Omics Applications

Case Study

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Chemical Risk Assessment

Exposure:

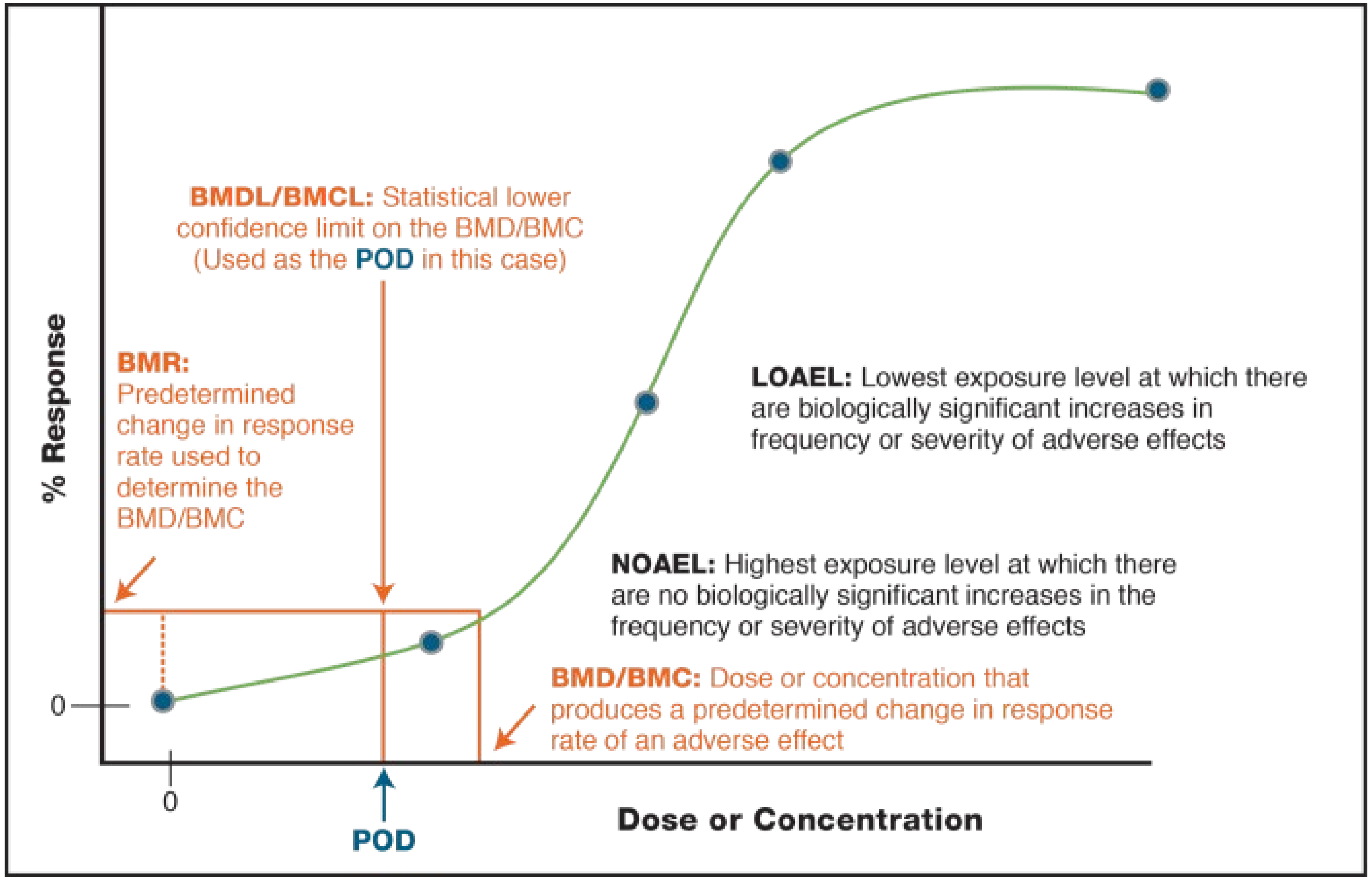
What concentrations occur in organisms or the environment?

Hazard/Effect:

What concentrations cause adverse effects to exposed organisms?

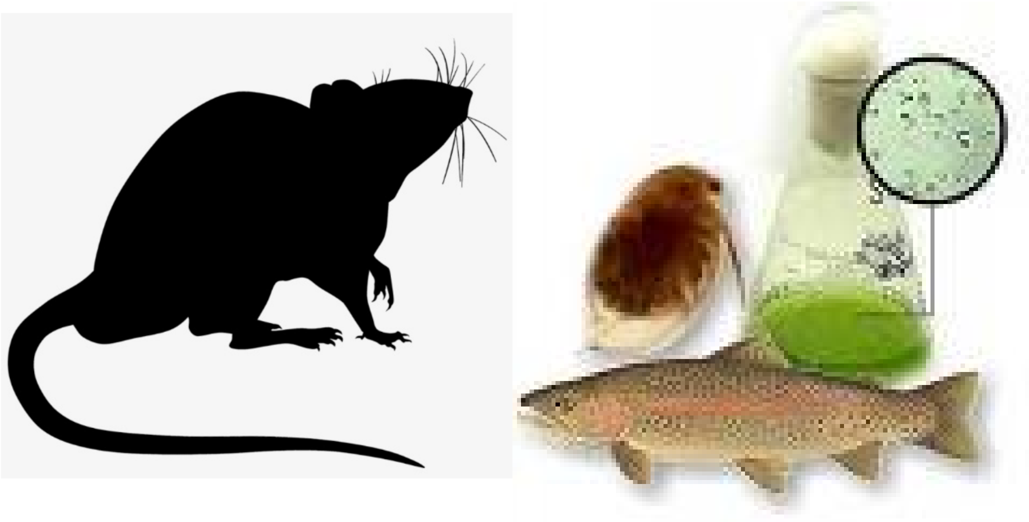
Safety:

At what concentration is there likely to be little or no hazard (adverse effects unlikely)?



Hazard/Safety Data

Toxicity Testing



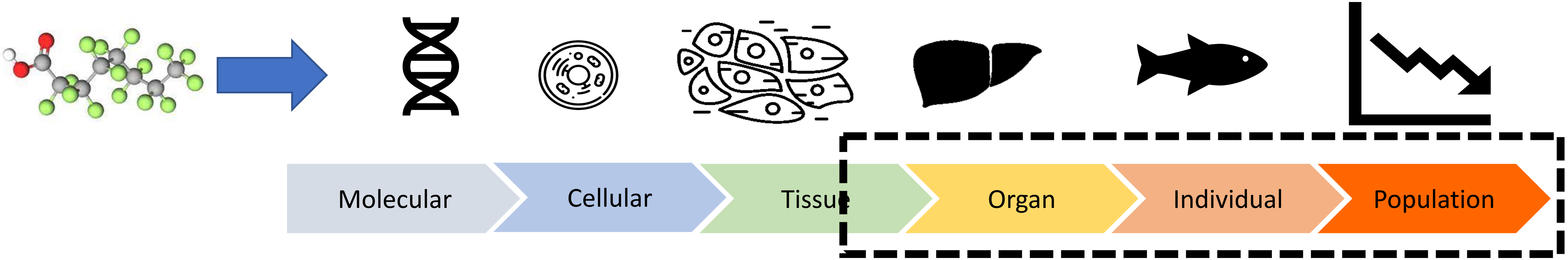
- Costly
- Time-consuming
- Animal intensive
- Lacking in mechanistic insight

Structure-based Prediction

- Requires understanding about what chemical properties/structural features are associated with toxicity.
- Understanding of mechanism(s) of toxicity relevant to different structural groups.
- Traditional models don’t work well for PFAS

Approach – NAMs

(New Approach Methodologies)



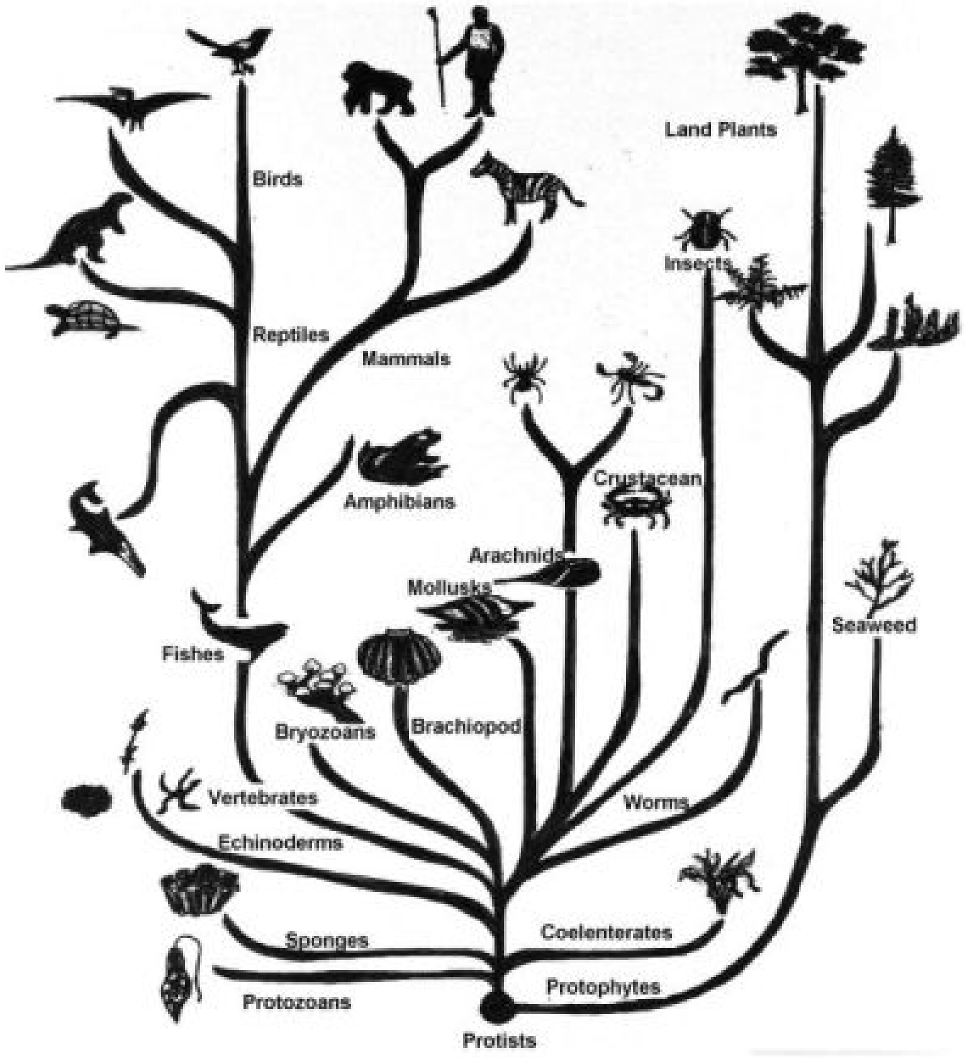
High throughput assays

- Smaller scale
- More rapid response
- Simplified systems
- Pathway coverage via batteries, multiplexing, or high content
- Dose-response more cost-effective

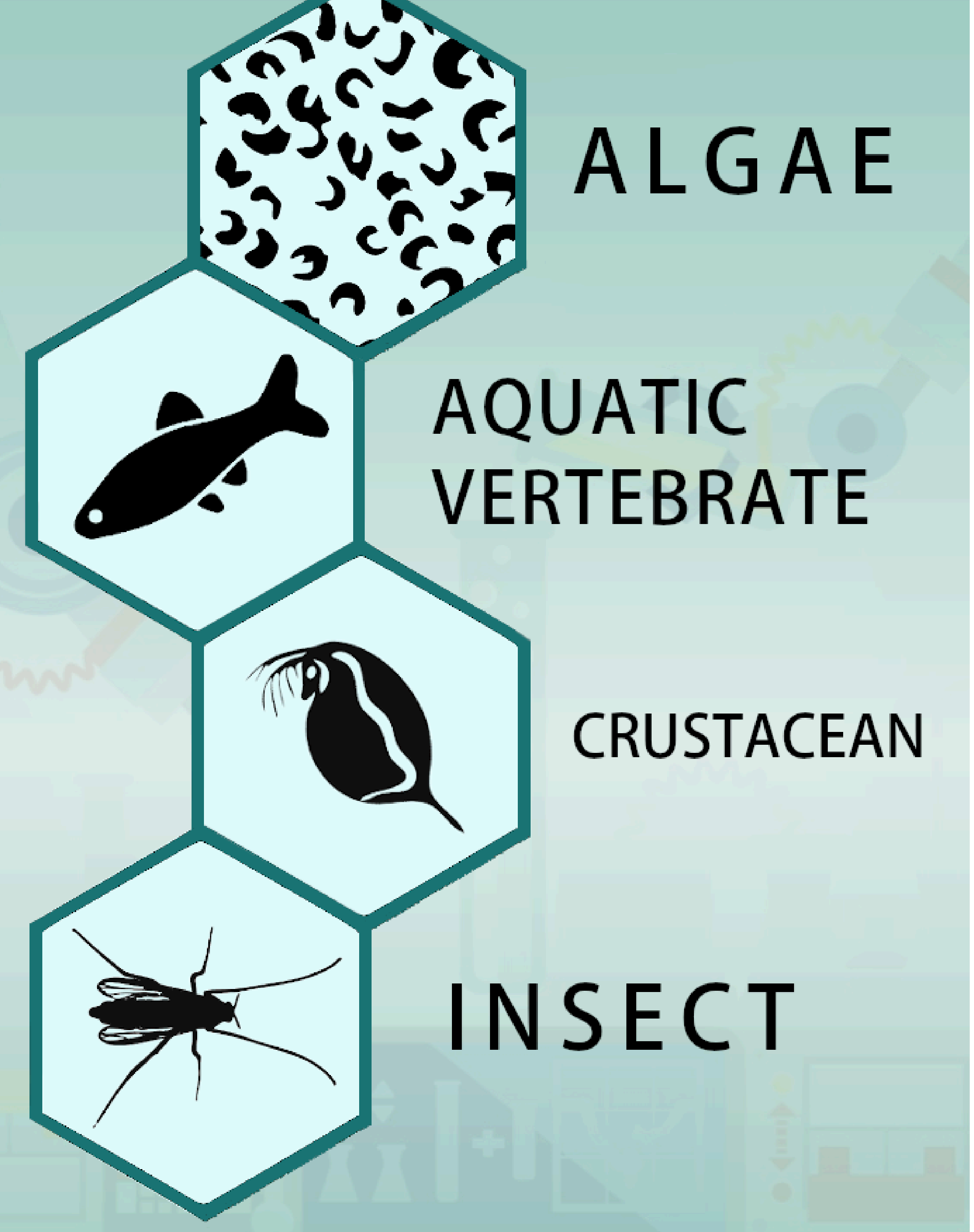
Direct observation of apical adverse effects

- Often slower, more latent response (especially when sub-lethal)
- Complex systems, integrate pathways
- Larger scales
- Dose response characterization is costly

Ecotoxicology Perspective



- Humans are just a tiny fraction of the biological diversity we are charged to protect.
- Many genes/pathways are conserved
- Unique physiology in other kingdoms, phyla, classes…
- How do we assure those pathways are covered?



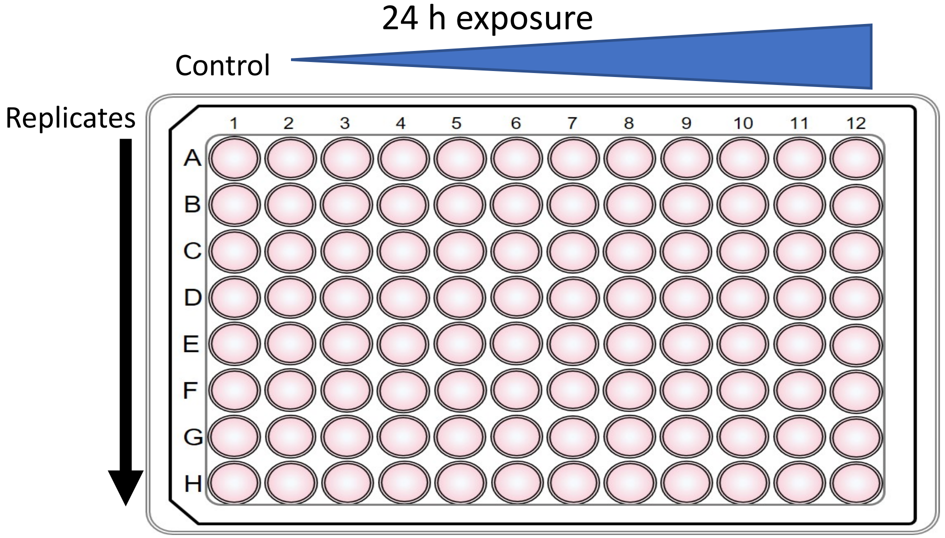
High throughput assays for three major trophic levels of aquatic ecosystems

- Primary producers (e.g., algae)
- Primary consumers (e.g., zooplankton, aquatic inverts)
- Secondary consumers (e.g., fish)

Commonly used for GHS classification and labeling of chemicals for environmental hazard

Aquatic organisms highly vulnerable to exposure

Eco HTP Assay Descriptions



Phenotypic anchoring

- survival
- behavior
- Photo pigments

Species	Guideline Test Method	Age at Start	Temp
Daphnia magna	850.1010 Aquatic Invert Acute Toxicity	72-hour	20° C
Pimephales promelas	850.1075 Fish Acute Toxicity	24-hour	25° C
Chironomus dilutus	850.1790 Chironomid Sediment Toxicity	3rd instar	20° C
Raphidocelis subcapitata	850.4500 Algal Toxicity	Log-phase	24° C

Exposures Design

- 1 ml deep 96-well plates
- 12 concentration – 8 replicates per concentration
- 1 individual per well (algae ~5 x 10^4 cells/ml)
- 24-hour static exposures
- phenotypic endpoints assessed
  - animals: survival and behavior
  - algae: cell viability & division, photopigments
- then after homogenization, RNA extracted for transcriptomics

Species	Time to Load Plate	RNA Qty per Well
Daphnia magna	~45 minutes	~1000 ng
Pimephales promelas	~30 minutes	~1500 ng
Chironomus dilutus	~60 minutes	~900 ng
Raphidocelis subcapitata	~10 minutes	~300 ng

Initial 10 Chemicals

Metals

- $CuSO_4$
- $NiSO_4$
- $ZnSO_4$

Selective Serotonin Reuptake Inhibitors (SSRI)

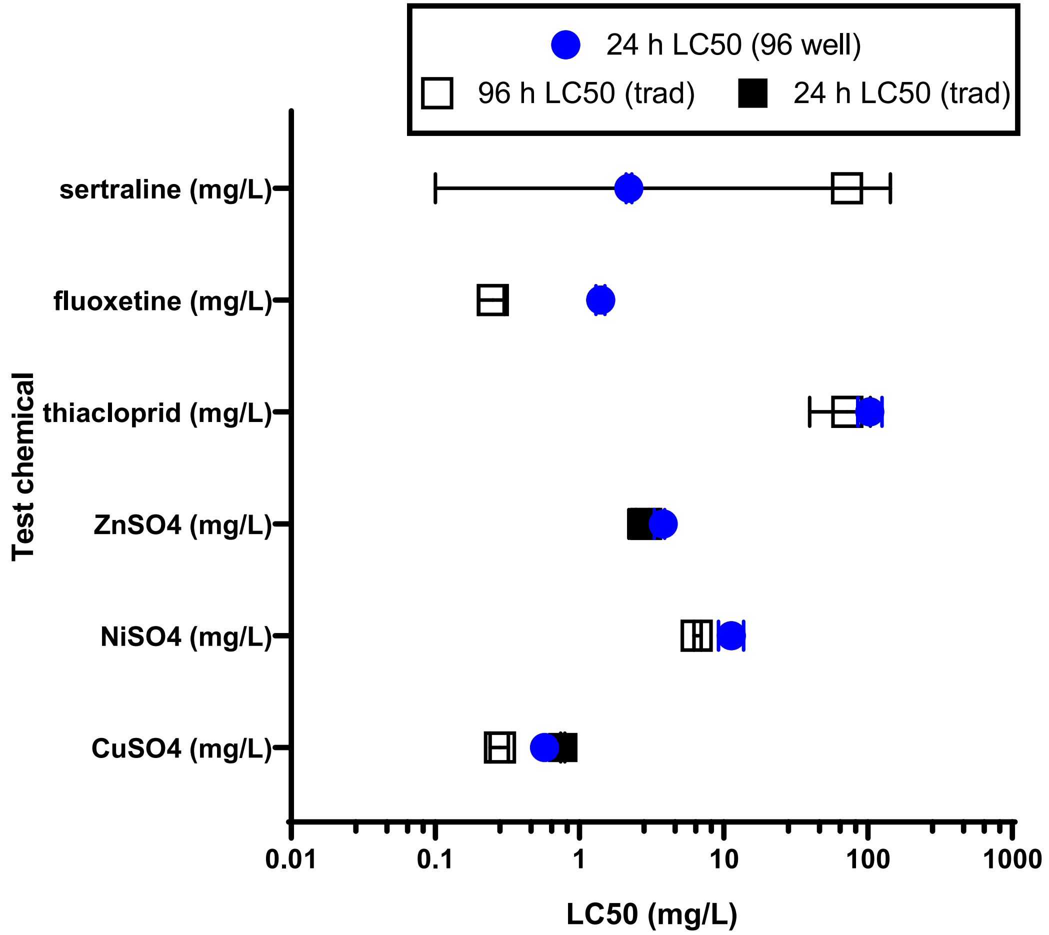
- Fluoxetine
- Paroxetine
- Sertraline

Neonicotinoids

- Clothianidin
- Imidacloprid
- Thiacloprid
- *Flupyradifurone*

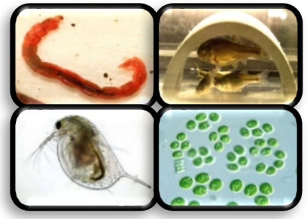
Assay design

How does the 24 h, 96 well plate format compare with the traditional 24 or 96 h LC50 in a tank or beaker?



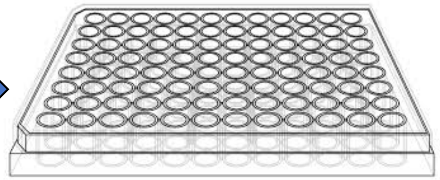
- Four of 10 chemicals were not toxic at the maximum concentration tested
- 24 h LC50s in 96 well plate format closely matched those in traditional tank/beaker format
- 24 h LC50s generally > 96 h LC50s as expected.
- 96 well format does not appear to be markedly altering overall sensitivity

Incorporating transcriptomics as assessment endpoint



Load organisms

24 h exposure

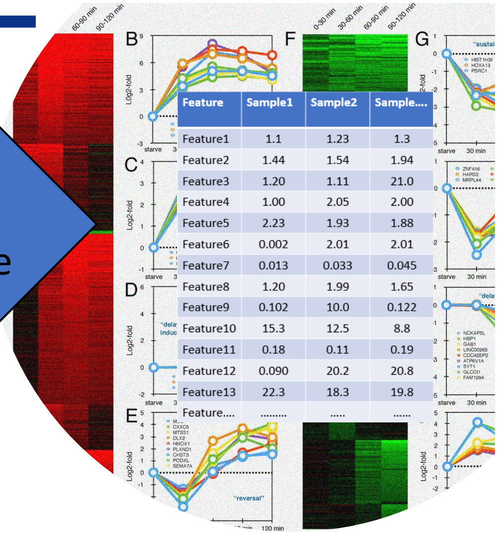


Phenotypic anchoring

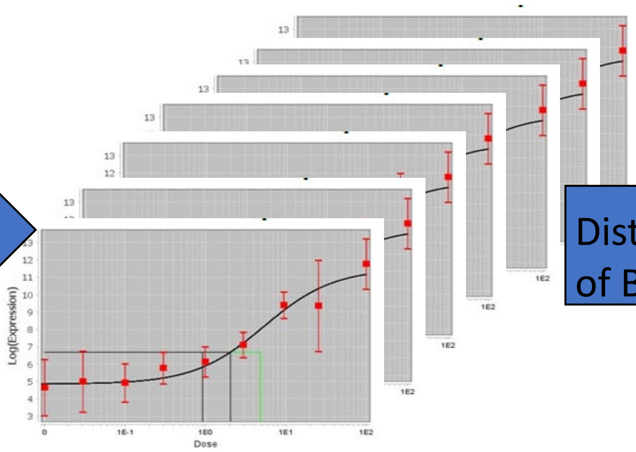
Homogenate /extract

RNA

Whole transcriptome

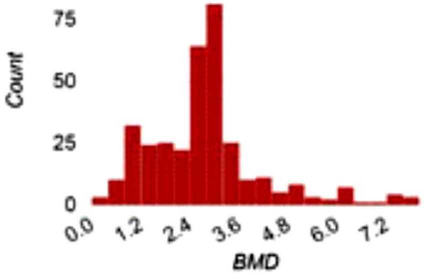


Curve fitting



BMD Express

Distribution of BMDs



10th centile BMD

Point of departure

[tPOD]

APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

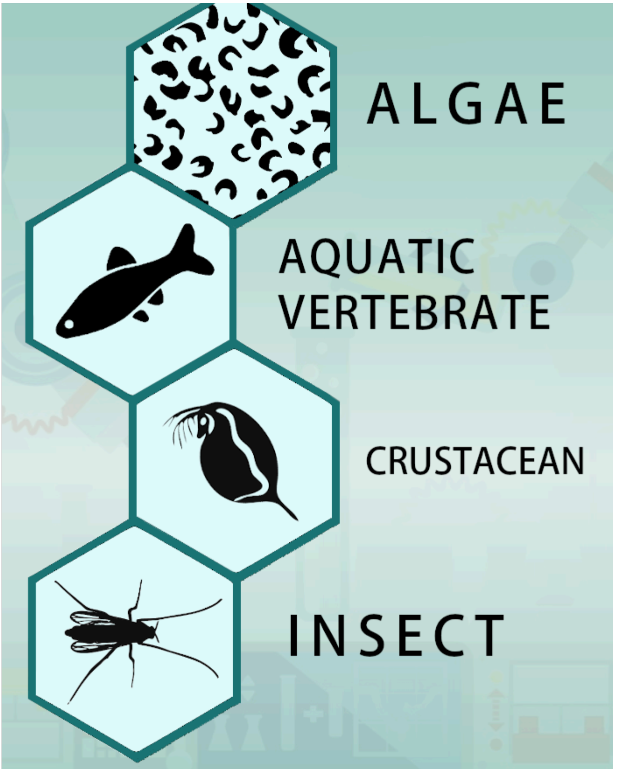
APCRA Case study: Transcriptomics-based PODs for Ecotoxicology

1. Generate transcriptomic PODs for ≈ 20 chemicals
    - Initial focus on fathead minnow
    - Parallel assays with additional taxa – for future analyses

2. Compare tPODs with available acute and chronic toxic toxicity data

3. Compare tPODs with in vitro-derived PODs

Eco-HTTr Research at EPA



Assay Optimization

- How many replicate wells (animals)?
- How much genome coverage?
- Assay acceptance criteria?

Reliable point of departure [tPOD] with defined uncertainty range

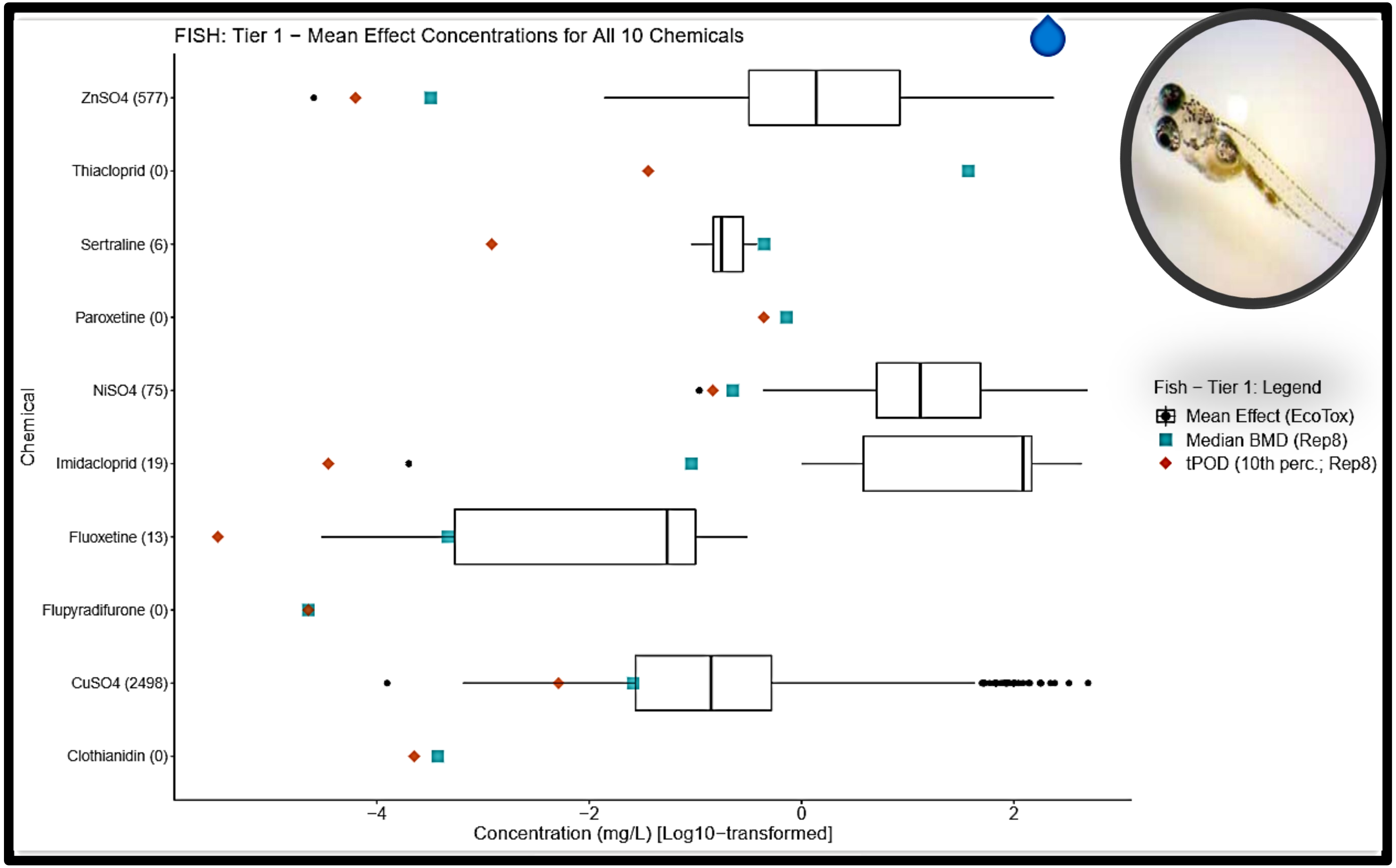
Assay Evaluation

[tPOD] ≤ [Most sensitive chronic endpoint]
Effective provisional, protective value

[tPOD] <<< [Most sensitive chronic endpoint]
Overly conservative

[tPOD] > [Most sensitive chronic endpoint]
Not protective

Comparison with In vivo, **Adverse Effect** Concentrations (Fish)



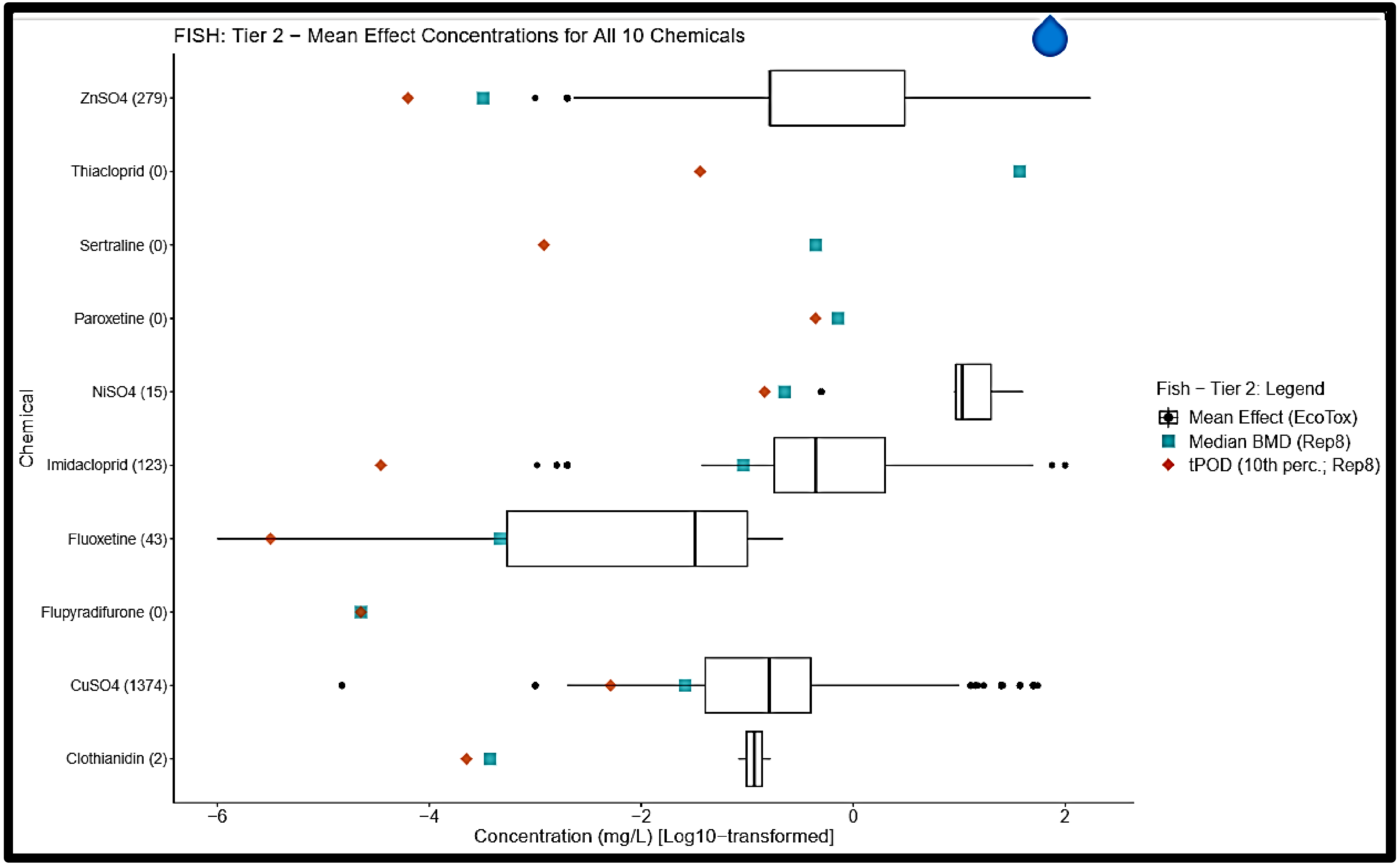
tPODs were generally more sensitive than apical adverse effect concentrations.

tPOD based on median BMD were less protective

In some cases 2 orders of magnitude more protective

Still in the process of more detailed QA of the ECOTOX records with lower effect conc.

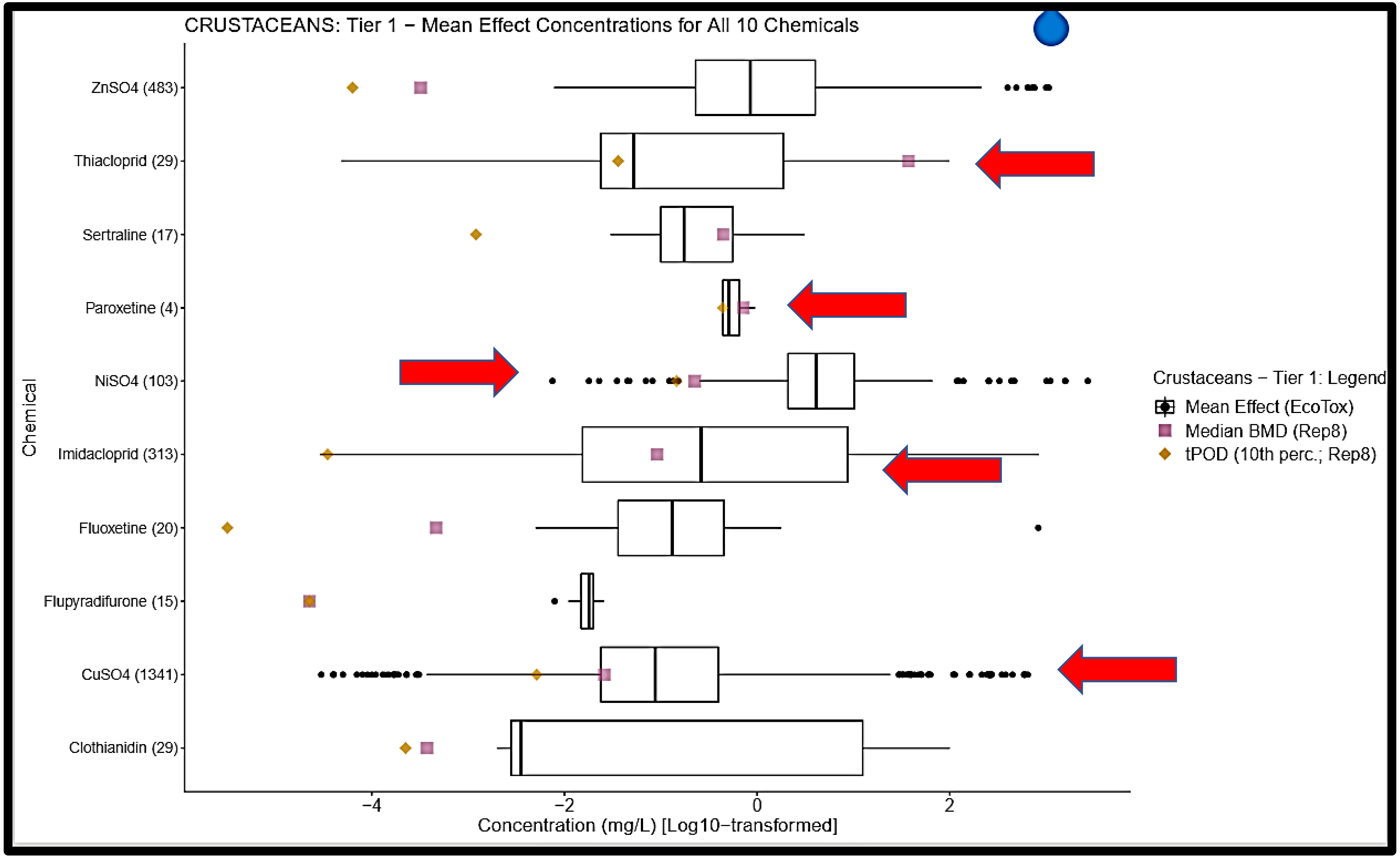
Comparison with In vivo, **Biological Effect** Concentrations (Fish)



tPODs still generally more sensitive in vivo biological effect concentrations.

tPOD based on median BMD were less protective

Fish-based tPODs are not protective of all aquatic organisms



Preliminary Evaluation

- Preliminary data suggest tPOD is promising as a lower bound estimate of toxicity to fish.
- Appears more conservative than ½ log – different regulatory programs will need to weigh in on whether too conservative (need to test more chemicals).
- There does appear to be a need for taxa-specific tPOD determinations

Molecular Biomarkers

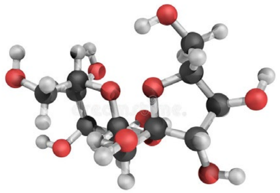
Connectivity Mapping

US EPA, Center for Computational Toxicology and Exposure, Great Lakes Toxicology and Ecology Division

Current Limitations

- Cumulative Risk
  - Chemical in water or tissue
  - Interactions - Mixtures
  - Nonchemical stressors e.g. DO
- Lamp post
  - Look for what you **can** look for
- Apical endpoints
  - Uninformative
  - Read-across
  - Prediction of mixture effects

Biomarker Library





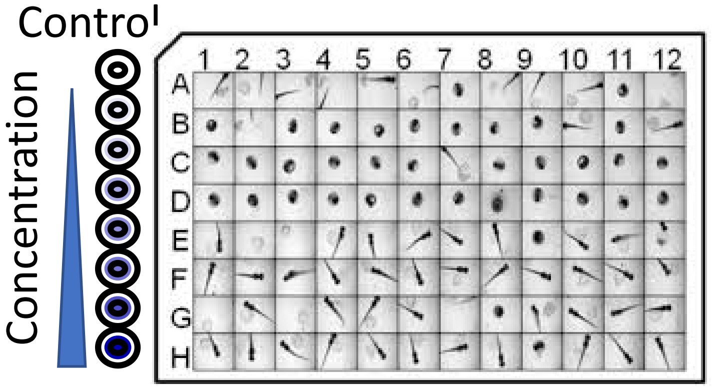

Estrogenic

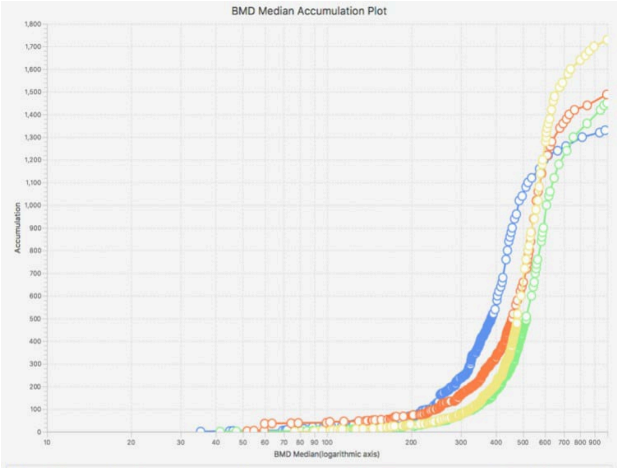
Neurotoxic

Hepatotoxic

Thyroid active

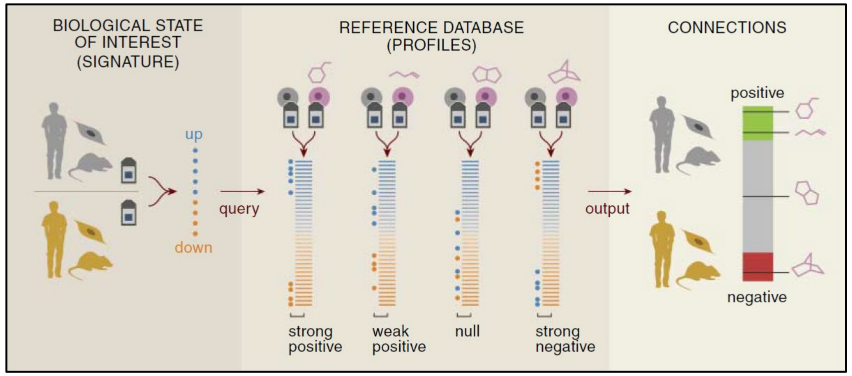
Omics-based Biomarkers





Points of Departure

NCBI



Connectivity Mapping (CMAP)

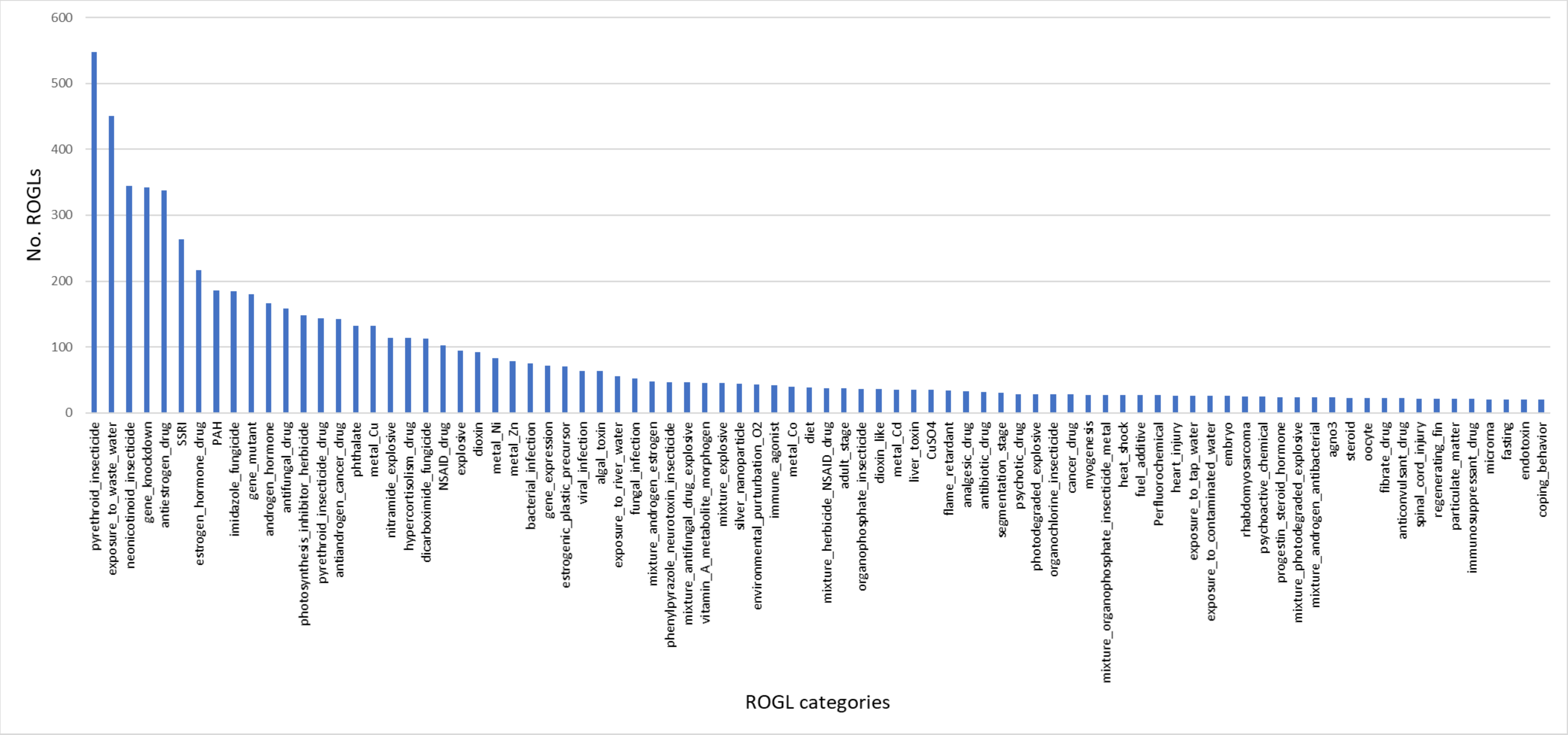
Justin Lamb et al. Science 2006;313:1929-1935

Scaled up ROGL Library

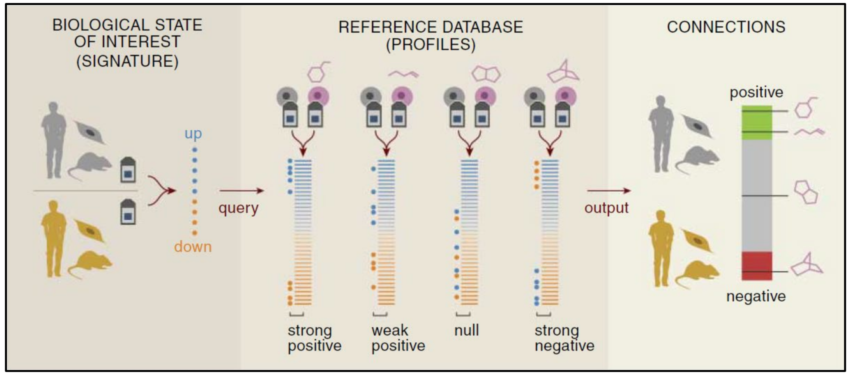
Metrics	Current Effort 2021
No. studies/datasets (GSE)	**450** = 70 (FHM) + 153 (ZF Affy) + 227 (ZF Agilent)
No. samples	**11639** = 4222 (FHM) + 2147 (ZF Affy) + 4440 (ZF Agilent) + 830 (RNAseq)
No. microarray platforms	**42** = 9 (FHM Agilent) + 5 (ZF Affy) + 28 (ZF Agilent)
Profiling technology	array & RNAseq
NO. ROGLs	**8021** = 7191 (array) + 830 (RNAseq); **4491** sets (combo of platform/chemical/dose/duration/ tissue/lifestage)
NO. sets of query signatures	**1188**
Signature cross-mapping	Ensembl ZF gene orthologs; EPA FHM genome
Performance across platforms/species	Much better

780 RNA-seq datasets

ROGL Categories by Chemical/Biology Applications/MOAs

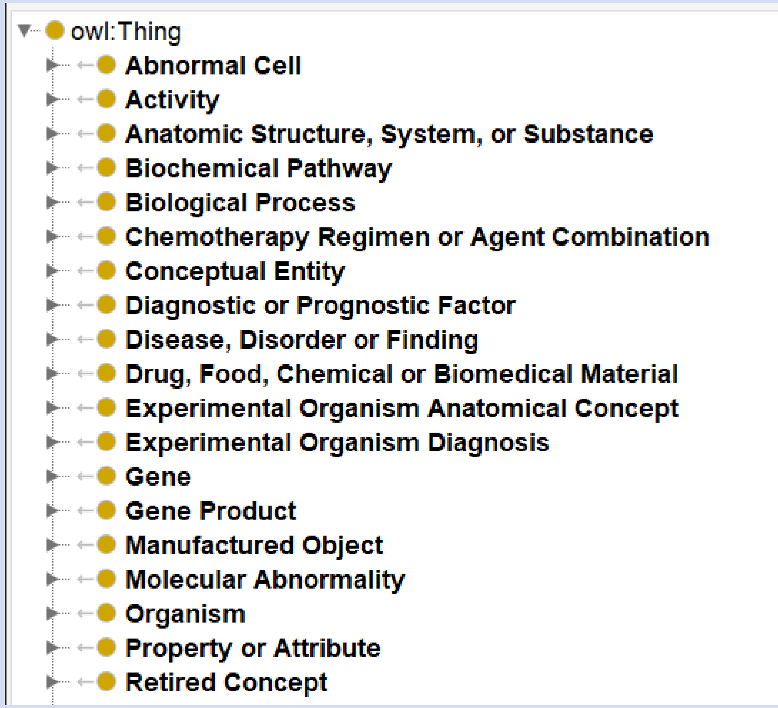


*among the 259 categories, only those >= 20 are displayed



CMAP: Effects-based linkages

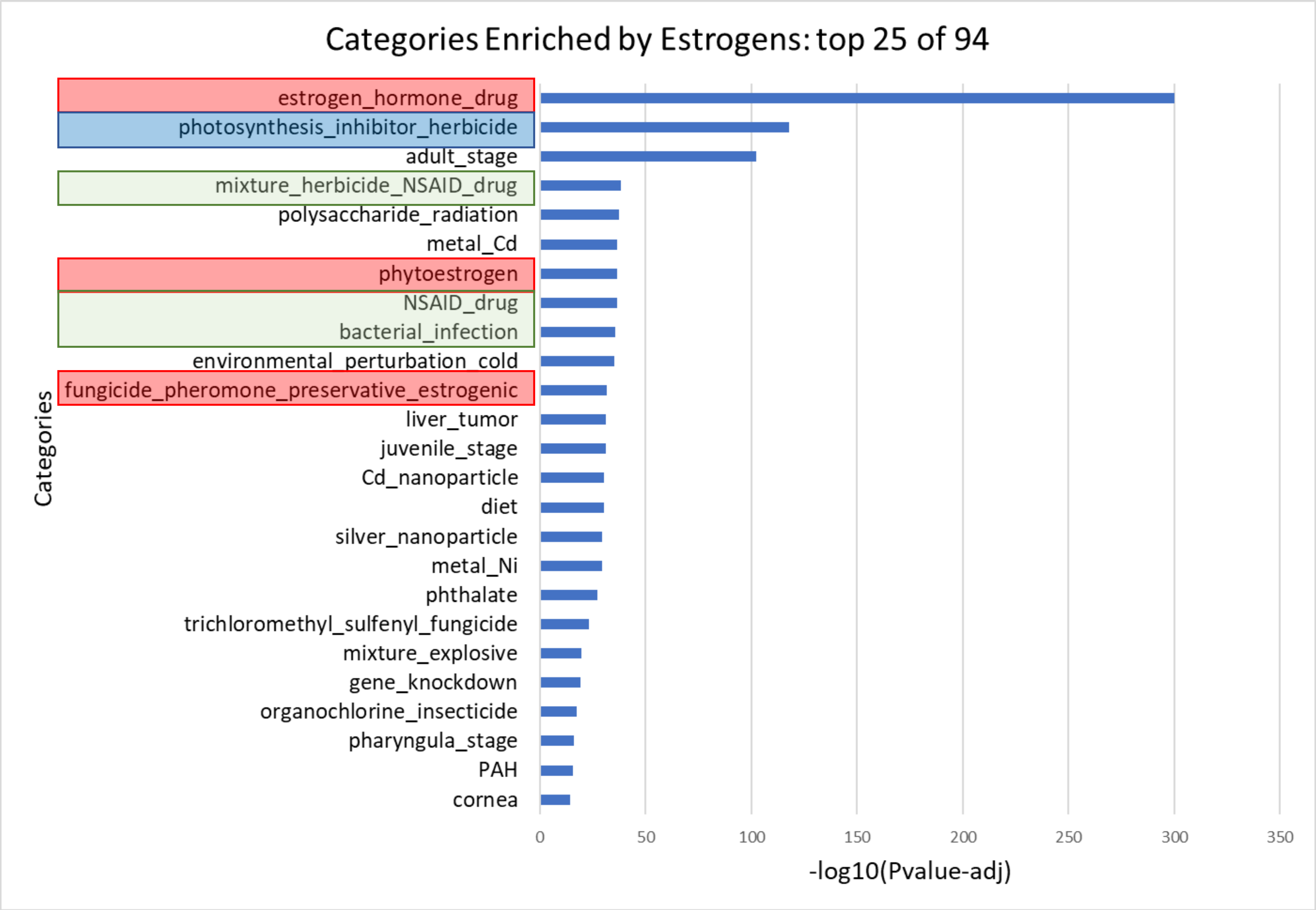
NCIT -National Cancer Institute Thesaurus

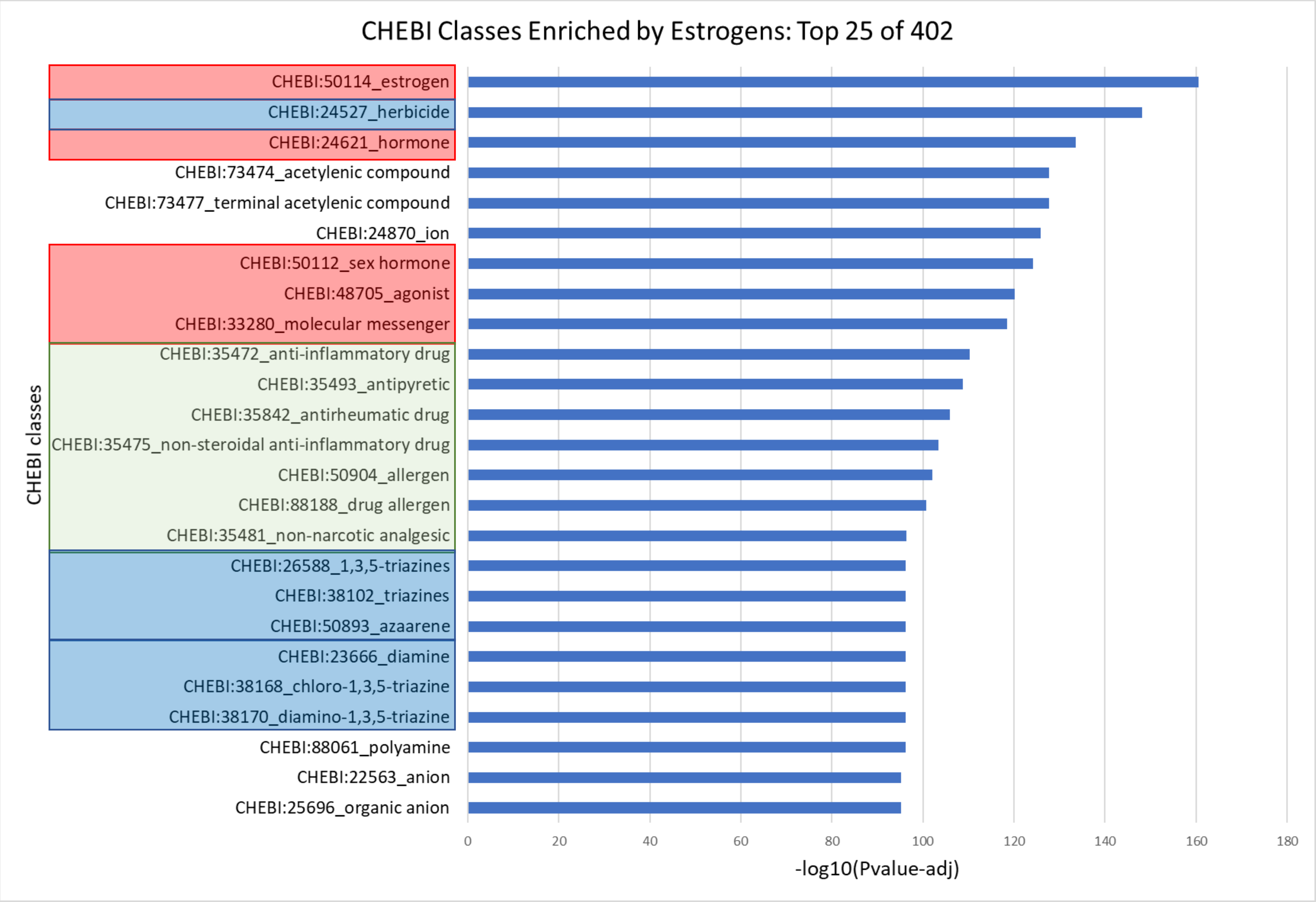


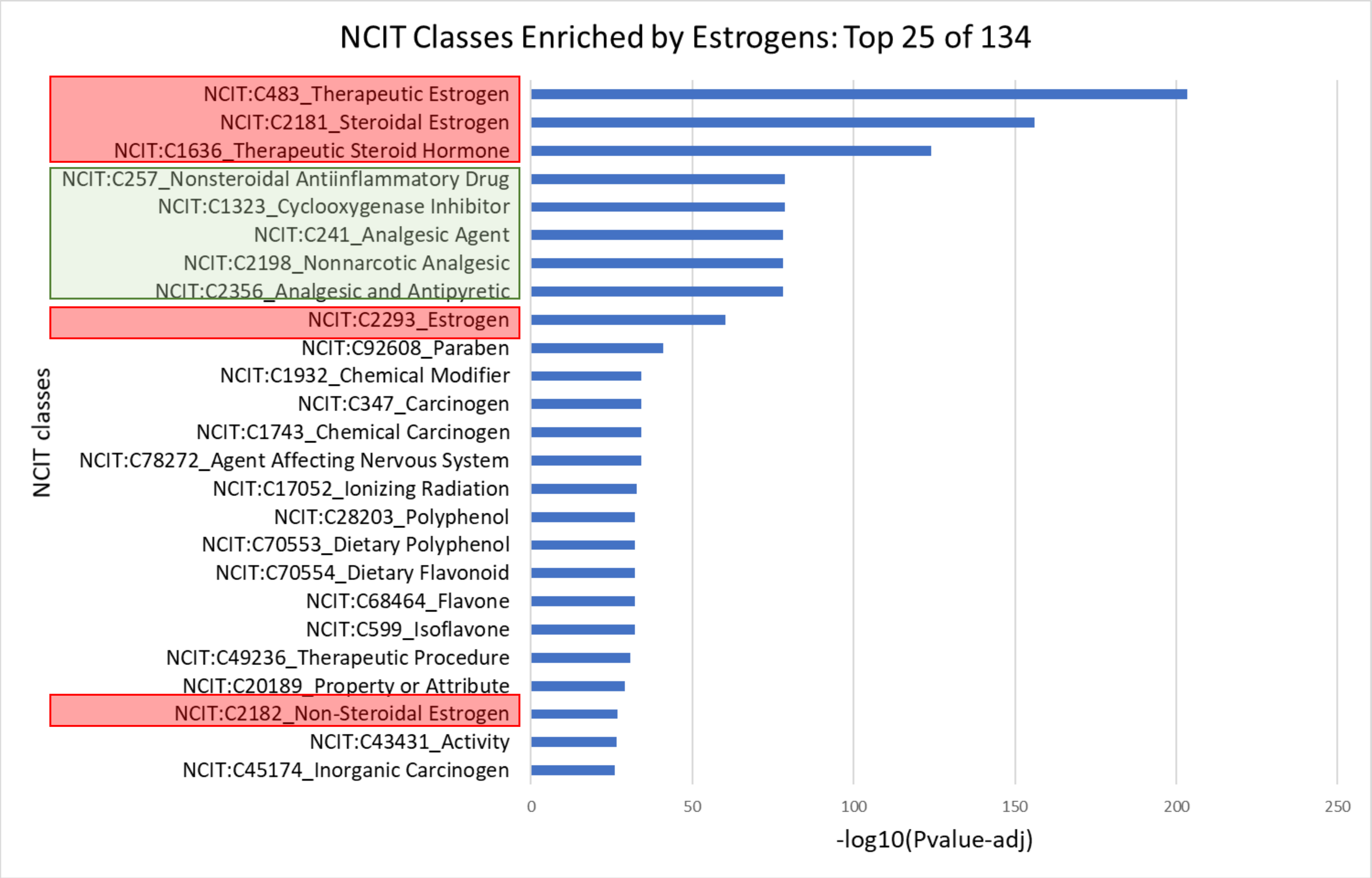
CHEBI – Chemical Entities of Biological Interest

- Structure
- Role
  - Chemical context (ligand)
  - Biological context (hormone)
  - Application (pesticide)
- Subatomic Particle

- Ontologies
  - Controlled vocabulary
  - Maintained by experts in the field
  - Evaluated and edited
- Enrichment
  - Discovery
    - Structural moieties
    - Biological connections
      - Roles
  - Weight of Evidence
    - Do the effects-based linkages reflect enrichment


Categories Enriched by Estrogens: top 25 of 94
Categories
estrogen_hormone_drug
photosynthesis_inhibitor_herbicide
adult_stage
mixture_herbicide_NSAID_drug
polysaccharide_radiation
metal_Cd
phytoestrogen
NSAID_drug
bacterial_infection
environmental_perturbation_cold
fungicide_pheromone_preservative_estrogenic
liver_tumor
juvenile_stage
Cd_nanoparticle
diet
silver_nanoparticle
metal_Ni
phthalate
trichloromethyl_sulfenyl_fungicide
mixture_explosive
gene_knockdown
organochlorine_insecticide
pharyngula_stage
PAH
cornea
0
50
100
150
200
250
300
350
-log10(Pvalue-adj)


CHEBI Classes Enriched by Estrogens: Top 25 of 402
CHEBI:50114_estrogen
CHEBI:24527_herbicide
CHEBI:24621_hormone
CHEBI:73474_acetylenic compound
CHEBI:73477_terminal acetylenic compound
CHEBI:24870_ion
CHEBI:50112_sex hormone
CHEBI:48705_agonist
CHEBI:33280_molecular messenger
CHEBI:35472_anti-inflammatory drug
CHEBI:35493_antipyretic
CHEBI:35842_antirheumatic drug
CHEBI:35475_non-steroidal anti-inflammatory drug
CHEBI:50904_allergen
CHEBI:88188_drug allergen
CHEBI:35481_non-narcotic analgesic
CHEBI:26588_1,3,5-triazines
CHEBI:38102_triazines
CHEBI:50893_azaarene
CHEBI:23666_diamine
CHEBI:38168_chloro-1,3,5-triazine
CHEBI:38170_diamino-1,3,5-triazine
CHEBI:88061_polyamine
CHEBI:22563_anion
CHEBI:25696_organic anion
CHEBI classes
0
20
40
60
80
100
120
140
160
180
-log10(Pvalue-adj)



NCIT Classes Enriched by Estrogens: Top 25 of 134
NCIT:C483_Therapeutic Estrogen
NCIT:C2181_Steroidal Estrogen
NCIT:C1636_Therapeutic Steroid Hormone
NCIT:C257_Nonsteroidal Antiinflammatory Drug
NCIT:C1323_Cyclooxygenase Inhibitor
NCIT:C241_Analgesic Agent
NCIT:C2198_Nonnarcotic Analgesic
NCIT:C2356_Analgesic and Antipyretic
NCIT:C2293_Estrogen
NCIT:C92608_Paraben
NCIT:C1932_Chemical Modifier
NCIT:C347_Carcinogen
NCIT:C1743_Chemical Carcinogen
NCIT:C78272_Agent Affecting Nervous System
NCIT:C17052_Ionizing Radiation
NCIT:C28203_Polyphenol
NCIT:C70553_Dietary Polyphenol
NCIT:C70554_Dietary Flavonoid
NCIT:C68464_Flavone
NCIT:C599_Isoflavone
NCIT:C49236_Therapeutic Procedure
NCIT:C20189_Property or Attribute
NCIT:C2182_Non-Steroidal Estrogen
NCIT:C43431_Activity
NCIT:C45174_Inorganic Carcinogen
NCIT classes
0
50
100
150
200
250
-log10(Pvalue-adj)

Acknowledgements

CMAP

- Rong-lin Wang
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- Mackenzie Morshead
- John Hoang