# Linking Changes in Endocrine Function to Apical Effects: Critical Role of the AOP Framework*

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*Content does not necessarily reflect US Environmental Protection Agency (USEPA) position or policy.

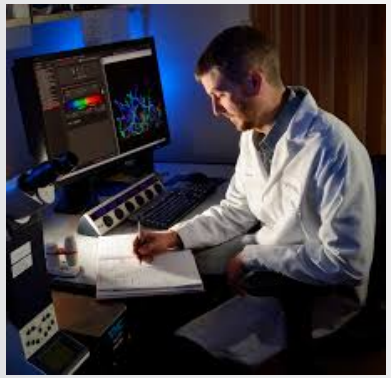
# A Brief History of the EDC Issue

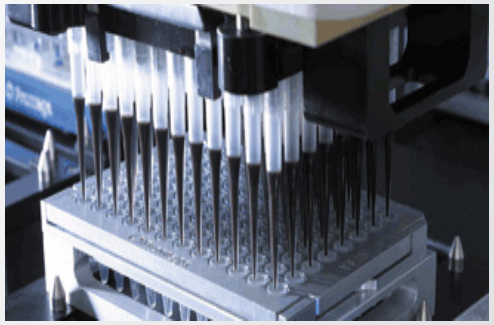

- In mid-90s EDCs become highly  visible human health/eco concern
- Testing/screening programs proposed to support regulatory activity worldwide (e.g., EDSTAC in US)
- Concept of tiered-testing for EDCs becomes widespread
  - Progressively more resource-intensive measurements
- Development/validation of novel in vivo assays measuring endocrine and apical effects (e.g., OECD VMGs)
- Realization that routine deployment of these in vivo assays not feasible
  - Too many chemicals (e.g., 10,000 for EDSP)
- Emphasis shifts to implementing New Approach Methodologies (NAM) to identify endocrine-active chemicals

# What are NAMs?

*Approaches—new and old—enabling rapid, cost-effective collection of data useful for predicting potential biological effects*

- In silico models relating structure to potential activity
- In vitro (incl. HTP) assays focused on specific molecular targets
- Short-term in vivo assays with endpoints (molecular, biochemical) predictive of perturbation of specific pathways
- Bioinformatic techniques supporting data integration/interpretation





# One Example of a NAM for EDC Screening

Environmental Science & Technology

Article

pubs.acs.org/est

## Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model

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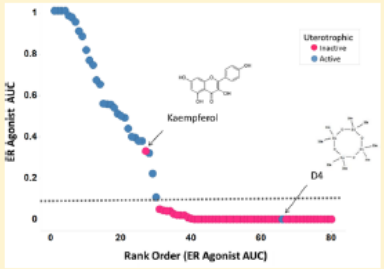
Supporting Information

ABSTRACT: The U.S. Environmental Protection Agency (EPA) is considering high-throughput and computational methods to evaluate the endocrine bioactivity of environmental chemicals. Here we describe a multistep, performance-based validation of new methods and demonstrate that these new tools are sufficiently robust to be used in the Endocrine Disruptor Screening Program (EDSP). Results from 18 estrogen receptor (ER) ToxCast high-throughput screening assays were integrated into a computational model that can discriminate bioactivity from assay-specific interference and cytotoxicity. Model scores range from 0 (no activity) to 1 (bioactivity of 17$\beta$-estradiol). ToxCast ER model performance was evaluated for reference chemicals, as well as results of EDSP Tier 1 screening assays in current practice. The ToxCast ER model accuracy was 86% to 93% when compared to reference chemicals and predicted results of EDSP Tier 1 guideline and other uterotrophic studies with 84% to 100% accuracy. The performance of high-throughput assays and ToxCast ER model predictions demonstrates that these methods correctly identify active and inactive reference chemicals, provide a measure of relative ER bioactivity, and rapidly identify chemicals with potential endocrine bioactivities for additional screening and testing. EPA is accepting ToxCast ER model data for 1812 chemicals as alternatives for EDSP Tier 1 ER binding, ER transactivation, and uterotrophic assays.




Computational model based on data from 18 HTP assays with known/potential ER-active chemicals

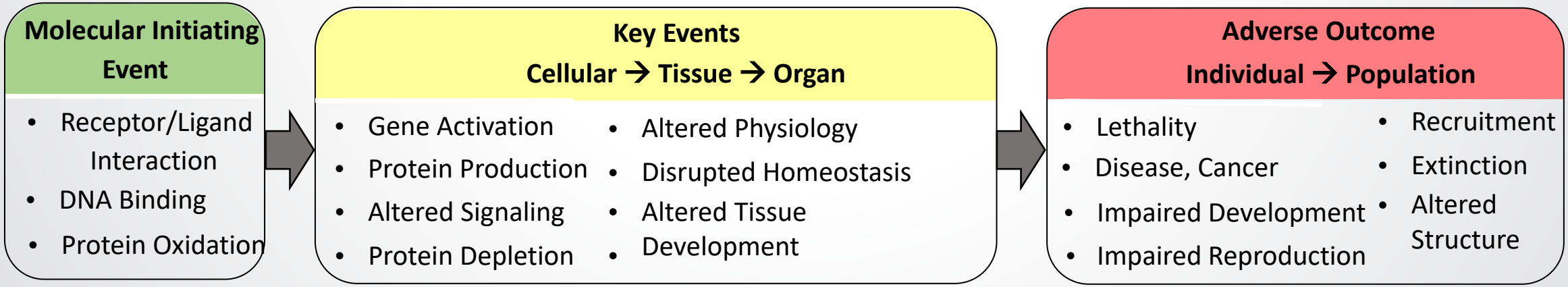
Designed as possible alternative for in vivo screening assays to rapidly estrogenicity

Used to screen ca. 2000 mostly data-poor chemicals

Work ongoing to develop comparable systems for other endocrine modalities

# Relating Changes in Endocrine Status to Apical Effects: Role of the AOP Framework

- Acceptance of NAMs for screening depends on **plausible linkages to adverse effects**
- Many authorities **require causal associations** between altered endocrine activity and negative apical effects to define an EDC
- "An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment." (Ankley et al. 2010. Environ. Toxicol. Chem. 29:730-741)



# Attributes of the AOP Framework

- Establishes **causal linkages** between mechanistic endpoints and apical outcomes
  - Systematically-organized, transparent and documented
  - Scientifically credible
- "Story line" and terminology as basis for communication

*Also*,

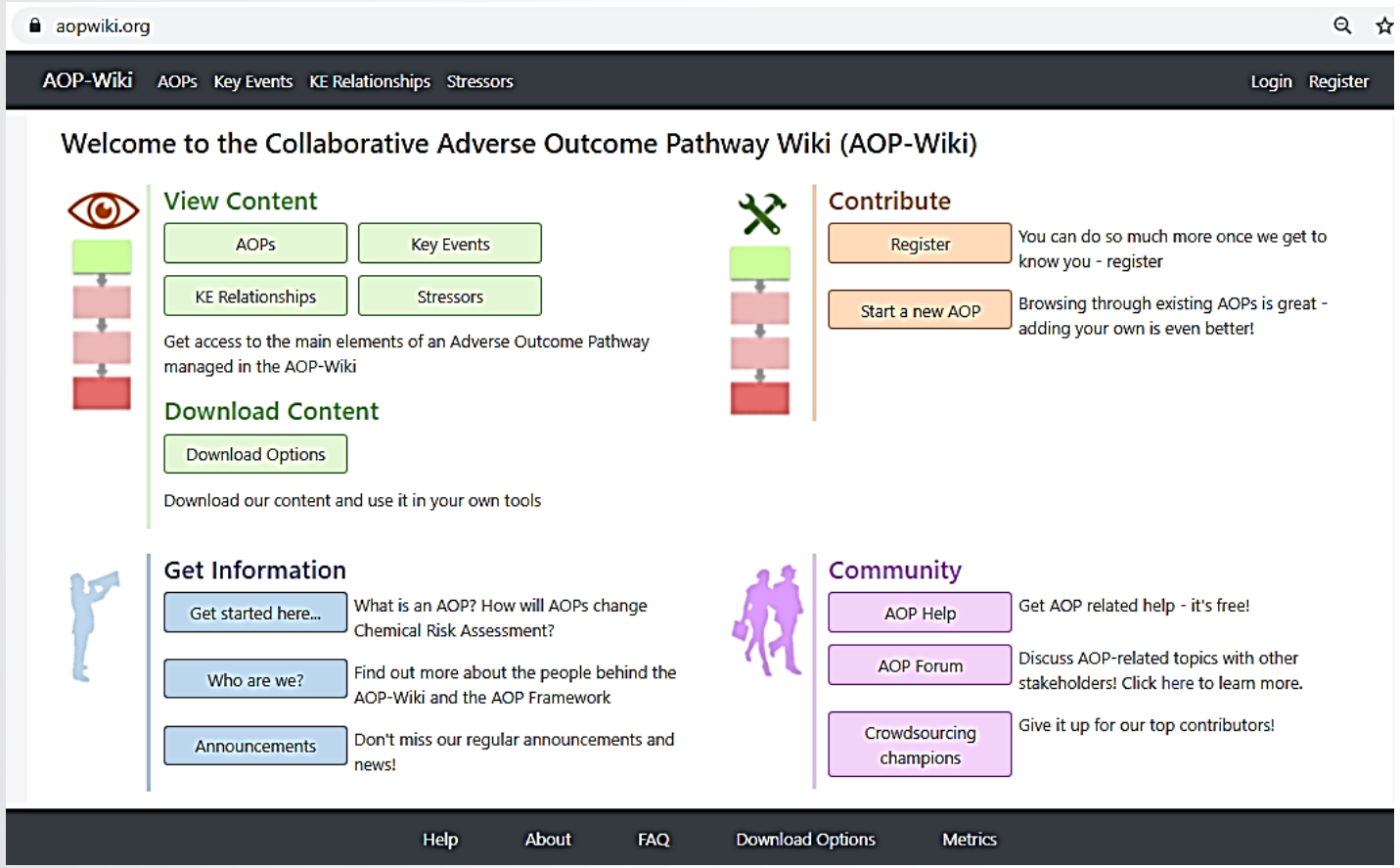
- Promotes a chemical "agnostic" approach to pathway perturbation, supporting evaluations based on biological similarity (categorization, read-across)
- Enables assembling and understanding complex data in the context of pathways, networks and systems
  - Informs assay development
- Provides basis for quantitative prediction of apical effects based on NAM data

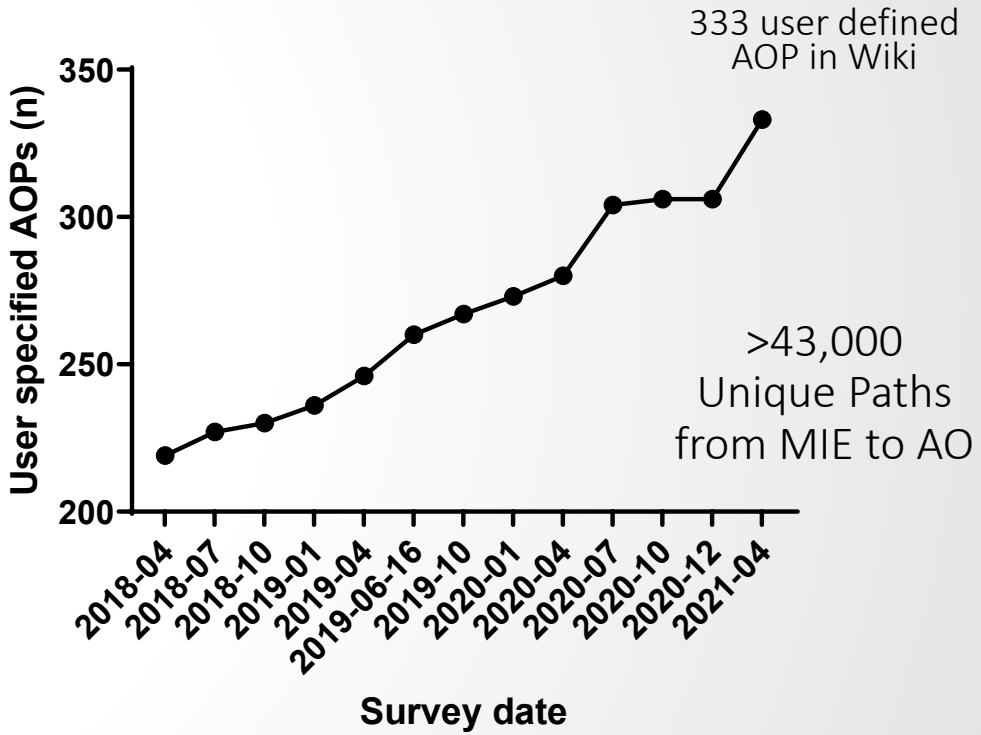
# International Formalization of the AOP Framework

- 2012 launch of OECD AOP development programme
- 2013 OECD Guidance on Developing and Assessing AOPs
  - Introduce standardization and rigor to AOP development
  - Conventions and terminology
  - Information content of an AOP description
  - Weight of evidence (WoE) evaluation based on modified Bradford-Hill criteria
- 2014 AOP-Wiki 1 public release – Users' Handbook 1st Edition
- 2014 Principles of AOP development guidance
- 2017 AOP-Wiki 2.1 public release
- 2018 User's Handbook 2nd Edition
- 2021 AOP Wiki 2.4 release (mobile version)
- 2021 First AOP Reports journal article (*Environ. Toxicol. Chem.)*

# AOP-Wiki: An Open Access Resource

aopwiki.org



# AOP-Wiki and EDCs

- Many of initial AOPs published Wiki (and/or open literature) focus on estrogen, androgen and thyroid (EAT) pathways
  - Regulatory concerns, amenability to AOP development (e.g., defined MIE)
- Currently about 15% of 330+ AOPs in Wiki focus on EAT pathways
- About 10% of AOPs in Wiki specifically for fish, with ca. 50% related to EAT
  - MIEs: activation of estrogen and androgen receptors; inhibition of enzymes producing sex steroids and thyroid hormone
  - AOs: developmental inhibition; reduced reproduction; population decreases
- Nearly 25% of fish AOPs subjected to advanced technical review, including several endorsed by OECD as potentially suitable for regulatory uses



# Select Recent Papers Describing AOP Application(s) to E(A)DC Screening/Testing


Browne et al. 2017. Application of adverse outcome pathways to US EPA's endocrine disruptor screening program. *Environ. Health Perspect.* 125 (9).096001.

Coady et al. 2017. Current limitations and recommendations to improve testing for environmental assessment of endocrine active chemicals. *Integ. Environ. Assess. Manag.* 13, 302-316.

Coady et al. 2019. When are adverse outcome pathways and associated assays "fit for purpose" for regulatory decision-making and management of chemicals? *Integ. Environ. Assess. Manag.* 15, 633-647.

Conolly et al. 2017. Quantitative adverse outcome pathways and their application to predictive toxicology. *Environ. Sci. Technol*. 51, 4661-4672.

Fitzgerald. 2020. Adverse outcome pathway bridge building from research to regulation. *Chem. Res. Toxicol.* 33, 849-851.

Knapen et al. 2020. Toward an AOP network-based tiered testing strategy for the assessment of thyroid hormone disruption. *Environ. Sci. Technol.* 54, 8491-8499.

Matthiessen et al. 2017. Recommended approaches to the scientific evaluation of ecotoxicological hazards and risks of endocrine-active substances. *Integ. Environ. Assess. Manag.* 13, 267-279.

McCardle et al. 2020. Critical review of read across potential in testing for endocrine related effects in vertebrate ecological receptors. *Environ. Toxicol. Chem.* 39, 739-750.

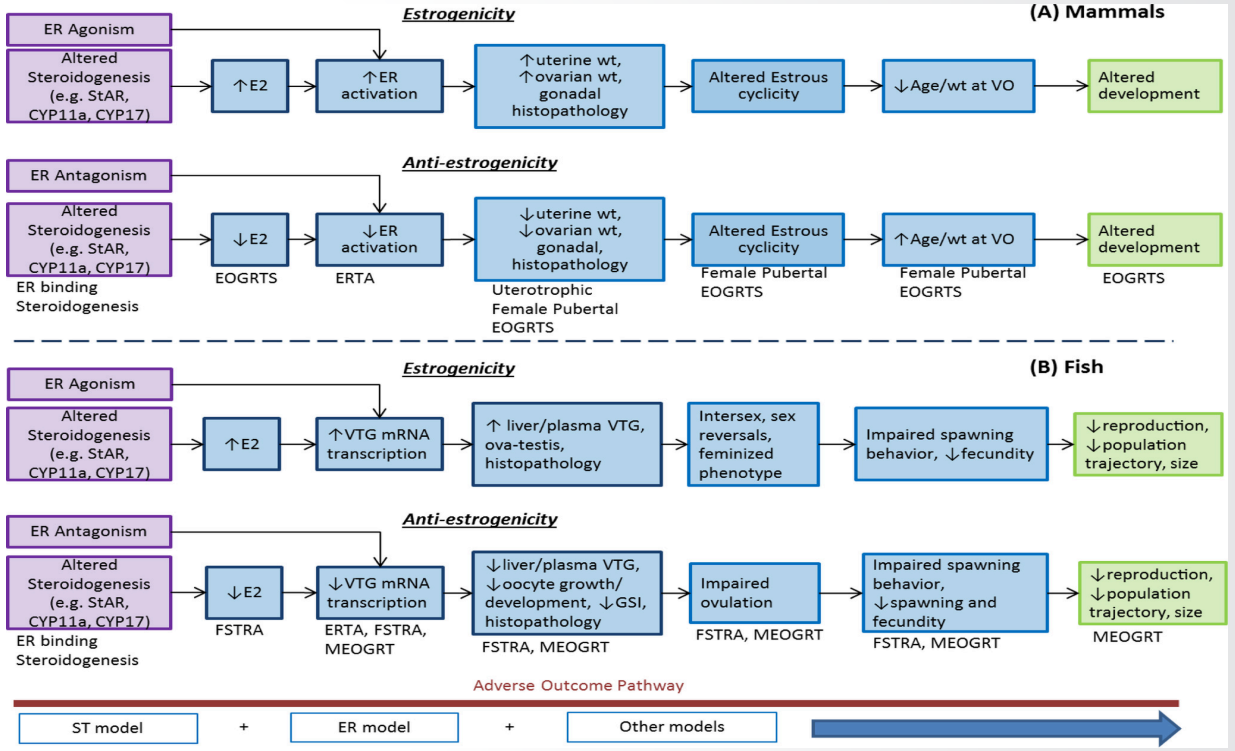
Noyes et al. 2019. Evaluating chemicals for thyroid disruption: Opportunities and challenges with in vitro testing and adverse outcome pathway approaches. *Environ. Health Perspect*. 127, 095001.

# AOPs and EDCs: Linking Screening and Testing Data for Estrogens to Apical Effects


Vol. 125, No. 9

**Application of Adverse Outcome Pathways to U.S. EPA's Endocrine Disruptor Screening Program**

Browne et al. (2017)
https://doi.org/10.1289/EHP1304




- USEPA EDSP tiered testing strategy employs assays at multiple biological levels of organization

- AOP framework used to demonstrate linkages among estrogen-oriented assay results to apical effects in mammals and fish

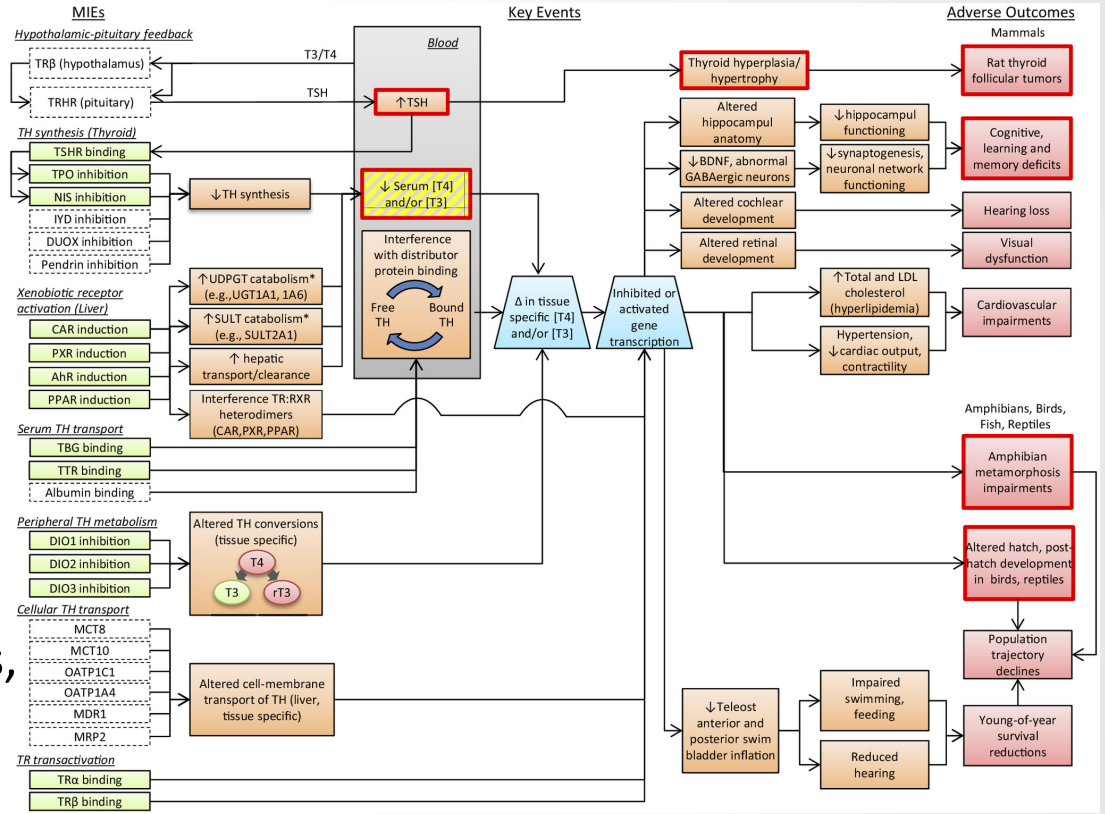
- Provides foundation for ongoing NAM-based prioritization for (anti) estrogenic chemicals

# AOPs and EDCs: Identifying Thyroid Axis Targets for Development of NAM Assays


Vol. 127, No. 9

**Evaluating Chemicals for Thyroid Disruption: Opportunities and Challenges with In Vitro Testing and Adverse Outcome Pathway Approaches**

Noyes et al. (2019)
https://doi.org/10.1289/EHP5297




- Regulatory mandate to identify chemicals with potential to impact thyroid-mediated processes (e.g., behavior in mammals, metamorphosis in amphibians, fish)

- Thyroid AOP network elements conserved across vertebrates, including multiple MIEs that can be “captured” by existing and new in vitro HTP assays

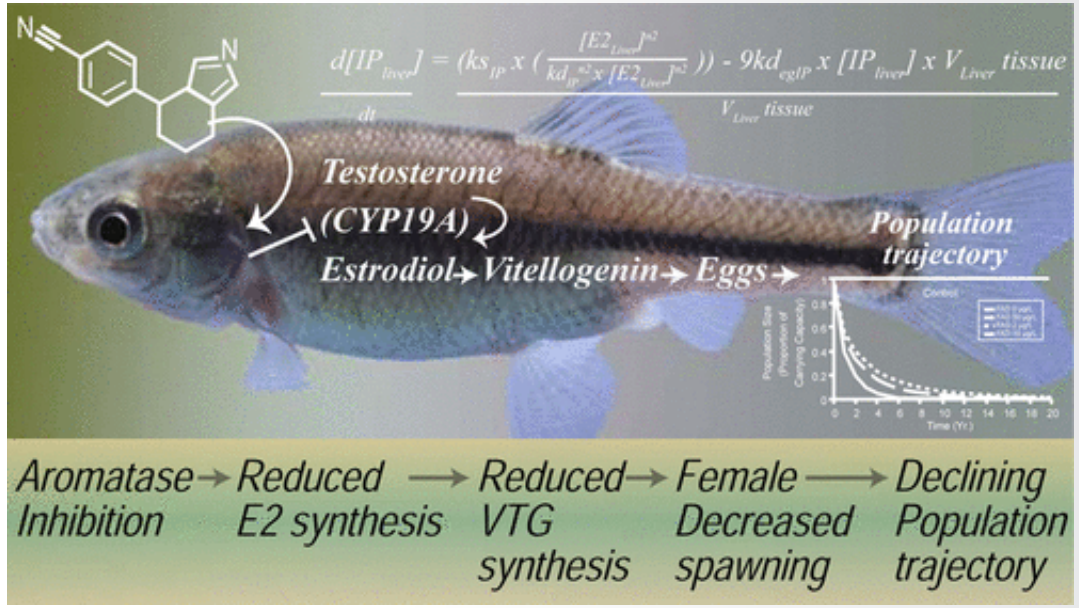
- Thyroid HTP assay suite used to screen large number of diverse and class-specific (e.g., PFAS) chemicals for possible thyroid activity to support prioritization

# AOPs and EDCs: Quantitative Prediction of Apical Effects from NAM Data

Environmental Science & Technology
Vol. 51, No. 98, 4661-4672

**Quantitative Adverse Outcome Pathways and Their Application to Predictive Toxicology**

Conolly et al. (2017)
https://doi.org/10.1021/acs.est.6b06230

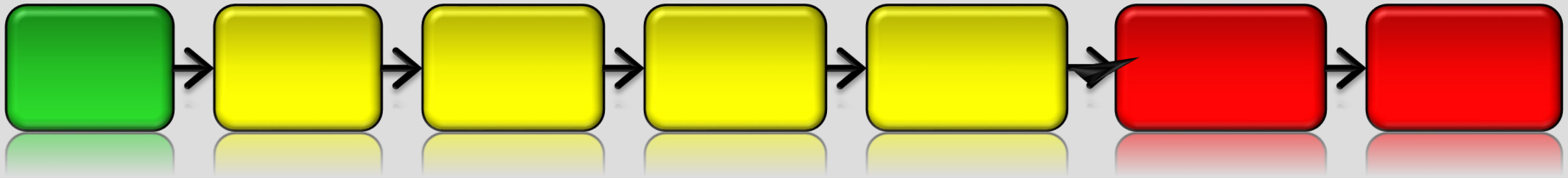



Inhibition of steroidogenic enzymes pathway of concern for most screening/testing programs, with particular emphasis on CYP19 aromatase (T→E2)

In vitro (incl. HTP) assays measuring aromatase inhibition exist, with 100s of chemicals tested

Multi-component qAOP developed linking relative degree of aromatase inhibition to reproductive (embryo) production and population status described (based on AOP#25 from AOP Wiki)

Illustration of  qAOP prediction application using HPT data for an untested conazole fungicide (iprodione)



AOP framework has multiple applications related to E(A)DC screening and testing

Thank you for your attention, questions?