Risk Assessment in the 21st Century

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Disclaimer/COI Statement

The views expressed in this lecture are those of the presenter and do not necessarily reflect the views or policies of the US Environmental Protection Agency.

The presenter has no conflicts of interest to declare relative to the materials presented.

Attribution and Thanks

- Thanks to Steve Edwards and John Wambaugh for their willingness to share material regarding adverse outcome pathways and computational exposure, respectively
- The science presented represents collaborative, transdisciplinary efforts from the US EPA/ORD:
  - National Health and Environmental Effects Research Laboratory
  - National Exposure Research Laboratory
  - National Center for Computational Toxicology

What is the Problem?

- For past ~50 years, risk assessment heavily depended on animal testing for hazard identification and dose-response assessment
- With increasing dependence on chemical tools, legacy approaches no longer sufficient
  - Decades needed to test ~90,000 chemicals currently in commerce
  - Increasing pressure to reduce animal use for toxicology testing
  - Uncertainty in extrapolating animal test data to humans
  - Need to expand beyond human and consider sentinel species within an ecosystem

Proposed Solutions

- NAS -- *Toxicology Testing in the 21st Century: A Vision and a Strategy* (2007)
  - Chemical characterization using computational and experimental approaches
  - Toxicity pathway identification and testing using high throughput approaches, preferably with human-based platforms
  - Targeted *in vivo* testing as necessary to reduce uncertainty
  - Dose-response assessment using in vitro testing and extrapolation modeling (IVIVE)
  - Population-based and human exposure data using biomarkers and biomonitoring
  - Fit for purpose testing based on problem formulation

Proposed Solutions

- NAS - *Exposure Science in the 21st Century: A Vision and a Strategy* (2012)
  - Measure internal exposure through non-targeted metabolomics and PBPK modeling
  - Identify exposure biosignatures or bioindicators
  - Identify modifiers of internal exposure
  - Computational approaches (modeling)

Proposed Solutions

- NAS – *Using 21st Century Science to Improve Risk-Related Evaluations* (2017)
  - Integrated, transdisciplinary, systems-based approach
    - Experimental and computational toxicology and exposure science
    - Molecular epidemiology
    - Statistics
    - Social sciences)
  - Use new data streams to inform specific aspects of risk assessment, *e.g.*, chemical and site-specific assessments
  - "Fit for Purpose," performance=based validation approaches

Progress?

- Numerous large efforts being sponsored by various agencies
  - US EPA's ToxCast andExpoCast
  - Tox21 and ES21 Consortiums (US Federal Government Agencies)
  - Integrated Approaches to Testing and Assessment (OECD)
  - Safety Evaluation Ultimately Replacing Animal Testing (SEURAT)
  - Human Early-Life Exposome (HELIX)
- Substantial increase in new technologies and data streams to screen/prioritize potential toxicants across multiple platforms
  - High throughput and high content assays
  - Advances in genomics, proteomics and metabolomics
  - Organotypic culture systems to introduce increased biological complexity
- Computational models

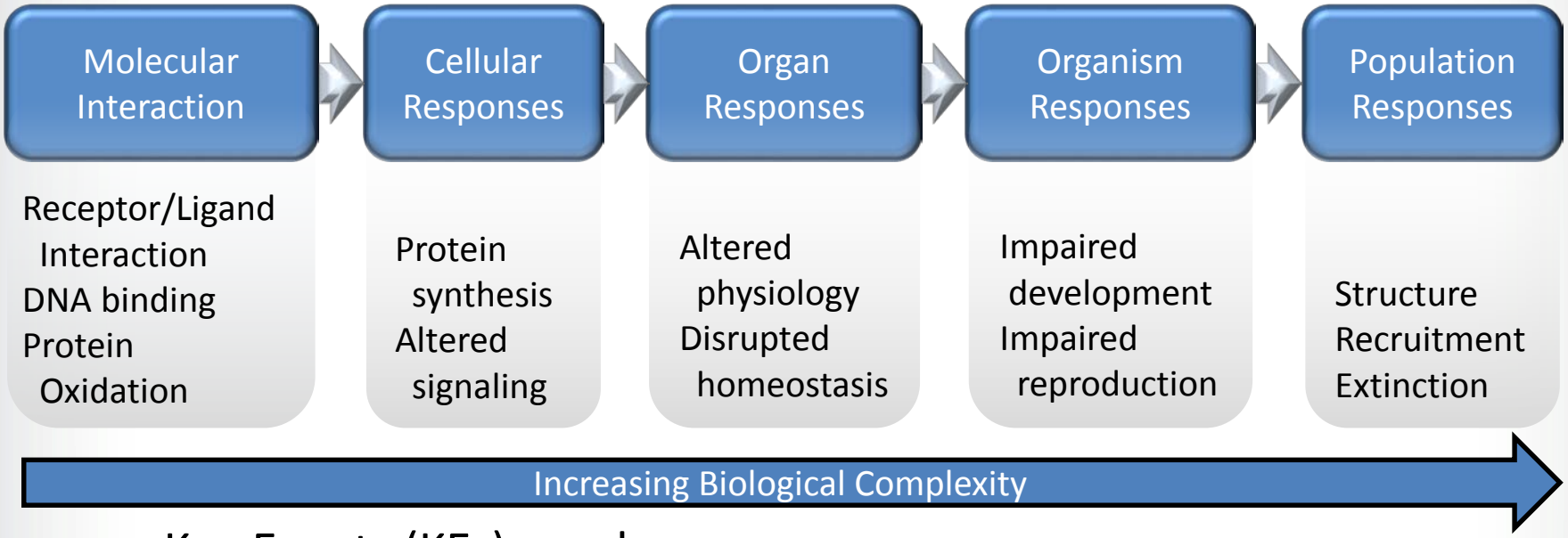
Progress?

- Toxicology is in a period of transition between more traditional approaches and TT21 approaches
- Path forward to use TT21 approaches in risk assessment and regulatory decision making is unclear
- Magnitude of the challenge in achieving TT21 goals remains substantial
- *Where are we?*

Adverse Outcome Pathways

- Problem
  - Characterizing biological context of TT21 assays is major obstacle to risk assessment acceptance
  - Can we develop suite of assays to mimic spectrum of events from initiation to adverse outcome, *i.e.*, toxicology pathways?
- Adverse Outcome Pathway (AOP) concept
  - AOP = conceptual, systems-based framework to explain chemical perturbation from a molecular initiating event (MIE), progressing through a sequence of key events (KE) to a specific adverse outcome
  - AOPs are chemically agnostic, defined by the adverse endpoint, not the MIE/chemical interaction

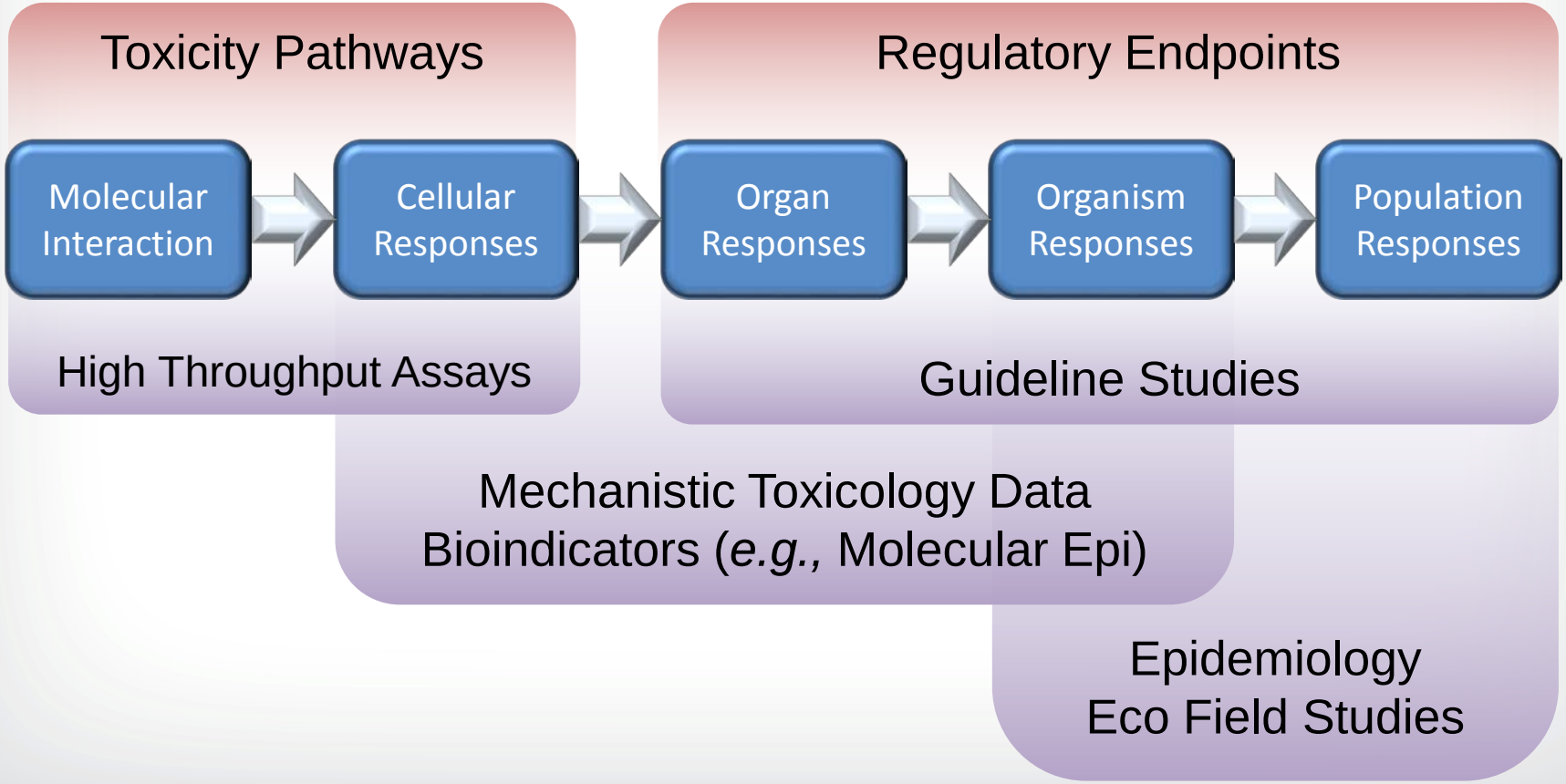
AOP Components



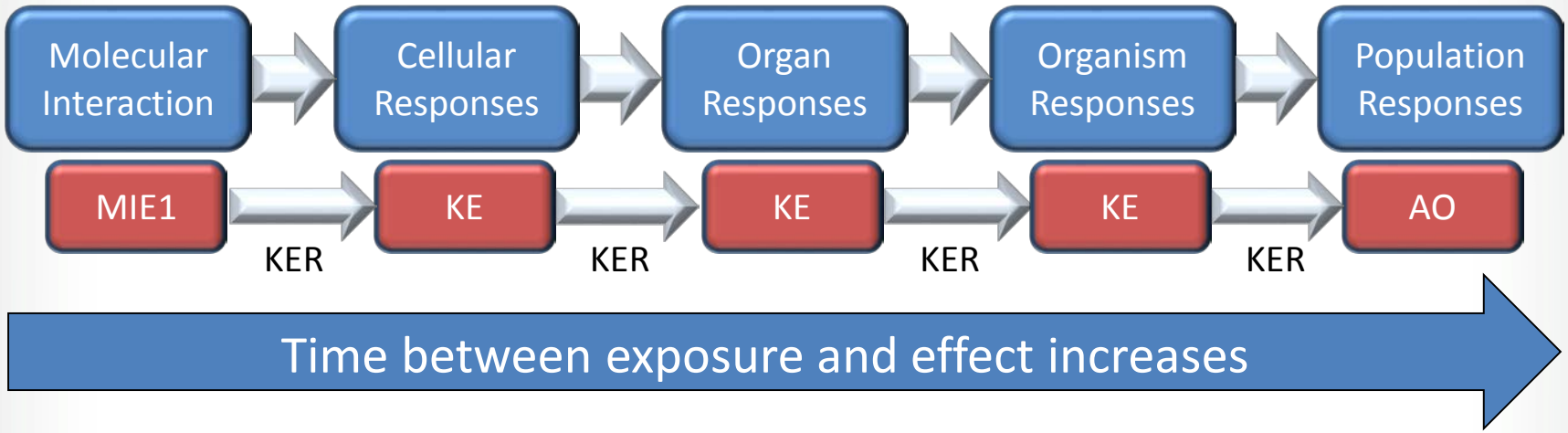
- Key Events (KEs) - nodes
  - Represent change in biological state
  - Measurable and essential for progression
  - MIE: Initial point of chemical interaction
  - Adverse Outcome (AO): Adverse outcome of regulatory significance
- Key Event Relationships (KERs) - edges
  - Connections between two key events
  - Critical for assembling evidence in support of the AOP

AOPs

Provide Understanding & Scaffold for Data



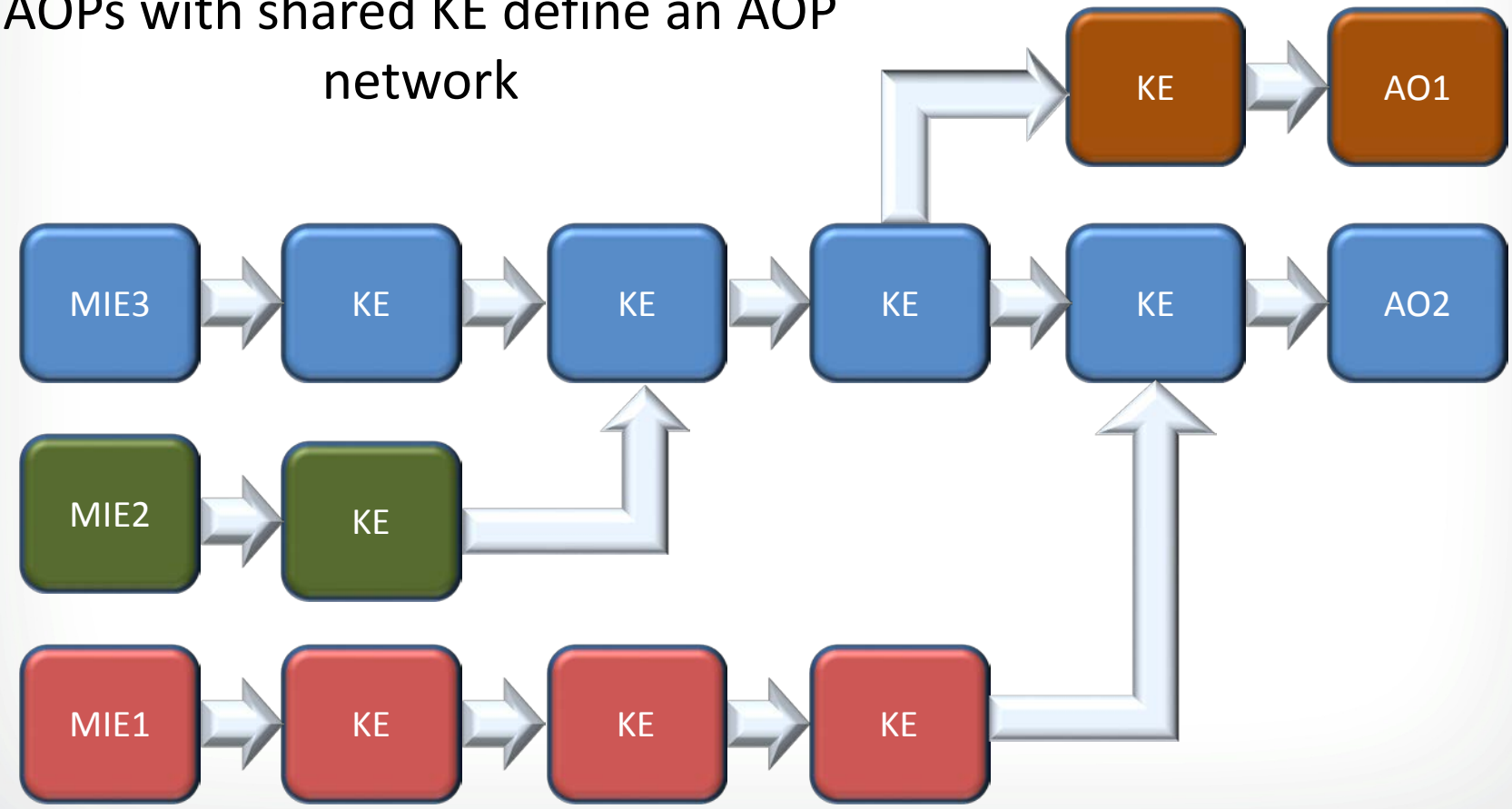
Predictivity of AOPs



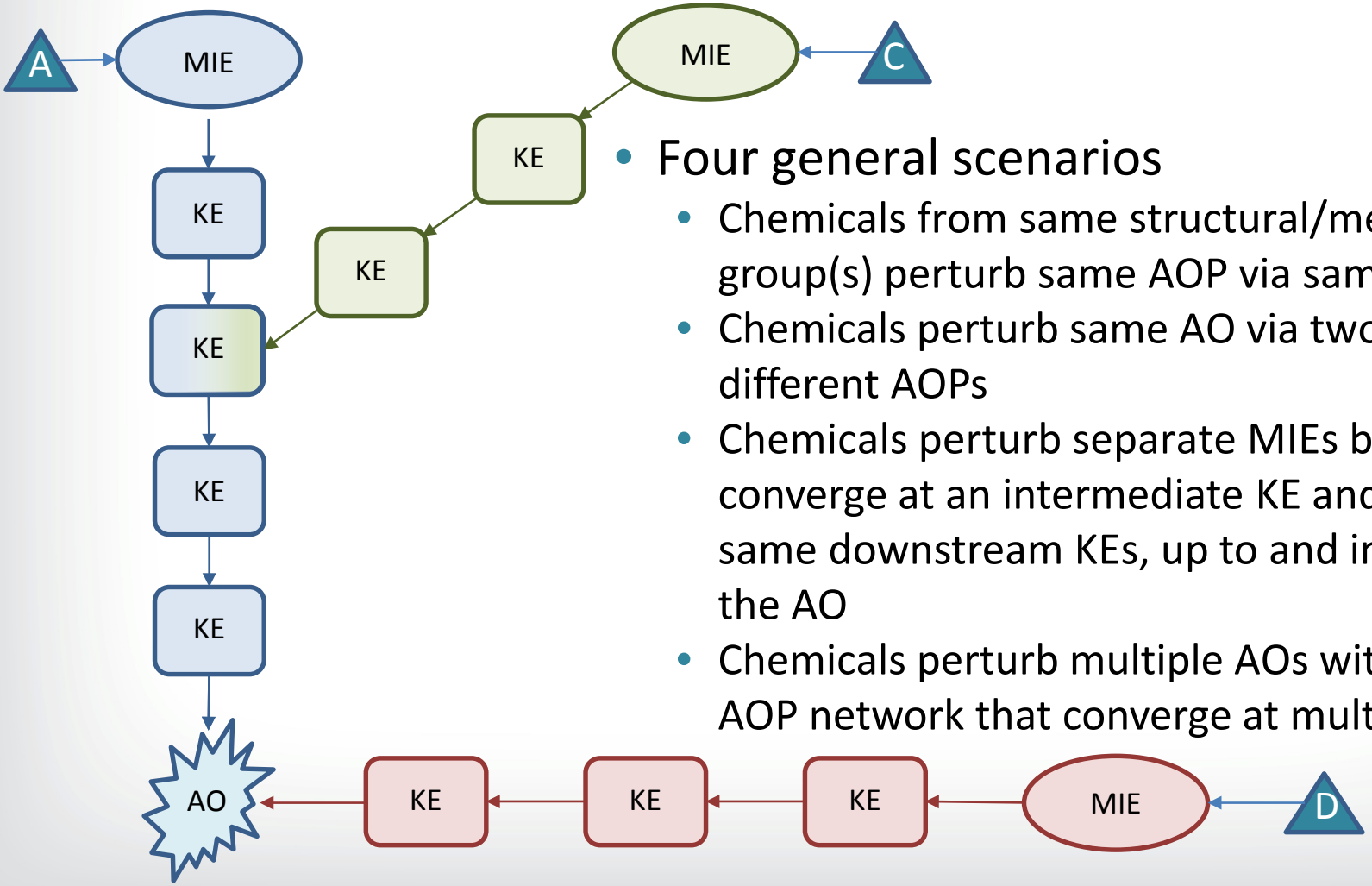
- Predictive model requires
  - Evidence supporting the KERs between KE and the AO
  - Definition of KE dose-response relationships
  - Quantitative understanding of the downstream KERs
  - Quantitative understanding of modifying factors that influence downstream KEs & KERs (*e.g.*, genetic polymorphisms, epigenome differences, life stage differences)

AOP Networks

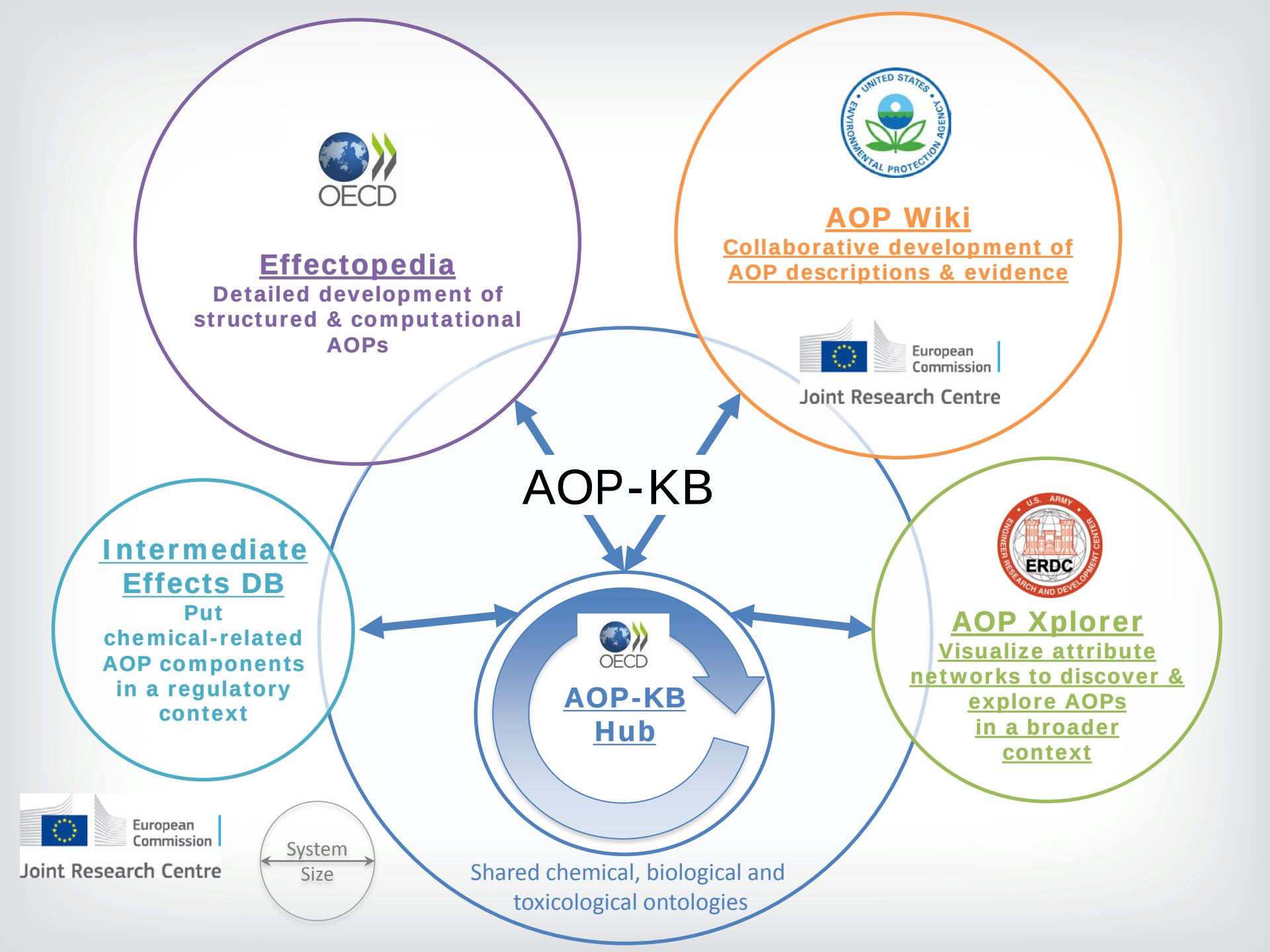
AOPs with shared KE define an AOP network



AOPs and Mixtures



- Four general scenarios
  - Chemicals from same structural/mechanistic group(s) perturb same AOP via same MIE
  - Chemicals perturb same AO via two, or more, different AOPs
  - Chemicals perturb separate MIEs but converge at an intermediate KE and initiate same downstream KEs, up to and including the AO
  - Chemicals perturb multiple AOs within an AOP network that converge at multiple points


OECD
Effectopedia
Detailed development of structured & computational AOPs
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
AOP Wiki
Collaborative development of AOP descriptions & evidence
European Commission
Joint Research Centre
AOP-KB
Intermediate Effects DB
Put chemical-related AOP components in a regulatory context
OECD
AOP-KB Hub
U.S. ARMY ENGINEER RESEARCH AND DEVELOPMENT CENTER
ERDC
AOP Xplorer
Visualize attribute networks to discover & explore AOPs in a broader context
Shared chemical, biological and toxicological ontologies
European Commission
Joint Research Centre
System
Size

AOP Wiki

- As of January 1, 2017, there were 187 AOPs listed in the AOP Wiki (https://aopwiki.org)

- Examples of MIE include nuclear receptors, AhR, CAR, ion channels, transporters, neurological receptors (e.g., glutamate receptor), enzymes

- AO examples include population loss, steatosis, cancer, reproductive dysfunction, developmental toxicity, neurotoxicity, kidney toxicity

Progress in applying AOP System Framework to Risk Assessment and Regulatory Decision Making

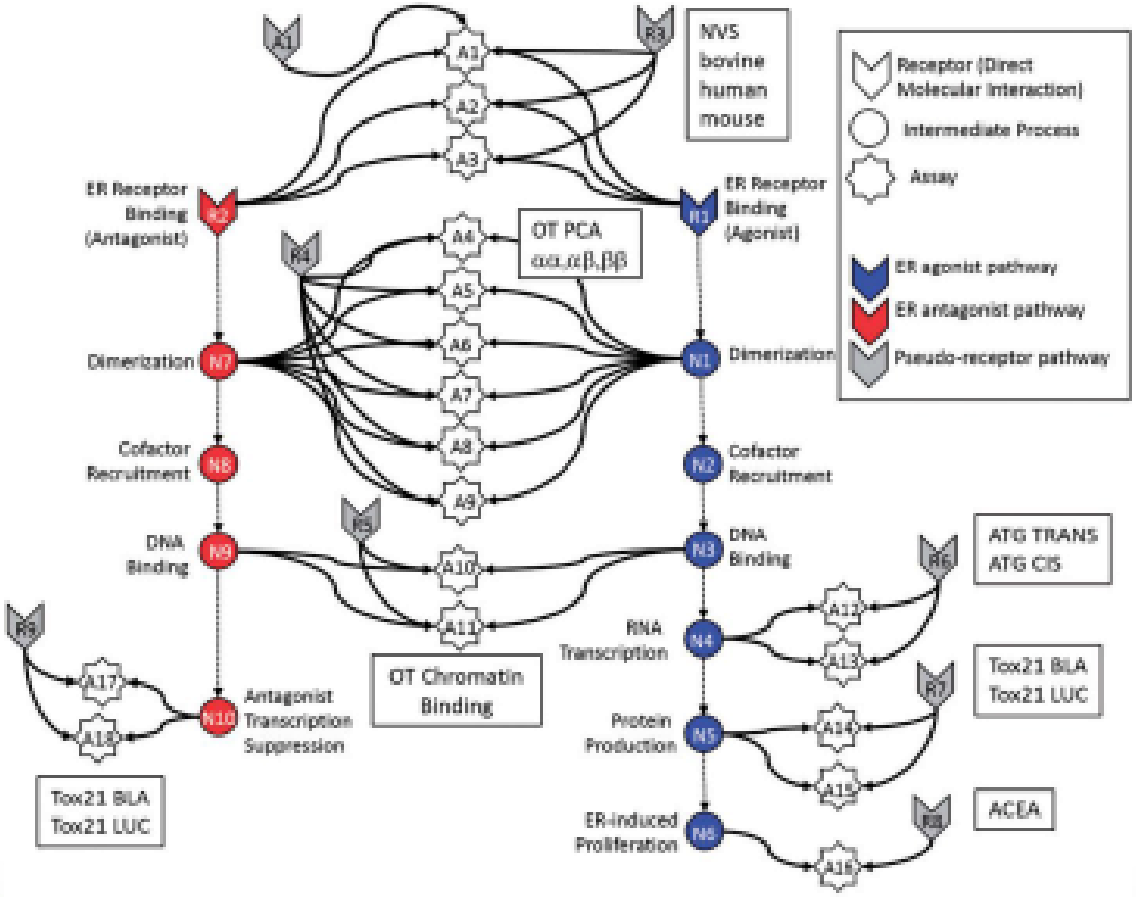
EDSP Problem

- Food Quality Protection Act (FQPA) passed 1996
  - Mandated EPA develop, validate and apply test systems to screen for endocrine disrupting chemicals (EDSP)
  - Approximately 10,000 chemicals identified as needing testing
- Two-tiered approach
  - Tier 1: Eleven *in vitro* and *in vivo* screening assays
  - Tier 2: Five *in vivo* assays
- Tier 1 testing complete on 67 chemicals and analysis ongoing
- Additional 107 chemicals identified and Tier 1 testing initiated on small number
- *Given above pace, >100 years would be necessary to complete task*

Success Story: ER Model

- Taking advantage of ToxCast, 18 assays identified that, in both an agonist and antagonist mode, represent a quantitative AOP resulting from disruption of ER signaling:
  - ER binding (3, ERα and ERβ)
  - ER dimerization (6, ERα and ERβ)
  - ER dimer chromatin binding (2, ERα only)
  - ER-dependent transcriptional activation (6, ERα only)
  - ER-dependent cell proliferation (1, ERα > ERβ)
- Concentration-response from 0.01 µM to 100 µM
- Output is simple linear, additive integration of dose response data across assays, normalized against 17β-estradiol (score = 1.00)
- Scores <0.001 = negative
- Scores >0.001 and <0.10 (AC_{50} ≈100 µM) considered inconclusive
- Scores ≥0.10 = positive

ER Model



From Judson *et al. Toxicol Sci* 148:137-154, 2015

ER Model

- Performance-based validation – agonist mode only
  - 40 chemicals previously validated in at least 2 independent in vitro assays
    - 28 agonists, 12 inactive
  - 103 chemicals previously validated with in vivo uterotrophic assay
    - 43 confirmed in at least 2, independent assays
    - 60 in OECD guideline-like study
    - 55 agonists, 48 inactive

Performance	In Vitro Reference Chemicals	In Vivo Reference Chemicals
Accuracy	0.93 (0.95)	0.83 (0.88)
Sensitivity	0.93 (0.93)	0.89 (0.86)
Specificity	0.92 (1.00)	0.77 (0.90)

( ) Analysis excluding inconclusive scores

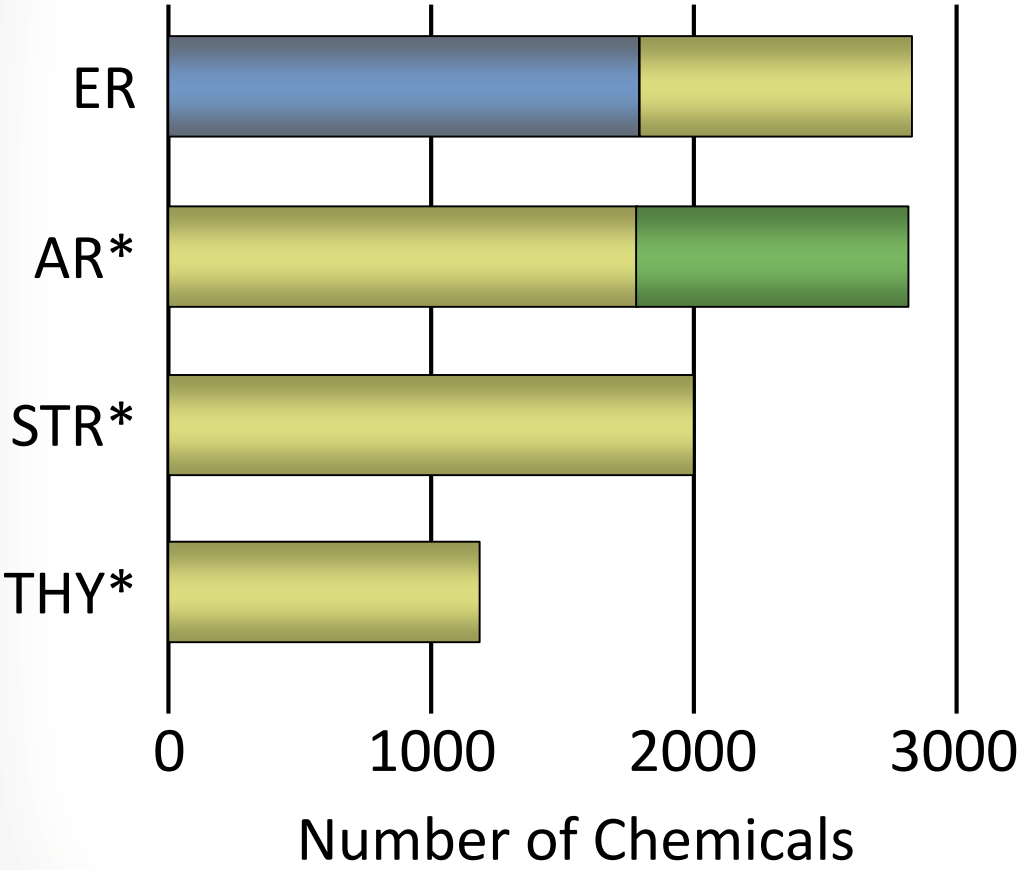
From Browne *et al. Environ Sci Technol* 49:8804-8814, 2015

EDSP Pivot

Current Tier 1 Assays	Alternative Tier 1 Assays
ER Binding	ER Model
ER-Dependent Transactivation	ER Model
Uterotrophic	ER Model
AR Binding	AR Model
Hershberger	AR Model
Aromatase	STR Model
Steroidogenesis	STR Model
Female Rat Pubertal	ER, THY, STR Models
Male Rat Pubertal	ER, THY, STR Models
Fish Short Term Reproduction	ER, AR, STR Models
Amphibian Metamorphosis	THY Model

From Juberg *et al. Toxicol Sci* 155:22-31, 2017

EDSP Pivot



Current

Anticipated phase 1

Anticipated phase 2

* Models under development

From Juberg *et al. Toxicol Sci* 155:22-31, 2017

Human risk assessment requires an understanding of not only the hazard, but also an estimate of the concentration of the chemical to which humans are exposed

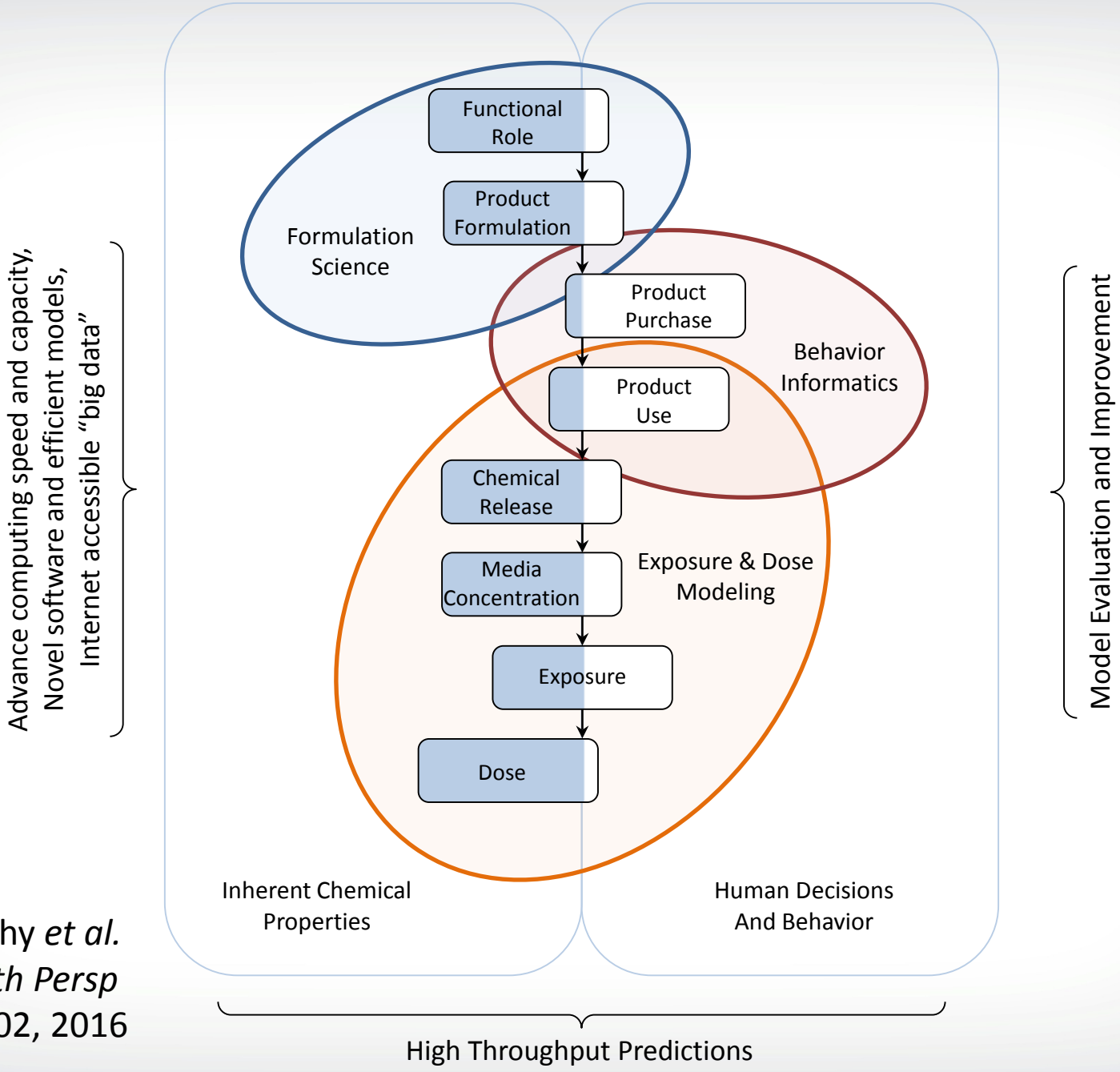
Hazard + Exposure = Risk

Exposure

- Decades needed to assess exposure to existing chemicals in commerce using traditional approaches
- Advent of computational exposure science to reliably and quantitatively predict exposure. Integrates advances in:
  - Chemistry
  - Computer science
  - Mathematics
  - Statistics
  - Social/behavioral sciences
  - Technologies for data collection

Exposure

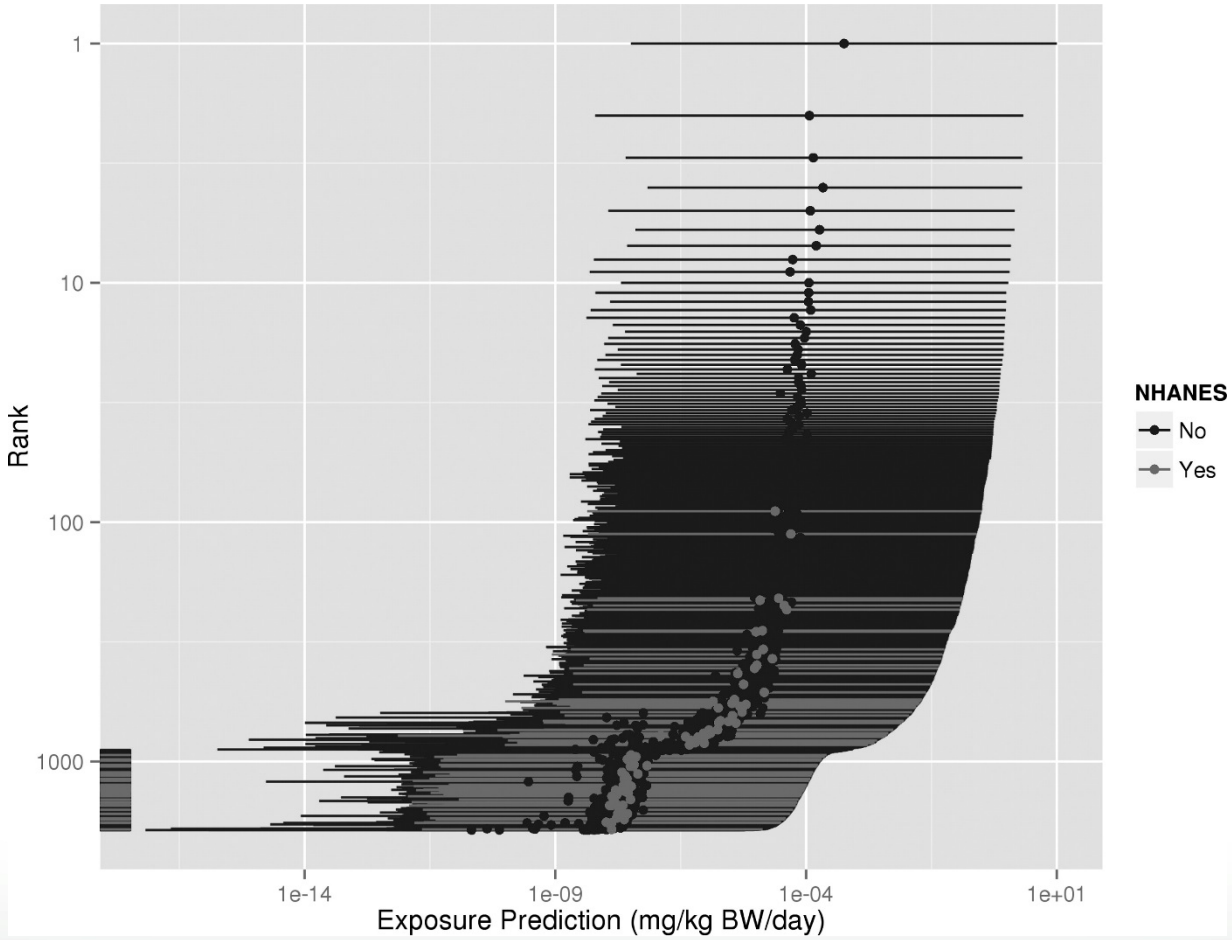
- Exposure models being developed based on:
  - Potential toxicant chemical/physical properties
  - Real or inferred/predicted chemical use information and production volume
  - In a set of NHANES urine samples, 50% of exposure variance explained by 4 use-dependent dichotomous variables and 1 continuous variable
    - Industrial use
    - Pesticide inert
    - Pesticide active
    - Consumer use
    - Production volume
- Although these variables are unknown for majority of chemicals in commerce, approaches have been developed to infer or deduce these data



From Egeghy *et al.* *Environ Hlth Persp* 124:697-702, 2016

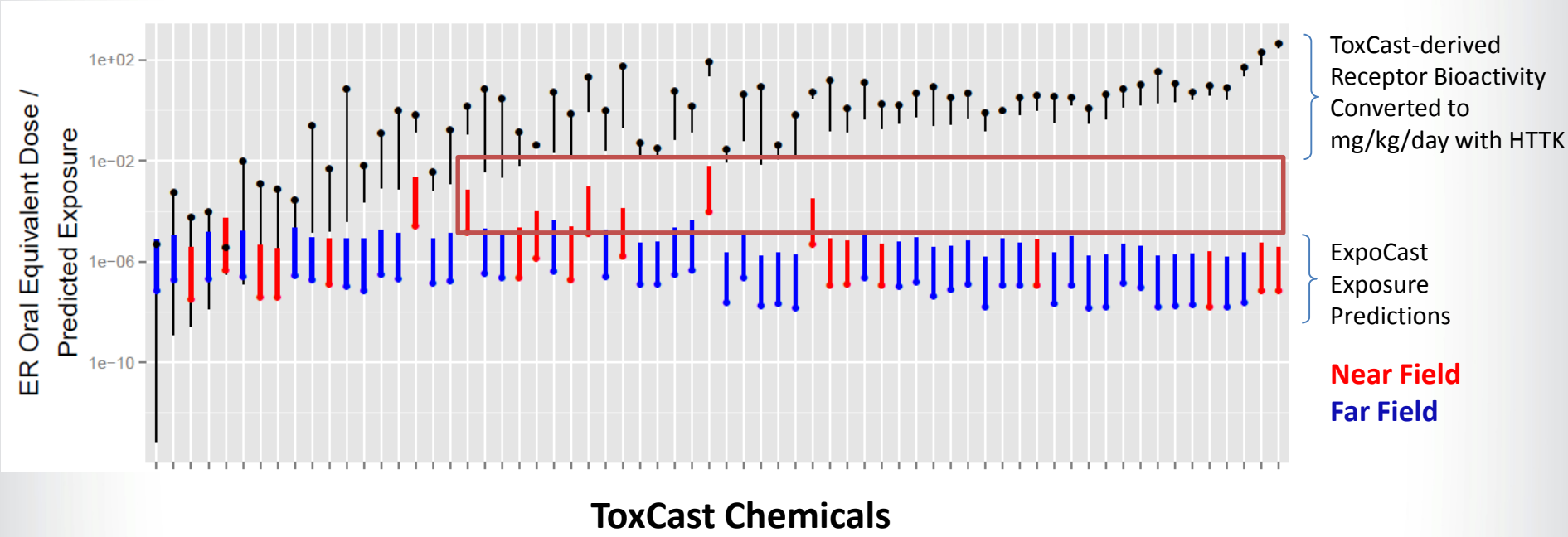
Predicting Exposure

Near-field Predictions (with 95% CI) on 1936 chemicals



From Wambaugh *et al. Environ Sci Technol* 47:8479-8488, 2013

HTT Risk Prioritization



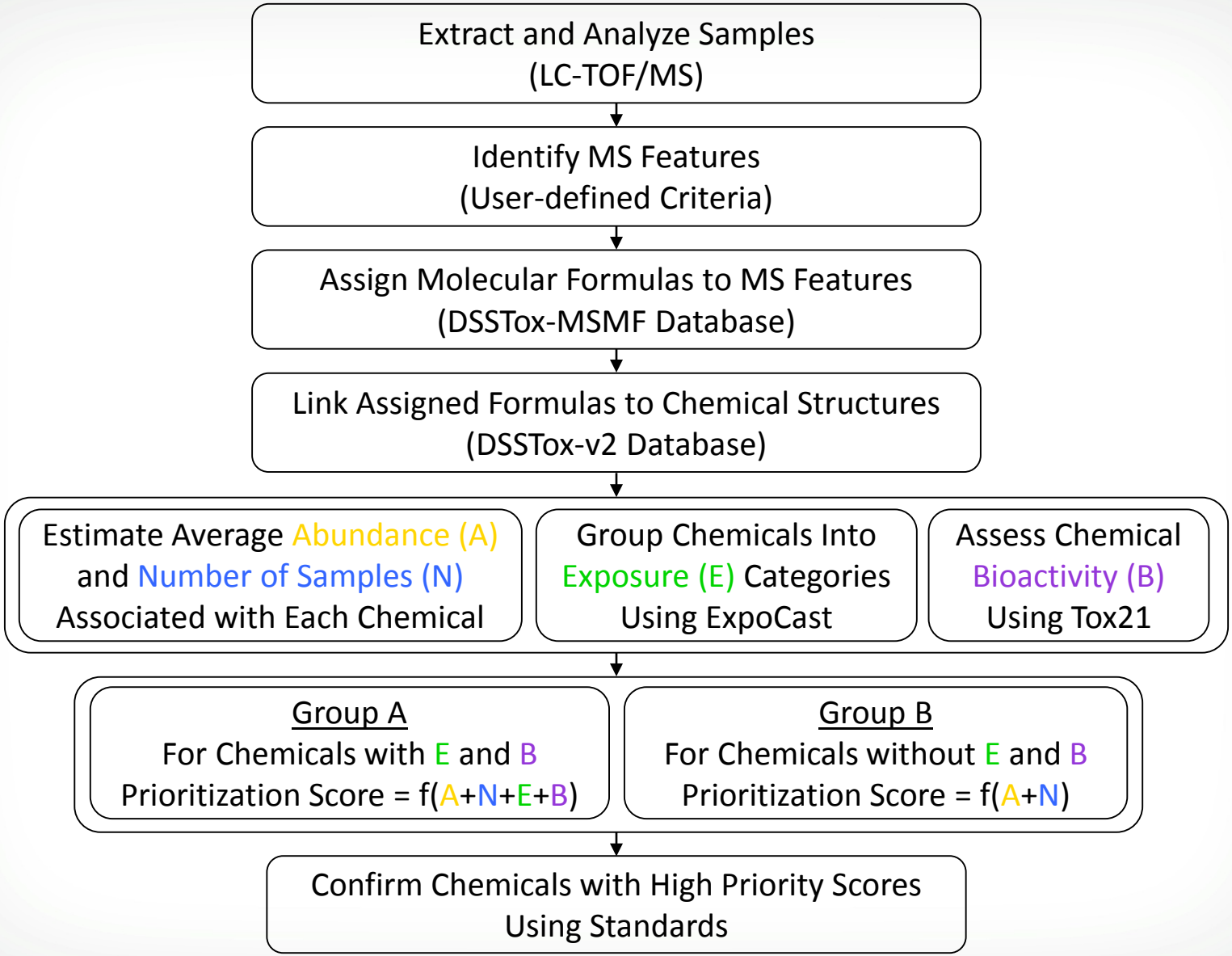
- When MOE is sufficiently high, can eliminate from further evaluation

From: December, 2014 FIFRA Scientific Advisory Panel, "Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening"

Non-Targeted Analysis

- Recent proof of principle study combining high-resolution mass spectrometry, exposure prediction (ExpoCast), and high throughput in vitro bioactivity (ToxCast) data (Rager *et al. Environ Int* 88:269-80, 2016)

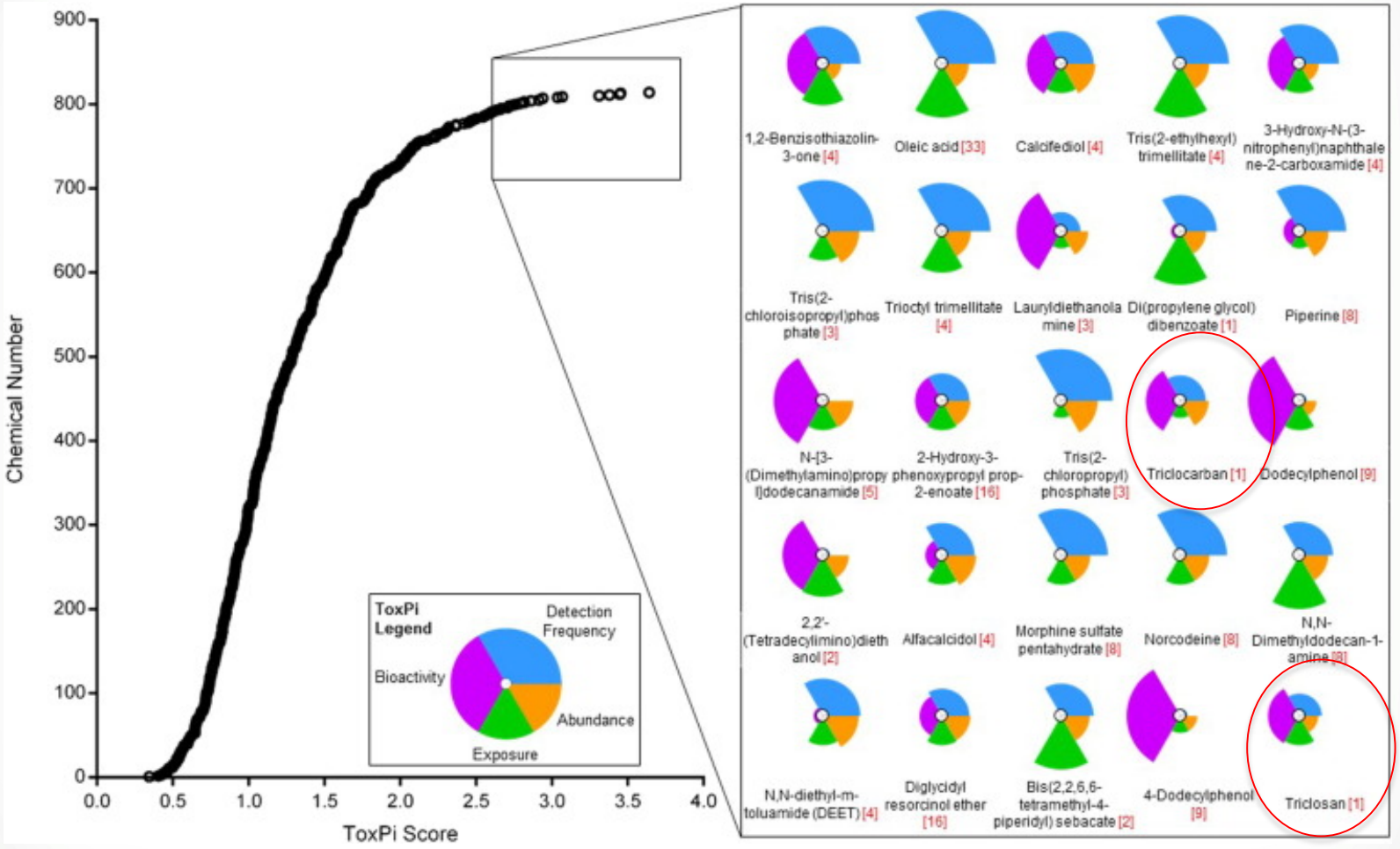
- Analysis of 56 household dust samples



(From Rager *et al. Environ Int* 88:269-80, 2016)

Non-Targeted Analysis

- On average, 5000 molecular features identified per dust sample
- Matching to EPA's Distributed Structure-Searchable Toxicity (DSSTox) database identified 978 chemical formulas mapping to 3228 possible chemicals
- Possible chemicals prioritized based on abundance, detection frequency, exposure prediction (ExpoCast), and bioactivity estimates (Tox21), using ToxPi scores
  - Bioactivity restricted to AhR, AR, ER, NFκβ, and PPARγ pathways



(From Rager *et al. Environ Int* 88:269-80, 2016)

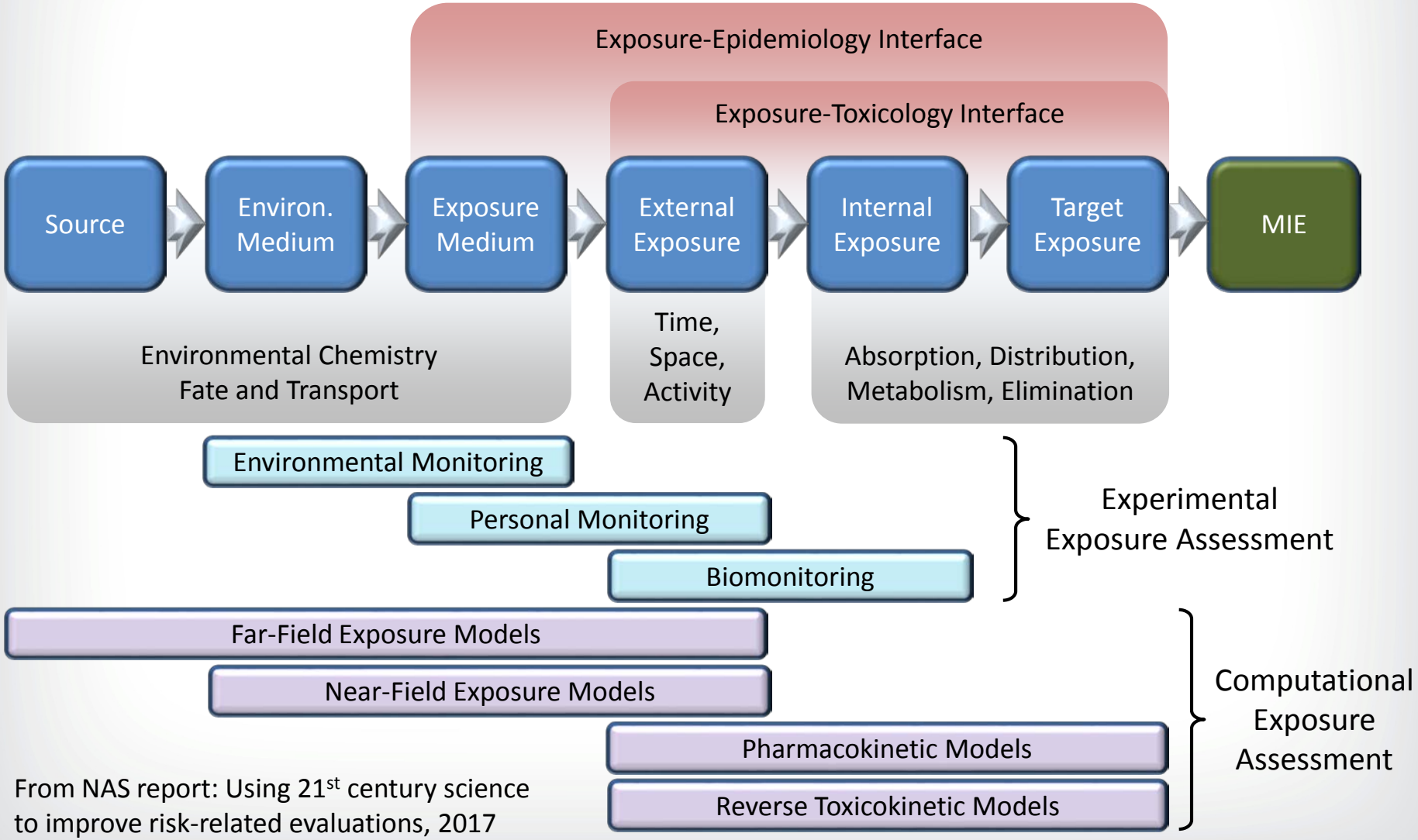
Non-Targeted Analysis

- Possible chemicals with relatively high exposure and/or bioactivity scores further assessed using 100 chemical standards
  - *33/978 (3.5%) chemicals identified*
- Much work to be done to improve specific chemical identification

AEP

- Aggregate Exposure Pathway (AEP) (Teeguarden *et al. Environ Sci Technol* 50:4579-86, 2016)
  - Systems-based framework to organize exposure and toxicokinetic data
  - Covers scope of exposure science
  - Potential for integration with AOP via molecular initiating event(s), completing source to outcome continuum

AEP



From NAS report: Using 21st century science to improve risk-related evaluations, 2017

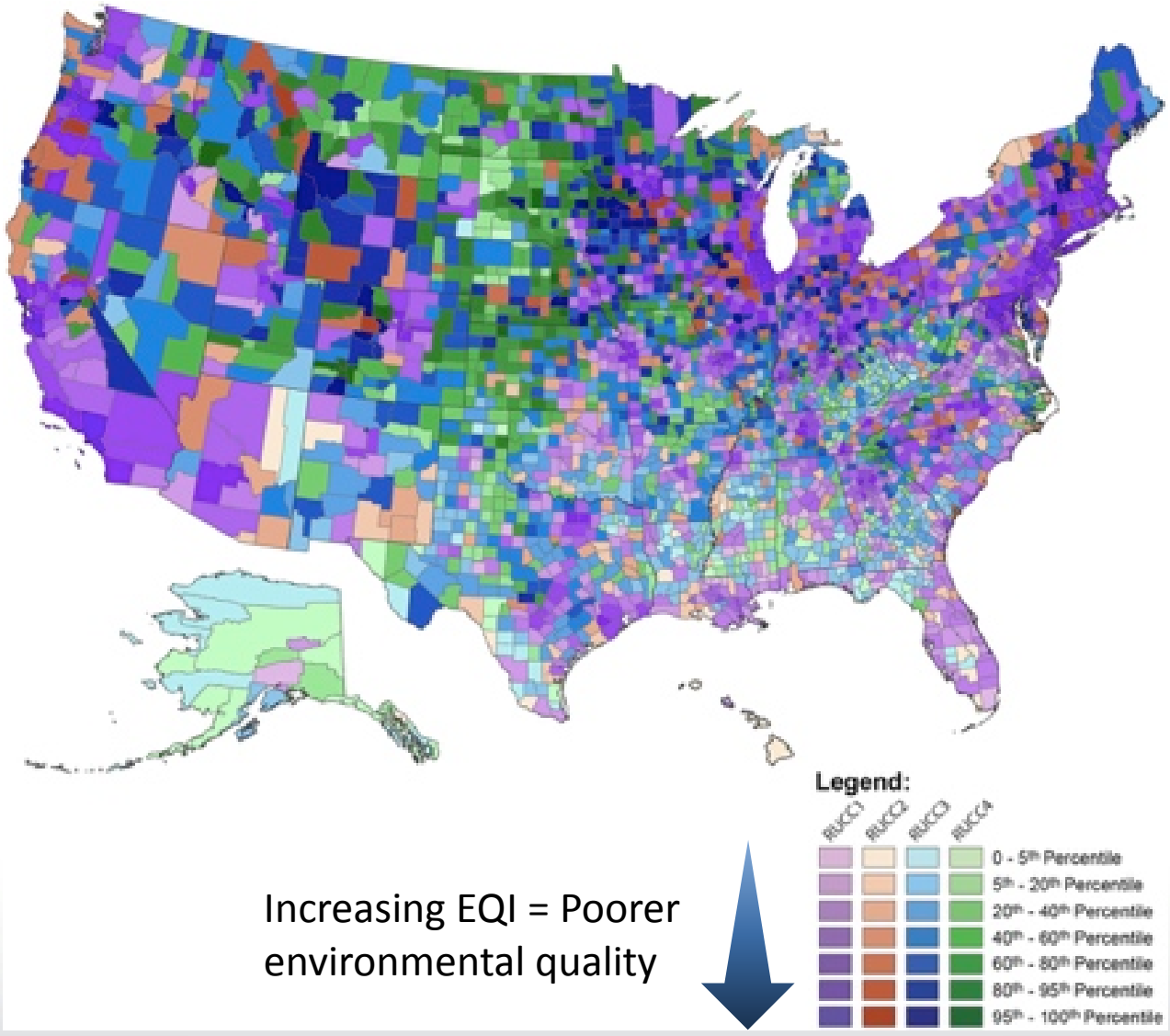
Risk Assessment & Public Health

- Interdisciplinary effort (toxicology, exposure science, epidemiology, engineering, social sciences, “omics”) to develop information and tools to facilitate better decision making by State and Community Health & Environmental Officials
  - Human Well Being Index
  - EnviroAtlas
  - Stormwater Calculator
  - StreamCAT
  - ~~Community Vulnerability~~ Index (wildfire smoke)
  - Environmental Quality Index

Risk Assessment & Public Health

- How can we quantitatively link environmental quality to human health outcomes?
- Development of Environmental Quality Index (EQI) (Messer *et al.* *Environ Hlth* 13:39, 2014)
  - Data on environmental quality in 5 domains
    - Air (e.g., Air Quality System and National Scale Air Toxics Assessment)
    - Water
    - Land
    - Built environment
    - Sociodemographic
  - Data reduction using Principal Components Analysis to derive domain-specific indices and overall EQI
  - Temporal (2000-2005) and geographic (US county level) restriction
  - Domain-specific indices and overall EQI stratified by 4 rural-urban continuum codes

Risk Assessment & Public Health



Risk Assessment & Public Health

- Studies associating EQI to specific human health outcomes
  - Preterm birth (Rappozza *et al. Environ Hlth* 14:50, 2015)
    - Strongest associations with air and sociodemographic domains
  - Overall mortality (Jian *et al. Environ Health Persp*, Oct 7 epub)
  - Cancer (in press)

- EQI associations with health outcomes
  - Measures impact of cumulative exposures to real-world mixtures
  - Given limited resources, can guide most effective public health interventions
  - Hypothesis generating

Challenges/Opportunities

- Exposures seldom involve single chemical exposures. How do we effectively address the risk of real-world, simultaneous exposure to multiple chemicals?
  - Does AOP framework and/or EQI offer viable approaches?
- Cumulative exposure
  - Need to better understand consequences of chronic, low-dose and multiple sequential exposures
    - EQI can point us in right direction
- Better understanding of toxicant susceptibility variation across population subgroups, as well as impact of life-stage differences
- Greater attention to ecological integrity and integration with human health outcomes

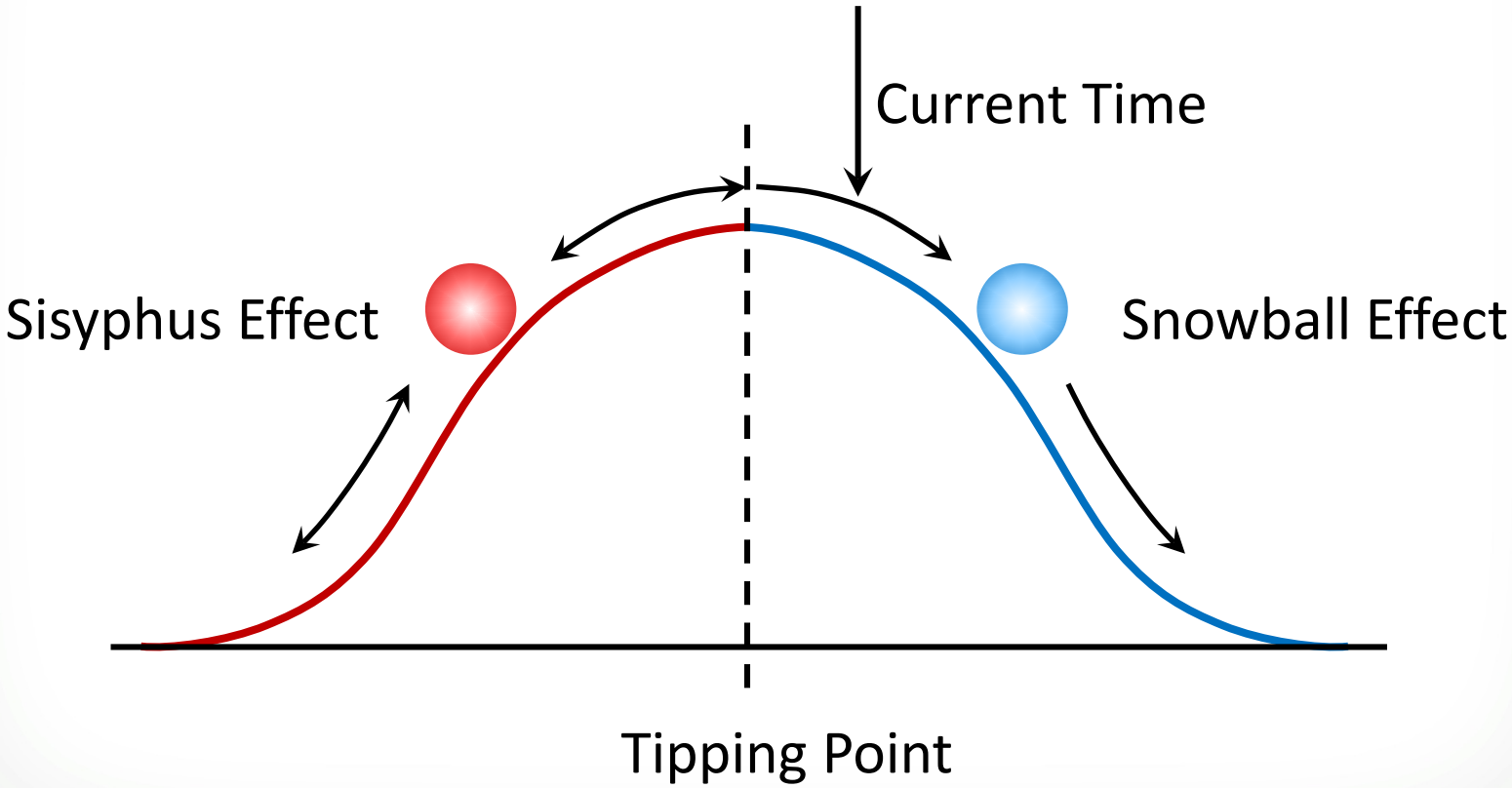
Challenges/Opportunities

- Integration of ADME prediction into high throughput and computational approaches
  - AEP Framework
- F.R. Lautenberg Chemical Safety for the 21st Century Act
  - Requires safety review of new chemicals **BEFORE** allowed on market
  - *Health-based assessment versus combined health and economic evaluation*
  - *Explicitly requires protection of vulnerable populations*
  - Limits claims of confidential business information
  - *Requires reduction in animal testing, therefore encourages TT21 approaches*
  - Prioritization and expedite testing of persistent and bioaccumulative carcinogens

Summary

- Evolution of mode of action framework into a chemically agnostic, adverse outcome pathway (AOP)
  - Systems-based data framework that facilitates integration of modifiable factors (*e.g.*, genetic variation, life stages), understanding of networks, and mixtures
- AOPs drive development of predictive models for risk assessment based on assembly of high throughput assay, mechanistic and molecular epidemiology daga representing AOP key elements
- Birth of computational exposure science capable of large-scale predictive exposure models
- Although still in its infancy, development of non-targeted analysis to begin addressing exposome
- Systems-based AEP integrating exposure, toxicokinetics and AOPs into a comprehensive framework

Progress?



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