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Toxicology and *In Vitro* Alternative Methods: Analyzing Effects of Oil Spill Dispersants Using Rapid, *In Vitro* Tests for Endocrine and Other Biological Activity

Richard Judson
EPA National Center for Computational Toxicology

National Academies of Sciences, Engineering and Medicine
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Office of Research and Development
National Center for Computational Toxicology

Outline

- Introduce Rapid Risk Assessment
- Details on work on dispersants
- Broader RapidTox approach

RapidTox: Supporting Rapid Risk Assessment

- There are 50K-100K unique chemicals in commerce to which we are exposed
  - ~5000 (<10%) have repeat-dose animal toxicity studies
  - ~1500 have risk assessments – e.g. “safe levels” defined
  - IRIS level risk assessment takes 3-10 years
- This drives the need for rapid “screening-level” risk assessments using “New Approach Methods” (NAM)
  - Available *in vivo* data
  - *In vitro* assay data
  - Models
    - Analogy models (QSAR, Read-across) – does my chemical look like some other chemicals with data?
    - *Ab initio* – *in vitro* to *in vivo* extrapolation

Gulf Oil Spill EPA R&D Charge

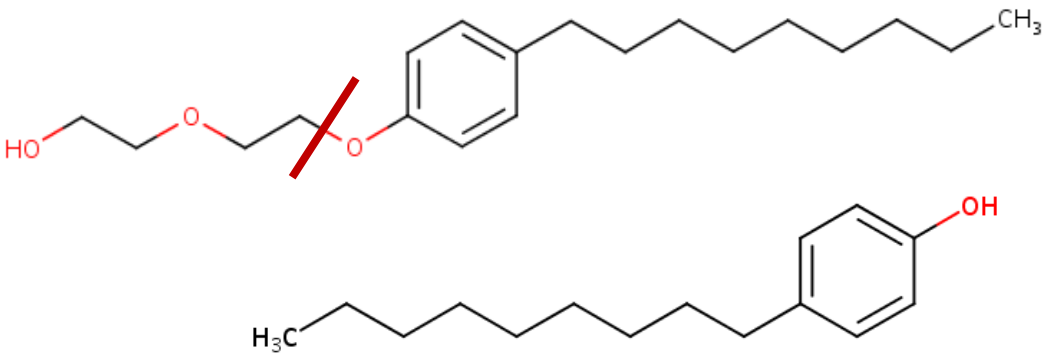
- Dispersants were going to be used and EPA was in charge of authorizing which one(s) to use
- Ideally, use the most effective formulation with the lowest toxicity

- Toxicity metrics
  - LC50 for mysid shrimp and silverside minnow
  - LC50 for cell culture (human)
  - Endocrine effects (estrogen, androgen, thyroid receptors: ER, AR, TR) – relevant to fish and humans
  - Broad *in vitro* activity screen

- Return results in < 6 weeks

Why worry about ER activity?

- Some dispersants were rumored to contain nonylphenol ethoxylate (NPE)
- Environmental breakdown product is nonylphenol – a weakly potent estrogen mimic
- Large quantities in coastal aquatic breeding grounds could have population-wide effects on reproduction



Nonylphenol ethoxylate (NPE)

Nonylphenol

Constraints

- Dispersant formulations are proprietary
- Manufacturers did not make them public
- EPA regulatory offices knew basics of formulations but could not legally let researchers know any of this information
- Manufacturers sent samples to EPA, but EPA could not send to other parties to test (e.g. universities)
- Google searching uncovered a cached page suggesting one dispersant contained NPE

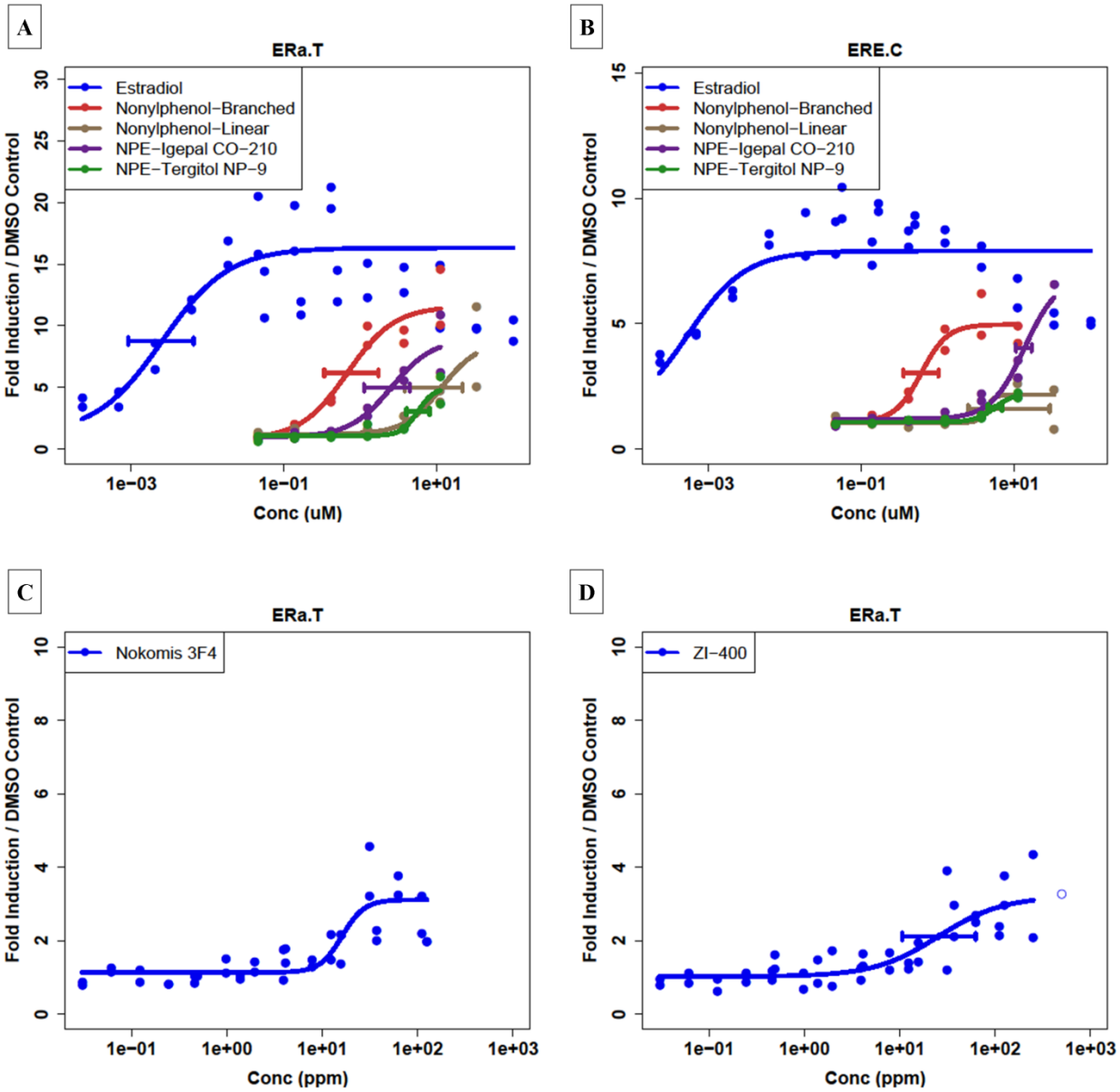
The Dispersants (spill started April 20)

Sample Name	Volume Received	Comments	Date Received	Manufacturer/ Source
Corexit 9500	1 L	hazy yellow	11-May-10	Nalco
JD 2000	10 ml	clear yellow	27-May-10	Ethox Chemicals, LLC
DISPERSIT SPC 1000	10 ml	clear amber	27-May-10	Polychem
Sea Brat #4	10 ml	hazy yellow	27-May-10	Alabaster Corp
Nokomis 3-AA	10 ml	clear light color	27-May-10	MAR-LEN Supply inc.
Nokomis 3-F4	10 ml	clear light color	27-May-10	MAR-LEN Supply inc.
ZI-400	25 ml	clear yellow	29-May-10	ZI Chemical
SAF-RON GOLD	500 ml	silver iridescent	4-June-10	Sustainable Environmental Technologies, Inc.

In Vitro Assay Technologies Used

- Competitive binding (Novascreen)
  - Cell-free
  - Dispersants seem to have denatured proteins, given non-specific results
- ER/AR reporter-gene assays (NIH NCATS / NCGC)
  - Agonist and antagonist mode
  - Quantitative cytotoxicity
- Collection of 81 nuclear-receptor-related assays (Attagene)
  - Includes AR, ER, TR
  - Other xenobiotic response pathways
  - Quantitative cytotoxicity
  - HepG2 (liver) cell line
  - CIS and TRANS modes (TRANS is more sensitive)

Concentration-Response Profiles



Control Data

Igepal and Tergitol are non-ionic surfactants

ERa.T=Attagene ERa TRANS
ERE.C=Attagene ERa CIS

CIS efficacy less than half TRANS efficacy for reference compounds

Dispersant Test Data

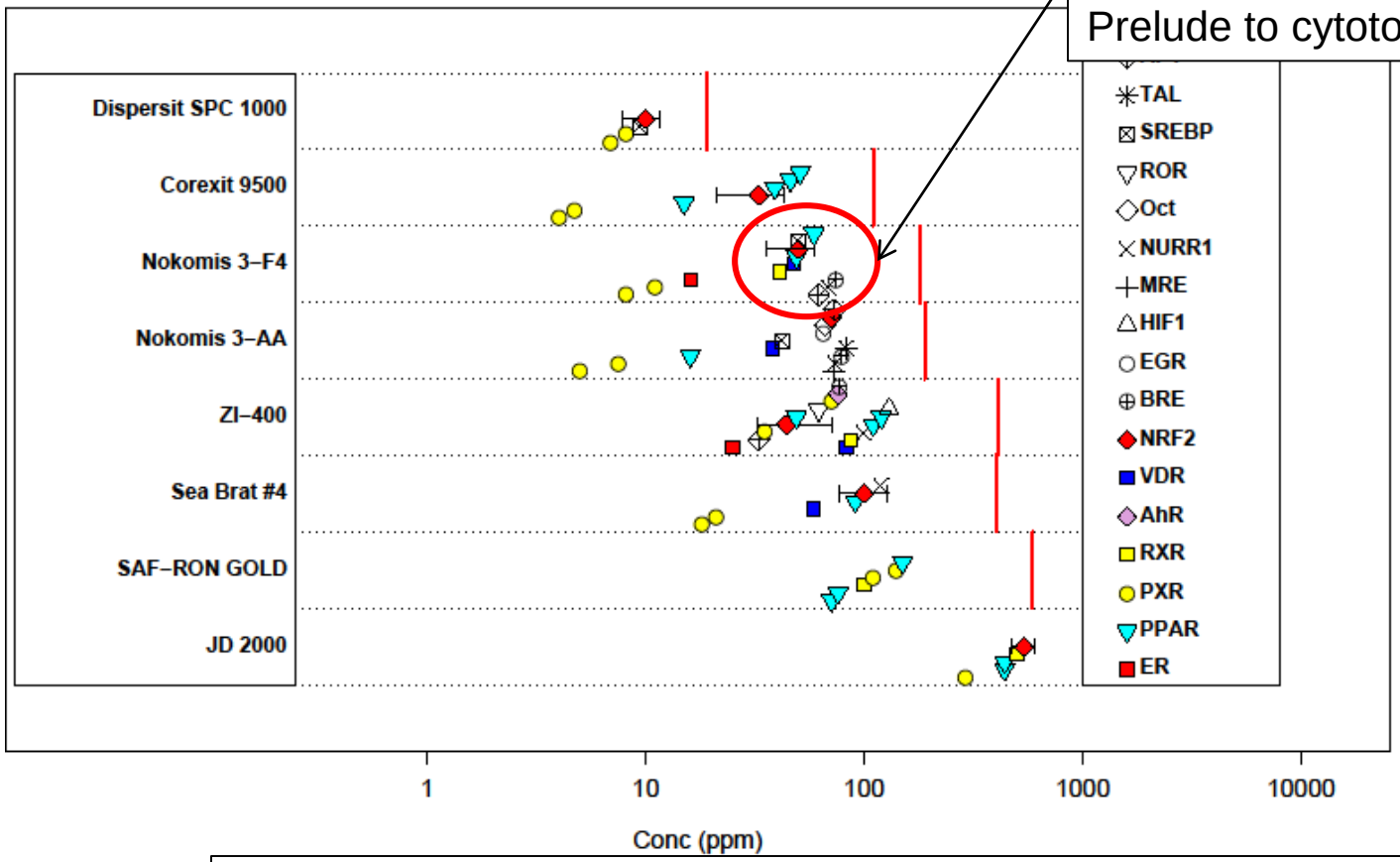
TRANS assay efficacy near detection threshold for these dispersants, and CIS is below

Further Nuclear Receptor Analysis

- Attagene runs 81 nuclear receptor-related endpoints in 2 multiplexed assays
- Relatively quick and inexpensive
- Many related to xenobiotic response
  - ER/AR/TR
  - PPAR(a,d,g)
  - CAR / PXR / RXR
  - AHR

Nuclear Receptor Results

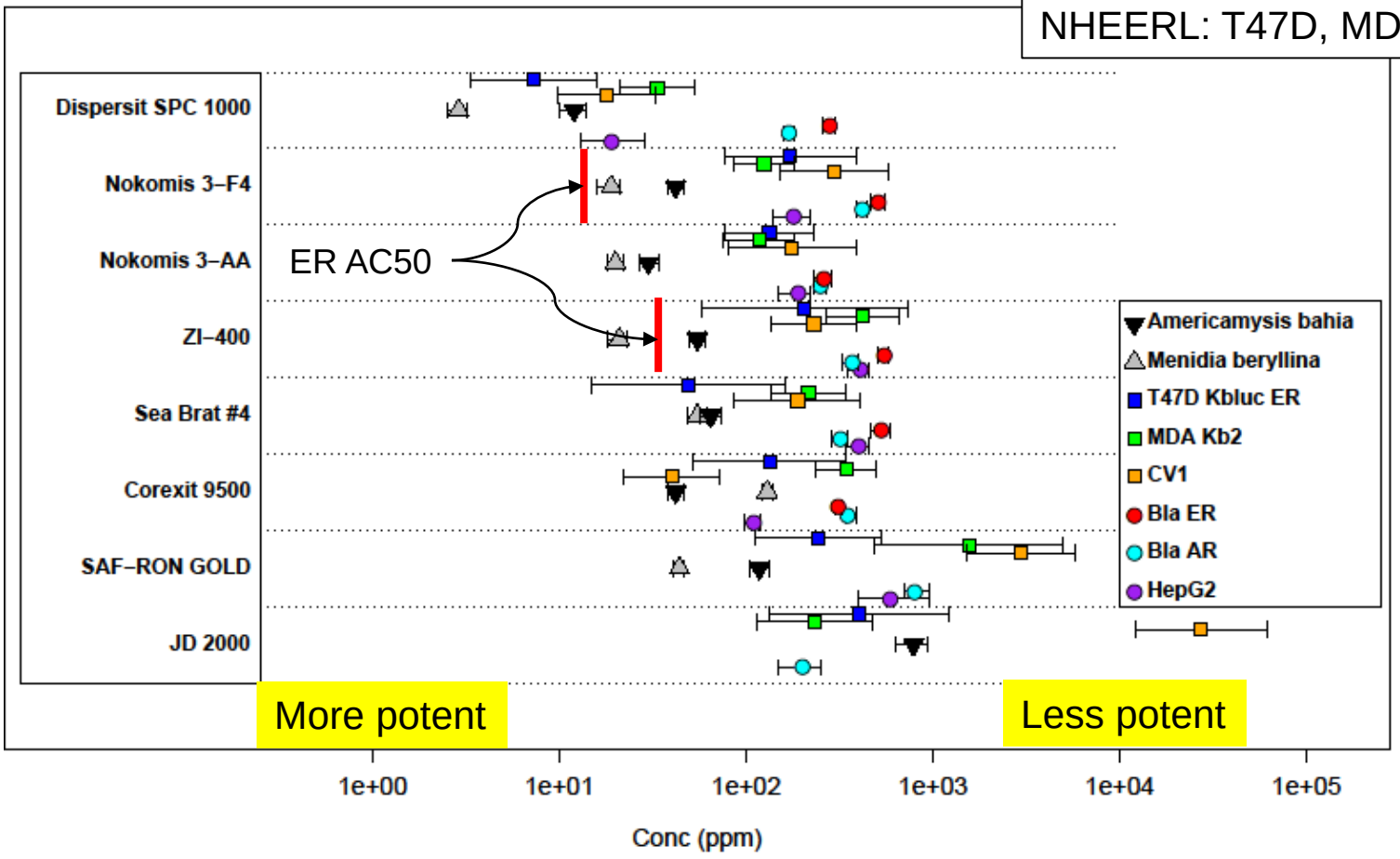
Multi-assay pile-up
Indication of generalized cell stress
Prelude to cytotoxicity



Little specific activity seen except for PXR/ PPAR
Consistent with Xeno-sensing

Dispersant Cytotoxicity Results

Attagene: HepG2
NCGC: Bla ER/AR
NHEERL: T47D, MDA, CV1



Mysid shrimp
Silverside minnow

Dispersant Conclusions

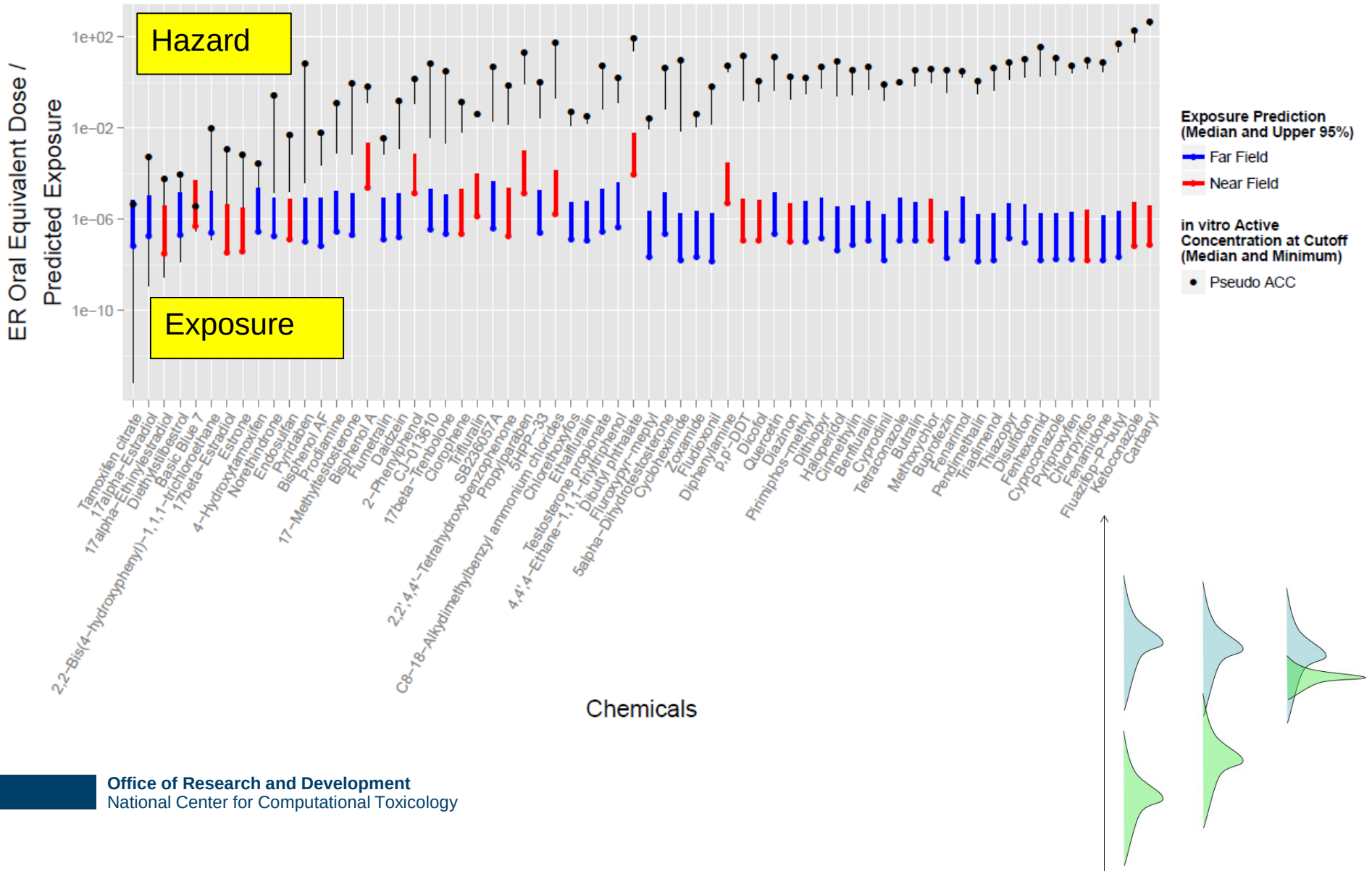
- Weak evidence of ER activity in 2 dispersants
  - Seen in single, perhaps over-sensitive assay (1 of 6)
  - Not of biological significance
  - Consistent with presence of NPE
  - Activity only at concentrations >> seen in Gulf after dilution
- No AR activity
- No ER activity seen in Corexit 9500
- Corexit is in the middle of the pack for cytotoxicity
- No worrisome activity seen in other NR assays

Broader Rapid Risk Assessment Applications

- Prioritization
  - Endocrine Disruptor Screening Program – 10,000 chemicals to be screened for endocrine activity
  - TSCA inventory – 25,000 chemicals to be prioritized for detailed risk assessment

- Rapid Risk Assessment Potential Opportunities
  - 2014 Elk River spill 4-methylcyclohexanemethanol: What effects might this have, and what is the safe level for drinking water?
  - Superfund sites – EPA finds 10s-100s of chemicals without risk assessment values – what chemical(s) should drive the cleanup?
  - Developing concerns over perfluorinated compounds in the environment

Example of using predicted hazard and exposure to prioritize further testing: ER activity



Tools of RapidTox

- Large databases of public *in vivo* data (ToxRefDB, ToxValDB)
- *In vitro* data on ~10,000 chemicals of environmental interest (ToxCast)
- *In vitro* toxicokinetic data to convert *in vitro* potencies to *in vivo* "points of departure"
- Databases of exposure-related information
  - Biomonitoring
  - Environmental monitoring
  - Chemical formulation and chemical-product use data
- Multiple models of hazard, exposure and toxicokinetics
- Public dashboards
  - https://comptox.epa.gov
  - https://actor.epa.gov
- Expertise to use all of these tools in rapid response situations

National Center for Computational Toxicology

United States Environmental Protection Agency

NCCT Staff
Rusty Thomas
Kevin Crofton
Keith Houck
Ann Richard
Richard Judson
Tom Knudsen
Matt Martin*
Grace Patlewicz
Woody Setzer
John Wambaugh
Tony Williams
Steve Simmons
Chris Grulke
Katie Paul-Friedman
Jeff Edwards
Chad Deisenroth
Joshua Harrill
Rebecca Jolley
Jeremy Dunne

NCCT Postdocs
Todor Antonijevic
Audrey Bone
Swapnil Chavan
Kristin Connors*
Danica DeGroot
Jeremy Fitzpatrick
Dustin Kapraun*
Agnes Karmaus*
Max Leung*
Kamel Mansouri*
Andrew McEachran
LyLy Pham
Prachi Pradeep
Caroline Ring*
Kate Saili
Eric Watt*
Todd Zurlinden

NCCT
Nancy Baker
Dayne Filer*
Parth Kothiya*
Sean Watford
Indira Thillainadarajah
Robert Pearce
Danielle Suarez
Doris Smith
Jamey Vail
Risa Sayre
Nathan Rush

NTP Collaborators
Warren Casey
Nicole Kleinstreuer
Mike Devito
Dan Zang
Rick Paules
Nisha Sipes

NIH/NCATS Collaborators
Menghang Xia
Ruili Huang
Anton Simeonov

* Graduates