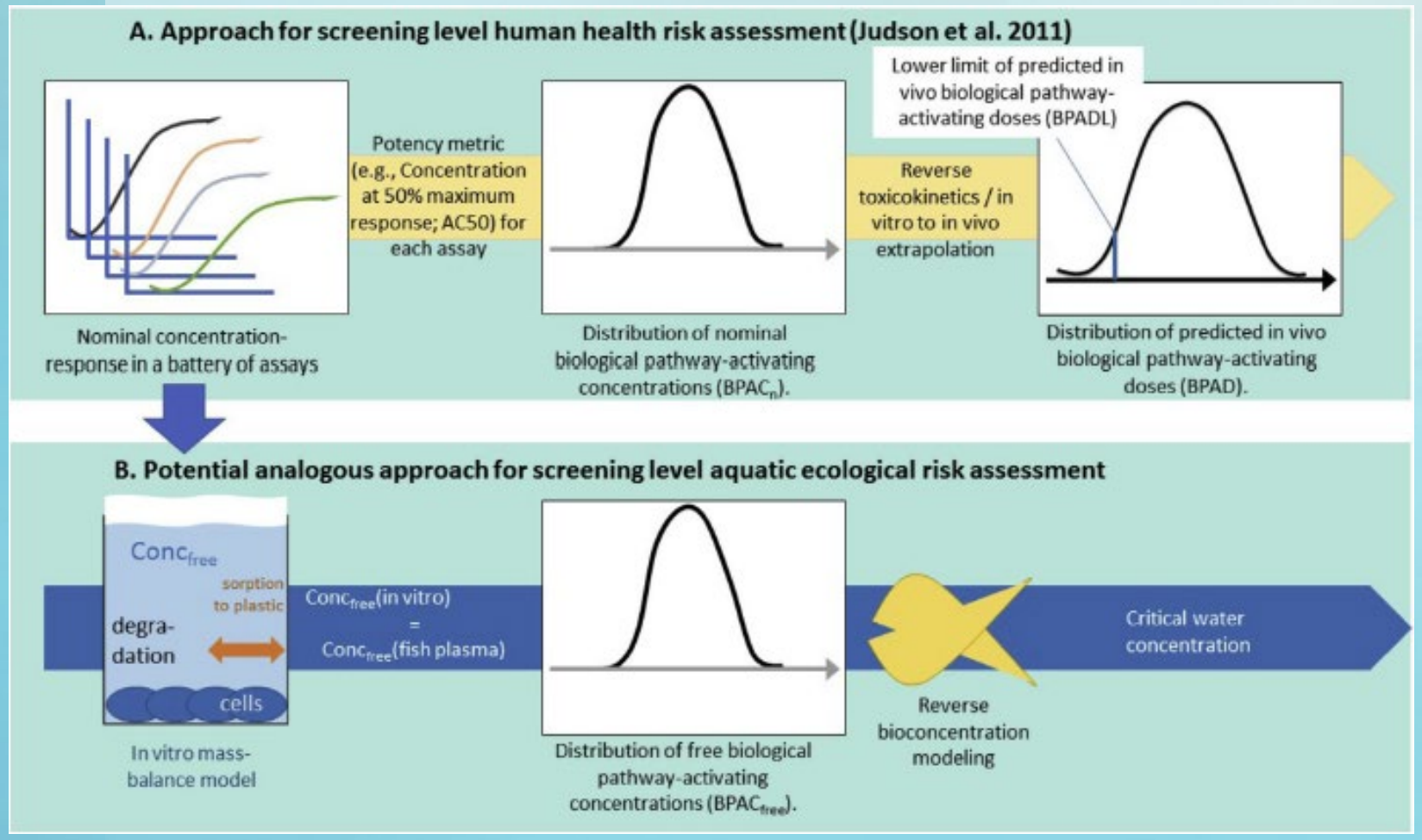


https://aopwiki.org/aops/25



- Simple to follow graphical and narrative format
  - Supported by scientific literature and evidence
  - Searchable, globally accessible, and transparent

3) Computational extrapolation models that could predict which exposures may result in adverse changes

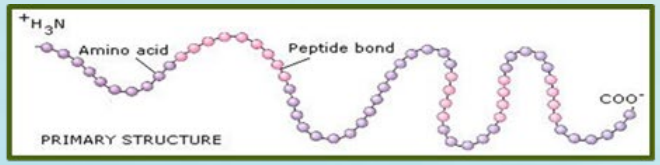


Extrapolating in vitro effect concentration to equivalent human plasma or environmental concentration.

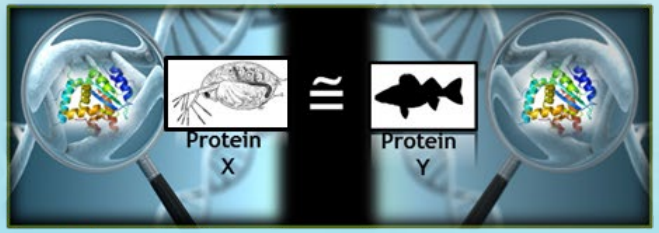
3) Computational extrapolation models that could predict which exposures may result in adverse changes

https://seqapass.epa.gov/seqapass/

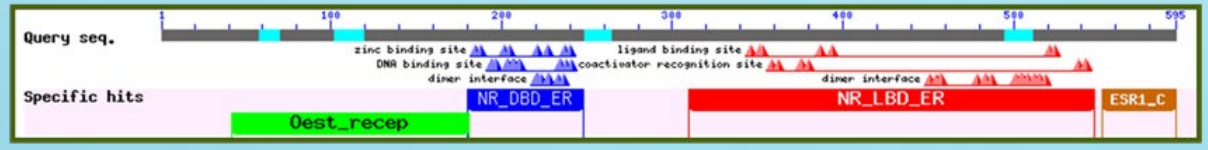
Primary Amino Acid Sequence Alignments



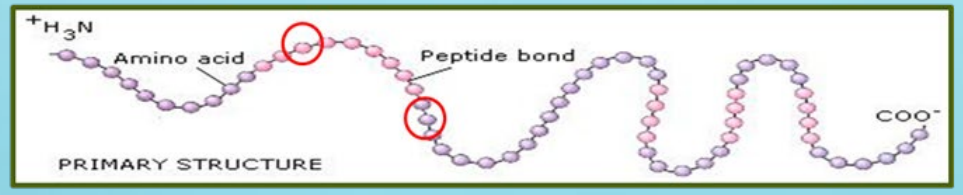
Ortholog Candidate Identification (RBH)



Conserved Functional Domain Alignments



Individual Amino Acid Residue Queries

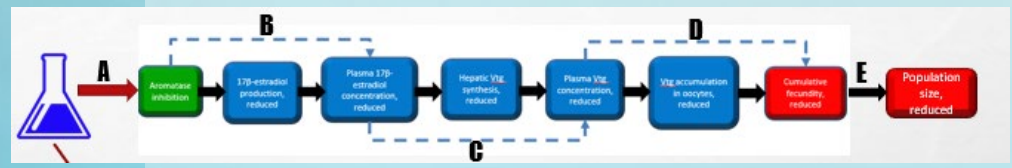


Tertiary Protein Structure Considerations

	Query Protein [b]SeqAPASS Cut-off	CYP19A1 46.79
Vertebrates	Mammalia	Yes *(111 of 121)*
	Actinopteri	Yes *(137 of 147)*
	Amphibia	Yes *(8 of 10)*
	Aves	Yes *(65 of 67)*
	Chondrichthyes	Yes *(4 of 5)*
	Coelacanthiformes	-
	Crocodylia	Yes *(4 of 4)*
	Lepidosauria	Yes *(6 of 6)*
	Myxiniformes	-
	Testudines	Yes *(7 of 7)*
[a]Invertebrates	Anthozoa	No *(0 of 5)*
	Arachnidia	No *(0 of 15)*
	Bivalvia	No *(0 of 10)*
	Branchiopoda	No *(0 of 2)*
	Branchiostomidae	No *(0 of 2)*
	Gastropoda	No *(0 of 6)*
	Insecta	No *(0 of 172)*

3) Computational extrapolation models that could predict which exposures may result in adverse changes

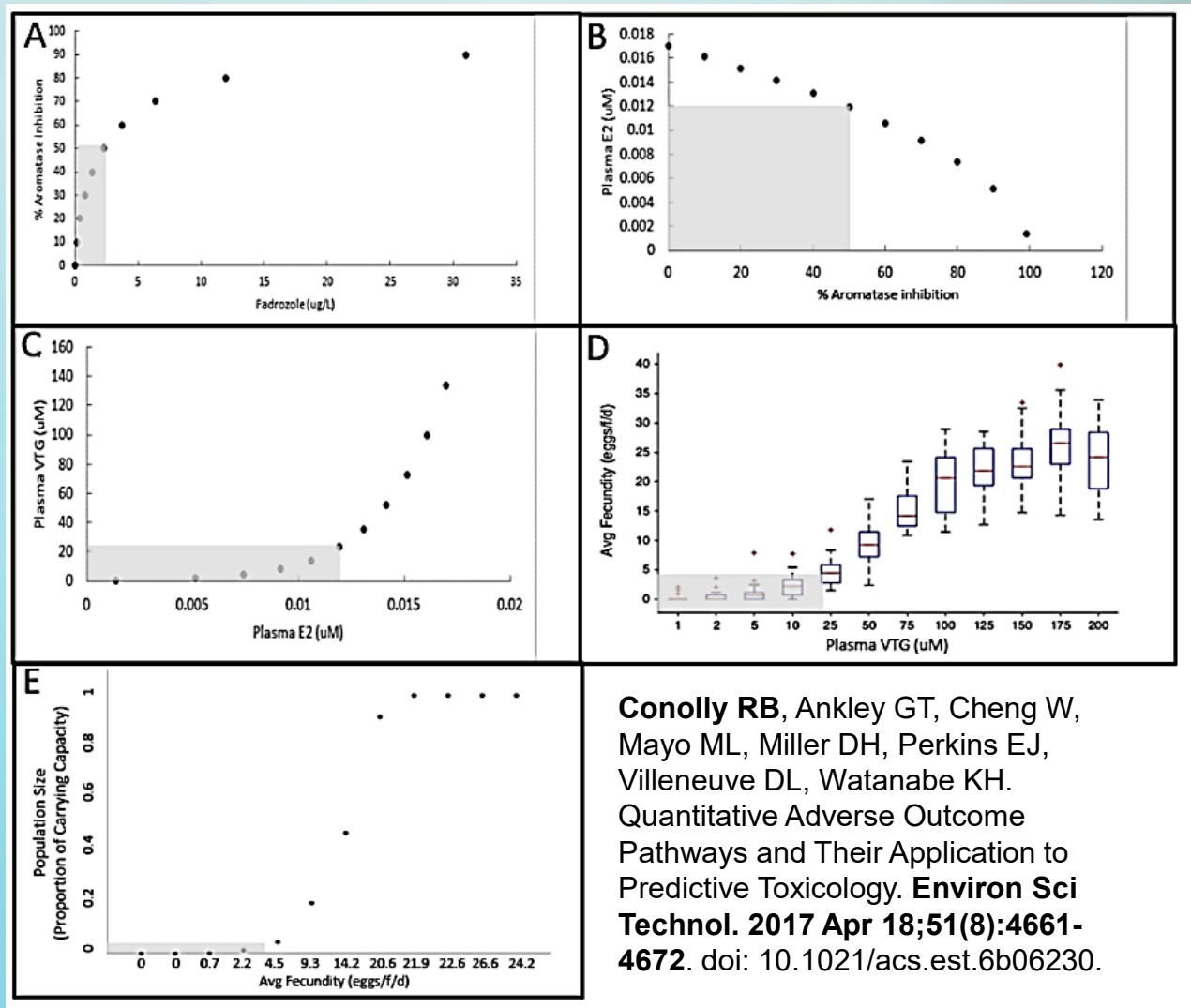
Quantitative AOP construct



HPG axis model (Cheng et al. 2016)

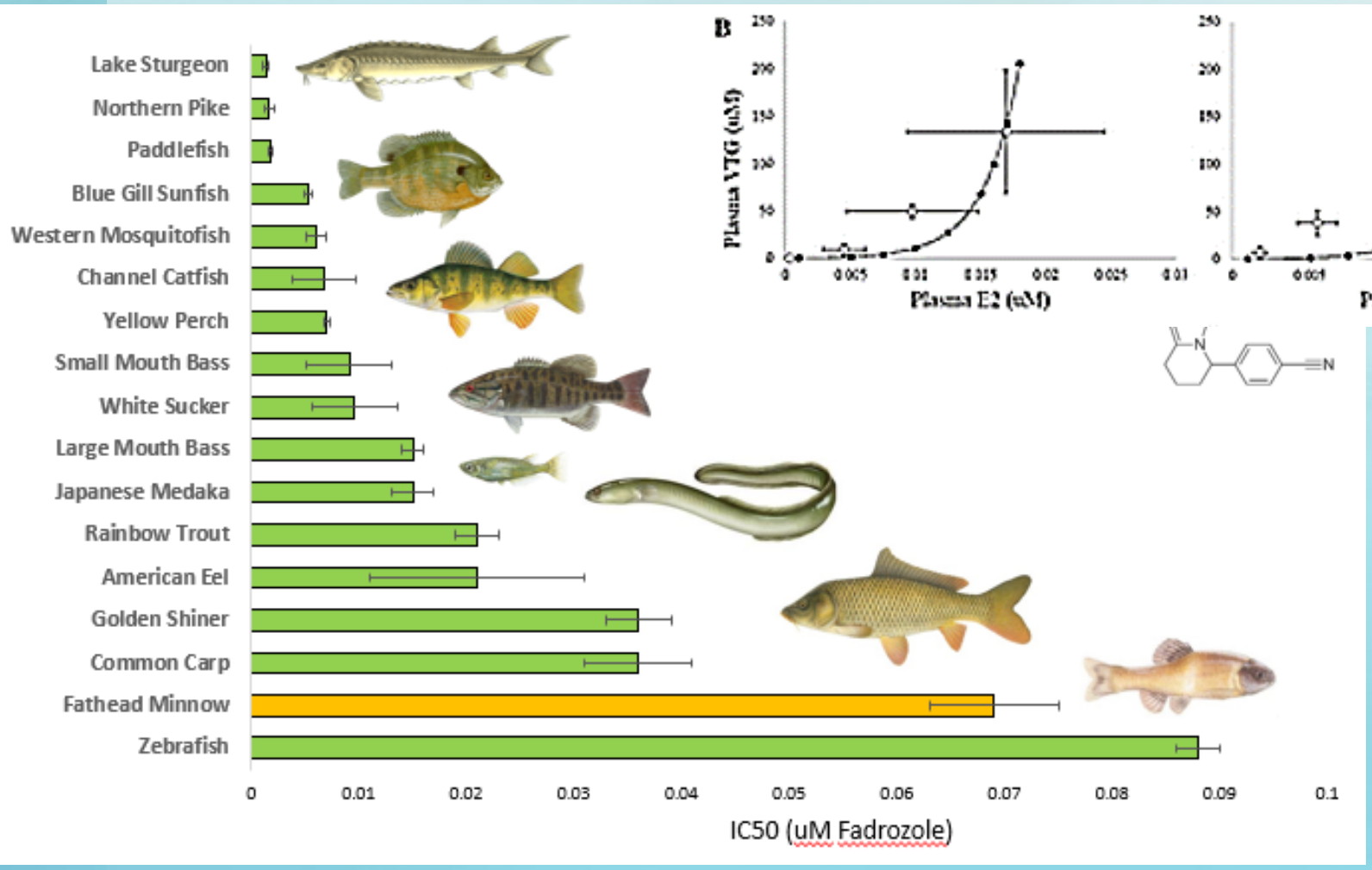
Oocyte growth and dynamics model (Watanabe et al. 2016)

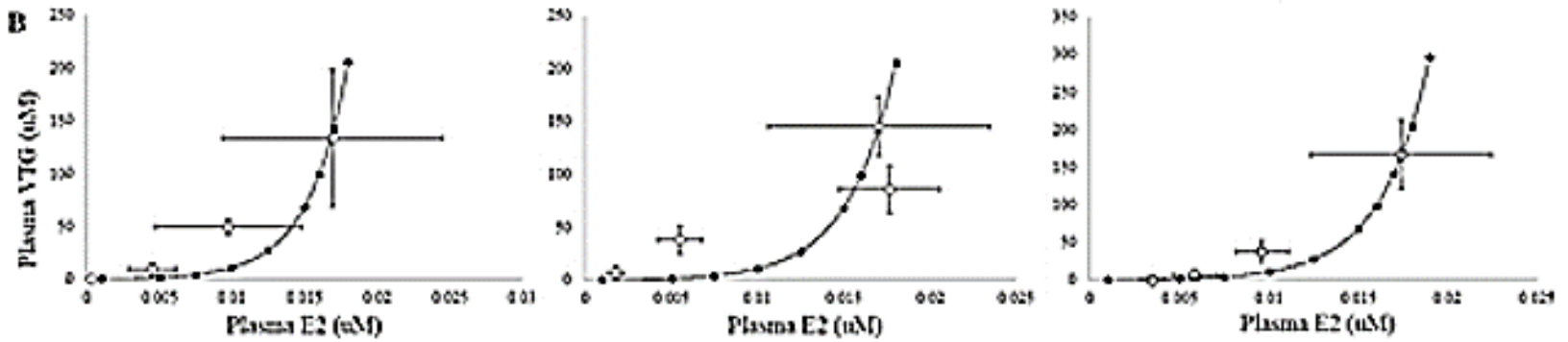
Population matrix model (Miller and Ankley, 2004)



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.

3) Computational extrapolation models that could predict which exposures may result in adverse changes





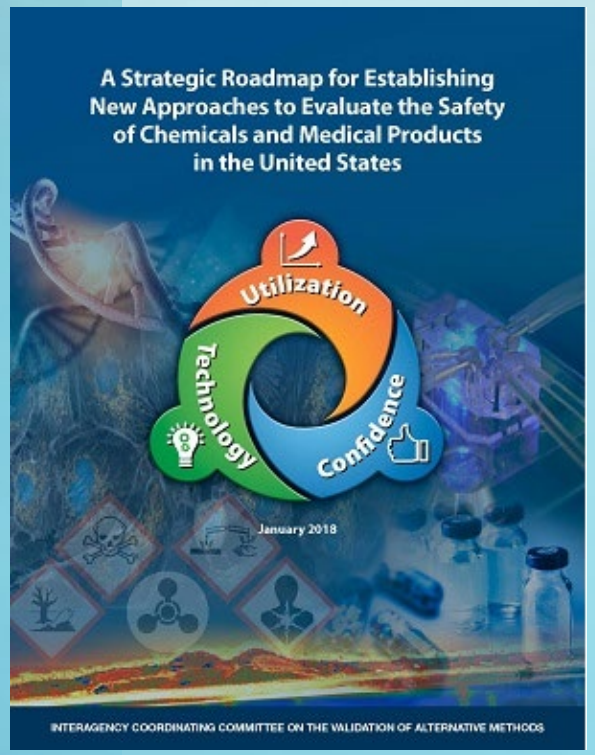
In vitro sensitivity of cyp19a1a from different fish to reference aromatase inhibitor.

Adjusts qAOP model to improve predictive accuracy

Doering et al. 2019, Toxicol Sci 170:394-403; Doering et al. 2019, Environ Sci Technol 53 10470-78

4) Infrastructure to support the basic and applied research to develop the assays and models

Technologies do not implement themselves and obstacles are not resolved without effort (Kleinstreuer)



16 US Federal Agencies

SEURAT-1

Towards the Replacement of in vivo Repeated Dose Systemic Toxicity Testing

s◡luti◡ns

Large scale European Projects and consortia

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

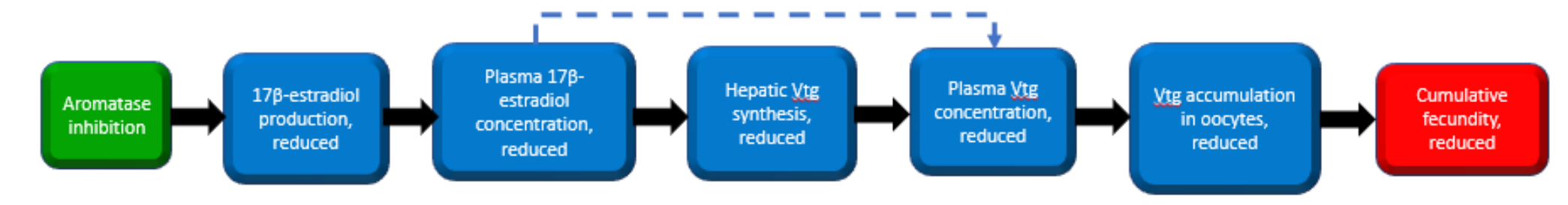
NCCT (≈25) → CCTE (≈250)

Accelerating the Pace of Chemical Risk Assessment

APCRA

International efforts

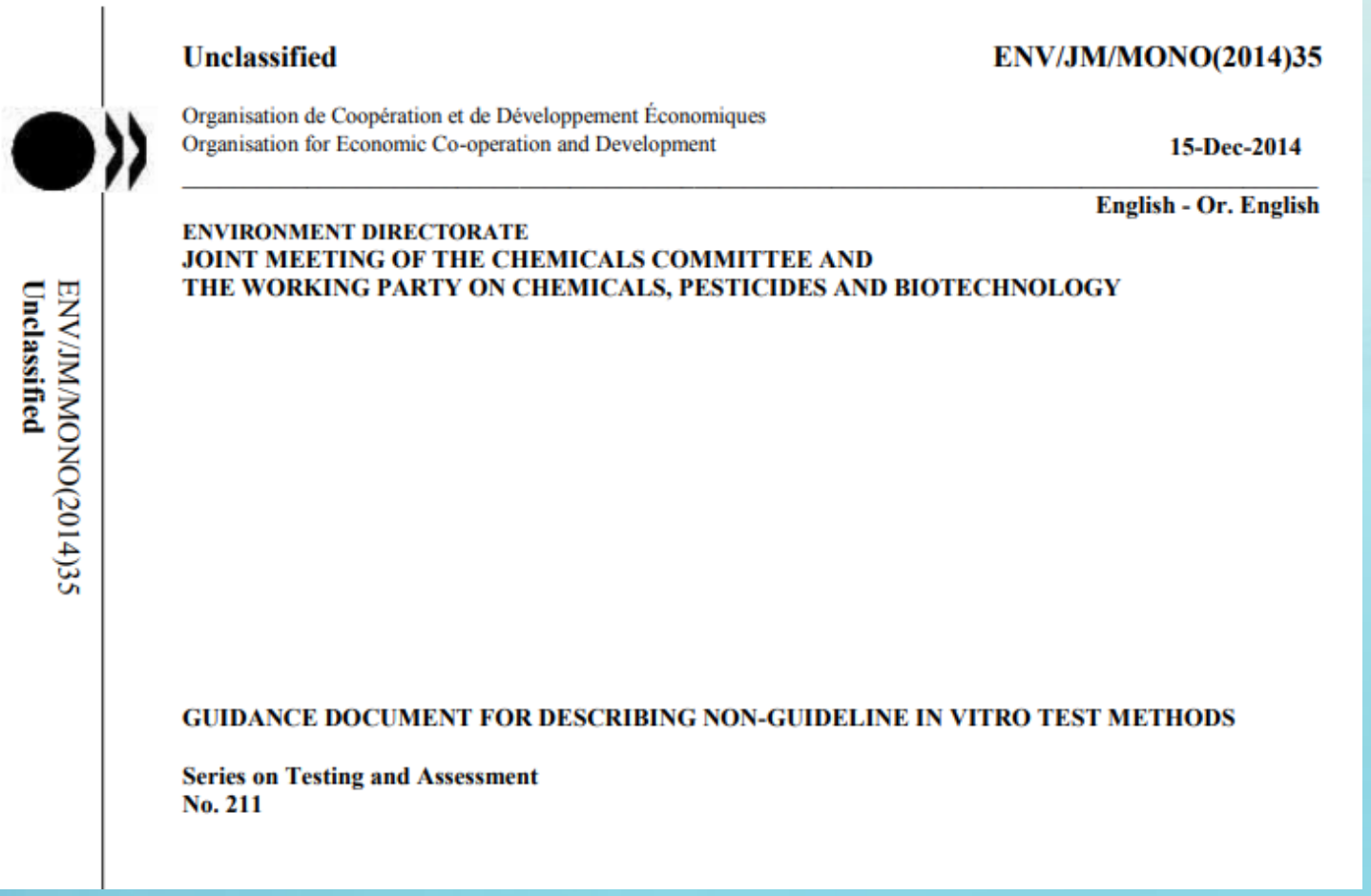
4) Infrastructure to support the basic and applied research to develop the assays and models



- US EPA, ORD – Four divisions
- US Army Corps of Engineers
- Pacific Northwest National Laboratories
- Five academic institutes

- 58 different co-authors

5) Validation of the assays and data for incorporation into guidance regarding their interpretation and use



Unclassified ENV/JM/MONO(2014)35

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

15-Dec-2014

English - Or. English

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

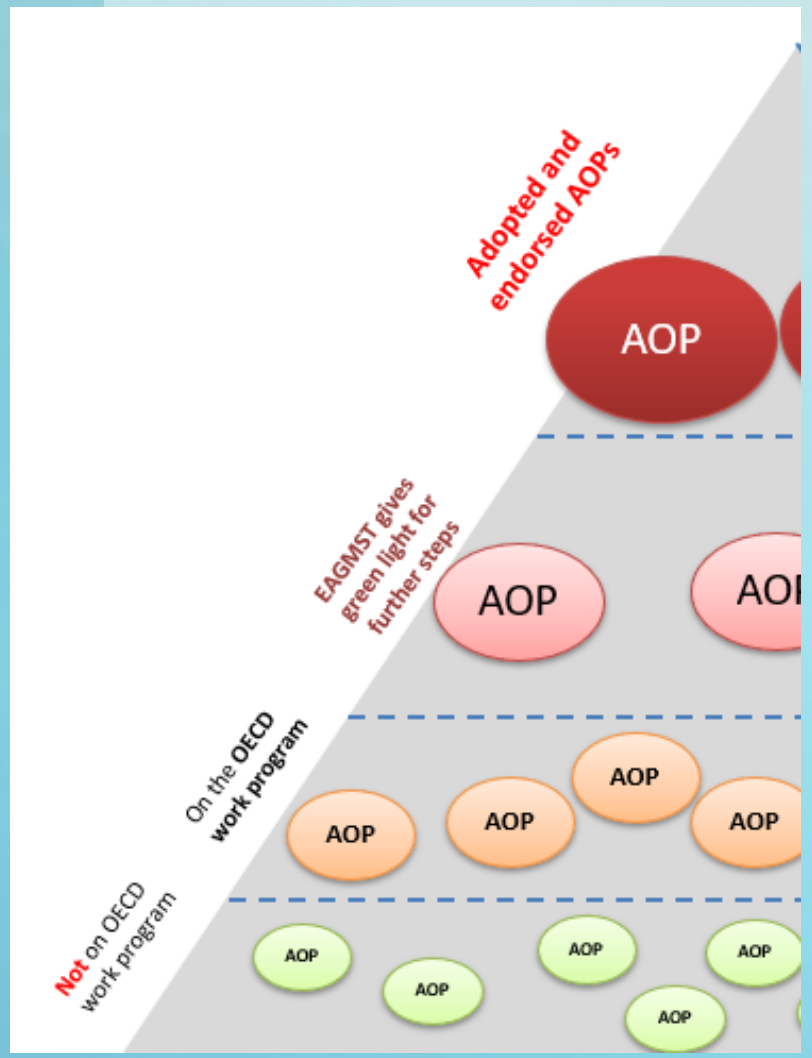
ENV/JM/MONO(2014)35
Unclassified

GUIDANCE DOCUMENT FOR DESCRIBING NON-GUIDELINE IN VITRO TEST METHODS

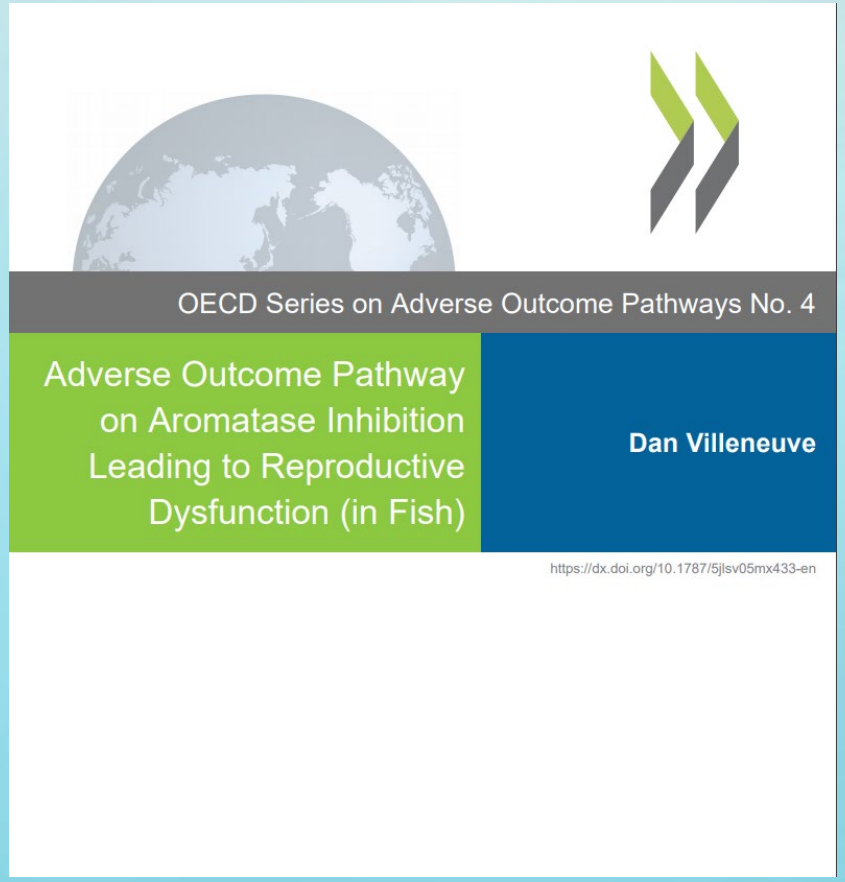
Series on Testing and Assessment
No. 211

OECD 211 Guidance Document for Describing Non-guideline in vitro Test Methods

5) Validation of the assays and data for incorporation into guidance regarding their interpretation and use



OECD AOP Development Programme

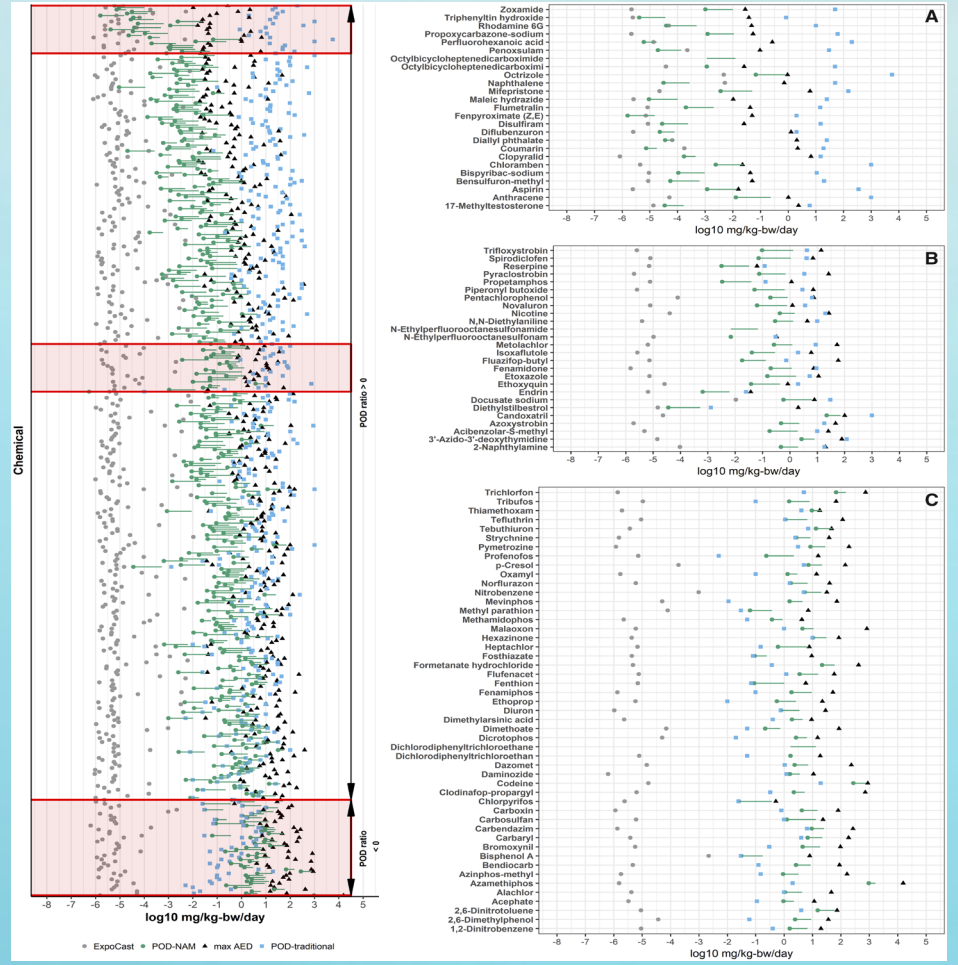


https://doi.org/10.1787/2415170X

6) Evidence that the new approaches are adequately predictive of adverse outcomes

448 chemicals

POD_{NAM} Vs POD_{trad}



NTP Research Report on National Toxicology Program Approach to Genomic Dose-response Modeling; NTP RR 5; April 2018

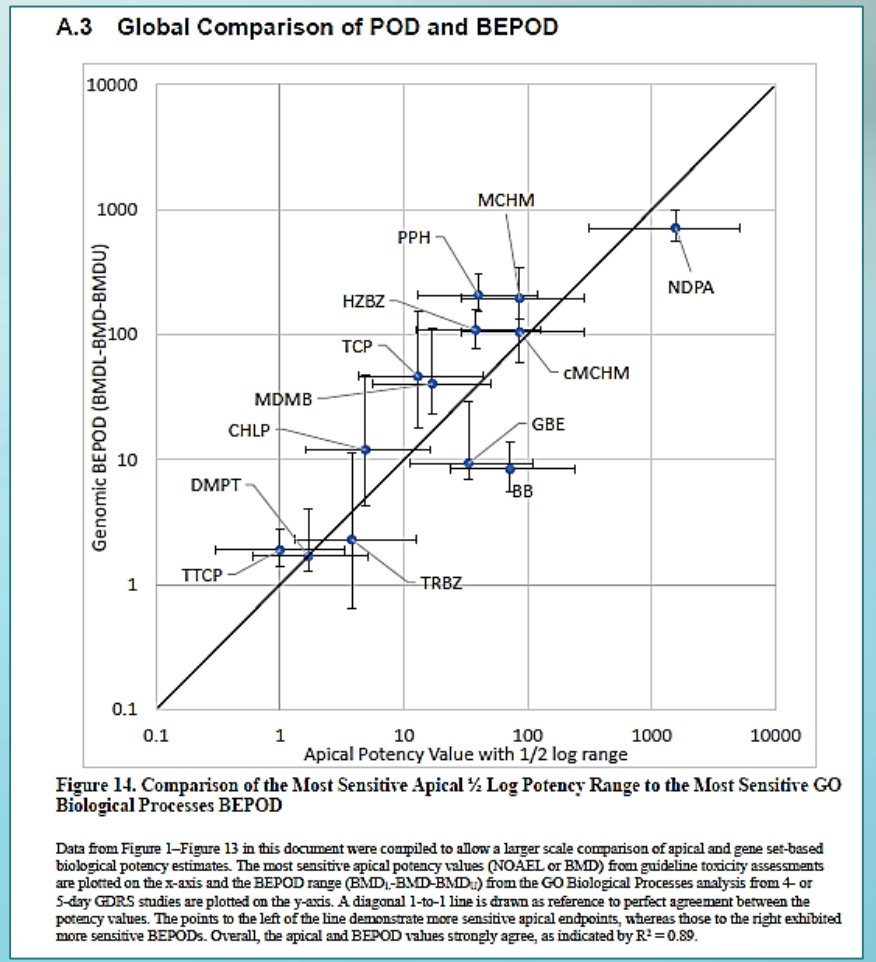


Figure 14. Comparison of the Most Sensitive Apical ½ Log Potency Range to the Most Sensitive GO Biological Processes BEPOD

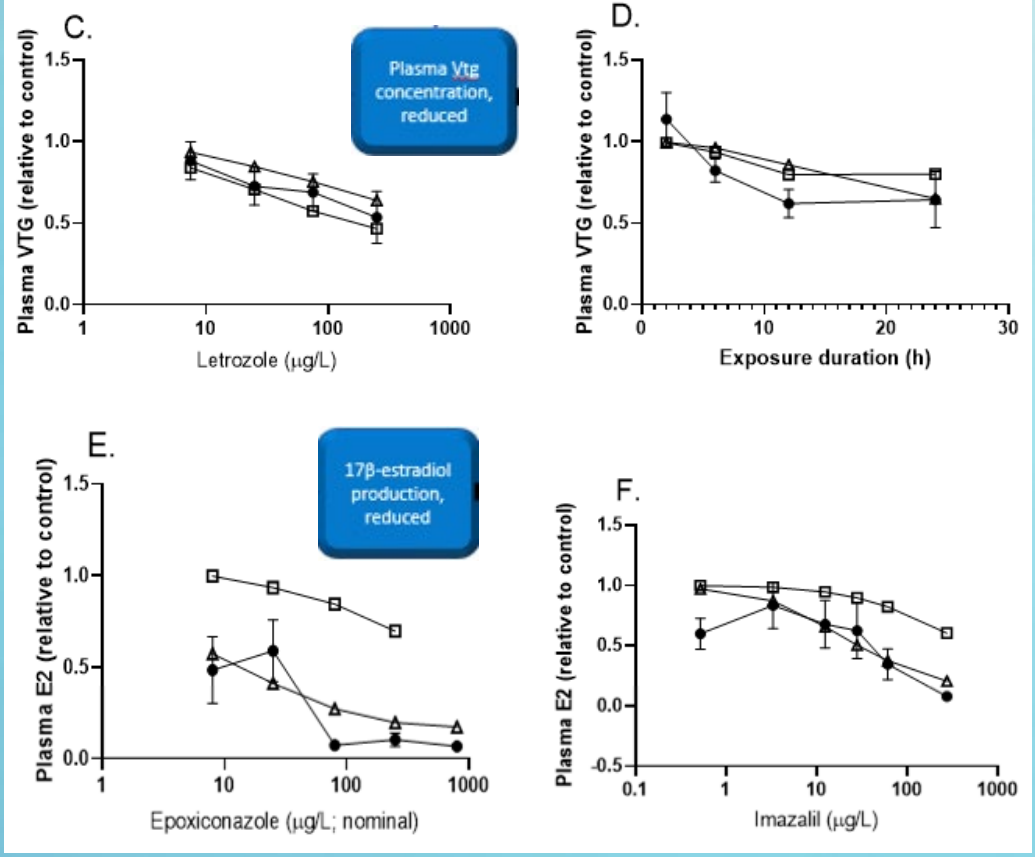
Data from Figure 1–Figure 13 in this document were compiled to allow a larger scale comparison of apical and gene set-based biological potency estimates. The most sensitive apical potency values (NOAEL or BMD) from guideline toxicity assessments are plotted on the x-axis and the BEPOD range (BMD_L-BMD-BMD_U) from the GO Biological Processes analysis from 4- or 5-day GDRS studies are plotted on the y-axis. A diagonal 1-to-1 line is drawn as reference to perfect agreement between the potency values. The points to the left of the line demonstrate more sensitive apical endpoints, whereas those to the right exhibited more sensitive BEPODs. Overall, the apical and BEPOD values strongly agree, as indicated by $R^2 = 0.89$.

Paul Friedman et al. Toxicol Sci. 2020; 173(1):202-225. doi: 10.1093/toxsci/kfz201.

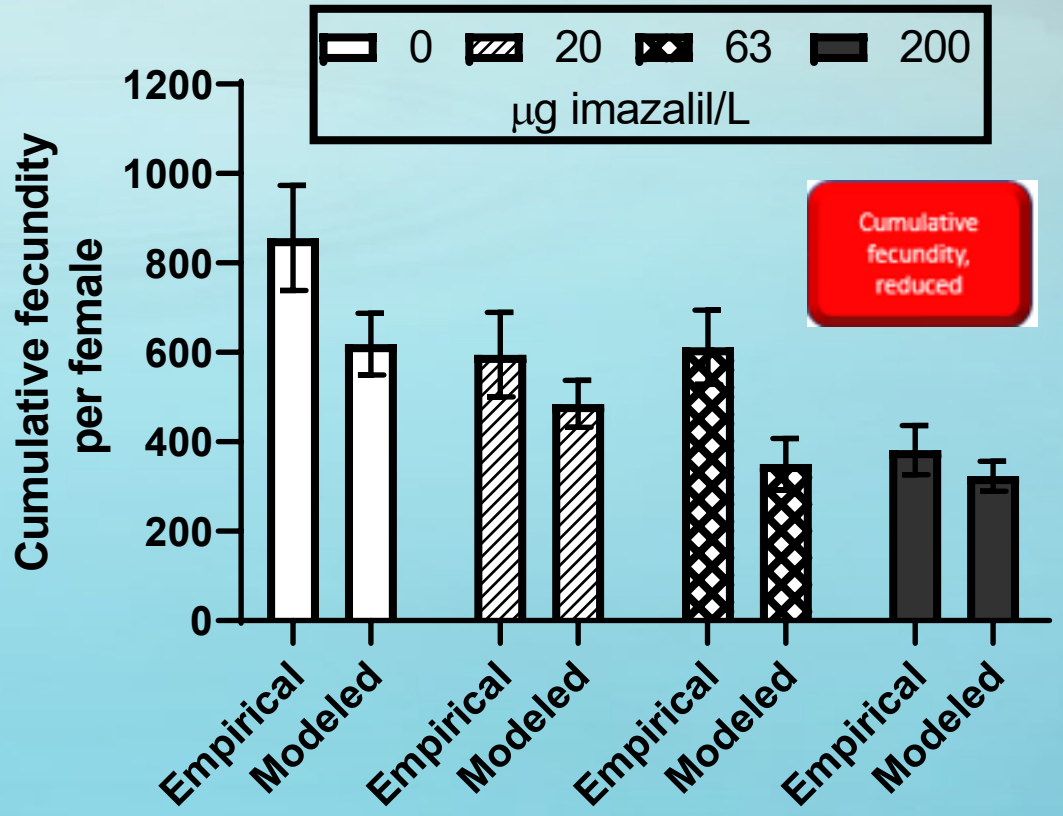
6) Evidence that the new approaches are adequately predictive of adverse outcomes

Empirical
Modeled (water conc.)
Modeled (plasma conc.)

Predicted DRTC for Key Events



Predicted apical effect (cumulative reproduction)



- By no means comprehensive, but considerable progress has been made in all six critical areas.

1) development of appropriate suites of HTS assays

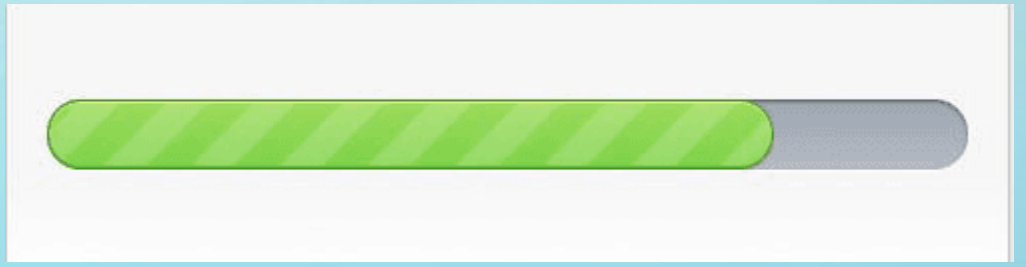
2) availability of targeted in vivo data to complement and provide an interpretive context for the HTS results

3) computational extrapolation models that could predict which exposures may result in adverse changes

4) Infrastructure to support the basic and applied research to develop the assays and models

5) validation of the assays and data for incorporation into guidance regarding their interpretation and use

6) evidence that the new approaches are adequately predictive of adverse outcomes



STATE OF APPLICATION OF HTS TO ENVIRONMENTAL RISK ASSESSMENT (circa 2018)

Risk assessors have expressed awareness of the HTS data themselves and conceptual recognition of the many ways in which those data might be used in the future

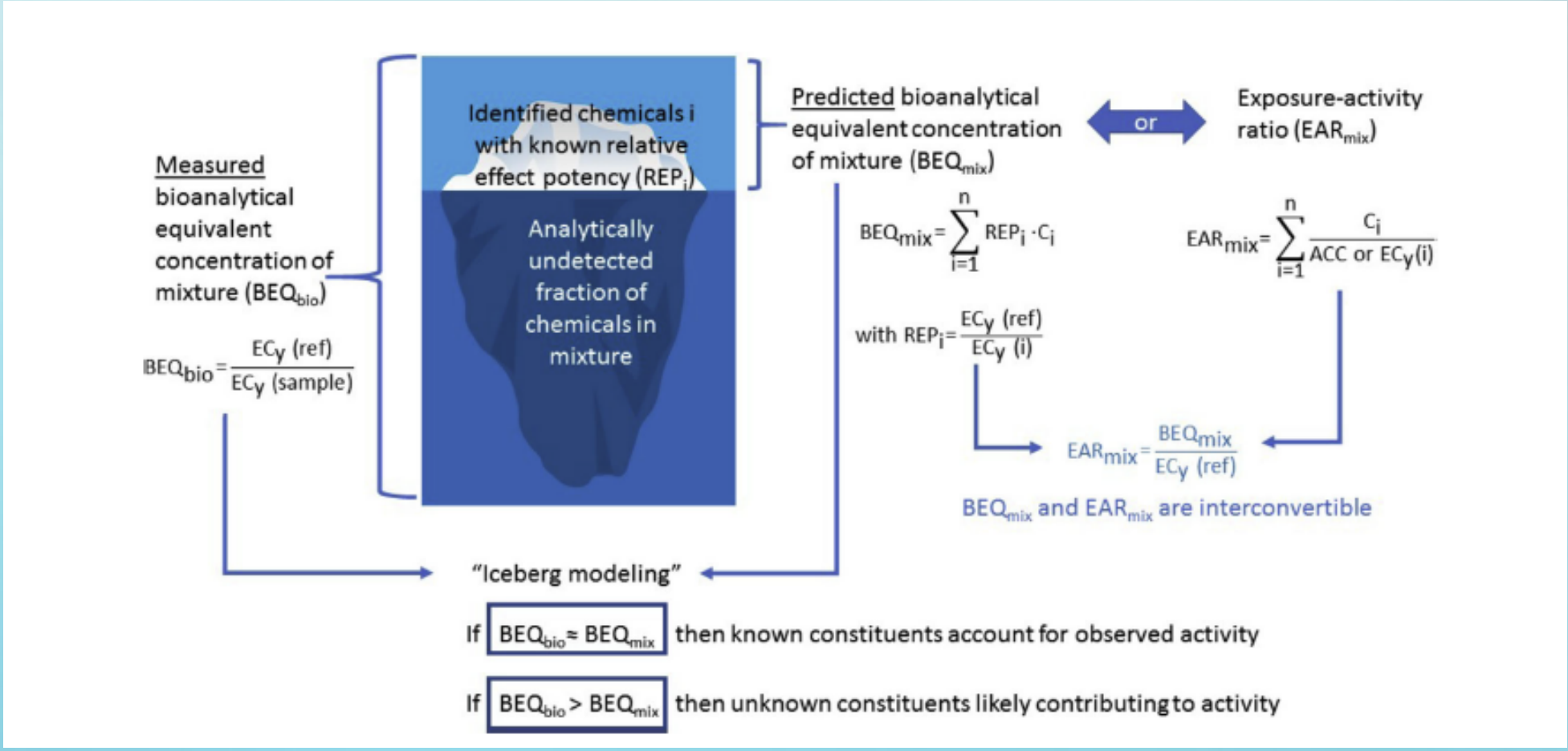
Adoption and acceptance into the chemical risk-assessment process have been limited

Well-developed AOPs to aid interpretation of these data do not exist in most cases, and even where they do exist, they provide only a qualitative connection,

Legacy nature of much of the legislation and regulatory structures under which risk assessments are conducted

Do not feel confident in how to interpret and extrapolate those data, and it remains unclear how to place the data into proper context for decision-making

BROADENING THE SCOPE



Great potential for the application of HTS data and assays for retrospective assessments, environmental monitoring, and complex mixtures.

Progress toward the NRC Vision

The committee believes that with a concerted research effort, over the next 10 years high-throughput test batteries could be developed that would substantially improve the ability to identify toxicity hazards caused by a number of mechanisms of action.

Those results in themselves would be a considerable advance.

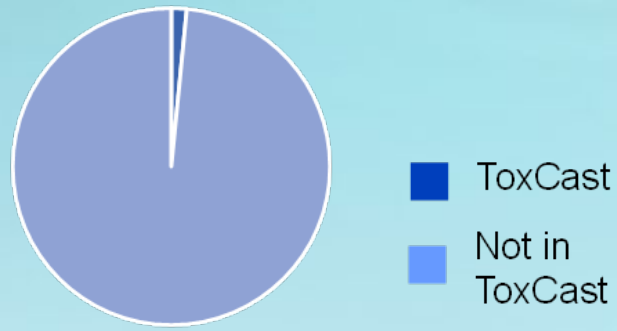
The time for full realization of the new test strategy, with its mix of in vitro and in vivo test batteries that can rapidly and inexpensively assess large numbers of substances with adequate coverage of possible end points, could be 20 or more years. (p. 63)

Global support for this ongoing transformation in chemical safety assessment appears strong

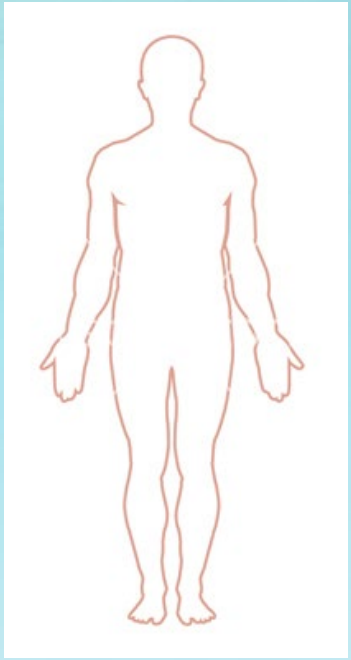
Six Opportunities for Impact

1. HTS assays for nonmammalian physiology

ToxCast
Gene Coverage

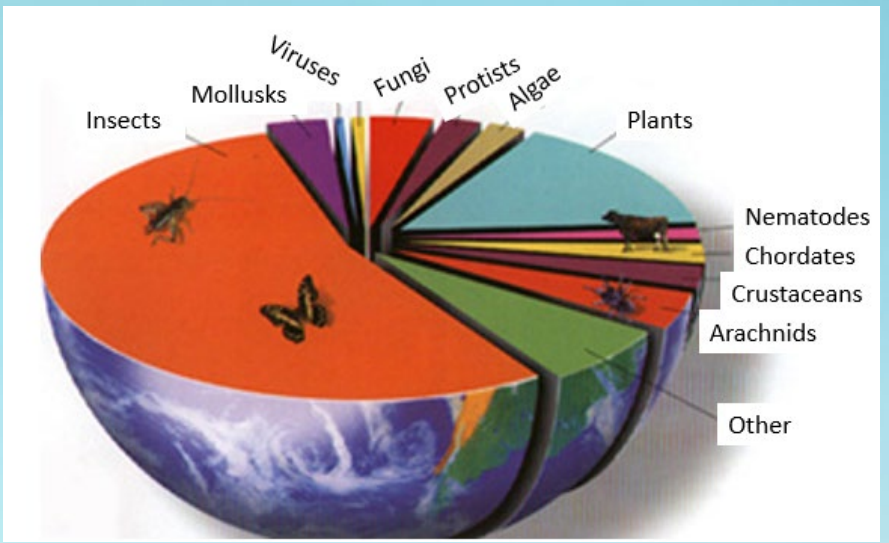


HTP
Transcriptomics

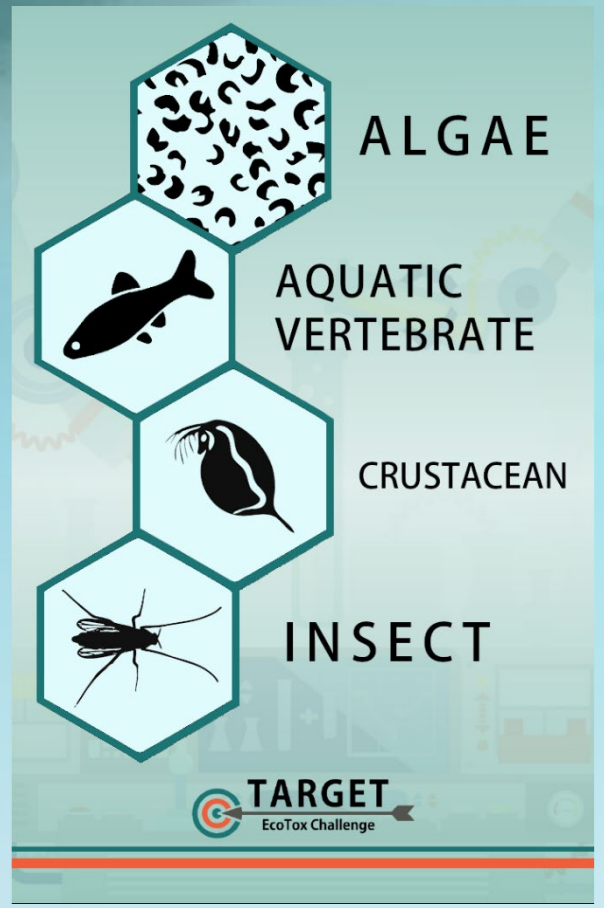


Entire human genome

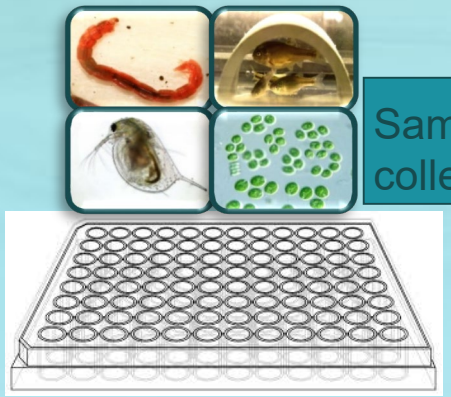
Still just a tiny slice of life on the planet



1. HTS assays for nonmammalian physiology



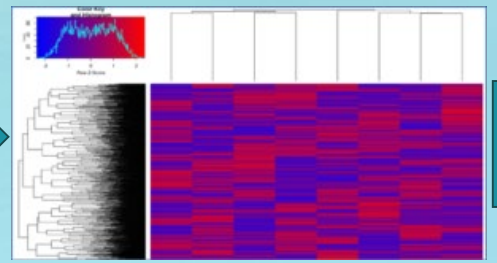
HTP Assays



Sample collection

100s of chemicals
10 concentrations
4 species
replication
10,000 + samples per yr.

Detection

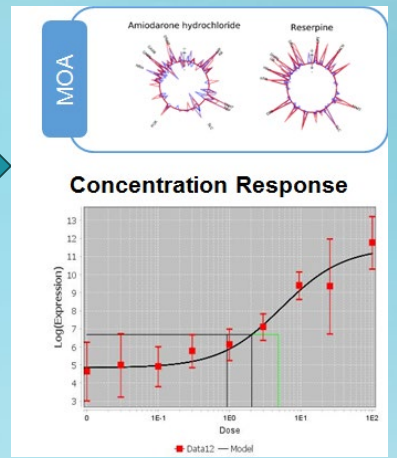


Commercially available
Low cost (<$50/sample)
High quality
Maximal coverage

TARGET EcoTox Challenge

Data analysis

Standardized data analysis

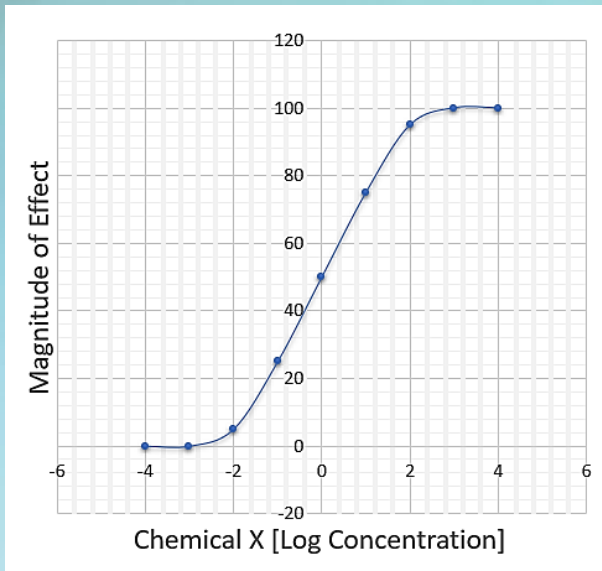


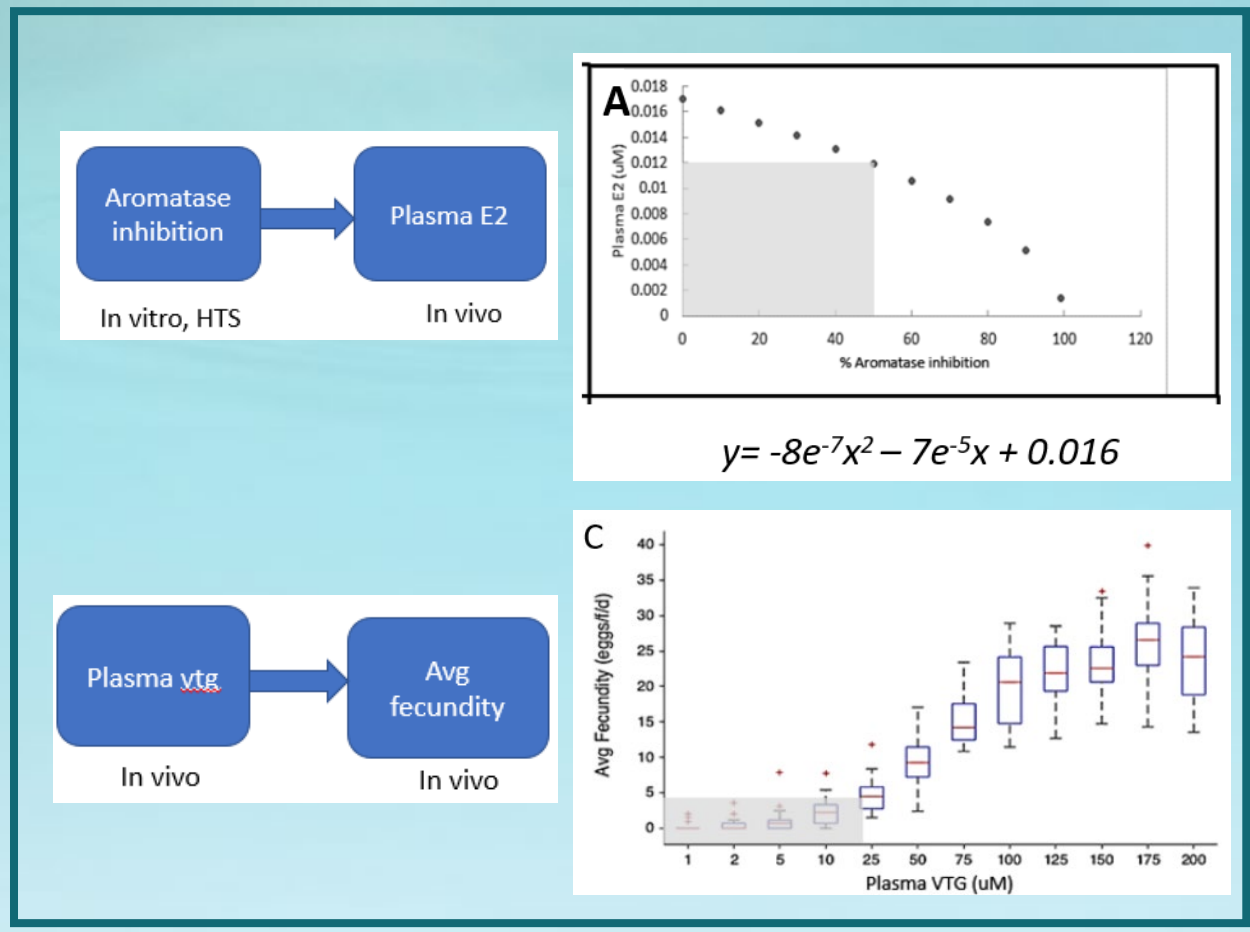
Informative for chemical safety decision-making

2. Response–response, not just dose–response

- Little skepticism that HTS data can be generated
- Quantitative extrapolation along AOPs is currently limited by a lack of data that address the critical question: ***how much perturbation of a key event is too much?***
- How much change in some upstream biological response (i.e., an early key event in an AOP) is needed to evoke a defined level of downstream biological effect (e.g., eliciting a later key event in an AOP) and under what conditions.
- Many toxicities are associated with competing damage and repair processes throughout the life of an organism – not simple to define a “tipping point”

2. Response–response, not just dose–response



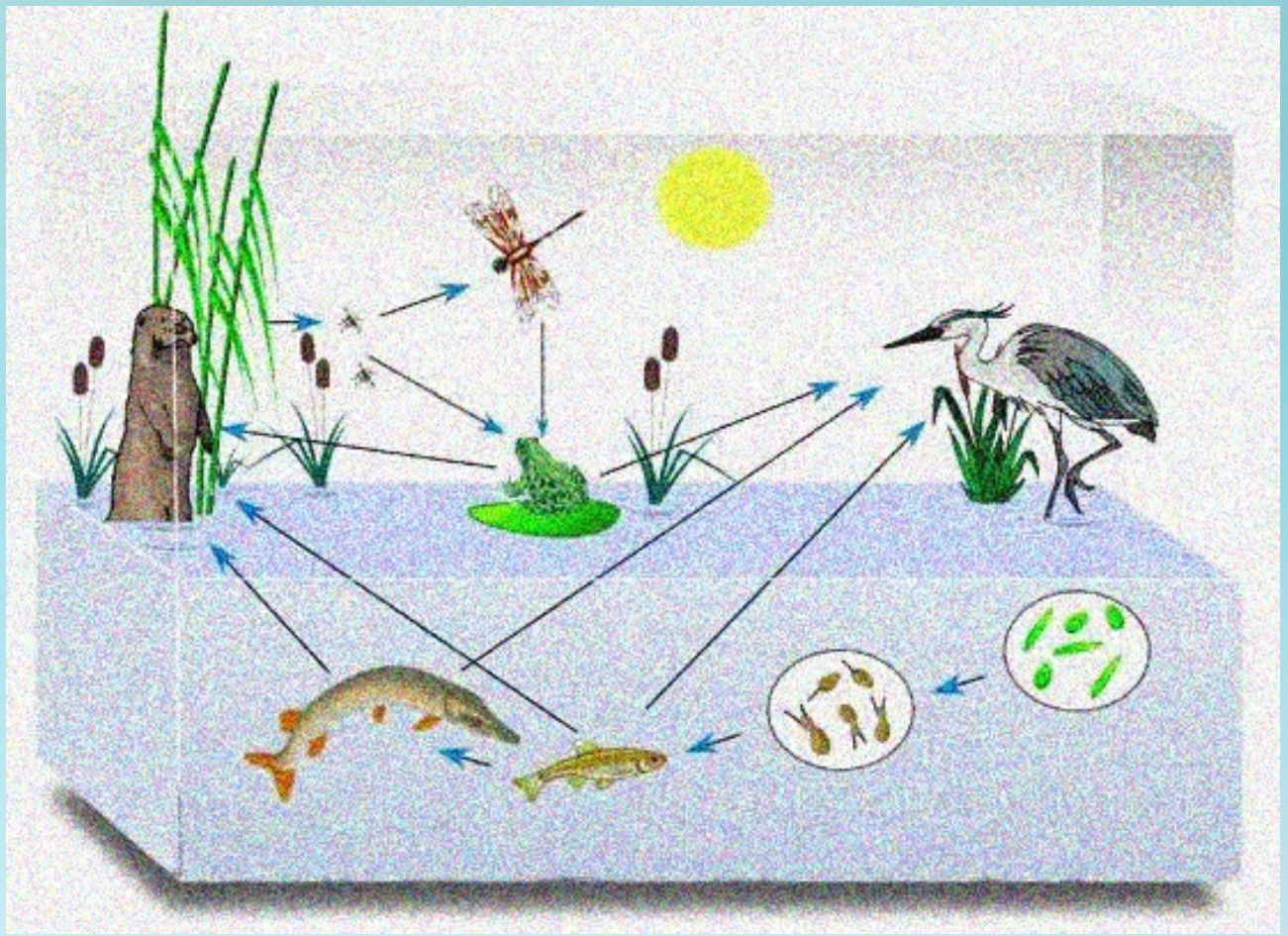


- **Conolly et al.** Environ Sci Technol. 2017 51:4661-4672.
- Hassan et al. Toxicol Sci. 2017 Nov 1;160(1):57-73
- **Foran et al.** *ALTEX.* 2019 . 36: 353-362.

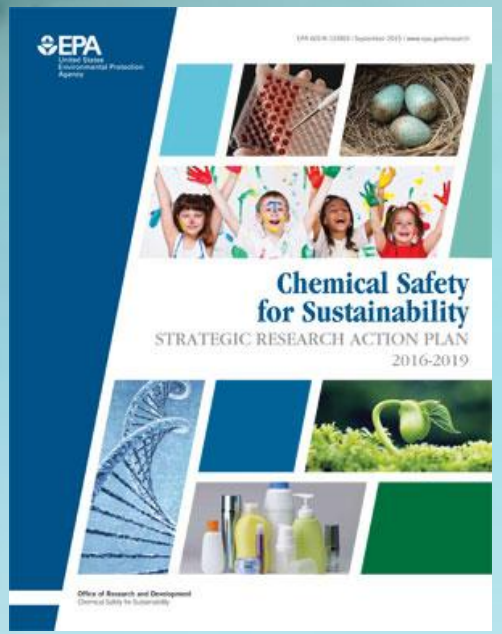
3. Ecosystem relevance

AOPs have typically been extended to the individual or population level.

Can’t ignore the effects that impacts on one component of the ecosystem may have on others.



3. Ecosystem relevance



CSS.4.6.4	Development and application of ecosystem level projection models coupled with AOPs
CSS.6.4.5	Development of ecosystem level projection models coupled with adverse outcome pathways and results from the Sequence Alignment to Predict Across-Species Susceptibility tool for fish and bees.
CSS.6.1	Develop and apply models to translate data from submitted studies into input for models that estimate population- and landscape-level impacts of pesticide use.

4. Enhanced international coordination

- International collaboration and coordination is quite good in some arenas
- Application of HTS in environmental monitoring has not been one of those:
  - relatively limited amount of interaction and awareness between various global efforts to develop the approaches, models, tools, terminologies to support the application of HTS in environmental monitoring.
- Global scientific societies (e.g., SETAC) and organizations (e.g., OECD) are uniquely positions to help coordinate a global research strategy.
- Providing developing countries access and training related to HTS technology and its applications

5. Accessible testing infrastructure

- Most HTS to date supported by large scale government contracts or individual laboratories testing a library of chemicals in a single assay.
- At present, it is generally not practically feasible for an individual investigator, manufacturer, or regulatory body to rapidly have a chemical or sample screened through a well established and validated battery of HTS assays.

5. Accessible testing infrastructure

- Establishment of one or more certified, accessible, HTS testing facilities would also have substantial benefits for validation and mutual acceptance of data.

- Creative public–private partnerships
  - Expand the amount of data in the public domain while protecting CBI
  - E.g, through substantially discounted pricing for users who agree to make their data public

- Guidelines, performance-based measures, controlled vocabularies, and databases that could be used to establish the quality, and comparability of low- to medium-throughput assays that would be **accepted as comparable to those available through commercial HTS services**.

- Genomics core facilities provide a model

6. Aggregation of ecological exposure data sets

- Temporal and spatial variability of exposure concentrations in the field and their intersection with the different life histories, behaviors, and physiological attributes of different species is an aspect of ecological risk assessment that cannot be ignored

- Human exposure and absorption, distribution, metabolism, and elimination data are being aggregated through sources like the USEPA's Chemistry Dashboard

- To date, there are no ongoing parallel efforts to aggregate ecological exposure data or relevant parameters needed to develop robust toxicokinetic models for a wide range of vertebrate and invertebrate wildlife and plants.

https://www.bio.vu.nl/thb/deb/deblab/add_my_pet/
An example of the type of aggregation that could be done for TK parameters

"The committee envisions that the new knowledge and technology generated from the proposed research program will be translated to noticeable changes in toxicity-testing practices within 10 years".

"Within 20 years, testing approaches will more closely reflect the proposed vision than current approaches."

(p. 79)

- Progress feel slows.
- Tendency to focus on the limitations and challenges that remain.
- SETAC FTM was an opportunity to take stock of the progress made
- Optimism about the progress made.
- Continue to grow the community that can nurture and strengthen our chemical safety evaluation system.

Acknowledgements

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SETAC Office: Tamar Schlekat, Nikki Mayo

Presenters and participants

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