Recent Progress Towards Reducing Animal Use & Adopting New Approach Methods at EPA's Office of Pesticide Programs

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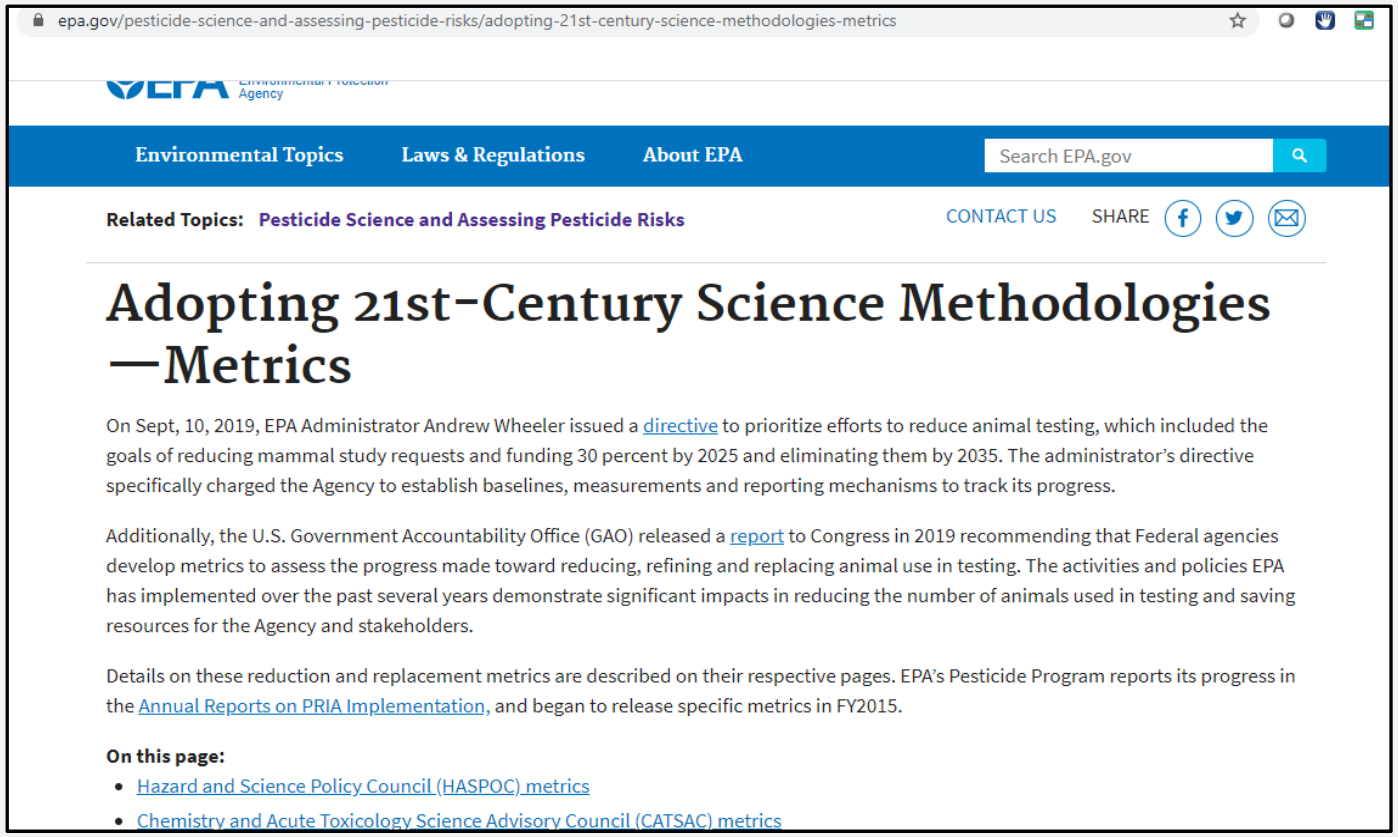
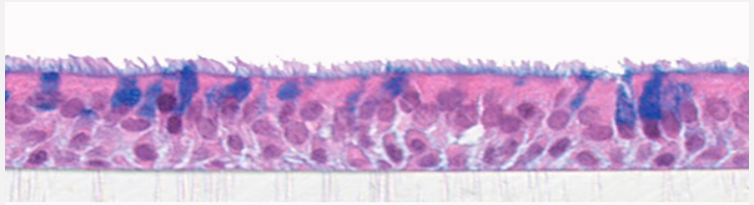
Progress on Metrics: EPA-OPP's New Webpage



- Main page: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-new-approach-methodologies
- Metrics: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/adopting-21st-century-science-methodologies-metrics

Inhalation Risk Assessment

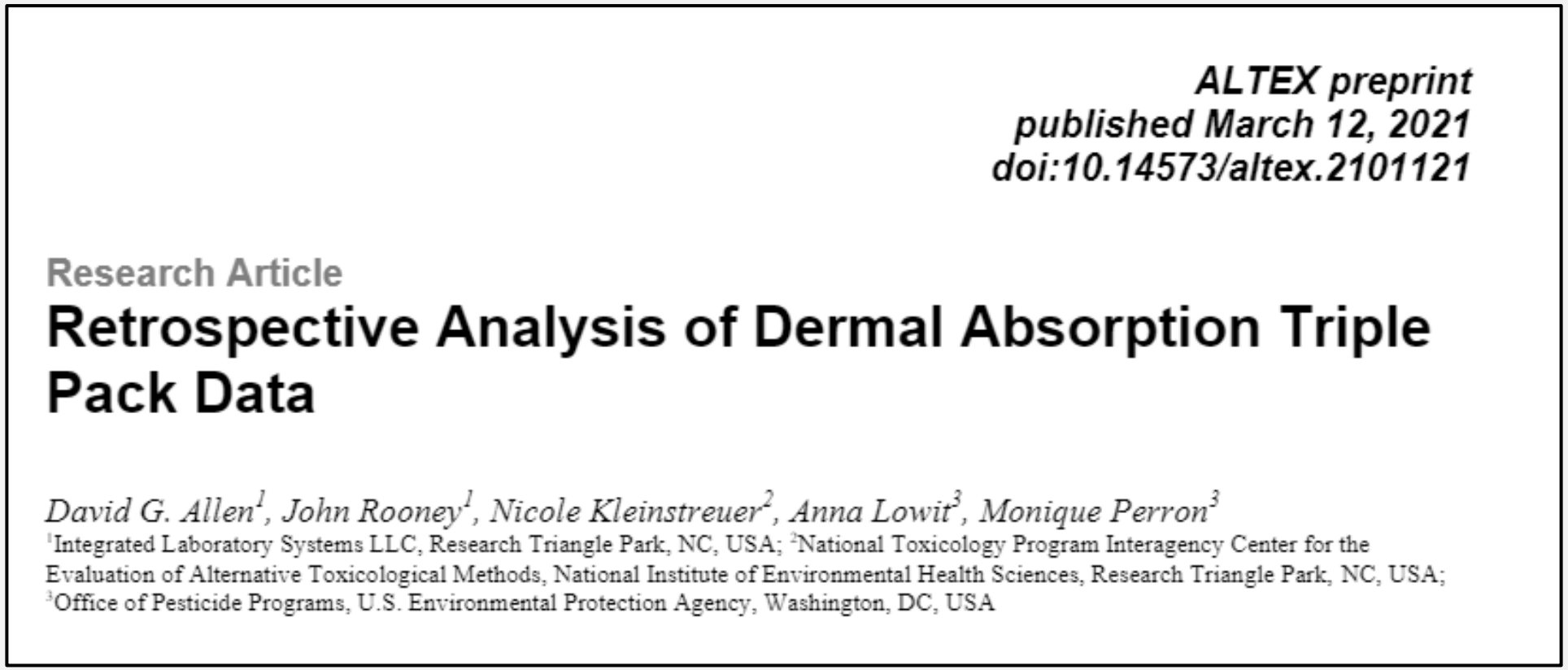
- Proposal for refining inhalation risk assessment using a 3D human airway epithelia reconstituted in vitro model initially presented to EPA in 2014 by Syngenta Crop Protection
- Agency recognized the value of the proposal for chlorothalonil, as well as other respiratory contact irritants and encouraged further development
- Collaborated with National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) for review
- Convened FIFRA SAP meeting in December 4-7, 2018 to evaluate the proposed approach
  - First time a point of departure for risk assessment will be derived using in vitro data for a pesticide
  - Potential use for other contact irritants, as well as other chemicals that cause portal of entry effects in the respiratory tract
- SAP report released in April 2019: No panelists supported using the laboratory animal study



FIFRA SAP on NAMs for Extrapolation: OP Case Study

- In September 2020, OPP convened FIFRA SAP on "Use of Non-Animal Studies to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment"
  - *in vitro* data for 16 OPs to potentially reduce reliance on default risk assessment uncertainty factors in favor of more refined data-derived factors.
  - ORD is working to develop a NAM for evaluating developmental neurotoxicity & is evaluating *in vitro* to *in vivo* extrapolation methodology. OPs are being used as a case study.
  - SAP Report was published in December, 2020
  - https://www.epa.gov/sap/use-new-approach-methodologies-nams-derive-extrapolation-factors-and-evaluate-developmental

Dermal Absorption "Triple Packs"



ALTEX preprint
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Research Article

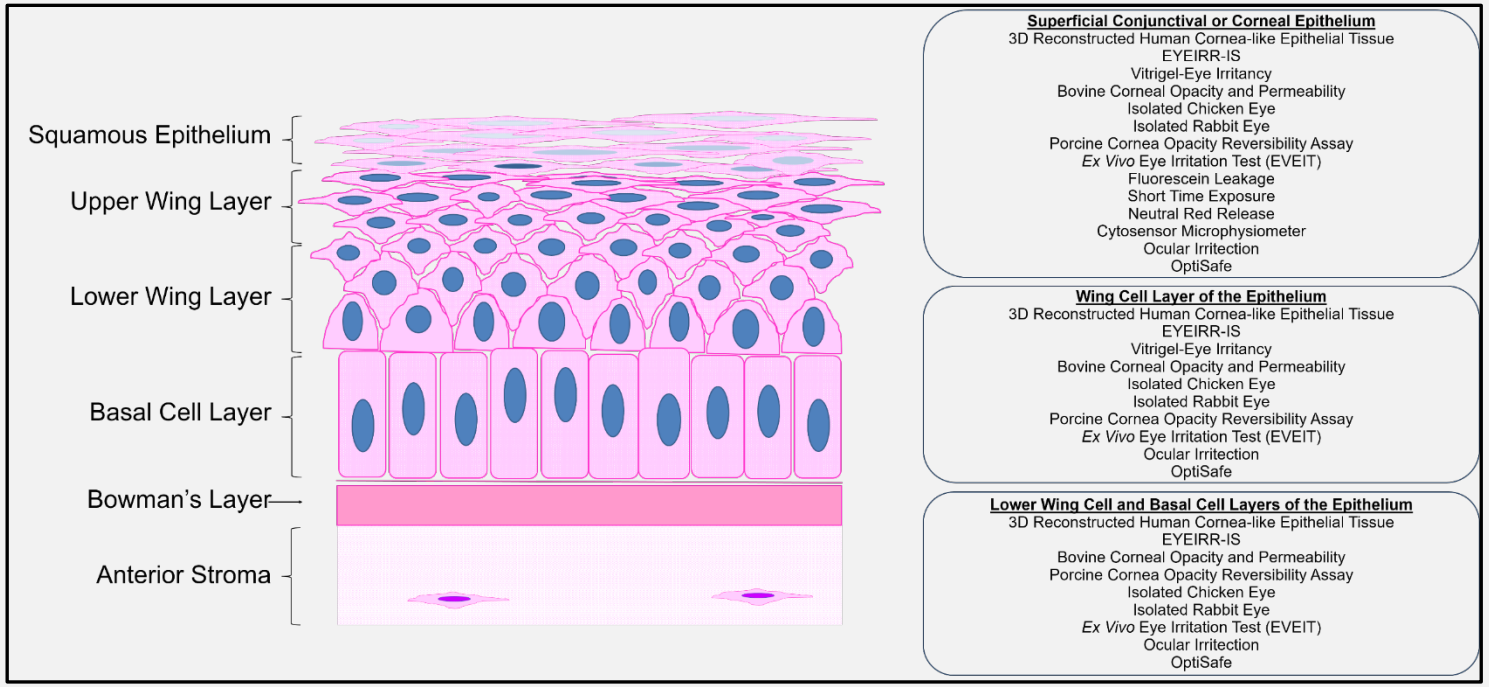
Retrospective Analysis of Dermal Absorption Triple Pack Data

David G. Allen[1], *John Rooney*[1], *Nicole Kleinstreuer*[2], *Anna Lowit*[3], *Monique Perron*[3]
[1]Integrated Laboratory Systems LLC, Research Triangle Park, NC, USA; [2]National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA; [3]Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC, USA

- Retrospective analysis of human *in vitro*, rat *in vitro*, and rat *in vivo* studies using similar protocols (e.g., same test material, doses)

Eye Irritation

- Clippinger, et al (2021) Human-Relevant Approaches to Assess Eye Corrosion/Irritation Potential of Agrochemical Formulations. Cutaneous and Ocular Toxicology. In press.
  - Provides evaluation of the human relevance of the in vivo rabbit study and in vitro assays.
  - Comparison of human, rabbit, porcine, chicken, and bovine corneas
  - Describes strengths and uncertainties of the in vivo and in vitro assays
  - Proposes an adverse outcome pathway for eye irritation
  - Concludes that many in vitro/ex vivo methods are equivalent or scientifically superior to the rabbit test and can be used now



MOLECULAR INITIATING EVENT
- binding to cell surface proteins
- binding to cytoplasmic proteins / enzymes
- surfactant intercalation and disruption of cell membrane lipid bilayer organization
- solvent dissolution of cell membrane proteins or lipids
- solvent precipitation of cell proteins
- alkaline saponification of membrane lipids
- acid precipitation of cell proteins, enzymes and nucleic acids
- oxidative damage to cell membrane lipids
- oxidative changes in cell proteins
- solvent dissolution of nucleic acids
- solvent dissolution of cytoplasmic components
- precipitation of nucleic acids
- binding to DNA and/or RNA

→

CELLULAR RESPONSE
- chemical antagonism of vital enzymes, nucleic acids
- cell stress responses
- breakdown of the tight junctions
- activation of matrix metalloproteases
- changes in cell surface markers and cell-to-cell and cell-to-basement membrane adhesion molecules, desmosomes / hemidesmosomes / anchoring proteins
- breakdown of cell membrane integrity
- release of chemokines and cytokines (e.g., IL-1α, TNFα)
- induction of secondary cytokines
- neural dendrites trigger TRPV1-type nociceptive response
- changes in cell metabolism/respiration
- changes in normal functional phenotype
- necrotic or apoptotic damage leading to cell death

→

ORGAN RESPONSE
- increased corneal or conjunctival permeability/loss of barrier function
- conjunctival hyperemia and discharge
- swelling of the conjunctival tissues / swelling of the eye lid tissues
- epithelial tissue swelling
- sloughing and loss of epithelial tissue layers
- corneal/stromal swelling and oedema, and swelling-related corneal opacity
- corneal opacity due to cellular/molecular denaturation/coagulation
- induction of wound healing response and basal cell regeneration/turnover
- inflammatory response and neutrophil migration
- induction of fibrosis, panus, and neovascularization
- loss of endothelium

→

ORGANISM RESPONSE
- increased corneal or conjunctival susceptibility to xenobiotics
- pain and nociceptive responses
- induction of lachrymation
- transient or permanent loss of visual acuity

SAB Consultation on NAMs for Chronic/Cancer Testing

- Science Advisory Board consultation on June 23-24, 2020 on "New Approach Methods and Reducing the Use of Laboratory Animals for Chronic and Carcinogenicity Testing"
  - https://yosemite.epa.gov/sab/sabproduct.nsf//LookupWebProjectsCurrentBOARD/2D3E04BC5A34DCDE8525856D00772AC1?OpenDocument
- Topics organized around the 3Rs
  - Reduce: Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP)
  - Replace:
    - Division of the National Toxicology Program (DNTP) of National Institute of Environmental Health Sciences (NIEHS): carcinogenicity initiative to develop efficient, fit for purpose approaches to characterize the potential for environmental exposures to cause or contribute to the development of cancer in humans
    - HESI to consider NAM-based approaches to replace chronic/carcinogenicity testing in mammals by use of omics-based points of departure
    - ORD case study to use NAMs on selected pesticides with established MOAs
  - Refine: use of kinetically derived maximum doses instead of traditional maximum tolerated dose

Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP)

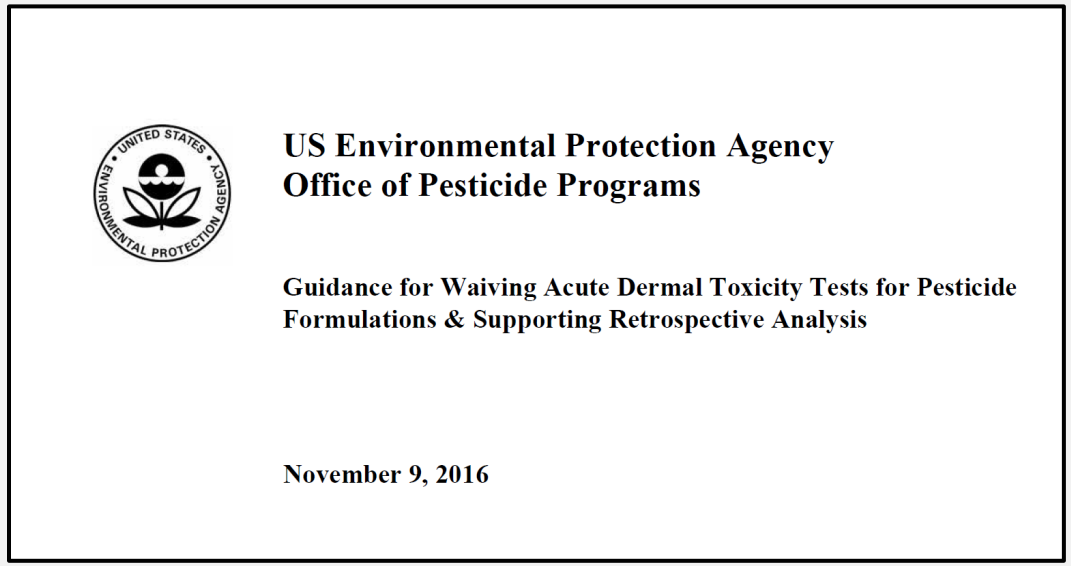
- Collaborative project to develop a waiver framework for pesticides:
  - Project led by PETA-ISC with contributions from Canada PMRA, APVMA, ORD, BASF, Corteva, Syngenta, OPP-HED (Brazil ANVISA has recently joined)
  - Retrospective & prospective case studies have been developed as part of the WOE development
  - Manuscript being developed for publication
    - Incorporates comments from SAB

Draft Carcinogenicity Waiver Reporting Framework

I. Purpose of this Analysis
II. Study Waiver Requests
    1. Use Pattern and Exposure Scenarios
    2. Physical-Chemical Properties
    3. ADME and Toxicokinetics
    4. Toxicity
        4.1 Acute Toxicity
        4.2 Subchronic Toxicity
        4.3 Evidence of Hormone Perturbation
        4.4 Evidence of Immune Suppression
        4.5 Genetic Toxicity
        4.5 Special Studies and Endpoints
    5. Evidence of Chronic Toxicity from Related Chemicals
    6. Proposed Points of Departure, and Prospective Risk Assessments
    7. Conclusion
    8. References

Acute Dermal Pesticide Toxicity Testing

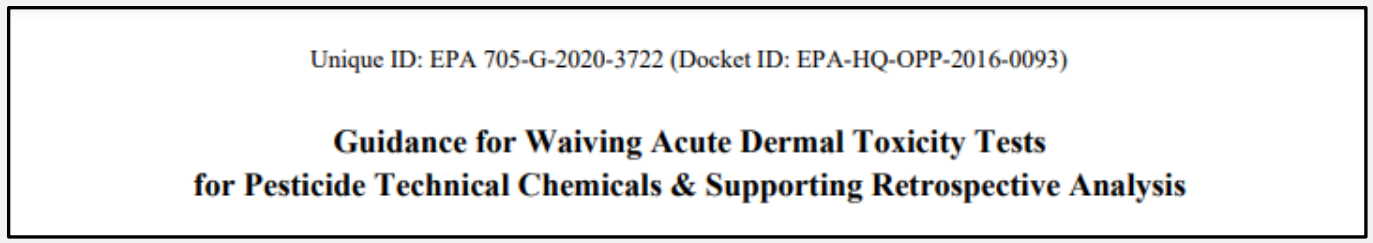
- Collaboration between EPA & NIEHS-NICEATM
- Analyzed the relative contribution of data from acute oral and dermal toxicity tests to pesticide hazard classification and labelling
  - Pesticide formulations, 2016
  - *Active ingredients, 2020*
- https://www.epa.gov/pesticide-registration/bridging-or-waiving-data-requirements



US Environmental Protection Agency
Office of Pesticide Programs

Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations & Supporting Retrospective Analysis

November 9, 2016



Unique ID: EPA 705-G-2020-3722 (Docket ID: EPA-HQ-OPP-2016-0093)

Guidance for Waiving Acute Dermal Toxicity Tests
for Pesticide Technical Chemicals & Supporting Retrospective Analysis

Avian subacute/acute risk retrospective

- EPA-OPP ecological risk assessments use both acute oral and sub-acute dietary studies to assess acute risks to birds (the endpoint that results in the highest risk quotient drives the risk conclusion)
- Question: Can we confidently assess acute risk for birds using a reduced suite of effects studies focusing on the single oral dose protocol?
  - How often have subacute dietary risk quotients (RQs) quantitatively driven risk assessment conclusions?
- Partnership with PETA-ISC
- Bottom line results are that 99% (118 of 119) of all subacute dietary studies for new use assessments did not change risk conclusions already reached using oral dose-based RQ's.
  - In most cases (there are some exceptions) a robust avian acute risk assessment can be conducted without the sub-acute dietary studies.
- Hilton, G.M., Odenkirchen, E., Panger, M., Waleko, G., Lowit, A., Clippinger, A.J. 2019, Regulatory Toxicology and Pharmacology, 105: 30-35, https://doi.org/10.1016/j.yrtph.2019.03.013
- *Policy finalized in February, 2020*
  - https://www.epa.gov/sites/production/files/2020-02/documents/final-waiver-guidance-avian-sub-acute-dietary.pdf

Fish Bioconcentration Single Dose Study Data Evaluation Guidance

Refinement of required studies

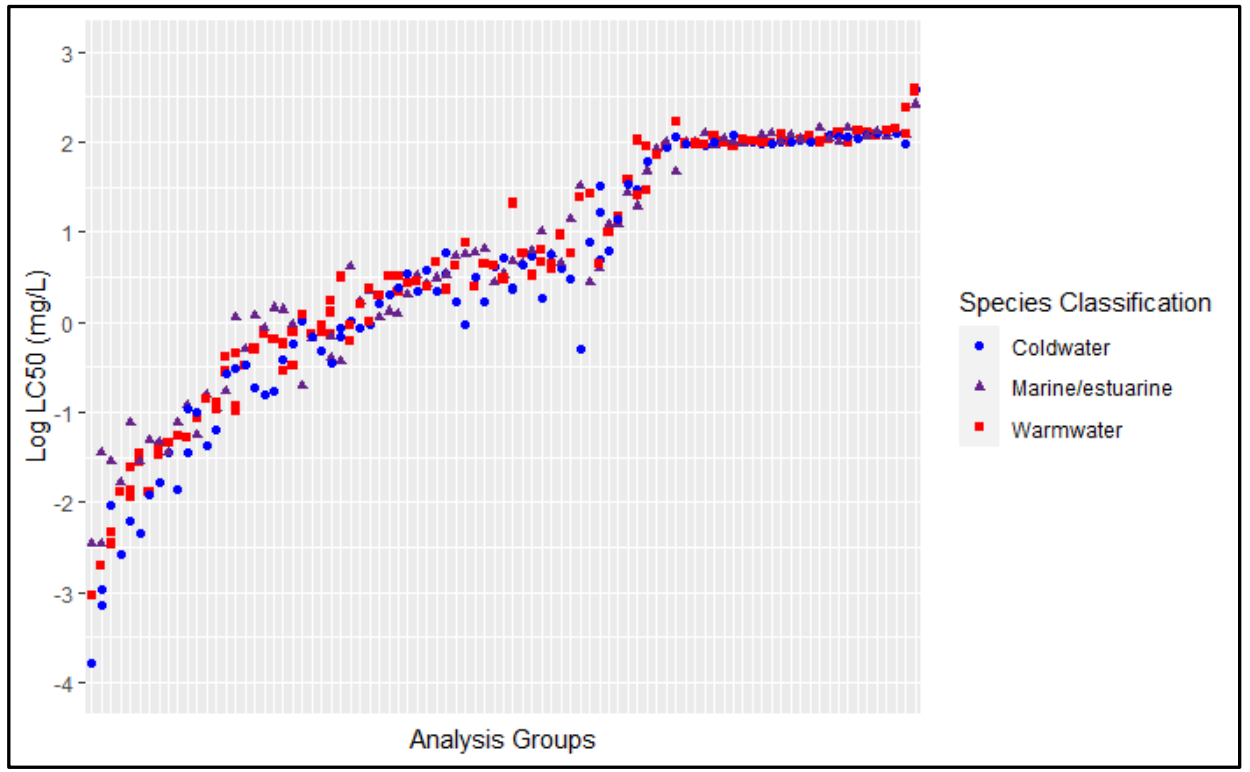
- Existing OCSPP guideline specifies using at least two test concentrations to establish BCF
- Question: Can we reduce the number of concentrations and still obtain data acceptable to characterize a fish BCF?
- Bottom line results are that conditions were identified under which a single concentration test can be used in lieu of multiple concentrations
- Policy finalized in July 2020: **https://www.epa.gov/sites/production/files/2020-07/documents/bcf-study-july-15-2020.pdf**

Fish Acute Retrospective

- Pesticide registration data requirement (40 CFR Section 158) for an acute LC50 test on 3 species (commonly rainbow trout, bluegill sunfish, and sheepshead 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?
    - Focus on conventional pesticide active ingredients newly registered by EPA for the years 1998-2016.
- Collaboration with NICEATM

Fish Acute Retrospective

- Almost 800 studies representing tests initially collected (active ingredients, formulations, and degradation products)
- Exclusion criteria: unacceptable studies; multiple active ingredient formulations; isomeric mixes; chemicals without a study with at least one each of a cold freshwater fish, warm freshwater fish, and estuarine/marine fish
- Final Dataset: 87 chemicals/formulations with at least one each of a cold freshwater, warm freshwater, and estuarine-marine fish
- Manuscript under development



QSAR for Rat Acute Oral LD_{50}

Replacement of required studies

- Collaborative Acute Toxicity Modeling Suite (CATMoS)
  - Being developed by NIEHS-NICEATM and ICCVAM
  - 35 Participants/Groups from around the globe representing academia, industry, and government contributed to the development
- Goal
  - OPP is working with NICEATM & Humane Society to evaluate applicability for pesticides as a potential replacement of the rat acute single oral dose study for establishing the effects endpoint in ecological risk assessment
- Products (Ongoing)
  - Peer–reviewed publication anticipated 2021

Summary & Looking Forward

- Progress in the 3Rs requires:
  - collaboration across many sectors
  - transparency & use of peer review
- In 2021-2022, we expect continued progress on:
  - Implementation of DNT NAM battery
  - Expanded use of 3D in vitro human lung tissue models
  - Adoption of NAMs for the Endocrine Disruptors Screening Program
  - QSAR evaluation for acute lethality
  - Retrospective analyses on subchronic dog and avian reproductive toxicity studies
  - Evaluation of various aspects of fish testing

Up and Coming Efforts

- Fish Acute to Chronic Ratio Retrospective Analysis

  Reduction of required studies

  - Partner with NICEATM
  - Beginning data extraction chronic studies
  - *Question*: Evaluate if an ACR can be used in place of chronic studies

Up and Coming Efforts

- Avian Reproduction Retrospective Analysis

  Reduction of required studies

  - Partner with PETA-US
  - Just beginning planning
  - Similar to acute fish retrospective in scope
  - *Question*: Can we confidently assess chronic risk for birds using a reduced number of species tested?

- Aquatic Organism QSAR
  - Updates to ECOSAR (Ecological Structure Activity Relationship) model