The Importance of Standardized Toxicological Methods for Aquatic Organisms

Combined with New Approach Methodologies (NAMs)

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A Little Background

- The NASEM literature review found extremely limited data on the toxicity of UV-filters on various aquatic organisms (animals and plants)

- Oxybenzone = most data rich (Ecamsule = most data poor, N=4)
  - 123 toxicity values
  - 38 species
    - 4 fish
    - 21 invertebrates (8 coral)
    - 11 algae
    - 2 bacteria

- NASEM report to spark a flurry of research, let's ensure it produces data we can use in a consistent, defensible, and reproducible manner
  - Species lacking standard test protocols will be investigated
  - Standardized data can still be obtained

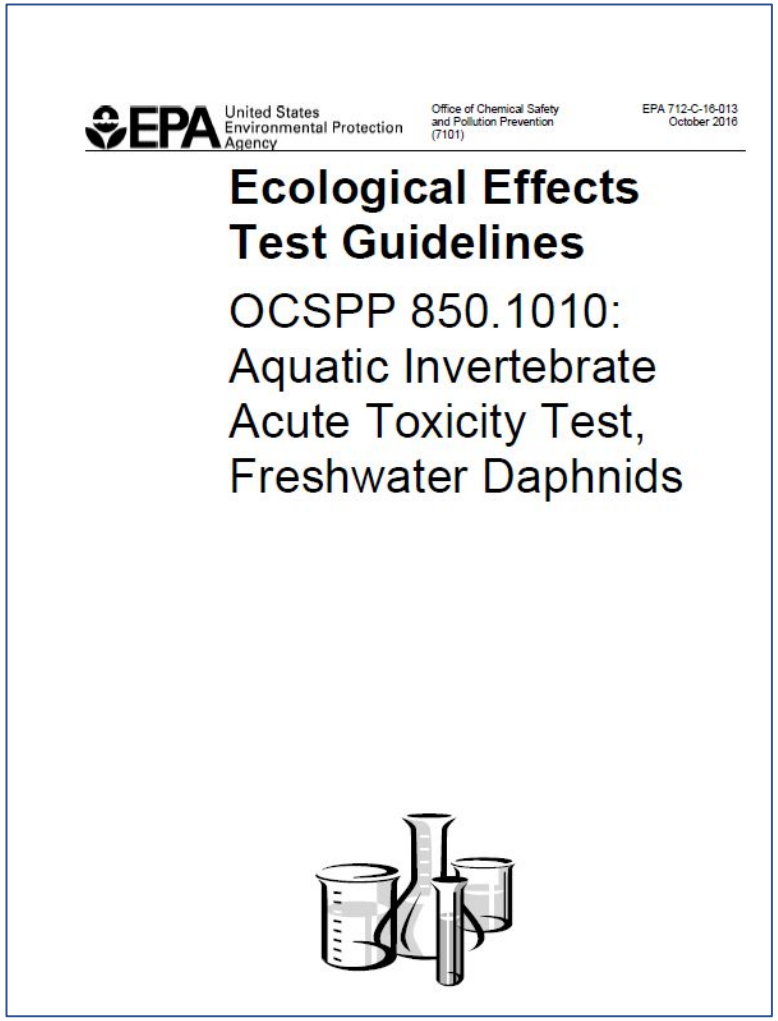
Discussion Goals

- Brief overview of what it means to be a “standardized” test method
- How are data from these protocols used?
  - Tier 2 Ecological Risk Assessment
- Limitations of standardized tests
- Looking toward the future:
  - Higher tiered assessments
  - Standardized endpoints + New Approach Methodologies (NAMs)

Availability of Standard Methods

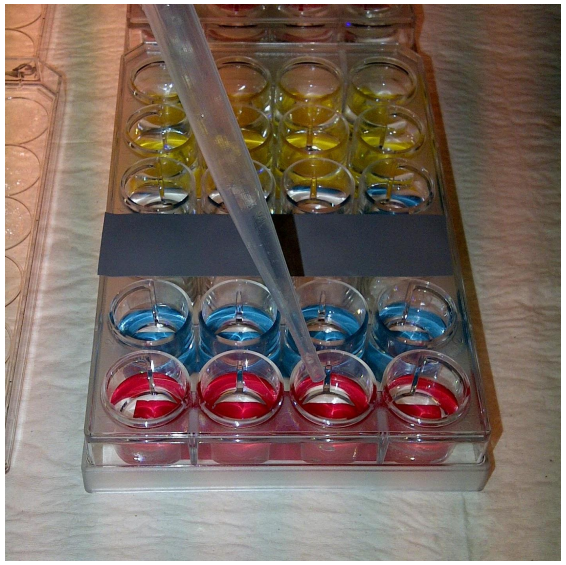
- Internationally accepted standards
  - US EPA
  - Organisation for Economic Co-operation and Development (OECD)
  - American Society for Testing and Materials (ASTM)
- Test Types:
  - Acute & chronic aqueous (freshwater & saltwater)
  - Chronic sediment (freshwater & saltwater)
  - Terrestrial (worms, plants, mammals, birds)
  - Microbial
- Aquatic taxa:
  - Fish
  - Amphibians
  - Crustaceans
  - Molluscs
  - Algae

Don't see what you need?
Testing a species without a formal "standard method" doesn't have to completely re-invent the wheel



What Do Aquatic Toxicity Tests Look Like?

- Replicated, controlled laboratory studies
- Single stressor (i.e., UV-filter)
- Expose organism to range of concentrations
- Static, static renewal, or flow through
- Ideally analytical chemistry to confirm each concentration



Single replicate 24-well plate small-scale exposures with fish embryos. Each well row housed fish exposed to a unique concentration.

Single replicate table of large-scale fish exposures at the EPA Gulf Breeze Laboratory. Each tank housed fish exposed to a unique concentration.

What is a Test “Endpoint”?

- Survival, growth, reproduction?
- LC50, EC50, EC20 (Lethal/Effect concentration of given percentage)
- NOEC/LOEC (No/lowest observable effect concentration)?

Endpoint = Exposure duration + Statistic + Biological effect

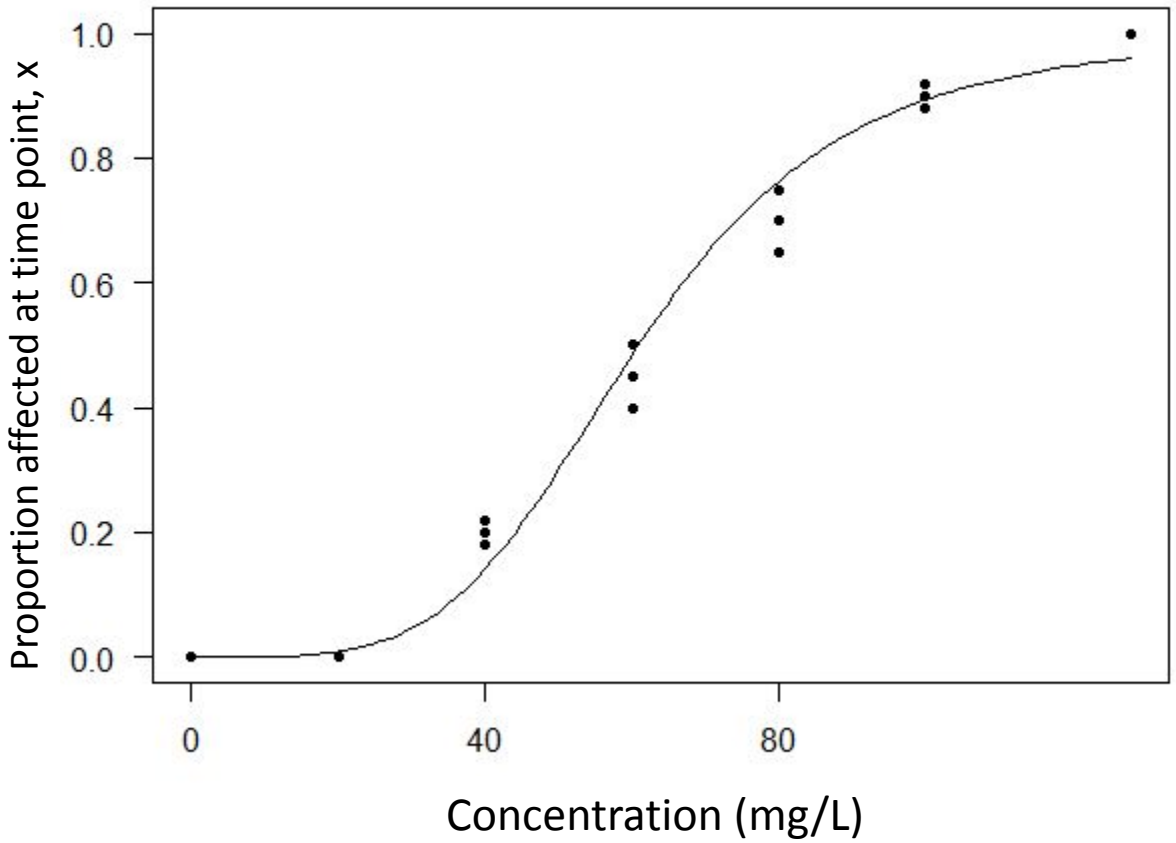
Endpoint examples:

- 96 h LC50 (lethality)
- 28 d EC20 for growth
- 270 d NOEC/LOEC for reproduction

Aquatic Test Data

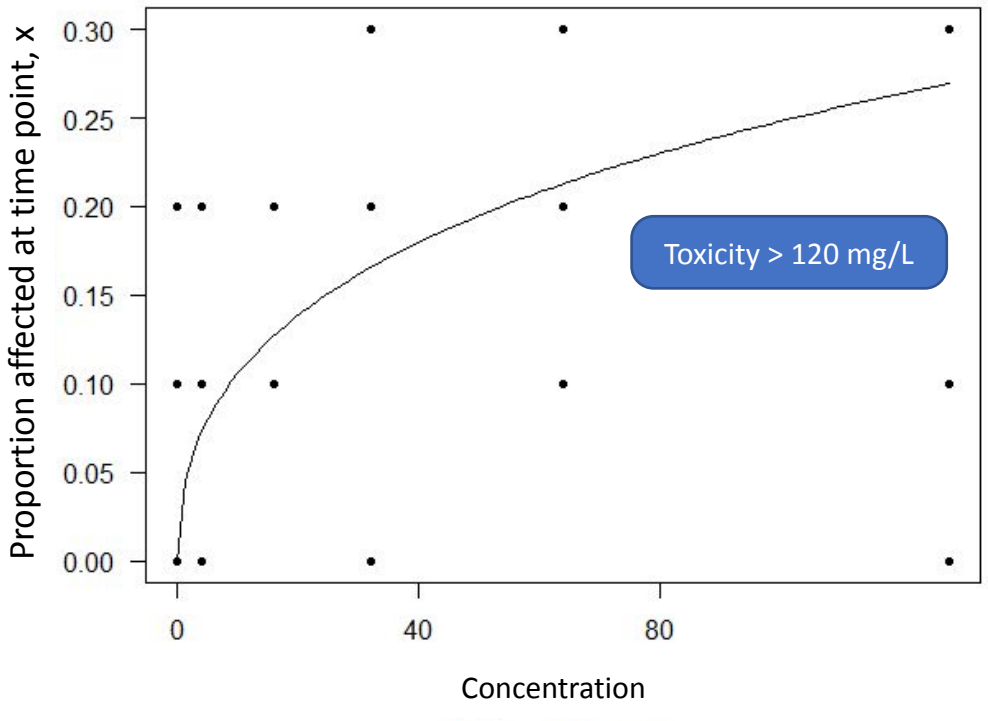
Concentration Response Curve
The relationship of biological response with concentration and time

- Influenced by:
  - Exposure duration
  - Concentration ranges
  - Test design
- Data needs to be conclusive
- Clear and significant relationships

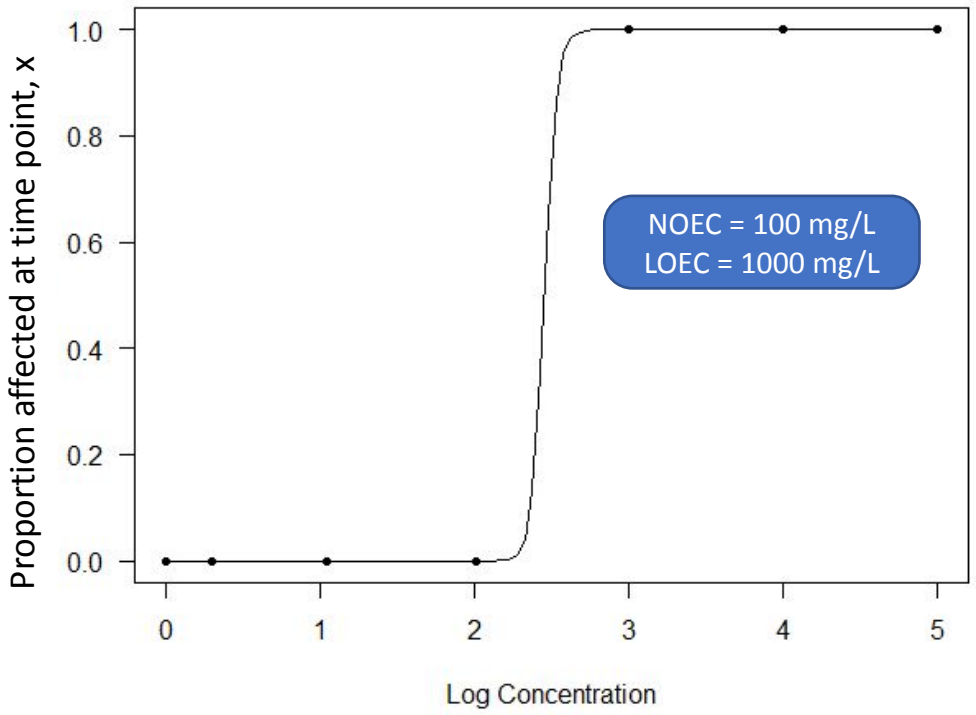


Pilot, Range-finding, and Inclusive Studies

Pilot study:
Work out kinks in the methods to reduce variability across replicates and ensure definitive test



"Range finding" study:
Conducted to identify a range in which effects are most likely to occur



What does it mean to be “standardized”?

- Exposure duration
- Biological response
  - Can range from immobilization to population growth rate, depending on species
- Age/life stage
- Test conditions
  - Temperature
  - Dissolved oxygen
  - Salinity
  - Hardness
  - pH

Within a species/taxa

Within a chemical

- Active ingredient (%)
- Analytical methodologies
- Normalizations

Across test reports

- What constitutes a “successful” test?
- Data analysis = statistic
- Open-ended toxicity values (i.e., > 100 mg/L) are way too common

What Does it Mean to be "Standardized"?
Acute vs Chronic

Acute toxicity

- Short term (24 - 96 hr*)
- Endpoint
  - Lethal (LC) or effect concentration (EC) of 50%
  - e.g., 96-h LC50, 48-h EC50
- Pros:
  - Quick, resource effective
  - Most common & abundant toxicity test data
- Cons:
  - Minimal information
  - Lack ecological relevance

Chronic toxicity

- Longer term (> 7 days*)
  - Early life stage (ELS)
  - Partial life cycle (PLC)
  - Life cycle (LC)
- Traditional statistics
  - NOEC/LOEC – not associate with % effect
  - EC20
- Biological responses
  - Growth, reproduction, lethality, biomass
  - Histopathology, behavior
  - Multi-omics, Adverse Outcome Pathways (AOPs)
- Pros: exposure durations and endpoints more ecologically relevant
- Cons: more resource intensive

*Duration of tests driven by test species life history

Common Test Species

Species/Taxa

- Amenable to culture in laboratory systems
- Bias towards smaller individuals and life stages
- Not always the most sensitive

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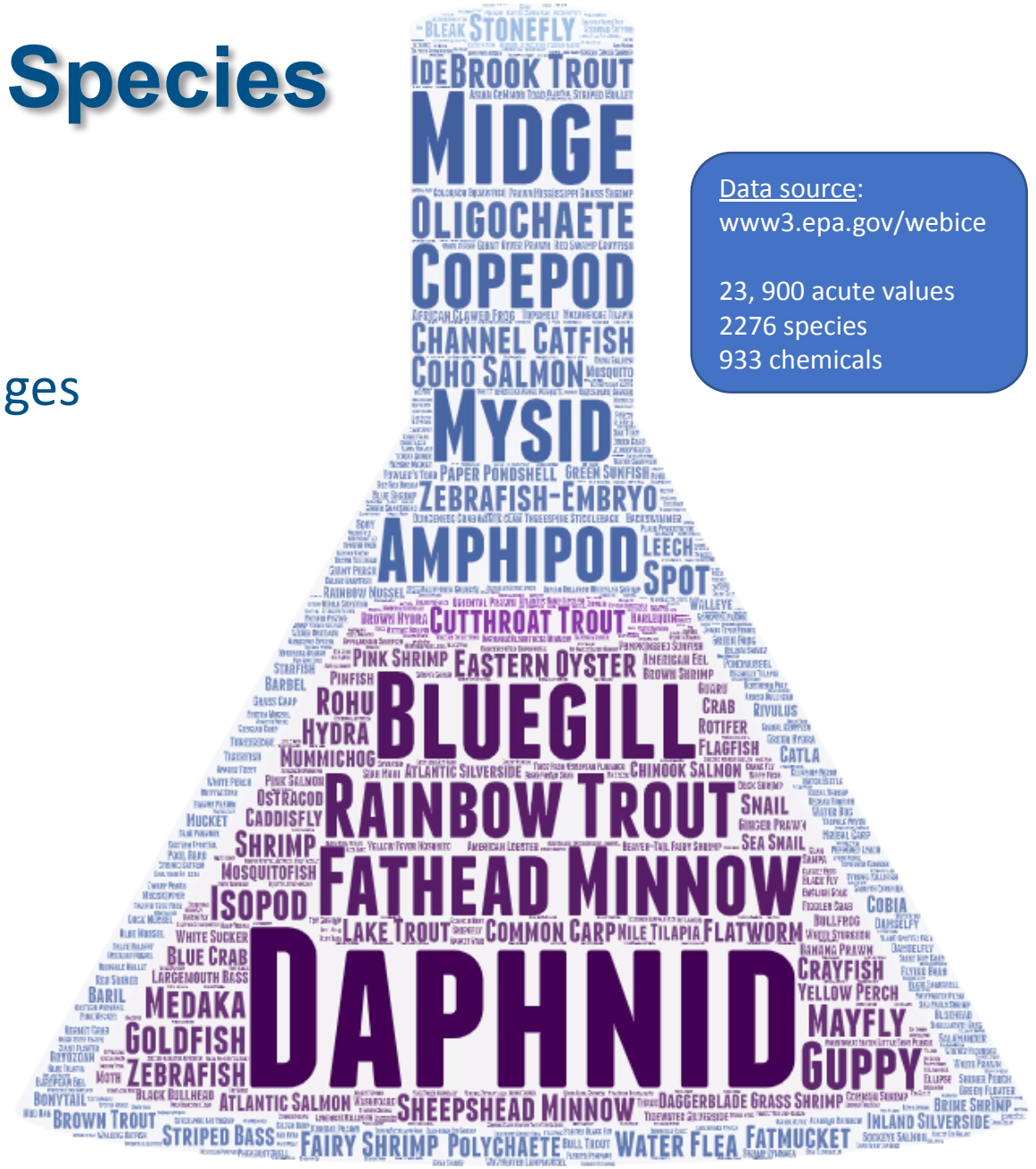
from the gallery of szymek drobniak



Data source:
www3.epa.gov/webice

23, 900 acute values
2276 species
933 chemicals

Discussion Goals

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- Looking toward the future:
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Toxicity Data Use: General

Data are used to understand:

- How a chemical may adversely affect an organism
- Which taxa/communities are most at risk
- Where should limited resources be invested for refined understanding of environmental hazards
- What mitigation efforts may reduce environmental impact

Data are applied to:

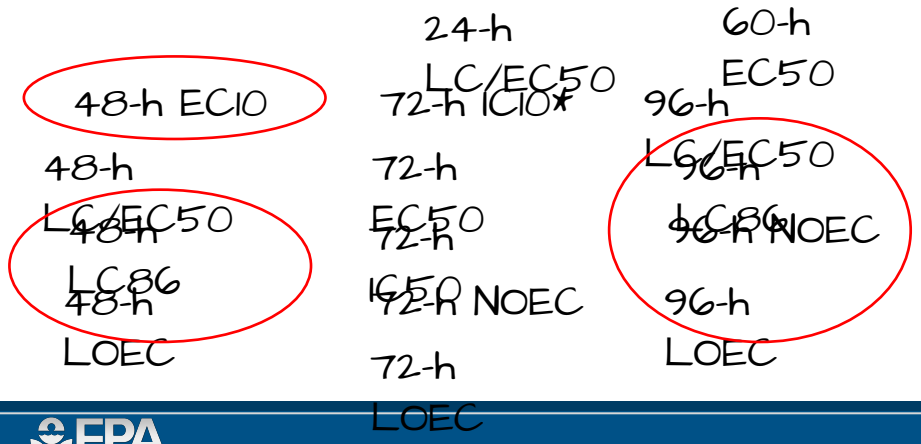
- Establish registered use of chemical that results in *de minimus* environmental impacts
- Establish water quality standards that guide State/Tribe permits and environmental restoration
- Assess impacts of accidental chemical release into to the environment

Example Application: Oxybenzone

Most data rich of all UV filters: 123 toxicity values, 38 species

Acute values

- 68 values, 28 species
- 7 open ended/inconclusive
- 24 -96 h
- Duration of exposure often, but not always, consistent within a species/taxa



Chronic values

- 54 values, 20 species
- 29 open ended/inconclusive

Exposure duration (d) = 7, 10, 12, 14, 20, 21, 28, 30, 42, 60
Statistic = EC5, EC10, EC20, EC50, ICx*, NOEC, LOEC

Biological response:

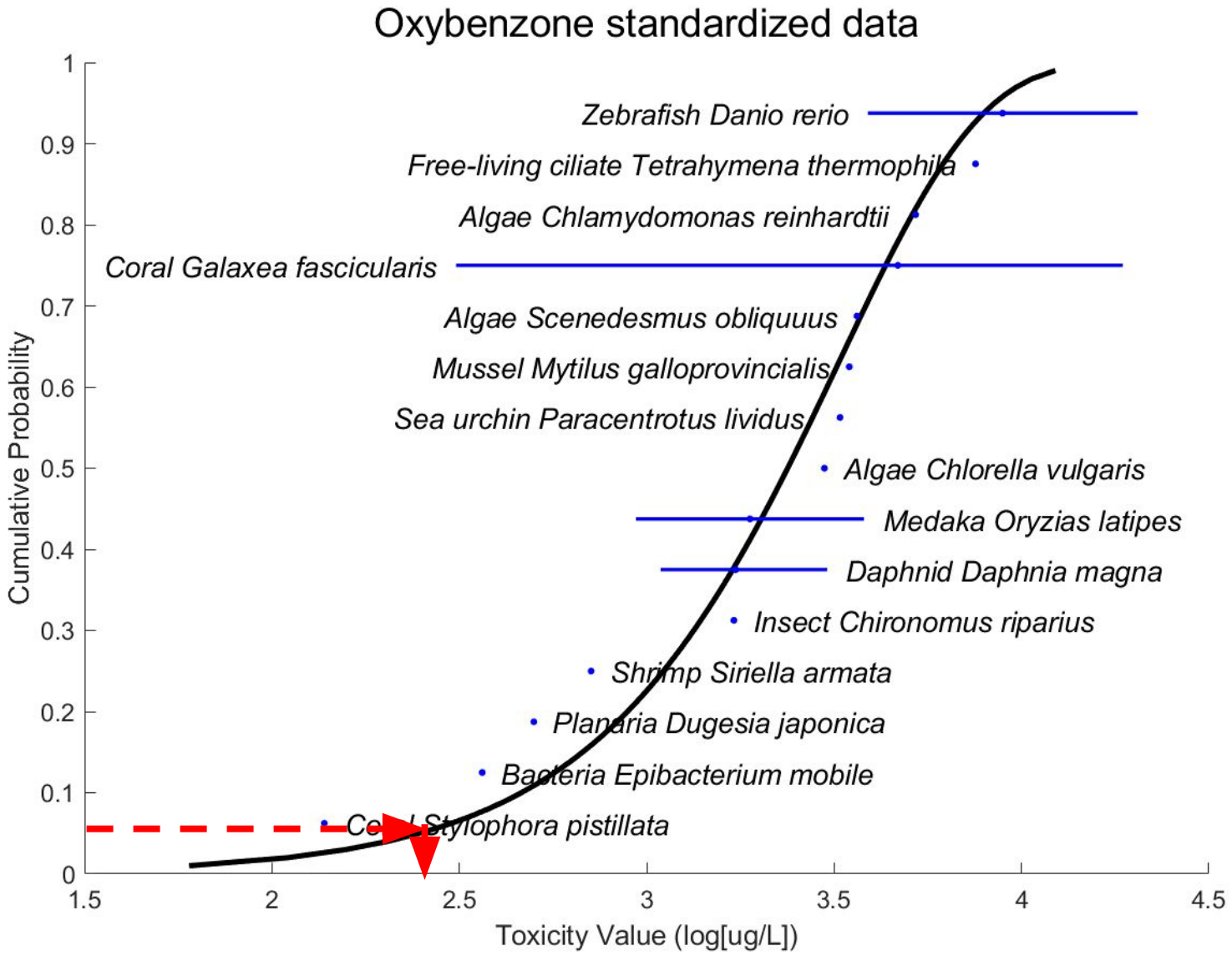
All introduce variability and uncertainty into data application and interpretation

*IC = Inhibitory concentration

Toxicity Data Use: Species Sensitivity Distributions (SSDs)

- Cumulative probability distribution
- HC5 = Hazardous concentration of the 5th percentile
  - Metric used as the basis for many risk determinations
  - Compared to measured/estimated environmental concentrations
- Acute and Chronic

- Identify which species are the most (or least) sensitive
- Estimate a concentration at which most taxa would be protected
- Can apply different approaches to adjust for uncertainties



SSD with Standardized Data: Oxybenzone

Standardized endpoints

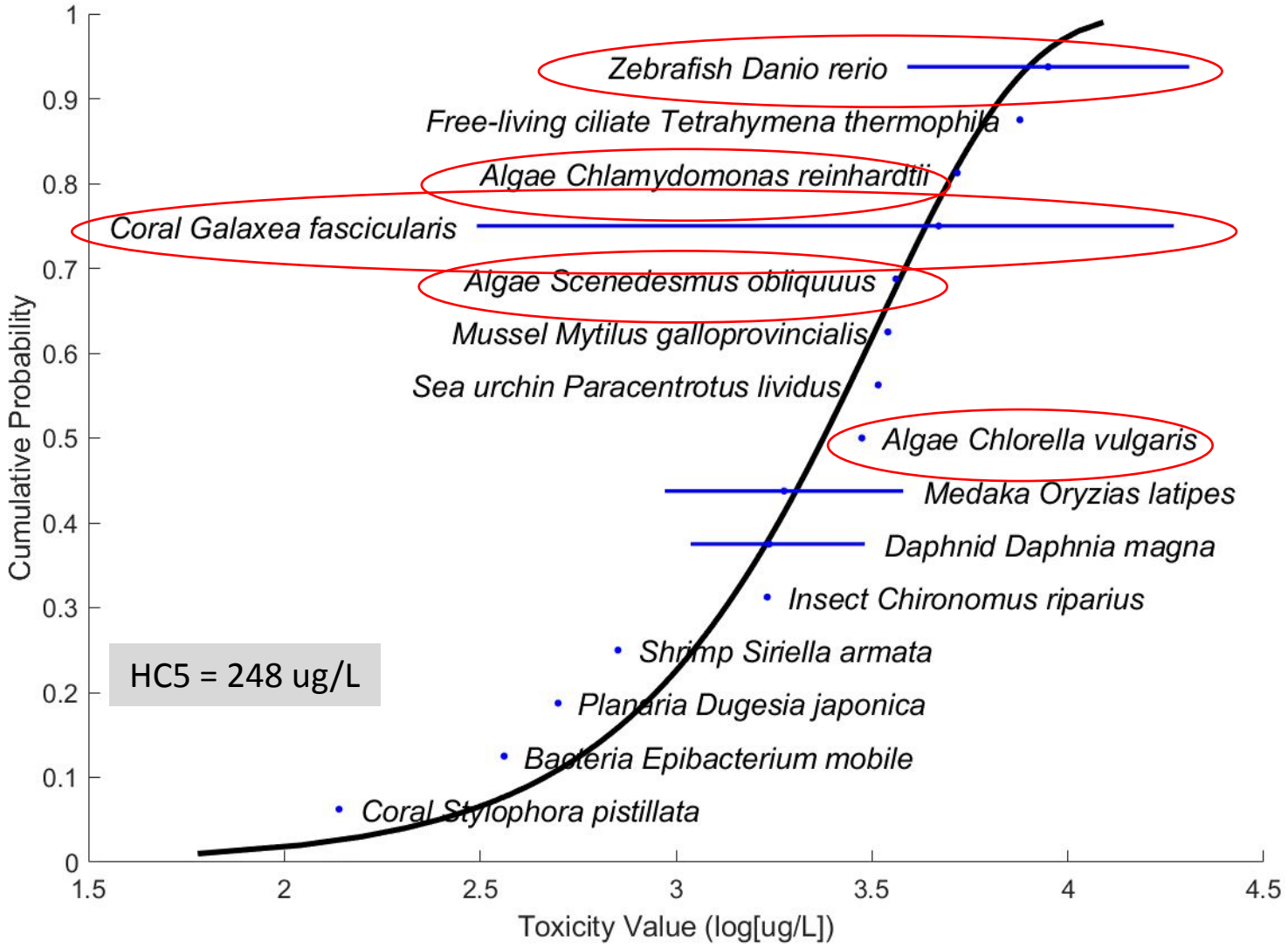
- All values EC/LC50
- 15 species, 36 test results (53% of 68 values)

Standardized species

- Algae: 96 h EC50
- Daphnid: 48 h EC50
- Mollusc larvae: 48 h EC50
- Insects, mysids: 96 h LC50
- Fish: 96 h LC50

Non-standardized species

- Sea urchin: 48 h EC50
- Free-living ciliate: 24 h EC50
- Planaria: 96 h LC50
- Bacterium: 60 h EC50
- Coral:
  - Planulae: 24 h EC/LC50
  - Adult fragments: 96 h EC/LC50

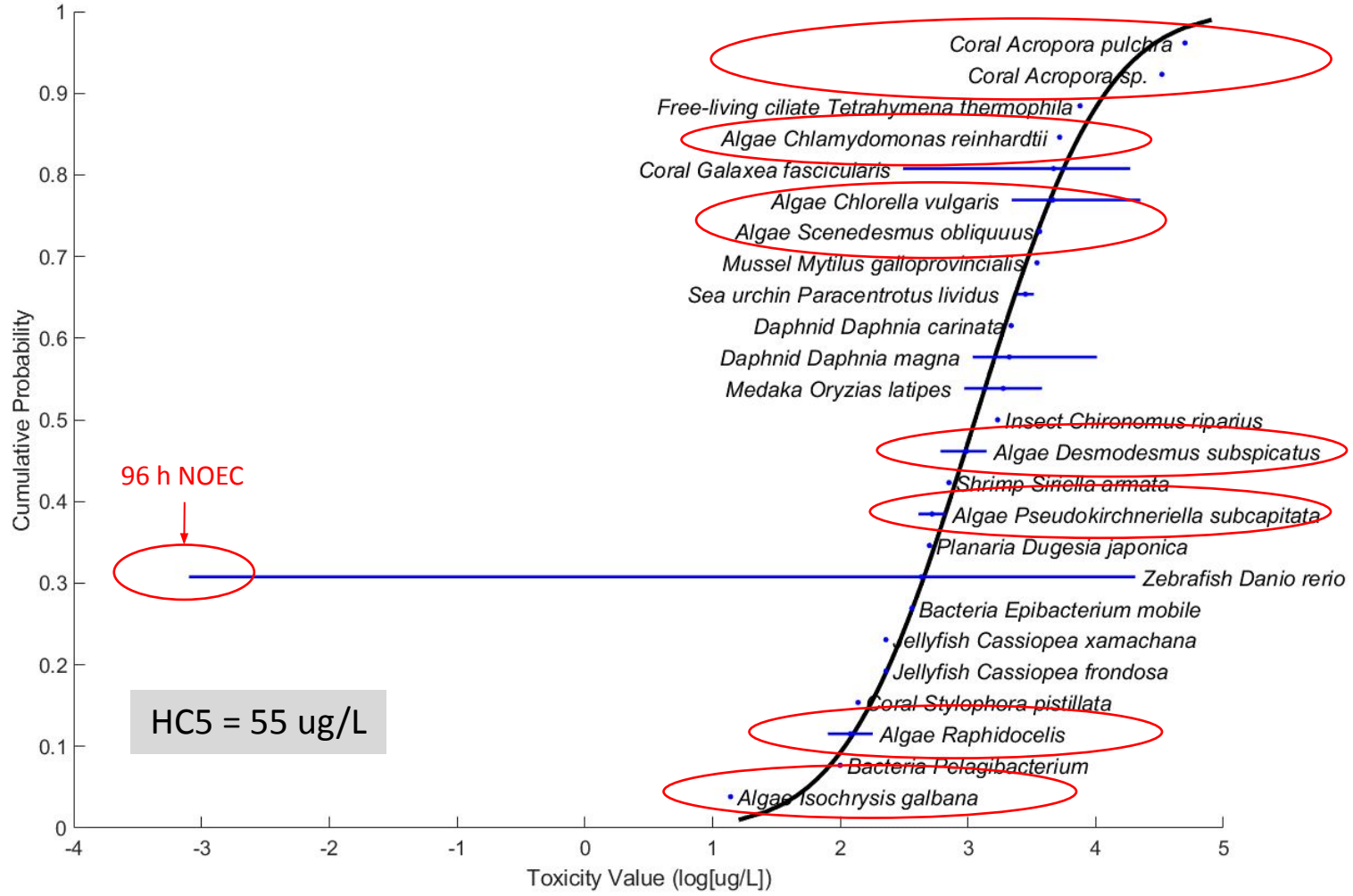


SSD with All Data: Oxybenzone

Included all values except open ended
- 25 species, 61 datapoints
- Excluded open-ended values (i.e., > 100 ug/L)

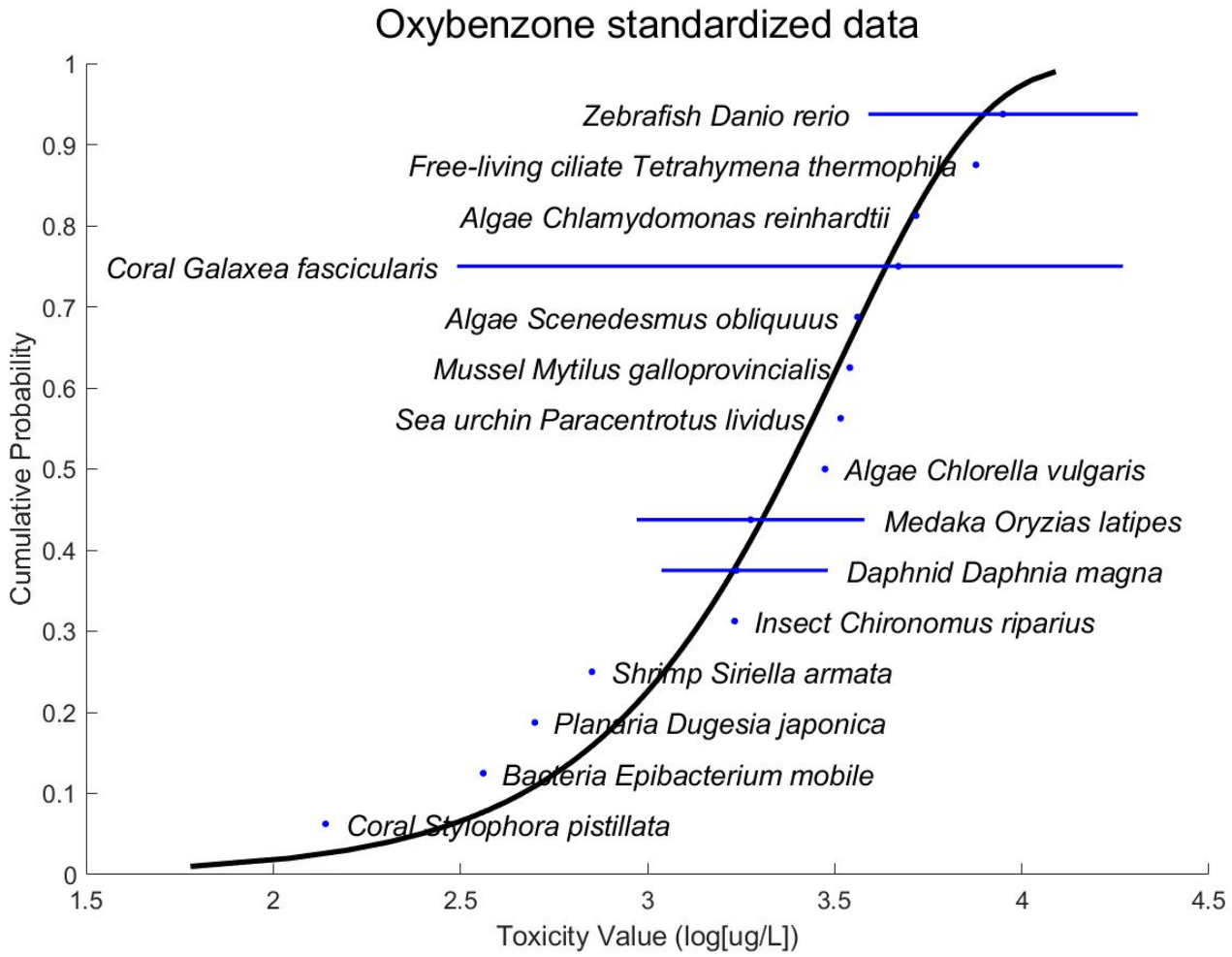
<u>What we added</u>:
- Coral least sensitive = LC86 (48 & 96 h)
- Sea urchin = EC10, EC50
- Zebrafish
  - least sensitive with standardized data
  - 48 - 96 h NOEC, LOECs,
- Jellyfish = 72-h LOEC
- Daphnids = 24 – 48 h
- Algae = NOECs, LOEC, IC10s, IC50s
- Bacteria = 48 h LOEC

<u>Bottom line</u>:
- We added a lot of noise
- Don't know relative species sensitivity



Why Standardized Data Are Important

- Reduces the variation in interspecies and inter-chemical comparison
- Prioritization of mitigation and management
  - Misrepresentation of relative species sensitivity may misguide decisions
- Standardization within EPA
  - Non-standardized endpoints not often used quantitatively
  - A LOT of thrown out data
- #1 DEFENSIBILITY



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Limitations of Standardized Tests

- Limited species with methods developed
- Limited interpretation of standardized endpoints
- Limited environmental realism

Nonstandardized lab tests contain all these limitations PLUS incompatibility, incongruence with other data

- Interspecies extrapolation from test species
- Lab-to-field extrapolation
  - Environmental exposure
  - Variability of biotic and abiotic environment
- Individual to population

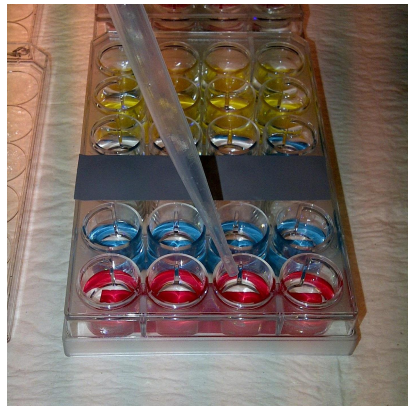

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Photo credit: Charles LoBue/EPA

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- Next steps
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Toxicity Testing of UV Filters without Formal Standards

- Develop *transferrable* test designs
  - Developing tests for non-standard organisms should be transferrable to other facilities
  - “Standardization” = Reproducibility, Reproducibility, Reproducibility

- Endpoints
  - More is more
  - ECx, NOEC, LOECs for widely accepted exposure durations (acute, chronic)
  - Consensus on endpoints for non-standard species (e.g., coral)
    - Endpoints don’t need to be the same as other species
    - Do need to be consistent across tests
    - Non-apical endpoints without quantitative links to apical endpoints difficult to defend

Higher Tiered Risk Assessments

- Improve environmental realism
- Methods and models for laboratory-to-field extrapolation
  - Includes extrapolation from standard endpoints to relevant ecological endpoints
- Improve inter-species extrapolation
  - Identify species amenable to controlled laboratory studies
  - Develop approaches for robust interspecies extrapolation
    - From ‘omics to traits-based approaches
  - Improves understanding of sensitivity of untested species

Photo credit: Cheryl Hankins/EPA

- Integration of laboratory data with New Approach Methodologies (NAMs)

Thoughts for Moving Forward

- Gain applicable toxicity knowledge
  - Screening sensitivity assays
  - Prioritization of chemicals and organisms
- Avoid “minimal publishable unit”
  - Repeat studies if needed
  - Publish useable data
- Develop NAMs compatible with toxicity tests
  - Marine environments
  - Abiotic variability
  - Interspecies extrapolations
  - Look ahead to higher tiered assessments

Photo credit: Cheryl Hankins/EPA

Questions?

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