



# Exploring Potential Reductions in Fish Testing in a Regulatory Context

Michael Lowit, Ph.D.

Senior Scientist

US Environmental Protection Agency, Office of Pesticide Programs

# Introduction



## 3Rs

- *Reduce* animal use
- *Replace* laboratory animal studies: Implementation of *in vitro*, *in chemico* or computational approaches
- *Refine* study protocols to reduce suffering

# Some driving forces.....



## Ethics & Animal Welfare



## Efficiency



## Public Health (Human Relevance, Improved science)

## Expectations



# Background



- US EPA's Office of Pesticide Programs (OPP) regulates use of all pesticides in the United States and establishes maximum levels for pesticide residues in food
- Federal statutes allow EPA to require data and relevant information from pesticide registrants
- 40 Code of Federal Regulations (CFR) Part 158 outlines data requirements for pesticides

<https://ecfr.federalregister.gov/current/title-40/chapter-I/subchapter-E/part-158>

# Background



- Unlike industrial chemicals, to register a pesticide in the US, substantial toxicology and exposure testing is required
  - Cost to register a new pesticide is >\$100 million
  - To register a new conventional pesticide, 10,000-15,000 animals are used
    - Rats, mice, rabbits, dogs, guinea pigs, birds, fish & invertebrates
- OPP is working with multiple national/international organizations and numerous stakeholders to:
  - Evaluate the toxicology studies conducted for pesticides & identify those studies that do not impact decision making for public health and the environment
  - To advance the use of new approach methods (NAMs) in regulatory risk assessment

# Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM)



- In 2000, Congress passed the ICCVAM Authorization Act and established ICCVAM as a permanent committee administrated by the National Institute of Environmental Health Sciences (NIEHS)
  - Comprised of 16 Federal regulatory and research agencies that require, use, generate, or disseminate toxicological and safety testing information
  - ICCVAM facilitates the development, validation, and regulatory acceptance of test methods that replace, reduce, or refine the use of animals in testing
  - National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) of the NIEHS provides scientific and operational support for ICCVAM technical evaluations and related activities

Agency for Toxic Substances and Disease Registry • Consumer Product Safety Commission • Department of Agriculture • Department of Defense • Department of Energy • Department of the Interior • Department of Transportation • Environmental Protection Agency • Food and Drug Administration • National Institute for Occupational Safety and Health • National Institutes of Health • National Cancer Institute • National Institute of Environmental Health Sciences • National Library of Medicine • Occupational Safety and Health Administration • National Institute of Standards & Technology



# Data Requirement Flexibility



- Flexibility in implementing 40 CFR Part 158 data requirements (§ 158.30):
  - *Waivers* may be granted as permitted by 40 CFR Part 158.45
  - Additional data beyond the 158 data requirements may be important to the risk management decision (§ 158.75), *alternative approaches* can be accepted, and other data can be used

# Guiding Principles for Data Needs for Pesticides



- *Purpose:* provide consistency in the identification of data needs, promote and optimize full use of existing knowledge, and focus on the critical data needed for risk assessment
  - <https://www.epa.gov/pesticide-registration/guiding-principles-data-requirements>
  - “...ensure there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision....”
  - “...avoid unnecessary use of time and resources, data generation costs, and animal testing.”



# 2016 OPP's Goal to Reduce Animal Testing



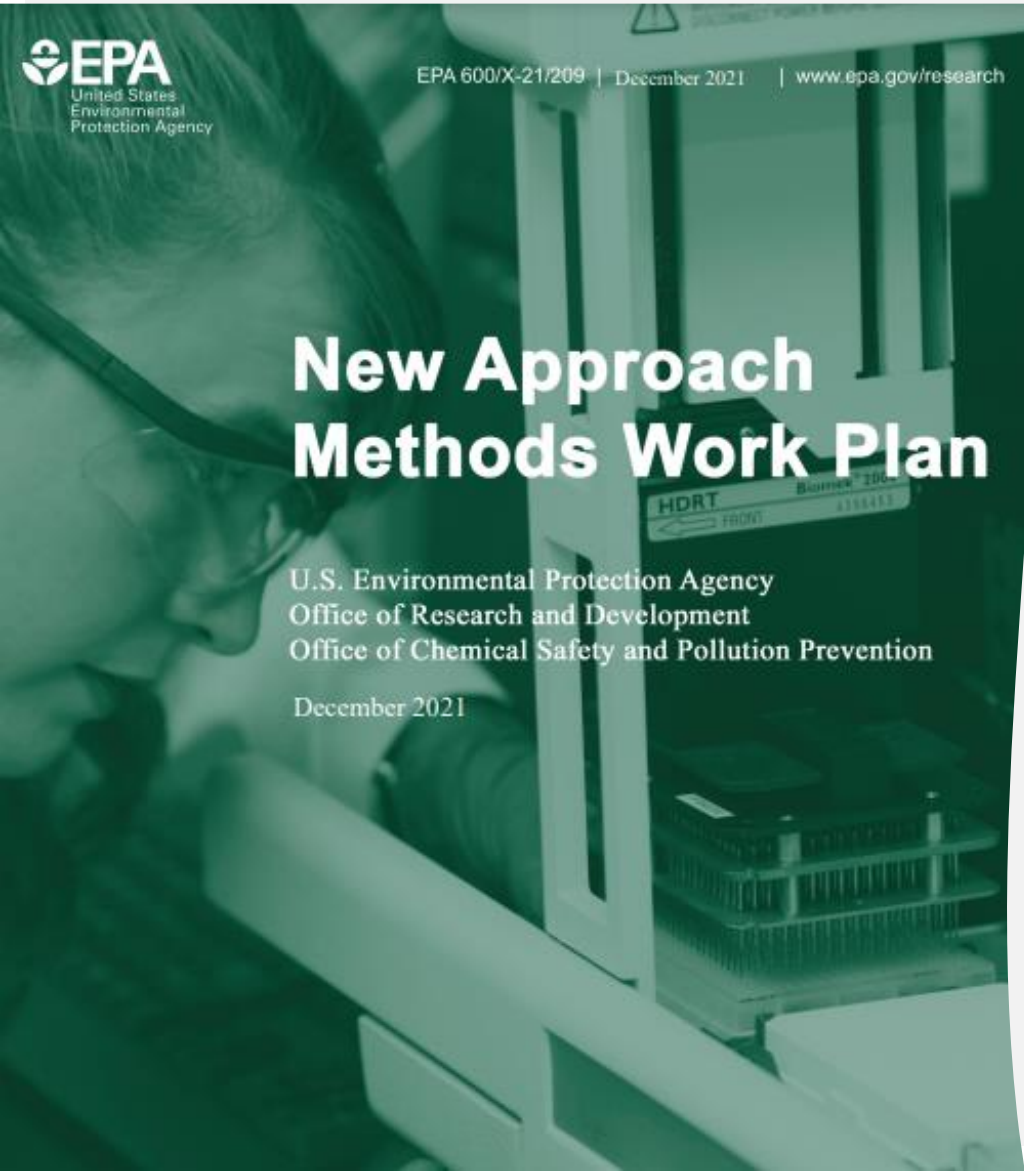
- 2016 Letter to Stakeholders on OPP's Goal to Reduce Animal Testing from Jack E. Housenger, Director
  - <https://www.regulations.gov/document/EPA-HQ-OPP-2016-0093-0003>
  - Working in partnership with other governmental entities, industry and non-governmental organizations (NGOs), and need continued robust participation and support to achieve our mutual goal
  - Activities fall under three main objectives:
    - Critically evaluating which studies form the basis of OPP decisions
    - Expanding acceptance of alternative methods
    - Reducing barriers such as challenges of data sharing among companies and international harmonization to adopting alternative methods in the US and internationally

# 2019 Directive from EPA Administrator



- Host conferences on the state of the science on development and use of NAMs to provide a forum for presentations in the field
  - First conference held in December 2019
    - Conference report: <https://www.epa.gov/chemical-research/conference-summary-state-science-development-and-use-new-approach-methods-chemical>
  - Second conference held in October 2020
    - Conference report: <https://www.epa.gov/chemical-research/summary-second-annual-conference-state-science-development-and-use-new-approach>
  - EPA plans to initiate the process for the next EPA NAMs Conference in 2022
- Develop a work plan for reduction of animal testing using NAMs while remaining protective of human health and the environment

# EPA's NAMs Work Plan



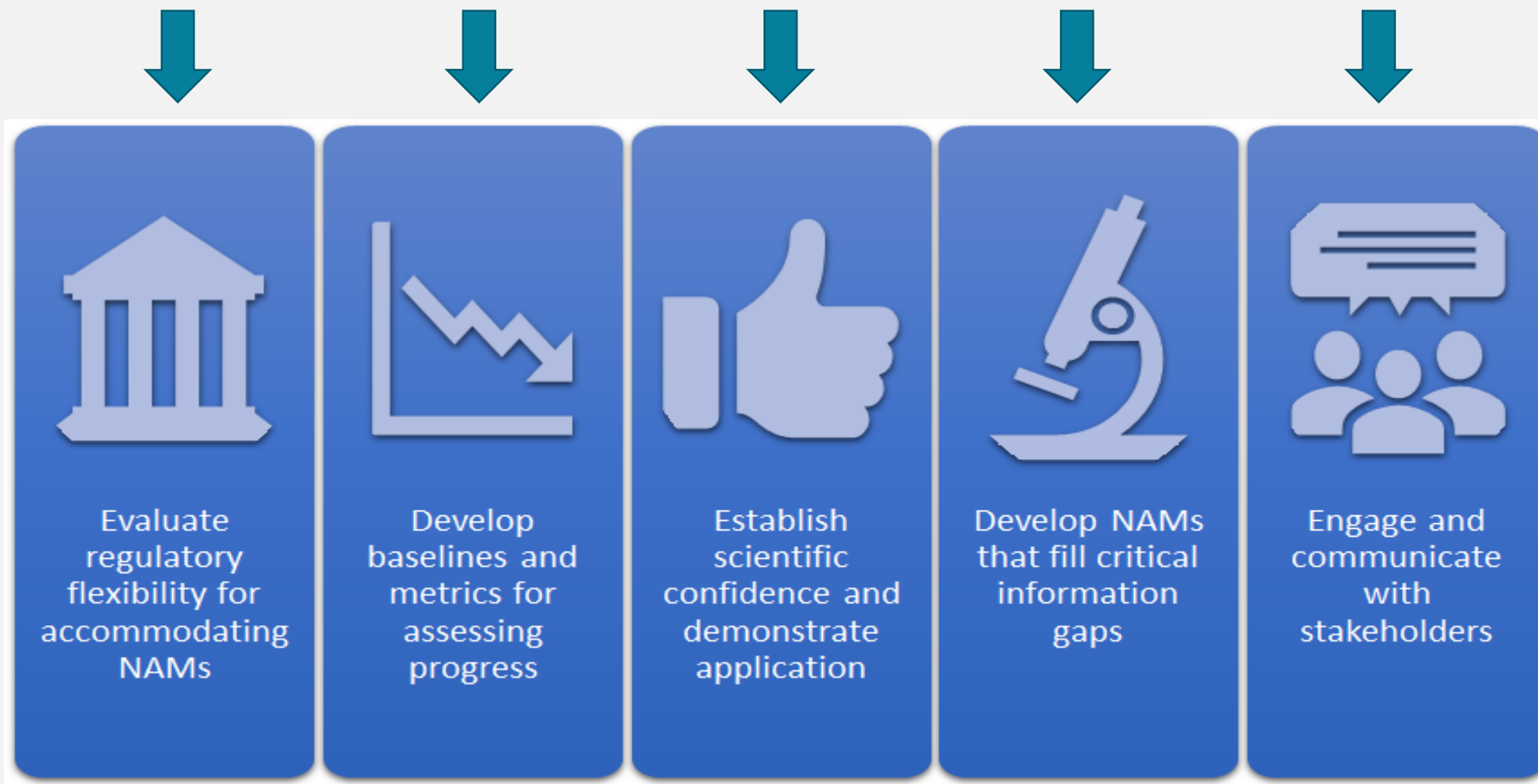
- Original work plan was released in June 2020
  - Laid out the Agency's objectives and strategies
- Committed to regularly reviewing the work plan and acknowledge the work plan will evolve as EPA's knowledge and experience grows, and as outside experts offer their perspectives and contributions
- EPA's work plan was recently updated in December 2021
  - [https://www.epa.gov/system/files/documents/2021-11/nams-work-plan\\_11\\_15\\_21\\_508-tagged.pdf](https://www.epa.gov/system/files/documents/2021-11/nams-work-plan_11_15_21_508-tagged.pdf)
  - Main objectives and strategies were left unmodified

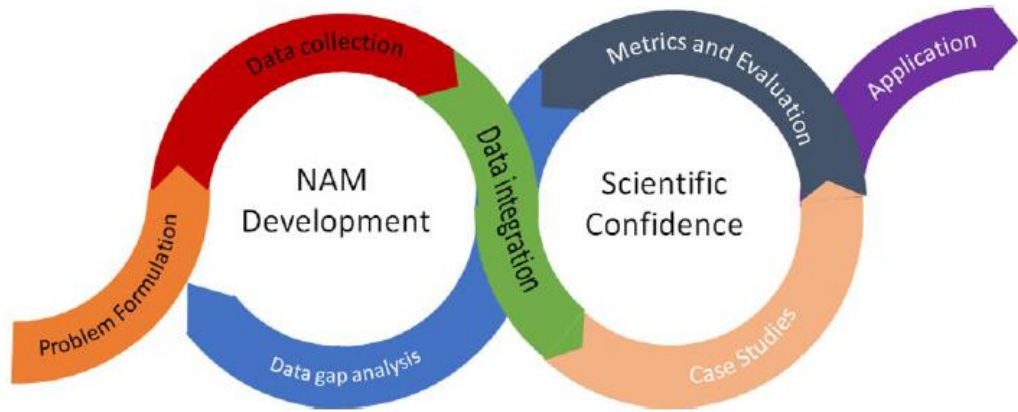
# NAMs Work Plan Roadmap



- Identifies **five objectives** for achieving the reduction goals while ensuring that the Agency's regulatory, compliance, and enforcement activities, including chemical and pesticide approvals and Agency research, remain fully protective of human health and the environment
- Discusses the short- and long-term strategies EPA will deploy to accomplish the objectives, working across offices and with stakeholders
- Reinforces that the work plan represents a snapshot in time and will need to continue to evolve as EPA's knowledge and experience grows

# 5 Objectives for the Agency

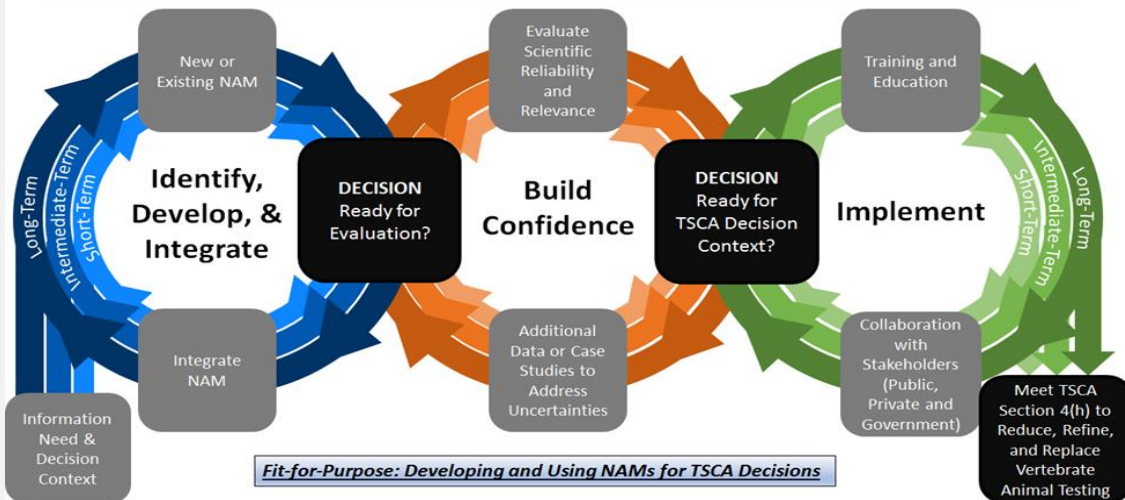




EPA NAM Workplan:

[https://www.epa.gov/system/files/documents/2021-11/nams-work-plan\\_11\\_15\\_21\\_508-tagged.pdf](https://www.epa.gov/system/files/documents/2021-11/nams-work-plan_11_15_21_508-tagged.pdf)

Fig. 1 Core Components of EPA Strategic Plan to Develop and Implement New Approach Methodologies (NAMs) in TSCA



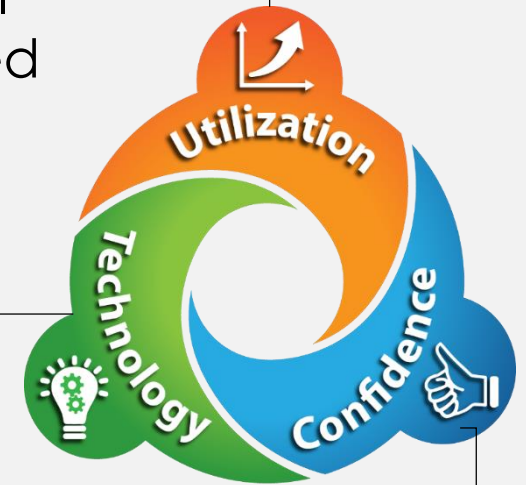
TSCA Strategic Plan:

<https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/strategic-plan-reduce-use-vertebrate-animals-chemical>

Encourage the adoption and use of new methods and approaches by federal agencies and regulated industries

Help end-users guide the development of the new tools needed to support their needs

Foster the use of efficient, flexible, and robust practices to establish confidence in new methods



ICCVAM Strategic Roadmap:

<https://ntp.niehs.nih.gov/whatwestudy/niceatm/natl-strategy/index.html>

# Projects Completed, Ongoing, and Proposed in EFED



- The Environmental Fate and Effects Division (EFED) is considering NAMs in the context of ecotoxicity and ecological risk
- Goal is to achieve reductions in the number of animals used without reduction in the quality of the ecological risk assessment process
- Focus on a variety of approaches from all three perspectives
  - Refine existing study protocols or their standard evaluation to allow for fewer animals required for a study
  - Reduce the number of studies and associated tested animals
  - Replace existing animal-based studies with other approaches

# Fish Acute Retrospective



## Reduction of required studies

- *Background:* OPP ecological risk assessments use studies with cold freshwater fish, warm freshwater fish, and saltwater fish to assess acute risks to fish
  - Pesticide registration data requirement (40 CFR Section 158) for an acute LC<sub>50</sub> test on 3 species (commonly rainbow trout, bluegill sunfish, and sheephead minnow)
  - Acute toxicity testing for a single chemical can use 200 or more fish
- *Question:* Is there a consistently more sensitive fish across all compounds and can we reduce data sets to two or even one fish study?
  - Collaboration with NICEATM
- Products (Ongoing)
  - Peer-reviewed publication anticipated 2022



# Fish Acute Retrospective



- *Dataset (initial)*: Focused on conventional pesticide active ingredients newly registered by EPA for the years 1998-2016
  - Over 700 studies representing tests on 348 substances (active ingredients, product formulations, and transformation products)
  - 181 pesticide active ingredients
- *Dataset (curated)*:
  - Excluded studies that would not be acceptable for quantitative risk assessment
  - Binned studies by active ingredient and specific formulation
  - Excluded chemicals without a study with at least one each of a cold freshwater fish, warm freshwater fish, and saltwater fish

# Fish Acute Retrospective



## Final dataset

- 110 analysis groups:
  - Each group represents a single chemical or formulation bin with at least one each of a cold freshwater, warm freshwater, and saltwater fish
  - Some contain > 3 species
- Mode of action representation:
  - 39 fungicides, 36 herbicides, 27 insecticides, and 6 other MOA
- Species representation:
  - Cold freshwater fish: 99% rainbow trout
  - Warm freshwater fish: 64% bluegill sunfish
  - Saltwater fish: 95% sheepshead minnow



# Fish Acute Retrospective



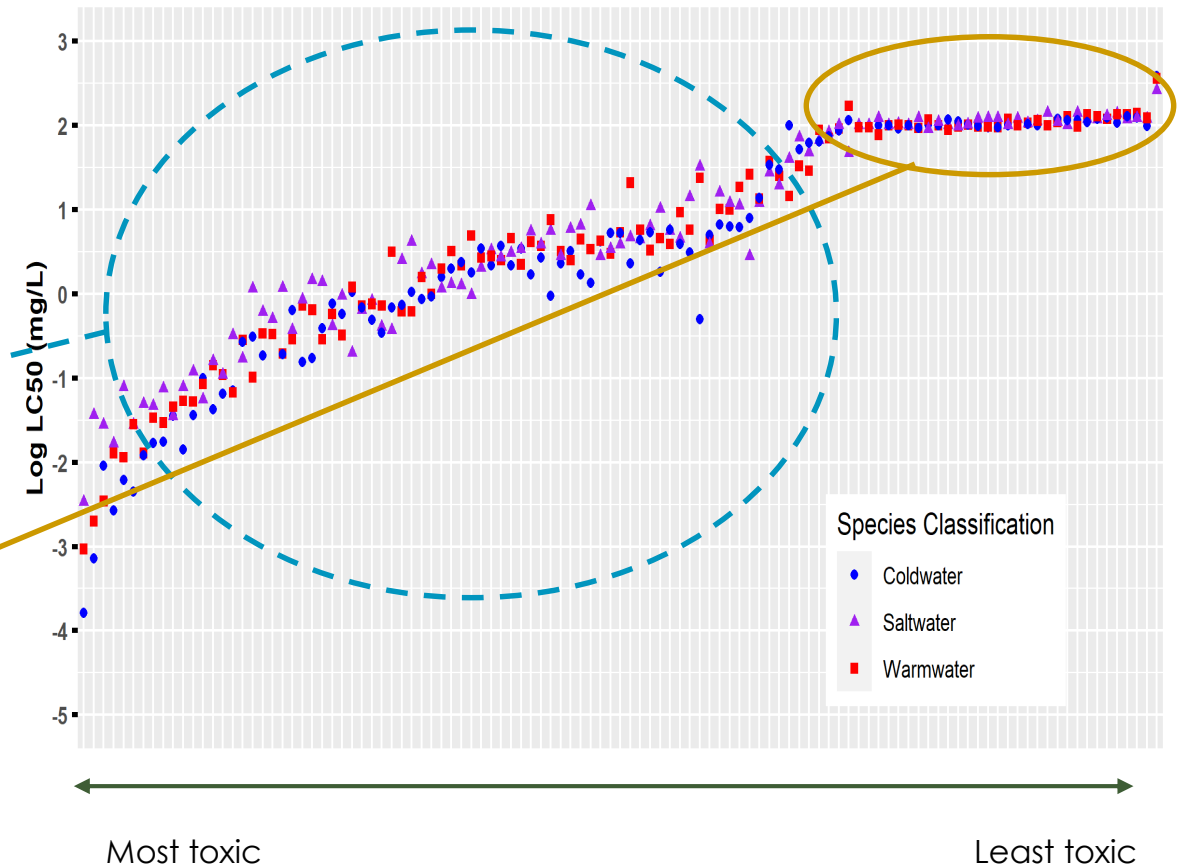
## Which species is most sensitive?

- The most sensitive species could be determined for only a subset of the 110 analysis groups, due in large part to some chemicals showing no toxicity to tested fish at limit test concentrations

**60 non-equivalent:** the most sensitive species category (cold, warm, saltwater) can be determined

**40 equivalent:** for risk assessment purposes we would consider the three species categories as equivalent because all three produced  $> LC_{50}$  values without mortalities

**10 unclear:** something about the combination of  $LC_{50}$  values makes it uncertain which of the three species groups is most sensitive but at least one of the  $LC_{50}$  values is definitive

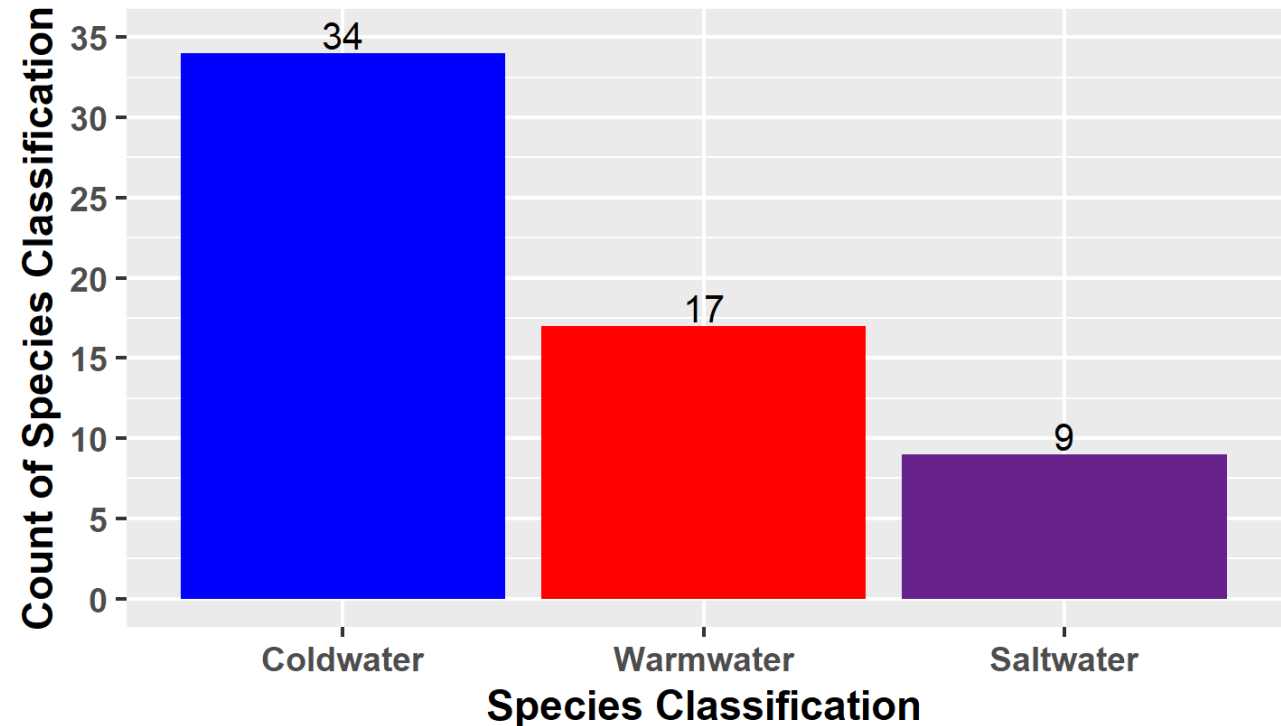


# Fish Acute Retrospective



## Species sensitivity

- Count of analysis groups for which a species type was most sensitive (60)
  - Cold freshwater = 34
  - Warm freshwater = 17
  - Saltwater = 9
- No clear pattern in terms of mode-of-action
- Hazard classification: freshwater fish (cold and warm) tended to be more sensitive than saltwater fish to the most toxic chemicals in the dataset



# In Closing



- OPP is committed to reduced animal testing burden without compromising the quality of the risk assessment
- Progress in the 3Rs requires:
  - collaboration across many sectors
  - transparency & use of peer review
- ICCVAM Ecotoxicology Workgroup  
<https://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam/wg/index.html>



**Email: [NAM@epa.gov](mailto:NAM@epa.gov)**