EPA Perspectives on Applying the 3Rs to Ecological Risk Assessment in Support of the Registration of Pesticides

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The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

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

Slide thanks to Warren Casey

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 17 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 • Department of Veterans Affairs

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.*"

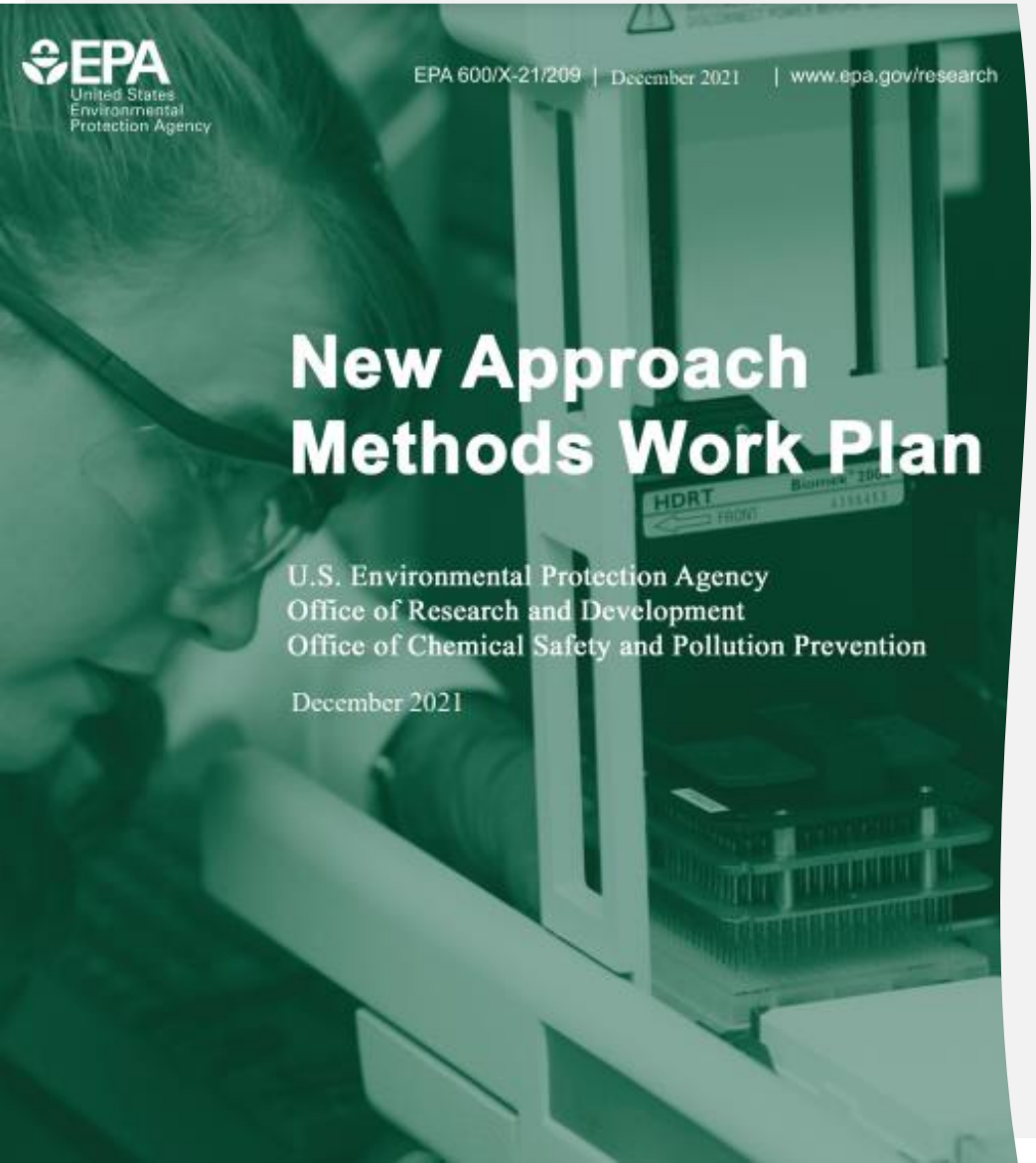
2016 OPP's Goal to Reduce Animal Testing

- 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 three conferences held between December 2019 and October 2022
    - Conference reports: www.epa.gov/chemical-research/past-conferences-state-science-development-and-use-new-approach-methods-nams
  - EPA is hosting the fourth EPA NAMs Conference on November 5-6, 2024 (hybrid)
    - Registration: https://www.epa.gov/chemical-research/epa-nams-conference
- 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
  - Main objectives and strategies were left unmodified

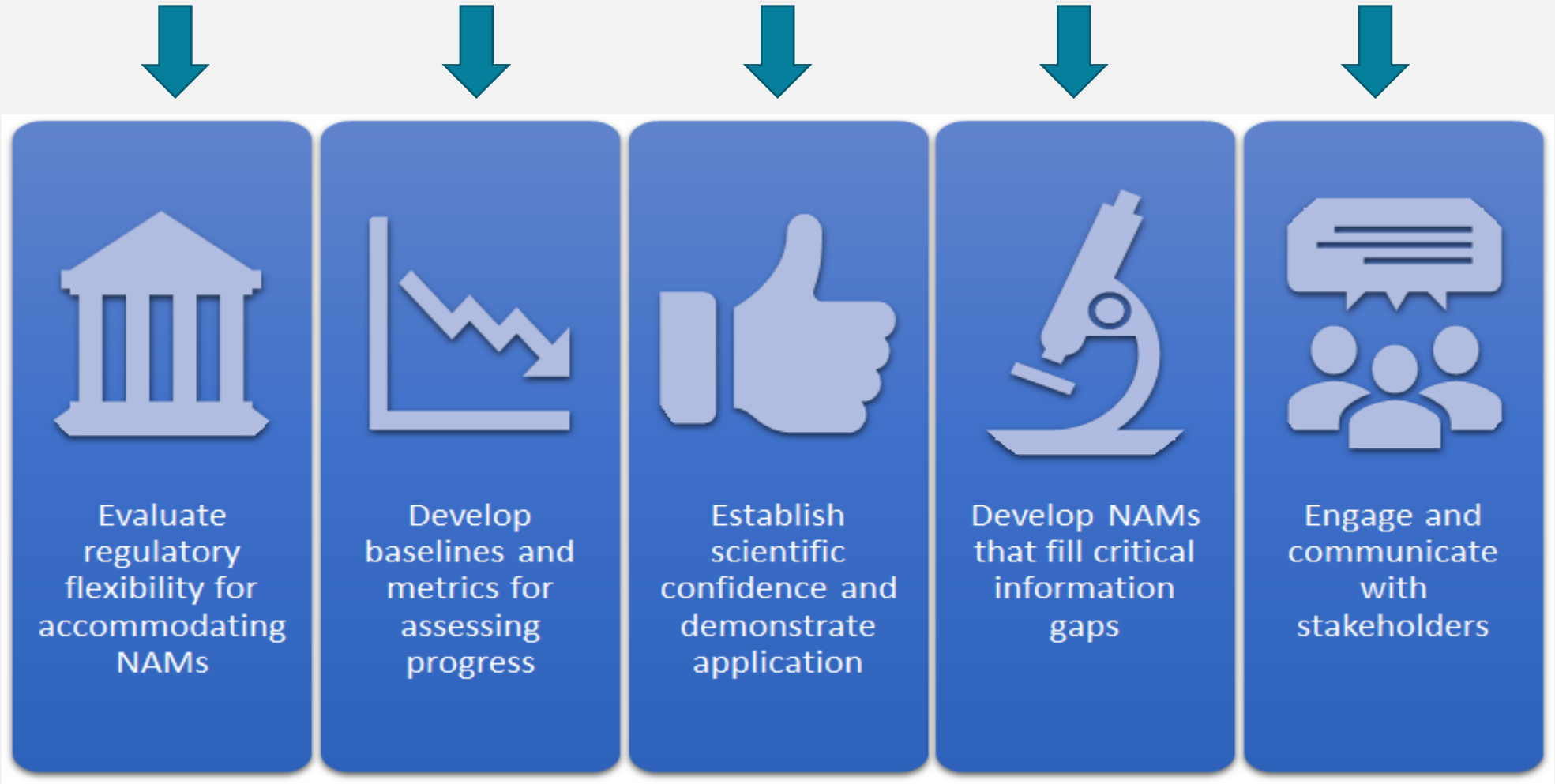
NAMs Work Plan Roadmap

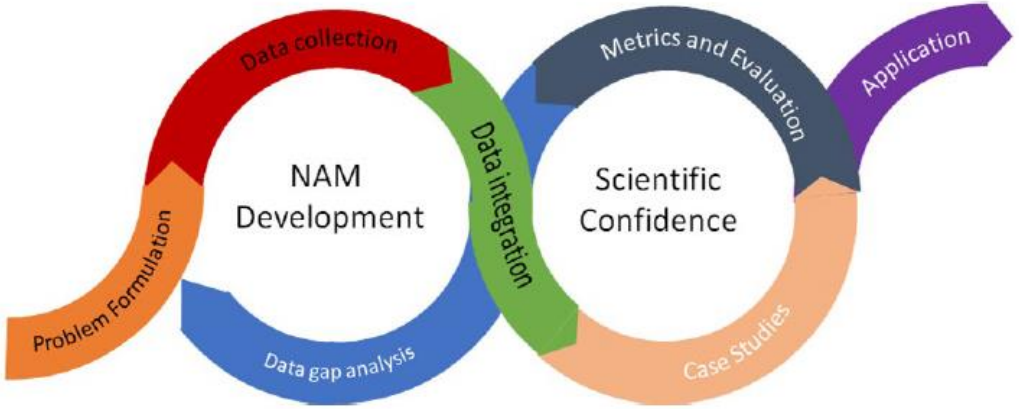
- Identifies **five objectives** for achieving the reduction goals while ensuring that the Agency's regulatory, compliance, and enforcement activities, including chemical and pesticide approvals and Agency research, remain fully protective of human health and the environment

- Discusses the short- and long-term strategies EPA will deploy to accomplish the objectives, working across offices and with stakeholders

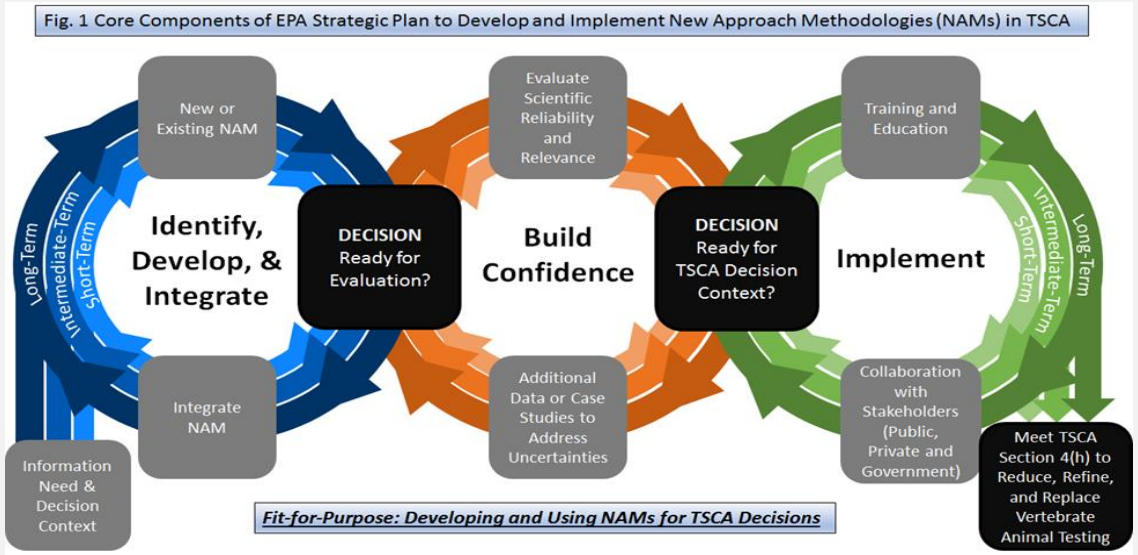
- Reinforces that the work plan represents a snapshot in time and will need to continue to evolve as EPA's knowledge and experience grows

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

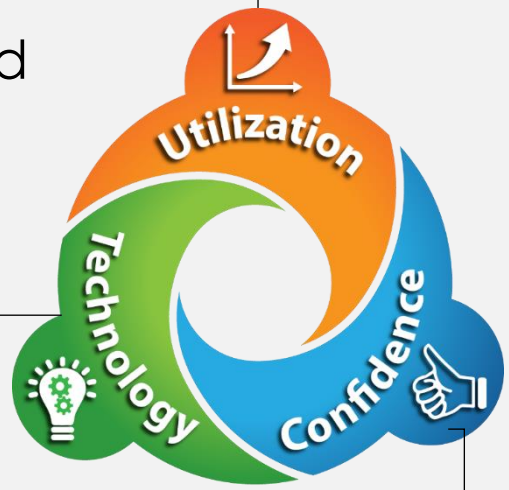


TSCA Strategic Plan: https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/strategic-plan-reduce-use-vertebrate-animals-chemical

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 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

Select Projects

Reduction of required studies

- Avian subacute/acute risk retrospective
- Fish acute retrospective
- Avian reproduction retrospective

Replacement of required studies

- QSAR for rat acute oral LD_{50}

Refinement of required studies

- Fish bioconcentration single-dose study data evaluation guidance

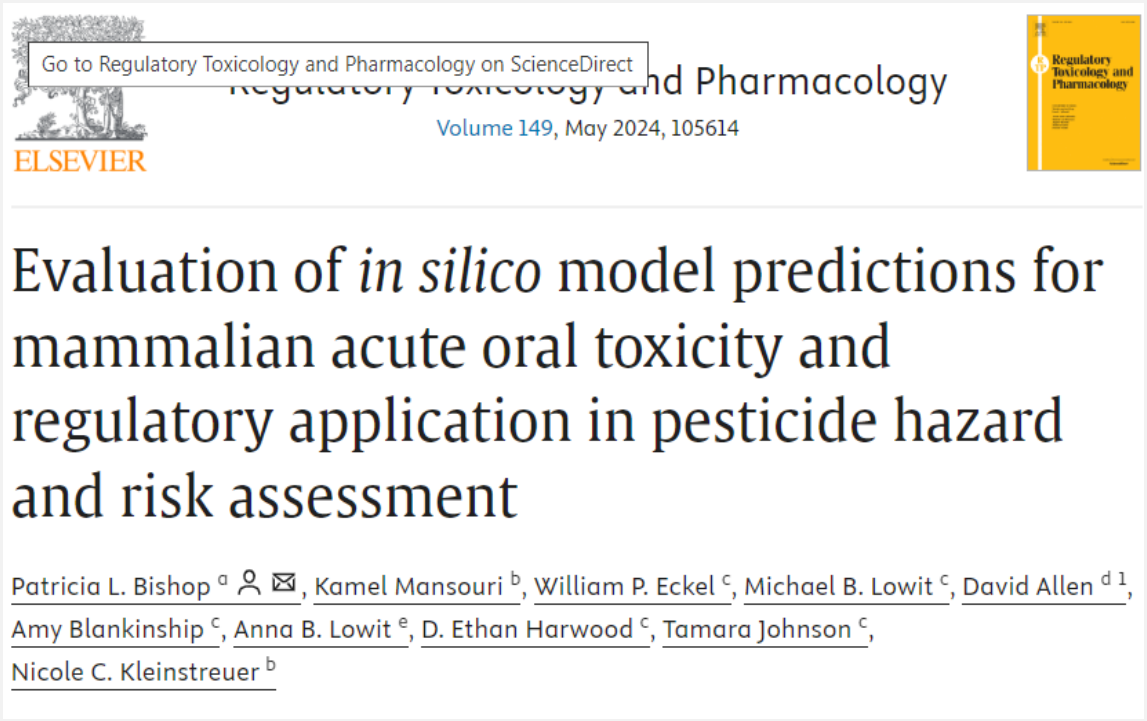
Aquatic organism quantitative structure-activity relationship (QSAR)

- Updates to ECOSAR (Ecological Structure Activity Relationship) model

QSAR for Rat Acute Oral LD_{50}

Replacement of required studies

- Collaborative Acute Toxicity Modeling Suite (CATMoS)
  - Developed by NIEHS-NICEATM and ICCVAM
  - 35 participants/groups from around the globe representing academia, industry, and government contributed to the development
- Goal
  - OPP worked with NICEATM & Humane Society to evaluate applicability for conventional pesticides as a potential replacement of the rat acute single oral dose study for establishing the effects endpoint in ecological risk assessment
- Products
  - Bishop, P.L., Mansouri, K., et. al. (2024). Regulatory Toxicology and Pharmacology, 149 https://doi.org/10.1016/j.yrtph.2024.105614



Background: EPA Use of the Acute Oral Toxicity Test

- OPP uses data specified in 40 CFR Part 158 to make regulatory decisions regarding the effects of pesticides on human health and the environment
- An *in vivo* rat acute oral toxicity test that results in the determination of an LD_{50} value is performed for all pesticide technical grade active ingredients (TGAIs) as well as end-product formulations
- Pesticide is assigned to an acute oral toxicity category based on the LD_{50} value ranging from Category I to Category IV
- Toxicity category determines the precautionary statements placed on the pesticide label for acute human exposure
- LD_{50} is also used by EPA as a surrogate for acute oral toxicity to all mammalian wildlife
- Potential risks to wildlife are determined by comparing a body-weight adjusted LD_{50} for different size classes of mammals to the estimated acute exposure dose of the pesticide via food items

EPA Toxicity Categories

EPA acute oral toxicity categories based on LD_{50} values and associated label precautionary statements and signal words

Category	Oral LD_{50} (mg/kg)	Precautionary Statement	Signal Word
I	≤50	Fatal if swallowed	Danger
II	>50 – 500	May be fatal if swallowed	Warning
III	>500 – 5,000	Harmful if swallowed	Caution
IV	>5,000	No statement required	None required

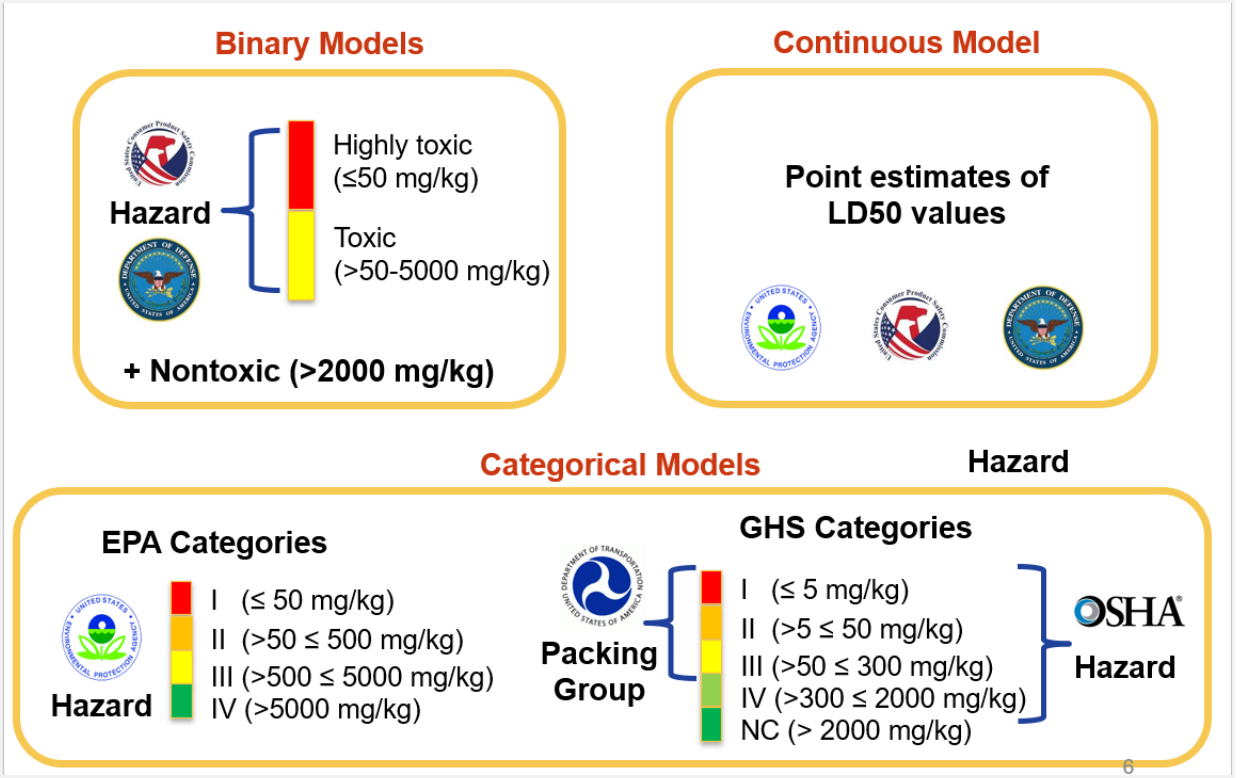
LD_{50}s may be determined to be definitive, i.e., reported as a specific dose, or non-definitive usually based on a limit test where a single dose of 2,000 or 5,000 mg/kg is administered resulting in little or no sign of toxicity. Limit tests are reported as >2,000 mg/kg or > 5,000 mg/kg

What is the Collaborative Acute Toxicity Modeling Suite (CATMoS)?

- *In silico* predictive tool for estimating acute oral toxicity based on molecular structure
- Result of an international collaboration sponsored by NICEATM to build *in silico* models that predict acute oral toxicity
- CATMoS composed of a suite of consensus models that combined results of individual models contributed by collaborators
- Models built by using chemical structures and available rat acute oral toxicity values for nearly 9,000 chemicals (training) and tested using about 3,000 chemicals

What does CATMoS do?

- CATMoS can predict endpoints according to regulatory needs: binary (toxic, non-toxic), discrete LD_{50} values, and categorical (US EPA toxicity categories and GHS toxicity categories)
- A 95% confidence interval of ± 0.24 $\log_{10}$ mg/kg was established based on the variability in experimental LD_{50} values
- Incorporating this inherent variability into the CATMoS predictions helps build confidence and allows the user to understand the relationship of predictions to reported *in vivo* data



Project Question

How well would CATMoS perform in the chemical space of conventional pesticides in predicting acute oral toxicity LD_{50} values relative to *in vivo* empirical LD_{50} values of TGAIs for pesticides registered by the EPA?

Approach to Comparative Analysis

EPA provided initial list of 195 pesticide TGAIs registered in the US or evaluated for import tolerances from 1998 to 2020; some pesticides excluded for various reasons - 177 final total

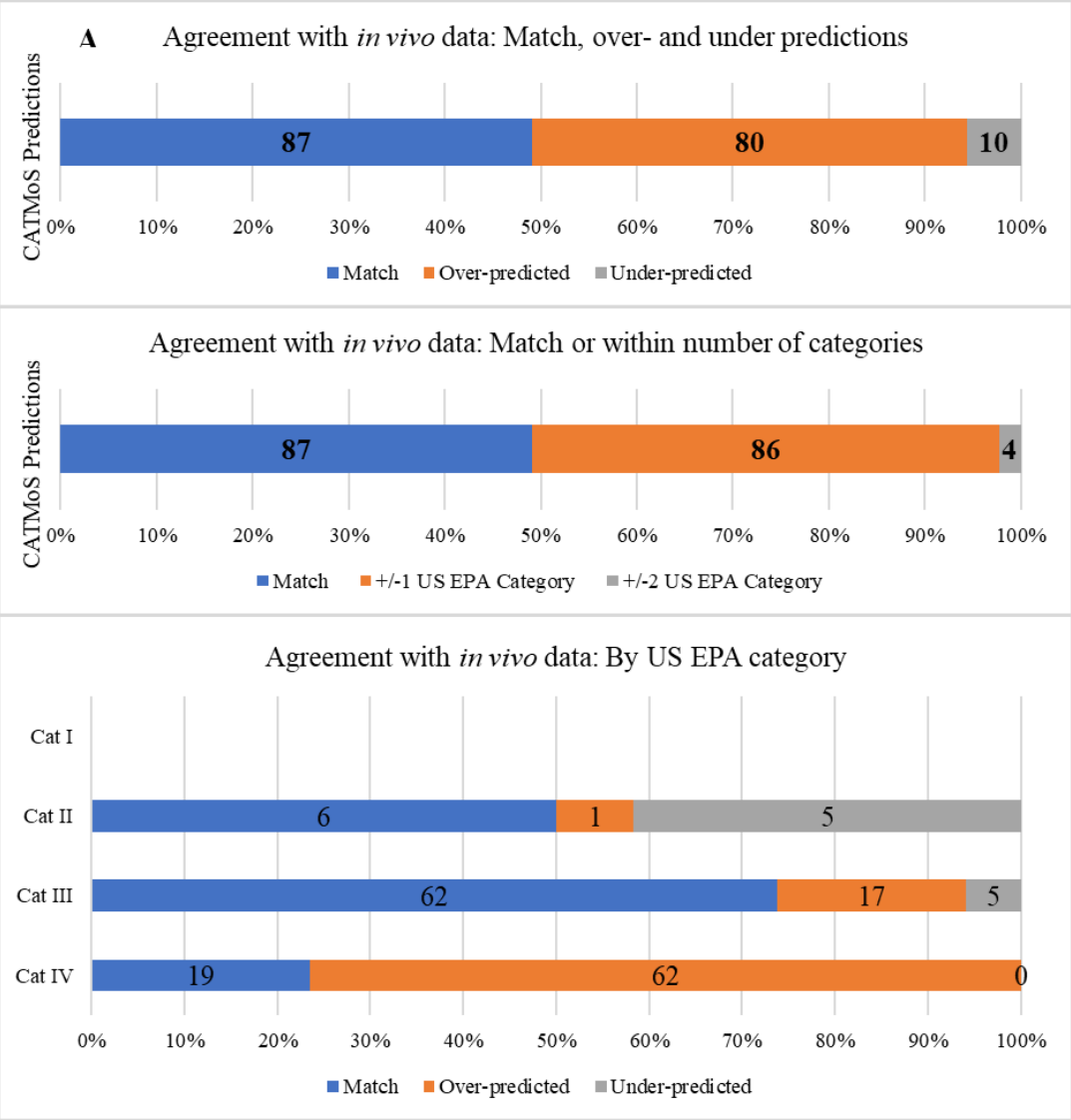
Broad pesticide coverage: 67 fungicides, 56 herbicides, 46 insecticides/acaricides, three nematicides, four plant activators/growth regulators, and one reptilicide

Empirical *in vivo* LD_{50}s conducted under EPA test guideline (OPPTS 870.1100) obtained from publicly available human health and ecological risk assessments conducted by EPA

CATMoS model was run using TGAI chemical structures; prediction outputs of both toxicity category and discrete LD_{50} value along with 95% confidence interval

The accuracy and reliability of the model predictions were assessed relative to the empirical data

Toxicity Category Results

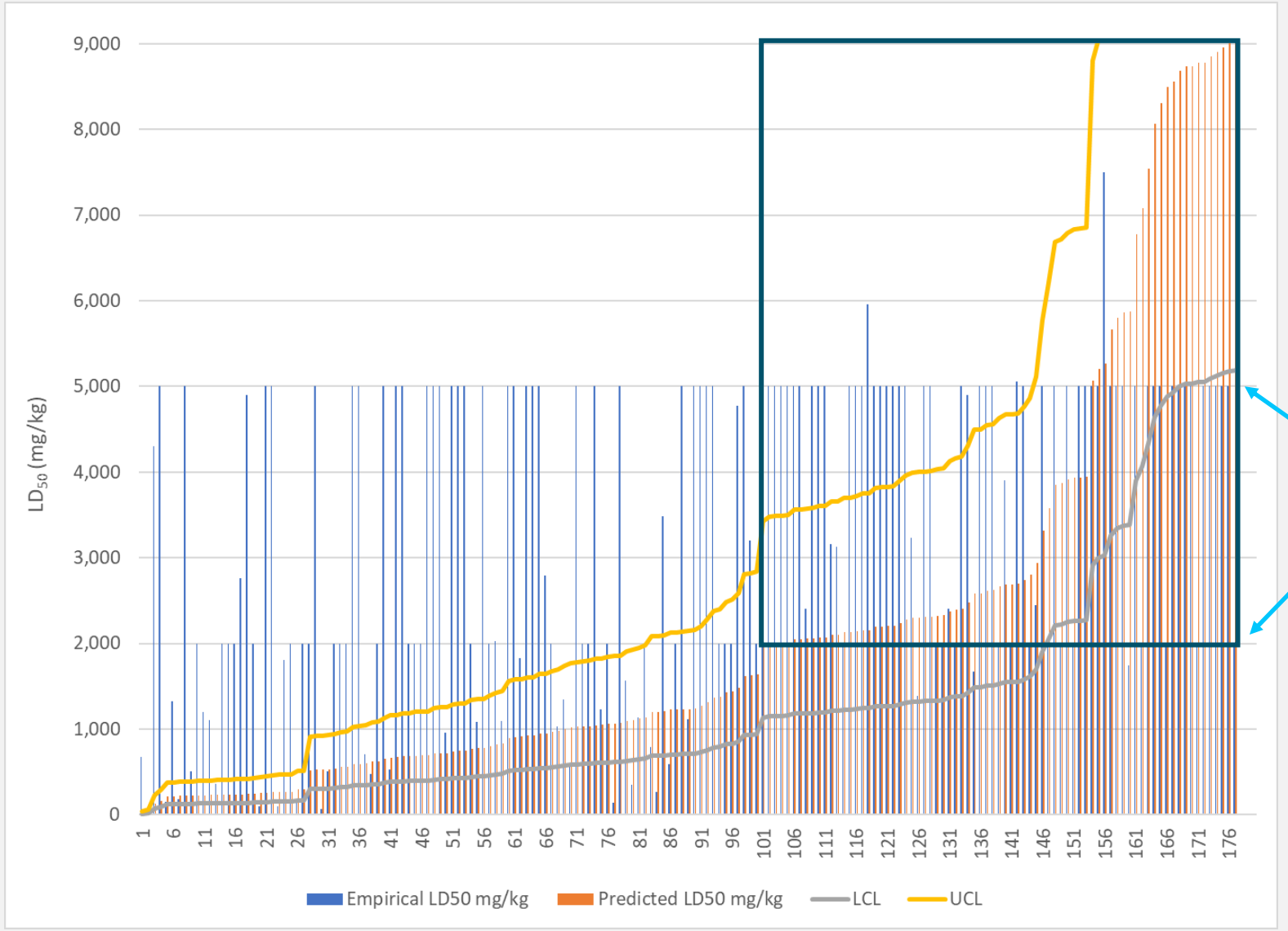


Toxicity Category based on CATMoS Prediction	Number of predictions	Toxicity Category based on Empirical *In Vivo* Test Data			
		I	II	III	IV
I (<50 mg/kg)	2	-	1	1	-
II (50-500 mg/kg)	25	-	**6**	16	3
III (>500-5,000 mg/kg)	126	-	5	**62**	59
IV (>5,000 mg/kg)	24	-	-	5	**19**
III and IV combined	150	-	5	145	

LD_{50} Discrete Value Comparisons

- More accurate prediction of LD_{50} needed for quantitative risk assessment than for acute toxicity categories
- Applied confidence interval of +/- 0.24 $\log_{10}$ mg/kg derived during the CATMoS project development that represents the variability in animal data
- Calculated upper and lower confidence limits of the CATMoS prediction using this value
- Determined whether the empirical *in vivo* value fell within the confidence interval of the CATMoS prediction

LD_{50} Discrete Value Comparisons



Empirical values reaching exactly 2,000 or 5,000 mg/kg are limit tests

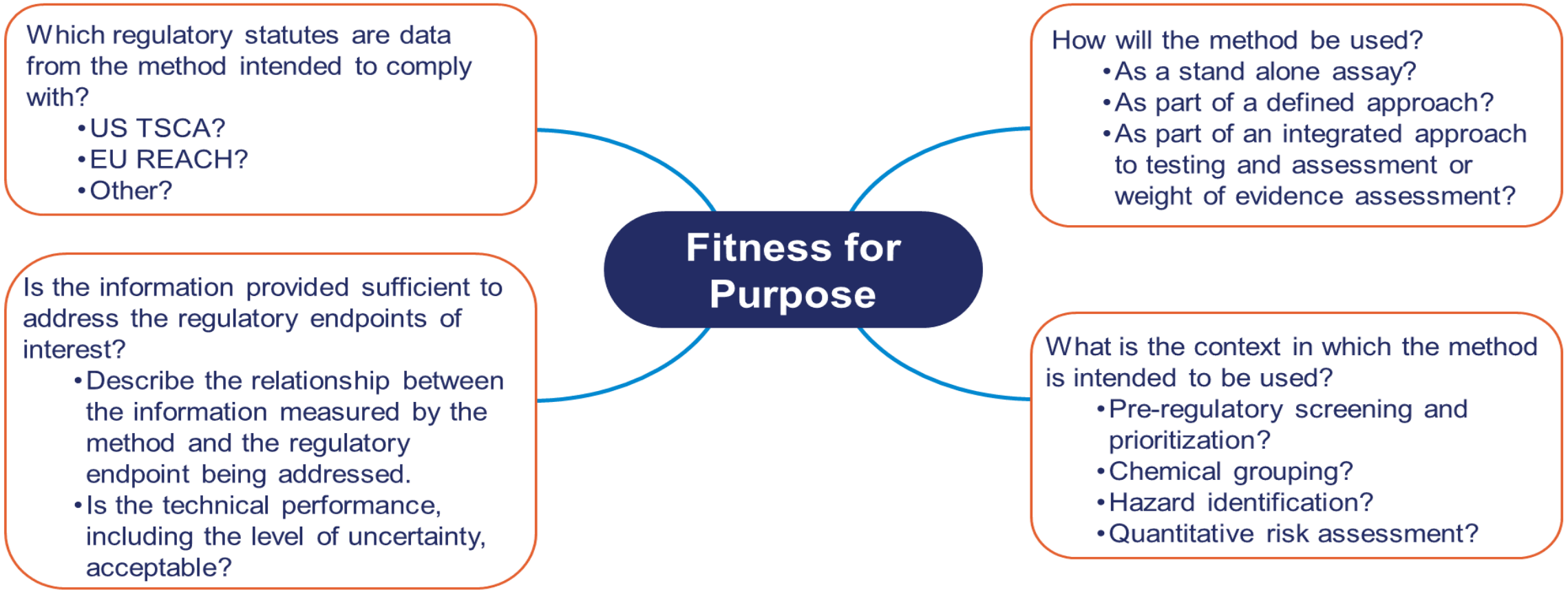
Overall Findings

- CATMoS predictions showed good agreement (97%) with empirical values for toxicity categories III and IV combined (i.e., predictions fell into either category)
- Use of CATMoS estimates for toxicity might result in a more stringent label warning than an animal test would require (e.g., category III warning when *in vivo* data indicate category IV)
- When predicting toxicity categories, 145/150 pesticides with CATMoS values of >500 mg/kg also had empirical values >500 mg/kg suggesting a high degree of confidence; however, 5/150 were category II pesticides that were under-predicted by CATMoS, thus predictions close to 500 mg/kg might require additional evidence to support the prediction
- Too few chemicals with empirical values in categories I and II to draw useful conclusions about CATMoS predictions

Overall Findings (continued)

- CATMoS estimates of discrete LD_{50} values of $\geq$2,000 mg/kg appear to be reliable
- For the highest assessed exposure scenario (small mammal eating short grass), use of a >2000 mg/kg-bw CATMoS LD_{50} estimate would result in "low risk" potential (non-listed species) for application rates below about 10 pounds per acre
- The results support potentially relying on CATMoS predictions in lieu of *in vivo* testing in some cases depending on considerations such as the LD_{50} prediction and the proposed application rate
- The results of this analysis can help inform whether CATMoS can be used to estimate acute oral toxicity from pesticides for purposes of identifying toxicity categories and assessing risk to wildlife
- Possible further work could focus on methods for estimating LD_{50}s of pesticide formulations, which can consist of more than one TGAI in addition to other ingredients, and which account for most of the LD_{50} tests submitted to EPA each year

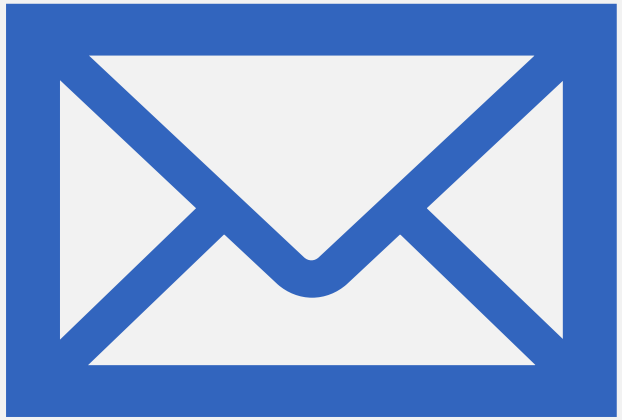
Fitness for Purpose



Extracted from Van der Zalm, AJ; Barroso, J; Browne, P; Casey, W; Gordon, J; Henry, TR; Kleinstreuer, NC; Lowit, AB; Perron, M; Clippinger, AJ. 2022. A framework for establishing scientific confidence in new approach methodologies. Archives of Toxicology.

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



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